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Im Gedenken an jene, die zu früh gegangen sind.

„Wer glaubt, etwas zu sein, hat aufgehört, etwas zu werden.“

Sokrates

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List of Abbreviations

AC	alternating current
AFM	atomic force microscopy
AgNP	silver nanoparticle
ALD	atomic layer deposition
APCVD	atmospheric pressure CVD
AuNP	gold nanoparticle
CMC	carboxymethyl cellulose
CTAB	cetyltrimethylammonium bromide
CTAC	cetyltrimethylammonium chloride
CVD	chemical vapor deposition
CVI	chemical vapour infiltration
DC	direct current
DCCA	desiccating controlling chemical additives
dH₂O	deionized water
DOS	density of states
E-beam	electron beam
ECD	electrochemical deposition
EDG	ethyl diglycol
EG	ethylene glycol
EtOH	ethanol
FTIR	Fourier-transform infrared
HFCVD	high frequency CVD
HiPIMS	high-power impulse magnetron sputtering
HOMO	highest occupied molecular orbital
HPPMS	high-power pulsed magnetron sputtering
ICB	ionized cluster beam
IL-6	interleukin 6
IR	infrared
LB	Langmuir-Blodgett
LbL	layer-by-layer
LFA	lateral flow assay
LPCVD	low pressure CVD
LSP	localized surface plasmon
LSPR	localized surface plasmon resonance
LSW	Lifshitz–Slyozov–Wagner
LUMO	lowest unoccupied molecular orbital
MIF	metal island film
MO	molecular orbital
MOCVD	metal organic CVD
NP	nanoparticle
OLED	organic light emitting diode
PAH	poly(allylamine hydrochloride)
PCD	physical vapor deposition
PdNP	palladium nanoparticle
PE	polyethylene

PECVD	plasma-enhanced CVD
PEG	polyethylene glycol
PEI	polyethyleneimine
PEM	polyelectrolyte multilayer
PET	polyethylene terephthalate
PETG	polyethyleneterephthalate glycol
PML	Polymer Multi-Layer
PMS	pulsed magnetron sputtering
PMVE-MA	poly(methyl vinyl ether-alt-maleic acid)
POC	point-of-care
PPR	particle plasmon resonance
PSA	pressure-sensitive adhesive
PSS	poly(styrene sulfonate)
PSS-MA	poly(4-styrenesulfonic acid-co-maleic acid)
PUR	polyurethane
PVD	physical vapor deposition
PVOH	polyvinyl alcohol
PVP	polyvinylpyrrolidone
R2R	roll-to-roll
REA	resonance enhanced absorption
RF	radiofrequency
RH	relative humidity
RHS	REA humidity sensor
RHSC	REA humidity sensor coating
RT	room temperature
SDS	sodium dodecyl sulfate
SPP	surface plasmon polariton
SPR	surface plasmon resonance
TEC	triethyl citrate
TEOS	tetraethyl orthosilicate
TPU	thermoplastic polyurethane
TRIS	tris(hydroxymethyl)aminomethane
TRIS base	same as TRIS
UV	ultraviolet
VOC	volatile organic compound
w/o	without

1 Introduction

Resonance enhanced absorption (REA) coatings are multilayer nanostructures that act as optical interference filters. As a result they exhibit bright colors, which may be tuned by variation of composition and thickness of individual layers. A broad range of potential applications of REA coatings and of substrates functionalized with these structures – herein further called REA materials – has been identified in the past decade. Especially for rapid tests and indicators they represent interesting novel platforms. To succeed in implementing REA materials in commercial analytical systems reliable performance must be guaranteed – a challenging task considering the inherent fragility of nanostructures. In addition, reproducible medium- to large-scale fabrication methods are required to decrease production costs to a competitive level.

Two REA-based sensor concepts were in the focus of this doctoral project – REA humidity sensors and REA immunoassays. Due to methodological problems REA humidity sensor materials could only be fabricated manually so far and their optical and sensor properties were generally neither reproducible nor stable. REA immunoassays were first described in 1998, but their limited sensitivity hampered the conversion of the technology into commercial assays.

Accordingly the central aims of this project were

- developing procedures for the preparation of REA humidity sensor materials, which yield reproducible quality and sensor performance and can be automated,
- improving the stability of these materials and their sensor properties and
- increasing the sensitivity of REA immunoassays via optimization of fabrication procedures or secondary enhancement technologies.

REA structures typically comprise several very dissimilar building blocks. Hence, their manufacture involves a variety of synthesis and deposition technologies. The first part of this thesis provides an overview of nanomaterials employed in REA coatings, their properties and methods for their preparation. Moreover, the physical basics of REA are introduced and the mode of operation of REA humidity sensors and REA immunoassays is discussed. The experimental part covers the progress achieved in assembly, stability and performance of the two assay systems.

2 Nanomaterials

2.1 Definitions

In principle, a material may be considered a nanomaterial, if a single unit of the material has a size of 1 to 1000 nm in at least one dimension. In most (legal) definitions a size range of 1-100 nm – also referred to as “nanoscale” – is preferred, however [1].

On 18 October 2011 the European Commission adopted the “Recommendation on the definition of a nanomaterial”, which defines the term “nanomaterial” and in addition the terms “particle”, “aggregate” and “agglomerate” as follows:

“2. ‘Nanomaterial’ means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

3. By derogation from point 2, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

4. For the purposes of point 2, ‘particle’, ‘agglomerate’ and ‘aggregate’ are defined as follows:

(a) ‘particle’ means a minute piece of matter

(b) ‘agglomerate’ means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;

(c) ‘aggregate’ means a particle comprising of strongly bound or fused particles.”

(European Commission, 2011, p. 3 [2])

Commonly, nanomaterials are further subdivided based on the number of dimensions, in which the size of the material is not confined to the nanoscale:

0D-nanomaterials: The size of the material ranges from 1-100 nm in all three dimensions (e.g. clusters, nanoparticles).

1D-nanomaterials: The material is larger than 100 nm in one dimension (e.g. nanotubes, nanorods, nanowires).

2D-nanomaterials: The material is nanosized in only one dimension (i.e. films with a thickness of 1-100 nm e.g. nanolayers, nanofilms, nanocoatings).

Larger units of solid materials i.e. materials not sized between 1-100 nm in any dimension are commonly referred to as bulk – with the exception of so-called “3D-nanomaterials”:

3D-nanomaterials: The size of the material itself does not necessarily range between 1-100 nm in any dimension, but it consists of equiaxed nanosized grains (e.g. polycrystals) [3].

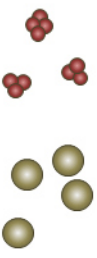
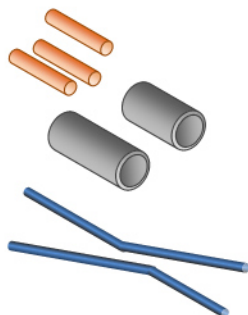
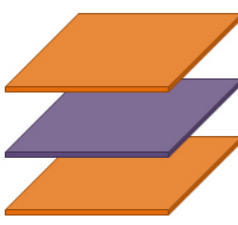
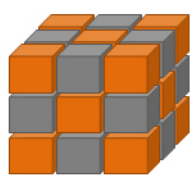
0D	1D	2D	3D
clusters, nanoparticles	nanowires, nanotubes, nanorods	films, coats	polycrystals
			

Figure 1 | Types of nanomaterials (modified from [4])

2.2 Preparation of Nanomaterials - Top-Down & Bottom-Up

Strategies pursued in preparing nanomaterials and nanostructures can be divided in two categories:

- In **top-down approaches** such as milling, nanosized matter is prepared from larger units of the respective material – generally by destructive or dissociative forces.
- **Bottom-up methods** rely on the arrangement of smaller units such as atoms or molecules into larger assemblies – a typical example being the wet-chemical synthesis of nanoparticles.

Nanocoatings and thin films are commonly generated in bottom-up processes such as physical vapor deposition (see 2.4.3). Etching, which represents a classical top-down method, can be employed to structure nanolayers e.g. for the fabrication of electric circuits [5].

For the successful implementation of nanomaterials in technologies and products two key requirements must be fulfilled:

- The nanomaterials must show sufficient thermal, chemical and structural stability. Some nanostructures exhibit pronounced metastability, which can occasionally be sufficient. Otherwise passivation and stabilization can be required.
- The synthesis (and passivation) process must be reproducible and scalable [6]. Unfortunately, most methods for the preparation of nanomaterials that have been developed so far are not suited for large-scale production or do not yield stable nanomaterials [7].

2.3 Nanoparticles

2.3.1 Definition

In accordance with the recommendation of the European Commission the term “nanoparticle” herein refers to particles that range in size between 1 to 100 nm in all three dimensions. Alternatively the terms “clusters” and “colloids” often occur in literature, but are rarely defined precisely. According to Pradeed et al. the term colloid describes a dispersion of one phase, either solid or liquid, in a second phase, which does not separate or sediment spontaneously. Colloidal particles typically exhibit a diameter of 10^{-6} to 10^{-9} m [8]. A range of definitions can be found for the term “cluster”. For instance, McGraw-Hill Dictionary of Scientific & Technical Terms defines clusters as aggregates of three to a few thousand atoms (or molecules) with properties intermediate between single monomers and bulk material [9]. Often, clusters are considered to be smaller than colloids (see e.g. [10]). Nevertheless, the definitions of both types of particles generally overlap and both commonly include but are not restricted to nanoparticles. Hence, all three terms will be used alternately in this thesis if appropriate and may be considered as partially interchangeable.

2.3.2 Properties

2.3.2.1 Surface & Quantum Effects

It is a major advantage of nanoparticles (NPs) that several of their physical and chemical properties can be modified by varying their size without having to change their chemical composition [11]. Two groups of properties can be distinguished scaling differently with particle diameter:

- 1) Features that are governed by the fraction of atoms at the particle surface scale smoothly with size.
- 2) Quantum effects are related to the electronic structure of atom ensembles (e.g. the filling of orbitals) and therefore show discontinuous behavior.

Surface effects: The fraction of atoms at the surface of a particle, the so-called dispersion (F), is inversely proportional to the particle radius. It increases with decreasing particle size. Surface atoms differ from atoms in the bulk by having fewer direct neighbors. Thus, they are connected to the particulate by fewer chemical bonds. Due to this lower coordination number they show less cohesion to the remaining ensemble than atoms within the particle. In return, they are more affine to external binding partners. Atoms at edges and corners of particles are

usually even less stabilized than in-plane surface atoms. For this reason they are even more reactive and are commonly missing in single crystals in thermodynamic equilibrium. Apart from affecting the chemical activity the cohesive energy between atoms determines the melting temperature of individual parts of the ensemble. Accordingly, the melting point of the outmost layer is lower than that of the core [12]. The overall melting point of a particle scales inversely with its size [13]. However, small particles show a special melting behavior. They do not exhibit a sharp melting point like bulk materials. The melting process rather spreads over a certain temperature range which broadens with decreasing diameter [12].

Quantum effects: As mentioned, quantum effects are governed by the electronic structure of atom ensembles. The electrons in isolated atoms exist in spatially localized atomic orbitals representing discrete energy levels. In atom ensembles (e.g. molecules) these orbitals combine or “overlap”. In the simplest case, the H_2 molecule, each hydrogen atom contributes one 1s orbital to the molecule. Two “delocalized” molecular orbitals (MOs) are formed that span the whole H_2 molecule: one so-called bonding orbital with lower energy than the two individual atom orbitals and one anti-bonding orbital with higher energy. The two electrons provided by the respective H atoms are redistributed over the MOs in a way that the potential energy of the molecule is minimized. Thus, both electrons occupy the bonding orbital, whereas the anti-bonding orbital remains empty. The same principle applies for larger molecules, clusters and bulk materials but the orbital structure is more complex in these cases. Several atomic orbitals form one molecular orbital. Hence, the density of energy levels or “density of states” (DOS) in the individual MOs increases with the number of involved atoms. In parallel, the MOs become slightly wider (see Figure 2). As a result the distances between them decline. For optical and electronic processes the distance between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) -also termed the HOMO-LUMO energy gap – is most interesting [14]. In bulk matter it is generally referred to as Kubo gap (and the numerous overlapping atomic orbitals in bulk materials are said to form extended electronic band structures instead of MOs).

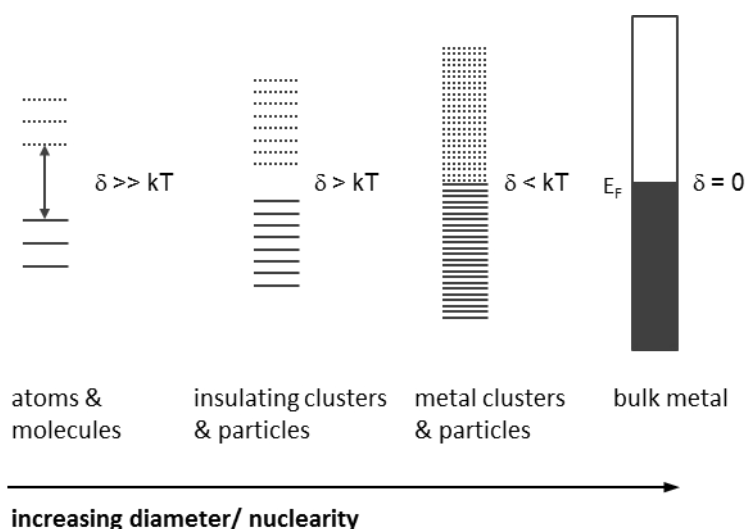


Figure 2 | Evolution of band gap and density of states with increasing particle diameter The density of states within molecular orbitals increases with the number of atoms in the cluster while the band gap between HOMO and LUMO (also called Kubo gap, δ) narrows (E_F = Fermi energy) (modified from [12])

The Kubo gap describes the energy difference between the highest occupied state in the valence band and the lowest unoccupied state in the conduction band. If it equals thermal energy, electrons can get excited into the conduction band above a certain threshold temperature, which makes electronic conductivity possible. With increasing number of atoms in an ensemble this energy gap narrows and can even become zero. The size-dependency of the absorption and emission wavelength of fluorescent semiconductor NPs is an illustrative example for this process. As demonstrated e.g. by Eychmüller [15] fluorescent emission of colloidal CdSe–CdS core–shell NPs can be tuned from blue (as emitted by particles of a diameter of 1.7 nm) over green and yellow to red by increasing the diameter by just a few nanometers. (Particles of a diameter of 6 nm already emit in the red range of the visible spectrum.) Of course, light absorption in such clusters - which are commonly referred to as “quantum dots” - follows a similar size-dependence. The color of metal colloids also changes as a function of size. However, this effect is not based on the electronic band structure of the clusters but on a phenomenon called “surface plasmon resonance” [12]. It is discussed separately in section 2.3.2.2.

Ionization potentials and electron affinities of NPs also scale discontinuously with cluster size as a result of the stepwise filling of orbitals with electrons. In this regard, small metal NPs exhibit pseudo-atom character as reported by Roduner [16]. He demonstrated that a strong odd-even effect is observed in the size-dependent variation of the electron affinity of small Au clusters (consisting of 1 to 70 Au atoms). Clusters consisting of an odd number of atoms generally show higher electron affinity than clusters with even atom number comprising only one more atom or one less, while the overall the electron affinity increases with increasing cluster diameter. This correlation is similar to the relationship of electron affinity and atomic number Z observed for the elements in the periodic table. Ionization potential and electron affinity govern the tendency of clusters to donate electrons in bond formation or redox

reactions. Together with the cohesive energy of surface atoms they therefore determine the size-dependence of the catalytic activity of NPs.

As described, the discontinuous scaling behavior of quantum effects can typically be observed clearly at small particle sizes. With increasing number of atoms in one cluster, discontinuities in the band structure of a cluster become more and more blurred. The scaling behavior of quantum effects appears to follow a smooth slope then. Thus, it becomes more difficult to distinguish from the scaling behavior of surface effects the larger the particle size [12].

2.3.2.2 Surface Plasmon Resonance

As mentioned above, light absorption by metal NPs is governed by a physical phenomenon termed surface plasmon resonance. A prominent example of this effect is the bright red color of gold NPs (AuNPs) in stained glass windows of churches [17]. Surface plasmons are coherent oscillations of conduction electrons at metal-dielectric interfaces that can be induced by the electromagnetic field of incident radiation [18]. In the bulk these plasmons propagate as surface waves along the interface between the metal substrate and the surrounding medium. They create an electric field that is strongly enhanced at the metal surface, but decays exponentially when penetrating into the surrounding medium. Thus, it extends only a few hundred nanometers into the dielectric and is very sensitive to changes in the refractive index within this region [19]. Electron oscillations and associated electromagnetic field together are also referred to as surface plasmon polariton (SPP) [20]. In NPs the field of incoming electromagnetic waves also induces the displacement of conduction electrons relative to the positively charged cores in the metal, which are considered as being immobile. This polarization creates a restoring force in NPs as it does in the bulk. Dipolar oscillations result – both within the metal and at the interface to the dielectric. However, surface plasmons cannot migrate in NPs due to the small size of the particles. They remain stationary and are therefore termed localized surface plasmons (LSPs). Surface and bulk plasmons in small particles are coupled. Thus, the charge density motion covers the entire volume of the individual clusters [19]. In the immediate environment of the NPs the oscillating electrons induce a polarization of opposite direction. This polarization decreases the restoring force and thereby dampens the electron oscillation. As a result, the particle plasmon resonance (PPR) is shifted to a lower frequency than that of the incoming waves (i.e. a higher wavelength). The extent of dampening can be fine-tuned via the dielectric constant of the medium surrounding the particle. The lower the dielectric constant the less the polarization induced in the medium i.e. by decreasing the dielectric constant in the environment of particles their resonance wavelength can be blue-shifted. If the dielectric constant of the surrounding medium is increased, PPR occurs at longer wavelengths. The shape of the PPR spectrum is determined by the NP dimensions and their size relative to the wavelength of the exciting radiation. If the particles are much smaller than the wavelength of the incident light or electron beam the electromagnetic field is uniform across each particle and dipole-type oscillations are excited. The corresponding PPR spectrum is characterized by a single, narrow peak. With increasing size, the electron

displacement across the particles becomes inhomogeneous. Multipole resonances (e.g. quadrupole, octopole) occur and several peaks appear in the PPR spectrum [21].

Surface plasmons can be excited by surface plasmon resonance i.e. by resonant interaction of conduction electrons with an electron or photon beam [19]. The conduction electron oscillation in the alternating field of the exciting radiation resembles a forced harmonic oscillator. Resonance only occurs if the exciting radiation matches plasma frequency of the substrate ω_p , which basically represents the resonance frequency of the forced harmonic oscillator [22]. It is correlated with the dielectric function of the substrate $\epsilon_r(\omega)$ also referred to as “relative permittivity”

$$\epsilon_r(\omega) = \epsilon_r' + i\epsilon_r'' \quad (1)$$

The frequency-dependent dielectric function describes the response of materials to applied electromagnetic fields with the (angular) frequency ω . ϵ_r' and ϵ_r'' are correlated with the refractive index n and the extinction coefficient κ according to

$$n^2 - \kappa^2 = \epsilon_r' \quad (2)$$

and

$$2n\kappa = \epsilon_r'' \quad (3)$$

In principle surface plasmon resonance can occur at any frequency, for which the real part of the dielectric function is less than zero. The quality of the resonance is determined by the value of the imaginary part then [23]. The relationship between the dielectric function $\epsilon_r(\omega)$ and the plasma frequency ω_p can be described by the so-called Drude model, which is a fundamental model in optics [24]. It is based on the assumption that most properties of metals can be explained by the behavior of their conduction electrons. These are considered as independent and quasi-free. For an ideal free electron gas ω_p and $\epsilon_r(\omega)$ are correlated according to

$$\epsilon_r = 1 - \frac{\omega_p^2}{\omega^2 + i\gamma\omega} \quad (4)$$

The scattering frequency γ , sometimes also expressed by the scattering time $\tau=1/\gamma$, reflects all scattering processes occurring in the material [23]. The Drude model basically covers absorption processes, which result in the excitation of electrons from an occupied to an unoccupied state within the conduction band. These so-called intraband transitions occur in the infrared-visible range of the electromagnetic spectrum. However, if the energy of the incident radiation is higher than a given threshold energy, also interband transitions (i.e. transitions from the valence band to the conduction band) are possible. For simple metals such as alkali or trivalent metals, this threshold energy lies outside the optical domain of the electromagnetic spectrum (covering near UV, visible and near IR range). Thus, their optical properties in the visible range may quite accurately be interpreted by the Drude model. In the

case of noble metals interband transitions are possible in the near UV and visible range and calculations based on the Drude model deviate from the real situation [25]. To consider the contribution of these interband transitions to the metal polarizability the term ε_0 is added to the formula

$$\varepsilon_r = \varepsilon_0 - \frac{\omega_p^2}{\omega^2 + i\gamma\omega} \quad (5)$$

as described by Derkachova et al. [26]. In the bulk, however, not only the frequency plays a decisive role for the excitation of a surface plasmon. The momentum of the incident radiation must also be equal to that of the plasmon. The two parameters are correlated via the so-called dispersion relation, which is different for free-space photons and SPPs. At the same frequency the momentum of a photon in the dielectric environment is always lower than that of the SPP in the metal substrate. Hence, neither direct resonant excitation by incident light is possible in the bulk, nor can SPPs radiate from smooth metal surfaces into the surrounding medium in the form of photons. In order to couple light into bulk metal, the momenta of photons and SPPs need to be matched, which can be achieved by the use of prisms or via diffraction on gratings or defects on the metal surface. In contrast, surface plasmons in NPs can be excited by photons of appropriate frequency irrespective of their momentum. Likewise, LSPs can also decay with the emission of light [27]. The excited plasmon oscillation can be damped non-radiatively by absorption and/ or radiatively by resonant scattering. Absorption, which relies on electron-phonon interactions, is the only damping mechanism occurring in small NPs (e.g. Ag particles of <30 nm). In larger particles both processes contribute to the extinction of incident radiation and the importance of resonant scattering increases with size. At very large particle diameters (e.g. 75 nm in the case of Ag) light extinction is primarily mediated by scattering, while absorption plays only a minor role [21]. Even if small, NPs of simple metals including Pb, Hg, Cu and Cd do not show strong color effects, however, because their resonance frequencies are located in the UV range. As mentioned, the coinage metals including Au, Ag and Cu exhibit different behavior. Due to interband transitions they absorb electromagnetic radiation in the visible range of the spectrum. Moreover, these metals are characterized by strong near-field enhancement, because the imaginary part of their dielectric constant, which determines the energy losses occurring in the polarization process, is very small. Apart from the dielectric properties of their base material, also particle size and shape, interparticle interactions and, as explained, the local environment affect the resonance frequencies of NPs [19]. Interparticle interactions are observed if two excited particles are brought into close proximity to each other. If the distance between them is reduced to less than their diameter, charge oscillations induced in the individual particles couple. As a result the plasmon resonance of each particle splits in two resonances, a longitudinal and a transverse mode. The longitudinal mode i.e. the charge oscillations along the interparticle axis (see Figure 3) are dampened because surface charges on one particle face opposite surface charges on the other particle. This reduces the restoring force in both particles and shifts the longitudinal mode to lower energies (i.e. higher wavelengths) [28]. In contrast, the charge oscillations perpendicular to the interparticle axis, the so-called transverse mode, are enhanced and thus blue-shifted. The strength of the

coupling is affected by the dielectric constant of the surrounding medium and increases with decreasing interparticle separation. Thus it can also be employed to measure distances in the nanometer range [29].

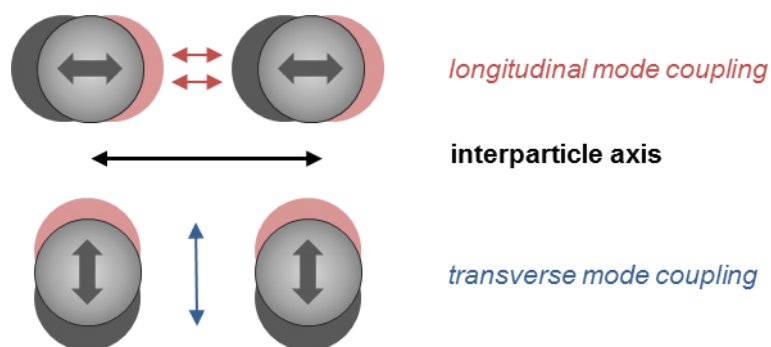


Figure 3 | Interparticle coupling Longitudinal mode coupling decreases the restoring force in the interacting particles. The resonance frequency is lowered (red-shifted) (top). Charge oscillations perpendicular to the interparticle axis are enhanced by transverse mode coupling. The resonance frequency is shifted to higher values (blue-shifted) (bottom) (modified from [28]).

2.3.3 Synthesis of Nanoparticles

In the past two to three decades a variety of top-down and bottom-up methods for the preparation of NPs has been developed [30] as illustrated in Table 1.

TOP DOWN	Solid phase methods	<i>Grinding methods</i>	Dry grinding
			Wet grinding
		<i>Mechanochemical methods (mill & ultrasonic wave)</i>	
		<i>Mechanical alloying method</i>	
BOTTOM UP	Solid phase methods	<i>Chemical methods</i>	Chemical vapor deposition (CVD)
			Thermal decomposition method
		<i>Physical methods</i>	Physical vapor deposition (PVD)
	Liquid phase methods	<i>Liquid/liquid methods</i>	Chemical reduction method
			Indirect reduction method (via e.g. photoreduction, gamma rays, ultrasonic waves)
			Spray drying
			Spray pyrolysis
			Solvothermal synthesis
		<i>Sedimentation methods</i>	Sol-gel method
			Co-precipitation method
			Hydrolysis

Table 1 / Nanoparticle preparation methods (modified from [31])

The nanoparticles employed in this thesis were primarily prepared by wet-chemical bottom-up techniques. Thus, the following chapters will focus on those synthesis routes and will illustrate how shape and size of the generated particles can be controlled in order to tune their chemical and physical properties.

2.3.3.1 Nucleation and Growth in Solution

Wet-chemical synthesis of NPs is achieved by assembling dissolved ionic, atomic or molecular units (generally referred to as “monomers”) into solid structures through various chemical reactions. Thermodynamically the process is subdivided in nucleation and growth phase.

As defined by Mullin [32], nucleation is a process whereby a new phase is generated from one phase. When nuclei form homogeneously throughout the parent phase one speaks about primary or homogeneous nucleation. In contrast, secondary or heterogeneous

nucleation occurs at structural inhomogeneities such as container surfaces, impurities or bubbles. Depending on the free energy of the formed nuclei they either grow or are re-dissolved. The free energy of a nucleus ΔG is determined by its surface energy γ , its radius r and the free energy of the bulk material ΔG_v according to

$$\Delta G = 4\pi r^2 \gamma + \frac{4}{3}\pi r^3 \Delta G_v \quad (6).$$

ΔG_v is dependent upon the Boltzmann constant k_B , the temperature T , the supersaturation of the solution S (i.e. the monomer concentration in the solution) and the molar volume of the monomer v according to

$$\Delta G_v = \frac{-k_B T \ln(S)}{v} \quad (7).$$

The surface energy γ is always positive, whereas the free energy of the bulk material ΔG_v is always negative. At a certain particle radius ΔG thus reaches a maximum, the critical free energy ΔG_{crit} (see figure below). The respective radius, also termed critical radius r_{crit} , defines the minimum size at which a particle can survive in solution without being re-dissolved. [33]

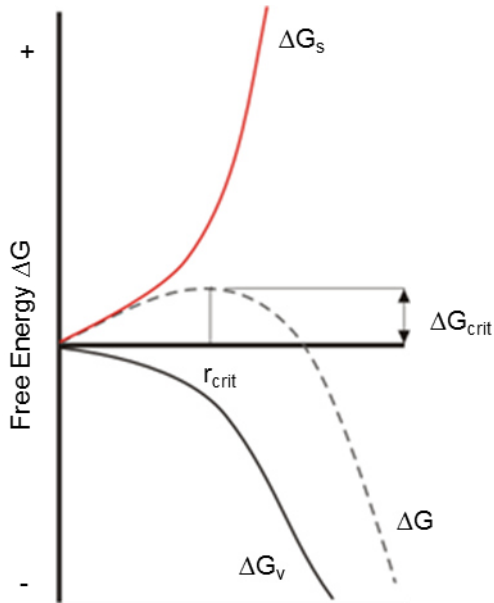


Figure 4 / Free energy of nucleus in solution (modified from [33])

Structural inhomogeneities in the parent phase facilitate nucleation. Nuclei assembling on support surfaces do not adopt a spherical shape, however, but grow into caps that form a spherical contact angle θ with the substrate. Depending on the affinity between nucleus and support, which governs the size of θ , the surface energy of the growing nanoparticle is reduced. Hence, the critical free energy required for heterogeneous nucleation ΔG_{crit}^{hetero} is lower than the critical free energy required for homogeneous nucleation ΔG_{crit}^{homo} according to

$$\Delta G_{crit}^{hetero} = \phi \Delta G_{crit}^{homo} \quad (8)$$

The correction term ϕ is a function of the contact angle θ :

$$\phi = \frac{(2 + \cos \theta)(1 - \cos \theta)^2}{4} \quad (9)$$

Thus, heterogeneous nucleation is thermodynamically favorable [33].

Stable nuclei enter the growth phase, which determines the final size, size distribution and shape of the prepared particles. The growth process is governed and can be limited by two factors: the diffusion of the monomers to the nucleus surface and the rate of reaction of the monomers at the surface. At high monomer concentration the growth rate is diffusion-limited and the monomers are immediately precipitated onto the particle surface when reaching it. This type of growth is commonly desirable in the preparation of monodisperse NPs i.e. disperse particles of uniform size. Surface reaction-limited growth is observed at low monomer concentration and influences particle shape [30].

2.3.3.2 Nucleation and Growth Models

The first model on the wet-chemical synthesis of NPs was proposed by LaMer in 1950 [34]. According to this theory NP formation can be subdivided into three sequential stages, pre-nucleation, nucleation and growth. In the pre-nucleation stage, the monomer concentration in the solution is increased rapidly [33]. In open systems, this is achieved by adding the monomer from outside the system using e.g. a constant rate pump. In closed systems, monomers are created within the system via chemical conversion of a precursor compound [35]. When a critical monomer concentration is reached, “burst-nucleation” occurs, which consumes monomers at a high rate. Their concentration drops significantly until almost no further nucleation is possible. The newly formed nuclei then enter the third stage and undergo diffusion-controlled growth [33]. The growth phase determines the final size distribution of the generated particles [36]. A prominent model for diffusion-controlled growth was suggested in 1900 by Ostwald [37]. In the so-called Ostwald ripening process, nanoparticle growth is determined by the size-dependent solubility of nanoparticles. The model assumes that smaller nuclei dissolve due to their higher surface energy and thereby provide monomers for the growth of larger particles [33]. The inverse process was described by Lee et al. in 2005 [38]. They observed so-called “digestive ripening” i.e. the growth of smaller particles at expense of larger ones. Another model for nucleation and growth, the Finke-Watzky two-step mechanism, was also described more recently [39]. In this model, slow continuous nucleation and autocatalytic growth, which is not diffusion-controlled, occur simultaneously. This mechanism was observed in the formation of transition metal NPs via reduction by hydrogen [33].

2.3.3.3 Nanoparticle Stabilization

Due to Van der Waals interactions unprotected NPs tend to aggregate and precipitate. To keep dispersions monodisperse they can be stabilized electrostatically or sterically via modification of the particle surface. Electrostatic stabilization is commonly achieved by adding counterions (such as citrate or chloride in the case Au NPs). Thereby an electrical double layer is created on the particle surface. Coulombic repulsion between counterion layers on individual particles counteracts coagulation. Alternatively the particles can be coated with polymers or surfactants. These confer steric stabilization by entropic as well as osmotic effects. As the interparticle distance shrinks, the configurational freedom of the adsorbed molecules decreases. The resulting reduction in entropy is energetically unfavorable and thus prevents agglomeration. In addition, polymer and surfactant molecules are osmotically active. Their concentration increases the narrower the interparticle space becomes. This elicits a net flux of solvent molecules into the interparticle region, which keeps the coated particles apart [40].

2.3.3.4 Liquid Phase Synthesis Methods

In order to introduce basic concepts of nanoparticle preparation in solution some common liquid phase synthesis methods are described in the following. The list is by far not exhaustive, of course.

Precipitation & co-precipitation: Precipitation is one of the oldest methods for the preparation of ceramic powders and still commonly used for the synthesis of oxide NPs [41]. Depending on the employed reactants, different precipitation methods are distinguished: Direct precipitation, for example, is induced by adding a precipitant, which neutralizes the precursor solution. In homogeneous precipitation a precipitant is not required, because a decomposition product of a precursor takes over the role. In the co-precipitation method multi-component materials are generated by addition of a precipitant to a mixed-salt solution [42]. Some precipitation reactions directly yield oxides. In others, a precursor is generated, which must then be processed further, e.g. by drying and calcination, to convert it into the desired oxide. Calcination is a solid-state heat treatment that makes oxysalts (e.g. carbonates, hydroxides) decompose into oxides and gas. If coarse powders are calcined at the correct temperature, i.e. just above their decomposition temperature, internal stresses can lead to fracturing of the particles. Then fine materials can be obtained [43]. In contrast, calcination of nanopowders commonly results in at least partial agglomeration. It is thus generally not suited for the preparation of monodisperse oxides [41].

Sol-gel: Sol-gel processes are a group of chemical techniques, which allow for fabricating solid inorganic non-metallic materials (glasses, ceramics) at relatively mild conditions (see also 2.4.4.3). Solutions of monomers, oligomers, polymers or colloidal particles serve as starting materials in these reactions. The term “sol-gel” refers to the so-called “sol”, a solution of solid colloidal particles, and the gel that are obtained as intermediates during the

conversion of the precursors into the desired end product [44]. Typically a sol-gel process involves the following steps:

- 1) Preparation of a homogeneous precursor solution, typically in an organic solvent that is miscible with water or the reagent used to induce sol formation
- 2) Addition of this reagent – e.g. water with HCl for oxide ceramics – to the precursor solution leading to conversion of the precursor solution into a sol
- 3) Transformation of the sol into a gel by polycondensation (upon or without induction)
- 4) Shaping of the gel (or viscous sol) into the desired form such as thin film, fiber, spheres or grains
- 5) Conversion of the shaped gel into a solid ceramic material by sintering at temperatures which are typically lower than those required for melting larger pieces of ceramic material of the same type together

To obtain NPs, the process is interrupted after step 2 and the formed colloids are precipitated (see Figure 5).

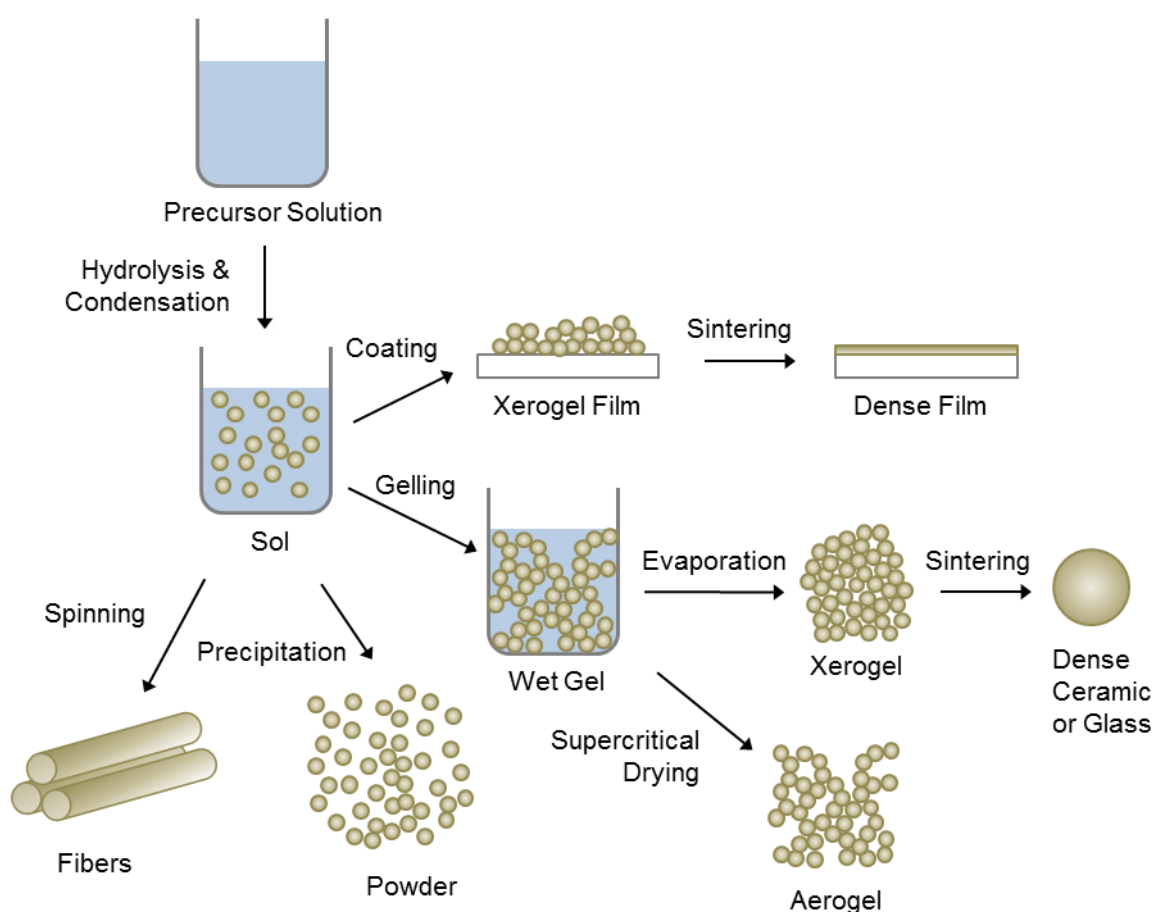


Figure 5 | Preparation of ceramic materials via sol-gel chemistry (modified from [45])

Sol-gel chemistry is most commonly employed for synthesizing oxide NPs. However, also carbides, nitrides and sulfides can be prepared by this approach [41]. In aqueous sol-gel processes inorganic metal salts or metal-organic precursors (mostly alkoxides) serve as

precursors. Generally amorphous particles are yielded in these reactions. Non-aqueous sol-gel chemistry allows for the fabrication of NPs with crystal morphologies, but is less popular [45].

Solvothermal & hydrothermal method: In the solvothermal method, nanoparticle formation is induced either by decomposition of one or more precursor species or by chemical reaction between precursors. In contrast to the previously described methods, the process is performed in an enclosed vessel at elevated pressure and at temperatures above the boiling point of the employed solvent [46]. Due to the higher solubility and reactivity of metal salts and complexes under these conditions, many inorganic materials can be prepared via the solvothermal method at significantly lower temperatures than required for solid-state reactions. In contrast to the products of sol-gel and coprecipitation reactions, solvothermally prepared NPs are typically crystalline. Thus, the approach has been investigated intensively for the preparation of nanocrystalline TiO_2 , an important photocatalyst for the decomposition of toxic compounds [41]. In principle, the term solvothermal can be used independent of the composition of the processed solution. Approaches, in which the solvent is water, are generally referred to as hydrothermal processes, however. The chemical nature of the precursors and the solvents is decisive for size and morphology of the generated NPs. Via hydrothermal synthesis processes, crystalline oxides but also hydroxides and oxyhydroxides can be synthesized [47]. In contrast to sol-gel and co-precipitation, a high-temperature calcination step is not always required in hydrothermal oxide syntheses. This represents a major advantage, because solid-state processing of nanoparticles commonly bears the risk of aggregation [48]. In solvothermal processes organic solutions are employed. These reactions allow for the preparation of crystalline oxides and highly porous silicates (e.g. zeolites). Moreover, crystalline non-oxide materials of technical interest such as carbon nanotubes, carbides, diamonds, chalcogenides and nitrides can be synthesized [7].

Polyol method: In this synthetic route a high-boiling alcohol is employed as solvent and reducer at the same time. It represents a promising technique for the preparation of both metal and oxide NPs [49] and offers good control over particle size and shape. Polyols like polyethylene glycol [50] or 1,2-hexanediol [51] are capable of dissolving inorganic metal precursors due to their high dielectric constants. Furthermore, they can reduce the precursors at elevated temperatures. Occasionally, they can act as stabilizers in the polyol process as well [50]. If not, additives such as oleic acid or oleylamine can be added to the precursor solution to control particle growth and prevent aggregation [51]. By seeding the solution with foreign particles, nucleation and growth phase can be separated completely from one another. That way, the particle size can be controlled more precisely than in the direct approach and uniform nanoparticles are obtained [50].

Reduction method: Chemical reduction of metal cations represents the most common technique for the preparation of metal NPs in solution. The process is facile and can yield large quantities of metal particles of various shapes within a short time [31]. In the direct reduction approach a reducing agent is added to an aqueous or non-aqueous metal precursor solution, which mediates reduction of the dissolved metal cations to atoms. These

metal atoms are insoluble in the solvent and thus assemble into nuclei. Most commonly gaseous H_2 , solvated ABH_4 (A standing for an alkali metal), hydrazine hydrate or hydrazine dihydrochloride are employed as reducing agents [41]. Sodium citrate or ascorbate are frequently used for the reduction of silver ions [52]. Capping or dispersing agents are generally added to prevent aggregation, but often they also affect the morphology of the prepared particles. Moreover, particle size and shape can be fine-tuned by varying reducing agent, solvent, reaction time and process temperature [31] (see also 2.3.3.5). Reduction can also be induced by other means than reducing agents: Radiation-assisted reduction, for instance, offers the advantage that fewer impurities are introduced in the particle preparations. A prominent example of this approach is the preparation of silver NPs by photoreduction i.e. by exposure of the precursor solution to UV or visible light. Electrochemical reduction techniques [41] and bio-based approaches relying on the action of microorganisms have also been described in context with nanoparticle preparation [52], but are of minor importance so far.

2.3.3.5 Controlling Nanoparticle Morphology

As mentioned in the previous sections, nanoparticle size and shape can be fine-tuned in liquid phase preparation by varying process parameters (pH, temperature, pressure), solvent(s) and precursor(s). Many of the underlying mechanisms have been elucidated meanwhile. This strongly facilitates the development of new synthesis protocols for particles with special morphology. Nevertheless, the best suited reagents and process parameters can usually not be predicted but need to be identified in a laborious trial-and-error process [36].

Seeding, template growth or capping agents can often improve synthesis results. Capping agents influence cluster growth by selectively adsorbing to their surface [30]. In some cases, they stabilize NPs of a certain size and preparations with very narrow size distribution are obtained [36]. In other cases the adsorbed capping agent molecules lower the surface energy of individual facets of the clusters. The growth of these facets is promoted thereby and particles with special (anisotropic) shapes are yielded. Commonly surfactants like cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), polyvinylpyrrolidone (PVP) and sodium dodecyl sulfate (SDS) are used as capping agents [30]. Seeded growth has proven an effective strategy if large NPs with narrow size distribution are aspired. Seeds for the synthesis of noble metal particles are typically prepared by fast reduction in the presence of a stabilizer such as sodium citrate. Afterwards a mild reducing agent is usually used to grow the seeds into clusters of the desired size. In this second step of the preparation process, fresh precursor is repeatedly supplied to the reaction solution in small doses only. It is reduced relatively slowly due to the weak reducing agent. Thereby de-novo nucleation is avoided [36]. Seeds are generally significantly smaller than the final particles and therefore do not affect their shape. Template growth works by the same principle but employs larger particles. Thus, the morphology of the final particles strongly depends on the initial shape of the templates [30].

2.4 Nanolayers & Nanocoatings

2.4.1 Overview

Nanolayers and thin films make it possible to design the interface between an object and its environment without significantly changing its bulk properties, its shape and dimensions. They may be used to render surfaces self-cleaning or scratch-resistant, to optimize the light reflection properties of a surface or provide it with sensor properties. Such functional coatings are nowadays in the focus of attention in many industrial branches including electronics, machine building, car and aircraft manufacturing or human health care [53]. This has stimulated the development of a wide range of coating technologies (see Table 2), each one having characteristic advantages and limitations in terms of potential substrates and substrate geometries, deposition rate, control over nanolayer properties and materials that can be deposited. The coating methods, which were most relevant for this thesis, are discussed in the following paragraphs.

GAS PHASE DEPOSITION	<i>Physical vapor deposition (PVD)</i>	Evaporation
		Sputter coating
		Ion beam assisted deposition
		Ion plating
		Ionized cluster beam (ICB) deposition
	<i>Chemical vapor deposition (CVD)</i>	Atmospheric pressure CVD (APCVD)
		Plasma-enhanced CVD (PECVD)
		Low pressure CVD (LPCVD)
		High frequency CVD (HFCVD)
		Metal organic CVD (MOCVD)
		Chemical vapour infiltration (CVI)
		Atomic layer deposition (ALD)
LIQUID PHASE DEPOSITION	<i>Plating</i>	Galvanic plating/ electroplating
		Electroless plating
	<i>Chemical solution deposition</i>	
	<i>Chemical bath deposition</i>	
	<i>Sol-gel deposition</i>	
	<i>Langmuir-Blodgett coating</i>	
	<i>Monolayer self-assembly</i>	
	<i>Polymer grafting</i>	
	<i>Layer-by-layer (LbL) deposition</i>	
DEPOSITION FROM AEROSOL	<i>Spray coating</i>	Pneumatic spraying
		Ultrasonic atomization
		Electrostatic spraying

Table 2 | Thin film preparation methods

2.4.2 Growth Modes

The formation of solid thin films is governed by the interplay of thermodynamics and kinetics. Thermodynamically, the observed growth processes can be explained via the surface energies of substrate and adsorbed materials and the interfacial energy between them. From the kinetic point of view, thin film growth is a non-equilibrium process. Rate-limiting steps determine how film components adsorb to the substrate. Generally, three principal growth modes are distinguished:

- 1) Frank-van der Merve mode: The deposited atoms or molecules bind more strongly to the substrate than to each other. As a result, a continuous monolayer develops. Only after completion of this layer the next one starts to form.
- 2) Volmer-Weber mode: Binding between film components is favored over adsorption to the substrate. Thus, the deposited atoms or molecules aggregate in separate three-dimensional islands reaching thicknesses of several atomic layers. These islands can eventually coalesce into a continuous thin film.
- 3) Stranski-Krastanov mode: This growth mode basically represents a combination of the previous two modes. First, one or a few monolayers are generated. Then separate islands nucleate upon them [54, 55].

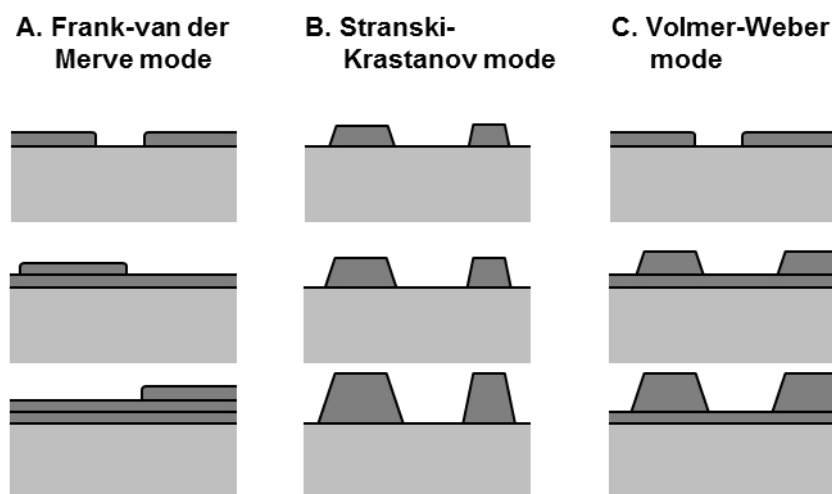


Figure 6 | Growth modes A) Frank-van der Merve mode B) Volmer-Weber mode C) Stranski-Krastanov mode (modified from [55])

2.4.3 Gas Phase Deposition Technologies

In vapor deposition thin films are prepared by depositing atoms or molecules from the gas phase. Two groups of coating technologies are distinguished by the way the desired film material is transferred into the gas phase, the involved mass transport processes and the film growth mode:

- 1) In physical vapor deposition (PVD) techniques the film material is vaporized from solid form and condensed on the substrate as thin film.
- 2) In chemical vapor deposition (CVD) the substrate is exposed to one or more vaporized precursors, which react or decompose on its surface generating the desired coating. Their deposition can be activated either thermally or by plasma discharge [56].

Gas phase deposition and especially vacuum coating processes allow for the preparation of a broad range of high purity coatings. The properties of the obtained films are generally well reproducible and can be varied in a very controlled way by changing coating parameters [57]. Evaporation coating and sputtering are the best known and industrially most important PVD methods [56].

2.4.3.1 Evaporation Coating

In high-vacuum evaporation the desired film material is transferred into the gas phase by thermal vaporization. The process is performed at very low pressure, typically in the range of $\sim 1 \times 10^{-5} - 1 \times 10^{-9}$ mbar. Thus, the concentration of gaseous contaminations in the system is very low. Collisions with gas molecules are rare, so hardly any scattering occurs. Hence, the vaporized material is transported to the substrate on a straight trajectory [58]. The distribution of the deposited material is governed by the angle between this trajectory and the substrate surface as well as by the distance of source and substrate. For this reason, the substrate is generally mounted on a moving fixture and rotated about one or two axes during coating to homogenize deposition. In most systems the evaporation unit is positioned at the bottom of the coating chamber. Thus, the vapor flows upwards. Some materials can also be evaporated in the inverse direction or sidewise, however, if the source is constructed appropriately [59].

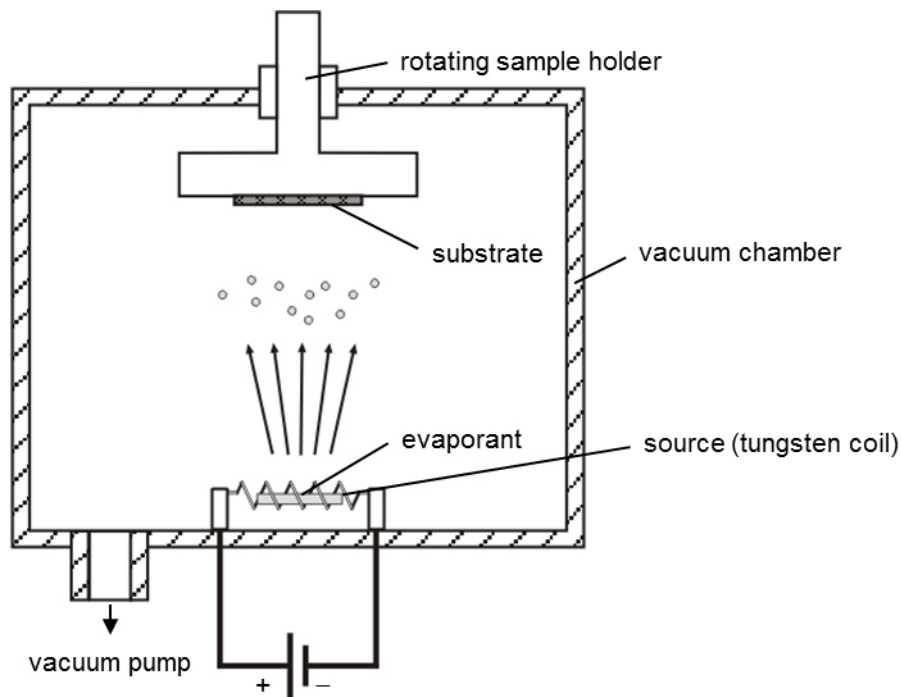


Figure 7 | Evaporation coating system (schematic drawing) (modified from [60])

Nowadays, evaporation coating is employed industrially for manufacturing a range of special coatings including interference layers, mirror coatings, permeation barrier films on flexible packaging (for the protection of products against moisture or gas) or electrically conductive coatings [58]. The technology is compatible with roll-to-roll fabrication, which offers high productivity. Compared to sputtering (see next section) considerably higher deposition rates can be achieved. However, evaporation coating is less suited for the deposition of alloys and compounds (see below) [61].

Five different vaporization systems or sources are used for evaporation coating [62]:

- **Resistively heated sources:** Resistively heated sources are metallic and heated by applying high electrical current. The evaporant (= the material to be evaporated) is directly placed onto the source or heater. To prevent contamination of the deposited film the heater consists of an inert material with low vapor pressure such as tungsten, molybdenum or tantalum [63]. Directly heated sources are available in a variety of shapes and sizes to fit the requirements of the material to be deposited. Single- or multiple-strand tungsten wires can be bent in shape (loops, coils, pins) and are inexpensive enough for single use. Boats or boxes are easier to load and allow for the evaporation of powders. In indirectly heated sources the evaporant is positioned in a crucible, which electrically isolates it from the heater filament. Due to the increased thermal mass and the separation of heater and evaporant the evaporation rate is easier to control than in directly heated systems. Those systems typically lack any features that assist in reducing thermal gradients. Non-reproducible continuously-changing evaporation rates can result. Nevertheless, both types of resistance-heated sources are still frequently used due to their low complexity and simple handling [64].

- **Electron beam (E-beam) evaporators:** In E-beam systems a large potential difference is applied between an anode and a cathode (lab-sized systems: 3-10 kV, large commercial systems: up to 30 kV). Electrons are ejected from the cathode usually by a thermionic process that involves heating of the cathode (e.g. a tungsten filament). The electrons are accelerated towards the anode. Due to the large voltage difference they reach high energy levels. The electron flux is deflected and focused onto a small spot at the surface of the evaporant, which is positioned in a water-cooled hearth (commonly made from copper). Melting and vaporization of the material is controlled by rastering the E-beam over it using electromagnetic deflection coils [65]. Very high temperatures up to 3500°C [66] and a large range of power densities can be achieved with E-beam sources. Thus, it is possible to evaporate practically all kinds of metallic or ceramic materials in a controlled manner. Moreover, the technology is suitable for continuous production, because large quantities of material can be provided in the hearth and it can also be re-filled by feed mechanisms (e.g. rod-feed or wire-feed systems). Usually the evaporant material in contact with the hearth does not melt, which prevents contamination of the generated layer. However, it is rather difficult to maintain a constant evaporation rate in E-beam evaporation. In addition, transient excessive heating or entrapped gases can result in the ejection of droplets of evaporant material. These can deposit on the substrate and reduce the coating quality [65].
- **Cathodic arc evaporators & anodic arc evaporators:** In arc evaporation sources vaporization is achieved by bombarding the evaporant with gas ions (cathodic arcs) or electrons (anodic arcs). These are generated by arcing i.e. in repetitive gas discharges between electrodes operated at high current and low voltage [67]. Due to the high electron density in the process a large fraction of the evaporated atoms are ionized. Especially cathodic arc sources are simple in design and operation allowing for the construction of small to large-sized systems. Moreover, the melting phase in these systems is very brief. Practically no liquid is formed on the evaporant surface so it can be positioned in the evaporation system in any orientation. Arc evaporation exhibits the same major disadvantage as E-beam evaporation. During erosion small metallic droplets can be ejected from the source together with the vaporized material, which can render the generated coating rough and dull [65].
- **Induction heating evaporators:** An induction heating source basically consists of a crucible (holding the evaporant) that is concentrically surrounded by an inductor i.e. a water-cooled circular coil. Powered by an alternating current (AC) generator the inductor coil generates an alternating magnetic field. Thereby an eddy current is induced either in the crucible, if it is electrically conducting, or directly in the conducting evaporant. This results in Joule heating and eventually in vaporization. The major benefit of electromagnetic induction is that it acts through non-conducting medium. Thus, the inductor coil can be positioned outside a closed housing or chamber made from dielectric material that contains the object to be heated. Thus, chemically active, toxic or even radioactive substances can be deposited with this type of sources without contaminating the residual evaporation system [68].

Evaporation rate and temperature of the material to be deposited are determined by its vapor pressure, which therefore also governs the choice of the source type. Materials with high vapor pressure at low temperature, e.g. chromium, sublime and can be vaporized by resistive heating. Materials with low vapor pressure generally melt and evaporate. Hence, electron beam sources are more suited in such cases [69].

The dominant role of the vapor pressure in evaporation coating particularly affects the deposition of alloys, mixtures and compounds. Commonly, the individual metals in alloys exhibit differing vapor pressures and are thus transferred to the gas phase at different rates in evaporation coating. As a result, the composition of the generated thin films generally differs from the composition of the evaporant. Only in some cases this limitation can be overcome by the use of flash evaporation techniques, which allow vaporizing small amounts of material within a very short time [69]. When evaporating compounds their stoichiometry often changes too. Oxides tend to decompose. They dissociate completely or are deposited as lower oxides or non-stoichiometric oxides. Only rarely they form films that exhibit the same composition as the starting material. However, a few evaporant materials, including MgF_2 and KI, are vaporized as molecules. Their stoichiometry is preserved throughout the coating process [64].

2.4.3.2 Sputtering

Sputtering is a versatile PVD technique and one of the few thin film coating technologies, which are already employed in mass production. Industrial applications include e.g. decorative films, hard, wear-resistant coatings, low-friction surfaces or thin films for the electronic industry [70]. The method is based on the evaporation of the material to be deposited via bombardment with highly energetic ions generated in a glow discharge plasma [71]. The boiling point of the evaporant, which is provided in the form of a plate – the so-called “target” – is not relevant for this process. For this reason, even refractory materials can be processed and alloys and compounds can be vaporized and deposited without changing their stoichiometry. In principle, sputtering is applicable to practically all materials, out of which a target can be fabricated. It offers good deposition rates and generally yields high-purity layers. The substrate is not heated strongly, so the method is also suited for coating temperature-sensitive materials. The vaporized substance typically hits the substrate with higher energy than in other deposition techniques. Sputtered films therefore often show comparably high adhesion [72, 73]. The deposition process is rather isotropic i.e. evaporated atoms and molecules do not migrate to the substrate on a straight trajectory as in evaporation coating but with an angular distribution. This allows for good step coverage on rough surfaces, but can be a problem if directionality is required e.g. when coating structures with high aspect ratio [74] (see Figure 8).

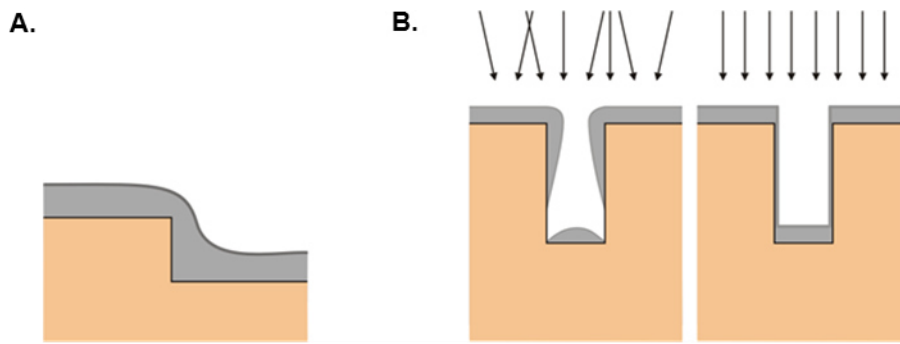


Figure 8 | Step coverage **A)** Isotropic deposition allows for good step coverage when sputtering moderately rough substrates (redrawn from [75]) **B)** Sputtering of high-aspect ratio steps ($>2:1$) results in excess deposition at the top corners of the steps but poor coverage of the sidewalls (left). Using directional deposition techniques, more homogeneous coatings may be generated (right) (redrawn from [76])

In direct current (DC) sputtering the target serves as cathode. The substrate is positioned opposite to the target on the anode [70]. After evacuation of the sputter chamber, a process gas is introduced. It is usually inert to avoid unwanted reactions with sputter source or target surface. However, it may also contain a certain amount of reactive gas if compound films need to be deposited. Generally Ar is employed as inert process gas. For reactive sputtering O_2 or N_2 can be added. A plasma is ignited by applying high voltage between the target (=cathode) and the anode. Even at ambient conditions gases comprise a small fraction of ions and free electrons (generated by background radiation). The electric field within the sputter chamber accelerates these electrons and ions in the process gas towards the cathode or the anode, respectively. On their way the electrons collide with neutral gas atoms, which decompose into further ions and electrons. The ions attracted by the cathode hit the target, which leads to 1) evaporation of target surface atoms and 2) ejection of secondary electrons. The secondary electrons are accelerated towards the anode and generate new ions and electrons. If the applied power is sufficiently high this avalanche process results in the ignition of a plasma. The plasma eventually attains a balance between charge losses and energy supply and reaches a rather constant temperature, density and distribution [77]. The vaporized target surface atoms form a metal gas, which fills the sputter chamber. In collisions with process gas atoms the metal particles lose some of their kinetic energy. Finally, they condense on surrounding objects such as the substrate and form a thin film [71]. In DC sputtering a uniform plasma can be established over a large area, so an accordingly large sputter target can be employed [70].

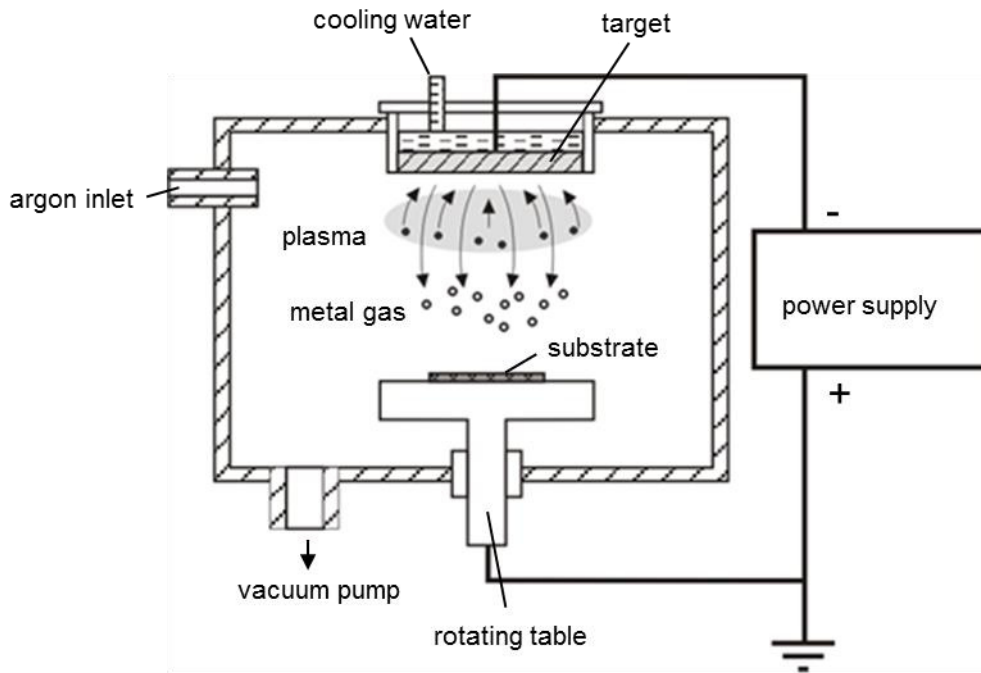


Figure 9 | DC sputter system (modified from [70])

However, the method is typically limited to conductive targets such as metals. When insulating materials are exposed to a DC plasma, positive charges quickly accumulate on the material surface during ion bombardment. These prevent further impingement of ions. As a result, the plasma breaks down [78]. Moreover, basic DC sputtering typically exhibits significant substrate heating and poor ionization efficiency in the plasma resulting in low deposition rates [71]. These limitations have led to the development of a range of alternative configurations: conventional or balanced magnetron sputtering (early 1970s), unbalanced systems (late 1980s), multi-source “closed-field” systems (early 1990s) and, most recently, high-power impulse magnetron sputtering (HiPIMS) or high-power pulsed magnetron sputtering (HPPMS) [70].

The invention of magnetron sputtering was a decisive step towards the establishment of sputtering as industrial coating technology. In standard DC sputtering setups, secondary electrons emitted from the target are accelerated away from the cathode and thus do not contribute sufficiently to maintaining the plasma. In magnetron sputter systems a magnetic field is generated near the target in order to trap the electrons there. A single magnet or a set of magnets is positioned below the center of the target serving as one pole. A ring of magnets around the outer edge of the target forms the second pole. The field lines of the resulting magnetic field are oriented parallel to the target. Secondary electrons emitted by the target are trapped in this field and are forced to circulate in the vicinity of the target, which increases the probability of ionizing collisions in the plasma. This increases the ionization efficiency and the ion bombardment of the target and results in higher sputtering and deposition rates. Due to the enhanced ionization efficiency magnetron sputtering can be performed at lower operating pressures and voltages than the standard DC process. In conventional or “balanced” magnetron sputtering systems, the plasma is confined near the

cathode, typically extending only about 60mm from the target. Objects positioned within this region experience ion bombardment during sputtering, which can interfere with film growth on their surface. Film properties and structure can be modified thereby allowing for e.g. the deposition of denser coatings. Outside this area the plasma density is commonly not high enough to influence film growth [71], neither is it sufficient to activate precursor gases for reactive sputtering. This limitation is overcome with unbalanced magnetron systems. Here a fraction of the secondary electrons can escape the magnetron field to maintain a dense plasma further away from the target. However, the flow of escaping electrons is not uniform and thus the generated plasma is not homogeneous either [70].

In contrast to the DC configuration, AC sputter systems (also called radiofrequency (RF) sputter systems) make it possible to deposit insulating materials. However, the efficiency of this configuration is typically low. In AC sputtering the potential on the target (and the counter-electrode) is periodically reversed. At frequencies below ~50 kHz the process gas ions are mobile enough to induce alternating DC mode-like discharges at the two electrodes, the total potential drop being located near the cathode. At higher frequencies the ion mobility is too low to allow such discharges, however. Then the applied potential is largely homogenous over the region between the two electrodes. Electrons are accelerated sufficiently for ionizing collisions to occur. The target is periodically bombarded with energetic ions, which causes sputtering. In between, electrons are transported to the target so that no charges can build up as would be the case in conventional DC sputtering. However, insulating materials often exhibit poor thermal conductivity and high coefficients of thermal expansion. Bombarding the sputter target with ions leads to significant heating. Large thermal gradients may thus be generated in dielectric targets, which can make them fracture [70]. Moreover, AC sputtering systems are generally difficult to scale up for industrial applications due to their complexity. As an alternative, reactive sputtering can be used to deposit dielectric coatings [71]. It is a popular technique for the fabrication of oxide, nitride, carbide, fluoride or arsenide thin films [79]. However, the reaction product also deposits on the sputter target during prolonged reactive sputtering. As a result, an insulating layer forms gradually. This so-called target poisoning can result in fluctuations in the coating process and defects in the sputtered film.

Pulsed magnetron sputtering (PMS) overcomes this and other limitations of the conventional reactive coating process. Other more advanced sputter systems like HiPIMS or HPPMS allow more time-efficient film deposition than standard DC setups, for they yield especially high sputtering rates [71].

2.4.4 Wet Chemical Deposition Technologies

Via gas phase deposition methods very pure nanocoatings can be obtained. Some of the processes are even well suited for large-scale production. However, many require high vacuum and costly equipment [80]. Moreover, the coating of substrates with complex geometry is often challenging. Wet chemical deposition techniques (also referred to as liquid phase deposition or chemical solution deposition techniques) offer higher flexibility regarding

substrate shape, substrate size and the number of substrates to be coated [81]. Moreover, the deposition process can be controlled precisely. The composition and structure of the deposited films can be fine-tuned by manipulating a range of reaction parameters such as precursor, additives and temperature. Upscaling is easier than for gas phase processes and the required equipment is generally inexpensive [80]. In some methods the substrate must remain immersed in the deposition solution throughout the whole coating process (e.g. electrodeposition, see 2.4.4.2). In other approaches, it is sufficient to spread the coating reagents homogeneously on the substrate surface to be coated. For this purpose one of the three application techniques described in the following section are frequently utilized.

2.4.4.1 Application Techniques in Wet Chemical Deposition

Dip coating: Under controlled process conditions (temperature, atmosphere composition) the substrate is immersed in the coating solution and withdrawn at defined speed. The solvent evaporates depending on its vapor pressure and the process conditions. Its solid contents are deposited as thin film. The thickness of the layer is determined by inertial and gravitational force, viscosity of the solution, surface tension, evaporation rate and withdrawal speed and angle.

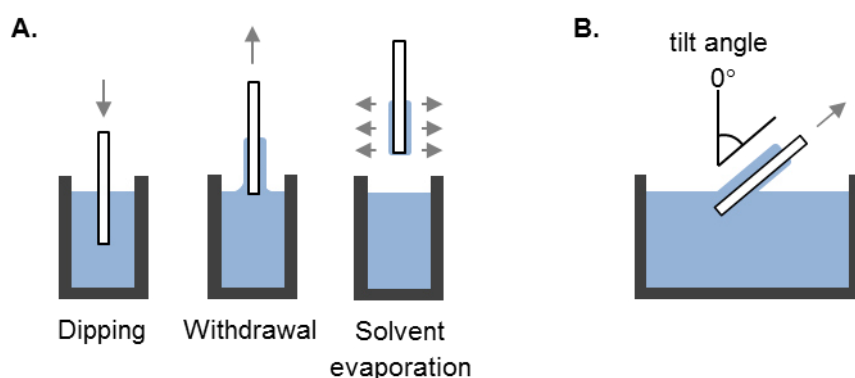


Figure 10 | Dip coating A) Dip coating process B) Withdrawal at non-zero tilt angle results in different layer thickness on the upper and lower side of the sample (redrawn from [82])

The tank holding the coating solution needs to be large enough so that the whole substrate can be immersed completely. This determines the solution volume required for coating and thus eventually the process costs. The immersed substrates must be clean and dry to avoid that the bath composition is changed or contaminants are carried in. Occasionally, the coating bath is stirred or agitated via a circulation to prevent the settling of reaction products or the formation of concentration gradients in the tank.

Dip coating is a very straightforward process. It may be performed with simple equipment and can even be automatized easily. In principle, the technique can be employed for substrates of various shapes. However, it provides only limited control over film thickness. On vertical surfaces the liquid film is generally thicker at the bottom than at the top, because the viscosity of the solution increases during draining as a result of solvent evaporation. Thick edges form, which can peel off or break after drying. The coating solution can also be

trapped in recessed structures and then drains only slowly or not at all. If large parts are coated and considerable amounts of solvent need to be evaporated, fire or toxicity hazards must be considered as well [83].

Spin coating: In spin coating, the substrate is rotated around an axis perpendicular to the substrate surface to be coated. For this purpose, the part is mounted on a spinner (see Figure 11). The coating process encompasses four stages: First, a defined amount of coating solution is deposited onto the substrate in the middle of the area to be coated (=deposition stage). The substrate is either static or rotated at low speed (ω) during this stage [84]. Then the substrate is accelerated to distribute the deposited solution, (=spin-up stage). The centrifugal force drives the coating solution outward over the substrate surface. The spreading of the solution is limited by the viscous force (or friction), which acts radially inward. The balance between the two forces determines the liquid film thickness. Typically, a significant amount of solution is spun off the substrate already during spinning up. In the last two stages of the process the substrate is spun at final spin speed (ω_f). The liquid film thickness is progressively reduced (=spin-off stage) and the applied liquid film is dried (=evaporation stage). The two stages occur simultaneously to some extent [85]. After spinning many coatings need to be dried further or solidified by “firing” (e.g. sol-gel coatings) [84]. Spin coating was originally developed for the modification of glasses for microelectronics and for coating parts with rotational symmetry such as lenses. The film quality is largely determined by the rheology of the coating solution i.e. its flow behavior during spinning. Viscosity and elasticity of the solution are most decisive in this context, but also some process parameters influence the rheological properties of the liquid: Temperature, ambient pressure, rotational speed and the concentration of dissolved components (e.g. polymers) or solid particles (in the case of sol-gel solutions) can, for instance, play a role in the spin-up stage [86]. Another critical factor to consider is the Reynolds number of the surrounding atmosphere. At certain rotation velocities pronounced turbulences (i.e. high Reynolds numbers) can occur due atmospheric friction, which can lead to coating defects and deprive the film quality.

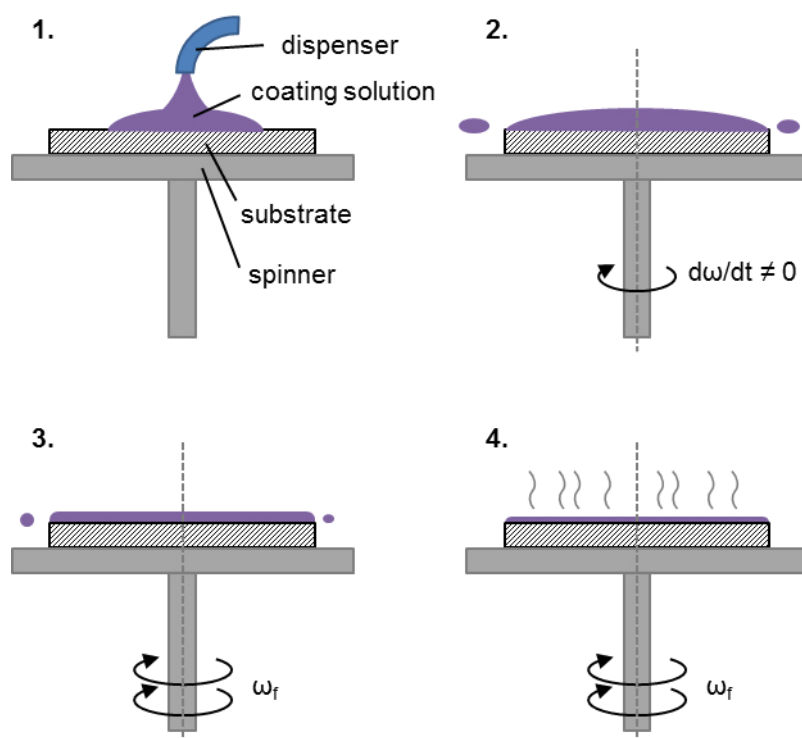


Figure 11 | Key stages of spin coating 1) deposition stage 2) spin-up stage 3) spin-off stage 4) evaporation stage (modified from [87] and [85])

Films prepared by spin coating are commonly more homogeneous in thickness than layers generated by dip coating – even if the substrates are non-planar. Furthermore, very thin coatings can be created [82]. Parts with pronounced surface structure are difficult to coat with either approach, however. Deep indentations usually interfere with the spreading of the solution in the spin-up stage. They can prevent complete coverage of the substrate and can lead to striation and thickness variation [88]. Furthermore, the size of the parts that can be handled is limited. As described, the substrate has to be rotated at high speed, which is not possible for large parts. Spin coating is also characterized by low utilization of material, as only 2-5 % of the deposited coating material is actually transformed into a film. 95-98 % is spun off and discarded. This can be unacceptable if expensive reagents (such as photoresists or organic light emitting diode (OLED) components) are to be deposited [85].

Spray coating: In industrial manufacturing, spray or aerosol coating methods are commonly employed for applying organic lacquers. However, it has also been studied extensively as an alternative to dip and spin coating for the preparation of thin films in the past few decades. Spray coating offers higher coating speed than dip and spin coating, is better suited for substrates with irregular shape and compatible with in-line processing. Significantly less coating solution is wasted in this coating method than in the previously described processes. Moreover, even coating formulations with short pot lives can be used [82]. In order to deposit the coating formulation (commonly a solution of precursors), it is atomized i.e. transformed into an aerosol with a droplet size of $<1\text{ }\mu\text{m}$ to 1 mm . Then it is directed towards the substrate surface. For atomization the liquid must be exposed to forces that overcome its surface tension. This can be achieved by the following techniques:

- Pneumatic spraying: The coating solution is passed through a two-fluid atomizer together with pressurized gas. At sufficiently high pressure the liquid stream is disrupted by the gas and the resulting aerosol is blown onto the substrate.
- Ultrasonic atomization: The liquid is atomized by exposing it to ultrasound with a frequency of a few tens of kHz to 1 MHz e.g. in a vessel surrounded by an ultrasound transducer ceramic. Alternatively, the solution can be pumped through an ultrasonically excited vibrating nozzle. The resulting aerosol is thrown onto the substrate using a carrier gas.
- Electrostatic spraying: High DC voltage (in the kV range) is applied between the substrate, which serves as ground electrode, and a capillary, through which the coating solution is pumped. Above a critical voltage at the outlet of the capillary atomization occurs. The as created droplets are directed towards the substrate by electrostatic forces.

Depending on the substrate temperature thin film formation occurs by different mechanisms. Vigié and Spitz [89] classified them into four categories:

- 1) Low substrate temperature: The liquid droplets hit the substrate and spread. The solvent evaporates leaving behind a dry precipitate film.
- 2) Elevated substrate temperature: The solvent evaporates before the aerosol reaches the heated substrate. Precipitate particles form, which deposit on the substrate surface.
- 3) Substrate temperature higher than in processes of category 2: During aerosol transport to the substrate the solvent evaporates and then the dissolved precursor vaporizes (or sublimes). The precursor vapor reaches the heated surface, where it undergoes a heterogeneous solid-gas phase decomposition. The process is therefore also referred to as “aerosol-assisted CVD”. Of course, suitable solvents and precursors are required for this process.
- 4) Very high process temperature: The solvent evaporates during aerosol transport to the substrate and the precursors thermally decompose in the gas phase. The resulting compounds deposit on the hot substrate surface in the form of fine solid particles.

All four types of spray coating are in principle interesting for sol-gel deposition (see 2.4.4.3) [90]. Polymer films are commonly fabricated by processes of the first category (see [91, 92]). However, spray-deposited coatings frequently exhibit non-uniform thickness and defects such as incompletely merged droplets and pinholes [91, 93]. As described above, the temperature of the substrate determines the extent of solvent evaporation during aerosol transport. Hence, it represents one of the parameters that can be varied in order to optimize deposition. Moreover flow rate, distance between substrate and nozzle outlet, pressure, spray duration, the number of times the surface is sprayed and the solute concentration affect the quality of spray-deposited thin films [91].

2.4.4.2 Electrochemical Metal Deposition

Electrochemical metal deposition (ECD) methods (or “electrodeposition” methods) belong to the oldest techniques used for the metallization of surfaces [94]. Today, decorative or protective films and multilayer coatings created by electrodeposition are indispensable for a broad range of industrial branches from mining to electronics and aerospace. In contrast to gas phase deposition methods, ECD techniques offer the possibility to coat objects with arbitrary three-dimensional geometries and generally require less complex equipment. Hence, these processes are also increasingly adopted in nanotechnology [95].

In ECD, the substrate is immersed in an electrolyte i.e. an aqueous metal salt solution or a salt melt. From this electrolyte metal ions are reduced by the uptake of electrons. Based on the source of these electrons two groups of ECD techniques are distinguished:

- 1) electroplating
- 2) electroless metal deposition

In **electroplating** the substrate is part of an electric circuit. Deposition is mediated by direct current. The electrons are provided by the anode. The reduced metal atoms deposit onto the substrate, which acts as cathode. Naturally, sufficient conductivity of the substrate is a prerequisite of this method. Generally pure metal layers (out of e.g. copper, nickel, zinc, tin, chromium, silver or gold) are deposited by electroplating [94]. However, alloy coatings can also be generated. These offer features that may not be yielded with single metals. Co-deposition of two or more metals is possible if their standard reduction potential is similar in the selected plating bath. Alternatively, a complexing agent can be used to adjust the reduction potential of the individual metallic ions to a common value for a certain set of reaction parameters. The composition of the resulting alloy can be tuned by adjusting the reaction conditions including current density, temperature, agitation and electrolyte composition (especially the concentration of the more noble metal ion, pH-value and additives) [96]. The properties of electroplated films also depend strongly on pre- and post-treatment of the substrate [94]. Contaminants, organic and inorganic films on the target surface can interfere with the bonding of the reduced metal atoms and can even prevent their deposition. Extrinsic soils can be transferred onto the substrate during processing or can originate from the general environment. Intrinsic inorganic films, e.g. oxide layers, typically form on metallic surfaces upon reaction with environmental gases. They can be protective such as the native oxide film on aluminum surfaces or detrimental like ferrous or ferric oxide on iron and steel. Pre-treatment procedures in electroplating commonly aim at the removal of these contaminations while inflicting minimum damage to the object to be coated. In physical cleaning procedures such as glass bead peening, ultrasonic agitation or brush abrasion kinetic energy is introduced for this purpose. Chemical cleaning methods rely on the interaction of the contaminations with active materials in the cleaning solution. Extrinsic soils are commonly detached by surface-active agents under mild conditions. In contrast, intrinsic films are dissolved with aggressive chemicals that often react with the below metal as well. Porous substrates are particularly challenging as residues can be trapped in pits and

cavities. During plating these residues can decompose or vaporize and can thereby provoke blisters in the coating [97]. Electroplating is typically employed to improve the mechanical stability and corrosion resistance of substrates (e.g. electrical contacts, electronic parts) and for decorative purposes [94]. Moreover, it is becoming increasingly appealing for micro- and nanotechnology as it is a low-energy process that can be performed in water close to room temperature. Using masks, intricate 3D-structures may be grown via plating, which is exploited e.g. in microelectronics for the fabrication of complex copper interconnects [95].

In **electroless deposition** the required electrons are provided by a reducing agent in the electrolyte, which is oxidized while reducing the metal ions in the solution. The redox reaction must be confined to the substrate surface to prevent that metal precipitates form everywhere in the coating bath. Frequently complexing agents are added. They decrease the concentration of the free metal cations in the solution and thereby stabilize the electrolyte. The substrate surface must counteract this stabilizing effect to induce metal deposition. Typically, it catalyzes the reduction process by mediating electron transfer between ions and reducing agent. Several metals including Ag, Au, Pd, Cu and Ni are catalytically active for their respective ions, i.e. Ag can induce electroless deposition of Ag films from a corresponding bath. Other materials, especially dielectrics, need to be activated by “seeding” with disperse catalytically active metal deposits. These seeds act as starting points for subsequent continuous metallization and should be homogeneously distributed in order to yield uniform films. Apart from deposited metal atoms, films generated via electroless metallization also contain bath components and reaction products. If Ni is deposited from a sodium hypophosphite bath, for instance, phosphorus atoms are integrated into the Ni coating, which therefore actually represents a NiP film. Electroless deposition offers significant advantages over electroplating: Selective, localized deposition is possible, even on non-conductive substrates (if activated). In addition, the technique generally yields more homogeneous layer thicknesses. A major drawback of the approach is the instability of electroless coating baths compared to the electrolytes employed in plating. Contaminations, defects in container walls or carry-overs of activation solution can induce uncontrolled deposition. Cleaning and degreasing of the substrate are similarly important for electroless deposition as for electroplating. Often the same techniques are employed. Well-attached seeds are also crucial for good film adhesion on non-autocatalytic materials. Plastics are popular raw materials in many industrial areas due to their low density and high chemical stability, but their optical appearance, mechanical resistance and UV-stability are typically moderate. These limitations can be overcome at least partially by electroless metallization. Fittings and handles on furniture and or in the automotive area are commonly metalized for this reason. Other applications include the generation of conduit paths or electrical contacts on plastic circuit boards and the metallization of plastic housings of electronic instruments. Compared to metal housings their plastic equivalents are lightweight and more cost-efficient. However, they only provide electromagnetic shielding if covered with metal coatings. These are able to protect the encapsulated instruments against external fields and can also dampen fields created within the devices [94]. Like electroplating, electroless deposition techniques generally represent low-energy processes. Thus, they are compatible with soft materials and

self-assembled structures and therefore interesting for nanotechnology research [95]. As substrates need not be contacted electrically, even minuscule substrates including micro- and nanoparticles can be functionalized via electroless deposition.

2.4.4.3 Sol-Gel Deposition

Via sol-gel deposition ceramic thin films with high purity and high optical quality may be fabricated at comparably low temperatures [82]. Conventional methods for the preparation of ceramic materials and coatings such as melting or firing typically require temperatures of up to 1600°C. In contrast, gels of ceramic particles prepared by sol-gel deposition can be compacted to solid ceramics by heat treatment (=sintering) at temperatures of 400-1000°C [44]. The technique can be employed to coat small substrates as well as large surface areas. The coating solution is most commonly applied to the substrate by dip-coating, spray coating or spin-coating [82] (see 2.4.4.1).

As explained in 2.3.3.4, a sol-gel process typically involves five steps: 1) Preparation of the precursor solution, 2) induction of sol formation, 3) transformation of the sol into a gel, 4) shaping of the gel and 5) conversion of the shaped gel into a solid ceramic material. Step 3, the formation of an inorganic gel or network, requires the interconnection of the sol components and thus depends on the structure of the sol. It can occur via two principal routes:

- A) Molecules in the precursor solution grow into macromolecules and finally fuse into an infinite network. This process also yields so-called polymerized gels. It is commonly observed in the acid-catalyzed hydrolysis and condensation of tetraalkyl silicates.
- B) Alternatively, gels can be formed via aggregation of colloids from a colloidal sol. A fairly stable sol is required as intermediate in this mechanism. Otherwise precipitation rather than aggregation occurs [82].

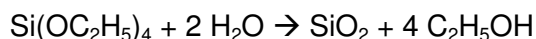
Several parameters influence the individual reactions of the sol-gel deposition process including:

- the composition of the precursor solution – especially the concentrations of the components
- the amount of reagent that is added to induce the deposition process (e.g. water in aqueous sol-gel chemistry)
- type and concentration of the employed catalyst
- further additives such as chelating agents (e.g. diketones) or desiccating controlling chemical additives (DCCA)
- the order, in which the reagents are added
- the schedule of reagent addition and mixing, because slowly reacting components or intermediates can exhibit aging (e.g. premature hydrolysis)
- mixing parameters such as efficiency of mixing, atmosphere or additional agitation e.g. by ultrasonic treatment

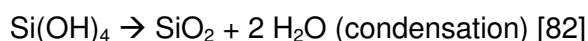
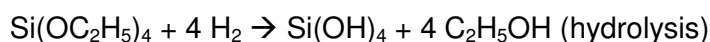
- the process temperature [44]

Immediately after formation of the gel, its pores are filled with the solvent of the precursor solution – generally water and/ or alcohol. It is therefore termed hydrogel, aquagel or alcogel. By drying, the solvent in the pores is replaced with air [82]. Depending on the desired microstructure of the dried gel different methods can be employed to remove the solvent. Xerogels are obtained upon natural thermal evaporation of the fluid. They exhibit small pores (≥ 2 nm) and their density is about 40-60 % of the density of the fired ceramic. Hypercritical evaporation in an autoclave yields networks with a porosity of ~95 %, so-called aerogels. By freeze drying cryogels are prepared. However, they are basically fine powders not suitable to fabricate monolithic ceramics [98].

Commonly used precursors in aqueous sol-gel processes include alkoxides i.e. organometallic compounds of the general form $M(OR)_n$, where M represents a metal of valence n and R represents an alkyl group (C_xH_{2x+1}). The conversion of alkoxide molecules into sol-gel networks is based on two groups of reactions: hydrolysis and condensation. Hydrolysis occurs upon reaction of an alkoxide (e.g. M-OR) with water and results in the formation of a metal hydroxide (M-OH). By condensation two metal hydroxide molecules (M-OH + HO-M) can combine to give a metal oxide (M-O-M). A typical example of a sol-gel process based on alkoxides is the synthesis of silica glass from tetraethyl orthosilicate (TEOS):



Individual reactions:



2.4.4.4 Langmuir-Blodgett Deposition

In the classical Langmuir-Blodgett (LB) deposition process water-insoluble amphiphilic molecules with hydrophilic head group and hydrophobic tail are dissolved in an organic, water-immiscible solvent (e.g. chloroform). Droplets of the solution are deposited onto a pure water surface, where they spread into a film. Upon evaporation of the solvent the amphiphilic molecules form a monomolecular layer. The hydrophilic moieties in this film are oriented towards the water surface, while the hydrophobic tails protrude into the gas phase. By the use of a moveable barrier the ratio of area per molecule in this so-called Langmuir layer can be controlled. With decreasing area per molecule the amphiphilic molecules start to interact and self-organize. Due to their surface-active properties the surface tension of the water subphase decreases. With progressive compression (exerted via the barrier), the amphiphiles (mainly their hydrocarbon chains) increasingly repel each other. As a result, the film exerts a force against the surrounding barriers. It may be considered as a two-dimensional analogue of a pressure and is therefore termed surface pressure (π). The

surface pressure is commonly monitored during monolayer compression and plotted against the area per molecule. This surface pressure (π) – area (A) isotherm is generally used as the main indicator for the formation and the properties of the amphiphile layer. At sufficiently high density the amphiphilic molecules show strong enough lateral cohesion so that the layer can be transferred like a carpet onto a solid support. Hydrophobic substrates are coated by immersing it at controlled speed (=“downstroke”) into the trough holding subphase and Langmuir layer. Transfer to a hydrophilic substrate is generally achieved by pulling it out of the subphase i.e. by emersion or “upstroke” [99] (see Figure 12). During the transfer process the Langmuir film must be compressed further to keep the surface pressure constant. In addition, the transfer ratio i.e. the reduction in Langmuir layer surface divided by the area of the substrate that has been coated is commonly monitored. It can be adjusted by varying experimental conditions. Generally, a transfer ratio of one (or close to it) is preferred. It is supposed that at this value the transferred film resembles most closely the pre-formed monolayer [100]. A hydrophobic surface can be rendered hydrophilic by depositing an amphiphile film according to the described process and a hydrophilic surface can be rendered hydrophobic. Repeated upstroke-downstroke cycles allow for depositing multilayers in a head-to-head and tail-to-tail configuration also called Y-type (see Figure 12, bottom). Although these Y-type stacks are most common, it is also possible to deposit multilayers by either repeated downstroke or upstroke (X-type and Z-type respectively). Even mixed deposition types (XY-types) have been reported. Temperature, pH, and composition of the subphase together with surface pressure and deposition speed apparently influence, which deposition type can be realized. However, the involved transfer mechanisms have not been fully clarified so far [99].

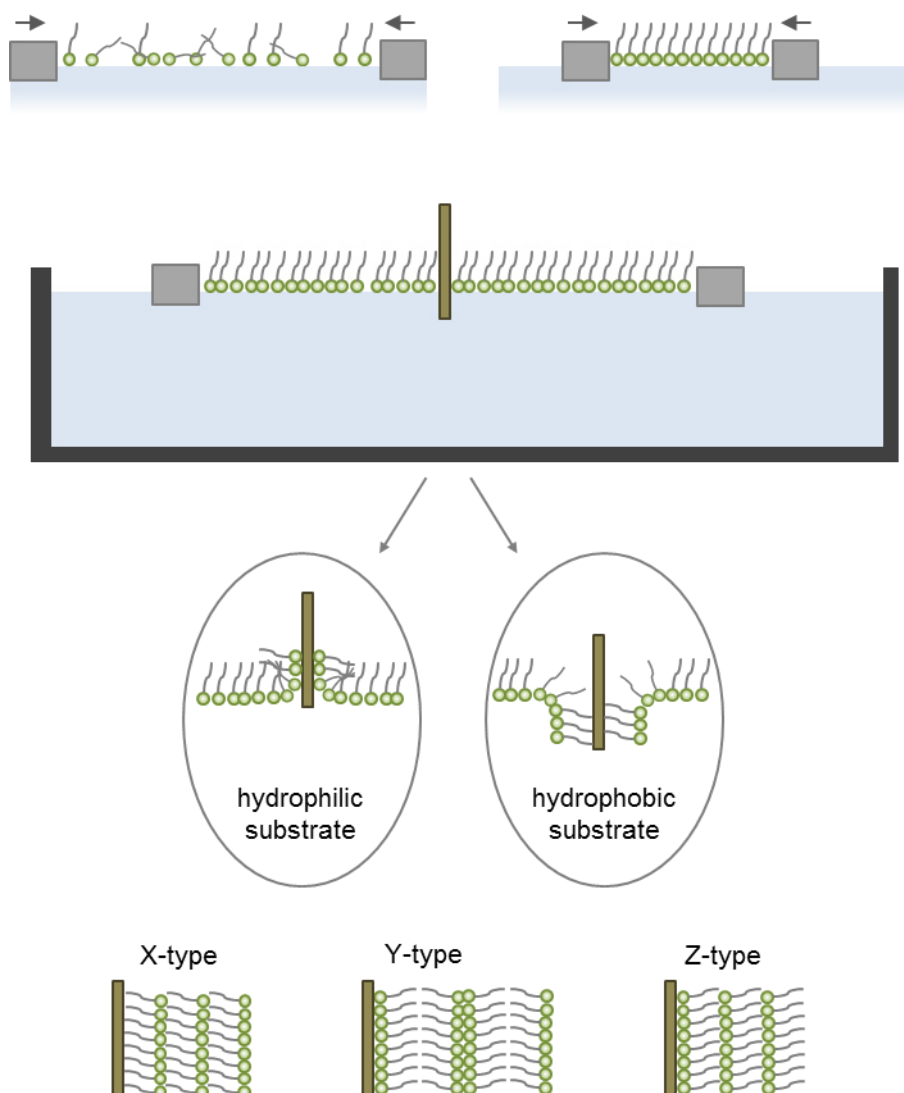


Figure 12 | Transfer of Langmuir-Blodgett layers onto solid supports (modified from [99])

LB deposition offers precise control over the arrangement of the molecules at the subphase-air interface and thus over the structure and thickness of the Langmuir monolayer. The layer properties can be preserved during transfer to the substrate, but only if the process conditions are closely controlled. Even subtle changes in individual parameters e.g. the purity of the deposited material, subphase quality and purity, subphase temperature or amphiphile surface concentration can lead to significant variations in the film quality. Substrate and trough should therefore always be cleaned thoroughly and the process should be performed in a dust- and vibration-free environment if possible [99].

The LB method was developed for the deposition of monolayers and ordered multilayers of amphiphilic molecules and has mostly been employed for this purpose so far. In the field of nanobiotechnology it has been studied as a tool for the preparation of biomimetic membrane models, for instance [99]. Applications in sensors, optics or microelectronics (e.g. fabrication of organic diodes or organic conductors) have also been considered [101].

Unfortunately, LB deposition is a complex and slow process [102] and, as discussed, highly sensitive to environmental influence and contaminations. Still it could be demonstrated that the technology can in principle be up-scaled and automatized - at least for simple applications (see e.g. [103]). However, LB coatings generally show limited stability. As they are generated by chemisorption, they lack strong molecular interactions. Moreover, similar layers or functionalities can supposedly be created in a cheaper and faster way by other means, as suggested by Albrecht et al. [103]. So, in spite of promising developments and optimistic forecasts in early publications, LB deposition is still rather an experimental technique than an industrial coating method.

2.4.4.5 Monolayer Self-Assembly

Self-assembled monolayers (SAMs) represent ordered assemblies of surfactant molecules typically consisting of head group, backbone and end group. The layers can in principle be grown from solution as well as from the gas phase [104]. Hereafter only the first fabrication method will be discussed.

Monolayer self-assembly relies on chemisorption i.e. the spontaneous adsorption of a surfactant to a substrate, to which the head group of the surfactant shows specific affinity. The bound molecules form a densely packed monomolecular film. Their backbones and end groups self-organize and thereby define the film structure [104]. The process is driven by lateral interactions e.g. van der Waals forces, π -stacking and hydrophobic interactions. In comparison with the Langmuir-Blodgett technique, monolayer self-assembly is relatively easy. The substrate, which should be cleaned well, is immersed into an organic solution of the molecule of interest. It can be necessary to incubate the substrate in this coating solution for minutes to days depending on the substrate surface, the nature of the molecule and its concentration in the solution (typically 10 mM to 10 μ M). The substrate is taken out of the deposition solution then and rinsed extensively to remove non-bound molecules [105].

Alkanethiol deposition on Au surfaces is a classic example of monolayer self-assembly. Other possible substrates for thiol compounds include Ag, Cu and Fe and Ni but also semiconductor surfaces [104]. Silanes can be coated onto titanium, silicon and aluminum oxide surfaces. Organic acid residues allow chemisorption to metals or metal oxides [102].

No special equipment is required for this technique so it is very interesting for industrial applications. External factors have minor to no influence on film formation. Moreover, small amounts of surfactant solution can be sufficient to cover large areas [105]. The surface properties of SAMs can be adjusted by modifying the structure and the end groups of the deposited molecules. In more complex coatings such films can also be used as mediators for the immobilization of additional layers [104]. The major limitation of SAMs is the moderate stability and robustness of the films. As in LB layers, adhesion and cohesion in SAMs are mediated by comparably weak forces. Moreover, the technique is generally restricted to specific head group-substrate pairs [102].

2.4.4.6 Layer-by-Layer Deposition

Layer-by-layer (LbL) deposition is based on the sequential adsorption of complementary multivalent molecules on the substrate surface (see e.g. Figure 13). Their binding and cohesion can be mediated by electrostatic as well as non-electrostatic interactions such as hydrogen bonding, covalent bonding, hydrophobic, charge transfer or host-guest interactions, coordination chemistry, specific biological interactions or stereocomplexation. A variety of coating techniques can be employed for LbL assembly including spraying and spin-coating, yet dip-coating is still most commonly used to date. To yield homogeneous and reproducible films, washing steps are commonly performed between individual deposition cycles. In the case of dip coating, this prevents the contamination of coating baths by carrying-over coating solution from previous deposition steps. Moreover, weakly adsorbed layers are stabilized in the washing steps. The LbL method is not restricted to a special type of surface and can thus be used for modifying a large range of substrates almost irrespective of their size (bulk or particulate material), shape (porous or planar) and surface chemistry. LbL deposition is also exceptionally versatile regarding the building blocks that can be incorporated into the multilayer assemblies. In their pioneering work from 1966 Iler et al. first demonstrated the technique by the fabrication of multilayers from sequentially adsorbed positively and negatively charged colloidal particles [106]. Decher and Hong adapted the approach for the preparation of polyelectrolyte multilayers in the early 1990s [107]. Since then many different compounds and materials have been tested successfully for LbL deposition e.g. metal oxides, nucleic acids and even nanotubes. The nature of the building blocks can be varied from one deposition cycle to the next, whereby additional functionalities and capabilities can be integrated. Moreover, multilayer assembly can even be combined with other deposition processes such as sol-gel [102] or Langmuir-Blodgett coating [108]. Thus, a variety of materials can be integrated in LbL-layers including polymers, proteins, polysaccharides, viruses, NPs and colloids. Unfortunately, LbL deposition requires large amounts of coating solutions, especially if the substrates are bulky. Moreover, the process is relatively time-consuming. Due to its simplicity and versatility it is nevertheless an appealing technique for surface functionalization [102].

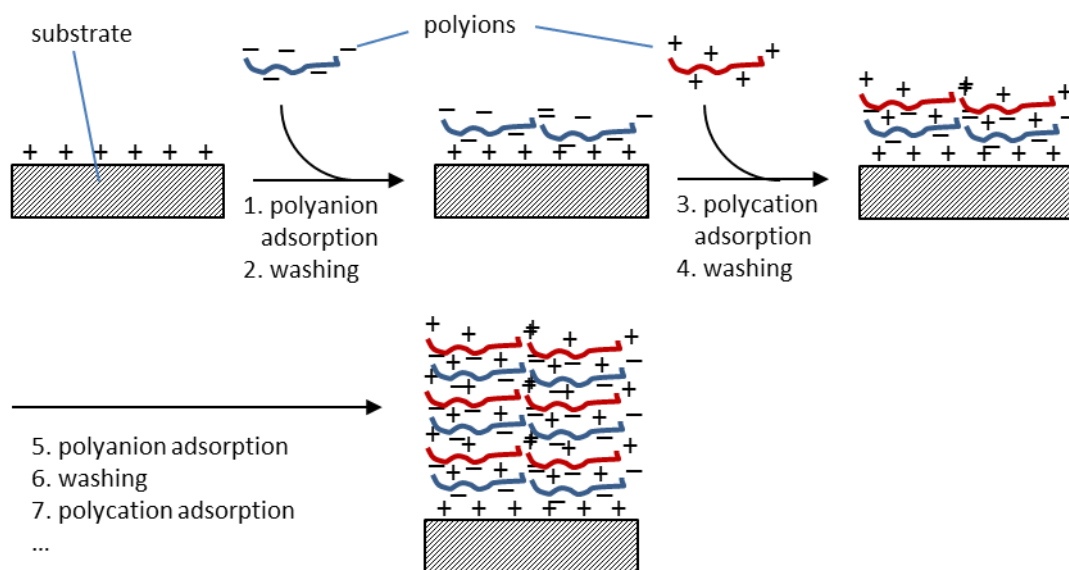


Figure 13 | LbL deposition of multilayer thin film via electrostatic interactions (modified from [102])

2.4.5 Polyelectrolyte Multilayers

Among the numerous assembly mechanisms that can be exploited for LbL deposition, electrostatically mediated binding represents the most prominent and best studied one. Via this type of interaction multilayer films with well-controlled composition, structure and thickness can be assembled. This is typically achieved by alternating immersion of a charged substrate in dilute aqueous solutions of oppositely charged molecules. Multilayers of polyanions and polycations, also termed polyelectrolyte multilayers (PEMs), can be obtained that way (see Figure 13). PEMs have been studied intensely over the last two decades [102]. Applications in sensors, functional coatings and selective membranes have been considered and investigated. To adsorb the first layer, a clean, hydrophilic substrate with non-zero surface charge is brought in contact with a solution of a polyelectrolyte of opposite charge [109]. This is most commonly done by dip-coating nowadays, but also spin-coating and spray-coating have already been tested for the purpose [102]. If dip coating is used, the substrate is commonly incubated in the coating solution for a certain period of time (typically 20 min). Excess polymer and loosely bound molecules are removed by washing with water then. Optionally the substrate can be dried before it is incubated in the second polyelectrolyte solution. For successful layer assembly the polyelectrolyte building blocks must exhibit a minimum charge density. Furthermore, each adsorption step must lead to surface charge overcompensation i.e. inversion of the net surface charge of the sample. Only then the next polymer layer can be assembled [109].

2.4.5.1 Growth Mechanism

In spite of more than two decades of research in the field, the mechanisms driving LbL assembly of PEMs have not been fully elucidated yet. Naturally, electrostatic interaction is considered to play a central role. However, it has been observed that even polymers with very low charge density can be used successfully as building blocks for PEMs, which

indicates that other types of interactions also contribute to the adsorption process [102]. The internal structure of the assembled polymer layers has been shown to depend on a range of process parameters including the salt concentration in the polymer solution, the polyelectrolyte concentration, the charge density of the polyelectrolytes, polymer rigidity and molecular weight. By variation of these parameters the conformation of the adsorbed molecules can be tuned from extended to rather globular. Thereby the layer thickness may be adjusted [109] as depicted in Figure 14.

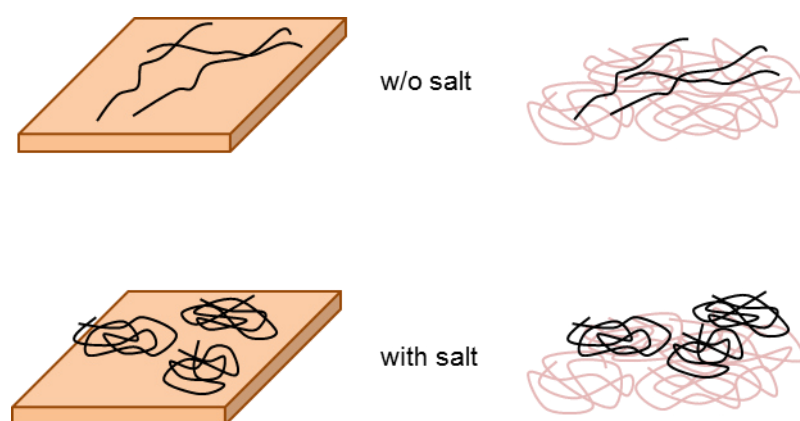


Figure 14 | Polyelectrolyte adsorption in dependence of salt concentration in coating solution
(modified from [109])

This led to the assumption that the polymer molecules largely retain their solution conformation after binding and do not rearrange significantly in favor of a thermodynamically more stable structure. In the so-called “hit-and-stick” model coil reorganization is even completely neglected, as several bonds would have to be broken simultaneously to allow restructuring. This is considered improbable. Although the hit-and-stick model can qualitatively explain the significance of preparation parameters for the structure and thickness of PEMs, it certainly draws a very simplified picture of the real adsorption process [109].

In fact, different binding regimes can be observed depending on the composition of the coating solutions and on properties of the polyelectrolytes and the substrate surface. When PEMs are prepared on solid substrates, the first polymer layer is deposited onto a “hard” surface, for instance. The higher the surface charge of the substrate the more polymer molecules can be bound. At low salt concentration, polyelectrolyte chains tend to adsorb in a flat conformation due to repulsive interaction of individual chain segments. Some segments of the flat chains, however, extend as loops and tails into the z-direction and thereby provide the surface charge overcompensation required for the next polymer layer to bind. Upon pH variation or addition of salt, internal charges are shielded partially. Thus, flexible polymer chains can adopt a more compact conformation. Surface charges are partially shielded as well so that short-range attractive interactions dominate the binding process. Hence, the chains can adsorb in a more “loopy” form causing higher surface charge overcompensation. The first polyelectrolyte layer creates a “soft” surface, onto which the subsequent layer is deposited. As the first layer is flexible and exhibits a vertical charge distribution, the binding

process of the second layer differs significantly from the assembly of the first layer. Instead of simply adsorbing on top, segments of the deposited polyelectrolyte molecules interpenetrate into the first layer and entangle with the loops and tails extending from it. Interactions between the opposite charges of the two types of polyions mediate adhesion. Due to this interpenetration and entanglement of chains a higher amount of polymer molecules is necessary to achieve charge overcompensation in the second than in the first one. Those segments of the second layer, which are buried in the below layer, are apparently required to compensate internal charges there. Solely the remaining segments are available to compensate surface charges and to extend as loops and tails into the surrounding solution in order to provide sufficient charge overcompensation. In multilayer systems interpenetration is apparently not limited to neighboring layers (as illustrated in Figure 15). In poly(styrene sulfonate) (PSS)/poly(allylamine hydrochloride) (PAH) multilayer structures, for instance, a segmental distribution of individual layers of 1-2.5 bilayers along the surface normal have been measured [109].

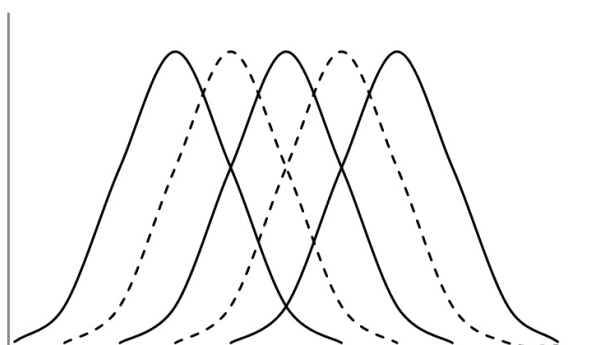


Figure 15 | Polyelectrolyte LbL assembly Concentration of polyanion (solid lines) and polycation segments (dashed lines) along the surface normal as suggested by Decher [110] (redrawn from [109])

Depending on the polyelectrolyte system and the deposition parameters different growth regimes can be observed in polymer multilayer assembly:

- Polyion pairs with strong intermolecular interactions typically exhibit *linear growth* i.e. mass and layer thickness linearly increase with the number of deposited layers [102]. Occasionally, the first few layers represent an exception in that fewer molecules are adsorbed or the thickness increase is lower than expected. It is hypothesized that the surface functionality multiplies over the first deposition cycles in these cases. Only after several layers the growth regime becomes independent of the original surface charge. In addition, adsorption on to the hard surface of the substrate supposedly limits the thickness of the first layer. The surface roughness and thus also the layer thickness most probably increases with the number of layers that bind to soft surfaces until finally both parameters become constant [109].
- In contrast, polyelectrolyte pairs that show weaker interactions e.g. pairs consisting of at least one polypeptide or polysaccharide tend to grow exponentially. The *exponential growth* regime is less well studied than the previously described linear growth. The underlying mechanisms are still not fully understood. Currently, the film

roughness and the pronounced diffusion of the polycationic molecules within the multilayer structures are considered to be decisive factors. According to the model proposed by Lavalle et al. [111] exponential growth occurs if at least one of the polyelectrolytes comprised in the multilayer assembly can freely diffuse inwards and outwards within the film. Diffusion out of the multilayer is supposed to be hindered by an energetic barrier at the film surface, which prevents film dissolution. If neither of the two polyelectrolytes diffuses, linear growth is observed.

It has also been reported that certain polyelectrolyte combinations can exhibit a transition from exponential to linear growth with increasing number of adsorbed layers. Film restructuring has been suggested to progressively hinder diffusion in these systems, so that eventually the increase in film thickness becomes linear [102]. The inverse transition, from linear to exponential growth, can apparently be triggered for some polyion pairs by increasing the salt concentration in the coating solutions as demonstrated by e.g. Guzmán et al. [112].

2.4.5.2 Hydration of PEMs and Swelling Behavior

X-ray reflectivity, neutron reflectivity and Fourier-transform (FTIR) infrared spectroscopy measurements revealed that PEMS typically exhibit strong hydration even at ambient conditions. For PSS/PAH systems a water content of 10-20 % has been reported, for instance [113]. The water of hydration determines the solvent environment of the polymer molecules and thereby controls the local molecular interactions. Hence, it plays an important role for the internal dynamics and the stability of the polyelectrolyte assemblies. When brought in contact with water or humid air PEMs can absorb additional water and can swell substantially- especially if the polyelectrolyte building blocks are weakly associated. If multilayers are prepared from salt-free solutions, incubation in salt solutions can even induce restructuring of the films due to competition between the salt ions and the polyion charges. Thereby polyelectrolyte bonds can be released, which allows for further water uptake and even stronger swelling. In addition to the strength of internal interactions and the ionic strength of the surrounding solution, the nature of the terminating layer of a multilayer assembly can affect its swelling behavior [109]. As reported by Wong et al. multilayers composed of the weak polycation PAH and the strong polyanion PSS exhibited strong variation in their water absorption capacity depending on whether PAH or PSS was deposited last [114]. It was hypothesized that PSS top layers allowed for better water uptake because they assembled in a more coiled conformation and were more hydrophilic than terminating layers out of PAH. However, Schönhoff et al. suggested that the absorption behavior of PAH/PSS multilayers is governed rather by the sign of the surface potential than by the hydration properties of the outermost polymer layer itself. According to their model, changes in the water mobility within the PEM structure, which can be correlated with the potential of the terminating layer, represent the main reason for the observed variations in swelling behavior [115]. The porosity of the multilayer structure, which can be changed by the surface potential as well as by external conditions, is considered a major factor determining PEM permeability. Moreover, the surface potential has been observed to influence the dissociation equilibrium of weak polyelectrolytes in PEMs [109]. A terminating

polycation layer can increase the dissociation of carboxylic groups ($\text{COOH} \rightarrow \text{COO}^- + \text{H}^+$) in multilayer assemblies, while a terminating polyanion layer can shift the dissociation equilibrium towards the undissociated form [116]. In the second case, the internal charge density of the PEM is reduced. This can, in turn, influence the water mobility. Changes in the internal pH of polymer multilayer assemblies have been reported to have a similar effect. The internal pH of a PEM can be modified by immersing the substrate in an aqueous solution of differing pH. Based on the distribution coefficient of protons inside and outside of the multilayer, the internal pH is shifted until an equilibrium pH is reached. (Hence, the internal pH may differ from the pH of the surrounding solution.) It was demonstrated that an increase in the internal pH can lead to an increase in the dissociation of primary amino groups ($\text{NH}_3^+ \rightarrow \text{NH}_2 + \text{H}^+$) in PAH-containing PEMs. The dissociated protons can diffuse out of the multilayer assembly. The number of internal charges is decreased thereby. As described above, this can affect the mobility of the hydration water within the PEM [109].

3 Resonance Enhanced Absorption

Resonance enhanced absorption (REA) is an optical phenomenon, which has been reported by Holland and Hall in 1983 [117] and first comprehensively described by Leitner et al. in 1993 [118]. It is observed when a metal island film (MIF) or “metal cluster layer” is positioned over a reflective metallic surface that is coated with a thin translucent interlayer as depicted in Figure 16A. This setup acts as an interference filter. If the distance of MIF and mirror is within a certain range of typically 50-300 nm, the structure exhibits bright so-called resonance colors that differ strongly from the colors of the individual layers [118].

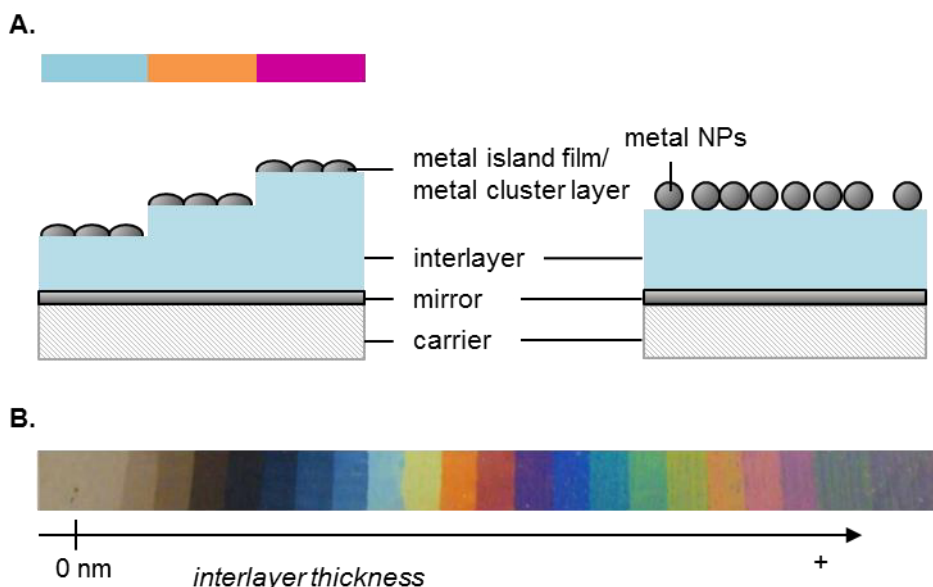


Figure 16 | REA setups **A)** Schematic drawings of REA setups with MIF created by PVD (left) and with cluster layer out of adsorbed NPs (right) **B)** Section of REA color palette (interlayer thickness increasing from left to right)

A MIF or metal cluster film is basically a monolayer of colloidal metal particles. Such films typically form in the early phase of thermal evaporation coating [118]. However, they may

also be created via sputtering, binding of NPs from solution or metal NP in-situ deposition. The interlayer may be a single layer or a multilayer structure consisting of chemically dissimilar sub-layers. It may be prepared from transparent ceramic material (e.g. SiO_2 , TiO_2) and/ or (high molecular weight) organic molecules such as polymers or proteins (internal data). For instance, Leitner et al. discussed REA by the example of a three-layer structure consisting of a silver cluster layer with a mass thickness of 3-7nm over a silver mirror layer separated by a quartz interlayer [118]. If the distance between mirror and cluster layer is close to zero, the reflectivity spectrum of REA coating mainly corresponds with that of the bare metal mirror and the cluster layer. At higher interlayer thickness the cluster composition mainly determines the colors that the REA coating adopts. In this doctoral project, cluster layers were most frequently prepared from palladium (Pd) by sputter deposition. Such REA structures go through a characteristic series of colors as the distance between mirror and cluster layer increases. At an interlayer thickness of a few nanometers, the REA coating first appears in shades of brown before it adopts a blue color (see Figure 16B). Then the color changes over light blue/ turquoise, yellow, orange and violet to another shade of blue. Hereafter, a similar series of resonance colors is passed repeatedly. Finally, the interlayer becomes too thick and resonance enhanced absorption does not occur anymore. The colors of metal mirror and cluster layer then dominate the appearance of the coating again (internal data).

3.1 Physical Background

The special interaction of REA structures with light is based on the ability of incident electromagnetic radiation to induce LSPs in the metal cluster layer as explained in 2.3.2.2. The resulting field passes the interlayer and is reflected by the mirror back to the MIF, experiencing a phase shift on the way. They interfere in the metal cluster layer (constructively or destructively) and thereby determine the reflection spectrum of the multilayer structure. Typically, narrow reflection minima are observed as the phase shift after double passing the interlayer is optimal only for a few wavelengths at a given interlayer thickness. The effect is most pronounced at the original absorption maximum of the MIF. It may enhance its absorption coefficient at this resonance wavelength at least 8 times [119].

Absorption, phase shift and reflection processes occurring in the REA multilayer structure are governed by the optical properties – i.e. the dielectric functions – of the individual components. The dielectric functions of metal mirror and interlayer are largely determined by their base material. In contrast, the dielectric function of the metal cluster layer strongly depends on its morphology. Determining factors are particle size, particle size distribution, particle shape and particle shape irregularities (as discussed in 2.3.2.2). They can often be controlled by preparation parameters. In the case of films deposited via thermal evaporation, mass thickness, deposition rate, type of substrate and temperature during deposition play a role. In addition, morphological changes and degradation processes sometimes occur after deposition due to thermal and chemical influences.

The interaction of metal cluster layer and mirror layer may be explained via two different theoretical models:

- 1) The CPS theory (developed by Chance, Prock and Silbey [120] considers the dipolar and multipolar interactions of polarizable particles with their image fields reflected from a flat metal surface. However, it does not take into account the interaction of individual particles (see 2.3.2.2). Thus, it is only applicable if the metal clusters forming the MIF are sparsely distributed and the distances between them are large compared to the distance between individual particles and the metal mirror. This situation occurs only in a few REA setups [118]. Dense metal cluster layers exhibit a shift in the reflectivity spectrum due to cluster-cluster interactions [119] (as explained in 2.3.2.2).
- 2) The so-called stratified medium theory may be employed if the metal cluster layer is rather dense. It may be modeled as a continuous layer with one effective dielectric function then. The stratified medium theory is based on the consecutive application of Fresnel's equations at every interface in a multilayer system. Light reflection and refraction occurring when a beam passes from one layer to the next are considered as well as the phase shift the electromagnetic waves experience upon reflection [118].

Application of the stratified medium theory to the three-layer structure schematically depicted in see Figure 16A reveals that the reflectivity spectra of REA structures primarily depend on two factors: 1) the absorption spectrum of the metal cluster layer and 2) the phase shift that the incident light undergoes when passing the interlayer two times. This phase shift results from three contributions: the phase of the polarization in the metal clusters relative to the driving field, the phase shift occurring upon reflection of the beam at the silver mirror and the phase retardation in the spacer layer [118].

The effective absorption intensity of a REA structure is directly proportional to the cluster density in the MIF [119].

3.2 Applications of REA Structures and Materials

Since the 1990s the potential of REA materials and setups for sensor applications and medical diagnostics is being studied. In 1998 REA-based sensors were introduced as novel (bio)analytical platforms [121]. Since 2004, the research and development company Attophotonics focuses on the development and improvement of biochemical REA sensors. A microfluidic diagnostic device employing the technology was developed by the scientific team of the company in 2009 [122]. This doctoral project was carried out in cooperation with Attophotonics and supported the research work of the company.

The project mainly dealt with two REA sensor concepts:

- 1) REA humidity sensor materials (RHS materials): In contrast to inert REA coatings, in which mirror and metal cluster layer are typically separated by a ceramic thin film, REA humidity sensor coatings are equipped with a ceramic/ polymer interlayer structure. The ceramic part of the interlayer defines the base color of the coating. On top of it, a polymer multilayer is assembled, which is capable of absorbing water molecules. If it does so - e.g. in contact with aqueous solutions or at elevated ambient air humidity – it swells strongly. As a result, the distance between mirror and metal cluster layer increases and the resonance color of the coating is shifted upwards the REA color palette (see Figure 17A). Ideally, the swelling reaction is fully reversible and the material returns to its base REA color in dry environment.
- 2) REA immunoassays: REA immunoassay chips are coated with mirror and distance layer and a bio-recognition layer on top. No metal cluster layer is applied. Hence, they do not exhibit a resonance color initially. To determine the presence or absence of an analyte of interest in a sample, the sample is mixed with a solution of analyte-specific antibody-metal NP conjugates (and mediator proteins if required). If the sample contains the analyte, analyte/antibody-NP complexes form. When the mixture is brought in contact with the REA immunoassay chip, these complexes are captured by the bio-recognition layer (either directly or via the mediator proteins) and a metal cluster layer forms on the chip surface. Thereby the REA structure is completed and the chip adopts a resonance color visualizing the positive detection of the analyte. The signal intensity is proportional to the concentration of the bound NPs. In principle, the technology is similar to classical lateral flow immunoassays and other systems based on bio-recognitive NP conjugates. Its primary advantage is the resonance effect of the REA structure. It provides an 8-fold increase of the absorption of certain wavelengths compared to the spectrum of an equivalent metal cluster layer alone [121].

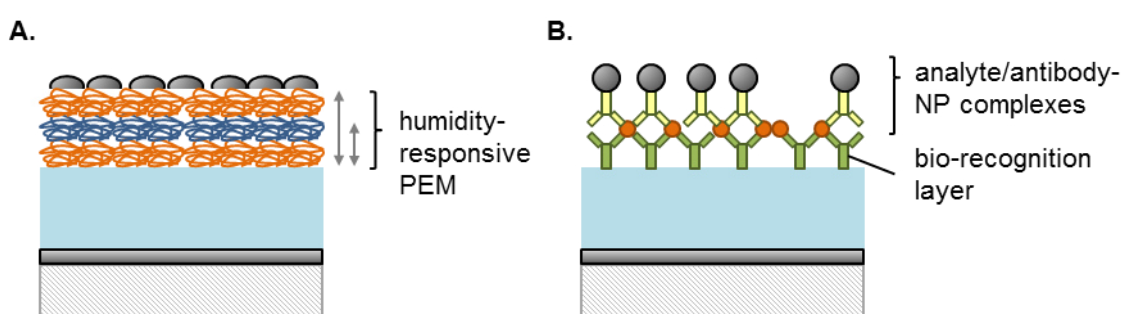


Figure 17 | REA sensor concepts A) REA humidity sensor (RHS) structure B) REA immunoassay

4 Liquid Phase Deposition of Homogeneous Polymer Monolayers for REA Humidity Sensor Materials

4.1 Introduction

REA humidity sensor coatings (RHSCs) represent multilayer assemblies of very dissimilar types of thin films. As described in the previous section, a typical RHSC comprises a mirror layer, a ceramic distance layer, a humidity-sensitive polymer multilayer assembly consisting of 5 (to up to 11 or more) polymer monolayers with alternating charge and a metal cluster layer or MIF on top. The reflectivity spectrum of a REA coating is determined by the optical thickness ($d_{\text{opt}} = \text{effective thickness } (d_{\text{eff}}) \times \text{refractive index } (n)$) of its interlayer. A minor increase in the optical thickness of a few tens of nanometers can already shift the absorption maximum and thus the color of a REA film visibly e.g. from light blue to yellow [118, 121]. Accordingly, the resonance color of an RHSC is very sensitive to thickness variations of distance layer and polymer multilayer structure. Depositing very uniform thin films is thus crucial for the preparation of RHS materials with homogenous optical appearance and reactivity. Typically, the mirror layer of an RHSC is created by PVD or electrochemical deposition. In the case of film substrates and larger flat objects the ceramic distance layer may be formed by PVD as well. In contrast, objects with pronounced 3D-structure and particulate substrates require a wet-chemical approach. Currently, sol-gel deposition of SiO_2 is preferred in this context. It yields layers of very homogeneous thickness even on large and 3-dimensional objects (see Figure 18). For film coating, the reaction can be performed in a roll-to-roll system, in which the substrate is spun off from one moving roll, is processed and is rolled up on a second roll. However, it may also be employed to coat a stack of loose pages or objects in an appropriate container.

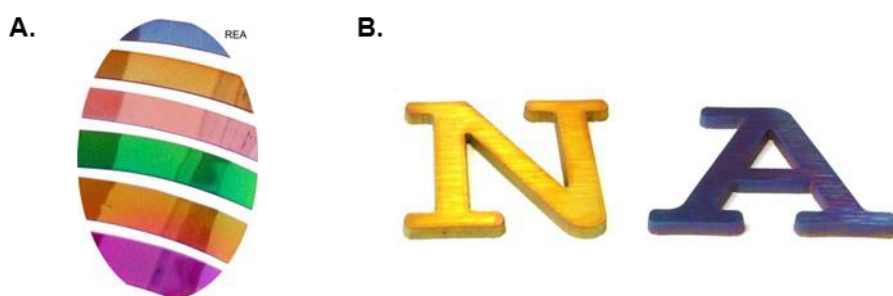


Figure 18 | SiO_2 -based REA colors on A) plastic film and B) stainless steel (Photos courtesy of Attphotonics Biosciences GmbH)

Depositing polymer layers with highly uniform thickness and molecular conformation to assemble the sensor part of the RHSC is a significantly more challenging task. REA materials may only be marketed successfully as smart labels, indicators and in rapid tests if they can be produced at low costs (around 500-2000 €/m²). The manufacturing costs are essentially determined by the productivity of the fabrication method and the required manpower. Hence, a deposition process is required that yields very uniform layers but is also compatible with larger substrate formats up to DIN A3 and suited for automation. Spin coating has already been employed successfully to prepare humidity sensor setups with high

quality PEMs (internal data). However, the method is impractical for coating large substrates as described in 2.4.4.1. CVD polymer deposition technologies (such as PECVD or the Polymer Multi-Layer (PML) technique, see [123]) may in principle be up-scaled to the desired substrate size and offer good control over polymer conformation, layer thickness and the deposition of alternating layers [124, 125]. However, the respective coating systems are quite cost-intensive and complex. For experimental purposes, the humidity-sensitive part of RHSCs has been prepared by LbL dip coating (see 2.4.4.6) so far. In this technique, the polymer layers are adsorbed by immersing the substrate alternately in solutions of two polyelectrolytes with opposite charge. In between the adsorption steps, the substrate is washed and dried to prevent contamination of the coating baths. Typically, the polyelectrolytes polyethyleneimine (PEI) and poly(methyl vinyl ether-alt-maleic acid) (PMVE-MA) were used as building blocks for the as-prepared PEMs. LbL dip coating is strikingly simple and would thus be very attractive for production. Unfortunately, the RHS materials prepared by this technique often exhibited inhomogeneous color and reactivity (see Figure 19).

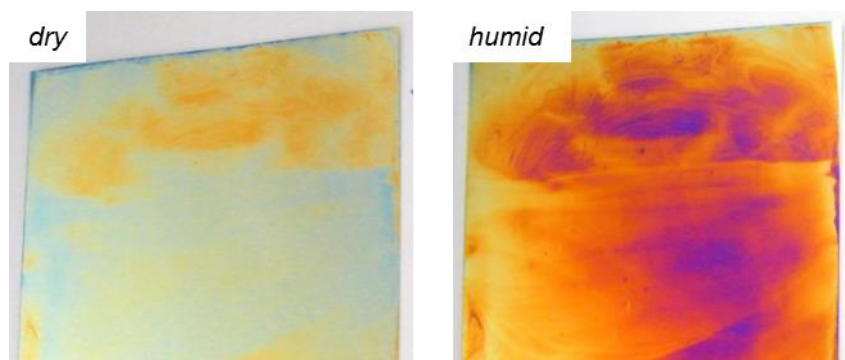


Figure 19 | Color inhomogeneities of REA humidity sensor coating prepared by dip coating
 RHS film prepared by dip coating at ambient (left) and elevated relative humidity (right)

To develop a manufacturing method from the manual coating procedure, it had to be improved significantly. For this purpose, the coating defects on RHS materials prepared by the dip coating approach were characterized optically and correlated with the coating process steps. Subsequently, it was attempted to suppress the formation of inhomogeneities by varying the composition of the employed coating and washing solutions and by modifying individual process steps. Based on the as gained understanding of the polymer deposition process a method for the automated deposition of polymer multilayers was developed.

4.2 Materials & Methods

4.2.1 Standard RHSC

To prepare a standard RHSC, a reflective substrate (commonly an aluminized polyethylene terephthalate (PET) film with a thickness of 175 μm (HiFi, #MEX12C)) was coated with a SiO_2 layer by sol-gel deposition. For this purpose the substrate was immersed in a solution of typically 0.1 M TEOS (Sigma-Aldrich, #131903) and 0.4 M ammonia (NH_4OH) (Carl Roth, #2610) in ethanol (EtOH) (Carl Roth, #T171)/ deionized water (dH_2O) ($\text{EtOH}:\text{dH}_2\text{O} = 4:1$) and

incubated in this solution at room temperature (RT) under shaking for 45-60 min. To obtain better adhesion between the SiO₂ and the first polymer layer, the SiO₂ layer was silanized as follows: A solution of 5 % (v/v) (3-glycidyloxypropyl)trimethoxysilane (Sigma-Aldrich, #440167) in EtOH was prepared and 10 ml of this solution per 86.4 ml TEOS solution were added to the TEOS solution. The substrate was incubated in the mixture under shaking for further 30 min. Then the TEOS/ silane coating solution was poured off and the substrate was extensively washed with dH₂O and dried with pressurized air using a blow-off gun. Following silanization, alternating layers of the polycation PEI (1.6 MDa) (BASF, Polymin P special) and the polyanion PMVE-MA (~216 kDa) (Sigma-Aldrich, #181099) were assembled by LbL deposition on the substrate starting with a PEI layer. Generally aqueous solutions of the two polymers of a concentration of 0.1 % (w/v) were used for layer deposition, but the composition was varied slightly in the course of the studies as described in 4.3. The individual formulations and their composition are listed in 4.2.2. For layer assembly the substrate was incubated in one of the two polymer solutions under shaking for 10-20 min. Then the coating solution was poured off and a washing solution containing a weak base (in the case of PEI) or acid (in the case of PMVE-MA) was added (see 4.2.3). The substrate was incubated in the washing solution on a shaker for 3-5 min, then rinsed 3-4 times with dH₂O and dried as described above. Typically, five polymer layers were deposited in total. Finally, the substrate was coated with a Pd (or less commonly a Ag) cluster layer by sputtering (see 4.2.5).

4.2.2 Polymer Coating Solutions

Initial experiments were performed with the following solutions:

Solution P1	0.1 % (w/v) PEI ($M_w = 1.6$ MDa) + 0.1 % (v/v) ethanolamine (Sigma-Aldrich, #15014) in dH ₂ O
Solution N1	0.1 % (w/v) PMVE-MA ($M_w \sim 216$ kDa) in dH ₂ O (prepared from stock solution: 5 % (w/v) PMVE-MA in dimethyl sulfoxide (DMSO) (Bussetti))

For competitive polycation adsorption the following solutions were used:

Solution P2	0.1 % (w/v) PEI (1.6 MDa) + 0.1 % (w/v) PEI (2 kDa) (BASF, Lupasol® G35) + 0.1 % (v/v) ethanolamine in dH ₂ O
Solution N1	see above

4.2.3 Washing Solutions for Polymer Deposition

Washing solution WP1 0.1 % (v/v) ethanolamine in dH₂O
(for polycation layer)

Washing solution WN1 0.1 % (v/v) acetic acid (Merck) in dH₂O
(for polyanion layer)

4.2.4 Pre-coating

It was tested whether more homogeneous polycation and polyanion layers could be adsorbed in the LbL deposition process if their adsorption was delayed. For this purpose, the substrate – generally an aluminized PET-film coated with SiO₂ and (3-glycidyloxypropyl)trimethoxysilane – was pre-coated with a water soluble reagent before each polymer adsorption step. The following pre-coating solutions were tested:

Solution A: 10 % (w/v) PVP (M_w = 360 kDa) (Sigma-Aldrich, #PVP-360) in dH₂O

Solution B: 10 % (w/v) poly (vinyl alcohol) (PVOH) (hydrolyzed, M_w = 30-70 kDa) (Sigma-Aldrich, #P8136) in dH₂O

Solution C: 10 % (w/v) PVP (M_w = 10 kDa) (Sigma-Aldrich, #PVP10) in EtOH

Solution D: 2 % (w/v) 2-hydroxyethyl cellulose (HEC) (M_w ~ 90 kDa) (Sigma-Aldrich) in dH₂O

Solution E: 5 % (w/v) polyethylene glycol (PEG) 4000 (M_w = 3500-4500 Da) (Herba Chemosan) in dH₂O

Solution F: 85 % (v/v) glycerol (Herba Chemosan) in dH₂O

One of the above solutions (A, B, C, D or E) was applied to the substrate by dip coating, roll blade coating or screen printing and dried at RT or at 50°C. For roll blade coating a silicon roll blade was used. For screen printing a loose PES HT 120/ 40 PW mesh (F. Huhn & Sohn GmbH) was employed. Alternatively, the substrate was covered with a thin film of solution F and incubated with this film for 5 min. Then PEI or PMVE-MA was deposited as described in 4.2.1.

4.2.5 Preparation of Mirror layers and Metal Cluster Layers

Sputtered Pd Metal Cluster Layers and Pd/Ag Alloy Mirror Layers

Pd metal cluster layers and Pd/Ag alloy mirror layers were deposited using a lab-scale DC sputter coater with a point target (circular or square with a diameter of ~5 cm) built by Attophotonics Biosciences GmbH (sputtering parameters listed in Table 3). The substrate was commonly placed on a rotating table at a distance of ~20 cm to the target. To improve the adhesion of Pd/Ag mirror layers on thermoplastic polyurethane (TPU) substrates (thickness: 100 µm)¹, they were generally pre-coated with a thin chromium adhesion layer.

¹ provided as a gift, the supplier may not be revealed

<i>Layer Type</i>	<i>Voltage</i>	<i>Current</i>	<i>Pressure</i>	<i>Deposition Time</i>
Cr adhesion layer	386 V	162 mA	9×10^{-3} mbar	10 min
Pd cluster layer	295 V	187 mA	$2.0\text{-}2.8 \times 10^{-2}$ mbar	45 s
Pd/Ag mirror layer	426 V	135 mA	$2.0\text{-}2.8 \times 10^{-2}$ mbar	3 min
Ag mirror layer	300 V	185 mA	$2.0\text{-}2.8 \times 10^{-2}$ mbar	2 min
Ag cluster layer	300 V	185 mA	$2.0\text{-}2.8 \times 10^{-2}$ mbar	25 s

Table 3 / Sputtering parameters

Aluminum Mirror Layers

200 nm thick aluminum mirror layers were deposited by thermal evaporation of Al wire in a BOC Edwards Auto 306 Vacuum Coater with rotating sample holder. A horizontal tungsten coil was used as evaporation source. To improve the adhesion of Al to TPU substrates, they were surface-activated by corona treatment using a FG2-1 corona unit from Ahlbrandt System (nominal generator output: 500 W, frequency: 50 Hz) at 70 % power and a feed speed of ~0.3 m/s before aluminum coating.

4.3 Results & Discussion

4.3.1 Defect Pattern Analysis

If the humidity-sensitive part of RHSCs was prepared by LdL dip coating, the coatings generally exhibited diverse patterns as shown in Figure 19. These apparently resulted from methodological problems in the LbL coating procedure. The REA phenomenon strongly facilitated investigations in this field. Analytical instruments with nanometer resolution were not required to measure the thickness of the polymer assemblies created by LbL coating, as they were generally incorporated in a REA structure. Hence, the thickness of the polymer assemblies could be assessed very precisely by comparing the color of the REA structure with REA color standards such as the palettes depicted in Figure 20. Such comparisons dominated this doctoral project and are regularly discussed within this thesis. To simplify the corresponding descriptions, REA colors resulting from low interlayer thickness will be referred to as “low REA colors” further on. In accordance, colors that occur at high interlayer thickness will be referred to as “high REA colors”. Colors will be compared by calling them “higher” or “lower” REA colors.

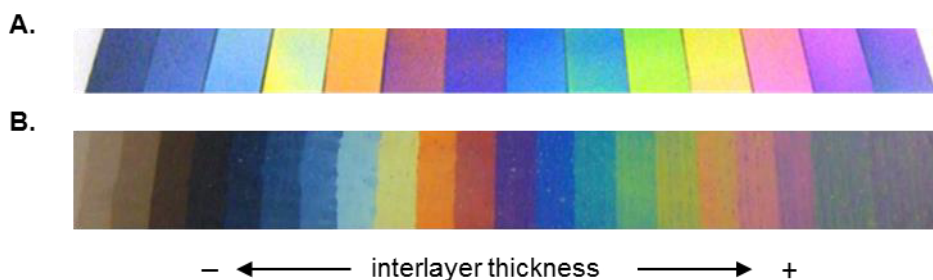


Figure 20 | REA color palettes A) Stripes of aluminized PET film coated with SiO_2 interlayers of increasing thickness and Pd cluster layer **B)** Aluminum plate coated with stripes of Al_2O_3 of increasing thickness and Pd cluster layer

The palettes were generally prepared from substrates with highly reflective metallic surface, onto which a ceramic interlayer (typically SiO_2 or Al_2O_3) was deposited (for details see 5.2.1). The interlayer thickness was increased from segment to segment to obtain different resonance colors. On top, a metal cluster layer (from Pd or Ag) was applied to complete the REA setup. Of course, the refractive indices of the interlayers of these palettes differ from the refractive indices of the analyzed polymer thin films (barrier-type Al_2O_3 : $n \sim 1.65$ -1.7 [126], sol-gel deposited SiO_2 (non-annealed): $n \sim 1.15$ -1.25 [127], crystalline PEI: $n \sim 1.42$ [128], PMVE-MA: no data available). Thus, the absolute thickness of the polymer assemblies within RHSCs could not be determined by comparing their color with the depicted palettes. Fortunately, their absolute thickness was not of interest, however. Analyses focused on thickness variations. These could be detected with ease by the help of the REA phenomenon. Comprehensive screenings of coating defects on sheets of 10 x 20 cm with nanometer resolution could thus be performed within a short time. In the course of this study, the defects resulting from manual LbL PEM assembly could be distinguished into four types, which are listed in the following.

First Contact Defects

Wavy lines and linear patches parallel to the short edges of the substrate were frequently observed on REA humidity sensor films prepared by dip coating (see Figure 21).



Figure 21 | Linear defects on RHS film The depicted RHS film with blue base color exhibited yellow wavy lines (*) and linear patches (**) at both short ends.

The colors visible on such films were correlated with the intervals of the REA color palette depicted in Figure 20 to assess the interlayer thickness in the individual areas. The linear defects generally exhibited higher REA colors than the surrounding areas (see Figure 21).

This indicated that the polymer structures below the defects were thicker than the surrounding PEM. Hence, they apparently either comprised polymer molecules of more globular conformation than the surrounding regions or polymer aggregates. Washing could be ruled out as source for the line defects. The washing solutions utilized to remove unbound polymer molecules in between the dip coating steps were generally poured onto the middle of the substrate. Defects resulting from the washing steps would therefore have been patchy and would have exhibited random shapes. Apparently, the defects did not form during incubation in the polymer deposition solution either. If they had done, the defects would have been distributed over the whole surface. The line defects were limited to the two ends of the RHS films, however. In the LbL dip coating process, the films were generally immersed with one of the short edges (and not the long ones) first (see Figure 22). Therefore, it was most probable that the lines resulted from the first contact between the substrate and the polyelectrolyte solutions.

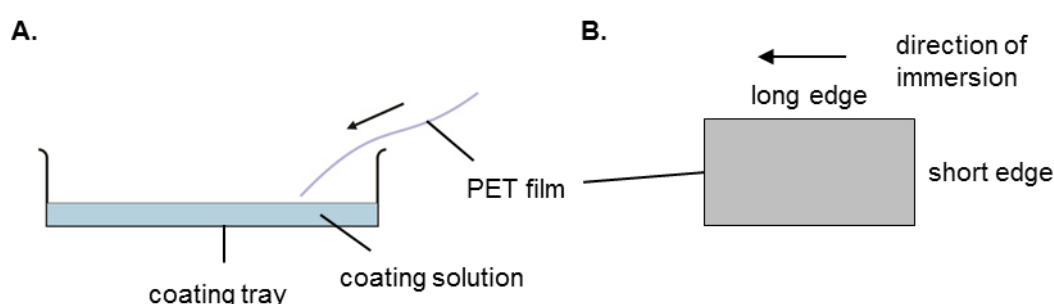


Figure 22 | Immersion of substrate in LbL dip coating (classical strategy) **A)** Schematic drawing of immersion process **B)** Schematic drawing of PET film substrate (The typical immersion direction is indicated.)

This finding was surprising. Separate trays were used for the adsorption of the anionic and the cationic polymer. The trays were rinsed with dH₂O and occasionally even cleaned with detergent, EtOH and dH₂O in between the coating steps. Hence, the solutions were unlikely to be contaminated in the course of the assembly process. The trays were not dried completely, however, because coating containers in a production machine cannot be dried completely either. Some droplets of water typically remained in the trays after rinsing. Contaminating the solution with clean dH₂O was not considered problematic, however, because aqueous polymer deposition solutions were used. (As described below, this assumption proved incorrect in the course of the project.) The working hypothesis on the formation of the linear defects was that a layer of polyelectrolyte chains with unusual conformation develops spontaneously at the interface between at least one of the two polymer coating solutions and the surrounding air. It was assumed that the polymer molecules within this layer are more globular than the molecules within the coating solutions. Upon immersion of the substrate, part of this interfacial layer is transferred onto the substrate in a Langmuir-Blodgett-like fashion. The fast sinking of the film into the coating solution and the resulting convection were supposed to disrupt the interface layer. Therefore, structurally altered molecules were only deposited on the ends but not in the middle of the film. The polymer solutions were generally filled into the coating tray shortly before the coating

procedure was started. Thus, the layer on the solution surface would have had to build up quite fast. The described working hypothesis implied that the contact between the substrate and the solution surface had to be prevented to avoid the formation of the described line defects. This led to the development of two techniques, whereby polyelectrolytes could be prevented from adsorbing before the substrate was fully immersed in the coating solution. The two techniques and their development are discussed in detail in 4.3.2 and 4.3.3. In the first approach, the substrate was pre-coated with a water-soluble agent before plunging it into the polyelectrolyte solutions. In the second strategy, low molecular weight polyelectrolytes were added to the polymer deposition solutions. These should compete with the high molecular weight polymers and delay their adsorption. Unfortunately, the strategy was only successful in the assembly of the polycation layers. However, the pre-coating technique helped to reveal that only the polycation formed first contact defects, not the polyanion. Therefore, the competition strategy (described in 4.3.3) was eventually sufficient to suppress the formation of first contact defects. It was well suited for regular use and was thus employed in all further experiments. First contact defects did not form anymore. Only then, further types which are discussed in the following sections could be identified. The hypothesis on the formation of first contact defects was corrected, when it was attempted to automatize the coating procedure. The respective investigations are reported in detail in 4.3.4. Nevertheless, the results must be mentioned here. Only then the formation of marbling defects and drying defects described below may be understood. It could be shown that the polymer molecules at the surface of the polycation deposition solution only adopted a different conformation than the polymer chains within, if the solution was mixed with pure water and not stirred properly. As mentioned, the coating trays were rinsed with dH_2O in between coating steps but were not dried. Hence, they generally contained a small amount of water, when fresh coating solution was poured in. Obviously, water does not mix immediately with the employed polymer solutions. It rather forms a second phase with lower polymer concentration, which floats on top of the deposition solution. At low concentration the polymer molecules apparently adopt a more globular shape. Upon immersion of a substrate, these polymer molecules are obviously deposited on its front end. Then the fast plunging of the substrate and the resulting convection promotes the adsorption of polymer molecules from within the deposition solution, which exhibit a more expanded, flat conformation. Apparently, the effect is stronger for the polycation. First contact defects never formed in the polyanion solution. Marbling defects, which are discussed below and apparently result from a similar process, could be correlated with polyanion deposition steps, however.

Marbling

Using the competition strategy described in 4.3.3 nearly completely homogeneous REA humidity sensor coatings could be created. Only occasionally, individual films showed marbled areas as depicted in Figure 23.



Figure 23 | Marbling defects in REA humidity sensor coating

It was found that such patterns only occurred if the coating tray was not dried carefully before depositing the polycation. The immersion strategy had been changed in the course of the investigations described in the above section and sections 4.3.2 and 4.3.3 to be able to better correlate coating defects with the immersion procedure. The substrate was not immersed by classical dipping (as depicted in Figure 22) anymore. Instead, the coating solution was spread over it from one end. For this purpose the coating tray was lifted on one end and the coating solution was poured in at the other end. The substrate was put into the empty, lifted part of the tray. Then the lifted end was lowered so that the solution flowed over the film. If the coating trays were not dried before the coating steps, the final RHS film exhibited marbling patterns as shown in Figure 23. Like the first contact defects described above, these marbling defects apparently result from concentration gradients within the polymer solution. When spreading the polyanion solution over the substrate it mixes only incompletely with residual water in the tray if present. Then the polymer concentration at the interface between substrate and deposition solution varies. No overlapping marbling patterns were observed. Apparently, they only form in one polyelectrolyte deposition step. In all PEI deposition steps, low molecular weight polycation molecules were added to the deposition solution, as described above. They delayed PEI adsorption and thereby prevented defects resulting from concentration gradients in the PEI solution. Hence, residual washing water in the coating tray should not have interfered with PEI assembly. Thus, marbling patterns most probably formed in one of the two PMVE-MA deposition steps. Currently, the causative thickness variations are considered to be restricted to the second PMVE-MA layer, because it is significantly thicker than the first one. This could be demonstrated by the following experiment: A standard RHS film was prepared according to the protocol provided in 4.2.1. After silanization, a strip was cut off the film (strip #0). The same was done after each polymer deposition step (strips #1-5). Finally, all strips were coated with a Pd metal cluster layer and their REA colors were compared to an aluminum-based REA color palette see Figure 24. (For details on the preparation of the palette see 5.2.1.)

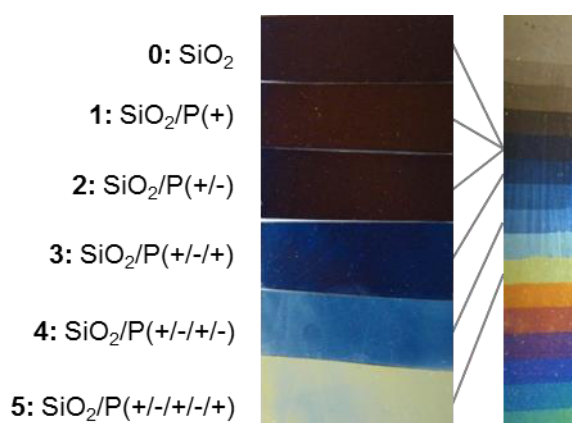


Figure 24 | Interlayer thickness increase in the course of polymer multilayer assembly The depicted six strips (left) were cut off a RHS PET film before (strip #0), in between (strips #1-4) and after (strip #5) the five polymer deposition steps. The strips were provided with a Pd metal cluster layer by sputtering. The resonance colors of the strips were compared to an aluminum-based REA color palette with linearly increasing interlayer thickness. Corresponding REA colors are indicated by lines.

The palette covered REA colors of very low to medium interlayer thickness ($d_{\text{opt}} = \sim 0\text{--}420\text{ nm}$) i.e. very low to moderately high REA colors. The interlayer thickness of each color on the palette differed from the interlayer thickness of the next color by the same value. Hence, the interlayer thickness increased linearly over the palette. The interlayer growth on the standard RHS film followed a different regime. The first three samples were cut off after silanization (strip #0), after the first PEI deposition step (strip #1) and after the first PMVE-MA adsorption step (strip #2). Nevertheless, all three strips exhibited nearly the same REA color. The interlayer thickness had apparently not increased visibly within the first two rounds of polymer deposition. Strip #3 was the first strip showing higher resonance color. It differed from the color of strip #1 by one interval of the REA color palette. In contrast, the fourth deposited polymer layer (strip #4) shifted the interlayer thickness of the RHS film by two color intervals. In the fifth polymer deposition step (strip #5), the interlayer grew to a similar extent. It was concluded that polymer molecules adsorb in a rather elongated shape in the first two rounds of polymer coating. Only in the latter three polymer coating steps, they can adopt a more globular conformation. Similar binding regimes have been reported earlier as discussed by Schönhoff et al. [109] (see also 2.4.5.1). It is hypothesized that the PMVE-MA molecules cannot adsorb in a pronounced 3-dimensional shape in the first polyanion layer (=the second layer of the PEM), independent of the polymer concentration in the deposition solution. The conformation of the deposited molecules can therefore not be affected visibly by water contamination. Hence, marbling defects presumably only develop during the second polyanion adsorption step. Unfortunately, this assumption could not be verified without doubt, because marbling could not always be generated intentionally.

Flow Defects (Large Area Thickness Variations)

Another group of defects, which will herein be further referred to as “flow defects”, was commonly observed on non-planar substrates. Insights into the causative processes could be gained by experiments on the coating of thermoplastic polyurethane (TPU) film. It was studied as alternative to PET films in the preparation of REA humidity sensors. The selected

TPU film did not carry a reflective layer like the aluminized PET used previously. Hence, it was first coated with a Pd/Ag mirror layer (Figure 25 A) or an Al mirror layer (Figure 25 B) (see 4.2.5). The 100 μm -thick film is very flexible, however, and generally deforms and rolls up during handling if not stabilized mechanically. Thus, TPU film pieces were mounted on frames before any kind of thin film was deposited onto them. The TPU film depicted in Figure 25A was mounted on a moderately flexible PET-frame, for instance, and then provided with sputter-deposited Pd/Ag mirror layer and a standard RHSC (see 4.2.1). Due to the differential thermal expansion coefficients of TPU film and PET frame the TPU corrugated, when it heated up in the sputtering process. It retained an undulated shape after cooling and in all subsequent coating steps.



Figure 25 | Flow defects on corrugated TPU REA films **A) TPU RHS film** The prominent areas (“hills”) exhibited a yellow resonance color indicating a locally thicker interlayer (= SiO_2 distance layer + PEM) (*... hills, #... valleys). **B) TPU REA film without (w/o) humidity sensor layer** The prominent areas of this sample also carried a thicker SiO_2 distance layer appearing in blue-violet on a yellow-pink background (*... hills, #... valleys).

After completion of the RHS coating it was found that the prominent areas of the film (“hills”) carried a thicker interlayer coating than the recessed parts (“valleys”) (see Figure 25A). To avoid these irregularities the PET frame was replaced by a wire frame in a subsequent test. The TPU film was only fixed to it at its two ends. Instead of a complete RHS coating a simple REA coating only comprising Al mirror layer, SiO_2 interlayer and Pd cluster layer but no polymer multilayer was applied. The SiO_2 distance layer was created in two sequential rounds of deposition using a coating solution with high TEOS concentration (0.4 M TEOS in $\text{NH}_4\text{OH}/\text{EtOH}/\text{dH}_2\text{O}$). However, the as-prepared film exhibited similar irregularities as the TPU RHS film (see Figure 25B). It is supposed that the convection speed in the immediate environment of the substrate strongly influences the deposition rate in SiO_2 sol gel deposition as well as in polymer self-assembly. It has been reported earlier that the nature of the deposition method can have a pronounced effect on the structure of the generated polymer coatings. In this context, Michel et al. suggested that shear forces might influence the conformation of adsorbing polymer chains [129]. Moreover, polyelectrolyte assembly might be accelerated if the deposition solution flows faster over certain regions of the substrate. Especially the replacement of the protective low-molecular weight polycation by the high-molecular weight species might occur faster. Additionally restructuring of the polyelectrolyte layers could be induced. The underlying processes were not investigated in detail, but the thickness of the SiO_2 / polyelectrolyte layer was frequently observed to mirror the flow of the

coating solution over the substrate in the coating tray. If the shape of the substrate or the coating tray provoked stronger convection at places, the deposited ceramic or polymer interlayer grew thicker there.

Drying Defects

When REA films were prepared manually, the substrate was dried after each round of polymer deposition using pressurized air. During drying, care was taken that the water film covering the RHS film after the last washing step was blown off the film quickly and completely and that dry areas were not wetted again. However, sometimes droplets splashed back onto already dried parts of the film or tiny amounts of washing water flowed back. This occasionally resulted in patterns like those depicted in Figure 26A. The depicted RSH film was dried after deposition and washing of the fifth polymer layer. Drying was nearly completed, when some water splashed back onto the film. Two water droplets had to be blown over the already dry film. The path of the droplets became visible after deposition of the metal cluster layer. It was marked by semicircular defects in the RHS coating. Their color indicated that the interlayer underneath was thicker than in the surrounding areas. In this context such thickness variations may in principle be caused by three processes

- 1) Deposition of contaminants or residual unbound polymer from the washing water
- 2) Local modification of the internal pH of the polyelectrolyte assembly due to the extraction of pH-active small molecules (here residual ethanolamine or acetic acid molecules from the washing solutions)
- 3) Restructuring of the polyelectrolyte layers upon rehydration

The defects found on the sample shown in Figure 26B support the third hypothesis. The depicted aluminized PET film was provided with a standard RHS coating in two phases: The SiO_2 distance layer and the five-layer polymer assembly were completed on one day. The sputtered Pd cluster layer was added on the morning of the following day. In between, the film was stored at RT in a cleanroom work bench lying on a paper towel on the perforated work plate. To avoid contamination and deposition of dust, it was covered with a tray turned upside down. The film had been dried after completion of the fifth polymer layer. However, its backside was still wet when it was placed onto the towel in the cleanroom bench and covered with the tray. After depositing the Pd layer on the second day, the RHS layer exhibited randomly colored spots. They resembled the holes in the work plate, on which the film had been stored, in size, shape and distribution. Apparently, the residual water on the backside of the film evaporated during the storage of the film and increased the humidity in the chamber formed by the tray and the work plate. This led to partial rehydration of the PEM on the REA film. Above the holes in the work plate the film was cooler than in the surrounding areas. Here the evaporated water condensed, which led to local variations in the rehydration speed and the speed of the secondary drying. Acid or base molecules may not be washed out by this type of rehydration nor may significant amounts of contaminants be deposited. Hence, the polyelectrolyte multilayer was apparently partially restructured overnight. As discussed in [109], the inner layers of polyelectrolyte assemblies are commonly

quite stable, because they interact strongly with neighboring layers. The outer layers exhibit higher flexibility, however, and are susceptible to rearrangements, which may result in the observed drying defects in RHS coatings.

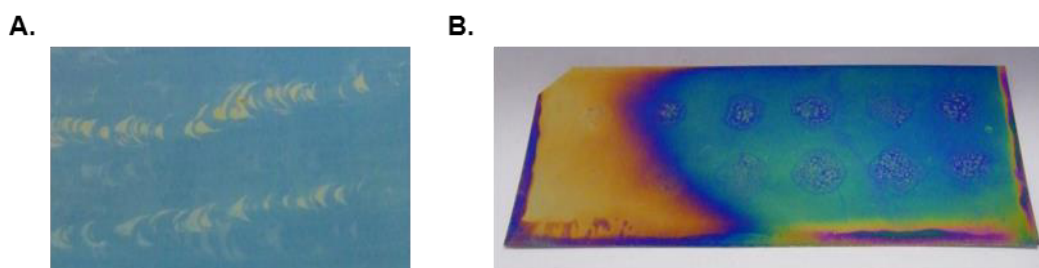


Figure 26 | Drying defects

4.3.2 Defect Prevention by Pre-coating

Initially it was attempted to delay the first contact of substrate and polymer solution by the help of a water soluble pre-coating. This coating should prevent polymers from adsorbing to the substrate during its immersion in the coating solution. Within the coating solution, the coating should dissolve and expose the substrate surface. Then, polymer molecules from within the solution should be able to bind. By the help of this coating, molecules from the solution surface with unusual conformation should be hindered from binding and first contact defects should be prevented. Several water-soluble binders were screened for this purpose, including polyvinyl alcohol (PVOH), polyethylene glycol (PEG) 4000, polyvinylpyrrolidone (PVP), and 2-hydroxyethyl cellulose (HEC). Also glycerol was tested as a “liquid pre-coating” (for details see Table 4 and chapter 4.2.4). High molecular weight PVP and HEC were found to completely block multilayer assembly, but low molecular weight PVP and PEG 4000 could be employed successfully for pre-coating. The formation of first contact defects could be prevented with both agents. However, the RSHCs prepared with pre-coating exhibited new patterns that retraced the spreading of the pre-coating solution on the substrate. Different application techniques were tested including screen printing, roll blade coating and dip coating, but all methods resulted in characteristic defects. Screen-printed films showed a grid pattern, for instance. Fortunately, most patterns created via pre-coating could easily be distinguished from defects that originated from the polyelectrolyte adsorption steps. Best results were obtained if PEG 4000-based pre-coating formulation was applied by screen-printing. If the substrate was pre-coated this way prior to each polymer adsorption step the resulting RHS film did not exhibit any first contact defects and only faint grid patterns resembling the employed screen. This made the pre-coating approach a valuable tool for further investigations on the polyelectrolyte multilayer assembly process. A critical question could be elucidated: Do first contact defects form in all five polymer deposition steps or is one of the two polyelectrolyte species primarily responsible for the observed inhomogeneities? To exclude defect formation during the adsorption of the cationic polymer, a PEG pre-coating was applied before the respective deposition steps. The polyanion layers were generated without PEG pre-coating. The as-prepared RHS film did not show any first contact defects at all, which indicated that these usually develop during polycation assembly.

Thus, first contact defects only had to be counteracted in three of five polymer layer assembly steps. The pre-coating technique was time-consuming, however, and left faint marks on the substrate. For large-scale RHS film production an alternative protective strategy was required.

<i>Solution</i>	<i>Composition</i>	<i>Result</i>
A	10 % (w/v) PVP (360 kDa) in dH ₂ O	no pattern of polyelectrolyte adsorption, total interlayer thickness lower, no humidity sensor effect
B	10 % PVOH (30-70 kDa) in dH ₂ O	no pattern of polyelectrolyte adsorption, poor reaction to humidity, drying pattern of PVOH visible on film
C	10 % PVP (10 kDa) in EtOH	no pattern of polyelectrolyte adsorption, pattern of pre-coating
D	2% HEC (~90 kDa) in dH ₂ O	no pattern of polyelectrolyte adsorption, total interlayer thickness lower, no humidity sensor effect
E	5% (w/v) PEG 4000 (3500-4500 Da) in dH ₂ O	no pattern of polyelectrolyte adsorption, only faint pattern of pre-coating if applied by screen printing
F	85% (v/v) glycerol in dH ₂ O	defects worse than w/o pre-coating, more patterns resulting from glycerol being washed away

Table 4 | Pre-coating strategy Tested pre-coating solutions and properties of obtained RHS coatings

4.3.3 Defect Prevention by Competition

A new approach was thus developed. It was inspired by the so-called Vroman effect, a phenomenon commonly described in context with the adsorption of proteins to surfaces. If a solution of different types of proteins is applied to a surface that permits adsorption, smaller molecules will generally bind faster due to their higher diffusion speed. Over time, they will be replaced by larger proteins, however, for these possess more binding sites per molecule and thus can attach more strongly. In order to obtain a similar effect in PEI layer assembly, a small-molecular weight variant of the polycation was added to the deposition solution (see 4.2.2). The composition of the polyanion solution was left unchanged. With these polymer solutions practically defect-free RHS films could be prepared by the standard immersion coating procedure for the first time (see Figure 27). Pre-coating was not required.

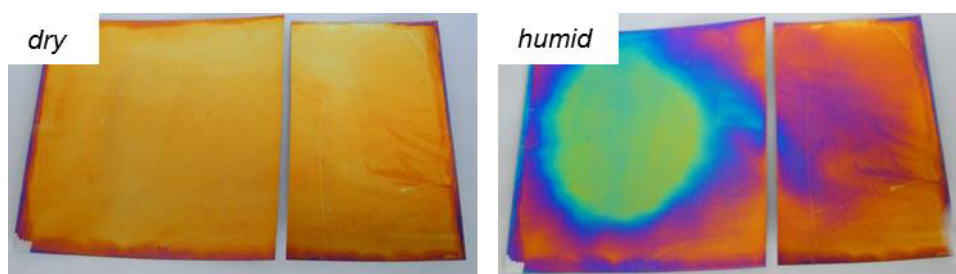


Figure 27 | RHS film prepared using mixture of high M_w and low M_w PEI

The competition approach is strikingly simple and thus attractive for automated manufacturing. In contrast to the pre-coating strategy it does not add process steps to the coating procedure. The underlying competition mechanism, which is supposed to suppress inhomogeneous assembly of the high-molecular weight polycation, is depicted in Figure 28.

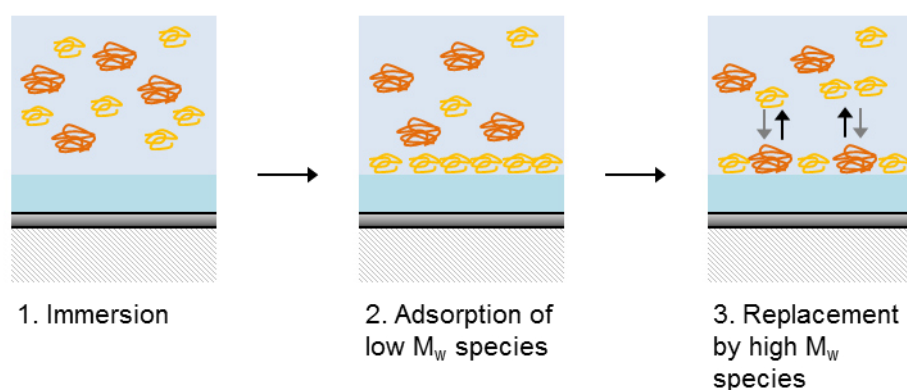


Figure 28 | Defect prevention by competitive polymer adsorption

4.3.4 Automation of SiO₂ and Polymer Deposition

4.3.4.1 Initial Considerations & Machine Design

At the start of this doctoral project, RHS films could only be prepared manually at a maximum format of about 20 x 20 cm. Larger substrates could not be handled without risking defects. To market the technology in the field of chemical indicators a higher productivity was indispensable. Therefore, Attophotonics was interested in developing a coating system, in which SiO₂ and polymer layers can be deposited on films of a size of at least 1 m². Based on the results of the defect analysis reported so far, several requirements for a suitable coating system could be defined:

1. Either substrate or surrounding coating solution must be agitated homogeneously. (In principle, it is possible to deposit the polymer layers by mere incubation without convection, but the deposition rate then decreases significantly. For depositing the single polymer layer, the substrate would have to be incubated for ~1 h. With agitation, 10-15 min of incubation are sufficient.)
2. Under agitation the substrate must be incubated with the SiO₂ deposition solution for at least 30-45 min and with the polymer deposition solutions for at least 10 min.

3. The substrate must be dried after depositing the SiO_2 distance layer and each single polymer layer to avoid concentration gradients in the coating solutions due to residual washing water.
4. The inner volume of the coating container should be as low as possible to minimize the consumption of reagents and washing water.

Originally a R2R coating system was considered. In principle, a broad range of additive and subtractive manufacturing processes can be performed in R2R systems including vacuum, thermal and solution-based methods [130]. It had also been demonstrated earlier that plastic films can be sol-gel-coated with SiO_2 in such a system. Instead of incubating the film in a tray for the required period of time, the coating solution was sprayed onto the substrate. The substrate was running through the machine horizontally at very low speed, so the sprayed-on wet film could react on the substrate for a sufficiently long time. The as prepared SiO_2 layers were of moderate quality, however, because the more volatile components of the deposition solution, EtOH and NH_4OH , tended to evaporate from the sprayed-on liquid film before the deposition reaction – taking 30-45 min – was completed. To assemble polymer layers in a R2R-coater on a film substrate, it would have to be pulled through a series of coating and washing baths. The film would have to be dried very carefully in between each adsorption step and before being rolled up at the end of the machine. A very long machine would be required to deposit all five polymer layers in line. In a short production line only one layer could be assembled per run. In such a setup, the substrate would have to be dried and rolled up after each polymer deposition step. Unfortunately, the polymer nanolayers are sensitive to mechanical contact. Hence, only the backside of the substrate could be dried by wiping. The functionalized side would have to be dried by a contactless method i.e. using pressurized air. Heating is not considered suitable for the purpose, for it might lead to inhomogeneous evaporation of washing water. Drying defects might result (see 4.3.1). Several other potential sources of coating defects were identified in the conceptual planning of a suitable R2R coater, but they shall not be discussed in detail. Overall, R2R production of RHS films turned out to be technically infeasible. Instead, the system depicted in Figure 29 was designed, which will be further referred to as “Endless Band Coater”: The substrate holder of the system comprises an endless band carrier belt made from silicon that is mounted on two rolls. The belt, onto which the substrate is fixed, is driven via the upper roll that is connected to a motor. The lower part of the substrate holder reaches into a narrow tray. The substrate is coated by cycling it through the tray, which is filled consecutively with the individual coating and washing solutions. The small inner volume of the tray minimizes the consumption of reagents. A casing surrounding substrate holder and tray reduces the evaporation of solvent from tray and wetted substrate.

In R2R systems larger substrates can be processed, of course. Nevertheless, an Endless Band Coater system of the size of a cupboard was expected to be suitable for the production of films at medium batch sizes.

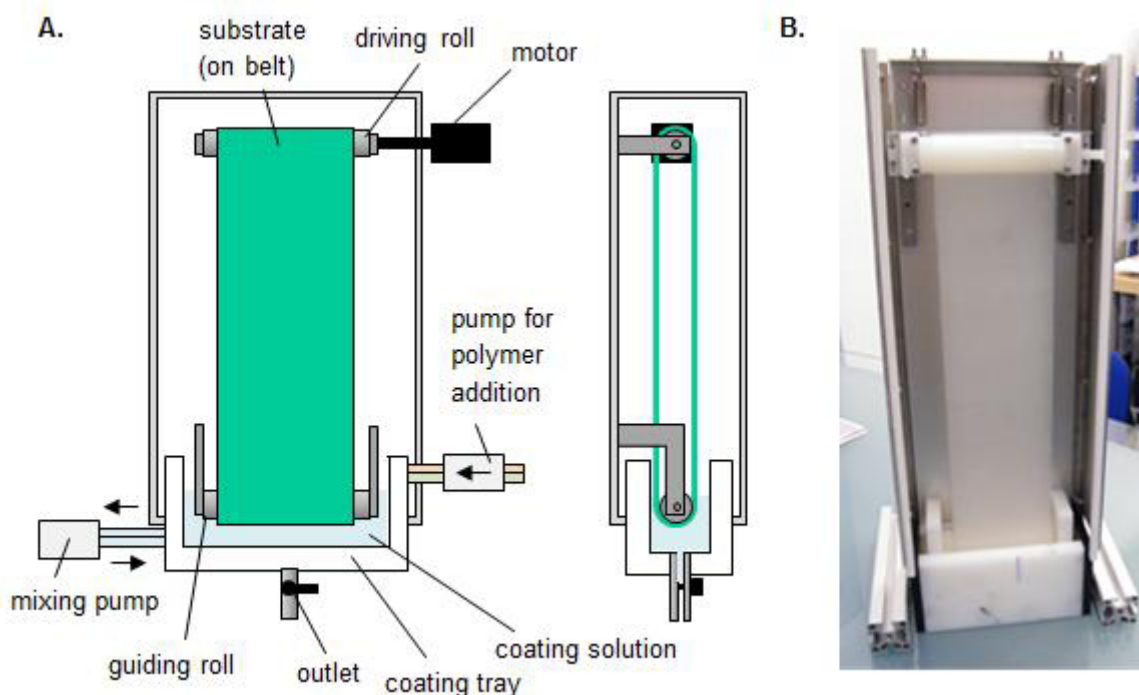


Figure 29 | Endless Band Coater for wet-chemical SiO₂ and polymer deposition **A)** Schematic drawing of basic components of the model system (pumping system not included) **B)** Model system with belt, partially completed

The compactness and simplicity of the system significantly facilitated machine and process development. A model system was constructed to assess the functionality of the setup (see Figure 29B). Films with a maximum size of 100 x 12 cm could be coated in this system.

4.3.4.2 Coating Tests with Endless Band Coater Model System

The model coater system was tested using standard SiO₂ deposition solution (see 4.2.1) and the polymer coating and washing solutions established for the competitive polymer layer assembly approach (solutions P2, N2, WP1 and WP2 see 4.2.2 and 4.2.3). The belt speed was commonly adjusted to 20-24 rpm. After each polymer deposition step, the substrate was washed and dried with pressurized air. To add the metal cluster layer the PET film was cut into pieces and sputtered with Pd as described in 4.2.5.

First tests aimed at preparing non-sensor REA films i.e. a single SiO₂ interlayer was deposited on an endless band of aluminized PET film. For this purpose the substrate was fixed on a silicon belt, which was mounted on the two rolls of the model coater (see Figure 29). As depicted in Figure 30A, a very homogenous coating could be created already at the first try. Assembling a uniform polymer multilayer turned out to be more challenging. A functional humidity-sensitive structure could be deposited, but color and reactivity of the RHS film were highly inhomogeneous (see Figure 30B).

A. PET/Al/SiO₂/Pd



B. PET/Al/SiO₂/Polymer(5x)/Pd



Figure 30 | First test of Endless Band Coater A) Non-sensor REA coating B) Complete RHS coating

Further investigations and coating tests revealed the reasons for the individual patterns found on the prepared RHS films. Three main defect types are discussed in the following table:

#	Sample	Discussion
1		<i>Observations:</i> Coating solution caught between the silicon belt and the substrate could generally not be removed completely in the washing steps. This led to contamination of the subsequently employed coating solutions. Precipitates formed and deposited on the substrate. This type of defect was most pronounced near the ends of the substrate, where the trapped solution leaked out most. <i>Conclusions:</i> No belt should be used, because the coating solutions cannot be prevented from entering the space between substrate and belt. The substrate has to be mounted on the rolls directly.
2		<i>Observations:</i> Two other common defects were splatters exhibiting higher resonance colors than the surrounding area, and lines running parallel to the moving direction of the film with lower resonance color. They resembled the drying defects discussed in 4.3.1. It was concluded that both, lines and splatters, resulted from drying the film with pressurized air. Splatters were exclusively found near the two ends of the substrate film. Supposedly, washing water from the backside of the film (possibly contaminated with residual

	polymer solution) was blown out from the gap between the two ends of the substrate onto the front side of the film. (The ends were connected with staples and nylon thread leaving a slit in between.) The blue, vertical line defects resembled traces of droplets running over a flat surface. To dry the substrate, it was generally cycled at low speed in one direction, while the washing water was blown off in the other direction. Typically, some droplets of washing water remained on the film after one full revolution. These were then blown over already dry parts of the film. Thereby the observed defects were created most probably. <i>Conclusions:</i> The two ends of the substrate should be glued together or connected by tape so that no gap is left between them. The drying process should be modified to avoid re-wetting of already dry parts of the film.
3	 <i>Observations:</i> After drying, the substrate was always set to the same position. Thus, it was always immersed in the coating solutions with the same part first. A line pattern perpendicular to the moving direction of the substrate formed on this segment. As described, the coated side of the substrate was dried after each polymer adsorption step. The backside and the coating tray were generally left wet, however. It is supposed that the line defects resulted from contamination of the coating solutions with washing water as observed earlier in manual coating tests (see 4.3.1). Apparently the water did not mix immediately with the polymer solution, but partially floated on top of the polymer solution and formed a layer with lower polymer concentration. Polymer molecules within this layer apparently adopted a more globular conformation than the chains in the remaining solution. Upon immersion of the substrate into the coating solution, these more globular molecules deposited on the film in the form of lines. <i>Conclusions:</i> To counteract the formation of such line defects, substrate, rolls and tray of the coating machine would have to be dried completely before each addition of fresh polymer solution. However, this is not feasible in medium- or large-scale production. Hence, an alternative strategy to avoid the formation of gradients in the polymer solution had to be developed (see below).

Table 5 | Polymer assembly in Endless Band Coater model system – Defect analysis

Based on the above defect analysis the model system and the coating process were adapted:

- 1) The belt was removed and the substrate was always mounted directly on driving and guiding roll.
- 2) The ends of the substrate were connected with a strip of PET film. It was glued across the gap between them to the back of the two ends.
- 3) The substrate has to be dried in two steps as illustrated in Figure 31: First, one half of the film is dried starting at the topmost position of the film, while cycling the film against the drying direction. Then the film is cycled the other direction and the second half of the film is dried starting at the height of the guiding roll.

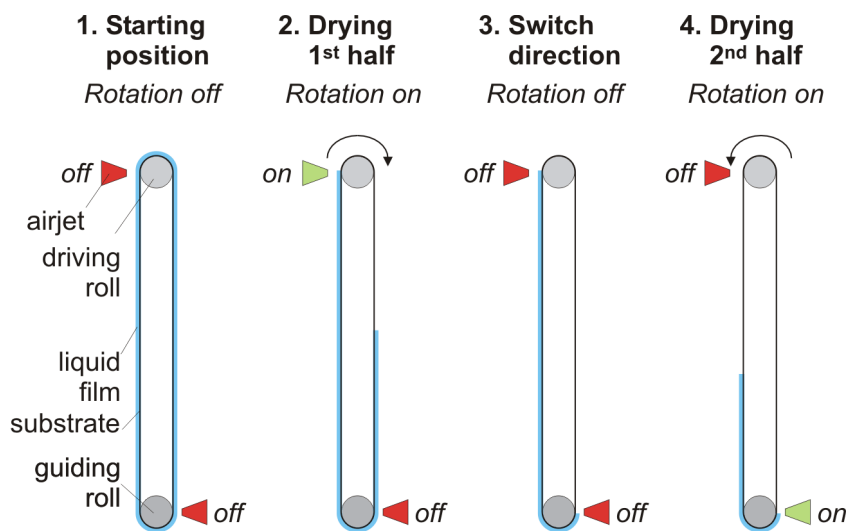


Figure 31 | Drying of substrate in Endless Band Coater

- 4) Pouring the polymer solutions into the empty coating tray generally resulted in the formation of coating defects due to residual washing water. Thus, the polymer solutions were mixed directly in the machine further on as described in the following section.

4.3.4.3 In-situ Mixing of Polymer Solutions

The Endless Band Coater model system was supplemented with a pump and tubing so that the polymer solutions could be pumped into the coating tray. Another pump was installed, by which the solution in the tray could be mixed. Polymer stock solutions with two-fold concentration compared to the previously employed solutions (i.e. 0.2 % (w/v) polymer in dH₂O) were prepared. SiO₂ was deposited wet-chemically as described earlier. Then the tray was filled with dH₂O, the substrate was immersed and the motor was restarted. While the PET film was cycling through the water in the tray, the first polymer solution was pumped into the water in the tray slowly. To accelerate mixing of added solution and water, the mix was circulated at the bottom of the tray via the secondary pump. Excess solution flowed out of the tray via a spillway. Polymer solution was added until the standard working concentration (i.e. 0.1 % (w/v) polymer in dH₂O) was reached. Then all pumps were stopped and the film was cycled in the mix for further 5 min. The washing process was not changed. However, the

substrate was not dried afterwards. The film was left cycling, the tray was filled with fresh water and the next polymer layer was added by in-situ mixing as explained. Only after the last polymer layer had been deposited and washed, the washing water was blown off with pressurized air. Then, the film was cut in pieces and was coated with a Pd cluster layer by sputtering.

As shown in Figure 32 the modified coating process allowed for the preparation of very homogeneous RHSCs. The coated films exhibited hardly any inhomogeneity. Only a few drying defects were found on those parts, where the pressurized air drying had been started (see Figure 33). Moreover, the in-situ mixing strategy rendered drying steps in between polymer adsorption steps obsolete, which strongly simplified the coating procedure and reduced the overall process time.

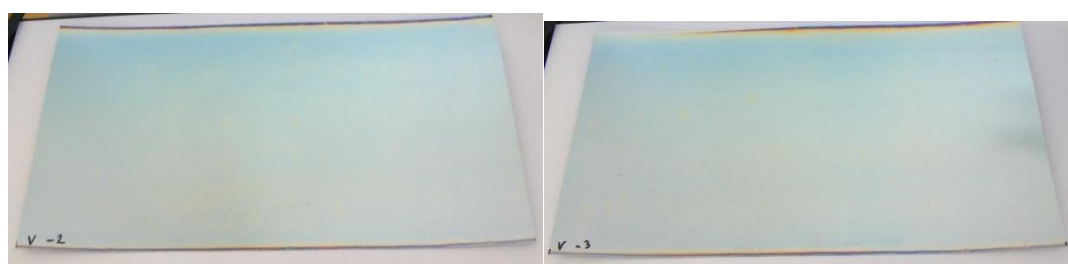


Figure 32 | Polymer multilayer assembly in Endless Band Coater via in-situ mixing method

The response of the film to humidity even exceeded that of RHS films prepared manually by the competition strategy described in section 4.3.3. In contact with humid air the resonance color of the film shifted immediately over a wide range colors from light blue up to dark green (see Figure 33). The color change was fully reversible. The swelling capacity of the sensor polymer multilayer decreased slightly within the first two days (as observed for RHS films prepared manually, see also chapter 5), but then remained constant over several weeks. (The long-term stability of the sensor layer was not tested.)

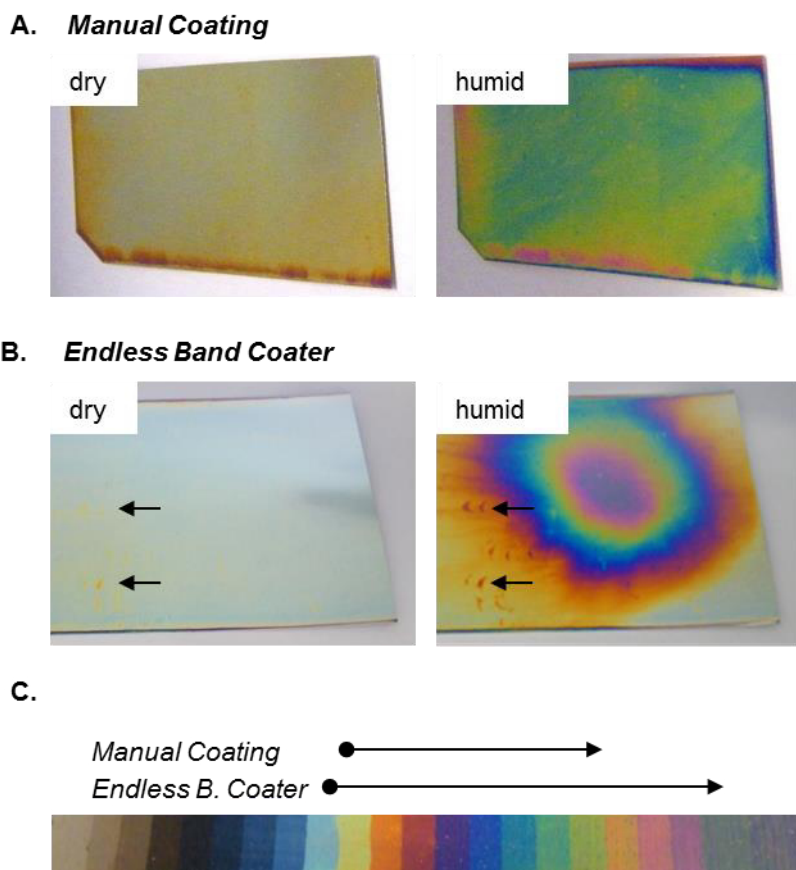


Figure 33 | Sensor response of RHS film prepared in Endless Band Coater compared to manually prepared RHS coating **A)** Manually prepared RHS film in dry state and in contact with humid air. **B)** RHS film prepared using Endless Band Coater model system: The color of the film was largely homogeneous apart from minor drying defects (indicated by arrows). In contact with humid air, the PEM of the RHSC swelled stronger than the manually prepared PEM. **C)** The color shift of the two films at maximum swelling is indicated above the aluminum-based REA color palette.

4.4 Summary & Outlook

The manual LbL deposition process employed for the assembly of humidity-sensitive PEI/ PMVE-MA multilayer structures could be improved significantly. At the beginning of this project, especially the polycation PEI adsorbed irregularly and thereby hindered the preparation of uniform REA humidity sensor coatings. Via addition of a low-molecular species of PEI to the coating solution, layer assembly could be delayed and thereby homogenized. Moreover, it could be demonstrated that the wet-chemical part of the RHS film fabrication process i.e. polymer LbL assembly and SiO_2 deposition can be automated. A coating system was developed, in which both processes can be carried out. A corresponding model coater was built for practical tests. Initially, contamination of the polymer deposition solutions, concentration gradients and interface effects led to coating defects. Hence, the model coater was modified and the method for adding the polymer deposition solutions to the coating tray of the machine was changed. The ready-made solutions were not pumped directly into the empty tray, anymore. Instead, the tray was filled with water and a stock solution of the required polymer was added slowly. Thereby the polymer concentration was gradually increased. Concentration gradients and interface effects were avoided. That way,

highly uniform RHS films with very good sensor performance could be prepared in the model coater. The coater was developed cooperation with Trapichler, who also scaled up the technology as described in his master thesis „Konstruktion einer Beschichtungsanlage zur Aufbringung nano-dünner Filme auf Folien“ [131]. He could demonstrate that the established coating procedure can also be successfully performed in a significantly larger semi-industrial coating system, in which films of a size of 2.4 x 0.3 m can be processed

5 Analysis of Aging of PEMs

5.1 Introduction

As demonstrated in chapter 4, the color of RHS materials changes reversibly in contact with aqueous solutions or highly humid air. This effect is based on the absorption of water molecules by the PEM between mirror and metal cluster layer, which results in the expansion of this part of the interlayer. Fresh RHS materials comprising a standard PMVE-MA/PEI five-layer sensor coating (see 4.2.1) typically respond at relative humidity (RH) values above 60-70 %. At 100 % RH their color shifts over a wide range of the REA color spectrum as depicted in Figure 34. If such RHS materials are stored for longer periods of time (≥ 6 months) at ambient conditions in the open air, their sensitivity to humidity diminishes. Higher RH values are required to elicit a color change then. Moreover, the PEM of such films expands significantly less at 100 % RH than the sensor layer of fresh films. The resonance color does not shift over up to 8-11 color values anymore, but merely over two (see Figure 34).

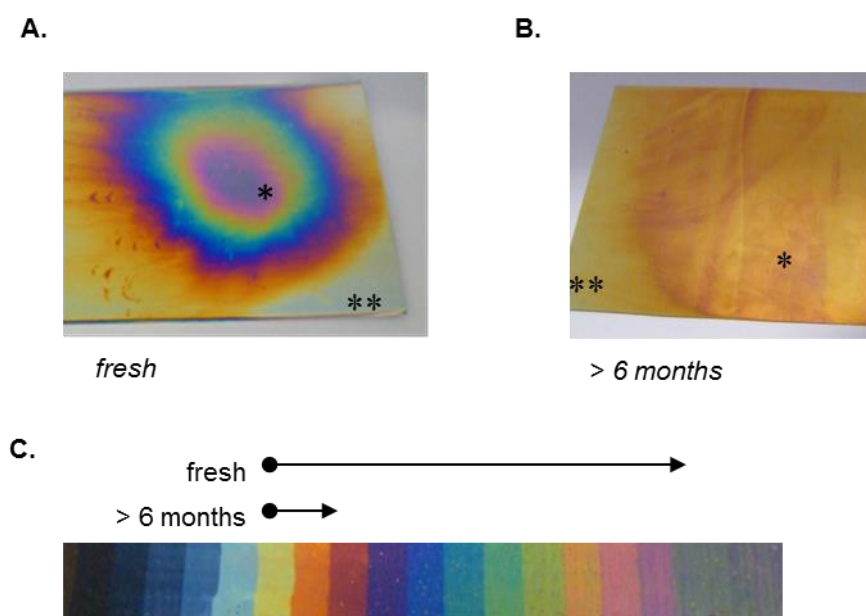


Figure 34 | Swelling capacity of fresh and old RHS materials **A)** Swelling test with fresh RHS film (dry and humid areas indicated by asterisks: *... humid area, **... dry area) **B)** Swelling test with old RHS film (~6 months) (dry and humid areas indicated by asterisks: *... humid area, **... dry area) **C)** Maximum range of color shift of fresh and old RHS films indicated by arrows above Al-based REA color palette

During storage, standard RHS materials apparently undergo changes that decrease the ability of their PEI/ PMVE-MA multilayer to swell and/ or to absorb water molecules. The reasons for this aging process were largely unclear at the start of this doctoral project. However, it had been found to occur at a slower rate if the RHS coating was covered with a self-adhesive, detachable polymer film or if the RHS material was stored in a closed vessel or plastic bag. Accordingly, interactions with the environment could be involved in the process such as

- gas exchange with the surrounding atmosphere
 - evaporation of film or coating components
 - penetration of atmospheric components into the multilayer structure
 - chemical reaction with atmospheric components
- deposition of material from the surrounding atmosphere (dust, fat particles)
- chemical modification of multilayer structure by UV irradiation

In parallel or as a result thereof, internal structural changes could occur. The swelling capacity of the PEM structure in RHS coatings is determined by the mobility of the polymer molecules it is composed of. As discussed by Klitzing et al. lateral diffusion can be observed in some types of PEM assemblies. It is typically restricted to the outer layers of a PEM and its extent depends on the preparation conditions and the types of polyelectrolytes in the assembly [132]. Hence, the responsive layer of RHS materials could in principle undergo slow restructuring even without external stimulus. Upon interaction with segments of the oppositely charged electrolyte diffusing chains might be trapped resulting in progressive stiffening of the PEM structure. Furthermore, corrosion processes could release ions from the metallic layers of the REA structure i.e. the mirror or the metal cluster layer. These ions might interfere with the water absorption capacity of the polymer layers. PEI has already been reported by Kislenco et al. to form complexes with copper(II), nickel(II) and cobalt(II) [133]. In summary, the following internal aging mechanisms were considered in the analysis of RHS film aging

- spontaneous gradual restructuring of the PEM i.e. gradual collapse, increasing interdigitation, progressive stiffening or sealing of the top polymer layer by restructuring
- crosslinking of polymer layers by Al^{2+} or Pd^{2+} ions generated by corrosion of mirror or metal cluster layer

The role of these processes as well as of the effects environmental aging factors was verified via a medium-term aging test and a variety of short-term aging and stress tests. The LbL deposition process, by which PEI/ PMVE-MA PEMs were assembled, was also reviewed. The role of components of RHS coatings in the aging process and the effects of individual process steps on the performance of RHS materials was assessed.

5.2 Materials & Methods

5.2.1 Analysis of Base Color and Maximum Swelling Capacity of RHS Coatings

The swelling capacity of the examined RHS films was determined by comparing their color in the dry state (= “base color”) and at maximally expanded PEM layer with color standards. Maximum swelling of PEMs of RHS film samples was induced by exhaling onto them through a tube with a diameter of ~3 cm and a length of 21 cm. To measure base color and swelling capacity, two types of color standards were used, namely:

Aluminum-based REA Color Palette

Stripes of aluminum oxide with increasing thickness (width: 3-5 mm) were generated on an aluminum plate (20 x 8 cm, 0.5 mm thickness, Glattblech natur, OBI) by anodizing in a boric acid electrolyte (pH 7.0). For this purpose, the lower 11.5 cm of the aluminum plate were immersed in the electrolyte and a voltage of 5 V was applied for 5 min. Then the plate was lifted by ~5 mm and the part, which remained immersed in the electrolyte was anodized at 10 V for 5 min. The plate was repeatedly lifted by 3-5 mm and anodized at the following voltages for 5 min each: 5, 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300 and 320 V. After completion of the last anodizing step, the plate was removed from the electrolyte. At some places it was covered with deposits of boric acid and foamed aluminum oxide, which had formed on the aluminum plate during anodizing at the interface of electrolyte and air. These deposits were wiped off with a soft wet towel. Then the plate was rinsed with EtOH and dH₂O and dried with pressurized air. Subsequently, the plate was sputter-coated with a Pd cluster layer as described in 4.2.5 and lacquered with a clear spray lacquer (Car's Rallye Klarlack glänzend, farblos, Dupli-Color), which did not change the resonance colors, to protect the plate from mechanical and chemical stress. The individual colors of the palette were indexed according to the voltage, which had been applied to generate the interlayer.



Figure 35 | Aluminum-based REA color palette with Pd cluster layer

PET-based REA Color Palette

PET-based REA color standards were assembled from strips of REA PET film with increasing interlayer thickness. The strips were prepared from aluminized PET film (HIFI, #MEX12C). They were provided with SiO₂ interlayer by wet chemical deposition according to the standard protocol described in 4.2.1. After completion of the SiO₂ coating, the strips were cut in two. One half of each strip was coated with a Pd cluster layer by sputtering, the other half with a Ag cluster layer as described in 4.2.5. The thickness of the SiO₂ interlayer on the individual strips was not measured directly. It was known to be proportional to the TEOS concentration in the SiO₂ deposition solution employed for the preparation of the layers and the number of SiO₂ deposition steps. Hence, the individual strips were indexed accordingly. (Example: If the interlayer was prepared in two deposition steps at a TEOS concentration of 500 µl/ 5 ml EtOH in the first step and 200 µl/ 5 ml EtOH in the second step, the color was indexed 700.) All strips were lacquered with the same clear lacquer as the aluminum color

palette. Then they were cut in shape and assembled on a laser-engraved polyethyleneterephthalate glycol (PETG) plate (thickness: 0.5 mm) (VIVAK PETG, Bayer).

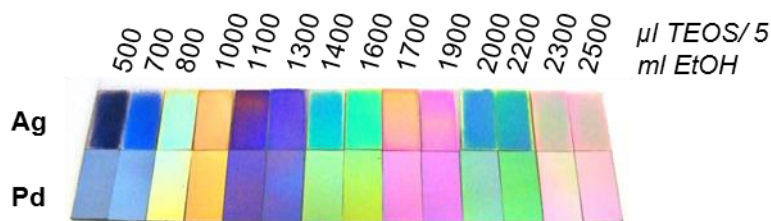


Figure 36 | PET-based REA color palette with Pd and Ag cluster layer

The swelling capacity of RHSCs was determined on the basis of the palette indices. For this purpose, the color values corresponding best with base color and color at maximum PEM expansion were determined. Then the first was subtracted from the latter.

5.2.2 Contact Angle Measurements

An aluminized PET film (HIFI, #MEX12C) was coated with a SiO_2 interlayer and a five-layer PEI/ PMVE-MA PEM according to the standard protocol see 4.2.1 (competitive approach). A metal cluster layer was not deposited on the film. The surface energy of the film was tested by the sessile drop technique within 48 h after preparation at the following time-points: 10 min, 45 min, 2 h, 3 h, 21.5 h, 24 h, 29 h and 48 h post preparation.

A professional sessile drop test setup was not available. Hence, a suitable setup was built in house. In contrast to commercial versions, a light microscope and not a digital camera with macro lens was used to examine droplet shape and contact angle.

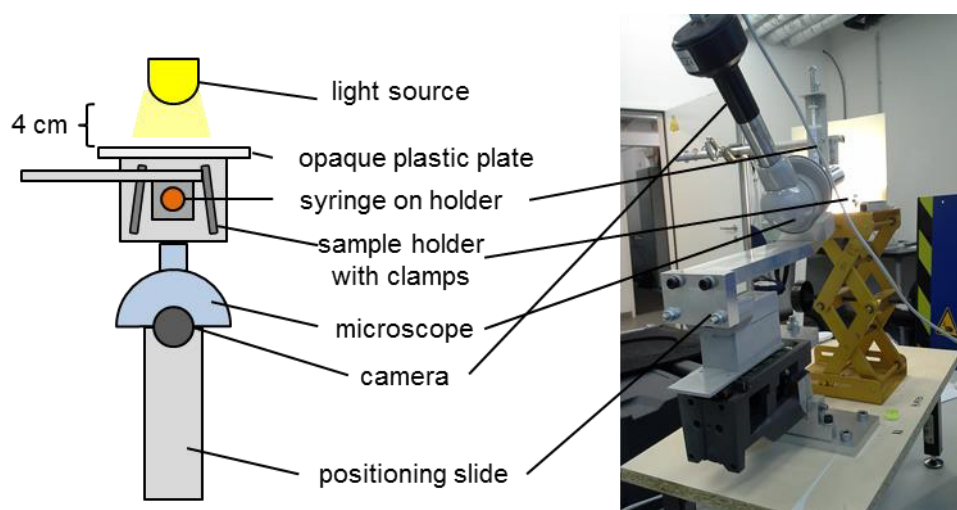


Figure 37 | Sessile drop test system Schematic drawing of setup (left) and assembled system (right)

As depicted in Figure 37, arm and head of a light microscope including eyepiece tube and revolving nosepiece with 4 x objective were mounted on a manual positioning slide (screw-

driven). A digital microscope camera with CMOS chip (ScopeTek, #DCM-900) was inserted into the eyepiece tube. The camera was operated with the corresponding software ScopePhoto 3.0. The sample holder was assembled out of an aluminum plate, onto which two microscope slide clamps were mounted. This plate was fixed on a lab jack. The sample holder was backlit by a LED light bulb (2700 K, 3.3 W, 250 lm, 50/60 Hz) (OSRAM, LED Star Classic P E14) through an opaque white plastic plate (thickness: 4 mm). The light source was positioned 4 cm behind the plastic plate. Lab jack and positioning slide were mounted on a small table with height-adjustable legs. A syringe (cannula: 0.3 x 12 mm, syringe volume: 1 ml) (Braun, Omnican 40) positioned above the sample holder was used to deposit droplets of dH₂O onto the material to be analyzed. (The cannula tip was blunted by grinding to reduce the distance required between cannula opening and sample surface.) The syringe was mounted on a height-adjustable holder and was operated manually. To improve dosing accuracy, the plunger of the syringe was manipulated via a screw. (To deposit a droplet, the screw was screwed into the holder, whereby the plunger of the syringe was pressed down.) A picture of the droplet was taken immediately after deposition. The contact angle of the droplet on the respective sample surface was determined manually and via the plugin DropSnake of the open source image processing program ImageJ [134]. At each time-point, three to four measurements were performed within ~5 min on different areas of the respective sample. From those data an average contact angle was calculated for each time-point.

5.2.3 Accelerated Aging under Thermal Stress at Elevated Atmospheric Humidity

Preparation of Samples

Stainless steel plates (steel type: 1.4301, various suppliers) with a thickness of 0.15 mm were degreased and cleaned with neutral detergent, EtOH and dH₂O and dried with pressurized air. Then they were provided with a RHS coating comprising SiO₂ interlayer, silane adhesion layer and a PEM consisting of five alternating layers of PEI and PMVE-MA as described in 4.2.1 (competitive approach). If required a top Pd cluster layer was deposited as described in 4.2.5. The moderately glossy surface of the steel plates served as mirror for the resonance setup.

Test Procedure

The tests were performed with stainless steel-based RHS plates, because the aluminum layer of standard RHS PET films would have corroded upon prolonged exposure to elevated humidity. This would have falsified results. Rectangular strips (0.5 x 2 cm) were cut off the plates. These strips were stuck into the necks of 15 ml Falcon tubes, the RHS coating facing inwards, using double sided tape. Depending on the desired treatment condition, the tubes were left empty or filled with 1.5 ml dH₂O or a reagent solution. They were left open or were closed with a screw lid and incubated at RT or 50-60°C for 43-72 h (see Table 6). Base color and maximum swelling capacity of the RHS coating of each sample was determined before and after the aging test as described in 5.2.1 using the aluminum color palette. The RHS

strips employed in heat stress test #1 were all cut from one RHS steel plate with Pd cluster layer, which was prepared 7 days before the stress test was started. The strips with and without Pd cluster layer that were tested in heat stress test #2 were cut from two different RHS steel plates. The plate with Pd cluster layer was prepared 9 days earlier than the plate without cluster layer. The test was started 1 day after the plate without cluster layer had been completed.

<i>Treatment Condition</i>	<i>Temperature</i>	<i>Added Solution</i>	<i>open/ closed</i>
Dry, RT	RT	-	closed
Dry, hot	50-60°C	-	open
Humid, hot	50-60°C	1.5 ml dH ₂ O	closed
Humid, acidic, hot	50-60°C	1.5 ml 1% (v/v) acetic acid in dH ₂ O	closed
Humid, basic, hot	50-60°C	1.5 ml 1% (v/v) ethanolamine in dH ₂ O	closed

Table 6 / Treatment conditions for accelerated aging tests

5.2.4 Treatment with Packaging Materials

To test the influence of packaging materials on RHSC aging, a RHS stainless steel plate with Pd cluster layer (see 5.2.3) was employed. A strip of the plate (0.4 x 5 cm) was placed into a PP petri-dish, the RHS coating facing upwards. The RHS coating on the strip exhibited a homogeneous base color and maximum swelling capacity before the experiment. Strips of the following materials (width ~ 4 mm) were placed next to each other across the steel plate strip at a distance of ~3 mm:

1. double-sided tape covered with siliconized PET-release liner (covered side facing the sample)
2. siliconized PET-release liner alone
3. standard paper towel
4. standard printing paper
5. standard printing paper coated with soft glue (UHU, UHU stic) (glue-coated side facing upwards i.e. not facing sample)
6. strip of RHS stainless steel plate (same as from which the sample was cut)

The petri dish was closed and the setup was incubated at RT for 20 h. Then the strips, which had been placed on the RHS sample, were removed from the sample and base color and maximum swelling capacity of the sensor coating on the sample were examined as described in 5.2.1.

5.2.5 Mid-term Aging Screening

Sample preparation and storage

12 different RHS films were prepared for an 84-day aging test (see Table 7). In all cases, aluminized PET-film (thickness = 175 μm) with a thin SiO_2 distance layer and a 3-glycidyloxypropyl)trimethoxysilane adhesion layer was used as substrate. Five different PEM types were deposited on pieces of this substrate: PskC, PskP, PmC, PmP, PP (for details see Table 8). The variant PmP was prepared with and without the plasticizer triethyl citrate (TEC) (Sigma-Aldrich, #27500). All other variants were only prepared with TEC. (All polyelectrolyte deposition solutions were prepared 24 h before use.) SiO_2 , silane and polymer deposition were performed according to the standard preparation protocol provided in 4.2.1. Finally a Pd or Ag metal cluster layer was deposited on the films by sputter coating (see 4.2.5).

#	Mirror	PEM	Plasticizer added to PEM*	Metal Cluster Layer
1	Al	PskC	+	Ag
2	Al	PskP	+	Ag
3	Al	PmC	+	Ag
4	Al	PmP	-	Ag
5	Al	PmP	+	Ag
6	Al	PP	+	Ag
7	Al	PskC	+	Pd
8	Al	PskP	+	Pd
9	Al	PmC	+	Pd
10	Al	PmP	-	Pd
11	Al	PmP	+	Pd
12	Al	PP	+	Pd

Table 7 | RHS film variants analyzed in 3-month aging test * -... no plasticizer added, +... 0.05 % (v/v) TEC added to polyelectrolyte deposition and washing solutions

PEM type	Polyelectrolyte deposition solutions	Composition
PP	Solution P1	0.1 % (w/v) PEI (1.6 MDa) in 0.1 % (v/v) ethanolamine/dH ₂ O
	Solution N1	0.1 % (w/v) PMVE-MA (~216 kDa) in dH ₂ O
PmP	Solution P2	0.1 % (w/v) PEI (1.6 MDa) (BASF) + 0.1 % (w/v) PEI (2 kDa) (BASF) in 0.1 % (v/v) ethanolamine/dH ₂ O
	Solution N1	see above
PmC	Solution P2	see above
	Solution N2	0.1 % (w/v) carboxymethyl cellulose (CMC) (700 kDa) (Sigma-Aldrich, #419338) in dH ₂ O
PskP	Solution P3	0.1 % (w/v) Polymine SK (BASF) in 0.1 % (v/v) ethanolamine/dH ₂ O
	Solution N1	see above
PskC	Solution P3	see above
	Solution N2	see above

Table 8 | Polyelectrolyte deposition solutions

If the respective PEM variant was prepared with the plasticizer TEC, it was added to both coating solutions at a concentration of 0.05 % (v/v).

For all PEM types the following two washing solutions were used:

Washing solution WP1 0.1 % (v/v) ethanolamine in dH₂O
(for polycation layer)

Washing solution WN1 0.1 % (v/v) acetic acid in dH₂O
(for polyanion layer)

If the respective PEM was prepared with TEC, it was also added to the washing solutions at a concentration of 0.05 % (v/v).

The twelve RHS films were prepared on the same day and cut into four pieces each on the following day. One strip each was directly used for swelling tests, the remaining strips were transferred to nine glass vessels with rubber sealing (inner volume: 220 ml). Each vessel was loaded with one strip of each RHS film type i.e. 12 film strips were put into each vessel. The strips were placed in vertical position next to each other into customized plastic rags assembled from PETG plates. The rags kept the films separate (distance between individual films: ~2 mm). Three of the vessels were left open and three vessels were closed after loading. In the remaining three vessels, samples should be stored at elevated CO₂ concentration. For this purpose, 5 g dry ice were transferred into each of them before loading. The vessels were incubated at RT until the ice had sublimed and the vessels had warmed to RT again. Then rags loaded with the 12 RHS films samples were plunged into the

vessels. Significant stirring of the gas within the vessels was avoided. All nine glass vessels were closed and packed into a cardboard box, which was stored in a dark room. During storage, temperature and humidity in the room were largely stable (19-21°C, 35-40 % RH). After 7, 28 and 84 days one vessel per storage condition was taken out. Base color and swelling capacity of the contained RHS films strips was determined as explained in 5.2.1.

5.2.6 UV Treatment of RHS Films

A RHS PET film without metal cluster layer was prepared according to the standard protocol described in 4.2.1 (competitive approach). UV treatment tests were performed on the following day. For these tests, illumination stencils were prepared (see Figure 38). Because black plastic film was not available, overhead transparencies were used as raw material. The transparencies were printed black using an office laser printer and stencils were cut out with a PC-controlled CO₂ laser system (LS100, Gravograph).



Figure 38 | Layout of illumination stencils

Three pieces of RHS PET film were covered with the stencils, which were fixed with clamps. One piece was incubated in the dark for 1h without UV treatment. The other two pieces were illuminated with short-wave UV radiation (253.7 nm) using a handheld UV source (4 W) (CH lighting, # F4T5/GL) for 30 min and 60 min respectively. Then they were provided with a Pd cluster layer by sputtering as described in 4.2.5. Base color and swelling capacity of the samples were determined the following day as explained in 5.2.1.

5.2.7 Treatment with Aluminum Salt Solution

Preparation of Samples

A stainless steel plate with a thickness of 0.3 mm was degreased and cleaned with a neutral detergent, EtOH and dH₂O and dried with pressurized air. Then a RHS coating was deposited comprising SiO₂ interlayer, silane adhesion layer, a PEM out of five alternating layers of PEI and PMVE-MA and a topmost Pd layer as described in 4.2.1 (competitive approach) and 4.2.5. The moderately glossy surface of the steel plates served as mirror for the resonance setup.

Test Procedure

The test solutions listed in Table 9 were prepared. Two pieces of were cut off a stainless steel RHS plate (~2.5 x 3.5 cm). Both were placed in a wet chamber. Droplets of all six test

solutions (10 µl each) were pipetted onto both steel plates. The plates were incubated in the wet chamber at RT for 1.5 h. Then the droplets were blown off with pressurized air. Base color and swelling capacity of the plate pieces were analyzed as described in 5.2.1 using the aluminum color palette. Subsequently, the two plate pieces were incubated in dH₂O, shaking, for 1 h to equilibrate the internal pH of their RHSC. The samples were dried with pressurized air then. Base color and swelling capacity of the plate pieces were measured again.

#	Composition	pH-value
1	0.2 % (w/v) Al ₂ (SO ₄) ₃ in dH ₂ O	4
2	1 % (w/v) Al ₂ (SO ₄) ₃ in dH ₂ O	3.5
3	0.2 % (w/v) Na ₂ SO ₄ in dH ₂ O	6.5
4	dH ₂ O, pH adjusted with H ₂ SO ₄ to pH=4	4
5	dH ₂ O, pH adjusted with H ₂ SO ₄ to pH=3.5	3.5
6	dH ₂ O, pH adjusted with H ₂ SO ₄ to pH=4.5	4.5

Table 9 | Test solutions for aluminum treatment tests

5.2.8 Storage under Dry Conditions

Preparation of Samples

see 5.2.7

Test Procedure

The stainless steel RHS plate was cut into pieces of ~2 x 3 cm. Base color and maximum swelling capacity of one of the pieces was determined as described in 5.2.1 using the aluminum color palette. The remaining pieces were put into separate polyethylene (PE) bags (inside dimensions: 6 x 10 cm), which were prepared from PE film by lamination. Silica gel desiccant bags (~10 g silica gel beads per bag, bead diameter: 2-4 mm), which were pre-dried for 3 h at 60°C, were put into half of the bags to create a dry atmosphere within (one dessicant bag per plastic bag). Then the bags were sealed by lamination and incubated at RT in the dark for >= 1 month (experiment #1: 30 days, experiment #2: 42 days). Base color and maximum swelling capacity of the RHS coating of the samples were determined after incubation as described in 5.2.1 using the aluminum color palette.

5.2.9 Replacement of Ethanolamine

RHS PET films without ethanolamine were prepared largely according to the standard procedure (see 4.2.1). Only the composition of the coating and washing solutions was varied slightly. In test #1, the standard polyanion deposition solution N1 and the corresponding washing solution WN1 were utilized (see 4.2.2 and 4.2.3). Instead of the standard polycation solution P1 and the corresponding washing solution WP1, the following solutions were employed:

Solution P1A	0.1 % (w/v) PEI (1.6 MDa) + 0.1 % (w/v) PEI (2 kDa) + 0.2 % (w/v) tris(hydroxymethyl)aminomethane (TRIS or TRIS base) (Sigma-Aldrich, #T87602) in dH ₂ O
Washing solution WP1A (for polycation layer)	0.2 % (w/v) TRIS in dH ₂ O

In test #2, humidifiers were added to both polymer and both washing solutions. Two types of RHS films containing different humidifiers were prepared:

Test #2

Sample #1

Solution P1B	0.1 % (w/v) PEI (1.6 MDa) + 0.1 % (w/v) PEI (2 kDa) + 0.2 % (w/v) TRIS + 0.302 % (w/v) sorbitol (ACM Herba Chemosan) in dH ₂ O
Solution N1B	0.1 % (w/v) PMVE-MA (~216 kDa) + 0.302 % (w/v) sorbitol in dH ₂ O
Washing solution WP1B (for polycation layer)	0.1 % (v/v) ethanolamine + 0.302 % (w/v) sorbitol in dH ₂ O
Washing solution WN1B (for polyanion layer)	0.1 % (v/v) acetic acid + 0.302 % (w/v) sorbitol in dH ₂ O

Sample #2

Solution P1C	0.1 % (w/v) PEI (1.6 MDa) + 0.1 % (w/v) PEI (2 kDa) + 0.2 % (w/v) TRIS + 0.1205 % (v/v) glycerol (Carl Roth, #3783) in dH ₂ O
Solution N1C	0.1 % (w/v) PMVE-MA (~216 kDa) + 0.1205 % (v/v) glycerol in dH ₂ O
Washing solution WP1C (for polycation layer)	0.1 % (v/v) ethanolamine + 0.1205 % (v/v) glycerol in dH ₂ O
Washing solution WN1C (for polyanion layer)	0.1 % (v/v) acetic acid + 0.1205 % (v/v) glycerol in dH ₂ O

5.3 Results & Discussion

5.3.1 Cross-reactions with Pressure-sensitive Adhesives & Soft Glues

As described above, RHS materials with PEI/ PMVE-MA PEM that were covered with self-adhesive, detachable protective film during storage were found to exhibit slower aging than equivalent films that were stored in the open air. Slower aging was also observed on parts of a film, which had been stored next to an object coated with soft glue (UHU, UHU stic). In this case, the RHS material had not been kept in direct contact with the glue. This suggested that volatile components of permanently soft glues are able to modify the swelling behavior of PEI/ PMVE-MA-type RHS coatings.

The hypothesis was verified via a short-term incubation test, which is schematically depicted in Figure 39. A strip of a RHS steel plate was placed into a petri dish. A strip of double-sided translucent PVC tape (Hi-Bond, #HB397F-9) was positioned across the RHS strip. The glue-coated side facing the RHS strip was covered with the PET release liner of the tape. The other glue-coated side was uncovered. At the other end of the RHS strip, a piece of printing paper coated with UHU stic was placed with the glue-coated side facing upwards, so that the glue did not touch the sample directly. Furthermore, a strip of the release liner of the double-sided tape, a strip of blank paper, a strip of paper towel and another strip of the RHS steel plate were placed across the sample to exclude artifacts from the printing paper, the release liner and materials, which the RHS sample had been in contact with earlier. The petri dish was closed and stored at RT for 20 h. Then the swelling behavior of the RHS sample was analyzed as explained in 5.2.1. The RHS film exhibited significantly higher swelling capacity left and right of the areas that had been covered with the self-adhesive film and the UHU stic-coated paper. The swelling behavior of the remaining RHS coating appeared unchanged. This confirmed the above hypothesis that certain glues can affect the swelling behavior of standard RHS coatings even without direct contact. No short-term effects of incubation with printing paper, paper towels or pieces of steel based RHS material were noticed.

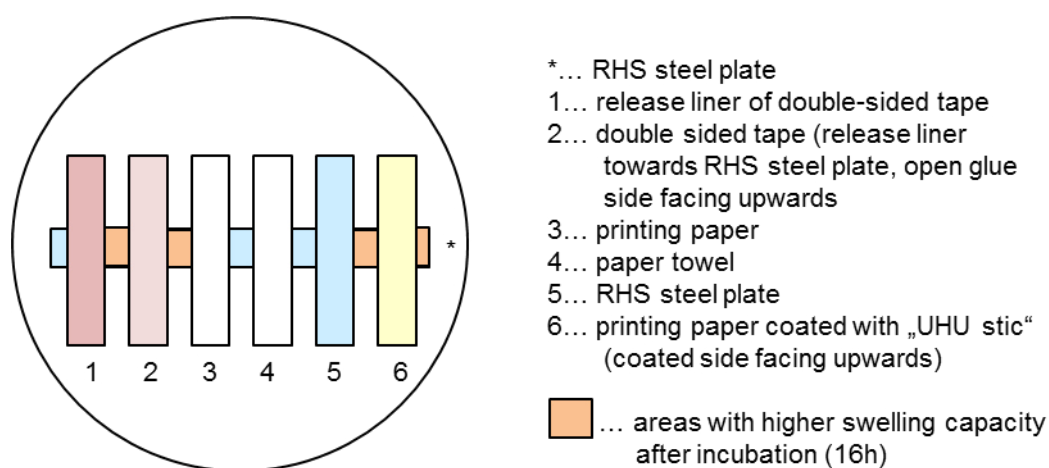


Figure 39 | Cross reaction test Schematic illustration of the test setup

Unfortunately, the exact composition of the tested double-sided tape and the soft glue “UHU stic” are not known. The component(s), which modified the swelling capacity of the RHS steel plate in the cross reaction test, could therefore not be identified without doubt. However, it is supposed that it is/ they are hydrophilic and might also be contained in the tested glues. In a patent application by UHU on a glue stick, it is mentioned that the stick may contain a variety of additives such as dyes, color indicators, perfumes, lubricants, plasticizers, or substances acting on the moisture content of the glue stick. Glycerol and PEG 200 are listed as examples, which are both strongly hydrophilic [135]. Adhesive tapes typically consist of a backing material and a so-called pressure-sensitive adhesive (PSA). This adhesive is commonly a mixture of an elastomer and a tackifier i.e. a plasticizer that makes the elastomer adhesive and fluid. Moreover, surfactants can be added to modify the surface tension of the adhesive in order to control its wettability and adhesion [136]. Both

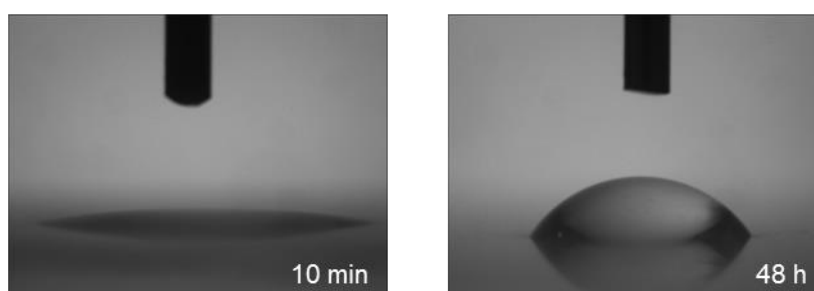
types of adjuvants could interfere with the absorption behavior of PEMs if they are sufficiently volatile. If possible, tapes, glues and adhesive materials were thus avoided in the aging and stress tests discussed below. If tapes had to be used, e.g. to fix RHS plates and films to vessels, control experiments were performed to exclude experimental artifacts caused by those tapes.

5.3.2 Decrease of PEM Surface Wettability

After completion of the PEM coating, RHS films were occasionally stored for several weeks without metal cluster layer. If such films were brought in contact with water or aqueous solutions, the respective liquid appeared to spread worse on their surface than on equivalent films with fresh PEM coating. Apparently, the wettability of RHS films changes during prolonged storage and the PEM surface becomes increasingly hydrophobic. It might form a barrier, which is difficult to pass for water molecules. Thereby, the reactivity of the RHSC could be reduced. The observed changes in the wettability of the PEM of RHSCs could thus play a major role in their aging. To assess the dynamics of these changes, the wetting behavior of RHS films without metal cluster was occasionally checked days to weeks after their preparation. Unfortunately, it was found that their wettability already decreased within hours after preparation. The phenomenon was studied in detail by the static sessile drop method. In this analysis, a droplet of test liquid (typically pure water) is deposited vertically onto the sample surface. The profile of the sessile drop is captured using a high-resolution camera or another type of optical system. Then the contact angles between test liquid and sample surface are determined [137]. Such drop shape analysis can in principle be done manually (see e.g. [138]). However, the accuracy of the as gained results strongly depends on the correct positioning of baseline and tangents at the contact points of substrate and droplet. Thus, image analysis software is generally employed for the purpose [137, 139]. As described in 5.2.2, sessile drop measurements were performed on strips of RHS film without cluster layer within the first 48 h after coating at defined time points. The photos of the individual sessile drop tests were evaluated manually and with the ImageJ plugin DropSnake [134]. At very low contact angles, the plugin could not trace the droplet correctly, because the contrast between droplet and background was too low. Therefore, the two functions depicted in Figure 40B deviate strongly at the first few measurement time-points. Nevertheless, both exhibit a similar shape. The contact angle between the test liquid dH₂O and the sample surface was low at the start (11-16°) and increased only slowly within the first 21 h of the analysis (see Figure 40). Between 24 h and 29 h after preparation a sigmoidal change of the wettability was observed, however. The measured contact angle increased strongly. Thereafter, the wetting behavior of the RHSC changed only insignificantly. The test confirmed previous observations. The wettability of RHS films without metal cluster layer decreases much faster than the sensitivity of standard RHSCs. For this reason, the two processes are not supposed to be correlated. The swelling capacity of RHSCs was repeatedly noticed to drop by approximately half an interval to 1 interval of the aluminum color palette depicted in Figure 35 within the first 48 h after preparation (see also 5.3.5). This might result from the observed decrease in wettability of ~40°. However, the swelling

capacity of old RHSCs is generally 6-9 intervals lower than that of fresh coatings (see Figure 34). The PEM surface would have to become ultrahydrophobic to reduce the swelling capacity as strongly. This has never been observed. Unfortunately, it cannot be determined, whether the PEM surface energy also changes in RHS films with metal cluster layer, because the metal cluster layer masks the underlying PEM in the sessile drop test. The wettability of standard sputter-deposited Pd or Ag cluster layer is generally significantly lower than that of fresh PEI/ PMVE-MA PEMs and was not observed to change significantly with time.

A.



B.

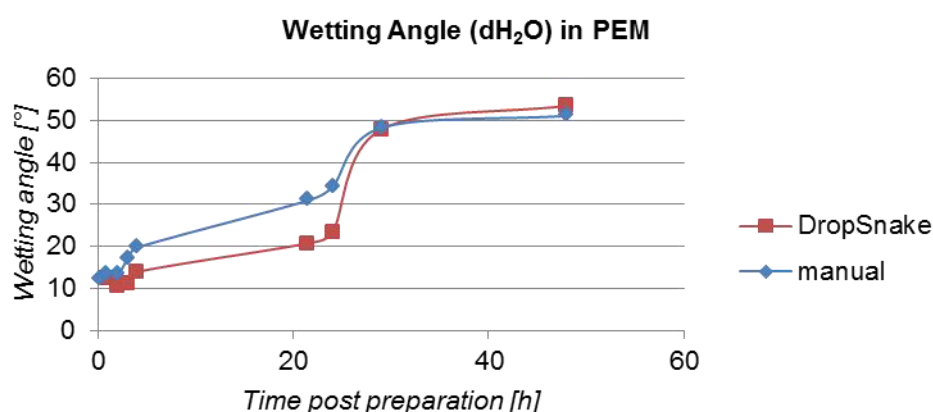


Figure 40 | Change of wettability of PEI/ PMVE-MA PEM monitored by sessile drop test (n=1)
A) Shape of dH₂O droplet on PEM 10 min (left) and 48 h after preparation of PEM (right) **B)** Wetting angle of dH₂O droplet on PEM within 48 h after PEM preparation (manual... wetting angle determined manually, DropSnake... wetting angle determined with ImageJ plugin DropSnake)

5.3.3 Effects of Thermal Stress & Elevated Atmospheric Humidity

The role of temperature, atmospheric humidity, pH and the metal cluster layer in RHSC aging was determined by accelerated aging tests. In these tests, pieces of RHS steel plates with or without Pd cluster layer were incubated at elevated temperature (50-60°C) under dry conditions and in highly humid neutral, acidic or basic atmosphere. For this purpose, the samples were either heated in an open vessel or in a closed vessel containing water or a solution of a volatile acid or base (acetic acid or ethanolamine respectively) as described in 5.2.3. After 43-72 h the swelling capacity of the samples was compared with the swelling capacity of reference samples i.e. pieces of the same RHS plates, which had been stored

under ambient conditions in closed vessels. (The tests were performed with steel-based RHS materials, because the aluminum mirror layer of standard RHS PET films would have corroded and dissolved during prolonged incubation at high humidity.)

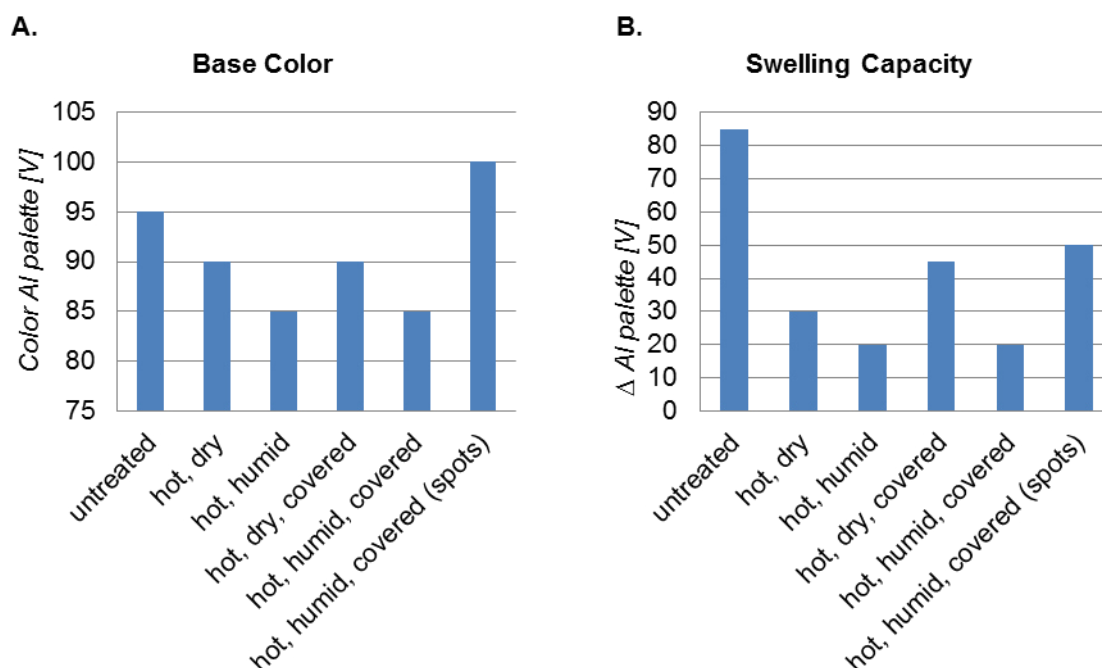


Figure 41 | Heat stress test #1 – Aging of RHS steel plate strips at elevated temperature in dry and humid atmosphere (n=1) **A)** Base color of individual samples post aging compared to Al-based REA color palette (color index according to anodizing voltage, = “Color Al palette [V]”) **B)** Swelling capacity of samples after aging (43 h): Base color and color at maximum expansion were compared to the Al-based palette. The difference of the corresponding color values of the palette was calculated (Δ Al palette [V] = color at maximum expansion [V]– base color [V])

In heat stress test #1, RHS samples with Pd cluster layer were exposed to thermal stress in dry or humid atmosphere. Two of the strips were covered with detachable, adhesive protective film (NITTO, SPV series) to verify the previously observed stabilizing effect of this film. The samples were stressed for 43 h. Then base color and swelling capacity were determined as explained in 5.2.1. The aging of the RHS structure was found to be strongly accelerated by heating and was fastest if the samples were incubated at elevated temperature and high humidity (see Figure 41). Moreover, the treated samples exhibited a lower base color than the untreated reference sample. Apparently the interlayer thickness of the stressed samples decreased in the course of the stress test. This supports the hypothesis that polymer chain restructuring plays a role in the aging of RHS coatings and that the PEM progressively loses its swelling capacity due to a flattening or collapse of the polymer layers. Only some spots on the covered sample stored in hot, humid atmosphere exhibited a different behavior. In the course of the aging test, the protective film had been detached nearly completely from this sample by condensing water. For this reason, the sample exhibited largely the same degree of aging as the strip, which had been stored non-covered in hot, humid atmosphere. At those spots, where the adhesive film had remained stuck to the covered sample, a protective effect was observed, however. While the base

color of the surrounding areas had dropped (from 95 to 85 V), the covered spots had adopted a higher resonance color (100 V). Their swelling capacity was also significantly better (30 V) than that of the surrounding RHSC. Most probably, the substances in the glue of the adhesive film, which can improve the swelling capacity of RHS coatings (see 5.3.1), were washed out by condensing water in hot, humid atmosphere and diffused into the PEM of the covered spots. The sample covered with protective film, which was aged in hot, dry atmosphere, adopted the same base color as the non-covered strip stored at the same conditions. However, its swelling capacity remained better by 15 V. This confirmed that the sensitivity of RHSCs can be stabilized by covering them with adhesive film. Probably, the film affected the swelling behavior of RHSCs less in hot, dry atmosphere than in humid atmosphere, because part of the responsible components evaporated. (To create a hot, dry atmosphere the tube was left open. In contrast, the tube for hot, humid incubation was closed with a screw lid to maintain a constant relative humidity within.)

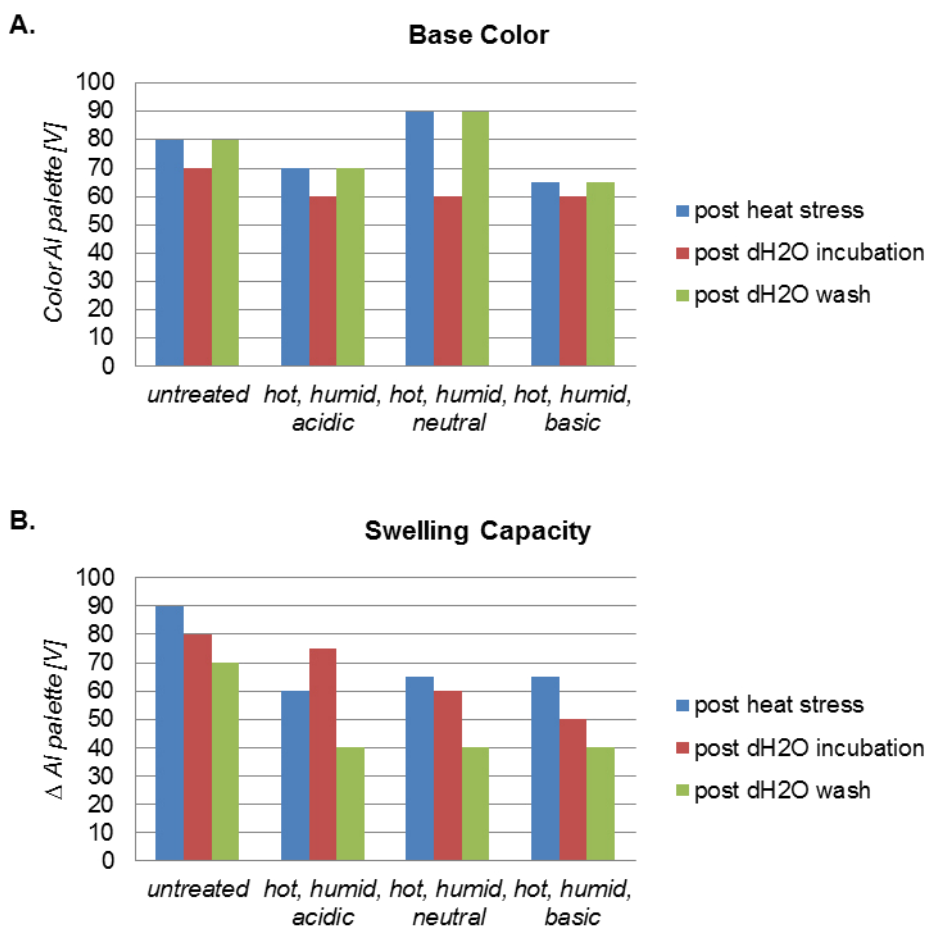


Figure 42 | Heat stress test #2 – Aging of RHS steel plate strips at elevated temperature and different atmospheric pH (n=1) Base color and swelling capacity of the samples were analyzed directly after the stress test (46 h) (=“post heat stress”). Then a droplet of dH₂O was deposited on each strip and the strip was incubated briefly at RT in a wet chamber. The droplet was removed and base color and swelling capacity of the water-treated areas were examined (=“post dH₂O incubation”). Finally, the strips were rinsed with water and both parameters were determined again (=“post dH₂O wash”). **A)** Base color of RHS steel strips after heat stress in presence of different pH-active molecules **B)** Swelling capacity (Δ = color at maximum expansion – base color) of RHS steel strips after stress test compared to Al-based REA color palette

Heat stress test #2 (incubation time: 46 h) aimed at determining whether pH-active molecules in the hot atmosphere affect the aging process (see Figure 42). For this purpose, strips of RHS steel plates were exposed to small amounts of evaporated acetic acid or ethanolamine at elevated temperature and humidity (see 5.2.3). The base color of the individual samples varied strongly after the stress test as depicted in Figure 42. It was supposed that acid or base molecules had diffused into the PEM of the samples treated with acetic acid and ethanolamine fumes. These apparently changed the internal pH of the respective RHSCs. It had been observed earlier that the internal pH of RHSCs can be shifted by incubation in mildly acidic or basic aqueous solutions. As a result the PEM of the coatings shrinks or expands (see Figure 43). Thus, it was attempted to reset the color of the aged coatings by incubation in water. It should extract pH active molecules and equilibrate the internal pH of all coatings. A droplet of dH₂O was deposited on each sample and the samples were incubated at RT for 30 min. Indeed, the as-treated areas of the individual samples

adopted the same base color after removal of the water droplets. They also exhibited different swelling capacities. After rinsing each strip with water, this effect was abolished, however. The samples adopted different colors again, but showed the same swelling capacity. Apparently, the aging test had also induced permanent structural changes. Heat stress test #1 had led to the conclusion that heating in combination with high humidity speeds up conformational changes in the PEM of RHS coatings and/or enhances gas exchange processes with the surrounding atmosphere, which modify the responsiveness of the PEM. However, the degree of expansion of the polymer molecules in the assembly is dependent on the internal pH of the PEM also at high humidity. Hence, it was expected to influence restructuring processes. Heat stress test #2 demonstrated that pH-active substances can also enter the PEM from the gas phase and change the internal pH of RHSCs like liquid acidic or basic media. Apparently, they do not play a role in the aging process itself, however. They only affected the thickness changes the PEM underwent during the performed stress test. The swelling capacity off all stressed samples was equally decreased in the course of the test.

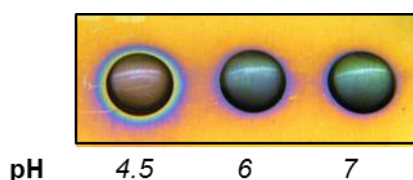


Figure 43 | Effect of pH-value on PEM expansion A RHS steel plate was incubated with droplets of 0.05 % (v/v) acetic acid in dH₂O (pH ~4.5) (left), 0.05 % (w/v) sodium acetate trihydrate (C₂H₃NaO₂ · 3 H₂O) in dH₂O (pH ~6) (middle) and 1 % (w/v) C₂H₃NaO₂ · 3 H₂O in dH₂O (pH ~7) (right). Within 10 min after droplet deposition, the areas covered by the droplets adopted different colors (from left to right: pink, blue, blue-green).

Another surprising finding was that the base color of the film incubated in hot, humid atmosphere without pH-active substances shifted to a higher value in the course of the test (from 80 to 90 V). In all comparable experiments, the interlayer thickness of RHSCs had been decreased by such a treatment. However, the PEM deposition process had not been optimized, when the RHS steel plate for this experiment was prepared. It is possible, that the PEM was compacted unintentionally during preparation (e.g. by a contamination that slightly shifted its internal pH). The compaction might have been reversed by incubation in elevated humidity.

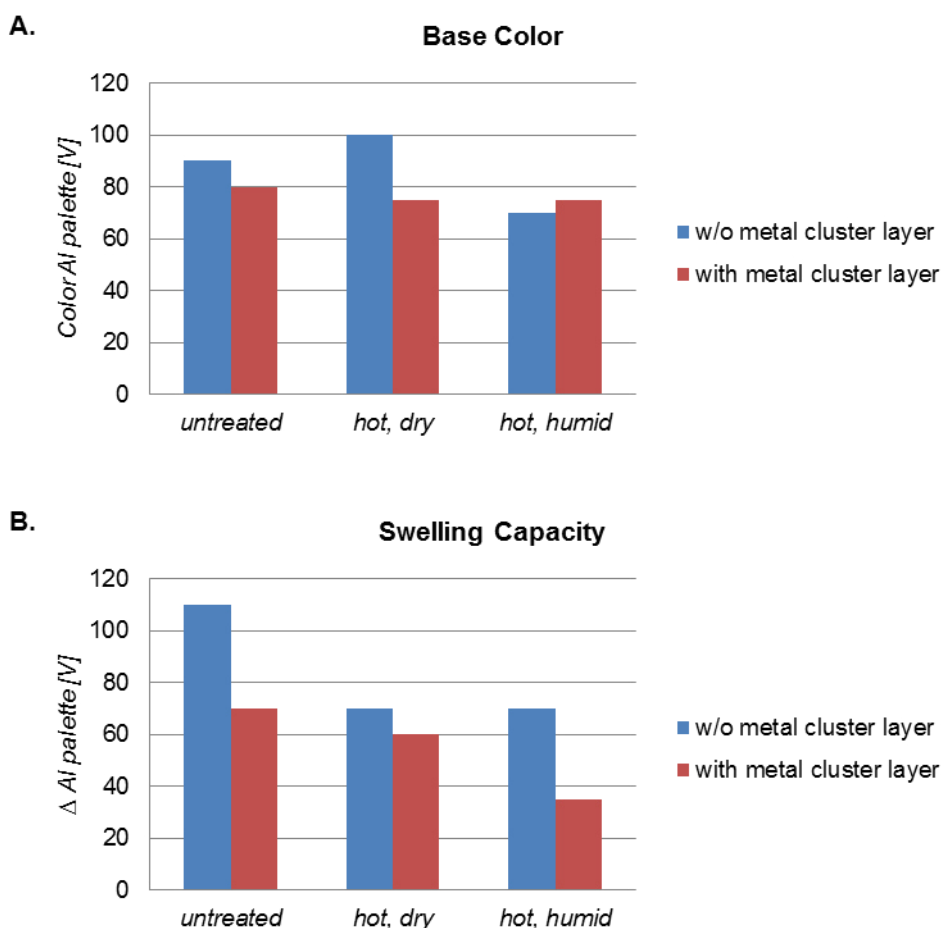


Figure 44 | Heat stress test #3 – Aging of RHS steel plates at elevated temperature with and w/o Pd cluster layer (n=1) **A)** Base color of samples after stress test (72 h) compared to AI-based REA color palette **B)** Swelling capacity (Δ = color at maximum expansion [V] – base color [V]) of samples after stress test

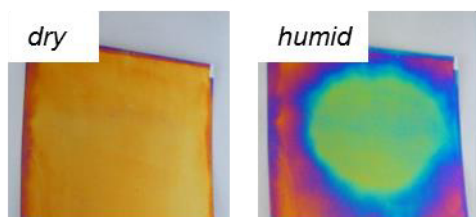
In heat stress test #3 steel-based RHS strips with and without Pd cluster layer were exposed to thermal stress in dry and humid atmosphere for 72 h to investigate the role of the cluster layer in RHSC aging. The results are summarized in Figure 44. Interestingly, RHS strips without Pd cluster layer aged equally at hot, humid conditions and in hot, dry atmosphere. The strips with Pd cluster exhibited the same aging behavior as the samples in heat stress test #1. The swelling capacity of the Pd-coated sample incubated in hot, humid atmosphere was decreased most. In contrast, the swelling capacity of the samples without Pd cluster layer decreased equally in dry and humid hot atmosphere. Apparently the Pd cluster layer enhances the aging of RHS coatings. The underlying process is unclear, however. The stainless steel substrate of the treated samples did not exhibit any signs of corrosion and the Pd layer did not appear fainter than the cluster layer on the untreated samples. Nevertheless, it cannot be excluded that Pd cluster layer and steel substrate formed a galvanic cell with the wet PEM acting as electrolyte. As a result, one of the two might have been oxidized to a minor extent. The as-generated ions might have interacted with the PEM layer. Without Pd counter electrode, the stainless substrate should not corrode significantly. Nonetheless, the swelling capacity of samples without cluster layer decreased strongly in the course of the stress test. Hence, ion contamination is most probably not the major cause of RHSC aging.

5.3.4 Plasticizer Addition

The results of the above experiments supported the assumption that RHSCs gradually lose swelling capacity by conformational changes within the PEM structure. These might lead to stiffening and/or hydrophobization of the polyelectrolyte molecules. In bulk polymer materials, stiffening is typically counteracted by adding plasticizers. It should be tested, whether such agents can also enhance the long-term stability of RHSCs. Potential fields of application of RHS materials include drug and food packaging. Their components should thus not be toxicologically critical. Triethyl citrate (TEC) is a food-safe plasticizer and did not induce unwanted reactions like polymer aggregation when added to PEI or PMVE-MA deposition solutions. It was thus tested as stabilizing agent for RHSCs.

In the respective experiments, RHSCs were prepared according to the standard protocol, but TEC was added to both polymer and both washing solutions. Thereby, leaching of TEC during the first washing step of each polymer deposition cycle – the 3-5 min incubation in acidic or basic solution – should be avoided. However, no plasticizer was added to the deionized water used in the second washing step of each cycle. Typically, the substrate was rinsed with water for a maximum of 30 s in this step, so no significant leaching was expected. RHSCs with PEI/ PMVE-MA multilayer prepared with TEC generally exhibited a slightly higher base color than standard PEI/ PMVE-MA-based coatings. Nevertheless, their maximum swelling capacity was equivalent. The effect of TEC on alternative PEM assemblies was also tested. PEM assemblies composed of PEI and carboxymethyl cellulose (CMC) were modified strongly by the plasticizer. Without TEC, PEMs of this type are thin compared to standard PEI/ PMVE-MA assemblies as demonstrated in Figure 45 and Figure 46. They swell only moderately at elevated humidity

A. PEI/PMVE-MA w/o TEC



B. PEI/CMC w/o TEC



C.

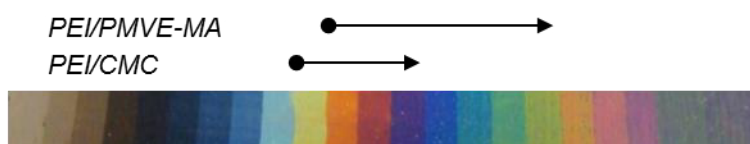


Figure 45 | Comparison of PEI/ PMVE-MA and PEI/ CMC RHS coatings w/o TEC The depicted films were coated with equally thick SiO₂ layers before the individual PEMs were assembled on them. **A)** Standard PEI/ PMVE-MA RHSC in dry state and at elevated humidity **B)** PEI/ CMC RHSC w/o TEC in the dry state and at elevated humidity **C)** Base color and swelling capacity of the depicted standard RHSC and the PEI/ CMC-type RHSC w/o TEC are indicated with arrows above the Al-based REA color palette.

By addition of TEC to polymer deposition and washing solutions, the dry thickness of PEI/ CMC PEMs is increased only slightly, but the swelling capacity is enhanced extremely (see Figure 46). It could not be determined, whether TEC was actually integrated into the PEMs prepared in this study and if so, to which extent. However, the higher base color of the films prepared with the plasticizer confirmed that it at least modified the conformation, which the polymer molecules adopted during LbL assembly. Apparently, they bound in a more globular shape in the presence of TEC and thus formed a thicker multilayer structure.

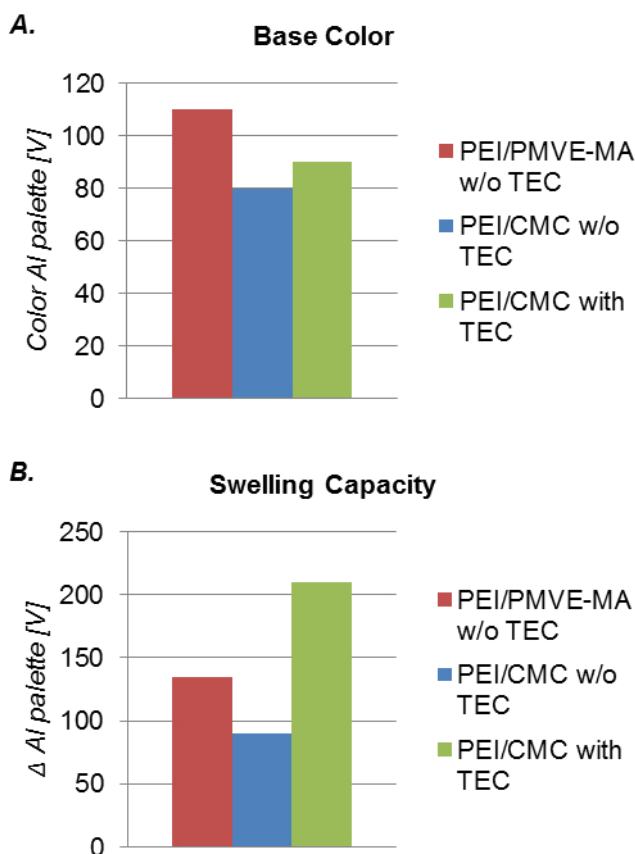


Figure 46 | Base color and swelling capacity of standard PEI/ PMVE-MA RHS coating and PEI/ CMC RHS coatings prepared w/o and with TEC (n=1)

5.3.4.1 Further Observations & Improvements of Preparation Process

The stability and maximum swelling capacity of RHS materials, which had been employed in earlier experiments (including the experiments described in 5.3.3.), had varied from batch to batch. One factor that appeared to influence coating homogeneity and PEM stability was the age of the polymer deposition reagents employed for coating. PEMs prepared from polymer solutions, which were several days old, were observed to swell less in contact with elevated humidity than PEMs prepared from fresher solutions. The old solutions did not show any visible signs of degradation or bacterial growth, but they smelled slightly rotten compared to fresh ones. It was hypothesized that components of the solutions decomposed into volatile components during storage. Especially polymer solutions containing TEC appeared to change fast. It was thus decided not to use polymer solutions and washing solutions for longer than 72 h after preparation for the assembly of PEMs. If required, TEC was added 30 min to 1 h before use in order to minimize any adverse effects of the plasticizer. However, very fresh solutions (prepared 1-2 h before use) did not always perform well either, although the polymers appeared completely dissolved. As discussed by Miller-Chou et al., the process of polymer dissolution is not as instantaneous as the dissolution of non-polymeric materials. It occurs in two steps called solvent diffusion and chain disentanglement [140], which can take days in the case of very high molecular weight polymers (>1 MDa). If reproducible polymer solutions are required, it is therefore recommended to let the polymer dissolve at

least overnight [141]. The effects of dissolution time on RHS performance were not systematically investigated or verified. Instead, it was decided to use polymer deposition solutions not earlier than 22 h after preparation. Moreover, they were stirred at least 30 min immediately before use.

Minor variations in the preparation process also appeared to affect the performance of RHSCs. Sputter-coating was noticed to interfere with PEM stability, for instance. For shipment or long-term storage, RHS films or plates were occasionally covered with a protective detachable adhesive plastic film (NITTO, SPV series). Usually the adhesive film could be detached from RHS materials without damaging their coating. If the protective film was applied to the RHS material and taken off for repositioning directly after sputtering, however, the PEM delaminated from the SiO₂ interlayer. In consequence, PEM and cluster layer were pulled off together with the adhesive film. Moreover, freshly sputtered RHS materials appeared to lose their swelling capacity if exposed to highly humid air directly after sputtering. In all experiments described hereafter, RHS materials were therefore incubated for at least 30 min at RT before testing their swelling capacity or further processing.

5.3.5 Mid-term Aging Test

A 84-day aging test was performed to evaluate the effect of TEC on the gradual loss of swelling capacity that PEMs in RHSCs undergo under ambient conditions. In addition, the importance of two other factors potentially involved in the process should be investigated: the concentration of CO₂ in the surrounding air and/or the composition of RHS coatings i.e. the composition of the PEM and the type of metal cluster layer deposited on top of it. For this purpose, a range of RHS films with different PEM composition was prepared. Apart from the two, already established polymer pairs PEI/ PMVE-MA and PEI/ CMC two further combinations were tested: Polymin SK²/ PMVE-MA and Polymin SK/ CMC. All films were cut in two and the two parts were provided with different metal cluster layers (either silver or palladium, see Table 7). To verify whether TEC prevents or slows down aging, RHSCs with PmP PEM (= PEM out of PEI/ PMVE-MA) were prepared with or without TEC. The aging of the two film types was compared in the analysis. PEMs containing the polyanion CMC are not sufficiently humidity sensitive without plasticizer. Hence, these were always prepared with TEC.

² POLYMIN SK is the trade name of a modified polyethyleneimine polymer sold by BASF. It differs from unmodified polyethyleneimine in that the primary amine groups are typically substituted to 20-25 % and preferably to 100 % with N-acetyl groups [142].

The films described above were cut in ten pieces each. The swelling capacity of one piece of each film type was determined one day after preparation. The remaining strips were stored under three different conditions for up to three months:

- in an open vessel
- in a closed vessel
- in a closed vessel at elevated CO₂ concentration

Glass vessels were utilized for the test. In contrast to plastic vessels they are inert and gas-tight. No plasticizers evaporate from glass vessels. If they are closed, there is no gas exchange between the inside of the glass and the environment either. The vessels were stored in the dark under constant temperature and humidity (ranging from 19 to 21°C and from 35 to 40 % RH). Thereby, heating by sunlight and UV irradiation – factors which could have influenced the aging of the RHS films – were avoided. All samples had to be prepared freshly at the same day. Hence, only a limited number of samples could be prepared overall. Duplicates were therefore not included in the screening. Swelling capacity measurements were only performed on four days of the test period. Thus, the statistical relevance of some results is certainly questionable. Nevertheless, the test provided valuable further information on RHSC aging. As described, Ag- and Pd-coated RHS films were included in the aging test. The cluster composition slightly influences the resonance color that is reflected from a REA structure at defined interlayer thickness. Thus, base color and swelling capacity of the samples was determined according to the method described in 5.2.1 using the PET-based REA color palette (see Figure 36). It comprises two equivalent sets of REA color strips, which differ only in the cluster layer: one set is coated with Pd clusters, the other one with Ag clusters. The palette was more suited for the experiment than the previously used Al-based palette. The latter was coated with Pd only. The main disadvantage of the PET-based REA color palette was that the interlayer thickness increase from one color value to the next was not perfectly linear. The interlayer strips on the Al-based palette were generated by anodizing. In this process, the thickness of the generated layer can be controlled very accurately. In contrast, the interlayers on the segments of the PET-based palette were prepared by wet-chemical SiO₂ deposition, which is slightly less reproducible. Still, the PET-based palette was accurate enough to examine changes in base color and swelling capacity of RHS films qualitatively.

Selected diagrams summarizing the swelling capacity of individual samples measured 1, 7, 28 and 84 days after preparation are depicted in Figure 47 and Figure 48. The swelling behavior PskP and PskC is not shown however. Already one day after preparation, these two RHS film variants responded only weakly to changes in atmospheric humidity or not at all. Obviously, the respective polymer combinations do not form suitable PEMs for RHS coatings. PskP and PskC films were thus excluded from further analyses. The aging of strips of type PmP- (PmP without TEC) with Ag cluster layer could not be characterized either, because their resonance color was extremely pale for unknown reasons. All remaining PEM types exhibited high maximum swelling one day after preparation. The swelling capacity of

most RHS film types decreased slightly within the first week. In contrast to earlier observations, however, assemblies of the standard polyelectrolyte pair PEI/ PMVE-MA did not exhibit significant aging within the following months. Surprisingly, the swelling capacity of the respective samples decreased only slightly or even improved slightly during 84 days of storage. Only the RHS films of type PmC (PEI/ CMC PEM) exhibited noticeable aging (see Figure 47). The PmC samples, which had been stored in closed vessels at elevated CO₂ concentration (both Ag- and Pd-coated), and the Pd-coated PmC film that had been stored in a closed vessel at ambient CO₂ concentration performed better after 84 days than the PmC samples stored in open vessels. Gas exchange may thus have played a role in the loss of swelling capacity of the PmC type films. This assumption is contradicted by the swelling behavior of the Ag-coated PmC sample stored in a closed vessel (Figure 48). It aged as strongly as the PmC strips stored in open vessels. This result might be an artifact. If it is not, it may suggest that the aging of PmC-type RHS films is determined by the availability of oxygen in the surrounding atmosphere in combination with the type of metal cluster layer on the film. The latter assumption is, however, only based on the performance of one sample and is likely to be disproved. The samples, which were stored at elevated CO₂ concentration, did not exhibit faster aging than samples of the same type, which were stored in closed vessels at ambient CO₂ concentration. Hence, it is considered unlikely that acidification by atmospheric CO₂ plays a role in the aging of RHS coatings. As mentioned, RHS films with PEI/ PMVE-MA PEMs did not age significantly in the course of the test. Therefore, the stabilizing effect of the plasticizer TEC in these films could not be judged. It was only found that the presence or absence of plasticizer did not affect their initial maximum swelling capacity. In the case of the PmC type films, TEC provided the polymer multilayer structure with a high initial swelling capacity but could not prevent its aging. Thus, it is considered improbable that it can stabilize other types of PEMs for a prolonged period of time. The aging of PmC PEMs was not accompanied by a change in the base color of the respective films. Hence, the polyelectrolyte layers within the PEMs did not shrink significantly during the twelve weeks of storage. In this regard, the aging PmC-type RHS films differed from the aging of PmP RHS steel plates at elevated temperature discussed in 5.3.3. Here, a slight reduction of the PEM thickness was observed.

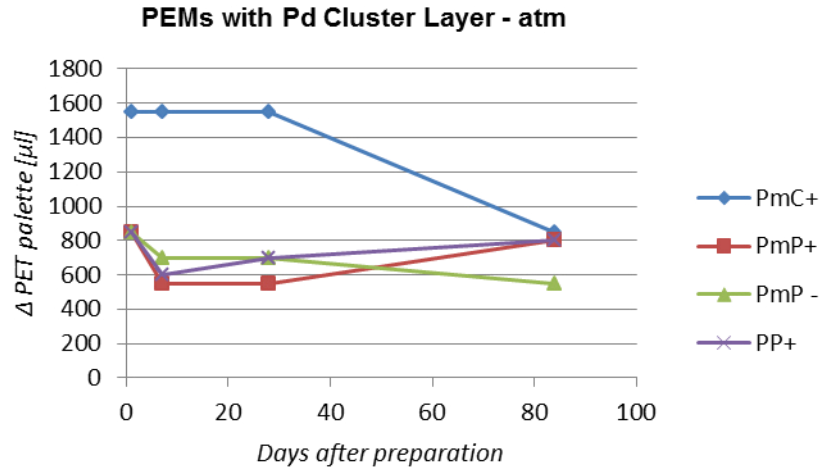


Figure 47 | Swelling capacity of PEMs with Pd cluster layer stored in open vessels (atm) within 1, 7, 28 and 84 days after preparation (n=1) (PmC+, PmP+, PP+ ... prepared with plasticizer TEC, PmP-... prepared w/o TEC) To determine the swelling capacity of a sample, base color and color at maximum expansion were compared to the PET-based REA color palette. The difference of the corresponding color values of the palette was calculated (Δ PET palette [μ l] = color at maximum expansion [μ l] – base color [μ l])

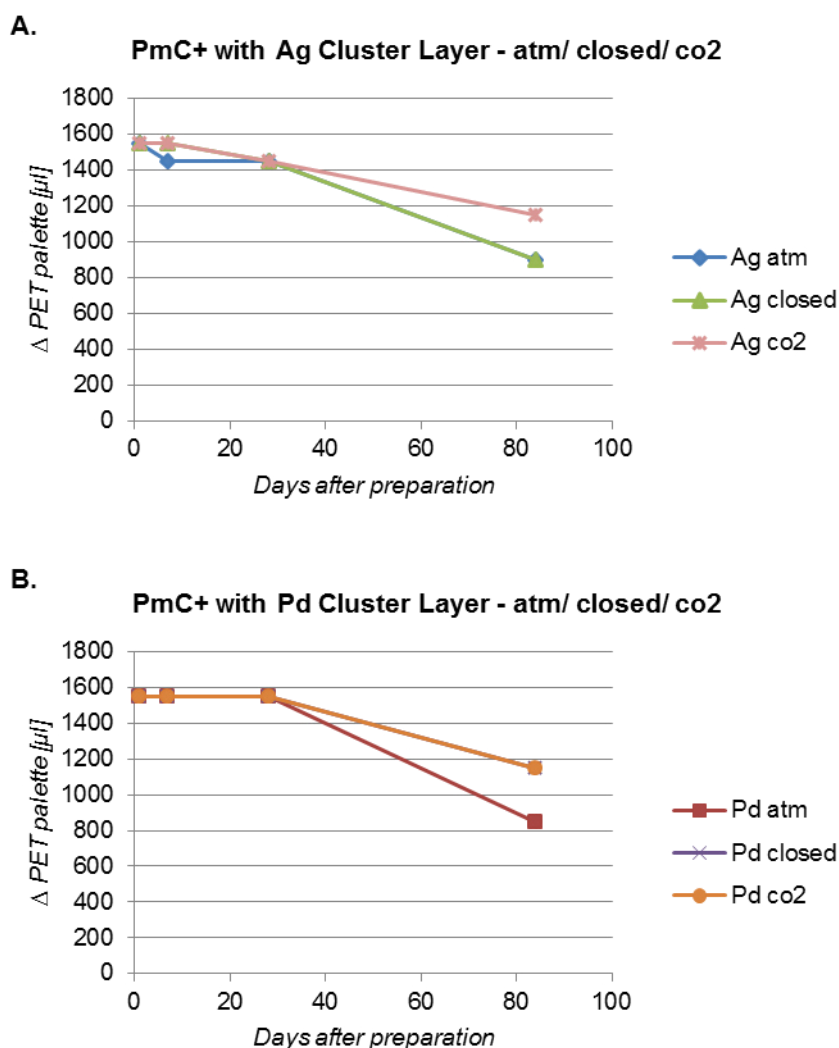


Figure 48 | Decrease of swelling capacity of PmC type PEMs with TEC (PmC+) and Ag or Pd cluster layer within 84 days after preparation at different storage conditions (n=1) The samples were stored at ambient conditions (atm), in closed vessels (closed) or in closed vessels at elevated CO₂ concentration.

Valuable results were obtained by the performed mid-term aging screening. Nevertheless, the experimental setup was not optimal. As described, only nine small glass vessels were utilized as storage containers, one vessel per storage condition (open, closed and closed + elevated CO₂ concentration) and planned incubation time (7, 28 and 84 days). Into each vessel, strips of all twelve RHS film types were packed close to each other. It is possible that ethanolamine, acetic acid or TEC evaporated from individual samples and synchronized the aging behavior of all samples. Thereby results could have been falsified. The gas exchange in the open glass containers was probably poor. Therefore, the storage conditions “atm” and “closed” (samples stored either in open or closed glass containers) did not differ significantly. Artifacts could have been avoided if samples had been stored separately or at greater distance in larger containers, so that evaporated substances could have diffused away from the samples. Moreover, the individual RHS film types should have been monitored over a longer period of time. Unfortunately, the experiment could not be repeated within this

doctoral project. Other analyses were performed to elucidate the processes, which slowly render standard RHS films insensitive to humidity.

5.3.6 UV Irradiation of RHS Coatings

As reported above, PEI/ PMVE-MA PEMs did not age significantly in the mid-term aging test within 84 days. RHS coatings of the same type had been observed to lose their sensitivity much faster before. Considering that the samples were stored in the dark in the mid-term aging test, UV illumination might be a crucial factor in RHSC aging. Hong et al. demonstrated that the surface of PEI layers can be modified by illumination with short wave UV radiation. They irradiated PEI SAMs at a wavelength of 254 nm for 1 h and thereby induced selective removal of amine groups at the surface of the polyelectrolyte layer [143]. If such a process also occurs at the surface of the topmost PEI layer of PEI/ PMVE-MA PEMs, it could change the internal pH of the assembly. This could modify both absorption behavior and swelling capacity of the whole PEM. Therefore, the effects of short-wave UV light on RHS films were tested. Films without metal cluster layer were utilized for the experiment, as metal clusters might have reflected or partially absorbed the radiation. Two pieces of RHS PET film were covered with stencils that partially shaded the RHS coating. The stencils were prepared from transparencies that were printed black using a laser printer. The samples were illuminated with UV light at a wavelength of ~254 nm for 30 min and for 60 min, respectively. The test setup differed from that of Hong et al. in that the light source was not placed at a distance of 0.5 cm to the sample. If light source and film were put so close to each other, the sample was strongly heated. As demonstrated in 5.3.3, thermal stress alone can significantly reduce the swelling capacity of RHS coatings. Sample and light source were therefore positioned 15 cm apart. At this distance, the light source did not significantly heat the sample even if it was irradiated for 1 h or longer. A reference sample was also covered with a stencil. It was not irradiated with UV light but stored in the dark for 1 h. Another reference sample was incubated in the dark without stencil to be able to identify cross-reactions of stencil and RHS coating. After irradiation or incubation in the dark, respectively, all samples were provided with a Pd cluster layer. Their base color and swelling capacity were analyzed the following day.

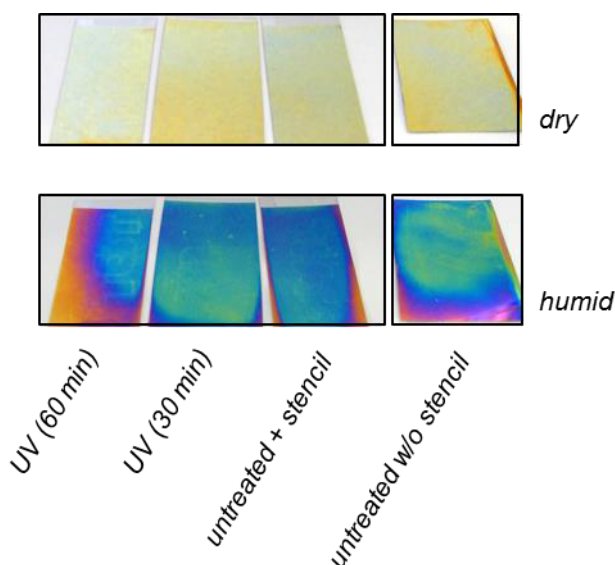


Figure 49 | REA PET film pieces after UV irradiation test

As demonstrated in Figure 49 no passivation was observed in this test. UV-treated and reference samples exhibited the same base color and the same sensitivity. At elevated humidity, the outlines of the stencils appeared on all samples. The swelling capacity had increased in these areas, not decreased, however. It is supposed that components of the binder of the black laser printing toner on the stencils had evaporated during the test and had interacted with the RHS samples. The test demonstrated that UV light of a wavelength of 254 nm does not have any observable effect on the swelling behavior of RHSCs. Nevertheless, it is possible (but considered rather unlikely) that the coatings are sensitive to UV light of other wavelengths.

5.3.7 Treatment with Aluminum Salt Solution

The mirror layer of standard PET-based RHS films typically consists of aluminum. On top a SiO_2 thin film is deposited by a sol-gel process for two reasons: On the one hand, it acts as distance layer determining the base color of the RHS coating. On the other hand, it protects the Al layer during PEM assembly. Al thin films are highly sensitive to alkaline agents. Thus, the mirror layer of standard RHS films would readily dissolve in the standard polycation deposition solution (pH~11) (see 4.2.2) if not shielded by a ceramic layer. Sol-gel deposited SiO_2 layers are porous, however. Thus, the mirror layer of RHSCs is still exposed to the polymer deposition solutions to a minor extent. Likewise, it can chemically interact with the PEM assembled on it. As suggested in 5.3.3, the mirror layer probably oxidizes within the RHS structure, because of electrochemical interactions with the PEM and the top metal cluster layer. If this is true, the generated Al^{2+} ions could diffuse through the SiO_2 into the PEM. PEI has already been reported to form complexes with other divalent ions (see 5.1). Complexation of PEI and Al^{2+} would supposedly lead to a stiffening of the PEM assembly and could thereby compromise its sensitivity.

Hence, several stress tests were performed, in which RHSCs were exposed to Al^{2+} containing solutions. Then their swelling capacity was compared to untreated coatings. The most informative test was performed on pieces of RHS steel plates using the six solutions listed in Table 10.

#	Composition	pH-value
1	0.2 % (w/v) aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$) in dH_2O	4
2	1 % (w/v) $\text{Al}_2(\text{SO}_4)_3$ in dH_2O	3.5
3	0.2 % (w/v) sodium sulfate (Na_2SO_4) in dH_2O	6.5
4	dH_2O , pH adjusted with H_2SO_4 to pH=4	4
5	dH_2O , pH adjusted with H_2SO_4 to pH=3.5	3.5
6	dH_2O , pH adjusted with H_2SO_4 to pH=4.5	4.5

Table 10 | Aluminum treatment test solutions

As listed, only two of the solutions contained Al^{2+} ions. The remaining solutions should reveal experimental artifacts caused by SO_4^{2-} and the pH-value of the test solutions. RHS steel plates were selected as substrates, because the RHS coating of these plates does not comprise Al. Hence, Al^{2+} ions could not be released from the substrate itself. Two pieces of a RHS plate were treated with droplets of all six test solutions as described in 5.2.7. Then swelling capacity and base color of the treated spots and untreated parts of the samples were examined. Afterwards, the plates were equilibrated in dH_2O to reset the internal pH of the RHSCs in the treated areas and the measurement was repeated. Both samples exhibited equal changes in base color and swelling capacity in response to the stress test, so average values did not have to be calculated.

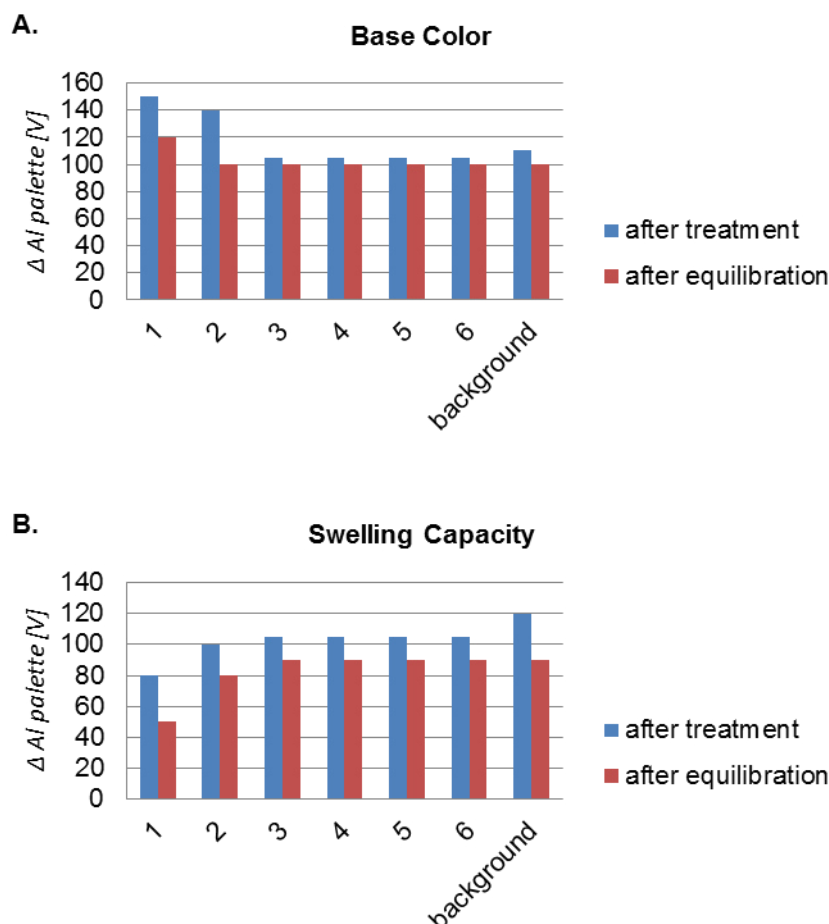


Figure 50 / Base color and swelling capacity of RHS steel plates after treatment with solutions of $\text{Al}_2(\text{SO}_4)_3$, Na_2SO_4 and H_2SO_4 ($n=2$) The Al-based REA color palette was used for measurements (1-6... spots on RHS plates treated with solutions 1-6; background... parts of the RHS plates not treated with any test solution) The results were equal on both RHS plates.

The obtained results (see Figure 50) indicate that Al^{2+} can indeed influence the sensitivity of RHSCs. Both $\text{Al}_2(\text{SO}_4)_3$ solutions decreased the swelling capacity of the treated samples, while equally acidic solutions of H_2SO_4 and Na_2SO_4 solution did not significantly interfere with RHSC reactivity. However, it is unclear, why the 0.2 % $\text{Al}_2(\text{SO}_4)_3$ solution affected the RHSCs more strongly than the five times more concentrated $\text{Al}_2(\text{SO}_4)_3$ solution. Further tests need to be performed to verify the results and elucidate, whether the pH-value of the test solution plays a role in the interaction of Al^{2+} ions and standard RHSCs.

5.3.8 Storage at Dry Conditions

As discussed in the next chapter (see 6.2.5), standard RHS coatings can be passivated by irradiating them with a CO_2 laser beam at low intensity. CO_2 lasers emit infrared light (typical wavelength: 10.6 μm), which is readily absorbed by water [144] and many organic materials including plastics. When absorbing the laser light, the irradiated materials are heated strongly. At sufficient energy density they melt, degrade and/ or vaporize [145]. It was demonstrated in this doctoral project that laser patterning inflicts only minor damage to the PET substrate and the aluminum mirror of RHS films (see chapter 6). The base color of the

irradiated areas remains the same or shifts to a slightly lower shade, which indicates that the PEM is not ablated in these areas. Nevertheless, its swelling capacity drops drastically. RHS PET films were also observed to passivate if they were hot-pressed at 200°C or incubated at 50-60°C for several days as discussed in 5.3.3. It was hypothesized that all these methods of heating caused evaporation of volatile components from the PEM layer, which are essential for water absorption and swelling. In principle, the solutions used for PEM assembly contain four volatile substances, which could be integrated into the polymer assembly: dH₂O, dimethyl sulfoxide (DMSO), ethanolamine and acetic acid. It is supposed that water molecules and at least traces DMSO, the acid and the base remain trapped between the polyelectrolyte molecules in RHSCs at ambient conditions and thus influence PEM structure or internal pH-value. During storage they might volatilize slowly leaving back less sensitive RHSCs. To determine, whether RHS coatings indeed comprise dH₂O and whether it plays a role in their aging, it was attempted to extract it from PEMs. For this purpose, pieces ~2 x 3 cm in size were cut off a RHS steel plate and packed separately into polyethylene (PE) bags together with silica gel desiccant beads and incubated in these bags for ~ 1 month. The bags were stored in the dark to avoid heating by sunlight. The color of the RHS steel plate pieces shifted slightly from yellow-orange (color shade “110 V” of AI color palette) to yellow (105 V), immediately after the PE bags were sealed by lamination. This demonstrated that the desiccant bags indeed reduced the relative humidity within the bags and also extracted humidity from the RHSCs. Reference samples, which were cut from the same RHS steel plate and packed into PE bags without silica gel, retained their yellow-orange base color. After 33 days, two of the three samples incubated with silica gel and two of the three reference samples were unpacked. Their base color and swelling capacity were determined as explained in 5.2.1 using the aluminum color palette depicted in Figure 35.

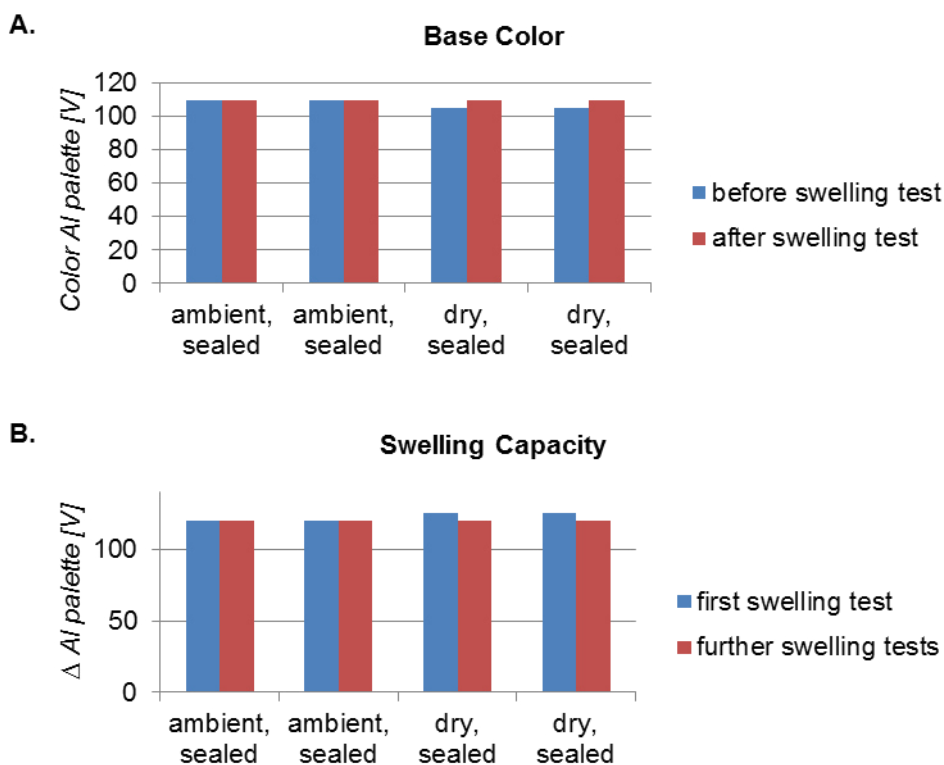


Figure 51 | Effects of storage of RHS steel plates in dry atmosphere ($n=1$)

The base color of the reference samples stored without silica gel was yellow-orange, while the samples stored in dry atmosphere exhibited the yellow base color they had adopted immediately after packing. Surprisingly, it shifted back to yellow-orange as soon as the samples were brought in contact with humid air. At condensing humidity, all samples swelled to the same maximum color. Hence, all samples exhibited the same swelling capacity after the first swelling test. The two samples that had not been unpacked after 33 days, one reference sample and one dried sample, were incubated in the dark for further 43 days before they were examined. They showed the same base color and swelling behavior as the previously tested samples then. The dry storage test was repeated with an incubation time of 38 days and equivalent results were obtained. It may thus be concluded that evaporation of water alone is not responsible for the aging of RHS coatings.

5.3.9 Replacement of Ethanolamine

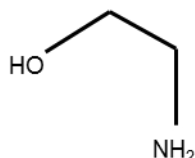
Ethanolamine fulfills two functions in the assembly of PEI/ PMVE-MA PEMs:

- 1) It is added to the PEI solution to render it slightly more alkaline. The higher pH-value supposedly makes the PEI molecules adsorb in a more globular conformation.
- 2) Aqueous ethanolamine solution is employed as washing solution in the PEM assembly process to remove unbound PEI molecules after deposition of PEI layers.

To determine, whether ethanolamine plays a role in the long-term stability of RHS coatings, it was replaced with an alternative base. The weak base tris(hydroxymethyl)aminomethane

(also called TRIS base or TRIS) was selected as substitute. TRIS is only slightly larger than ethanolamine (see Figure 53) and non-toxic. It also carries one amino group, but three OH-groups. In contrast to ethanolamine, TRIS has no vapor pressure at ambient conditions. Hence, it should not evaporate from films stored at RT.

Ethanolamine



Tris(hydroxymethyl)aminomethane (TRIS)

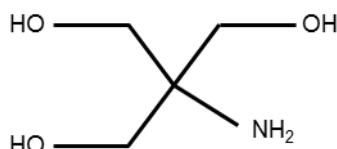


Figure 52 | Structural formulas of ethanolamine and TRIS

Two samples were prepared for the experiment as follows: An aluminized PET film was coated with a SiO₂ interlayer as explained in 4.2.1. The film was cut in two and a standard PEI/ PMVE-MA PEM was deposited on one half (4.2.1, competitive approach). On the other half, a PEI/ PMVE-MA PEM was assembled without using ethanolamine. Instead, TRIS was added to the polycation solution and the alkaline washing solution at the same molar concentration (see 5.2.9). Both pieces were sputter-coated with a Pd cluster layer. Base color and swelling capacity were tested half an hour later. The standard PEM prepared with ethanolamine was slightly thicker than the PEM prepared with TRIS, which resulted in a higher base color of the first. Both RHS coatings exhibited equivalent swelling capacity, however (see Figure 53). Two days later, the swelling tests were repeated with much different results. The standard PEM still responded similarly well to elevated humidity as on the day of preparation. The PEM prepared with TRIS had become nearly insensitive to humidity and swelled only slightly.

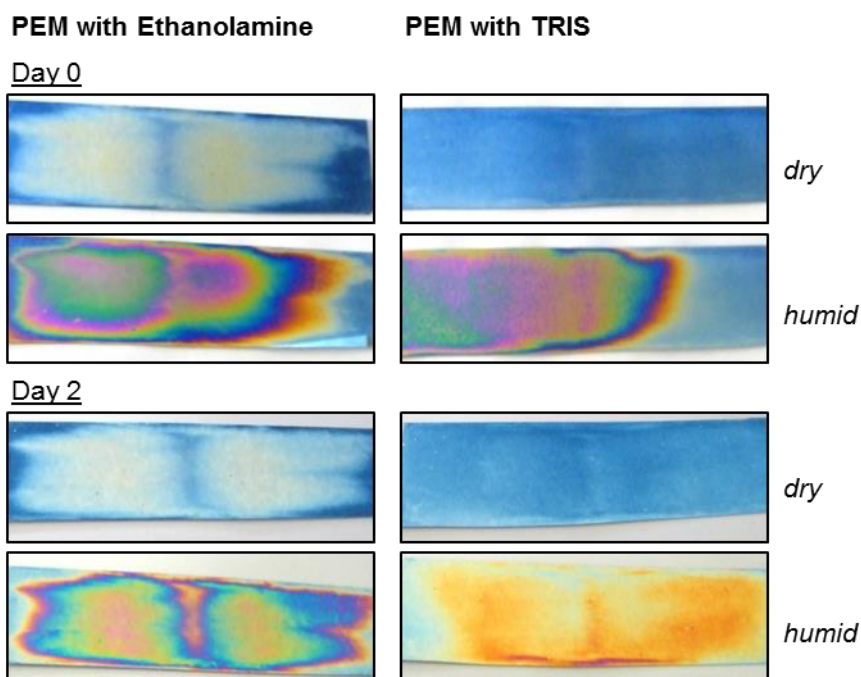


Figure 53 | Swelling capacity of standard PEM and PEM prepared with TRIS instead of ethanolamine The swelling behavior of the samples was characterized on the day of preparation (day 0) and two days afterwards (day 2)

In contrast to TRIS, ethanolamine is hygroscopic. From the above results it was concluded that it acts as a humectant in standard RHS coatings and assists the polymer assembly in absorbing humidity. This effect might be crucial for good performance of PEI/ PMVE-MA PEMs. To verify this assumption, PEMs were prepared with TRIS and hygroscopic agents. Two neutral humectants were selected for the purpose: sorbitol and glycerol. Another aluminized PET film with SiO₂ interlayer was prepared and cut in two. Both strips were coated with a PEI/ PMVE-MA PEM according to the standard protocol, but using alternative polymer and washing solutions. As described in 5.2.9, TRIS was added to the polycation deposition solution and the alkaline washing solution. Moreover, sorbitol or glycerol was added to all polymer and washing solutions at the same molar concentration as TRIS. The swelling capacity of the freshly prepared films was nearly as high as that of standard RHS coatings (standard PEI/ PMVE-MA PEMs: $\Delta = 110\text{-}120$ V, PEI/ PMVE-MA PEM with TRIS base and sorbitol or glycerol: $\Delta = 90\text{-}95$ V) (see Figure 54). As expected, they did not lose their sensitivity within two days after preparation but remained well reactive for at least 21 days. This result strongly supports the hypothesis that ethanolamine is essential for the performance of standard RHS coatings and that its evaporation is a major factor in RHSC aging. Ethanolamine apparently has two functions in assembled PEI/ PMVE-MA PEMs: it influences their internal pH and – probably more importantly – it acts as humectant and attracts humidity from the environment.

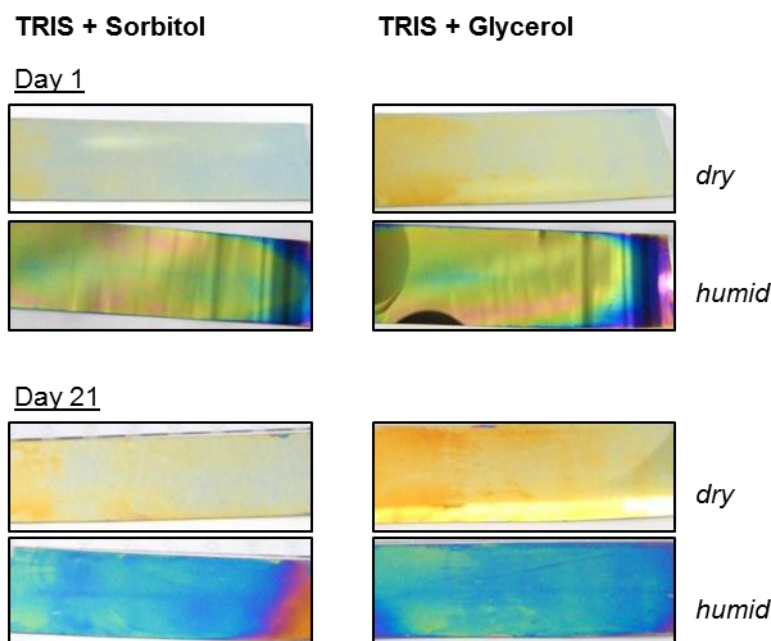


Figure 54 | Swelling capacity of PEMs prepared with TRIS base and humectants

5.4 Summary & Outlook

Standard RHS materials with PEI/ PMVE-MA PEMs were observed to lose their sensitivity and swelling capacity when stored at ambient conditions for >6 months. Several internal and external factors probably contributing to this aging process were investigated. It could be demonstrated that thermal stress, especially at elevated humidity, can impair the swelling capacity of the PEI/ PMVE-MA PEMs. In contrast, pH-active substances in the surrounding atmosphere, atmospheric CO₂ and short wave UV (~254 nm) did not affect the sensor properties of RHSCs. The role of other potential factors, such as the contamination of the PEM by metal ions, could not be determined clearly. The results of the performed screening are summarized in Table 11. Eventually, ethanolamine was found to be essential for the swelling capacity of RHS coatings. It is used in the preparation of PEI/ PMVE-MA PEMs to adjust the pH of the PEI deposition solution and to remove excess PEI after deposition of the polycation layers. Obviously, some ethanolamine remains in RHSCs after completion of the PEM and acts as humectant there. With a vapor pressure of 0.2 mmHg at 20°C [146] it is not highly volatile, but may slowly evaporate from the coating during prolonged storage. As described, it was replaced by a non-volatile weak base (TRIS) and a hygroscopic agent (sorbitol or glycerol) in PEM assembly tests. The as-prepared films exhibited similar sensor properties as standard RHS films. Unfortunately, the long-term stability of these films could not be evaluated systematically, so far. Hence, it could not be proven yet that PEI/ PMVE-MA PEMs containing a non-volatile humectant remain stable for a longer period of time than standard PEMs. After the completion of this screening only one alternative RHS film was prepared using a volatile base and a humectant with very low vapor pressure. However, this film retained its sensor properties for more than 1 year and could not be passivated by hot-stamping or lasering (unpublished data). The performed study provided first insights into the aging of RHSCs. Based on these results the PEM assembly process could be improved

significantly. Nevertheless, the aging of RHSCs needs to be investigated further to determine all involved factors and to reveal the causative chemical and physical processes.

<i>Screened internal & external factors</i>	<i>Effects on RHSC aging</i>
Thermal stress	yes
UV-treatment	not observed but probable
Contamination by metal ions released from mirror and metal cluster layer	unclear, probably minor effects on swelling capacity of PEM
Collapse of PEM	not at ambient conditions, occurs upon thermal stress
Change of surface energy of PEM	probably minor effects
Stiffening of chains	unclear (long-term stabilizing effect of plasticizer TEC for PmC type RHS coatings not observed, long-term stabilizing effect of TEC for PmP-type coatings could not be evaluated)
Atmospheric CO ₂	no
Evaporation of volatile substances from PEM	evaporation of ethanolamine from the PEM appears to be a major factor in the aging process

Table 11 | Effects of potential internal and external aging factors in the passivation of standard RHS coatings

6 Patterning of REA Coatings

6.1 Introduction

The Endless Band Coater discussed in 4.3.4 allows for reproducible fabrication of REA films of uniform color. REA films for other applications (e.g. immunoassays) can presumably be produced with the novel coater as well if other coating solutions are used. However, a major disadvantage of the system was that it only yielded unicolor coatings. There was no possibility to pattern the deposited multilayer structure i.e. to change its resonance color locally in order to generate text or symbols. However, graphic elements represent a valuable tool in sensor technology as they can be used to visualize test results in the form of symbols or to communicate the purpose of a sensor. Of course, labels or pictograms could simply be printed onto REA films using ink or varnish and conventional printing techniques, but sensor properties and resonance color of the coated areas would be abolished then. Hence, such prints would significantly reduce the functionality of the REA material, especially if they covered a large part of the REA coating. It would be preferable to pattern REA coatings by a technique that does not interfere with the special features of REA sensor coatings (resonance color, reactivity). Optimally, the respective technique should not significantly complicate the manufacturing process, so that production costs remain at an acceptable level. Nevertheless, generated patterns should be easily discernible and their layout should be customizable without much effort.

As reported in chapter 3, the absorption behavior and thus the color of a REA structure can be varied by modification of the thickness or the optical properties (transmission, refractive index) of individual layers. Hence, it would in principle be possible to obtain permanent patterns by selective adjustment of these parameters. To create text or symbols, which only appear in presence of the targeted analyte, the response of the sensor layer needs to be tuned locally.

In the patterning of thin films two approaches can be taken: Direct patterning methods allow for the selective deposition of material on the substrate. In post-deposition structuring continuous films are provided with patterns by selectively removing or modifying parts of the film. For either of the two strategies several patterning techniques have been developed already. Their suitability for a certain application is determined by the type of layer to be structured, the employed deposition method, the desired resolution and the required processing speed.

As mentioned, investigations on the upscaling of REA film fabrication focused on wet-chemical deposition techniques so far. The developed processes yield very homogeneous ceramic layers and PEMs with good sensor performance (see chapter 4). However, compatible patterning techniques were not at hand. In wet-chemical coating processes, substrates are commonly patterned using adherent masks. These can be prepared directly on the substrate surface by a range of methods: In photolithography, which is a mainstream technique for high-volume manufacturing in semiconductor industry, a photoresist film is

applied and exposed to light at predefined positions [147]. Positive photoresists are locally destabilized thereby. In the subsequent developing step, the exposed areas can be washed away with a solvent, the so-called “developer”. In contrast, negative photoresists harden upon exposure. The unexposed areas are removed in the developing step [148]. Photolithography offers very high resolution, but represents a multi-step process. In comparison, generating masks by printing is significantly more straightforward. Concerning resolution, classical printing techniques such as gravure, flexo or screen printing are inferior to photolithography, of course. However, they are more than sufficient for creating logos and pictograms that are visible with the bare eye.

In this doctoral project, direct patterning and post-deposition structuring methods were screened for RHSC patterning. Investigations focused on technically simple approaches suited for industrial production. Accordingly, only cost-efficient masking agents were tested, which can be processed by conventional printing techniques.

6.2 Materials & Methods

6.2.1 Masking with Nitrocellulose & SiO₂ Deposition

1 % (w/v) nitrocellulose in EtOH/ ethylacetate (5:1) was applied with a brush or a stamp. In some cases, the substrate was pre-masked with a stencil prepared from self-adhesive film to limit the spreading of the masking solution. The nitrocellulose mask was dried at RT for 5 min. Then, a SiO₂ layer was deposited as described in 4.2.1. To remove the mask, the substrate was incubated briefly in EtOH/ ethylacetate (5:1) and wiped with a soft towel afterwards.

6.2.2 TiO₂ Deposition

25 % (w/v) titanium(IV) oxysulfate hydrate (TiOSO₄ · xH₂O) (Riedel-de-Haen, #14023) was dissolved in dH₂O under heating (60°C). Then it was cooled to 4-6°C. To deposit TiO₂ layers, 400 µl of the 25 % (w/v) titanium(IV) oxysulfate solution was added to 60 ml pre-cooled dH₂O (4-15°C). The solution was mixed briefly and the substrate was incubated in it, shaking, for 25-45 min at RT. Then the coating solution was poured off. Pre-cooled dH₂O or 0.1 % acetic acid (Carl Roth, X895) in dH₂O were added into the coating tray to remove residual titanium(IV) oxysulfate. The substrate was incubated in this washing solution, shaking, for 1-3 min and rinsed with dH₂O at RT 2-3 times then. Afterwards, it was either dried with pressurized air using a blow-off gun or subjected to further coating steps without drying.

6.2.3 Masking with Eudragit L

Three Eudragit L-based masking solutions were employed in the course of this thesis:

- Masking solution 1 (EtOH-based): 5 % (w/v) Eudragit L100 (= Eudragit L powder) (Röhm GmbH) in EtOH.

- Masking solution 2 (EtOH-based): 5 % (w/v) Eudragit L100 + 0.5 % (w/v) ethyl cellulose (Sigma-Aldrich, #2365) in EtOH.
- Masking solution 3 (H₂O-based): 10 % (w/v) Eudragit L100 + 1 % (v/v) NH₄OH (Carl Roth, #2610) + 1 % TEC (Sigma-Aldrich, #27500) in dH₂O

Masking solutions 1-3 were applied onto the substrate surfaces either with a brush or a silicon stamp. Alternatively, masking solution 2 was applied by screen printing using white 100-48 mesh (SEFAR-PET). The print was dried for 1-10 min at 50°C. Then, a TiO₂ thin film was deposited as described in 6.2.2. To remove the mask, the substrate was washed either in 0.1 % (v/v) ethanolamine (Sigma-Aldrich, #15014) in dH₂O or in pure dH₂O for 0.5-10 min. The substrate was then either coated with a PEI/ PMVE-MA PEM or with SiO₂ by wet chemical deposition as described in 4.2.1 (competitive approach).

6.2.4 Chemical Crosslinking

Carbodiimide (CDI) crosslinking

Three CDI solutions were prepared:

- 1) 10 mM N-Cyclohexyl-N'-(2-morpholinoethyl)carbodiimide methyl-p-toluenesulfonate (Morpho CDI) (Sigma-Aldrich, # C10,640-2) in dH₂O
- 2) 10 mM Morpho CDI in DMSO (Bussetti)
- 3) 10 mM Morpho CDI in DMSO/ dH₂O (1:1)

Three pieces of RHS steel plates (prepared 4 days earlier) were incubated with 10 mM Morpho CDI in dH₂O, 10 mM Morpho CDI in DMSO or 10 mM Morpho CDI in DMSO/ dH₂O (1:1) in a wet chamber at RT for 1 h. Then the samples were rinsed with dH₂O and dried with pressurized air. One piece of RHS film was left untreated. The maximum swelling capacity of the 4 pieces was examined as explained in 5.2.1 using the Alu color palette.

Formaldehyde Crosslinking

To prepare 4 % (v/v) formaldehyde in dH₂O 0.8 g paraformaldehyde (PFA) (Carl Roth, # 0335) and 50 µl 0.5 M NaOH (Sigma-Aldrich, # 71694) were added to 20 ml dH₂O. The mixture was stirred at 50-95°C until the PFA had completely dissolved. The solution was filtered with qualitative filter paper (grade: 3h, 65 g/cm²) (Sartorius Stedim Biotech) and stored at 4°C until use. The solution was not used for longer than two weeks after preparation.

For crosslinking, pieces of RHS steel plates (prepared as described in 5.2.3), complete or without top metal cluster layer) were either incubated in formaldehyde solution or in formaldehyde vapor at RT for 1 h. For incubation in formaldehyde vapor, samples were placed on a glass slide in a petri dish. 200-500 µl of 4 % (v/v) formaldehyde solution were pipetted onto a piece of paper towel underneath the glass slide. Then, the petri dish was closed and incubated in the dark. Samples incubated in formaldehyde solution were rinsed

with dH₂O and dried with pressurized air using a blow-off gun after the treatment. Samples treated with formaldehyde vapors were used directly. Those without metal cluster layer were sputter-coated with Pd for 35 s. Then, the swelling behavior of all samples was examined as described in 5.2.1.

Glutaraldehyde Crosslinking

RHS steel plates (prepared as described in 5.2.3) were incubated with droplets (5 μ l) of glutaraldehyde (Sigma-Aldrich, #49630) solutions in dH₂O or dH₂O/ DMSO (see Table 12, page 124) for 30 min in a petri dish in the dark. Then the droplets were blown off with pressurized air and the base color and swelling capacity of the treated areas were examined as described in 5.2.1.

6.2.5 Laser Patterning of Polymer Multilayers

Laser patterning was performed with a PC-controlled engraving and cutting CO₂-laser system (LS100, Gravograph). Standard RHS films (prepared as described in 4.2.1, competitive approach) could be patterned well by a single laser treatment at 8-10 % power, 100 % speed and a resolution of 300 dpi. Minor to nearly no damage was inflicted to carrier and mirror layer at these settings.

6.3 Results & Discussion

6.3.1 Masking with Nitrocellulose

SiO₂ distance layers for REA films are usually deposited from an alkaline silane solution in EtOH/ dH₂O (see 4.2.1). The substrate, commonly a 50-200 μ m thick PET film, is flexible and bent repeatedly in the coating process. Thus, the primary requirements for a compatible masking agent were flexibility and stability at alkaline pH and in diluted EtOH. Nitrocellulose shows these properties, so it was selected for initial tests. The employed nitrocellulose solution (see 6.2.1) could be applied conveniently with a brush or by stamping. It dried quickly at RT (typically within <5 min). The as prepared masks were flexible and prevented SiO₂ deposition efficiently. If no suitable stamp was available to create the desired pattern, areas that should not be masked were covered with adhesive tape. The free areas were coated with nitrocellulose and dried. Subsequently the adhesive tape was detached and a layer of SiO₂ was deposited. To obtain logos with two colors, SiO₂ was deposited one more time after removal of the nitrocellulose mask. Then, a cluster layer was deposited either by sputtering or by adsorption. The samples depicted in Figure 55 were prepared by this method.



Figure 55 | Multicolored patterns on REA films Two-colored patterns were generated on REA films by direct patterning of SiO_2 layers using nitrocellulose masks. Metal clusters were either deposited homogeneously over the whole REA film surface (left) or only in the shape of a stick figure (right). In the second case surrounding area was protected by adhesive tape. Therefore the background is non-colored but silvery metallic.

Unfortunately, it turned out to be difficult to completely remove the nitrocellulose after completion of the SiO_2 coating step. Commonly, incubation in solvents did not suffice. Remnants of masking film had to be detached mechanically i.e. by wiping. Of course, this cleaning procedure usually inflicted damages to SiO_2 distance layer and aluminum mirror. Another main disadvantage of the process was that significant amounts of highly flammable solvents were released into the atmosphere during mask drying. Even more solvent had to be used for detaching the masking agent. Thus, the overall process was ecologically problematic. If it had been scaled up for manufacturing, special safety measures (e.g. explosion protection) would have been required in the production facility.

For this reason, further tests focused on aqueous masking agents. However, no water-soluble compound could be found that is sufficiently stable in dilute EtOH after drying. Thus, two alternative strategies were considered:

- 1) Using a masking agent that can be stabilized against dissolution via light-induced crosslinking: Respective water-based formulations are commercially available, e.g. as photo emulsions for screen printing. However, the composition of these agents is generally complex and is usually not revealed by the manufacturer. If cross-reactions with the employed coating solutions occur, the respective formulations can thus hardly be customized without support from the developer.
- 2) Development of a water-based method for the deposition of ceramic distance layers: If the precursor solution was free from organic solvent, EtOH-stable masking agents would not be required but water-stable agents could be utilized. To minimize the release of organic solvents during mask drying and removal, the respective agents should be soluble in weak or dilute organic solvents.

The standard SiO_2 deposition procedure (sol-gel reaction in dilute EtOH, see 4.2.1) produces a lot of organic liquid waste, which is problematic both ecologically and economically. Thus, there was demand for an alternative water-based method anyway. For this reason, the second strategy was selected.

6.3.2 Preparation of Ceramic Distance Layers by TiO_2 Deposition

More sustainable processes than the standard SiO_2 deposition process were tested for the preparation of ceramic interlayers. One of these processes was precipitation of TiO_2 from aqueous titanium oxysulfate solution. The titanium ion (Ti^{4+}) is small and has a valence of four, so its charge density is very high. For this reason, titanium ions react readily with water. The stability of the resulting titanium oxo species depends on pH and temperature of the solution. At RT they tend to precipitate as hydrated TiO_2 unless the pH of the solution is highly acidic. Titanium oxysulfate (also “titanyl sulfate”) (TiOSO_4) and titanium tetrahalides (e.g. TiCl_4) are frequently used as starting compounds for aqueous TiO_2 deposition. The respective precursor solutions are commonly prepared under highly acidic conditions. Precipitation is induced by increasing the pH of the solution or by heat treatment [149].

For this study, a deposition protocol derived from a patent by W.J. Marshall et al. [150] was tested. 25 % TiOSO_4 in dH_2O was employed as starting reagent (see 6.2.2). TiO_2 deposition was initiated by diluting one part of this solution in 150 parts of deionized water. To generate homogeneous layers, it was essential to keep the deposition rate low at the start of the coating phase. For this reason the deionized water was cooled (to 4-18°C) before adding it to the TiOSO_4 solution. The coating process was performed at RT. Thus, the temperature of the TiOSO_4 solution gradually increased with time and so did the deposition rate. After completion of the TiO_2 deposition, the substrate was rinsed with dH_2O and dried. The as-prepared TiO_2 layers were largely homogeneous, but frequently a few random patterns became visible after depositing a metal cluster layer on top (see Figure 56).



Figure 56 | Defects in TiO_2 coatings Thin layers of TiO_2 were deposited on the mirror layer of the depicted RHS films by precipitation from solution. The films exhibited irregularities (marked by asterisks), which either resembled marbling (left and middle) or small granular, rather homogeneously distributed aggregates.

Some patterns resembled the marbling defects of polymer multilayer coatings described in 4.3.1. Moreover, granular white precipitates were observed (see Figure 56, right). It was found that both types of TiO_2 patterns formed during the washing process. They were generated by the interaction of washing water and the thin film of TiOSO_4 solution that remained on the substrate after pouring off the coating solution. The washing water was not cooled, while the temperature of the coating solution was generally still lower than RT after 30 min reaction time. Upon contact with the washing water, the temperature in the residual coating solution film rose abruptly, which induced immediate, inhomogeneous TiO_2

deposition. This process could be prevented completely by using cold water (4-6 °C) for the first washing step. Homogeneous TiO_2 layers were obtained then (see Figure 57).

6.3.3 Development of Solvent-free Masking Technology

Compared to the standard SiO_2 coating method described in 4.2.1, the established TiO_2 deposition procedure yielded optically thinner layers i.e. the color shift per deposited layer was significantly lower (see Figure 57). Nevertheless, the new TiO_2 process was tested further, because it was compatible with a wider range of masking agents than the standard SiO_2 deposition protocol and less ecologically problematic.



Figure 57 | Comparison of SiO_2 and TiO_2 deposition protocols **A)** REA films with Al mirror layer, Pd metal cluster layer and either SiO_2 (left) or TiO_2 (right) distance layer **B)** The color of the two REA films is indicated above the depicted Al-based REA color standard

As mentioned earlier, the aspired masking technology should employ reagents containing low amounts of volatile organic compounds (VOCs). Therefore, the potential of Eudragit L as masking agent was investigated. Eudragit L is composed of anionic copolymers of methacrylic acid and methyl methacrylate. It is typically used for preparing controlled-release coatings on drugs. The agent is insoluble in acidic aqueous solutions but easily dissolved in aqueous media above pH 6.0 and EtOH. In initial tests, EtOH-based solutions were utilized (see 6.2.3, masking solution 1). Masks were applied manually using a brush and were dried at elevated temperature, before TiO_2 deposition was performed. Pure Eudragit L masks often exhibited insufficient stability and partially detached in the coating process. Reproducible results could be obtained only after adding the binder ethyl cellulose to the mask formulation (see 6.2.3, masking solution 2). After deposition of the TiO_2 distance layer, the mask could be detached nearly completely by incubating it in a weakly alkaline aqueous solution (typically 0.1 % (v/v) ethanolamine) or water. Using Eudragit L/ ethyl cellulose masks patterned non-sensor REA films with uniform colors could be prepared (see Figure 58). The viscosity of the solution was quite low. Hence, it might be well suited for flexographic printing. In this industrial printing process, the ink is applied to the substrate via a roll carrying a flexible printing plate (see Figure 59). The printing plate is structured and thereby determines the image that is printed. Generally, low-viscosity inks are utilized for this printing technique [151].



Figure 58 | Non-sensor REA film patterned by masking with Eudragit/ ethyl cellulose solution

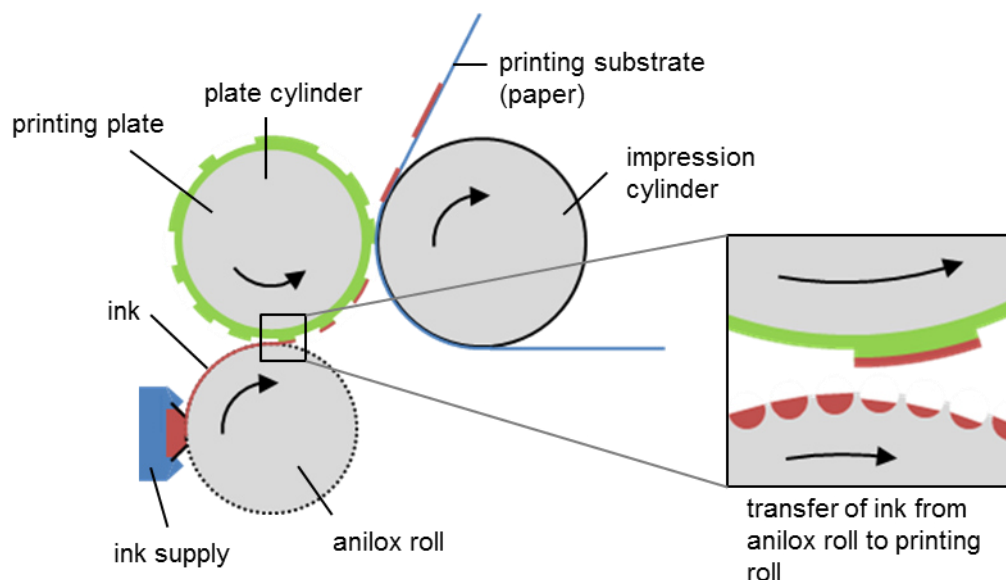


Figure 59 | Flexographic printing (schematic drawing) The ink is transferred from the ink supply to the printing plate via the so-called anilox roller or screen roller, which is evenly screened with cells. A doctor blade installed in the ink source helps filling the cells evenly with ink and stabilizes the printing process.

Further tests were performed with an aqueous masking formulation based on the recipe provided by the manufacturer of Eudragit L [152] (see 6.2.3, masking solution 3). It yielded more flexible, stable masks than the previously used EtOH-based solution. Adding ethyl cellulose was thus not necessary. The preparation of a complete RHS coating with patterned ceramic layer was attempted with the new masking solution. Surprisingly, the areas covered with Eudragit L during TiO_2 deposition eventually showed a higher resonance color than the non-masked parts of the substrate surface. In contact with humid air, the PEM in the previously masked areas swelled so strongly that the respective areas lost their resonance color (see Figure 60A). A similar phenomenon had been observed earlier in the development of the polymer deposition technology. When it was tried to slow down polymer adsorption by pre-coating substrates with protective, water soluble layers (see 4.3.2), PEMs with similar swelling capacity were obtained occasionally. In those cases, polymer remnants on the substrate interfered with PEM assembly. Apparently, Eudragit masks could not be completely detached via the established washing protocol either. Obviously, a thin layer of Eudragit L (with or w/o ethyl cellulose) generally remained on the masked areas and affected subsequent polymer adsorption. Attempts to wash off Eudragit L completely using EtOH failed. Instead of screening other organic solvents or other more aggressive cleaning agents, it was tried to passivate mask remnants by covering them with a ceramic layer. TiO_2 layers

generated from aqueous solution and SiO₂ layers prepared by the standard TEOS-based process were tested for that purpose. Eudragit L remnants could be passivated with either of the two coatings. Polymer multilayer coatings with normal swelling capacity assembled on top. The previously masked areas still exhibited a higher base color because of the Eudragit L thin film between mirror and passivation layer. However, the swelling capacity of the PEM was nearly equivalent in masked and non-masked areas (see Figure 60).

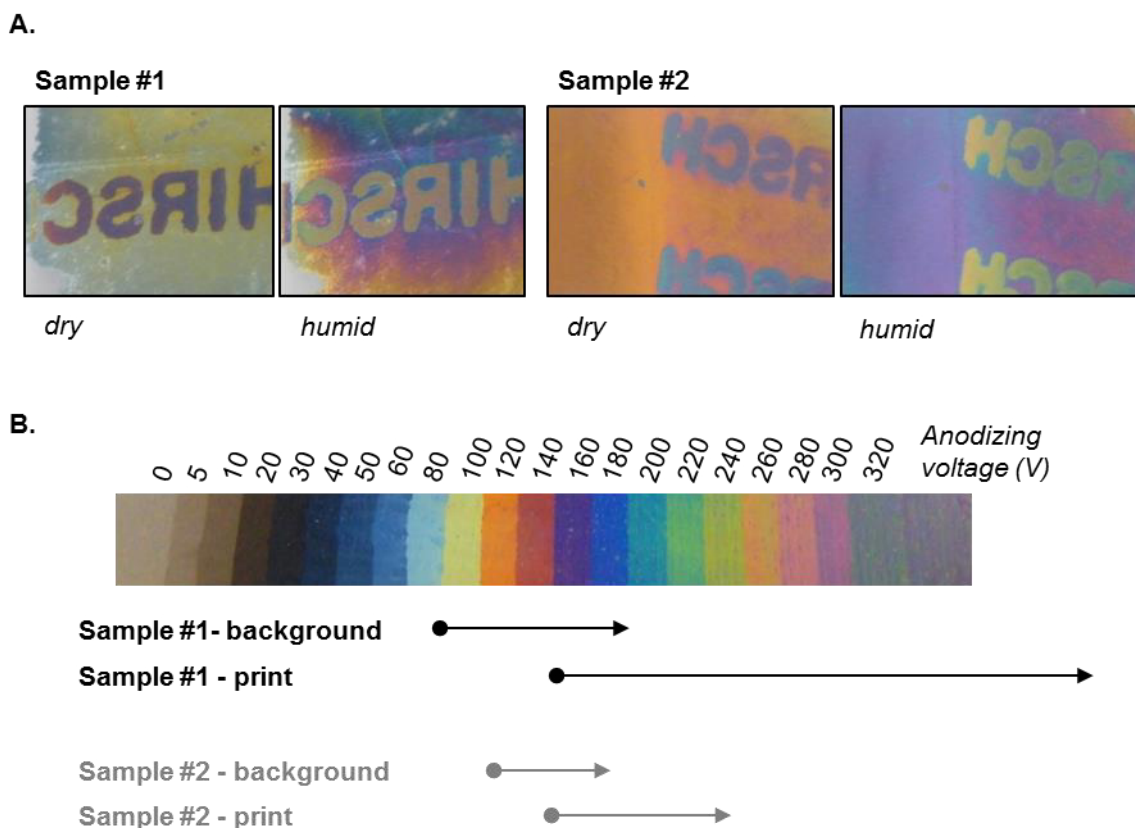


Figure 60 | RHS films patterned by masking with aqueous Eudragit L solution **A)** Sample #1: No ceramic passivation layer was deposited between patterned ceramic layer and PEM. The distance layer on top of the printed letters was significantly thicker than in the surrounding areas resulting in a violet-blue base color at ambient humidity (dry). At elevated humidity (humid), the interlayer thickness in the printed area increased above the maximum interlayer thickness, at which REA is observed. As a result, the resonance color vanished. Sample #2: A ceramic passivation layer was deposited after mask removal. The swelling capacity of the PEM on top of the created pattern was only slightly higher than the swelling capacity in the surrounding areas (background). **B)** The depicted Al-based REA color palette covers nearly the complete range of REA colors that can be created. Arrows indicate the color range, over which the background and the patterned areas of sample #1 & 2, respectively, swelled at elevated humidity.

To further increase the resolution and accuracy of the patterns generated with Eudragit L, the mask application technique has to be improved. Hence, screen printing was tested for depositing the aqueous masking formulation. Screen printing is a popular printing technology that offers high speed and versatility [153]. The principal components of a screen printing system are screen, squeegee (generally made from polyurethane or stainless steel) and substrate holder [154] (e.g. a vacuum table). The screen consists of a mesh, which is stretched over a frame. For printing, the screen is positioned above the substrate and ink is applied onto it. Using the squeegee or a floodbar the ink is spread over the screen and

pushed into the openings of the mesh. Screen and substrate are not in contact in this filling step. For the printing step, the screen is positioned atop the substrate. The squeegee is pressed onto the screen with sufficient force to make mesh and substrate touch. By pulling the squeegee over the screen, the ink in the openings of the mesh is transferred onto the substrate surface. A negative stencil determines the layout of the print. It blocks off parts of the screen while allowing ink to pass in other areas [155]. The printing quality depends on ink viscosity, mesh material, opening size and thread diameter, printing speed, angle and geometry of the squeegee and the distance between substrate and screen (the so-called “snap-off”). Usually high viscosity inks are processed in screen-printing, because they remain in the mesh openings after the filling step. Inks with low viscosity usually simply drip through [153]. EtOH-based Eudragit L solution would thus not be suited for this printing method. In contrast, the aqueous formulation was quite viscous. Indeed, screen printing tests with the formulation yielded quite accurate prints on aluminized PET film (see Figure 61A). However, the employed Eudragit L solution was not perfectly suited for screen printing. It foamed when the screen snapped off the substrate (see Figure 61B). For this reason, especially small and delicate structures could not be printed precisely. Usually defoamers are added to screen printing inks to suppress such bubble formation. Suitable adjuvants of this kind need to be identified for the Eudragit L solution. Then, REA films could be patterned quite ecologically using this industrial printing technology and Eudragit L as masking agent. Unfortunately, the development of a screen-printable Eudragit L masking formulation was beyond the scope of this doctoral project.

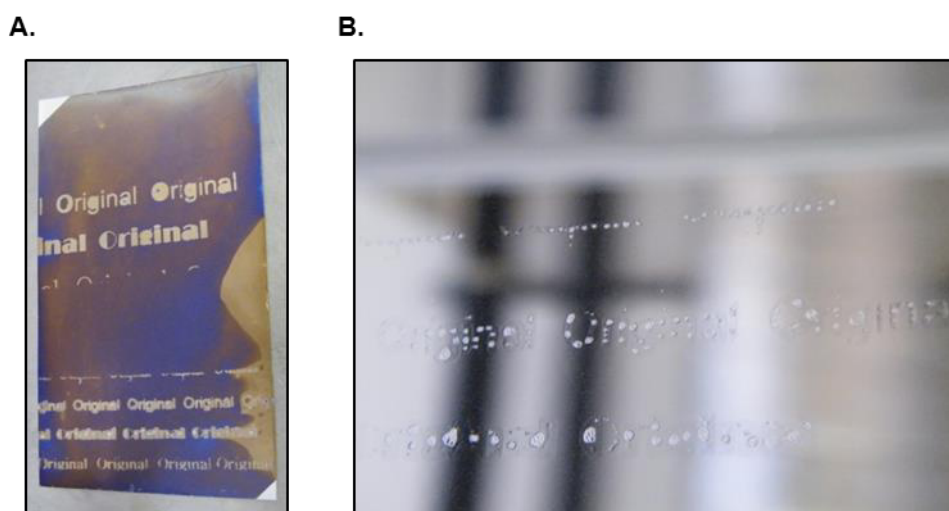


Figure 61 | Patterning of REA film by screen printing of a water-soluble lift-off mask **A)** An Eudragit L mask was applied by flat-bed screen printing. Then a TiO_2 layer was deposited, the mask was dissolved and the film was coated with a second layer of TiO_2 . Finally a Pd cluster layer was deposited by sputtering. **B)** The utilized aqueous Eudragit L solution foamed when applied by screen printing.

6.3.4 Patterning RHSCs by Laser Irradiation

As mentioned earlier, the reactivity of REA sensor coatings must be suppressed locally to generate patterns that become visible only upon interaction with the analyte. Earlier studies had revealed that RHS coatings can be passivated by brief hot stamping at temperatures

$\geq 200^{\circ}\text{C}$ (internal know-how of Attophotonics, data not published) (see Figure 62). The as-passivated areas exhibited nearly the same base color as surrounding untreated parts, but their swelling capacity was significantly lower.

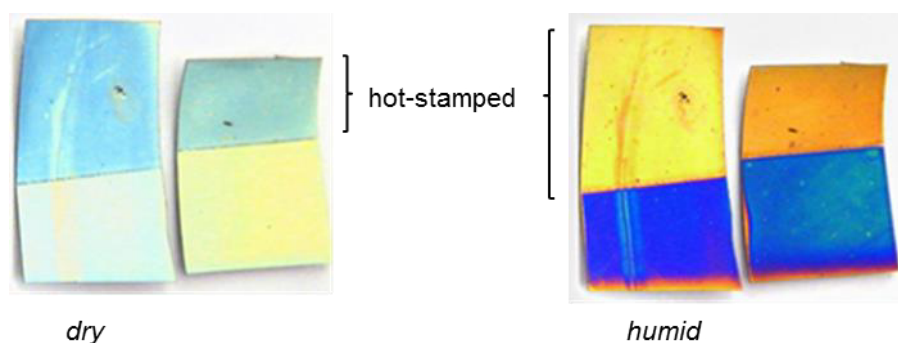


Figure 62 | Hot-stamped RHS PET films The upper half of the depicted RHS films samples was passivated by hot-stamping at 200°C for 2 s

The technique proved very useful for patterning metal-based RHS materials. Polymer-based REA materials are not that heat-stable, however. Especially thin RHS PET films usually deform in hot stamping when the hot metal die (= stamp) is pressed onto them. Brief but strong targeted heating of materials can also be achieved by laser irradiation. In this contactless technique the energy input into the substrate can be controlled much more precisely than in hot stamping. This minimizes the risk of melting and substrate deformation. Furthermore, size and shape of the treated area can generally be adapted easily via the laser control program. In hot stamping, a new die must be manufactured for each new layout. Thus, laser writing was thus considered an attractive alternative for the patterning of RHSCs.

Patterning tests were carried out using a PC-controlled engraving and cutting CO_2 -laser system (LS100, Gravograph). Intensity, resolution and speed of the laser beam were adjusted until the substrate, a RHS PET film (thickness: $175\text{ }\mu\text{m}$), could be heated without significantly eroding carrier material and mirror layer. Using the established settings a sample text (“test test”) was “written” onto the sample with the laser beam in scan mode (see Figure 63). The treated sample was incubated for 1 h at RT afterwards. Then base color and swelling capacity of its RHSC were examined. Indeed, the irradiated areas responded less to elevated humidity than the surrounding parts of the RHS films, while their base colors were nearly alike. The contours of the lasered text were well defined. The underlying substrate did not exhibit any deformation. Only the mirror layer was occasionally slightly eroded in the treated areas (see Figure 64).

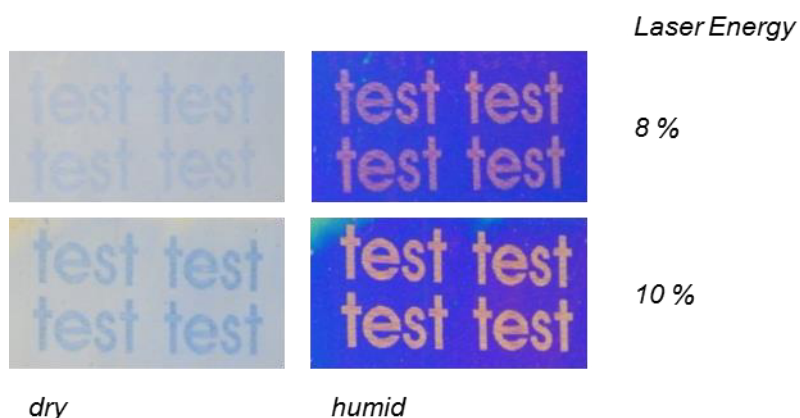


Figure 63 | Laser patterned RHS films Pattern introduced in RHS coating by CO₂-laser irradiation at 8 % energy (top) or 10 % energy (bottom) at ambient (dry) and elevated humidity (humid). In the dry state, the patterned area exhibited a slightly lower base color than the surrounding film (more pronounced on the samples patterned at higher energy). In contrast to the untreated areas, the laser-written text barely swelled at elevated humidity.

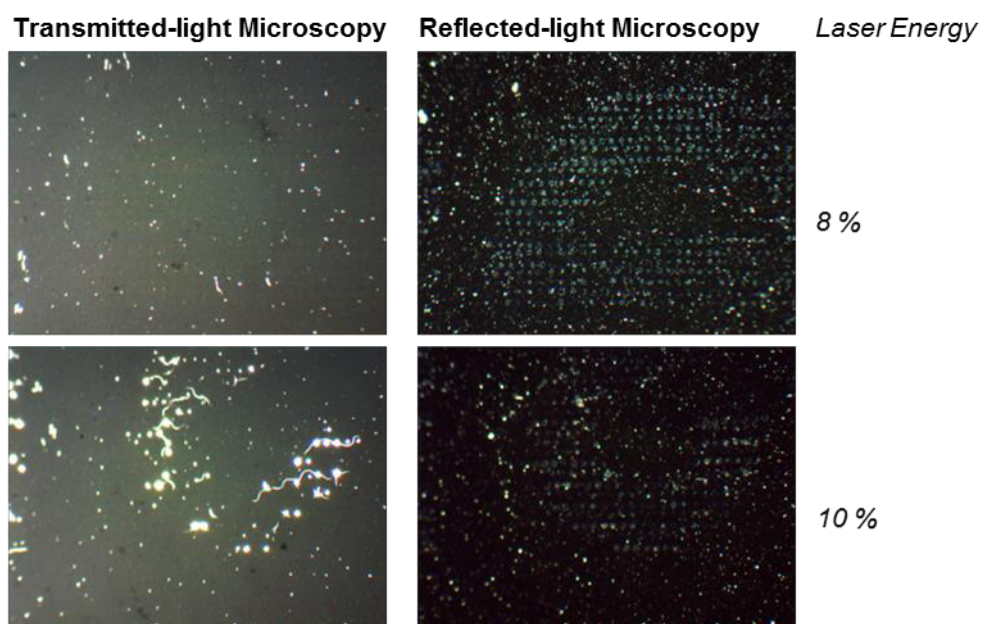


Figure 64 | Effects of laser patterning At 10 % laser energy, the color of the RHS coating was changed visibly (bottom, right). The introduced pattern appeared light blue on a black background in reflected-light microscopy. Transmitted light microscopy revealed that the mirror layer was slightly pierced too. The color change of the RHS coating was not correlated with the damages in the mirror layer. At 8 % laser energy, the mirror layer remained largely intact. Still the laser-treated spots exhibited a different color than the surrounding areas.

After the performance test, one of the samples was stored at RT in open air. Roughly two months after the first test its response to humidity was examined again. The maximum swelling capacity of the untreated areas of the film had decreased slightly. In contrast, the laser-treated areas had apparently regained some swelling capacity. The whole RHS coating exhibited nearly homogeneous swelling again. The pattern that had been introduced by laser irradiation had largely disappeared.

Based on the findings discussed in 5 diffusion processes within the PEM were considered the most probable explanation for the unexpected regeneration of the RHS material. In principle, PEI/ PMVE-MA PEMs can comprise four compounds that which are sufficiently mobile and can affect the swelling behavior of the polymer assembly, namely:

- H₂O
- ethanolamine
- acetic acid
- DMSO

So far, the concentration of these four substances in standard PEI/ PMVE-MA PEMs could not be determined. As described in 5.3.9 standard RHS coatings definitely contain at least small amounts of dH₂O and ethanolamine. Moreover, ethanolamine was found to play a crucial role in the aging of RHS coatings. It apparently acts as a humectant in the PEM and supports water absorption. It appears to gradually evaporate, however, if RHS materials are stored for several months without protective coating. Thus, it was attempted to replace it with non-volatile agents without compromising the sensor properties of PEI/ PMVE-MA PEMs (see e.g. 5.2.9). An ethanolamine-free PEI/ PMVE-MA PEM variant could be developed eventually. (Details on the preparation of this PEM type may not be revealed herein. The technology is currently under consideration for patenting.) However, the swelling capacity of this type of PEM could not be modified at all by laser irradiation. This indicates that standard PEI/ PMVE-MA PEMs are inactivated by thermal treatment, because heat makes ethanolamine evaporate from the assembly. Apparently, the base is crucial for the sensitivity of standard RHSCs. The finding supported the hypothesis discussed in 5.3.9 that ethanolamine evaporation is a major factor in the aging of standard RHS coatings. Unfortunately, it also demonstrated that only standard RHS coatings containing ethanolamine, which currently exhibit limited long-term stability, can be patterned by laser treatment.

6.3.5 Patterning by Chemical Crosslinking

Two chemical routes for passivating RHS coatings were examined as alternatives to laser patterning: carbodiimide- and aldehyde-mediated crosslinking.

1) Carbodiimides are among the most popular reagents for the conjugation of carboxylic acid (-COOH) and primary amino groups (-NH₂). They are so-called zero-length crosslinkers i.e. they mediate the direct conjugation of two chemical groups without becoming part of the crosslink themselves [156]. PEI carries primary amino groups. Carboxyl groups are present on PMVE-MA chains. Hence, it should be possible to connect neighboring PEI and PMVE-MA chains in PEMs by treating them with carbodiimide. The structure of standard PEI/ PMVE-MA PEMs was expected to stiffen upon crosslinking. As a result, their swelling capacity should decline. Water soluble N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide methyl-p-toluenesulfonate (also called Morpho CDI) was selected for respective experiments. Initially, crosslinking protocols were tested that had already been employed successfully for

the conjugation of proteins. Unfortunately, the swelling behavior of the treated samples was not modified significantly in these experiments. It was observed that the PEM layers adopted their most expanded conformation in contact with the aqueous crosslinking solutions. Even if bonds between the layers had formed in this state, they would probably not have prevented PEM expansion. They would rather have interfered with the collapsing of the polymer layers upon drying. DMSO does not elicit swelling (nor collapse) of PEI/ PMVE-MA PEMs of RHS films. Thus, crosslinking tests were repeated with Morpho CDI solutions in DMSO and DMSO/ dH₂O (1:1). Nevertheless, neither swelling behavior nor base color could be modified significantly in these tests. Probably, the employed CDI was too bulky to penetrate the metal cluster layer or invade the multilayer assembly of RHS samples. It is also possible that Morpho CDI could not pass the topmost PEI layer due to its positive charge. As described below, tests with formaldehyde, which were performed in parallel to CDI crosslinking tests, yielded more promising results. Hence, carbodiimides were not further considered as crosslinkers and the difficulties in the above passivation attempts were not investigated in detail.

2) Formaldehyde, CH₂O, is a gas, which rapidly dissolves in water. In aqueous solution, formaldehyde molecules react with H₂O to form methylene hydrate, HO-CH₂-OH [157]. Both, formaldehyde and methylene hydrate, readily react with various functional groups of biological macromolecules including amino, amido and thiol groups. If two groups of this type are situated in close proximity within a substrate, a crosslink – a so-called methylene bridge - can be formed. Formaldehyde rapidly penetrates biological substrates, but bridge formation occurs slowly compared to other fixation reactions (such as CDI- or glutaraldehyde-mediated crosslinking) [158]. In tissue fixation, initial binding of formaldehyde is claimed to be typically completed within 24 h after infiltration. The reaction is reversible, however. To achieve full, stable crosslinking, fixation must be continued for days to weeks [157].

In initial passivation tests, strips of RHS steel plates were treated with aqueous formaldehyde solutions in wet chambers. The swelling capacity of the treated samples was strongly reduced after 1 h of fixation, while their base color was shifted only slightly. Surprisingly, strips of RHS plates that had been stored in the incubation chamber together with the crosslinked samples but had not been treated directly with the formaldehyde solution showed a similar decrease in sensitivity.

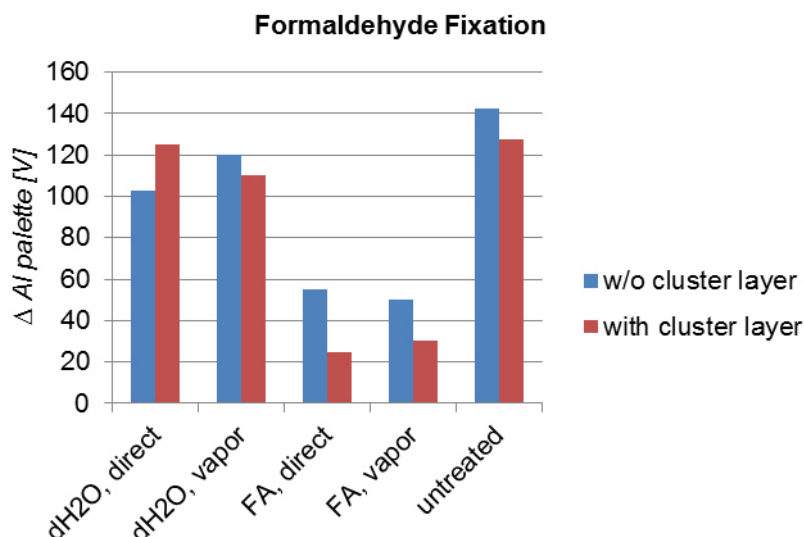


Figure 65 / Formaldehyde fixation of RHS coatings (n=1)

The control experiment summarized in Figure 65 confirmed that gaseous formaldehyde is released from aqueous formaldehyde solution into the atmosphere. If RHSCs are exposed to this vapor, they are inactivated similarly strongly as if treated with formaldehyde solution. In this experiment, strips of RHS steel plates with or without Pd cluster coating were incubated with droplets of dH₂O and formaldehyde solution in separate petri dishes for 1 h. Then the strips without metal cluster layer were coated with a Pd cluster layer by sputtering and the swelling capacity of all samples was analyzed. The swelling capacity of the strips that had been incubated with dH₂O droplets was only slightly decreased compared to untreated reference samples. The formaldehyde-treated strips exhibited significantly lower swelling capacity. Of course, the RHS coating of the strips had been passivated in the areas, onto which the formaldehyde solution droplets had been deposited (“FA, direct”). The surrounding areas, which had not been in direct contact with the solution (“FA, vapor”) were nearly equally inactive, however. Using evaporated formaldehyde for crosslinking turned out to be a convenient strategy for the passivation of RHS materials. Reproducible, fast fixation could be achieved by this approach, while defects resulting from direct water contact i.e. corrosion and water stains (for the latter see 4.3.1) could be avoided. To pattern RHS films or plates with formaldehyde, part of their surface was covered so that it could not interact with the formaldehyde gas in the atmosphere. At ambient humidity, formaldehyde-treated and untreated areas exhibited the same base color (see Figure 66). At elevated humidity, however, the formaldehyde-treated areas could be identified easily by their poor swelling capacity. Stencils made of detachable adhesive film were utilized for masking. Formaldehyde did not diffuse through the masks, so inactivation of the covered areas could be prevented. However, masks usually detached at least partially during the passivation process because of the high humidity in the incubation chamber. Hence, accurate patterns could not be created. Most probably, this problem can be circumvented by utilizing dry formaldehyde gas, however.

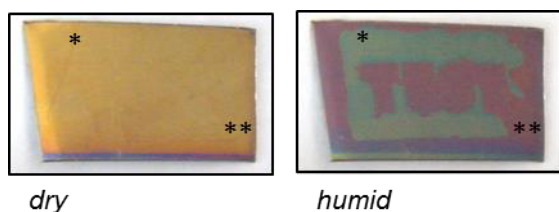


Figure 66 | Patterning of RHS materials with formaldehyde The patterned RHS steel plate is depicted in the dry state (dry) and after maximum swelling (humid) (*... native, **... formaldehyde-crosslinked)

Unfortunately, stress tests revealed that the passivation method described above does not yield stable crosslinks. It was demonstrated that RHS materials with formaldehyde-crosslinked coating regain their swelling capacity if they are incubated in humid atmosphere at 50-60°C for 48 h. (The test was performed as described in 5.2.3.) As mentioned above, it takes days to achieve irreversible crosslinking with formaldehyde. Hence, RHS coatings would probably have to be treated with formaldehyde for one week or more to yield permanent fixation. For this purpose, dry formaldehyde gas would be required. Several days of incubation in highly humid atmosphere would most probably lead to at least weak corrosion of mirror or metal cluster layer of any type of RHS material that can be prepared currently. Dry formaldehyde gas was not available for this doctoral project, however. It was thus tested, whether stable passivation could be obtained by the above protocol if patterned samples were stored at ambient conditions for several day prior to testing, so that the crosslinking process could be completed. For this purpose, a piece of RHS steel plate was partially covered with detachable adhesive tape and passivated with formaldehyde according to the established protocol and incubated at ambient conditions for 12 days. Then it was cut into pieces. Part of the samples was subjected to the 48h-stress test described above. Some were left untreated and served as references. Unfortunately, the same results were obtained as with freshly passivated RHS materials. The passivated strips, which were incubated at elevated temperature in humid atmosphere, regained their swelling capacity completely. Hence, another aldehyde, namely glutaraldehyde, was examined as crosslinking agent for RHSC patterning. Like formaldehyde, it interacted strongly with PEI/ PMVE-MA PEMs, if applied to RHS materials in aqueous solution. If samples were incubated only locally with droplets of glutaraldehyde solution, the surrounding areas were not passivated, however. In contrast to formaldehyde, glutaraldehyde could thus probably be used to print patterns on RHS materials. Unfortunately, the areas passivated with this aldehyde did not show the same base color as the untreated areas but a slightly higher shade. Apparently, PEMs were crosslinked in a slightly swollen state. As described earlier, PEI/ PMVE-MA PEMs do not swell in DMSO. It was thus attempted to prevent PEMs from swelling in contact with glutaraldehyde solution by addition of DMSO to the solution. The tested mixtures are listed in Table 12.

#	25 % (v/v) glutaraldehyde in dH ₂ O [μ l]	dH ₂ O [μ l]	DMSO [μ l]
1	10	20	25
2	10	15	30
3	10	10	35
4	10	5	40
5	10	-	45
6	5	-	45
7	3	-	47
8	1	-	49

Table 12 | Glutaraldehyde solutions in dH₂O/ DMSO

The strategy was not successful, however. Crosslinking only occurred if the water content in the employed glutaraldehyde solution was sufficiently high (at least 13.6 % (v/v), see Figure 67). Then the PEM was crosslinked in a slightly swollen state (solution #5). At lower water content, no passivation occurred (solutions #6-8). Best passivation results were obtained with solutions #4 and 5.

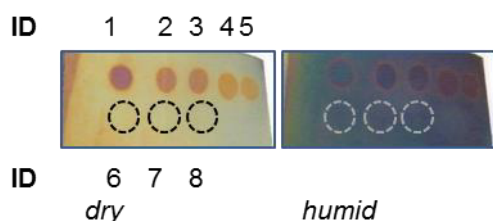


Figure 67 | Glutaraldehyde-mediated crosslinking of RHS steel plate pieces Pieces of RHS steel plate were incubated with solutions of glutaraldehyde in dH₂O/ DMSO with different dH₂O and glutaraldehyde content (see Table 12). The areas that were treated with crosslinking solutions but were not passivated thereby are indicated by circles. The ID of the solution deposited at the individual treated areas is noted above or below the respective area.

6.4 Summary & Outlook

Several methods for the integration of graphic elements in REA coatings were screened. It was found that permanently visible patterns could be generated by a lift-off approach. The desired symbols were painted or stamped onto the substrate with a masking agent. Then the substrate was coated with a ceramic interlayer. In the masked areas, the ceramic deposited on the masking agent and was detached together with it in the mask removal step. If the substrate was coated with a second continuous interlayer subsequently and a metal cluster layer was deposited on top, the previously masked areas exhibited a different resonance color than the surrounding substrate surface. Initially SiO₂ sol-gel coating was used to prepare the interlayer and nitrocellulose solution was employed as masking agent. Unfortunately, nitrocellulose could only be dissolved well in highly volatile and flammable organic solvents (mixtures of acetone and isopropanol or ethylacetate and EtOH isopropanol). The same solvents were required to remove the masks. Using nitrocellulose for industrial production would thus be ecologically problematic and an explosion-protected

printing facility would be required. The same problem would supposedly have been encountered with any other polymer that is sufficiently solvent-resistant to withstand the high EtOH concentration in the precursor solution used for SiO₂ sol-gel coating. For this reason, a method for the preparation of ceramic interlayer coatings in aqueous media was established. By precipitation of TiO₂ from acidic aqueous precursor solution ceramic interlayers of good quality could be created. A compatible masking agent was identified: Eudragit L (Evonik), a methacrylic acid – methyl methacrylate copolymer. It can be dissolved in EtOH or alkaline aqueous solutions, but is insoluble in acidic aqueous media [159]. EtOH-based or alkaline aqueous Eudragit L solutions were successfully employed to create masks on PET substrates. After drying, the substrates were coated with TiO₂ by the precipitation process mentioned above. Then, the masking agent was detached by washing in weakly alkaline solution. The approach was optimized for the patterning of REA PET films and can prospectively be up-scaled for production within a short time. As reported, the application of Eudragit L masks by screen printing has already been tested. Good results were obtained, although the deposited polymer solution foamed slightly, when it was transferred from the printing screen to the substrate. Prospectively, defoaming agents are required to optimize the established masking solution for screen printing. Compatible agents of this type must still be identified.

It was also investigated, whether RHS materials can be provided with graphics that appear only after interaction with water. Two passivation strategies were tested for the purpose, heat treatment and chemical crosslinking. It had been demonstrated earlier that RHS coatings lose their reactivity if exposed to thermal stress. Hence, laser irradiation was tested for RHSC patterning. It was found, that standard RHS coatings can indeed be inactivated locally if irradiated with a CO₂ laser beam of low intensity. Very accurate graphics could be prepared quickly and easily with this method. Unfortunately, stability tests described in detail in 5.3.9 suggested that RHSCs could only be passivated by heating because ethanolamine evaporates from the RHSC at elevated temperature. The weak base is present in the polycation solution and the washing solution used for preparing the PEI layers of standard RHSCs. It is apparently integrated into the PEM during its assembly. Obviously, the sensitivity of standard RHSCs decreases if ethanolamine is extracted. Ethanolamine also seems to evaporate gradually if RHS materials are stored at ambient conditions. As discussed in 5.3.9 this appears to be the main reason for RHSC aging. Thus, ethanolamine will prospectively be replaced with non-volatile agents that can take over its tasks in PEM assembly and stabilization. In this case, the established laser patterning process would not work anymore. For applications, in which long-term stability is not essential but graphics have to be integrated in the coating, RHS materials prepared with ethanolamine would be acceptable, however. Then laser patterning could be employed. Two variants of chemical crosslinking were also tested successfully for the selective passivation of RHS coatings: formaldehyde and glutaraldehyde-mediated crosslinking. Unfortunately, glutaraldehyde-passivated RHSCs were always arrested in a slightly swollen state, because crosslinking only occurred if the glutaraldehyde solution contained a certain amount of water. Standard RHSCs could not be crosslinked with glutaraldehyde solutions of a water concentration

below 13.6 % (v/v). In contrast, RHSCs could be inactivated very efficiently with formaldehyde gas and the base color of the treated areas was not changed. The areas, which should not be treated, were masked e.g. with an adhesive film. The approach is not as flexible and as fast as laser patterning, but might also work for RHS coatings that do not contain ethanolamine. (This still remains to be tested.) Formaldehyde-mediated passivation is not completely irreversible, however. If stored at elevated temperature and high humidity for two days, formaldehyde-passivated areas regained their swelling capacity. RHS materials are not supposed to be employed at such conditions. Hence, formaldehyde crosslinking is still considered a potentially feasible approach for generating graphics in RHS coatings that only appear at elevated humidity. If formaldehyde patterned RHS films were stored at ambient conditions and only transiently exposed to high humidity, their passivated areas were never observed to be reactivated. Nevertheless, the stability of formaldehyde crosslinking should be characterized better, before it is attempted to scale up this patterning method.

7 Wet-chemical Deposition of Metal Cluster Layers on RHS Materials

7.1 Introduction

For smart packaging applications, RHS films are converted to labels and attached onto products in this form. However, such labels cannot be handled in all production lines. This motivated the development of RHS pigments that may be processed via standard printing technologies such as screen or laser printing. Glass micro-platelets and steel pigments were selected as carriers for such RHS pigments. Processes employed for the fabrication of the individual layers of RHSCs were adapted to the new type of substrate. At the start of this doctoral project, all components of REA and RHS coatings could be deposited at fairly good quality via wet-chemical processes except for the top metal cluster layer. On large planar substrates such as polymer films this layer is commonly prepared by sputtering or high-vacuum evaporation coating. Sputter deposition is less directional than evaporation coating. Nevertheless, 3D-objects must be rotated in both techniques so that their surface is coated homogeneously. This is feasible for large substrates. Particulate materials, however, may not be agitated in PVD systems in a way that allows for the deposition of a uniform cluster layer on the entire surface of each particle. At the start of this doctoral project, RHS particles could only be attached to the desired substrate and sputter-coated afterwards. Then intense resonance colors were obtained (see Figure 68).

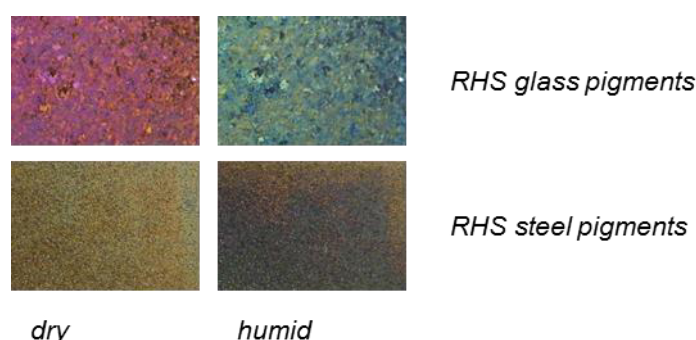


Figure 68 | RHS pigments sputter-coated with Pd

When depositing metal clusters from solution, substrate geometry is not as critical as in gas phase deposition. However, cluster properties are far more difficult to control. As discussed in 2.3.2.1 and 2.3.2.2, shape, particle size and size distribution, composition, refractive index and density of the deposited nanoparticles govern their interaction with incident light. Their scattering behavior is most decisive for the optical quality of the REA structures they are deposited on. Ideally, the metal cluster layer of a REA coating mainly exhibits specular reflection and direct transmission i.e. incident light is reflected and transmitted in one direction only (see Figure 69). Pronounced scattering at the NP layer results in diffuse reflection and transmission and quenches the visibility of the REA effect. Resonance colors appear dull and whitish then.

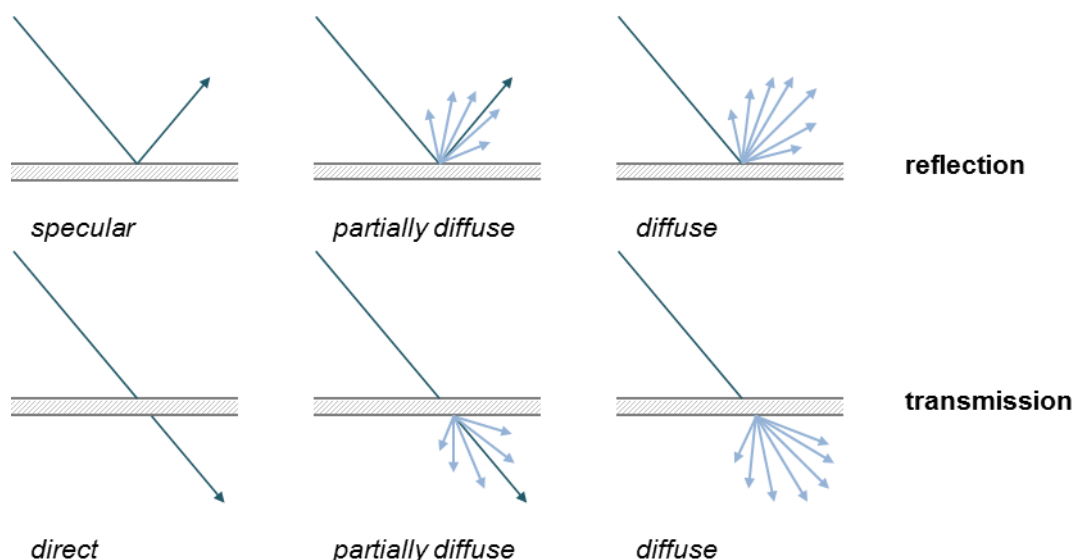


Figure 69 | Scattering in reflection and transmission (based on [160, 161])

It has been observed that the ratio of diffuse to specular reflection in REA coatings strongly depends on the diameters of the metal clusters in the cluster layer. Above a certain size limit (e.g. ~ 70 nm for Ag particles or ~ 40 nm for Au particles) scattering predominates and impairs the optical quality of the resonance structure. The light absorption behavior of the cluster layer is similarly important for the appearance of REA structures for it determines the palette of interference colors that may be generated (see 3.1). Like the scattering behavior it is determined by the cluster size, but also by cluster shape and composition (see 2.3.2). As described, REA is strongest at the absorption maximum of the metal cluster layer. Sputter-deposited Pd layers show nearly constant absorption over the whole visible range (see Figure 70A). Therefore they give rise to a broad range of equally strong REA colors as depicted in Figure 70B. Sputter-deposited Ag cluster layers primarily absorb in the green and the red range of the visible spectrum but only weakly in the blue range (see Figure 70A). Thus, they generate intense shades of blue and violet. Yellow and pink REA colors are also accessible with sputtered Ag clusters, but they appear significantly paler (see Figure 70B).

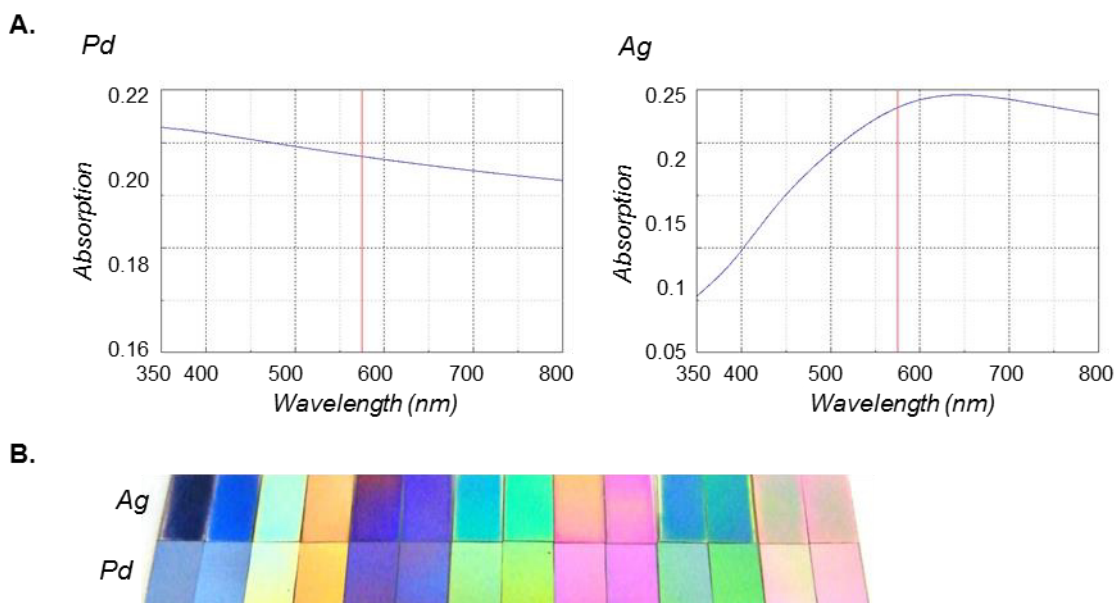


Figure 70 | Sputtered metal cluster layers **A)** UV/Vis absorption spectra of sputter-deposited Pd (left) and Ag (right) metal cluster layers **B)** REA color palette on aluminized PET film with sputtered Pd (bottom) and Ag (top) cluster layer (interlayer thickness increasing from left to right)

Thus, it was supposed that clusters deposited from solution also need to exhibit broad-band absorption or at least multiple LSP resonance (LSPR) peaks to perform well in REA coatings. Otherwise, they might not give rise to all colors of the REA palette. As explained in 2.3.2.2, spectra of small spherical metal particles comprise only one LSPR peak. In contrast, large particles exhibit higher-order resonances and therefore multiple peaks. Hence, they were expected to be more useful for REA unless showing too strong scattering due to their size.

Within this doctoral project, three strategies for the synthesis and deposition of metal nanoparticles on REA pigments were screened (see also Figure 71):

1. *Electroless in-situ deposition of clusters (w/o seeding)*: A layer of nanoparticles is generated directly on top of the interlayer by chemical reduction of precursors.
2. *Electroless in-situ deposition with seeding*: The interlayer is coated with pre-formed small catalytic nanoparticles by adsorption. Then the actual metal cluster layer is deposited from solution. The NP seeding was expected to allow more targeted deposition of the metal clusters and better control of their shape and distribution.
3. *Adsorption of nanoparticles from dispersion*: Nanoparticles are prepared in a preliminary step and deposited on the interlayer via adsorption. Interaction of synthesis reagents with components of the interlayer – especially the humidity-sensitive polymer multilayer – is prevented thereby.

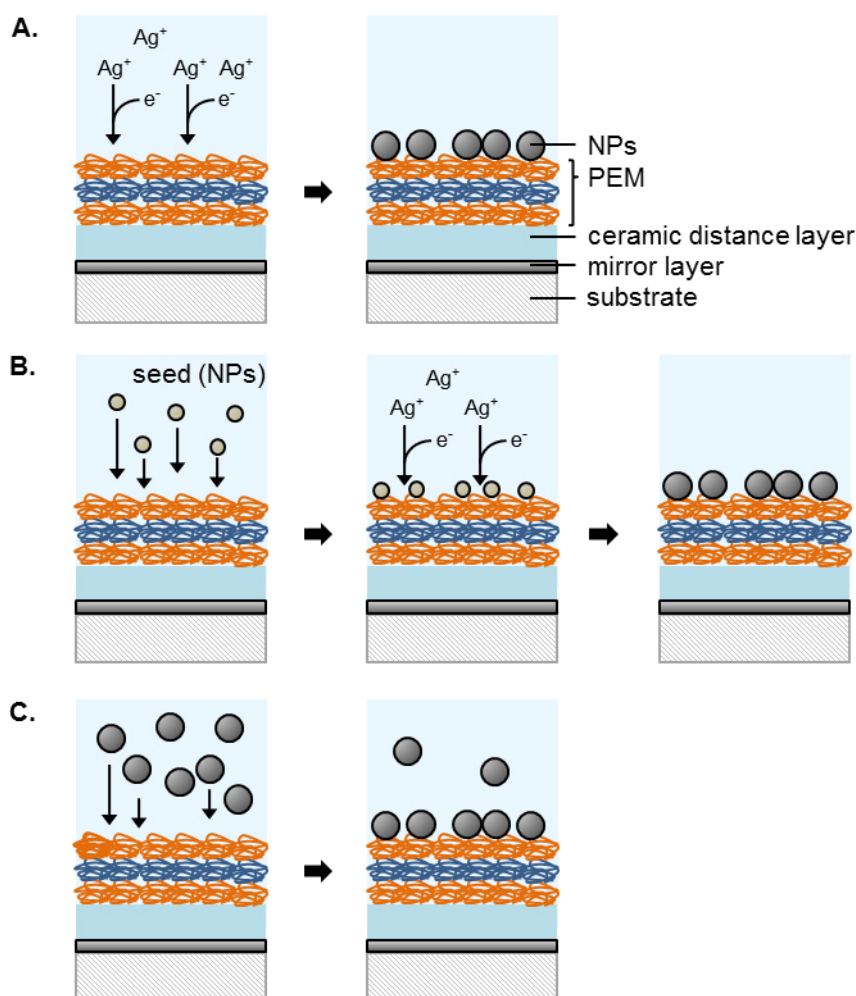


Figure 71 | Strategies for wet-chemical deposition of metal cluster layers **A)** Electroless in-situ deposition of clusters (w/o seeding) **B)** Electroless in-situ deposition of clusters with seeding **C)** Adsorption of nanoparticles from dispersion

7.2 Materials & Methods

7.2.1 Direct Electroless Deposition of Metal Clusters

Protocol DD1

Solution A

- 340 μl palladium diammine sulfite ($\text{Pd}(\text{NH}_3)_2\text{SO}_3$) solution in dH_2O (5 g palladium/ 100 ml) (ÖGUSSA)
- 500 μl 30 % (v/v) sulfuric acid (H_2SO_4) (Carl Roth, # X876) in dH_2O
- 1660 μl dH_2O
- 2500 μl DMSO (Bussetti)
- heated to 60°C under stirring until Pd salt completely dissolved
- 1 μl 5 % (w/v) poly(sodium 4-styrenesulfonate) (PSS) (1 MDa) (Sigma Aldrich, #527491) in dH_2O
- mixed
- + ~1.5ml RHS pigments w/o metal cluster coating (wetted with

DMSO/ dH₂O (3:2)) (glass-based, average diameter: 600 µm)
 + 1 ml 2.5 % (w/v) sodium hypophosphite monohydrate (NaH₂PO₂ · H₂O) (Sigma-Aldrich, # 30410) in dH₂O
 → vortexed
 → mixed by end-to-end rotation for 20 min

Protocol DD2

Solution A

15 mg palladium(II) acetate (98 %) (Pd(OCOCH₃)₂) (Sigma-Aldrich, # 20,586-9)
 1 ml DMSO

Solution B

9 ml ethylene glycol (>99 %) (Carl Roth, # 9516)
 +25 mg NaH₂PO₂ · H₂O
 + ~1.5ml RHS pigments w/o metal cluster coating (wetted with ethylene glycol) (glass, average diameter: 600 µm)

→ solution A injected

→ stirring for 1 h at RT

Functional metal cluster layers could be generated on RHS glass pigments with both protocols, DD1 and DD2. The swelling capacity of the underlying polymer multilayer coating was not impaired.

7.2.2 Ag Enhancement

Enhancement solution

10 µl 4.5 % (w/v) silver nitrate (AgNO₃) (ÖGUSSA) in dH₂O
 350 µl 15 mM citrate buffer, pH~4.1 (1 part 30 mM sodium citrate dihydrate (HOC(COONa)(CH₂COONa)₂ · 2H₂O) (Riedel-de Haën) + 1 part 30 mM citric acid ((HOOCCH₂)₂C(OH)COOH) (Merck) + 18 parts dH₂O)
 75 µl 2.5 % (w/v) hydroquinone (C₆H₄-1,4-(OH)₂) (Sigma-Aldrich, #H9003) in dH₂O
 → The substrate (wet (dH₂O) or dry) was incubated with/ in the solution until the desired density of Ag deposit was reached. Then the substrate was rinsed with dH₂O & dried with pressurized air (films) or in the oven at 50°C (particulate substrates e.g. pigments).

7.2.3 Preparation of Pd Seed with Stabilizer & Ag Enhancement

Protocol PNPSEED1.1

680 μ l palladium diammine sulfite ($\text{Pd}(\text{NH}_3)_2\text{SO}_3$) solution in dH_2O
 (5 g palladium/ 100 ml) (ÖGUSSA)
4000 μ l DMSO (Bussetti)
400 μ l 30 % (v/v) H_2SO_4 in dH_2O
→ heated to 60°C under stirring until Pd salt completely dissolved
+ 2 μ l 5 % (w/v) PSS (1 MDa) in dH_2O
→ mixed
+ 200 μ l 5 % (w/v) $\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$ in dH_2O
→ heated (60°C) under stirring for 2 h

100 μ l of the NP preparation were pipetted onto a strip of standard PEI/ PMVE-MA RHS film without cluster layer in a wet chamber. The film was incubated for 15 min. Then it was washed with water. The treated spot on the film exhibited a moderately dense cluster layer. Upon Ag enhancement (1 min 40 sec) (see 7.2.2) the spot adopted a strong partially scattering resonance color.

Protocol PNPSEED1.2

680 μ l $\text{Pd}(\text{NH}_3)_2\text{SO}_3$ solution in dH_2O (5 g palladium/ 100 ml)
 (ÖGUSSA)
1600 μ l 30 % (v/v) H_2SO_4 in dH_2O
→ volume adjusted to 2.5 ml with dH_2O
+ 2.5 ml DMSO
→ partial precipitation of palladium salt
→ stirred and heated to 50-60°C until precipitate completely dissolved
→ The further solutions were added under stirring:
5 μ l 5 % (w/v) poly(sodium 4-styrenesulfonate) (1 MDa) (Sigma
 Aldrich, #527491) in dH_2O
200 μ l 5 % (w/v) $\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$ in dH_2O (fresh)

→ The reduction reaction started within a few seconds after addition of the hypophosphite solution and occurred within about 2 min.
→ Stirring was continued for further 30 min.

The resulting dispersion was stable. The particles could not be pelleted even at 16200 g in a table-top centrifuge.

An aluminized PET-film with incomplete RHS coating (ceramic distance layer + 5 alternating layers of PEI/ PMVE-MA) was incubated in the dispersion for 10 min. Ag enhancement of the

adsorbed cluster layer was performed according to the standard protocol (see 7.2.2). The film exhibited a strong resonance color and good swelling capacity.

7.2.4 Preparation and Adsorption of Pd Seed in Organic Solution & Electroless Pd Deposition

Protocol PDNPSEED2

Solution A

10 mg $\text{Pd}(\text{OCOCH}_3)_2$
1 ml diethylene glycol monoethylether (Fluka, #32230)
→ vortexed for 10 min
→ heated to 60°C and incubated under stirring until the palladium salt was completely dissolved

Solution B

500 mg $\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$
49 ml ethylene glycol

→ Solution A was added to solution B under stirring.
→ Pd nanoparticle (PdNP) formation occurred within 5 min.

After 24 h of storage at RT, the as prepared NPs started to precipitate.

1 ml Ag-coated glass pigments (average diameter 600 μm , thickness: 4 μm) with incomplete RHS coating (ceramic distance layer + 5 alternating layers of PEI/ PMVE-MA) were incubated in the NP dispersion PDNPSEED2 under stirring for 10-15 min within 24 h after preparation. Then the pigments were washed with dH₂O and the adsorbed Pd cluster layer was enhanced as follows:

Solution A

120 μl 12.75 % tetrammine palladium (II) sulphate ($[\text{Pd}(\text{NH}_3)_4]\text{SO}_4$) (Johnson Matthey) in dH₂O
250 μl DMSO
→ volume adjusted to 2 ml with dH₂O

Solution B

174 mg ethylenediaminetetraacetic acid (EDTA) (Aldrich, #E26282)
200 μl 25 % NH_4OH in H_2O (Carl Roth, #2610)
→ volume adjusted to 7 ml with dH₂O
→ Solution A was added to solution B.
→ pH-value adjusted to pH 8.4-8.5 with 30 % H_2SO_4 in H_2O

Solution C

170 mg	$\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$
1 ml	dH_2O

→ Glass pigments coated with PdNP seed layer were stirred in solution B for 5 min. Then solution C was added to the solution and stirring was continued for 10 min.

→ After about 1-2 min a color change was noticed on the pigments. After 5-6 min release of gas was observed. After 10 min the pigments were washed (although the reaction might have continued) and dried.

The as-coated pigments exhibited a strong resonance color and good swelling capacity.

7.2.5 Deposition of AuNPs to PEI-coated REA Film by Adsorption

Two polymer coating solutions were prepared:

Solution A: 1 % (w/v) PEI (1.6 MDa) (Polymin P special, BASF) in dH_2O (pH = 10,3)

Solution B: 1 % (w/v) PEI (1.6 MDa) (Polymin P special, 1.6 MDa, BASF) in dH_2O (The pH-value of the solution was adjusted to pH = 7.5 by addition of acetic acid (100 %) (Merck).)

A piece of aluminized PET film (10 x 16 cm) was sol-gel coated with SiO_2 four times as described in 4.2.1 (4 x 5.5 ml TEOS/ 5 ml EtOH). Two pieces (1.3 x 1.2 cm each) were cut off this film (= sample #1 and 2). Both were transferred to a wet chamber. Sample #1 was covered with solution A (~150 μl) and sample #2 with solution B (~150 μl). The two pieces were incubated in the wet chamber at RT for 20 min. Then they were rinsed with dH_2O and dried with pressurized air using a blow-off gun. The samples were transferred back into the wet chamber and a droplet of gold nanoparticle (AuNP) solution (~30 μl) (OD = 30, average particle diameter: 40-47 nm, coated with anionic IgG³) was deposited onto each sample. The samples were incubated in the wet chamber at RT for 1 h. Then they were rinsed again with dH_2O and dried with pressurized air using a blow-off gun.

7.2.6 Synthesis of Nanoparticles for Adsorption

Details on the following synthesis protocols may not be revealed, because they are currently under consideration for patenting.

³ provided as a gift, the supplier may not be revealed

Pd Nanoparticles (PdNPs#1)

PdNPs were prepared from tetrammine palladium (II) sulphate (Johnson Matthey) in aqueous solution. Ascorbic acid (APONORM) was employed as reducer. Poly(4-styrenesulfonic acid-co-maleic acid) sodium salt (PSS-MA) (Sigma-Aldrich) and low molecular weight⁴ PVP (Sigma-Aldrich) served as stabilizers.

Ag Nanoparticles (blue) (AgNPs#1)

Blue AgNPs were prepared from AgNO₃ (ÖGUSSA) in ethyl diglycol (EDG) (Bussetti), which also served as reducer. Low molecular weight PVP (Sigma-Aldrich) was added as reducer and stabilizer.

Ag Nanoparticles (orange) (AgNPs#2)

Orange AgNPs were prepared from AgNO₃ (ÖGUSSA) in EDG (Bussetti). The solvent also served as reducer. Low molecular weight PVP (Sigma-Aldrich) was added as reducer and stabilizer.

Ag Nanoparticles (yellow) (AgNPs#3)

Yellow AgNPs were prepared from AgNO₃ (ÖGUSSA) in ethylene glycol (EG) (Carl Roth). N,N-diethylhydroxylamine (Sigma-Aldrich, #471593) was employed as reducer. Low molecular weight PVP (Sigma-Aldrich) was added as stabilizer.

7.2.7 Deposition of Ag and Pd Nanoparticles by Adsorption

Steel or glass based RHS pigments without metal cluster layer were stored in dH₂O or DMSO/dH₂O after PEM deposition. For cluster adsorption, the pigments were separated from the solvent by filtration using a suitable sieve or filter paper. If required, they were rinsed with the solvent of the nanoparticle dispersion they should be treated with. Then they were mixed with the respective dispersion. The concentration of pigments in the cluster solution was kept low enough, so that the pigments floated freely in the dispersion when stirred. The mixture was stirred at 300-600 rpm for 1-2 h. If all clusters in the dispersion were adsorbed to the RHS pigments but a sufficiently dense cluster coating was not obtained, the pigments were filtrated and transferred to a fresh aliquot of the cluster dispersion. As soon as the cluster coating was dense enough, the pigments were filtrated, rinsed with dH₂O and dried in the oven at 50-60°C.

⁴ classification of polymers by molecular weight according to [162]

7.2.8 Preparation of REA Color Palettes

The REA color palette strips were prepared in a similar process as the REA color palette employed in chapter 5 (see 5.2.1). Stripes of aluminum oxide with increasing thickness (stripe width: 3-5 mm) were generated on an aluminum plate (20 x 8 cm, 0.5 mm thickness, Glattblech natur, OBI) by anodizing in a boric acid electrolyte (pH 7.0). For this purpose, the lower 10.7 cm of the aluminum plate were immersed in the electrolyte and a voltage of 5 V was applied for 5 min. Then the plate was lifted out of the electrolyte by ~5 mm and the part, which remained in the electrolyte, was anodized at 10 V. The plate was repeatedly lifted by 3-5 mm and anodized at the following voltages: 5, 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300 and 320 V. After completion of the last anodizing step, the plate was removed from the electrolyte. At some places it was covered with deposits of boric acid and foamed aluminum oxide, which had formed on the aluminum plate during anodizing at the interface of electrolyte and air. The deposits were wiped off with a soft wet towel. Then the plate was rinsed with EtOH and dH₂O and dried with pressurized air. Subsequently, the plate was coated with SiO₂ (100 µl TEOS/ 5 ml EtOH), a silane layer and five-layer assembly of PEI and PMVE-MA (starting with PEI) as described in 4.2.1 (competitive approach). Then the plate was cut in strips of 5-10 mm and a metal cluster layer was deposited on each strip. This was done either by sputtering (Ag or Pd, see section 4.2.5 for sputtering parameters and instrument) or by adsorption of nanoparticles (Pd or Ag) (synthesis see 7.2.6). For nanoparticle adsorption, one of the aluminum strips was incubated in the respective colloidal solution at RT, either in a wet chamber without motion for 1 h or under shaking for 2.5 h. (Both absorption strategies yielded equivalently dense nanoparticle coatings.) Then the strip was rinsed with dH₂O briefly and dried with pressurized air.

7.2.9 Preparation of Samples for AFM Imaging & UV/Vis Spectroscopy

Glass slide pieces (1.9 x 1.3 cm, prepared from standard microscope slides (Carl Roth, #0656)) with sputter-deposited Cr mirror coating (sputter coater built by Attophotonics: 1.5 kW power supply, target size 125 x 300 mm; sputter conditions: 420 V, 749 W, 2.5×10^{-2} mbar, 1 min; sputter gas: Argon 5.0) were used as substrates for AFM imaging. They were coated with a five-layer PEI/ PMVE-MA PEM as described in 4.2.1 (competitive approach). Then the NP preparation that should be characterized, was adsorbed. For this purpose, a chip was incubated in the respective colloidal solution (pure or diluted) at RT, either in a wet chamber without motion for 1h or under shaking for 2.5 h. (Both absorption strategies yielded equivalently dense nanoparticle coatings.) Four chips were incubated in the four pure nanoparticle dispersions PdNPs#1, AgNPs#1, AgNPs#2 and AgNPs#3 (one chip per solution). Four chips were incubated in the following dilutions: PdNP#1 (1:10) in dH₂O, AgNP#1 (1:100) in EDG, AgNP#2 (1:100) in EDG, AgNP#3 (1:100) in EG. After nanoparticle adsorption, the glass chips were rinsed with dH₂O and dried with pressurized air.

Smaller glass slide pieces (2.7 x 2.5 cm, prepared from standard microscope slides (Carl Roth, #0656)) were used as substrates for UV/Vis spectroscopy. The pieces were coated

with a five-layer PEI/ PMVE-MA PEM and the nanoparticle types PdNPs#1, AgNPs#1, AgNPs#2 and AgNPs#3 were adsorbed from the pure dispersions as explained above.

To determine the UV/Vis absorption spectrum of the investigated nanoparticle dispersions in solution, they were diluted as follows: PdNP#1 (1:10) in dH₂O, AgNP#1 (1:100) in EDG, AgNP#2 (1:100) in EDG, AgNP#3 (1:100) in EG.

7.2.10 UV/Vis Spectroscopy

UV/Vis spectra were measured using a JASCO V-530 UV/Vis two-channel spectrophotometer (settings: band width: 2.0 nm, response: medium, measurement range: 800-350 nm, data pitch: 0.5 nm, scanning speed: 400 nm/min). All nanoparticle dispersion samples were measured against the corresponding pure solvents (dH₂O, EDG or EG). Cluster-coated glass slides were measured against bare glass slides.

7.2.11 AFM Imaging

For AFM imaging a table-top AFM of the type TT-AFM (AFM Workshop) was used (parameters: scan setup: scan rate: 0.5 Hz, scan lines: 256, scan size: 2.5 μ m, samples/pixel: 20, rotation: 0; z-feedback: gain: 1, proportional: 100, integral: 1000, derivative: 0). Cantilevers ACLA (AppNano) were utilized. All measurements were performed in the tapping mode.

7.2.12 Substrates & Reference Samples

For metal cluster deposition tests, films or particles with incomplete RHSC were used. The coating generally encompassed mirror layer, ceramic distance layer and five to seven polymer monolayers but no metal cluster layer. A sample of each RHS substrate that was employed in the screening was coated with a palladium cluster layer by sputtering to determine the attainable resonance color. The maximum swelling capacity of these reference samples was analyzed as well and the results were used as reference values for cluster deposition tests. Before sputtering RHS pigments, they were frequently attached to a glass slide with double-sided tape, so that they would not move in the sputtering chamber.

Comparing base color and swelling capacity to color palettes was not conclusive in early cluster deposition experiments, for the quality of RHS materials varied strongly. The investigations described in chapters 4 and 5 had not been performed yet. Thus, old polymer deposition solutions were occasionally utilized for PEM assembly and old RHS pigments and films (prepared several weeks or months before cluster deposition tests) were used as substrates for cluster deposition. The quality of wet-chemically deposited cluster layers was therefore only assessed qualitatively by comparing the respective samples to the corresponding sputter-coated reference samples. In the following, cluster layers are therefore frequently described rather imprecisely as having been very sparse, moderately dense and very dense i.e. equivalently dense as or denser than sputter-deposited layers. For better

understanding, these categories are illustrated in Figure 72. Similarly, the swelling capacity of RHS coatings with wet-chemically deposited cluster layer is often simply classified as “equivalent to” or “lower than” the swelling capacity of the RHS coating of reference samples.

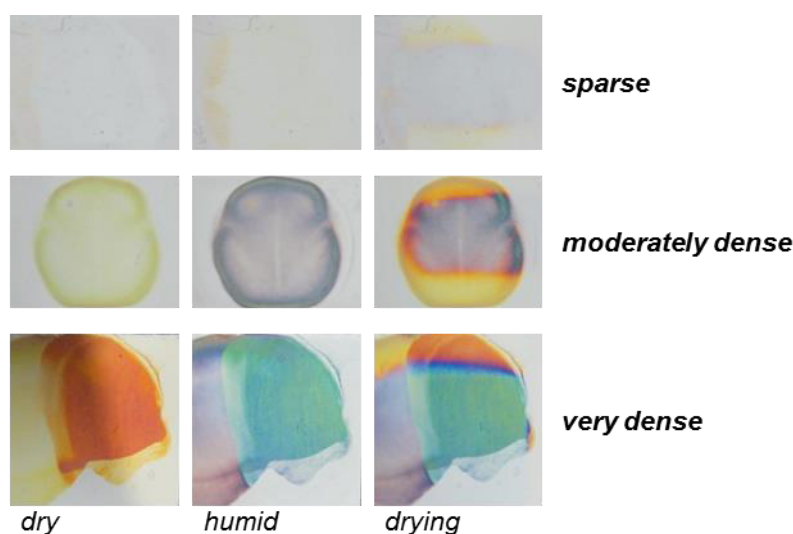


Figure 72 | Categories of cluster density The depicted samples were prepared from a standard RHS film without metal cluster layer. Three types of metal nanoparticles were adsorbed to pieces of the film. (dry... REA color at ambient humidity, humid... REA color at condensing humidity, drying... color shift during drying)

7.3 Results & Discussion

7.3.1 Electroless In-situ Deposition of Clusters w/o & with Seeding

Initially, it was attempted to directly generate metal clusters on RHS materials in solution. Two protocols for electroless Pd reduction were tested for the purpose (see 7.2.1). First, palladium diammine sulfite was utilized as precursor (protocol DD1). Sodium hypophosphite was employed as reducing agent. The deposition reaction was performed in an aqueous dilution of DMSO containing the anionic polymer poly(4-styrene sulfonic acid) (PSS). DMSO served as stabilizer for the growing Pd clusters. The polyanion should adsorb to the generated nanoparticles and mediate their binding to the topmost PEI layer of standard RHS coatings. RHS pigments without metal cluster layer were used as substrates for deposition tests. After the coating procedure, base color and reactivity of the samples were compared to the sensor properties of RHS pigments of the same batch coated with sputter-deposited Pd cluster layer. In the first test, a dense cluster layer formed on the sample pigments. Their swelling behavior was equivalent to that of the corresponding sputter-coated reference pigments. The result was not reproducible, however. The density of as-generated cluster layers varied strongly from experiment to experiment. Once, the cluster layer was so sparse that the substrate barely exhibited a resonance color. In another test, a very dense layer was obtained, but it suppressed the swelling capacity of the underlying PEM. Moreover, the reaction was rather inefficient. Shortly after addition of the reducer, the deposition solution started to darken indicating that nanoparticles and particle aggregates formed in solution. Only a fraction of the reduced palladium was actually deposited on the substrate. Similar

results were obtained with coating protocol DD2 using palladium (II) acetate as precursor. It could be reduced well by sodium hypophosphite and ethylene glycol. Part of the resulting Pd⁰ atoms were captured on the RHS pigments in the solution, but also a large amount of free Pd nanoparticles (PdNPs) formed. The coating procedure was tested several times using different glass pigment batches with equivalent RHS coating (Ag mirror layer/ SiO₂ layer/ 5-layer PEI/ PMVE-MA assembly). Unfortunately, cluster formation was as difficult to control as in tests with protocol DD1. Repeatedly, dense cluster layers were obtained without compromising the sensitivity of the coated RHS substrate. In some tests, however, nearly no clusters grew on the substrate surface.

It was hypothesized that cluster formation could be targeted better to the PEM surface by seeding the topmost polymer layer with catalytic nanoparticles. Cluster layers with good optical properties had been obtained earlier by seeding incomplete RHS films with a sparse sputter-deposited Pd cluster layer and enlargement the clusters by Ag enhancement (see 7.2.2). In this process, the sputter-deposited seed acts as catalyst for hydroquinone-mediated electroless reduction of silver nitrate. The sensor properties of as-completed RHS coatings were compromised only slightly by the enhancement reaction (see Figure 73).

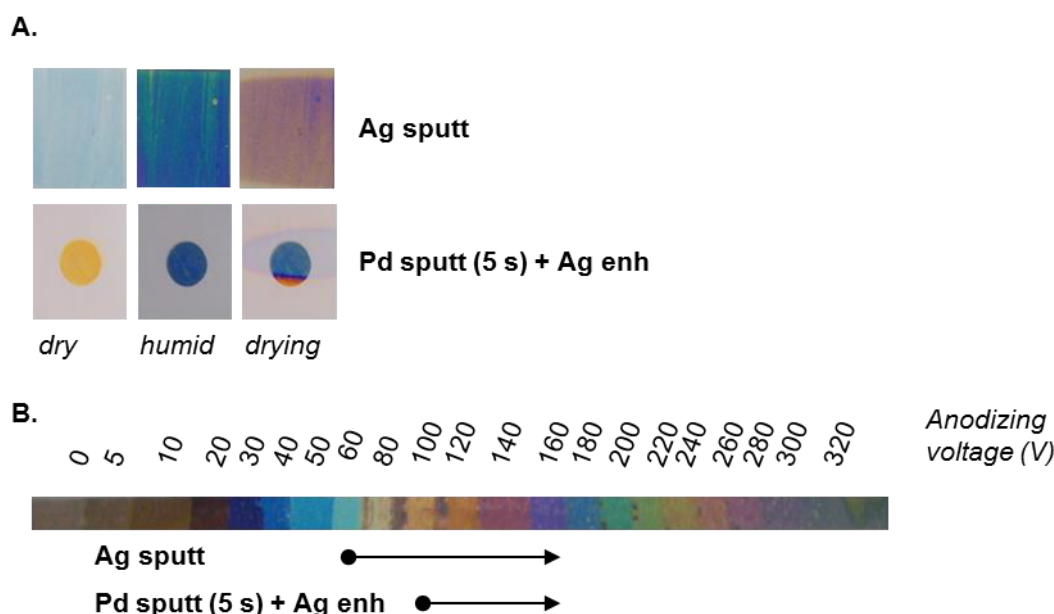


Figure 73 | Ag enhancement of sputter-deposited Pd clusters on RHS film **A)** Two strips were cut off a standard RHS film w/o cluster layer. One strip was sputter-coated with a Ag cluster layer (Ag sputt). The other strip was only sputter-coated briefly (5 s) with Pd. Then the Pd clusters were enlarged by Ag enhancement (15 s) (Pd sputt (5 s) + Ag enh) (dry... REA color at ambient humidity, humid... REA color at condensing humidity, drying... color shift during drying) **B)** The Al₂O₃ interlayer of the depicted Al-based REA color palette was prepared like that of the Al-based REA color palette described earlier. Then the palette was coated with a Ag cluster layer by sputtering. Arrows below the Al-based REA color palette indicate the color range, over which the color of the two depicted RHS film types and shifted at elevated humidity.

Ag enhancement of sputter-deposited Pd clusters on RHS film **A)** Two strips were cut off a standard RHS film w/o cluster layer. One strip was sputter-coated with a Ag cluster layer (Ag sputt). The other strip was only sputter-coated briefly (5 s) with Pd. Then the Pd clusters

were enlarged by Ag enhancement (15 s) (Pd sputt (5s) + Ag enh) (dry... REA color at ambient humidity, humid... REA color at condensing humidity, drying... color shift during drying) B) The Al_2O_3 interlayer of the depicted Al-based REA color palette was prepared like that of the Al-based REA color palette described earlier. Then the palette was coated with a Ag cluster layer by sputtering. Arrows below the Al-based REA color palette indicate the color range, over which the color of the two depicted RHS film types and shifted at elevated humidity.

As mentioned earlier, homogeneous metal layers cannot be deposited on particulate substrates by sputtering. Therefore, adsorption of pre-synthesized clusters from solution was tested as alternative seeding strategy. A variety of protocols was tested for the purpose, but not all of them will be discussed in detail here. Most promising seed dispersions were prepared by variants of deposition protocol DD1. First, the reduction reaction was performed without stabilizer (see 7.2.3, protocol PDNPSEED1.1). The resulting PnNPs adsorbed at moderate density to standard PEI/ PMVE-MA PEMs, but could be enhanced efficiently by electroless Ag reduction. The nanoparticle dispersion was instable, however. Aggregates formed within 20 min after preparation. As a result, nanoparticles adsorbed and partially precipitated on substrates in an uncontrolled fashion. To stabilize the generated nanoparticles and make them bind at higher density, poly(4-styrene sulfonic acid) was added to the preparation. In addition, the ratio of DMSO and dH_2O and the reducer concentration in the precursor solution were varied. It was found that all three factors strongly determined the stability of the dispersion and the adsorption behavior of the generated clusters. Highly stable NP preparations usually adsorbed at very low density to RHS substrates or did not bind at all. Instable dispersions aggregated shortly after preparation. Eventually, the preparation protocol was optimized by trial and error and stable seeds could be prepared from palladium diammine sulfite as described in 7.2.3 (protocol PDNPSEED 1.2). Tests with several other reducers, precursors and stabilizers failed, however. Frequently, stabilizers suppressed the catalytic activity of synthesized clusters. Moreover, unbound stabilizers often competed with the nanoparticles in the seeding process and prevented cluster binding. To remove excess stabilizers from such nanoparticle preparations, the particles were generally pelleted by centrifugation, the supernatant was discarded and the clusters were resuspended in fresh solvent. Of course, this method of purification is time-consuming. Moreover, it often impaired the quality of the nanoparticle dispersion. As described in section 2.3.3.5, nanoparticles prepared by electroless reduction are not monosized but exhibit a certain size distribution. Dispersions of particles with large size distribution usually had to be centrifuged at high speed (16200 g) in order to pellet all particles including the very small ones. Small clusters generally exhibit higher catalytic activity than larger particles and are thus valuable seeds (see 2.3.2.1). However, the large particles in the centrifuged dispersions were strongly compacted and aggregated if pelleted at high speed. As a result, pellets could not be resuspended completely after replacement of the solvent. To be able to avoid any purification steps, it was attempted to prepare nanoparticles in organic media without stabilizers. Indeed, some of the obtained dispersions proved highly efficient for seeding and could even be enhanced by electroless Pd reduction. One of the respective synthesis and enhancement

protocols are provided in 7.2.4. A drawback of the organic synthesis approach was that the preparations were only metastable and started to precipitate within 24h after preparation. Hence, this type of seed had to be synthesized directly before use.

Depositing cluster layers by electroless reduction using the described seeds and enhancement protocols was tested on several RHS pigment samples. In some cases, the strategy was successful, but overall it did not prove more reproducible than the direct approach. Eventually, the ratio between metal precursor and substrate surface was identified as one determining factor. If this ratio was adjusted well, cluster layers with good optical properties were obtained. If slightly less substrate was used in the reaction but the concentration and the volume of the precursor solution were maintained the same, the deposited metal layer became too dense and the polymer layers of the RHS coating were passivated. If slightly too much substrate was added to the reaction, the generated cluster layer was sparse and visible REA color were not generated. Unfortunately, the ratio between substrate surface and precursor could only be varied within a very narrow range without impairing the coating quality. Dosing particulate RHS materials precisely was difficult, however. Their polymer coating rendered the particles highly adhesive, so they could not be re-dispersed completely if they were dried. The particles were thus maintained in solution after polymer multilayer deposition. For electroless metal coating, the dispersions were filtrated or sieved in order to carry over as little solvent as possible to the metal precursor bath. Of course, the precise amount of residual solvent in the wet particle filtrate was not known and varied from experiment to experiment. Unfortunately, the exact amount of particles in the filtrate was not known either. At least nine different layers had to be deposited on raw particles to create mirror layer, ceramic distance layer and PEM. After most of these coating steps the particles had to be washed once or several times and filtrated or sieved. Commonly, an unknown amount of particles was lost in each sieving step. Therefore, the amount of particles, that was obtained after the last polymer deposition step varied strongly from batch to batch. Glass particles, which were frequently used as substrates, also tended to break during coating and washing, whereby their effective surface increased unpredictably. For these reasons, successful electroless metal deposition tests with particulate RHS materials could rarely be reproduced. Adapting electroless reduction protocols to new substrates was difficult as well. The amount of substrate that had to be added to the precursor solution had to be calculated on the basis of the surface of the individual particles. This only worked well for spherical substrates but was hardly feasible for irregularly shaped or brittle materials. For this reason, further investigations focused on cluster deposition by adsorption.

7.3.2 Adsorption of Nanoparticles from Solution

Effects of Polyelectrolyte Layer Structure on Nanoparticle Adsorption

Variations in the amount of substrate in cluster deposition reactions can only be compensated if the reaction is self-limiting. As described in the previous section, seed layers can be created on particulate RHS materials by adsorption of nanoparticles from solution.

Nanoparticles with charged surface can be captured efficiently by polyelectrolyte coatings of opposite charge, for instance. Tests on the adsorption of AuNPs to REA films revealed that the structure of the capture layer strongly influences nanoparticle binding. The respective investigations were performed in the course of the study described in chapter 8, but their results also supported developments discussed in this section. A PET-based non-sensor REA film was used as substrate for coating tests. Two pieces of the film were coated with PEI using one of the following PEI solutions: 1% PEI in dH₂O (pH = 10.3) (sample #1) or 1% PEI in dH₂O (pH = 7.5) (sample #2). Then AuNPs carrying an anionic protein coating were adsorbed to the samples (see 7.2.5). The AuNPs adsorbed at significantly higher density to sample #1 (see Figure 74). As described in 2.4.5.1 the pH-value of a polymer solution – amongst other factors - determines the 3D-structure that dissolved polymers adopt upon adsorption to a substrate. PEI adsorbs in a rather globular conformation under alkaline conditions, but in an expanded, flat shape at neutral pH (internal data). The above results indicated that PEI layers with pronounced 3D-structure can capture nanoparticles more efficiently than comparably flat PEI layers. Of course, strongly folded polyelectrolyte coatings enlarge the effective surface of flat substrates. Moreover, their charge density per surface area is significantly higher than that of 2-dimensional layers of the same polymer. Both properties support the adsorption of oppositely charged nanoparticles. Surface charges make nanoparticles repel each other and thereby prevent them from aggregating, but they also determine their adsorption behavior. Such particles adsorb competitively due to the mutual repulsion between them. Binding stops as soon as the surface is covered with a monolayer of clusters. The strength of the repelling forces defines the interparticle spacing in the monolayer. Dense nanoparticle coatings can only be obtained if the repelling forces are sufficiently compensated by the substrate surface. Due to their structure and charge density strongly folded polyelectrolyte layers can shield charged adsorbates better and can thus capture more clusters per surface area.

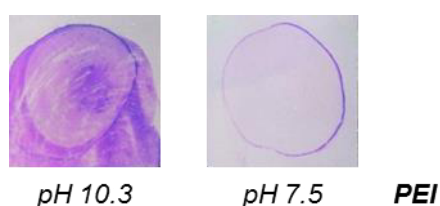


Figure 74 | Adsorption of AuNPs to PEI layers of different charge density Aluminized PET chips coated with a SiO₂ distance layer, a self-assembled monolayer of PEI deposited by at pH 10.3 (left) or pH 7.5 (right) and Au nanoparticles adsorbed from solution.

Development of Pd Nanoparticles for Adsorption

As demonstrated in 4.3.1, the topmost PEI layer of a five-layer PEI/ PMVE-MA PEM exhibits pronounced folding. At the beginning of this project, it was therefore supposed that nanoparticles with charged stabilizer coating would adsorb to the PEM fast and efficiently. In the seeding attempts described in 7.3.1 the contrary was observed, however. Frequently, free stabilizer molecules appeared to interfere with nanoparticle adsorption. With the nanoparticle preparations, which were developed for seeding, dense nanoparticle layers

could usually not be generated either (see 7.2.3 and 7.2.4). It was attempted to further reduce the stabilizer content in the respective precursor solutions, but the synthesized clusters then generally aggregated and precipitated. Apparently, the stabilization strategy had to be changed. The nanoparticle preparations tested so far had been stabilized with only one type of molecule, most often a negatively charged polyelectrolyte of low to high molecular weight. Instead, blends of uncharged and charged stabilizing agents were screened as substitutes further on. Mixtures of low molecular weight PVP and the slightly larger negatively charged polyelectrolyte poly(4-styrenesulfonic acid-co-maleic acid) (PSS-MA) exhibited best performance in these tests. PdNPs generated in an aqueous solution of the blend (see 7.2.6, PdNPs#1) were extremely stable. They could not even be pelleted by centrifugation at 16200 g. Nevertheless, they adsorbed well to RHS pigments and formed dense cluster layers as demonstrated in Figure 75.

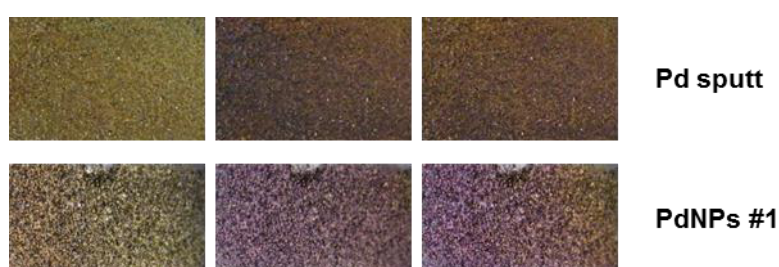


Figure 75 | Resonance colors generated by adsorbed and sputter-deposited Pd cluster layers
 RHS steel pigments (SiO_2 / 5 alternating layers of PEI & PMVE-MA) coated with Pd cluster layers by adsorption of nanoparticle type PdNPs #1 from solution (PdNPs #1) or deposited by sputtering (Pd sputt). (The depicted pigments were prepared from different RHS pigment batches.) The photos show the two pigment types in the dry state (dry), after maximum swelling of the PEM layer (humid) and during drying (drying).

RHS glass and steel pigments coated with PdNPs #1 were repeatedly compared to sputter-coated reference samples regarding base color and swelling capacity (see e.g. Figure 76). Generally, the swelling capacity of both pigment types was equivalent. However, the PdNP #1-coated glitter occasionally exhibited a higher base color. Probably, the shape of PdNPs #1 varied slightly from batch to batch resulting in aberrant resonance behavior. It is also possible, however, that the stabilizer modified the base color. As explained, the PdNPs generated in solution were stabilized with a polymer monolayer. Upon nanoparticle adsorption, the coating of the RHS pigments was thus thickened by one polymer layer. Hence, the distance of adsorbed clusters to mirror layer must have been slightly higher than that of sputter-deposited clusters to mirror layer. The actual reason for the observed base color variations remains to be elucidated, however. To examine the importance of PSS-MA as stabilizer the nanoparticle synthesis test was repeated without the polyelectrolyte. The optical properties of the resulting nanoparticles were equivalent to those of the Pd particles stabilized with PSS-MA and PVP. They also adsorbed efficiently but significantly less fast.

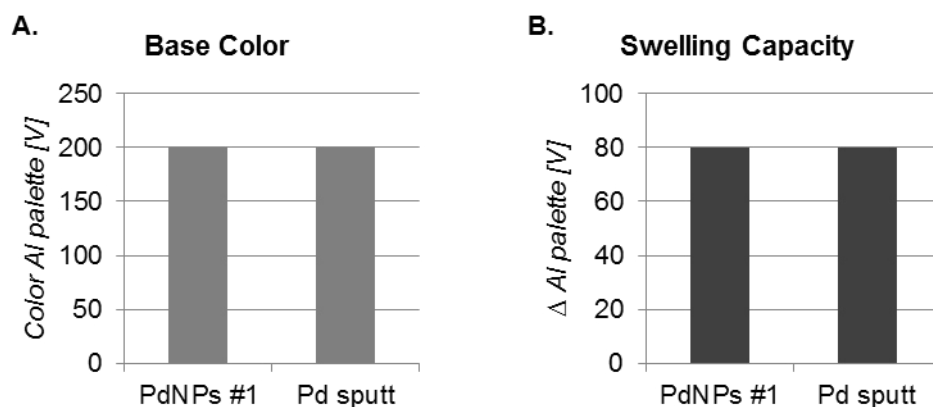


Figure 76 | Comparison of base color and swelling capacity of RHS pigments coated with PdNPs #1 and sputter-coated reference samples ($n=1$) RHS glass pigments (Ag mirror/ SiO_2 / 5 alternating layers of PEI & PMVE-MA) were coated with Pd cluster layers by adsorption of nanoparticle type PdNPs #1 from solution (PdNPs #1) or deposited by sputtering (Pd sputt). Then base color and swelling capacity were determined. The AI-based REA color palette was used for measurements.

Development of Ag Nanoparticles for Adsorption

Unfortunately, Pd is an expensive raw material (price on May 21st 2017: 24.50 US dollars per 1 g Pd). Cluster layers out of silver would be significantly more cost-efficient (price on May 21st 2017: 0.54 US dollars per 1 g Ag). Moreover, it is so far largely unclear, whether PdNPs are toxicologically problematic [163]. If released into the environment they might persist and have adverse effects. In contrast, the toxicology and environmental stability of Ag nanoparticles (AgNPs) has been studied intensively in the past decade [164, 165]. Considering that RHS pigments are supposed to be converted primarily to intelligent labels on packaging, the properties of all components should be well understood. Hence, further investigations focused on the synthesis of AgNPs with similar optical properties and adsorption capacity as the developed Pd clusters. PVP and PSS-MA had mediated their binding to PEI efficiently and were therefore also tested as stabilizer for Ag clusters. Several AgNP preparations with PVP or PSS-MA/ PVP coating were screened. In most synthesis tests yellow or yellow-green AgNP dispersions were obtained. One of the respective synthesis procedures is summarized in section 7.2.6 (AgNPs#3). If such yellow Ag particles were deposited on RHS pigments, their coating generally adopted a faint yellow-brownish or pink-brownish resonance color (see Figure 77). They shifted to pale lavender and blue shades at high humidity. RHS pigments sputter-coated with Pd or Ag cluster layer typically exhibit the following colors at increasing interlayer thickness: yellow $\rightarrow$ orange $\rightarrow$ violet $\rightarrow$ lavender (compare with the REA color standards depicted in Figure 70). These colors were not observed on RHS pigments coated with yellow AgNPs.

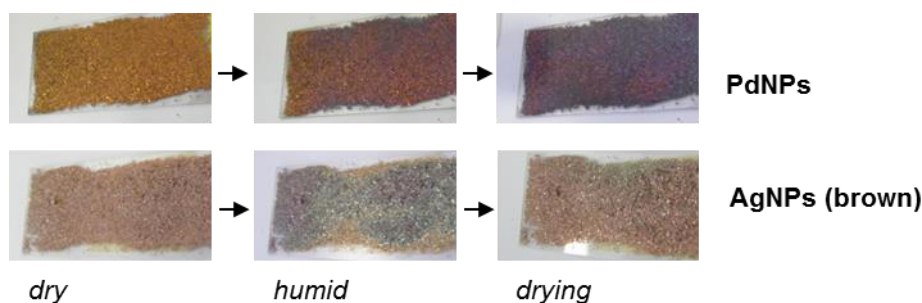


Figure 77 | Comparison of glass-based RHS pigments coated with PdNPs type PdNPs #1 or yellow AgNPs Glass pigments were coated with a Ag mirror layer, a SiO_2 distance layer and a five-layer PEI/ PMVE-MA assembly. Then either PdNPs with PSS-MA/ PVP stabilizer coating prepared in dH_2O or AgNPs with PSS-MA/ PVP stabilizer coating prepared in dH_2O were adsorbed from solution. The photos show the two pigment types in the dry state (dry), after maximum swelling of the PEM layer (humid) and during drying.

The shape of Ag nanoclusters strongly influences their absorption spectrum. While spherical or near-spherical AgNPs typically exhibit a yellow, orange or brown color (see e.g. [166]) triangular Ag nanoplatelets have been reported to appear blue [167, 168]. Sputter-deposited Ag cluster layers are also blue - not yellow as the particles tested so far. It was thus attempted to tune the color of the synthesized Ag particles towards a blue shade by variation of the growth conditions. Eventually, a AgNP dispersion with the desired color could be obtained by thermally induced electroless reduction of silver nitrate (AgNO_3) in ethyl diglycol (EDG) (see 7.2.6, preparation AgNPs #1). As depicted in Figure 78, RHS pigments coated with these nanoparticles exhibited similar REA colors as pigments coated with PdNPs by sputtering. As observed earlier for PdNPs #1-coated pigments, the AgNPs #1-coated pigments exhibited a slightly higher base color than the sputter-coated reference pigments. Their swelling capacity was also slightly increased by nanoparticle adsorption. Most probably, the stabilizer layer on the AgNPs modified the swelling behavior of the pigments. As described in 7.2.6, AgNPs #1 were coated with low-molecular weight PVP to prevent them from aggregating in solution. Apparently, the PVP layer expanded at elevated humidity like the PEM on the RHS pigments. Thereby, it increased the total swelling capacity of the RHS setup.

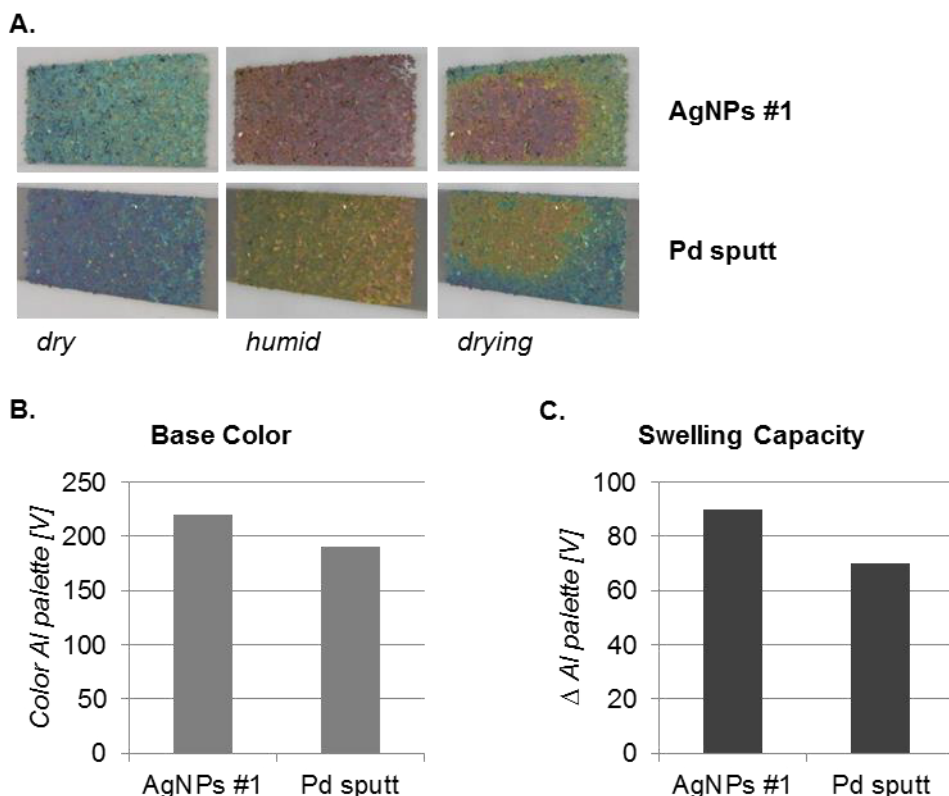


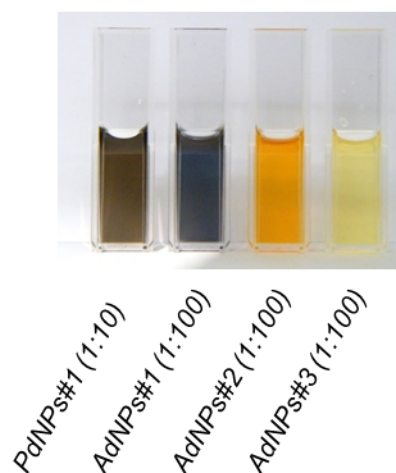
Figure 78 | RHS pigments with AgNP coating **A)** Glass-based RHS pigments (Ag mirror/ SiO_2 / 5 alternating layers of PEI and PMVE-MA) coated with AgNPs#1 by adsorption (AgNPs#1) or with Pd clusters by sputtering (Pd sputt). The photos show the two pigment types in the dry state (dry), after maximum swelling of the PEM layer (humid) and during drying (drying). **B-C)** Base color and swelling capacity of both pigment types were determined. The Al-based REA color palette was used for measurements.

Characterization of Resonance Properties

To verify whether PdNPs#1 and AgNPs#1 can generate equivalent resonance colors as sputtered Pd cluster layers over the whole REA color range they were tested on aluminum color palette strips. These color palette strips were prepared from an aluminum plate, which was coated with aluminum oxide by self-passivating anodization as described in 7.2.8. The thickness of the coating, which serves as interlayer for the REA effect, was increased stepwise over the length of the plate by increasing the anodizing voltage. After anodizing, the plate was coated with a thin SiO_2 layer and a five-layer PEI/ PMVE-MA PEM. Then strips were cut off and the nanoparticles of interest were adsorbed to them. Apart from the already mentioned PSS-MA/ PVP-coated PdNPs (PdNPs#1) and the blue AgNP preparation (AgNPs#1) two other Ag particle types were examined (see Figure 79). One was a yellow PVP-stabilized AgNP dispersion (AgNPs#3) prepared in ethylene glycol. The other sample was actually prepared according to the same protocol as the blue AgNPs, but a solvent batch was employed that was contaminated with traces of iron oxide. Due to these contaminations the Ag particles of this batch apparently adopted a different shape than the blue nanoparticle type and exhibited an orange color (AgNPs#2). Moreover, two color palette strips were coated with a Pd or Ag cluster layer, respectively, by sputtering. One strip was left uncoated

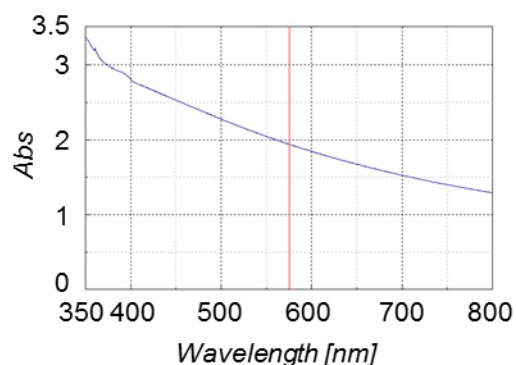
to be able to verify, whether clusters had been deposited successfully on the other strips (see Figure 80, page 149).

A.

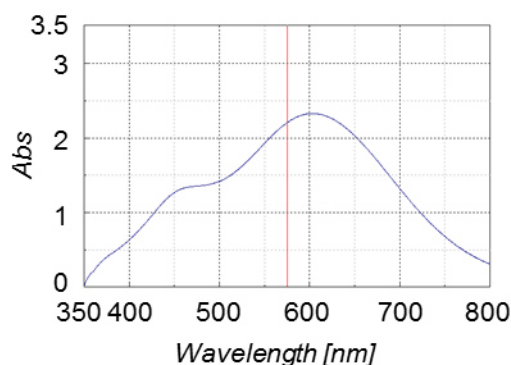


B.

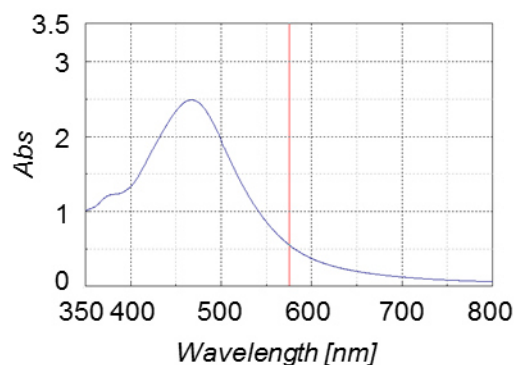
PdNPs#1 (1:10)



AgNPs#1 (1:100)



AgNPs#2 (1:100)



AgNPs#3 (1:100)

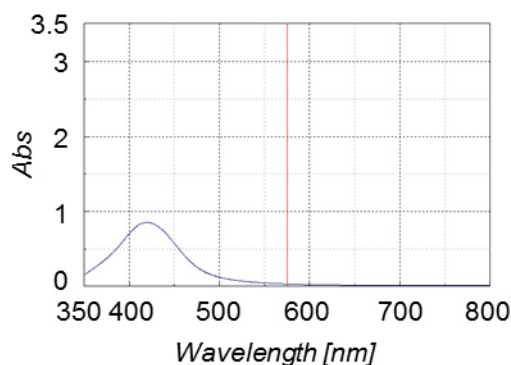


Figure 79 | Comparison of color and UV/Vis absorption spectrum of Pd and Ag nanoparticles A) Photos of dilutions of the tested PdNP and AgNP dispersions in the corresponding solvents (from left to right: PdNP#1 (1:10) in dH₂O, AgNP#1 (1:100) in EDG, AgNP#2 (1:100) in EDG, AgNP#3 (1:100) in EG) **B)** UV/Vis absorption spectra of all dilutions

The first color of the color palette strip with sputter-deposited Pd cluster layer was light blue-yellow, which is typically the base color of RHS coatings prepared according to the standard protocol (see 4.2.1, competitive approach). (The interlayer in this region was composed of

the thin native oxide layer on the aluminum plate, a SiO₂ layer and the PEM.) RHS coatings with this base color usually swell up to the shades of color palette strip #1 labeled with 80 and 100 V. The last color on strip #1 was pink-green, which is one of the last colors in the REA color palette. The color palette strip with sputter-deposited Ag cluster coating (Figure 80, strip #2) exhibited nearly the same colors as the Pd color palette strip, but at some intervals the colors appeared to be shifted by one interval. Most probably, these shifts results from different absorption spectra of Ag and Pd clusters. All color palette strips with adsorbed cluster layers exhibited less intense colors than their sputter-coated equivalents. This might appear surprising considering that intense resonance colors could be generated by depositing PdNPs#1 and AgNPs#1 on RHS pigments. In the course of this study, it was repeatedly observed, however, that nanoparticles can be adsorbed at higher density on particulate substrates than on films or plates. Even if the individual substrates carried the same absorption-mediating coating (e.g. a PEI/ PMVE-MA PEM) clusters deposited more densely on pigments. The reasons could not be verified so far, but it is assumed that the relative motion of substrate and nanoparticles during the coating process plays a role. Naturally, convection speeds up the diffusion of nanoparticles to a substrate. For this reason nanoparticles are generally adsorbed to RHS pigments and films under stirring or shaking. While pigments can move freely in the coating solution, however, films usually remain largely stationary in a shaking vessel. The coating solution mainly flows over them linearly. This might cause nanoparticles to deposit more slowly and at different distances than on pigments. Nevertheless, the nanoparticle layers on all color palette strips were sufficiently dense to generate visible resonance colors. It was found that layers of nanoparticle types PdNPs#1 and AgNPs#1 generated equally intense colors over the whole REA color range that was examined (see strips #3 & 4). The resonance colors of the strip coated with PdNPs#1 were shifted by 3-4 intervals to the right compared to strip #1, which was coated with Pd by sputtering. In contrast, the colors of strip #4 and strip #1 were not shifted relative to each other. This finding contradicted earlier results obtained in pigment coating experiments. As reported above, AgNPs#1-coated pigments and occasionally also PdNPs#1-coated pigments and exhibited slightly higher base colors than reference pigments sputter-coated with Pd. PdNPs#1 adsorbed at a slightly higher density than AgNPs#1 on the REA color palette strips, while both nanoparticle preparations formed similarly dense layers on RHS pigments. Probably, adsorption density plays a role in the relative color shift of RHS materials coated by adsorption and sputtering. The second and third investigated AgNP preparation (AgNPs#2 & AgNPs#3) generated dull orange-brown colors in the first intervals of the typical swelling range of RHS materials (0 V to 100 V as described above). The next few intervals were dull blue on both color palette strips. This explains why RHS pigments coated with these and similar nanoparticles did not adopt intense colors and why their color shift at elevated humidity appeared weak. Surprisingly, the performance of both particle types turned out to be much better at higher interlayer thicknesses. Above the typical swelling range the color palette strips coated with AgNPs#2 and AgNPs#3 showed the same colors as the strips coated with the PdNP preparation and the blue AgNP species. Thus, both nanoparticle types could in principle be employed for the preparation of RHS pigments if these were equipped with thicker interlayers. The main drawback of the AgNPs of type

AgNPs#3 was that they were metastable, however. On the aluminum color palette strip, they formed a dense cluster layer of suitable thickness. On the glass chips prepared for AFM imaging and UV/Vis spectroscopy they precipitated as mirror layer, however. This made it difficult to carry out the respective analyses. The stability the nanoparticle species must thus be improved, in order to obtain reproducible coating densities. Another surprising finding was that the two color palette strips #5 & 6 showed essentially the same colors but the palette of strip #6 (coated with AgNPs#3) was shifted to the left by four intervals compared to strip (#5 coated with AgNPs#2). Both nanoparticle species were stabilized with the same polymer (low molecular weight PVP), so the thickness of their stabilizer coating was not expected to differ so strongly. Their absorption spectra were similar as well (see Figure 79B).

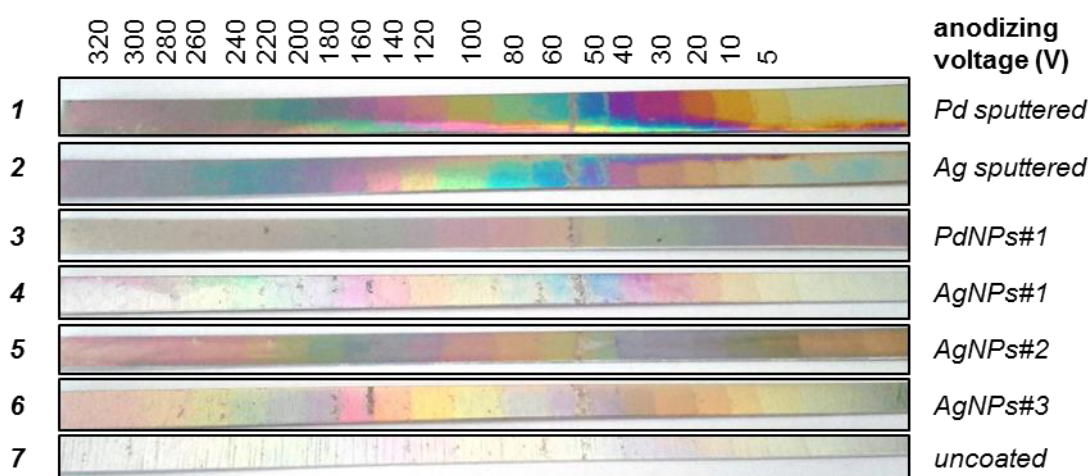


Figure 80 | Comparison of RHS colors of aluminum-based color palette strips coated with different Pd or Ag metal cluster layers The aluminum color palette strips carry an Al_2O_3 interlayer, deposited by self-passivating anodizing. The anodizing voltage was increased every 3-5 mm to obtain stripes of Al_2O_3 with increasing thickness. (The individual anodizing voltages are indicated above the topmost aluminum strip.) The strips were coated with a PEI/ PMVE-MA PEM. Then, either a Pd or a Ag cluster layer was deposited by sputtering or by adsorption from solution. Only one strip was not provided with a cluster layer (uncoated).

Eventually, atomic force microscopy (AFM) analyses provided a possible explanation for the different resonance properties of AgNPs#2 and AgNPs#3. The scans of preparation PdNPs#1 indicated that these particles were of rather spherical shape with an average aspect ratio of 1:1.1 (width : height) (see Figure 81 and Figure 82). The blue AgNPs AgNPs#1 appeared rather flat, platelet-like, in accordance with literature data [167, 168]. Unexpectedly, AgNPs#2 and AgNPs#3 were found to have a significantly different shape in spite of their similar absorption spectra. Whilst AgNPs#3 appeared almost spherical with an aspect ratio of 1:0.7, AgNPs#2 were obviously as flat as the blue AgNP species AgNPs#1. It also exhibited an aspect ratio of 1:0.2. The particles of preparation AgNPs#2 appeared slightly larger than the particles of type AgNPs#1. Unfortunately, the resolution of the AFM scans was not high enough to reveal further geometrical details of the two particle types AgNPs#1 and AgNPs#2. Thus, it could not be verified whether differences in their absorption spectra are caused by minor differences in their shape or whether they result from the iron oxide contaminations in the solvent of AgNPs#2, which might have been integrated in the particles. It would be interesting to further analyze the particles by transmission electron

microscopy to reveal the details of their resonance properties. These investigations would have exceeded the scope of this thesis, however. Still the AFM scans revealed at least one potential reason for the shift of the REA color palette of the color palette strip #5 coated with AgNPs#2 compared to strip #6 coated with AgNPs#3. Due to the spherical shape of the nanoparticles of type AgNPs#3 the resonance center of these particles is located significantly higher than the resonance center of species AgNPs#2 if the two particle species are adsorbed to REA coatings with equivalent interlayer thickness. The palette shift of strip #2 coated with PdNPs#1 relative to strip #3 coated with the blue AgNP species could have the same reason. Probably, the thickness of the stabilizer coating played only a minor role.

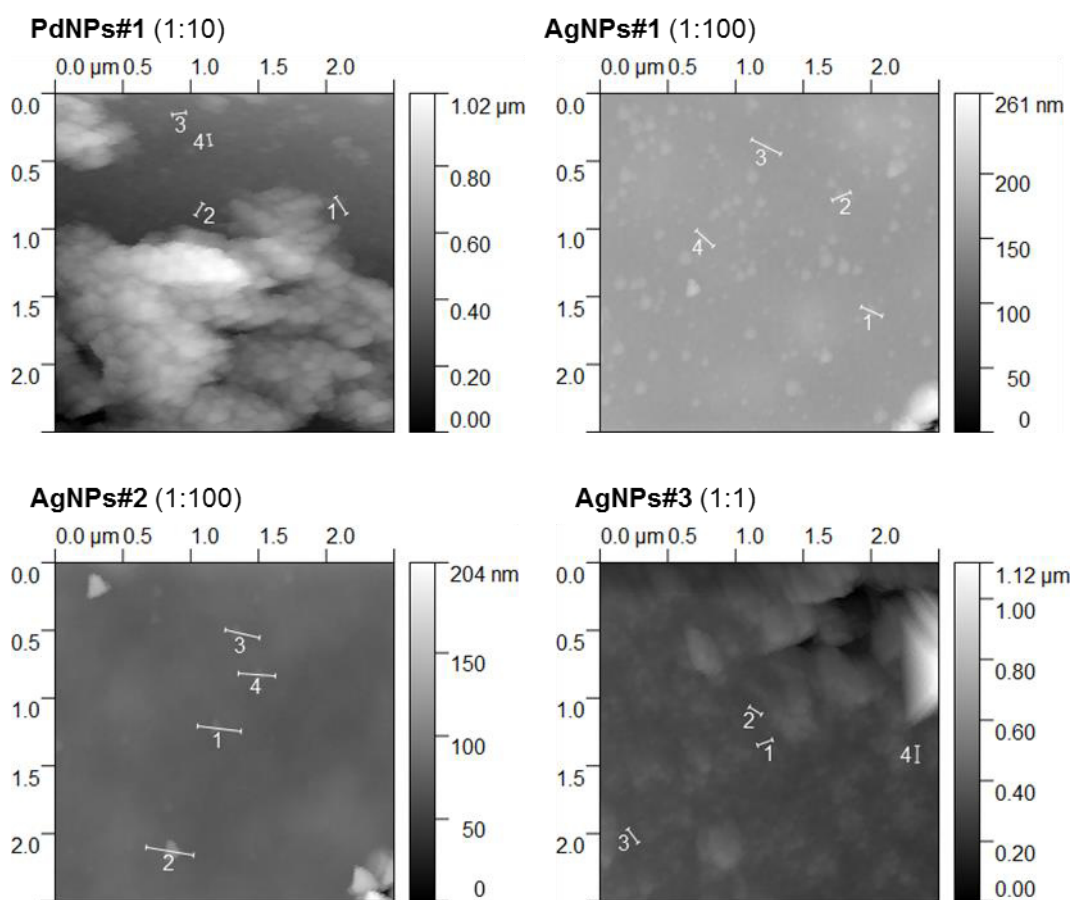


Figure 81 | AFM scans of Pd and Ag nanoparticles adsorbed to chromium-coated glass slides

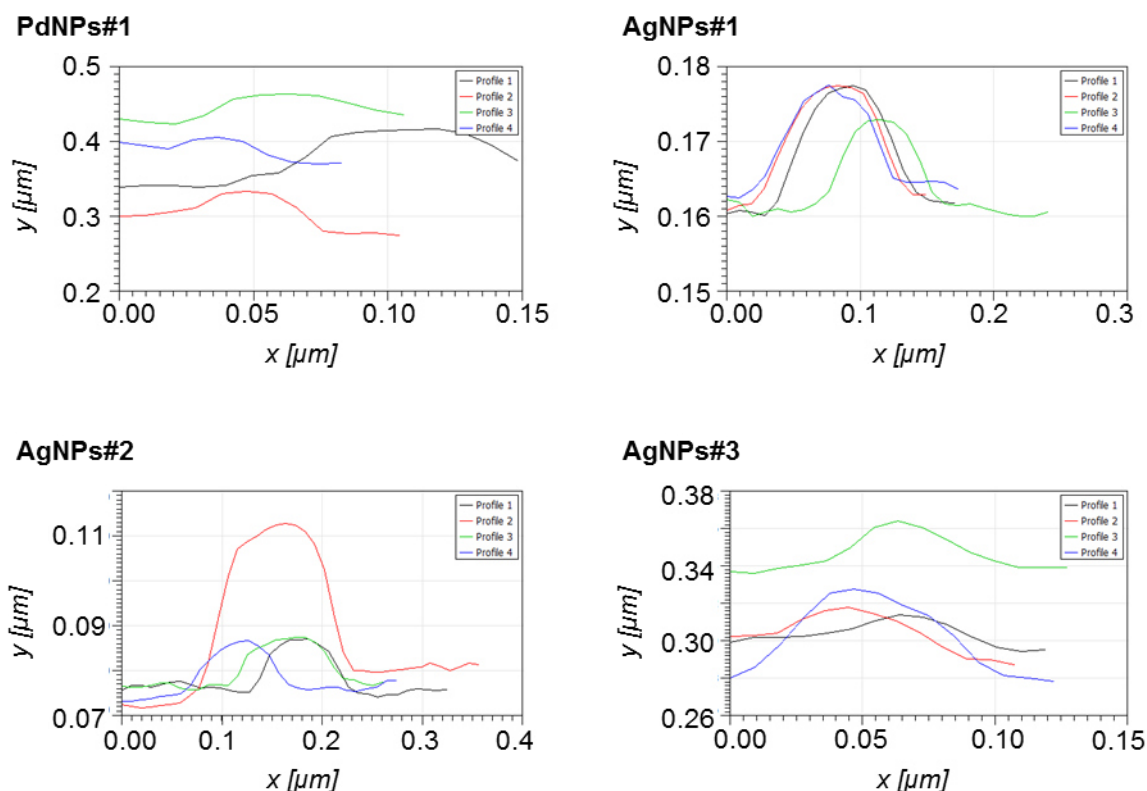


Figure 82 | AFM imaging - Height profiles of Pd and Ag nanoparticles

7.4 Summary & Outlook

Sputtering is a largely directional coating technique, so the deposition of a continuous metal cluster layer on particulate RHS materials by this method is not possible. To be able to prepare pigments with complete RHS coating, a non-directional cluster deposition technique had to be identified. Three wet chemical coating strategies potentially suited for the purpose were examined: direct electroless deposition of clusters, electroless deposition with pre-seeding and adsorption of pre-formed nanoparticles. Occasionally good results could be obtained with the first two strategies, but none of the respective coating protocols were found reliable and fully reproducible. The major drawback of both approaches – direct coating and seed-catalyzed coating – was that the rate of cluster formation was hard to control. Frequently, as-generated cluster layers were either too sparse to generate a resonance color or significantly too dense and mirror-like. In the second case, the resonance properties of the RHS coating and its swelling capacity were impaired strongly. In contrast, pre-formed nanoparticles were found to adsorb in a self-limiting way if the employed particles were sufficiently stable. The main challenge of this third coating strategy was to stabilize the particles, which should be adsorbed, to the required degree, while avoiding free stabilizer in the nanoparticle solution. In many tests unbound stabilizer molecules competed with nanoparticles for binding sites on the RHS materials and prevented cluster deposition. Eventually, protocols for the synthesis of a PdNP and a blue AgNP species could be developed. Both nanoparticle variants could be adsorbed at sufficient density to RHS materials and generated equivalent resonance colors as sputter-deposited Pd and Ag

clusters. The blue AgNP variant showed much better performance on standard RHS coatings than yellow and orange AgNP preparations that were also tested. With the developed Pd and Ag nanoparticle variants, complete RHS pigments can now be prepared in solution. Current investigations focus on integrating such pigments in intelligent labels for packaging. It could already be demonstrated that certain variants of RHS pigments can be processed by established industrial printing technologies such as laser printing and screen printing.

8 REA for POC-device

8.1 Introduction

The implementation of REA in bioassays has already been described in 1998 [121] and has been intensively studied since. In 2009, the research and development team of Attophotonics reported the development of a novel diagnostic platform, which combined the resonance color technology of the company with classical immunodetection and passive microfluidics based on capillary flow [122]. By the example of interleukin 6 (IL-6), an inflammation marker [169], the potential of this system for the detection of physiologically relevant human proteins was demonstrated. The working principle of the assay is similar to that of classical lateral flow assays (LFAs) (see Figure 83). The sample is applied at one end of the test device and migrates towards the detection area driven by capillary action. On the way, it redissolves a nanoparticle-conjugated probe and auxiliary reagents, which mediate the detection reaction. In presence of the analyte of interest analyte-probe-nanoparticle complexes form, which are captured at the detection area by immobilized capture proteins. At sufficiently high analyte concentration a colored symbol appears.

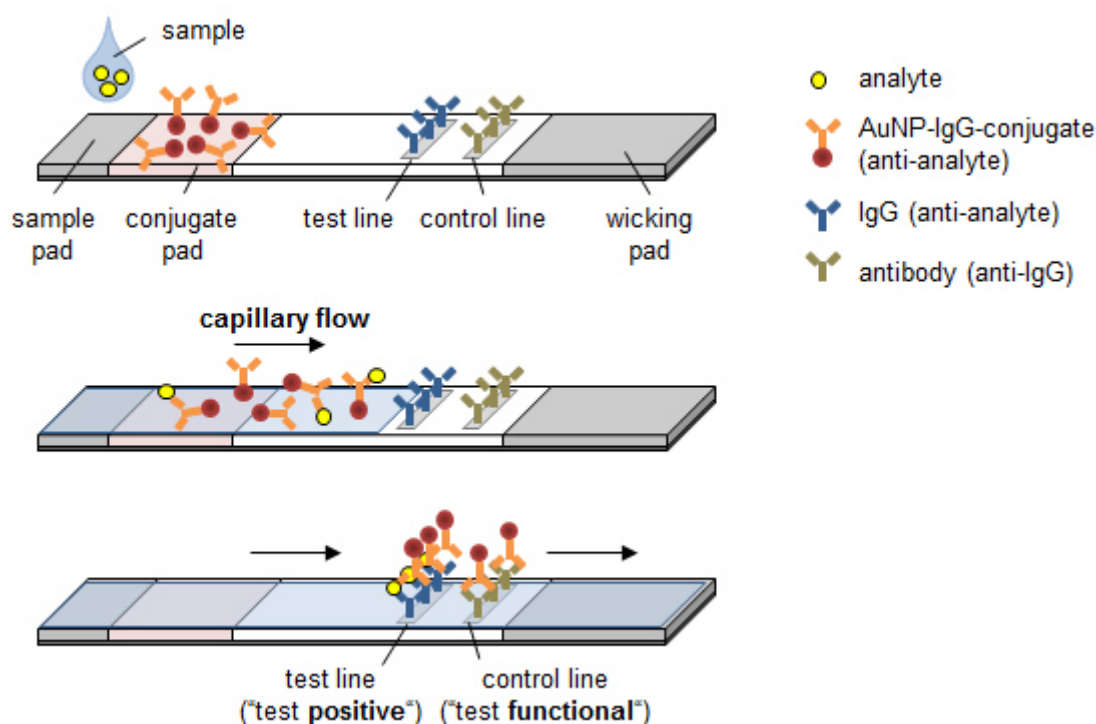


Figure 83 | Working principle of classical LFA

Classical lateral flow immunoassays employ porous membranes and tissues to transport the sample (e.g. urine) from the point of application to the detection unit. Generally, the latter also consists of a porous material (most often nitrocellulose), onto which detection antibody is spotted in the shape of a line or cross. LFAs are well suited for testing purified samples and urine, but liquids containing solid contaminants may block the porous components. In contrast, the assay device developed by Attophotonics is based on a microfluidic chamber comprising channels of a width of several hundred micrometers. Thus, it should allow the

analysis of samples containing impurities and cellular debris. The detection area, a REA chip, is not porous either but flat. It is usually fabricated from of a metal-coated PET film, onto which a translucent distance layer is deposited and capture proteins are immobilized. If analyte-probe-nanoparticle complexes are caught on the chip, the REA structure is completed and the chip adopts a pre-defined color.

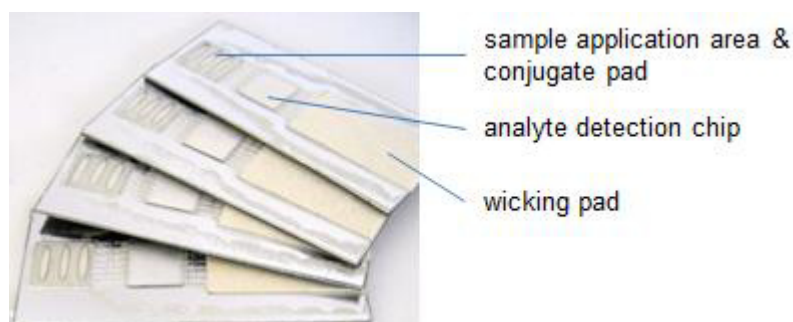


Figure 84 / Exemplary layout of REA microfluidic devices

Due to its 2-dimensionality the detection area in chip-based diagnostic devices is small compared to the surface area available for immobilization of capture proteins in porous lateral flow membranes. In consequence, the maximum number of binding sites that can be created on the chips is significantly lower and so is their capture capacity [170]. Therefore, signal enhancement is commonly required for high assay sensitivity. In the assay developed by Attophotonics this limitation is partially compensated by the REA phenomenon. Nanocluster layers incorporated in a REA structure exhibit up to 8-fold stronger absorption of certain wavelengths than equivalent nanocluster layers alone. For this reason sufficiently strong optical signals are obtained with the assay technology in several applications. In some cases, however, detecting the analyte of interest over the whole medically relevant concentration range is not possible without secondary enhancement. Assadollahi et al. demonstrated that the optical signal, which is generated by the capture of analyte-probe-nanoparticle complexes on the detection area, can be boosted by Ag enhancement [122], a process that was already mentioned in chapter 7. For Ag enhancement, an aqueous solution of silver salt, reducer and auxiliary reagents, which will further be referred to as Ag enhancement solution, is added to the assay device after the whole sample has passed the detection area. If nanoparticles are present on the detection area, they catalyze reduction and precipitation of metallic silver from the enhancement solution. As a result, empty spaces between the captured nanoparticles are closed. The metal cluster layer on the REA chip grows denser and thicker and the optical output of the assay device gains in intensity (see Figure 85).

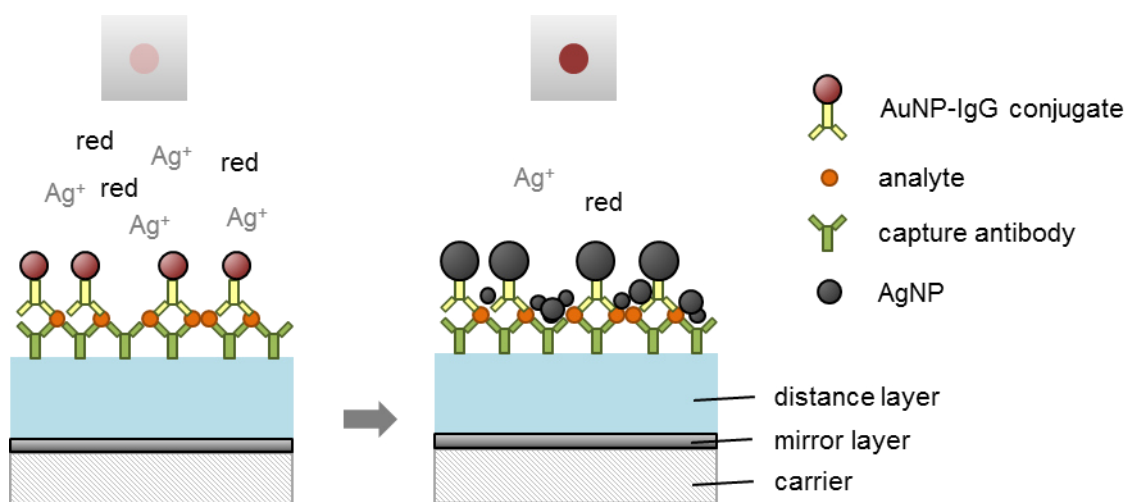


Figure 85 | Silver enhancement on REA chip

Unfortunately, ionic silver tends to precipitate as AgCl if added to biological fluids such as blood and urine for they generally contain high levels of chloride. Hence, the channels of the assay device of Attophotonics had to be rinsed with water preliminary to the enhancement reaction if biological samples were tested. Both processes, washing and secondary enhancement complicate handling. Thus, they are not compatible with rapid tests, especially not in the point-of-care (POC) sector. In contrast to classical laboratory testing, POC testing is supposed to be carried out close to the patient. Basically two categories of POC-tests can be distinguished. “Professional tests” such as critical care tests and assays for the detection infectious diseases, cardiac markers or diabetes are typically performed by skilled personnel. So-called “over-the-counter” devices i.e. for glucose monitoring or pregnancy testing are operated by the patients themselves. In both fields, simplicity and robustness are primary requirements [171]. For this reason, Attophotonics was interested in the development of a highly sensitive one-step REA bioassay system. It became another research topic of this doctoral project.

8.2 Summary of Results & Discussion

The results of the study on the optimization of microfluidic REA assays for POC testing were published in

M. Schlauf et al., “Immobilization Techniques and Integrated Signal Enhancement for POC Nanocolor Microfluidic Devices”, *Journal of Nanomaterials*, Volume 2015, 2015 [172] and

M. Schlauf et al., “Chloride-resistant In-situ Silver Enhancement for One-step Microfluidic Bioassays”, Poster presented at EUROPT(R)ODE XIII, 2016 [173],

which are both included in the Appendix of this thesis. As discussed therein, covalent and adsorptive immobilization techniques were screened initially to identify a fast and efficient method for the immobilization of capture proteins on REA bioassay chips at high density. Streptavidin, a robust capture molecule, which is frequently utilized in bioassays, was

selected as model protein for the study. Best and most homogeneous capture efficiency could be obtained by adsorbing streptavidin onto a high molecular weight PEI interlayer. The method was optimized and a deposition protocol could be developed, which is even considered suitable for large-scale production. The sensitivity of the as-prepared chips was significantly higher than that of the chips Att Photonics had employed before. Still, the assay signal had to be enhanced to reach low detection limits. As described, multiple assay steps should be avoided. Hence, possibilities to realize an integrated enhancement system for microfluidic REA devices were investigated. As reported in the above publications, a Ag enhancement formulation could be developed that operates even at chloride concentrations of up to 20 mM. With this solution, Ag enhancement of the optical signal on a REA detection chip can be performed without preliminary washing. All components of the reaction formulation are water soluble and can be provided in test devices in dry form. If positioned properly in the assay chamber, they can be released and mixed within the device upon sample addition and can thus mediate the boosting reaction without the requirement of further manipulation by the operator. The technology still requires further development but represents a first promising approach towards a highly sensitive one-step REA POC device.

The development was patented in Austria under the patent number AT516760 (A4) [174].

9 Outlook

REA color coatings represent appealing platforms for rapid diagnostic tests and indicators. This doctoral project focused on two REA-based sensor systems: REA humidity sensor (RHS) coatings and REA immunoassays.

A novel signal enhancement technology for microfluidic REA immunoassays was developed. It could be demonstrated, that all reagents required for the enhancement reaction can be integrated in microfluidic REA assays in dry form. Upon sample addition, they are automatically released and amplify the assay signal. The sensitivity of REA immunoassays could be increased significantly by this technology. However, the long-term stability of the enhancement reagents in microfluidic devices must still be characterized. The reservoir structures (e.g. tissues), onto which the reagents are spotted to integrate them in such devices must still be optimized as well. Moreover, techniques for the assembly of microfluidic devices containing such structures must be developed, which are suited for industrial manufacturing.

The project also yielded decisive advancements regarding the manufacturing of REA films and pigments. Novel wet-chemical techniques for the deposition of the humidity-responsive PEM and the metal cluster layer of RHSCs were developed. Both techniques are suited for semi-industrial production. RHS materials can now be produced at low enough costs to employ them as smart labels for humidity monitoring in packaging and technical instruments. The effects of internal and external factors on the performance and lifetime of RHSCs have also been examined. These investigations provided a basic understanding of the stability of the coatings. To make sure that they perform reproducibly in different environments, interactions between individual components and external influences still need to be studied in more detail, however. Especially the aging processes occurring in the humidity-responsive PEM assembly must be understood better. In addition, alternative building blocks for the PEM should be screened. Probably, a polyelectrolyte combination can be identified that does not depend on humectants for maintaining its swelling capacity. Future investigations will also deal with interlayers that react to other stimuli than elevated humidity. Protein layers that can be decomposed by specific medically relevant bacteria are currently under consideration, for instance.

However, stability will always be an issue when employing REA technology. As REA coatings are built from thin films, they are inherently fragile. Mechanical stress and corrosive agents can currently damage the coatings easily and severely. If RHSCs are touched with bare hands, for instance, sweat and sebum typically change the sensor behavior of the coatings and usually lead to mirror corrosion. In most prospective applications (e.g. in smart labeling), it cannot be excluded that REA sensor materials are manipulated without gloves or exposed to salts and pH-active substances. Thus, protective varnishes will have to be developed. Suited lacquers must guard REA sensor coatings against detrimental influences, but at the same time, they must allow interaction of the coatings with the analytes they are

supposed to detect. Respective water vapor-permeable varnishes for the reinforcement of RHSCs are currently being established on the basis of commercial formulations. Working out protective coatings that can be permeated by larger molecules (e.g. proteins) will be challenging, however.

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APPENDIX

ABSTRACT

Organic and inorganic thin films and multilayer structures thereof are powerful tools for the functionalization of surfaces. Nowadays, they find application in anti-reflective coatings and or as controlled release structures, for instance. This thesis deals with a special type of such functional multilayer coatings, so-called resonance enhanced absorption (REA) color coatings.

REA coatings are basically stacks of metallic, organic and ceramic thin films, which operate like interference filters. As a result, materials equipped with these coatings (= “REA materials”) exhibit characteristic, bleaching-stable colors. Variants of the coatings even change their color in response to external stimuli (such as humidity or pH-changes). The technology has proven useful in a broad range of fields, from design and architecture to medical analytics. However, the commercialization of REA coatings and materials has been impeded so far by the complex manufacturing process and the limited stability of the structures.

The aim of this doctoral project was to develop and optimize coating procedures for the large scale production of REA materials, especially of REA sensor films and pigments for humidity detection. In parallel, the stability of such REA humidity sensor materials (= “RHS materials”) should be improved. For this purpose, “aging” processes leading to a loss of sensitivity of the materials should be studied. In addition, new features should be worked out for REA materials and the sensitivity of REA-based bioassays should be enhanced.

A major success of the project was the development of an optimized process for the deposition of RHS coatings on thin plastic films. A corresponding coating system for medium-scale production of RHS films was designed, in which all required wet-chemical deposition steps can be performed. The causes of coating defects that occurred in the previously used coating process were revealed. Moreover, several factors involved in the aging of RHS coatings could be identified. A novel process for the deposition of a main component of REA coatings, the so-called metal clusters layer, on particulate materials was also worked out. To broaden the fields of application of REA and RHS films, patterning techniques for the two film types were established. In addition, a signal-enhancement technology for REA-based rapid diagnostic tests was developed, whereby the sensitivity of these assays could be increased significantly.

ZUSAMMENFASSUNG

Organische und anorganische Dünnschichten und daraus aufgebaute Mehrschichtstrukturen stellen vielseitige Werkzeuge für die Funktionalisierung von Oberflächen dar. Sie finden heutzutage z.B. in Anti-Reflex-Beschichtungen oder als Strukturen zur kontrollierten Freisetzung von Medikamenten Anwendung. Diese Doktorarbeit behandelt eine spezielle Art solcher funktionellen Mehrschichtaufbauten, sogenannte Resonanz-verstärkte Absorptions (REA)-Farb-Beschichtungen.

Bei REA-Beschichtungen handelt es sich prinzipiell um Stapel aus metallischen, organischen und keramischen Dünnschichten, die wie Interferenzfilter agieren. Aufgrund dessen zeigen mit diesen Beschichtungen ausgestattete Materialien (= „REA-Materialien“) charakteristische, bleichstabile Farben. Varianten dieser Beschichtung reagieren auf externe Stimuli (wie Feuchtigkeit oder pH-Änderungen) sogar mit einer Farbänderung. Die Technologie hat sich in vielen Bereichen, von Design und Architektur bis hin zur medizinischen Analytik, bereits als nützlich erwiesen. Allerdings behinderten der komplexe Herstellungsprozess und die begrenzte Stabilität von REA-Beschichtungen bisher deren Kommerzialisierung.

Ziel dieses Projekts war die Entwicklung und Optimierung von Beschichtungsverfahren für die großtechnische Herstellung von REA-Materialien, insbesondere von sensorischen REA-Folien und –Pigmenten für die Detektion von Feuchtigkeit. Parallel dazu sollte die Stabilität dieser REA Feuchtesensor-Materialien (= „RHS-Materialien“) verbessert werden. Hierfür sollten Alterungsprozesse, die zu einer Abnahme des Ansprechverhaltens dieser Materialien führen, untersucht werden. Außerdem sollten neue Funktionen für REA-Materialien und die Sensitivität von Sensoraufbauten, die auf REA-Technologie basieren, sollte gesteigert werden.

Ein bedeutender Erfolg des Projekts war die Entwicklung eines Prozesses für die Abscheidung von RHS-Beschichtungen auf dünne Plastikfilme. Ein entsprechendes Beschichtungssystem wurde entworfen, mittels dessen alle erforderlichen nasschemischen Abscheidungsschritte durchgeführt werden können. Die Ursachen für Beschichtungsdefekte, die beim vorherigen Beschichtungsprozess auftraten, wurden aufgeklärt. Darüber hinaus, wurden einige Faktoren, die bei der Alterung von Feuchte-Sensor-REA-Beschichtungen involviert sind, konnten identifiziert werden. Ein neuer nasschemischer Prozess für die Abscheidung eines Hauptbestandteils von REA-Beschichtungen, der sogenannten Metall-Cluster-Schicht, auf Partikelmaterialien wurde ebenfalls ausgearbeitet. Um die Einsatzbereiche von REA- und RHS-Folien zu erweitern, wurden Strukturierungstechniken für beide Folienvarianten etabliert. Darüber hinaus wurde eine Signalverstärkungstechnologie für REA-basierte medizinische Schnelltests entwickelt, wodurch die Sensitivität dieser Tests signifikant gesteigert werden konnte.

Immobilization Techniques and Integrated Signal Enhancement for POC Nanocolor Microfluidic Devices

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Abstract: Resonance enhanced absorption (REA) nanocolor microfluidic devices are new promising bioassay platforms, which employ nanoparticle (NP)-protein conjugates for the immunodetection of medically relevant markers in biologic samples such as blood, urine and saliva. The core component of a REA test device is a PET chip coated with aluminum and SiO₂ thin layers, onto which biorecognitive molecules are immobilized. Upon addition of a sample containing the analyte of interest, a NP- protein-analyte complex is formed in the test device that is captured on the REA chip e.g. via streptavidin-biotin interaction. Thereby a colored symbol is generated, which allows optical readout. Silver enhancement of the bound nanoparticles may be used to increase the sensitivity of the assay.

Herein demonstrate that adsorptive immobilization via a cationic polymeric interlayer is a competitive and fast technique for the binding of the capture protein streptavidin onto planar SiO₂-surfaces such as REA biochips.

Moreover, we report the development of a silver enhancement technology that operates even in the presence of high chloride concentrations as may be encountered in biologic samples. The silver enhancement reagents may be integrated into the microfluidic assay platform to be released upon sample addition. Hereby a highly sensitive one-step-assay can be realized.

1. Introduction

Modern point-of-care diagnostics strongly relies on low-cost, portable, easy-to-handle platforms especially in regions with limited resources and medical infrastructure.

Microfluidic devices also called μ TAS (micro total analysis systems) or lab-on-a-chip systems belong to the most promising technologies studied in this context. A major part of the respective research focuses on the combination of microfluidics with immunoassay technology being the most valuable tool for the detection of medically relevant markers. Compared to conventional immunoassays microfluidic systems offer efficient mass transport and a reduced surface to volume ratio. Thus fast analyses can be performed even with small sample volumes and significantly lower amounts of antibodies and reagents are required. [1] Detection strategies currently studied and employed for microfluidic platforms range from fluorimetric over colorimetric to electrochemical techniques (see e.g. [2]). The selection of the best suited technology is often a compromise between simplicity and sensitivity. Where quantification is an issue and low detection limits are most critical, electrochemical systems are generally the first choice. For quick tests and screenings, however, a non-instrumental read-out may be preferred, which implicates a colorimetric detection strategy.

Resonance enhanced absorption (REA) is an optical phenomenon already described by Leitner *et al.* [3] in 1993. In 1998, the application of the effect in a bioassay was reported [4]. Its potential as a novel (bio)analytical platform has been studied extensively since [5-7]. In 2009, our group developed a novel microfluidic diagnostic device combining REA technology with an innovative fluidic setup operating by passive capillary force. The manufacture of the device out of PET films by hot embossing or laser micromachining and the physical basics of REA were discussed in detail by Assadollahi *et al.* [8]. Current research focuses on the optimization of this system as versatile, fast and sensitive microfluidic POC one-step-immunoassay platform.

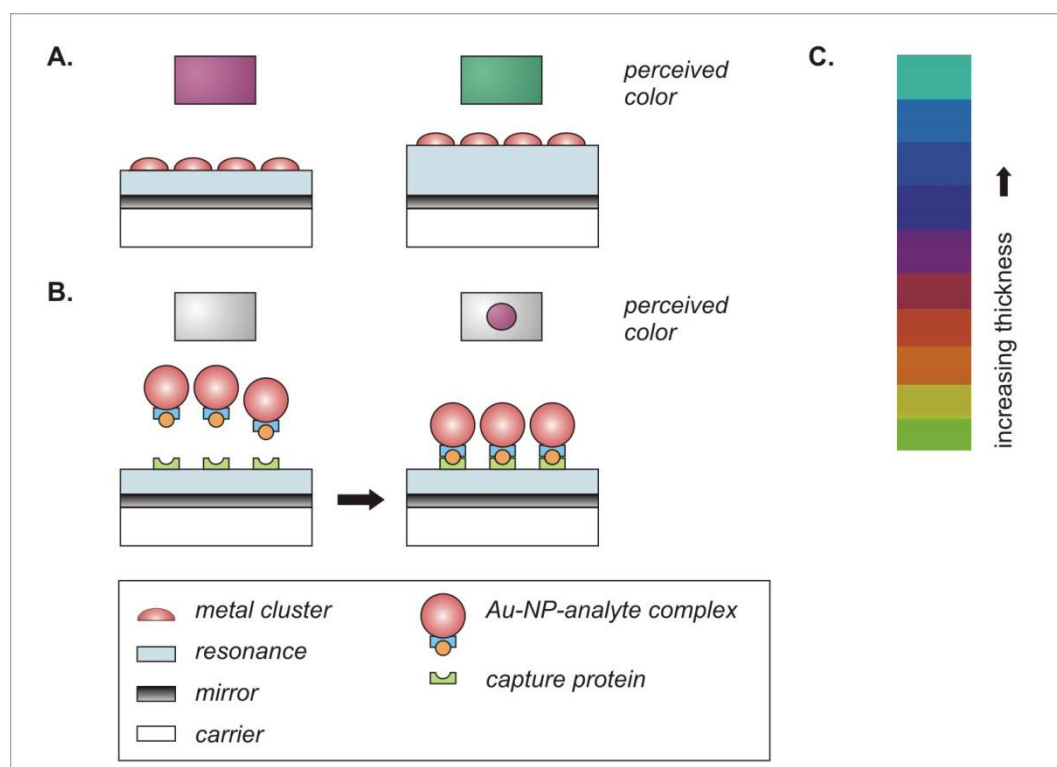
In brief, resonance enhanced absorption requires a four layer setup as depicted in Figure 1(A). It comprises a metal mirror layer, a transparent distance layer of nanometric thickness (<300 nm) and a (sub-)monolayer of metal nanoclusters.

The sandwich of nanocluster layer and mirror layer at a well-defined distance results in optical resonance. Compared to the absorption spectrum of the nanocluster layer alone this resonance effect causes an 8-fold or higher increase in absorption of electromagnetic radiation of certain wavelengths [5]. As a consequence, a color in the visible range of the electromagnetic spectrum is obtained (, which is commonly different from the color of the nanoclusters). The absorption maxima shift depending on the distance layer thickness, which allows the fabrication of REA materials of various colors and multicolored surfaces (see Figure 1(C)).

For analytical purposes REA chips without top metal cluster layer are used as platforms for colorimetric sandwich type immunoassays employing metal nanoparticle-conjugated probes (Figure 1(B)). If the antigen of interest is present in a sample, a complex comprising metal nanoparticle probe, analyte and – if required – additional mediator proteins is formed and binds to the chip surface. Thereby the REA structure is completed and a colored symbol appears indicating the positive detection of the analyte. The color intensity is proportional to

the concentration of the captured NPs. Like in traditional lateral flow assays (LFAs) all assay reagents are provided in the microfluidic cell in dry form and are released upon sample addition. Such REA immunoassays have already been used for the detection of HSA and IL-6 as reported by Assadollahi *et al.* [8] and Palkovits *et al.* [9]. Similar systems based on metal nanoparticle-conjugated probes have been developed by other groups recently (see e.g. [10-12]). However, those colorimetric tests rely solely on the light absorption of the captured nanoparticle layer. They do not profit from the signal enhancing effect of a REA structure, which may allow reaching even lower detection limits.

Figure 1. Classical REA Setup & Bioanalytical REA Assay (A) Schematic drawing of the classical 4-layer REA setup with two different resonance layer thicknesses. The colored squares above the metal cluster layers indicate the film color, which varies with resonance layer thickness. (B) Schematic drawing of a bioanalytical REA assay before (left) and after (right) binding of AuNP-analyte complexes to the capture protein layer. (Without top metal cluster REA materials exhibit the color of the base mirror layer - generally silver to gray.) The NP-analyte complex may comprise further mediator proteins, which are not depicted here. (C) **REA Color Spectrum** By stepwise increase of the resonance layer thickness from 0 to 300 nm a broad range of colors can be obtained.



In order to provide a flexible platform for a range of analytes, streptavidin should be introduced into the described microfluidic REA technology as capture reagent. Thereby already established immunoassays employing biotin-tagged antibodies for detection could be

combined with the device without the requirement for individual optimization of chip coating procedures.

For low-cost manufacture an efficient and simple technique for streptavidin immobilization must be employed, however. In order to identify a suitable method we performed a screening of published approaches and variations thereof and evaluated their efficiency. The four most successful techniques we identified are described herein. Basically there are three different approaches that can be taken in protein immobilization: (1) Adsorption or spotting is a strikingly simple technique, but commonly leads to random protein orientation. However, it has proven sufficient for various applications [13]. (2) Covalent immobilization limits the possible orientations of the immobilized protein and thereby can increase or at least homogenize the reactivity of the protein layer. It may, however, also decrease the reactivity if the employed technique entails molecule orientations that hide active sites from potential interaction partners. The choice of the immobilization chemistry depends on the reactive groups that are available or can be created on the protein and the substrate [14]. Recent developments even allow highly selective, oriented conjugation combining the advantages of protein tags with covalent methods [15]. (3) By immobilization via tags (e.g. myc, streptavidin-biotin interaction) highly oriented protein layers can be prepared - even from impure lysates or solutions with low concentration. [14]. However, employing tags for immobilization was not considered in this study as the streptavidin functionalities were required for antibody-antigen capture. Each streptavidin molecule provides four binding sites. The orientation of streptavidin after immobilization should therefore have only minor influence on its binding capacity. Thus, adsorptive as well as covalent immobilization techniques were considered suited for the preparation of streptavidin coatings on the SiO₂ surface of REA chips.

A major drawback of microfluidic systems is the limited number of binding sites that can be created on the commonly flat detection area compared to the generally porous substrates of LFAs. Recent publications suggest that a pronounced 3D-structure of the chemical linkers that are used to bind the immobilized molecules to the substrate can be helpful to obtain a high density of binding sites (as reviewed in [16]). Polyethylenimine (PEI) and carboxymethylcellulose (CMC) are two high molecular weight polymers that have been described previously as substrates for protein immobilization [17-19]. Due to its strong positive charge PEI was selected for adsorptive immobilization of streptavidin, which is, with a pI of ~5, negatively charged in the pH-neutral buffers used for chip preparation. CMC was tested as platform for carbodiimide-mediated covalent streptavidin immobilization. As there are several protocols available for the respective conjugation reaction, a screening was performed to identify suitable approaches. To determine the efficiency of the individual immobilization methods and the functionality of the generated streptavidin layers, all chips were tested in a standardized microfluidic immunoassay at defined analyte concentration. As depicted in Figure 1(B) the assay is based on the binding of a nanoparticle-conjugated probe to the analyte in solution and the subsequent capture of this complex on the REA chip surface. However, in

contrast to the illustration, a second, biotin-tagged probe is required to mediate the binding of the analyte-NP complex to the streptavidin layer on the chip. Two of the most competitive immobilization approaches that could be established are described herein. Chips prepared using CMC or PEI were compared with chips coated via adsorption of streptavidin to 3-APTES. Thereby it was evaluated whether polymer interlayers actually confer higher biorecognitive capacity to REA bioassay chips.

The limited number of binding sites in microfluidic systems may also be compensated by secondary signal enhancement reactions. Assadollahi *et al.* described how nanoparticle-catalyzed electroless deposition of metallic silver from solution can be used to boost sensitivity and signal intensity. Even layers of particles so sparsely bound that they are invisible to the eye may provide sufficient catalytic activity for this enhancement method. Optimally the deposited silver, which is generated by in-situ reduction of a precursor salt, forms a continuous film and closes the gaps between the captured antigen-antibody-nanoparticle complexes. An increase in signal intensity of up to 13-fold may be achieved thereby. Comparable strategies were employed by e.g. [20] and [21]. However, in all three cases the respective reaction was performed after the actual analyte detection in a secondary step. The silver enhancement solution had to be mixed freshly. Efforts to integrate the enhancement reagents in the assay platform to develop a true one-step device showed that the formulation of Assadollahi *et al.* was not compatible with the high chloride concentrations often occurring in biologic samples. Silver chloride precipitates formed, which prevented the reduction and precipitation reaction. Accordingly, integrated silver staining demands, first of all, the stabilization of ionic silver in chloride-containing solutions. Furthermore, it must be possible to provide the enhancement reagents – just like the immunoreagents – within the assay platform and store them over a prolonged period of time in this form. Their release, which induces the enhancement reaction, should at best not require additional manipulation by the person performing the assay. It should occur upon or shortly after introduction of the sample into the device. Considering these points a screening of complexing agents and silver staining protocols was performed. Eventually a formulation could be established, which operates stably in the presence of urine. It is entirely based on solid, water-soluble precursors and additives well suited for long-term storage and one-step release. Their performance in the already described REA bioassay system was tested and efficiency and morphology of the respective silver stainings were characterized, as reported herein. It could be demonstrated that the developed formulation is a promising first step towards an integrated enhancement system for nanoparticle-based microfluidic devices.

2. Materials and Methods

2.1. Preparation of REA Chips

A 100 μm thick, aluminum-metalized polyester film (Mitsubishi) was coated with a ~180–220 nm layer of SiO_2 as previously described [8]. (After addition of a metal cluster layer,

films of this resonance layer thickness exhibit a pink-violet resonance color, which provides optimal visibility.)

2.2. Streptavidin & Bioassay Reagents

Streptavidin and all reagents required for the bioassay part of the setup (i.e. purified analyte, gold nanoparticle (AuNP)-conjugated probe and biotin-tagged analyte binding protein) were kindly provided as a gift. Similar biotin-tagged mediator proteins and AuNP-conjugates are available from Sigma-Aldrich (e.g. Prod.no. G-0911 or P-6855). For bioassay experiments AuNP-mediator protein conjugate was used at an OD of ~10.9–11.8 in the final assay mix.

2.3. Streptavidin Coating

2.3.1. Protocol 1 (3-APTES/ Streptavidin)

A droplet of 3 μ l of 2 mg/ml streptavidin in 0.01 M Na-K-phosphate buffer, pH 7.0 was spotted in the middle of a 3-APTES (Sigma-Aldrich) coated chip. The chip was incubated in a wet chamber at RT for 1 h. The chip was washed by shaking in 1 x PBS + 0.1 % Tween 20 for 3 min. For blocking, the chip was incubated in 2 % BSA in 1 x PBS + 0.5 % Tween 20 for 1 h. The chip was again washed by shaking in 1 x PBS + 0.1 % Tween 20 for 3 min, rinsed with dH₂O and used directly for immunodetection.

2.3.2. Protocol 2 (PEI/ Streptavidin)

A REA chip was coated with PEI (1.6 MDa) by incubation in 10 % aqueous PEI solution. A 3 μ l droplet of 2 mg/ml streptavidin in 0.01 M Na-K-phosphate buffer, pH 7.0 was spotted in the middle of the PEI-coated chip. The chip was incubated in a wet chamber at RT for 1 h. Washing and blocking was performed as described in protocol 1.

2.3.3. Protocol 3 (3-APTES/ Activated CMC Na-salt/ Streptavidin)

CMC sodium salt (~700 kDa), carbodiimide and NHS were all purchased from Sigma-Aldrich. 0.45 % (w/v) CMC Na-salt in 0.01 M Na-K-phosphate buffer, pH 7.0 was activated with 200 μ M carbodiimide and 50 μ M NHS end-to-end rotating at RT for 2h. ~100–200 μ l of the solution were spread on the surface of a 3-APTES coated chip. The chip was incubated for 30 min in a wet chamber, rinsed with dH₂O and dried. A 3 μ l droplet of 2 mg/ml streptavidin in 0.01 M Na-K-phosphate buffer, pH 7.0 was spotted in the middle of the CMC-coated chip. The chip was incubated in a wet chamber at RT for 17–17.5 h. Washing and blocking was performed as described in protocol 1.

2.3.4. Protocol 4 (3-APTES / CMC Na-salt-streptavidin-conjugate)

0.45 % (w/v) CMC Na-salt in 0.01 M Na-K-phosphate buffer, pH 7.0 was activated with 200 μ M carbodiimide and 50 μ M NHS end-to-end rotating at RT for 2 h. Activated CMC was diluted in 0.01 M Na-K-phosphate buffer, pH 7.0 and mixed with streptavidin solution to yield final concentrations of 0.225 % (w/v) CMC Na-salt and 2 mg/ml streptavidin. The solution was incubated at RT for 17–17.5h. 3 μ l of the solution were spotted onto a 3-APTES coated chip. The chip was incubated in a wet chamber for 30 min. Washing and blocking was performed as described in protocol 1.

2.4. Analyte Detection via Microfluidic Assay

Instead of biological samples a standard solution of purified analyte was used. To exclude artifacts that could have resulted from incomplete dissolution of and mixing with the assay reagents (AuNP-conjugated probe, biotin-tagged analyte binding protein, further additives) the analyte solution was premixed with the respective reagents and incubated briefly before application to the microfluidic cell. Experiments were performed with such assay reagent mixes with an analyte concentration of 342 ng/ml since this was close to the detection limit of the most sensitive chip variants tested. Thus, it allowed distinguishing competitive from less successful immobilization approaches. The chip was placed in a microfluidic cell, the streptavidin-coated side facing a single channel of a width of 6-7 mm and a height of 5–15 μ m. The analyte was premixed with the assay reagents, incubated at RT for 1 min and applied to the microfluidic cell. After 3 min reaction time the assay was stopped by rinsing the chip with dH₂O. The chip was dried and the NP binding density was characterized.

2.5. Silver (Ag) Enhancement

PdNP-conjugated probe and standard human urine were kindly provided as a gift. For grafting the PdNP-conjugate was used at an OD of ~30. L-ascorbic acid (Ph. Eur.) and sodium hydroxide were purchased from Sigma-Aldrich, sodium thiosulfate pentahydrate from Carl Roth, citric acid from Merck Chemicals and silver nitrate from Ögussa. Ag enhancement tests were performed with the same microfluidic setup as used for the analyte detection experiments described in section 2.4.

For determination of the maximum enhancement factor of the established formulation the silver enhancement solution was freshly prepared for each test from stock solutions. Standard human urine was used as solvent. The working concentrations in the enhancement solution were 7.5 mM citric acid, 3 mg/ml NaOH, 2.5 mg/ml sodium thiosulfate, 2.25 mg/ml AgNO₃ and 6 mg/ml ascorbic acid. Pictures of PdNP-grafted REA-chips were taken before Ag enhancement. Then the chips were cut in two in the middle of the grafted area and one piece each was subjected to silver enhancement for 2 min. Pictures were taken and the efficiency of the silver enhancement was determined as described in section 2.7.

2.6. Grafting of REA-Chips with PdNP-conjugate

REA chips were coated with PEI using 1 %, 10 % or 50 % PEI solution in dH₂O to control PdNP-conjugate surface coverage. (Higher PEI concentration in the coating solution results in a higher capacity of the PEI coating to bind the NP-conjugate.) PEI-coated REA chips were spotted with 2 µl PdNP-mediator protein conjugate solution and incubated for 1.5 min in a wet chamber. Excess PdNP-conjugate was removed by rinsing with dH₂O.

2.7. Image Acquisition, Image Processing & Quantification of Signal Intensity

To investigate immobilization homogeneity, NP-analyte complex binding capacity and extent of silver enhancement it was necessary to measure VIS-light absorption at individual spots of the prepared chips. We found that conventional spectrophotometry was not suited for this purpose. Instead, photos of all chips were taken with a digital camera at constant angle, constant distance and constant illumination. Image processing software was used to subject all photos to a white correction. For quantification the color profile of the photos was converted to grayscale (8-bit). To evaluate the efficiency of the screened streptavidin immobilization protocols the gray value (in % black, *i.e.* black = 100%, white = 0%) of 4 spots in the center and 4 spots in the rim of the spotted area and 4 spots in the non-coated area (=background) was determined for each chip. The term “rim” herein refers to the more or less continuous, ring shaped structure at the outline of the spotted area of some chips, which clearly differed from the central region (=center) due higher signal intensity (see e.g. Figure 3(A), top row). Such ring structures were typically encountered on chips coated according to protocol 1. If no such structure was present or if it could not be clearly distinguished from the central area (see e.g. Figure 3(A), second row), the outmost region of the spotted area within 0.5 mm from the outline was considered as rim instead. (The typical diameter of the spotted area ranged from 5 to 8 mm.) Smear patterns, as described in Section 3.1., were not considered in the quantification if they could be clearly distinguished (due to position, shape and color intensity) from the spotted area, the rim and the background. Average values for REA color intensity at rim, center and background were calculated for each chip individually. The background gray intensity was subtracted from the values calculated for rim and center. Then the average values of all chips of one protocol were used to calculate overall average signal intensities and corresponding standard deviations for the individual protocols. For the determination of the enhancement factor of the developed silver enhancement protocol, the gray value (in % black) at two positions in the PdNP-spotted area of each chip was determined before Ag enhancement. The background signal was determined as described for the streptavidin immobilization tests. After Ag enhancement the gray value at the same two positions and the background signal were determined. After background subtraction the enhancement factor (EF) *i.e.* the ratio of the gray values before and after Ag enhancement was calculated.

2.8. AFM Imaging

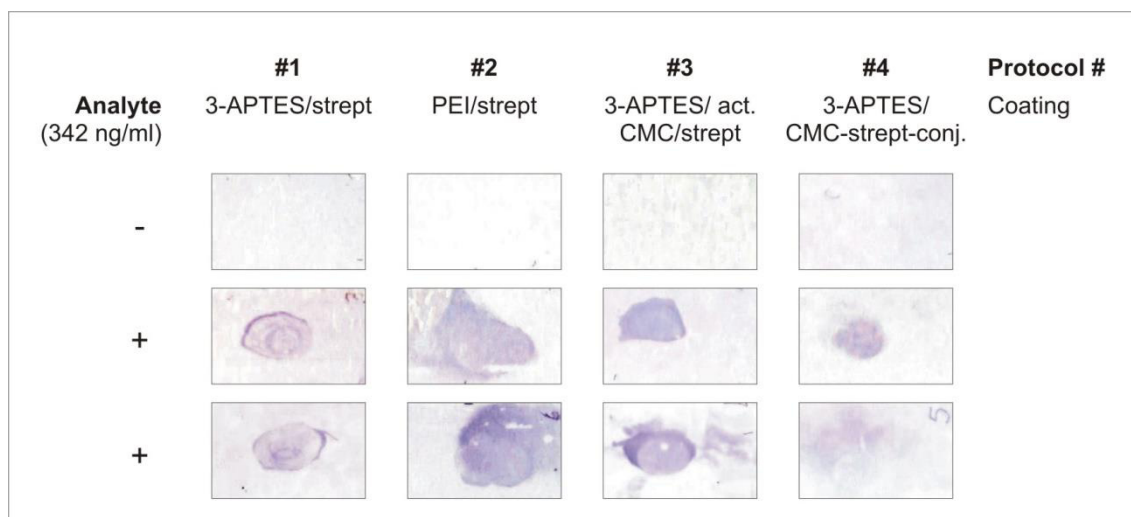
AFM imaging of bare polymer-coated REA chips, chips grafted with PdNP-conjugate and PdNP-conjugate grafted chips after Ag enhancement was performed using a TT-AFM WORKSHOP system with a 15 μm scanner and a Minus-K-vibration-isolation table in contact mode (SICONA-10 cantilevers).

3. Results and Discussion

3.1. Streptavidin Immobilization

Coating protocol 1, *i.e.* adsorption of streptavidin to a 3-APTES coated surface, resulted in high NP-analyte complex binding capacity at the outer rim of the spotted area (see Figures 2 and 3). In contrast, the spot center showed weak REA signal intensity suggesting a low streptavidin surface density. The phenomenon was described earlier in the context of protein microarrays and is supposed to be caused by the selective diffusion of proteins in droplets of protein solutions to the air-solvent-interface [22]. In experiments with other capture proteins we observed that the effect decreased with increasing protein concentrations (>4 mg/ml) and was practically abolished at a protein concentration of 8 mg/ml (unpublished data). Deng *et al.* [22] reported that the addition of detergent to the spotting solution could suppress ring formation in a covalent immobilization approach. However, there are concerns that such additives may interfere with adsorption processes and may thereby reduce the overall immobilization efficiency of adsorptive methods.

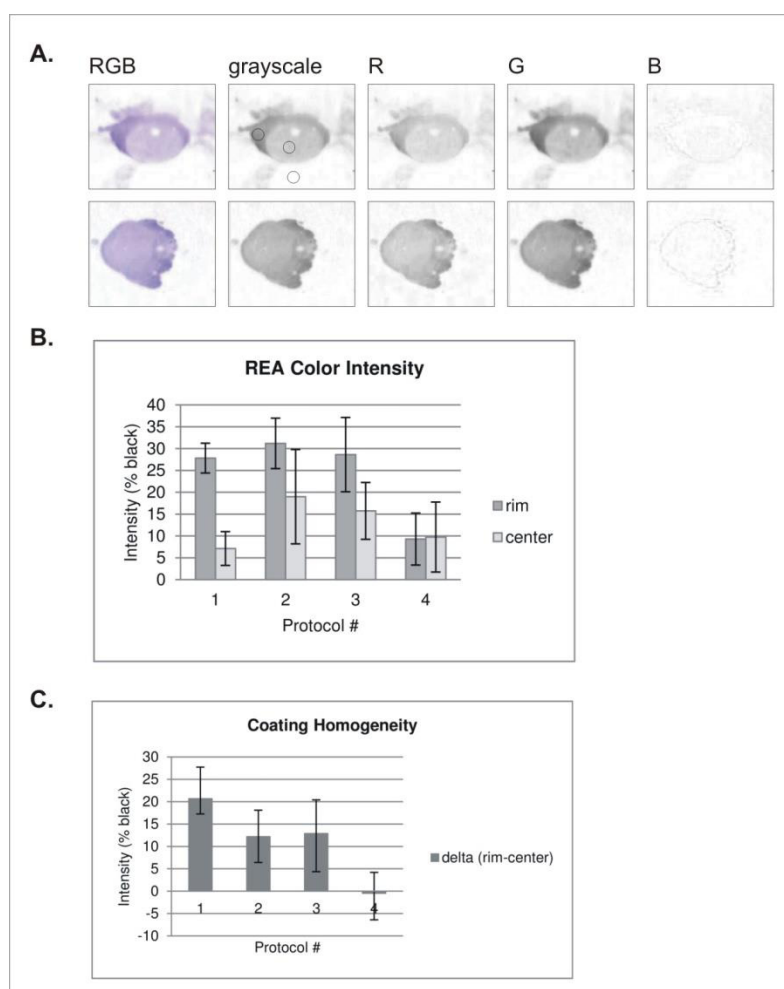
Figure 2. Streptavidin Immobilization - Protocols 1-4 After coating REA chips according to protocols 1-4, the chips were tested in a microfluidic cell with premixed assay reagent solution of constant analyte concentration (342 ng/ml, indicated by '+'). To analyze whether chips exhibited unspecific binding, instead of analyte solution the corresponding pure buffer was added to the assay reagent mix (-).



Chips coated by adsorption of streptavidin to a monolayer of high molecular weight PEI (protocol 2) exhibited a significantly higher NP-analyte complex binding capacity in the center of the spotted area (Figures 2 and 3) than protocol 1 chips, but weak ring formation was also observed. Additionally, at one side of the spotted area the chips generally showed a smear pattern with high signal intensity. Such patterns resulted from the adsorption of streptavidin within the seconds when the spotting solution was washed off the chip. This very brief (unintended) dynamic “application of protein” was apparently more efficient than half an hour of incubation. The effect may be used for other coating techniques, but unfortunately complicates spotting methods. Localized spotting of PEI instead of coating the complete chip cannot be employed to yield cleaner spot shapes either, because smearing is also observed here. Thus, when using coating protocol 2 clean circular spots may currently be achieved best by the use of masks. Preliminary experiments on lamination and lacquering techniques for mask application were performed. The suitability of precut commercial self-adhesive films and a variety of polymer lacquers was investigated. Masking could have improved the reproducibility of the spotting experiments as it would have allowed controlling the size of the contact area between chip and spotted protein solution. However, the tests suggested that components of the analyzed masks could interfere with structure or adsorptive properties of the polymer coatings used for immobilization herein. As such effects could not be excluded for any of the examined masking techniques we refrained from using masking in the present study. Structuring of the chip surface to create physical borders for the spotted solution i.e. by laser engraving was also considered. But any kind of chip surface structuring also affects the flow of the detection solution in the microfluidic test setup. This generally strongly interferes

with the performance of the assay leading to artifacts. It must therefore be stated, that there is currently no means to control the spot size in the studied immobilization techniques. However, spotting experiments were performed as reproducibly as possible. A defined volume of protein solution was spotted on each chip in the same way and the same area was covered during spotting. In the course of the subsequent incubation, the spotted solution tended to spread over the surface as a result of the hydrophilic properties of the chip surface. However, the extent of spreading was always comparable on chips of the same type (i.e. either pronounced – on PEI – or insignificant – e.g. on 3-APTES). Thus the sizes of the analyzed spots created by one and the same immobilization technique were similar even if not completely alike. The conformation of PEI and PEI derivative molecules in SAMs may be determined by adjusting pH and salt concentration of the employed PEI solutions [23]. However, coating with 10 % high molecular weight PEI in dH₂O already allows creating layers of highly globular molecules. Therefore we doubt that the structure or the charge density of the PEI base layer can be significantly optimized further. Solutions of much higher PEI concentrations are not useful for monolayer self-assembly, because their viscosity makes intense washing necessary. It has been observed that this can inflict considerable washing defects to the monolayer.

Figure 3. Quantification of REA Signal Intensity (A) Photos of REA chips after analyte detection are shown. For quantification the color profile of the images was converted to grayscale (8-bit) and the gray values at rim and center of the spotted area as well the value of the background were determined (indicated by black circles, for more detail see Materials & Methods). Quantification of the grayscale image was considered to correspond best with the actual REA color intensity and thus was preferred over measuring the contribution of one of the three channels red (=R), green (=G) and blue (=B). (B) REA color intensities at the rim and center of the spots on the tested chips prepared by protocols 1-4 are shown in % black (black = 100 %, white = 0%) (protocols 1 & 2: n = 4; protocols 3 & 4: n = 6). (C) The differences (delta) of average REA color intensities at rim and center [delta = intensity(rim)-intensity(center)] are shown in % black.

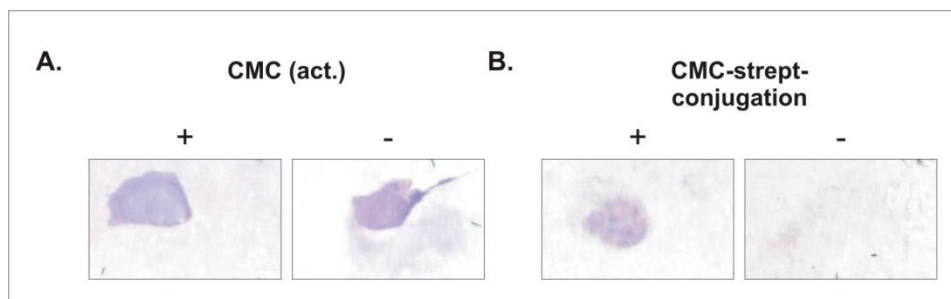


Layer-by-layer coating of 3-APTES and CMC followed by carbodiimide-mediated covalent protein immobilization (protocol 3) yielded spots with considerably high NP-analyte complex binding capacity. Ring formation was occasionally observed but was in general less pronounced than on chips coated according to protocol 1. However, the requirement of the CMC interlayer is doubtful. By incubation of streptavidin on 3-APTES coated chips without CMC interlayer for 17.5 h protein coatings with very good NP-analyte capture ability could

be created. The results were practically indistinguishable from the chips coated according to protocol 3 (see Figure 4(A)). In both approaches commonly thickening of the spotted coating solution occurred due to evaporation during incubation and in some cases even gel formation was observed. Most probably this led to increased protein concentrations in the coating solutions allowing more homogeneous immobilization - supposedly rather by unspecific adsorption than by covalent binding. However, protein immobilization via drying is generally not recommended. On the one hand, it bears the risk of protein denaturation. On the other hand, experiments with nitrocellulose coated chips showed that fast drying of the spotted solution entails deposition of salts (buffer components), which interferes with adsorptive immobilization and thus prevents homogeneous protein coating.

Protein immobilization protocol 4, conjugation of streptavidin to CMC in solution followed by monolayer self-assembly of the conjugate, is significantly less efficient and less reproducible than the three previously discussed approaches. Streptavidin immobilization is occasionally highly localized but sometimes the streptavidin binding sites appear to be distributed over large areas of the chip in a smear pattern. In a control experiment the CMC-streptavidin conjugate solution was replaced by a mix of non-conjugated CMC and streptavidin at the same concentrations and coating according to protocol 4 was performed. The resulting chips exhibited no visible NP-analyte complex binding capacity (see Figure 4(B)). Adsorption of free streptavidin molecules was obviously prevented by competition with CMC for positively charged groups on the chip surface. It can be concluded that streptavidin immobilization via protocol 4 is not the result of mere adsorption of streptavidin molecules. Efficient CMC-streptavidin crosslinking is critical for this approach and variations in the extent of CMC-streptavidin crosslinking in solution might be the reason for the poor reproducibility of this method.

Figure 4. Control Experiments - Protocols 3 & 4 (A) REA chip coated with streptavidin according to protocol 3 (left). 3-APTES-coated REA chip spotted with 2 mg/ml streptavidin solution and incubated at RT in a wet chamber for 17.5 h (right). Analyte detection was performed on both chips. AuNP-analyte complex binding capacity and the homogeneity of the binding was comparable. (B) REA chip coated with streptavidin according to protocol 4 (left). 3-APTES-coated REA chip spotted with 2 mg/ml streptavidin + 0.225 % CMC in 0.01 % Na-K-phosphate buffer, pH 7.0 and incubated at RT in a wet chamber for 30 min (right). Analyte detection was performed on both chips. The chip coated according to protocol 4 exhibited significantly higher AuNP-analyte complex binding.

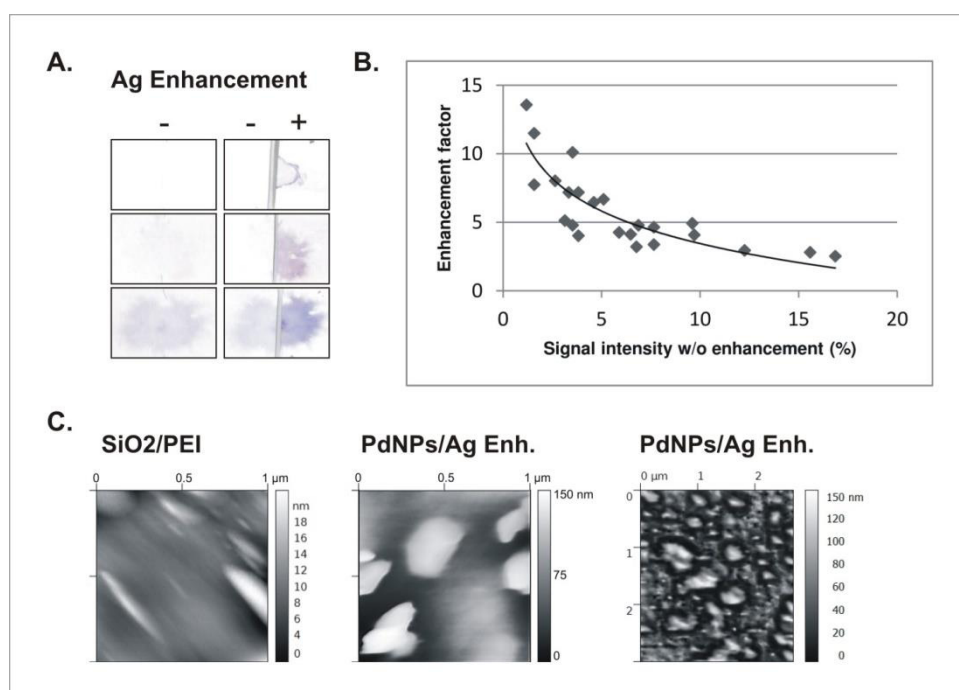


3.2. Chloride-resistant in-situ Silver Enhancement

In search of silver staining reagents with good performance in chloride-containing solutions several established protocols and known complexing agents for silver salts were screened including Tris base and histidine. Primarily, reagents were considered, which exhibit good solubility in aqueous solutions. This is most critical for the establishment of a robust in-situ enhancement technology as all reagents have to be provided in the assay platform in a stable form (preferably dry) and it must be possible to release them by addition of the sample without significant further manipulation of the test device. Regarding the stabilization of silver ions against precipitation with chloride, most promising results were achieved by the use of sodium thiosulfate. It was found that silver nitrate could be dissolved in a human urine sample (with a typical chloride concentration of 20 mM) by buffering it with citrate and addition of sodium thiosulfate to a concentration roughly equivalent to the desired molar silver nitrate content. At pH<7 such a solution was found to be stable for at least 1h. For Ag enhancement the mixture had to be activated by addition of a reducing agent. Glucose and ascorbic acid were considered as potential candidates here because both easily and quickly dissolve in water. Finally ascorbic acid was selected for it allowed good control of the reduction reaction. It was further observed that the efficiency and speed of the silver enhancement process and the stability of the solution strongly depended on the pH-value. We were interested in a fast enhancement process and therefore optimized an alkaline formulation (see Materials and Methods). Eventually, a silver enhancement protocol could be established, which employs exclusively highly water soluble reagents that can all be provided in an assay device in dry form: thiosulfate, citric acid and sodium hydroxide for stabilization and buffering and ascorbic acid as reducer. The efficiency of the developed formulation should be

characterized on REA-chips sparsely grafted with catalytic metal nanoparticles. Already at the start of the screening it had turned out that the AuNP-conjugated probe employed for the screening of streptavidin immobilization techniques did not act as good catalyst for silver reduction (independent of the tested enhancement protocol). The AuNP-conjugate was strongly stabilized against precipitation and unspecific binding (by inert proteins and polymers) which might have suppressed its interaction with the silver enhancement reagents. Therefore a less shielded PdNP-conjugate was used instead, which exhibited significantly higher catalytic activity but equivalent performance in bioassay applications. REA-chips were pre-coated with PEI and grafted with PdNP-conjugate by spotting. Chips with low, medium and high PdNP density were created. The silver enhancement reagents were dissolved in urine and the PdNP-grafted REA-chips were treated with this solution in a microfluidic cell for 2 min. The enhancement efficiency was determined by comparing REA color intensities before and after silver staining. (Only chips exhibiting a visible REA color spot before enhancement were considered in the quantification.) It could be demonstrated that the enhancement factor was especially high for weak signals i.e. if the PdNP density on the chip was low. Even very sparse and therefore invisible PdNP coatings exhibited a clearly visible REA color after treatment of the corresponding chip with the enhancement solution (Figure 5(A)). At low PdNP surface coverage an enhancement factor of up to 13 was observed (Figure 5(B)). With increasing PdNP density the signal enhancing effect decreased logarithmically. However, this is not considered too critical because visibility of the REA signal was already good for those PdNP coatings even before enhancement. AFM imaging revealed that the precipitated silver layer is not smooth but rather crystalline. This also resulted in increased light scattering under very bright illumination, which was not observed in the PdNP-grafted areas before enhancement. Generally the crystalline morphology is not problematic and does not diminish the signal enhancing effect of the silver coating. However, it is an indication that the enhancement reaction still requires fine tuning. Optimally stabilized silver enhancement yields slightly granular to smooth surfaces. Nevertheless, the performance of the developed silver enhancement formulation is competitive and, most importantly, it fulfills all requirements for the integration into microfluidic devices.

Figure 5. Thiosulfate-stabilized Silver Enhancement (A) PEI-coated REA chips grafted with PdNP-conjugated probe (low, medium and high surface coverage) before Ag enhancement (-) (left). The chips were cut in two and one half each was subjected to Ag enhancement (+) (right). (B) The enhancement factor (EF) strongly depended on the signal intensity before silver enhancement (i.e. the PdNP coverage density) ($EF = [\text{signal after Ag enhancement in \% black}]/[\text{signal before Ag enhancement in \% black}]$). (C) AFM Scans of REA chips before PdNP grafting (left) and after PdNP grafting and Ag enhancement (middle & right) (The grafted PdNP-conjugate could not be imaged due to the inherent roughness of the chip surface and the polymer layer and the small size of the PdNPs of ≤ 20 nm.)



4. Conclusions

4.1. Streptavidin Immobilization

Four different protocols for streptavidin immobilization on REA chips could be established and optimized. The techniques employ different chemistry and are based on two different immobilization approaches: adsorption and covalent immobilization respectively. They were compared regarding coating homogeneity and analyte capture capacity of the prepared protein layers. It was found that by adsorption to a monolayer of high molecular weight PEI the most homogeneous coatings could be generated within a short time. Thus, this method may be best suited for large scale chip manufacture. The only drawback of the approach was smear formation, which prevented the grafting of well-shaped protein spots. By addition of blocking

agents to the washing solutions or by use of a mask such random adsorption during the washing step effect may possibly be suppressed. Covalent immobilization to CMC monolayers (protocol 3) also yielded good results but turned out to be much more time consuming. Furthermore, control experiments suggested that immobilization was achieved at least in part by unspecific adsorption rather than by covalent binding and that the CMC base layer was not strictly required. In general, it was observed that linkers with pronounced 3D-structure are not absolutely necessary to generate protein layers with occasionally high analyte binding capacity. However, pre-coating with charged polymers significantly accelerated adsorption of proteins with opposite charge and has the potential to significantly improve capture protein density and coating homogeneity if short immobilization times are required.

4.2. Chloride-resistant in-situ Silver Enhancement

Silver enhancement reagents could be identified, which operate even in the presence of chloride and can thus be employed for silver staining in samples such as urine, blood or saliva. All required reagents are water-soluble and can be provided in dry form in a microfluidic cell. They can be released upon sample addition (preferably with delay) to react with the captured analyte-nanoparticle complexes on the REA-chip and enhance the assay signal. Initial tests in this context have already been performed. For this purpose the enhancement reagents were dried on hydrophilized polymer fleeces and integrated into a laser-structured microfluidic cell as described in Assadollahi *et al.* [8]. Although mixing of the reagents was not optimal, good signal enhancement could be achieved. Thus, optimization of release and mixing of the reagents will be the focus of future efforts towards this kind of integrated enhancement system.

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The authors declare that there is no conflict of interests regarding the publication of this paper.

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Chloride-resistant In-situ Silver Enhancement for One-step Microfluidic Bioassays

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INTRODUCTION

Resonance enhanced absorption (REA) nanocolor microfluidic devices belong to a group of new immunoassay platforms that rely on the biorecognition between an analyte of interest and a nanoparticle-conjugated probe and the binding of this complex to a biochip. The intensity of the as generated optical signal may be boosted by silver enhancement i.e. nanoparticle-catalyzed electroless deposition of metallic silver from solution [1]. However, ionic silver in the staining solution tends to precipitate upon mixing with biologic samples, for these generally contain high concentrations of chloride. Thus, preliminary to enhancement washing of the test device is commonly required. This complicates handling and is not acceptable for point-of-care assays. Here we report the development of a silver enhancement technology that operates even in the presence of high chloride concentrations as may be encountered in biologic samples. The silver enhancement reagents may be integrated into the microfluidic assay platform to be released upon sample addition. Hereby a highly sensitive one-step-assay can be realized.

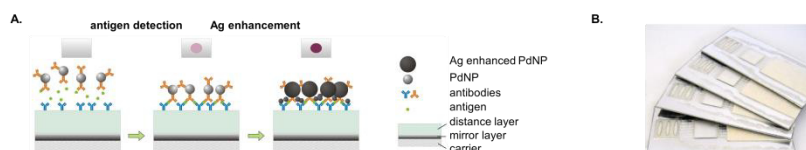


Figure 1 Resonance enhanced absorption (REA) nanocolor microfluidic devices A) Detection of antigen on REA biochip followed by silver enhancement to increase signal intensity B) Exemplary layout of REA microfluidic devices

METHODS

In search of silver staining reagents with good performance in chloride-containing solutions such as urine several buffering, complexing and reducing agents were screened. To allow the establishment of a robust in-situ one-step enhancement technology all reagents have to be provided in the assay platform in a stable form (preferably dry) and it must be possible to release them by addition of the sample without significant further manipulation of the test device. Thus, primarily reagents with good solubility in aqueous solutions were considered. Eventually the stabilization of silver ions against precipitation with chloride was best achieved by complexation with sodium thiosulfate in a citrate-buffered solution. Ascorbic acid was selected as reducing agent for it allowed good control of the silver deposition. The speed of the silver enhancement process and the stability of the solution depend strongly on the pH-value. We were interested in a fast enhancement process and therefore optimized an alkaline formulation.

The efficiency of the developed formulation was characterized on REA-chips sparsely grafted with antibody-conjugated palladium nanoparticles (PdNPs). Chips with low, medium and high PdNP density were prepared. The silver enhancement reagents were dissolved in urine (chloride concentration: 20mM) and the PdNP-grafted REA-chips were treated with this solution in a microfluidic cell for 2 min.

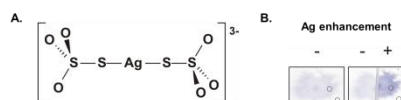


Figure 2 A) Thiosulfate complex of Ag^+ B) Quantification of enhancement efficiency The enhancement efficiency was determined by comparing REA color intensities before and after silver staining relative to the background color of the respective chips (as indicated by circles). (Only chips exhibiting a visible REA color spot before enhancement were considered in the quantification.)

The release of the enhancement reagents from dry form was tested using a specifically adapted microfluidic cell layout. The reagents were provided in two reservoirs on fleeces.

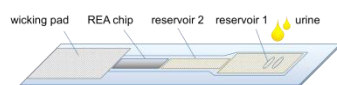


Figure 3 Microfluidic cell layout for reagent release tests

CONCLUSIONS

Aiming at the development of an integrated enhancement solution for one-step microfluidic REA devices we established a silver staining formulation that operates even at chloride concentrations of up to 20mM. All required reagents including buffer components, silver salt, complexants and reducer may be provided in test devices in dry form and may be released upon sample addition. An appropriate layout of the microfluidic ducts and the reagent reservoirs is most critical for timely dissolution and mixing and thus the determining factor for the successful implementation of the silver staining technology in one-step systems.

REFERENCES

- [1] Assadollahi S, Reininger C, Palkovits R, Pointl P, Schalkhammer T. Sensors, 9, 6084-6100 (2009)

RESULTS

It could be demonstrated that the enhancement efficiency of the developed formulation was especially high for weak signals i.e. if the PdNP density on the chip was low. Even very sparse and therefore invisible PdNP coatings exhibited a clearly visible REA color after treatment of the corresponding chip with the enhancement solution.

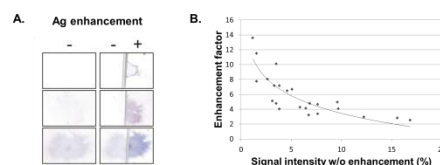


Figure 4 Enhancement efficiency A) PdNP-grafted REA chips before and after Ag enhancement B) The enhancement factor (EF) strongly depended on the signal intensity before silver enhancement. At low PdNP surface coverage an enhancement factor of up to 13 was observed. With increasing PdNP density the signal enhancing effect decreased logarithmically.

In-situ enhancement tests proved that all selected enhancement reagents can be provided in a microfluidic device in dry form and can be released by addition of an aqueous sample such as urine. However, the mixing of the reagents within the cell critically determines the enhancement quality.

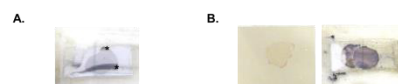


Figure 5 Release tests A) All enhancement reagents were provided in the microfluidic cell in dry form and were released by addition of urine. Incomplete mixing of the reagents in the microfluidic cell resulted in inhomogeneous enhancement (enhanced areas marked with asterisks). B) Only the reducing agent was provided in the microfluidic cell. Silver salts, stabilizers and buffering agents were pre-dissolved in urine. The signal intensity on the silver enhanced chip (right) was significantly higher than on the untreated negative control (left).