

MASTERARBEIT | MASTER'S THESIS

Titel | Title

Engineered Mesoporous Silica Nanoparticles as Drug Carriers for Sublingual
Delivery

verfasst von | submitted by

Sara Hoxhallari BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of
Diplom-Ingenieurin (Dipl.-Ing.)

Wien | Vienna, 2026

Studienkennzahl lt. Studienblatt |
Degree programme code as it appears on the
student record sheet:

UA 066 658

Studienrichtung lt. Studienblatt | Degree
programme as it appears on the student
record sheet:

Masterstudium Chemie und Technologie der
Materialien

Betreut von | Supervisor:

Univ.-Prof. Dr. Freddy Kleitz

Abstract

Sublingual drug delivery offers a non-invasive alternative for rapid systemic drug absorption, avoiding gastrointestinal degradation and hepatic metabolism. However, the short drug residence time at the absorption site, the low salivary volume required to dissolve the drug, and stability constraints limit the range of compounds amenable to sublingual administration. Mesoporous silica nanoparticles (MSNs) are promising drug-delivery carriers due to their high surface area, tunable particle size, porosity, and versatile surface chemistry. In this work, dendritic mesoporous silica nanoparticles (DMSNs) were engineered as potential carriers for sublingual drug delivery as a strategy to improve drug solubility, protect the drugs from enzymatic degradation and decrease cytotoxicity. DMSNs of 50 nm (**DMSN-50**) and 130 nm (**DMSN-130**) were synthesized to evaluate the effect of particle size interaction with buccal epithelial cells. To improve colloidal stability and ensure biocompatibility, **DMSN-50** and **DMSN-130** were functionalized with phosphonate moieties (**DMSN-50-PO3** and **DMSN-130-PO3**) or alternatively coated with liposomes composed of phosphatidylcholine, cholesterol, and PEGylated lipids (**L-DMSN-50** and **L-DMSN-130**). The physicochemical properties of the functionalized particles were characterized, and their biological performance was evaluated using *in vitro* cell viability and uptake studies, demonstrating improved cell viability upon treatment with functionalized particles, particularly those with lipid coatings, in agreement with efficient cell-particle interactions and potential internalization. To further address concerns about the limited biodegradability of silica nanocarriers, glutathione (GSH)-responsive materials were synthesized by incorporating tetrasulfide bridges into the silica framework, yielding mesoporous organosilica nanoparticles (MOSNs) and dendritic organosilica nanoparticles (DMOSNs). In order to promote particle degradation after drug release, these GSH-responsive linkages were designed to respond to high intracellular GSH concentrations within cells. At elevated GSH levels, mimicking intracellular conditions, the non-functionalized DMSNs remained as stable colloidal suspensions while the MOSNs and DMOSNs exhibited GSH-responsive aggregation, correlating with particle deformation and potential biodegradability. Overall, this study highlights the importance of structural and surface engineering of DMSNs nanocarriers to enhance their performance in sublingual drug delivery applications, as well as presenting a novel synthesis strategy for incorporating tetrasulfide bridges into dendritic mesoporous silica particles, leading to redox-responsive framework particles that may contribute to improved safety and degradability of silica nanocarriers in sublingual drug delivery applications.

Kurzfassung

Die sublinguale Arzneistoffapplikation stellt eine nicht-invasive Alternative zur raschen systemischen Wirkstoffresorption dar, da sie sowohl die gastrointestinale Degradation als auch den hepatischen First-Pass-Metabolismus umgeht. Allerdings begrenzen die kurze Verweildauer des Wirkstoffs am Resorptionsort, das geringe zur Auflösung verfügbare Speichelvolumen sowie Stabilitätsanforderungen die Anzahl geeigneter Wirkstoffe für die sublinguale Anwendung. Mesoporöse Silika-Nanopartikel (MSNs) gelten aufgrund ihrer hohen spezifischen Oberfläche, einstellbaren Partikelgröße, definierten Porosität und vielseitigen Oberflächenfunktionalisierung als vielversprechende Wirkstoffträgersysteme. In der vorliegenden Arbeit wurden dendritische mesoporöse Silika-Nanopartikel (DMSNs) als potenzielle Trägersysteme für die sublinguale Wirkstofffreisetzung entwickelt, mit dem Ziel, die Wirkstofflöslichkeit zu verbessern, die Substanzen vor enzymatischer Degradation zu schützen und die Zytotoxizität zu optimieren. DMSNs mit Partikelgrößen von 50 nm (**DMSN-50**) und 130 nm (**DMSN-130**) wurden synthetisiert, um den Einfluss der Partikelgröße auf die Interaktion mit bukkalen Epithelzellen zu untersuchen. Zur Verbesserung der kolloidalen Stabilität und Biokompatibilität wurden **DMSN-50** und **DMSN-130** entweder mit Phosphonat-Gruppen funktionalisiert (**DMSN-50-PO₃** und **DMSN-130-PO₃**) oder alternativ mit Liposomen beschichtet, bestehend aus Phosphatidylcholin, Cholesterin und PEGylierten Lipiden (**L-DMSN-50** und **L-DMSN-130**). Die physikochemischen Eigenschaften der funktionalisierten Partikel wurden umfassend charakterisiert, und ihre biologische Performance wurde mittels In-vitro-Zellviabilitäts- und Aufnahmeuntersuchungen evaluiert. Dabei zeigte sich eine verbesserte Zellviabilität nach Behandlung mit funktionalisierten Partikeln, insbesondere mit lipidbeschichteten Systemen, was mit effizienten Zell-Partikel-Interaktionen und einer potenziellen Internalisierung korrelierte. Zur weiteren Adressierung der limitierten Biodegradierbarkeit silikabasierter Nanocarrier wurden Glutathion (GSH)-responsive Materialien durch Einbau von Tetrasulfid-Brücken in das Silikanetzwerk synthetisiert, wodurch mesoporöse Organosilika-Nanopartikel (**MOSNs**) und dendritische Organosilika-Nanopartikel (**DMOSNs**) entstanden. Diese GSH-sensitiven Bindungen wurden so konzipiert, dass sie auf erhöhte intrazelluläre GSH-Konzentrationen reagieren und dadurch nach der Wirkstofffreisetzung eine Partikeldegradation begünstigen. Unter Bedingungen erhöhter GSH-Konzentrationen, die intrazelluläre Verhältnisse simulieren, verblieben nicht-funktionalisierte DMSNs als stabile kolloidale Suspensionen, während MOSNs und DMOSNs eine GSH-induzierte Aggregation zeigten, die mit Partikeldeformation und potenzieller Biodegradierbarkeit korrelierte. Zusammenfassend unterstreicht diese Studie die Bedeutung einer gezielten strukturellen und oberflächenchemischen Modifikation dendritischer mesoporöser Silika-Nanocarrier zur Optimierung ihrer Leistungsfähigkeit in der sublingualen Wirkstoffapplikation. Darüber hinaus wird eine neuartige Synthesestrategie zur Integration von Tetrasulfid-Brücken in dendritische mesoporöse Silikapartikel vorgestellt, wodurch redox-responsive Gerüstpartikel generiert werden, die zu einer verbesserten Sicherheit und Abbaubarkeit silikabasierter Nanocarrier in der sublingualen Arzneistofffreisetzung beitragen können.

Acknowledgement

I would like to sincerely thank Univ.-Prof. Dr. Freddy Kleitz for giving me the opportunity to be part of his research group in the Department of Functional Materials and Catalysis and for providing such an inspiring and supportive research environment.

I am especially grateful to my supervisor, Dr. Claudia Iriate-Mesa, for her continuous support, guidance, and mentorship throughout this thesis. Her valuable advice, constructive feedback, and encouragement were essential for the successful completion of this work.

I would also like to thank the Institute of Pharmaceutical Sciences at the University of Graz for the valuable collaboration. In particular, Dr. Atida Selmani, under whose supervision the experimental part of the liposome coating was carried out. I truly appreciate her support and guidance with the *in vitro* cell studies, which were crucial to this work. I would also like to express my gratitude to Univ.-Prof. Mag. pharm. Dr. rer. nat. Eva Roblegg for enabling this collaboration and for her support.

I would also like to thank the NMR Facility at the Faculty of Chemistry, University of Vienna, for providing access to the instrumentation and for their technical support. Furthermore, I gratefully acknowledge the Faculty Center for Nanostructure Research for the Raman measurements, especially Ass.-Prof. Dipl.-Ing. Dr. Dieter Baurecht for his support. I also thank Univ.-Prof. Mag. Dr. Herwig Peterlik for performing the small-angle X-ray scattering (SAXS) measurements and for his assistance.

My sincere thanks also go to all members of the Kleitz research group for their support, the scientific discussions, and the friendly working atmosphere.

Finally, I would like to thank my family and friends for their constant support, encouragement, and patience throughout this journey. Their belief in me has meant more than words can express.

Table of Contents

Abstract	i
Kurzfassung.....	iii
Acknowledgement	iv
List of Abbreviations.....	vii
1. Introduction	1
1.1 Peroral Drug Delivery Systems	2
1.2 Mesoporous Silica Nanoparticles in Drug Delivery	4
1.2.1 Tailored Nanocarriers for Enhanced Oral Bioavailability	4
1.2.2 MSNs for Efficient Drug Delivery	5
1.2.3 Synthesis Strategies for MSNs	6
1.2.3.1 Synthesis of DMSNs	8
1.2.3.2 Tailoring MSNs' Properties	8
1.2.3.3 Surface Functionalization.....	9
1.3 Liposomes in MSN-Based Drug Delivery Systems	11
1.3.1 Liposome-coated DMSNs	11
1.3.2 Cell Studies.....	12
1.4 Biocompatibility of MSNs	13
1.4.1 In Vivo Fate of MSNs: Distribution, Degradation, and Clearance	13
1.4.2 Strategies for Designing Biocompatible Nanocarriers	14
2. Objectives.....	16
3. Methods.....	17
3.1 Reagents and Materials	17
3.2 Material Synthesis.....	17
3.2.1 Synthesis of DMSNs at Room Temperature (DMSN-50)	17
3.2.2 Synthesis of DMSNs at 60°C (DMSN-130)	18
3.2.3 Functionalisation with THMP	18
3.2.3 Liposome Coating	18
3.2.3.1 Liposome Coating of DMSNs via Co-hydration with Liposome Thin-film	19
3.2.3.2 Cell Culture Studies	19
3.2.3.3 Rhodamine Labelling and Cellular Uptake Studies	20
3.2.4 Synthesis of MOSNs	21
3.2.5 Synthesis of DMOSNs	22
3.2.6 Biodegradability Studies	23
3.3 Material Characterization	24
3.3.1 Infrared (IR)- Spectrometry.....	24

3.3.2 Zeta Potential and Hydrodynamic diameter	24
3.3.3 Nitrogen Physisorption Isotherms (77K)	24
3.3.4 Thermogravimetric Analysis (TGA)	24
3.3.5 Solid-state Magic Sngle Spinning Nuclear Magnetic Resonance Spectroscopy (MAS NMR)	25
3.3.6 Transmission Electron Microscopy (TEM)	25
3.3.7 Confocal Raman Microscopy	25
3.3.8 Small-angle X-Ray Scattering (SAXS)	25
4. Results and Discussion	25
4.1 Synthesis and Functionalization of DMSNs	25
4.1.1 Synthesis of Dendritic MSNs (DMSNs)	26
4.1.2 Functionalization of DMSNs with THMP	28
4.1.3 Coating DMSNs with Liposomes	34
4.1.4 Cell Studies of the Liposome-coated DMSNs.....	40
4.1.4.1 MTS cell viability.....	40
4.1.4.2 Rhodamine Labelling and Cellular Uptake.....	42
4.2 Synthesis of Disulfide-bridged MSNs	46
4.2.1 Synthesis of MOSNs	46
4.2.2 Synthesis of DMOSNs	50
4.2.3 Biodegradability Studies.....	56
5. Conclusion and Outlook.....	59
5.1 Effect of Particle Size and Surface Chemistry on Particle-Cell Interactions	59
5.2 Development of Redox-Responsive Dendritic Organosilica Nanoparticles (DMOSNs)	59
5.3 Future Directions in the Development of Safer and Functional Dendritic Silica Nanocarriers	60
References	62

List of Abbreviations

ATR	Attenuated Total Reflectance
S_{BET}	Brunauer–Emmett–Teller specific surface area
BSA	Bovine Serum Albumin
BTES	Bis[3-(triethoxysilyl)propyl]tetrasulfide
BTEPDS	Bis(3-(triethoxysilyl)propyl)disulfide
CHO-HP	Cholesterol
CTAB	Cetyltrimethylammonium Bromide
CTAC	Cetyltrimethylammonium Chloride
ConA	Concanavalin A
CS-95	Phosphatidylcholine
DAPI	4',6-Diamidino-2-phenylindole
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle Medium
DMSN	Dendritic Mesoporous Silica Nanoparticles
DMOSN	Dendritic Mesoporous Organosilica Nanoparticles
DSPE-	1,2-distearoyl-sn-glycero-3-phosphoethanolamine- <i>N</i> -
PEG2000	[methoxy(polyethylene glycol)-2000]
DMHA	N,N-dimethylhexadecylamine
EDTA	Ethylendiaminetetraacetic acid
EtOH	Ethanol
FC4	Sodium Heptafluorobutyrate
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared Spectroscopy
GSH	Glutathione
GSSG	Oxidized Glutathione
HA	Hyaluronic Acid
HBSS	Hank's Balanced Salt Solution
HCl	Hydrochloric Acid
HOMO	High-Pressure Homogenization
IR	Infrared
LbL	Layer-by-Layer

LC-MSN	Liposome-Coated Mesoporous Silica Nanoparticles
LDH	Lactate Dehydrogenase
MAS NMR	Magic Angle Spinning Nuclear Magnetic Resonance
MOSN	Mesoporous Organosilica Nanoparticles
MSN	Mesoporous Silica Nanoparticles
MPTMS	3-mercaptopropyltrimethoxysilane
MTES	Methyltriethoxysilane
MTS	(3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
NaOH	Sodium Hydroxide
NEAA	Non-essential amino acid
NLDFT	Non-Local Density Functional theory
PBS	Phosphate Buffered Saline
PDI	Polydispersity Index
PEG	Polyethylene Glycol
PLGA	Poly (lactic-co-glycolic acid)
PS	Phosphatidylserine
SAXS	Small Angle X-Ray Scattering
SLN	Solid Lipid Nanoparticles
SUV	Small Unilamellar Vesicles
TEA	Triethanolamine
TEOS	Tetraethyl Orthosilicate
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
THMP	3-(Trihydroxysilyl)propyl Methylphosphonate
TR146	Human Buccal Epithelial Cell Line

1. Introduction

The sublingual route offers a compelling non-invasive strategy to enhance drug bioavailability by enabling rapid systemic absorption through the highly vascularized oral mucosa while bypassing gastrointestinal degradation and first-pass hepatic metabolism¹. Despite these advantages, its clinical application remains limited by physiological constraints, including the limited salivary volume available for drug dissolution, the short residence time due to continuous saliva washout, and the poor aqueous solubility and/or insufficient permeability of many drugs across the mucosal membrane. Furthermore, salivary enzymes and local pH conditions may compromise the stability of sensitive therapeutics, thereby restricting the applicability and therapeutic success of sublingual formulations to a narrow range of drug candidates, primarily small, moderately lipophilic molecules^{2,3}. These challenges underscore the need to develop advanced carrier systems that enhance local drug dissolution, prolong residence time, and improve biological compatibility without compromising safety, particularly for poorly water-soluble drugs and labile macromolecules such as peptides and proteins⁴.

Mesoporous silica nanoparticles (MSNs) are promising carriers for sublingual delivery applications due to their high surface area, tunable pore structure, and chemically versatile surface, which enable efficient drug loading, controlled release, and tailored surface functionalization to enhance mucosal interaction and cargo stability⁵. However, their performance in delivery formulations is strongly dependent on the precise control over particle size, pore architecture, and surface chemistry^{6,7}. The overall objective of this Master's thesis was to engineer MSNs nanocarriers with physicochemical properties suitable for sublingual delivery. The first part of this work focused on the synthesis of dendritic mesoporous silica nanoparticles (DMSNs), which are characterized by a radially oriented pore system. This open dendritic architecture offers high pore accessibility and large pore volumes, making DMSNs particularly attractive as carriers for both small molecules and larger biomolecules. By adjusting synthesis parameters, including temperature and the use of organic co-solvents and pore-swelling agents, particles of two sizes were obtained: 50 nm DMSNs (**DMSN-50**) and 130 nm DMSNs (**DMSN-130**). This enabled evaluation of the influence of particle size on the interactions of the DMSNs carriers with buccal epithelial cells.

After synthesis, surface functionalization strategies were implemented to address key limitations of pure DMSNs, like aggregation in physiological media and limited interactions with biological systems^{8,9}. First, phosphonate groups were introduced via post-synthetic grafting to improve colloidal stability while maintaining pore accessibility. Second, the particles were coated with liposomes composed of phosphatidylcholine, cholesterol, and PEGylated lipids. The liposome coating was included in the design to provide an interface similar to cell-membrane, leading to improved interactions with epithelial cells. The performance of the functionalized materials was evaluated *in vitro* using human epithelial cells (TR146 cells), demonstrating that surface engineering, including lipid coating, significantly enhanced the biological performance of the DMSNs compared to non-functionalized materials, including cellular uptake and metabolic activity.

The second part of this thesis focused on overcoming the limited biodegradability of DMSNs by developing redox-responsive organosilica analogues, thereby addressing a critical limitation that hinders the broader biomedical applications of silica-based delivery systems¹⁰. Tetrasulfide-

bridged mesoporous organosilica nanoparticles (MONs) were synthesized by co-condensation of tetraalkoxysilane precursors with sulfide-containing organosilanes, which enabled the introduction of cleavable bonds directly into the silica framework. This approach was further extended to the synthesis of novel dendritic mesoporous organosilica nanoparticles (DMOSNs) with large pores, which are required for potential high-weight biomolecule loading. The inclusion of sulfide bridges was motivated by safety considerations, aiming to destabilize the silica network under reducing conditions, such as elevated glutathione (GSH) levels. This is expected to improve the toxicological profile of MSNs, thereby supporting their safer and more effective translation into clinical drug delivery applications.

Before discussing the results of the tailored modifications of the DMSNs, a detailed understanding of the sublingual route itself is required. The following section therefore shows the key physiological features, advantages, and limitations of sublingual drug delivery.

1.1 Peroral Drug Delivery Systems

Delivering drugs effectively within the body depends strongly on both the physicochemical properties of the therapeutic agent and the chosen route of administration. For example, significant challenges arise when drugs exhibit poor aqueous solubility, limited membrane permeability, or chemical instability under physiological conditions. In these cases, selecting an appropriate administration route is critical for achieving effective systemic exposure. Pharmacokinetics describes how the body affects the drug after administration. This includes absorption, distribution, metabolism, and excretion¹¹. Thus, the choice of administration route plays a crucial role in determining a drug's pharmacokinetics. These routes can be broadly classified into parenteral routes (e.g., intravenous, intramuscular, subcutaneous) and non-invasive routes (e.g., oral, sublingual, buccal, topical, nasal, and rectal)¹². Among these, oral drug delivery remains the preferred method for systemic drug administration due to several advantages, including ease of administration and self-administration, which enhance patient compliance¹³. Furthermore, oral dosage forms are cost-effective and easy to produce on a large scale¹⁴. Despite these benefits, oral drug delivery routes face some challenges, particularly for drugs with poor gastrointestinal permeability or chemical instability, compared to parenteral routes, which provide higher bioavailability. This is because of the physicochemical challenges imposed by the gastrointestinal tract, including harsh stomach conditions, enzymatic degradation, intestinal mucus, and tight junctions. By the end of this process, a big portion of the drug is modified or inactivated, and only a small percentage of the drug actually reaches the systemic bloodstream¹⁵. In addition, the oral route is affected by the so-called first-pass metabolism, which occurs in the liver and intestinal walls. Due to the presence of hepatic and intestinal enzymes, the drug undergoes oxidation, reduction, or hydrolysis, usually carried out by cytochrome P450 enzymes¹⁶, and then conjugation reactions, where it is metabolized to water-soluble derivatives for elimination.

In contrast, the sublingual or buccal route, while remaining patient-friendly, ensures that drug absorption occurs directly through the oral mucosa, enabling systemic delivery while avoiding first-pass hepatic metabolism and gastrointestinal barriers¹. The oral mucosa (Fig. 1) consists of three main layers: a stratified epithelium that provides a protective barrier, an underlying lamina propria composed of connective tissue, and a vascularized submucosa that enables rapid drug absorption into the bloodstream¹⁷. Certain regions of the oral cavity, such as the floor of the mouth and the ventral surface of the tongue, exhibit high permeability due to dense vascularization, making them ideal sites for sublingual or buccal drug delivery¹⁸.

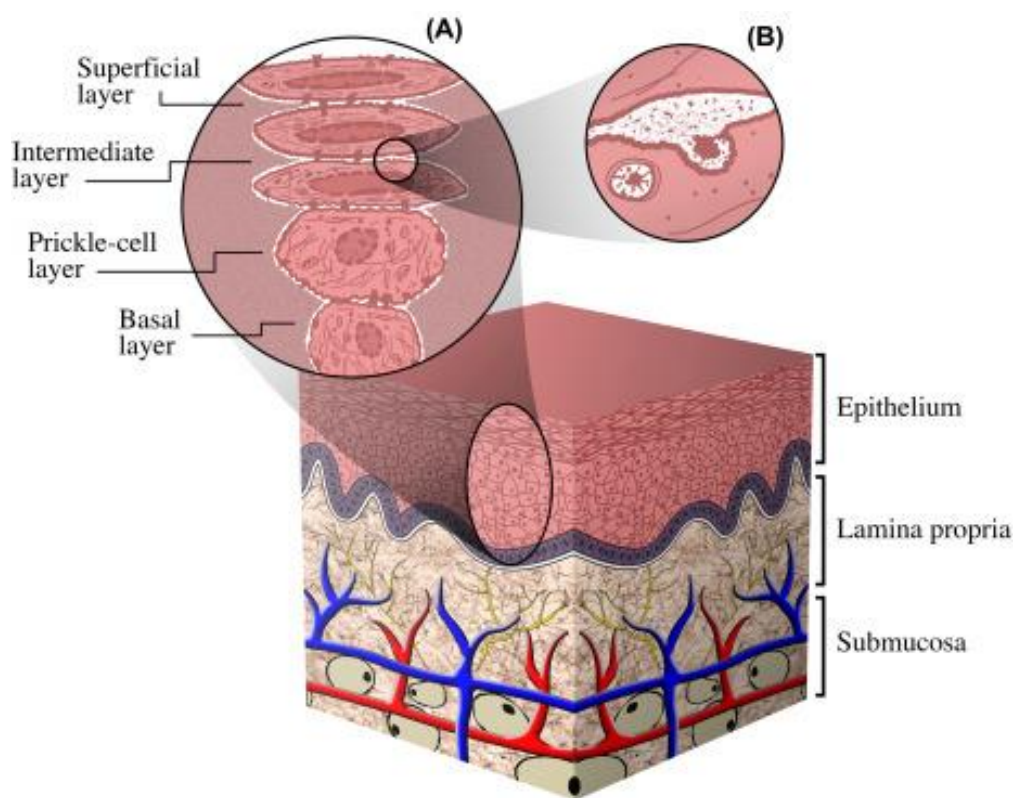


Figure 1. Anatomy of the oral mucosa and submucosa in the palatal region¹⁹. Reproduced with permission. Copyright © 2017 Elsevier.

However, only a limited number of drugs are compatible with this route, as it is associated with several barriers. The limited volume of saliva, approximately 1 ml in the sublingual area², requires drugs to have a high solubility in water to maintain an adequate concentration for absorption. Simultaneously, sufficient lipophilicity of the drug molecules is required to permeate the epithelial cell membranes effectively, narrowing down the number of suitable drug candidates for this delivery route, such as nitroglycerin, fentanyl, buprenorphine²⁰. Another challenge is the saliva washout, where the continuous production and clearance of saliva can prematurely remove the drug from the absorption site, significantly reducing retention time and bioavailability³. To address this, several studies have explored strategies to enhance mucoadhesion, including the use of positively charged polymers that can interact with the negatively charged mucosa. This strategy has enhanced the retention time of sublingual formulations. However, excessive mucoadhesion can risk drug entrapment within epithelial cells, hindering its effective transport through the epithelial cells²¹. Furthermore, proteases and esterases in saliva introduce an additional barrier to sublingual delivery systems by degrading susceptible therapeutic agents, such as peptides and proteins²². Although enzymatic concentrations are lower than in the gastrointestinal tract, their presence in the buccal compartment should still be considered to maintain drug stability and efficacy. Thus, enzymatic inhibitors can be used to protect the drug, but they add regulatory hurdles, particularly regarding toxicity and the risk of oral cavity irritation⁴.

In this context, drug formulations for sublingual administration must still be optimized to overcome biological and physicochemical barriers and thus exploit their advantages with respect to the traditional oral route. These include enhancing drug solubility in saliva, facilitating diffusion

through mucus, and increasing epithelial permeability, while avoiding enzymatic degradation. Several strategies, such as the incorporation of mucoadhesive polymers, permeability enhancers, and enzyme inhibitors, have been implemented to improve the effectiveness of sublingual formulations. However, these approaches usually rely on the inclusion of multiple additives in the formulations, each with potential side effects, including local irritation and long-term toxicity²³. This complexity has created a demand for drug carriers that address the diverse physiological barriers (*i.e.*, limited residence time, enzymatic degradation, and epithelial permeability) while minimizing toxicity. In this regard, MSNs are promising candidates, as they can be engineered to fine-tune their physicochemical properties to specific needs, including enhanced drug solubility, protection against enzymatic degradation, increased residence time at the absorption site, and reduced local toxicity. The use of MSNs for oral transmucosal delivery has been demonstrated in recent studies, for example, by incorporating MSNs into mucoadhesive buccal films, which resulted in prolonged mucosal residence, enhanced permeation, and improved systemic bioavailability of poorly soluble drugs such as eprosartan²⁴.

1.2 Mesoporous Silica Nanoparticles in Drug Delivery

1.2.1 Tailored Nanocarriers for Enhanced Oral Bioavailability

Since the early 1990s, several nanoparticles (Fig. 2) such as Poly(lactic-co-glycolic acid) (PLGA), liposomes, and solid-lipid nanoparticles (SLNs)²⁵ have been used to encapsulate active pharmaceutical ingredients to achieve a targeted and sustained drug release, improve their solubility, and protect them from enzymatic degradation, with specific applications in sublingual delivery^{26,27}. Furthermore, surface modification of these nanoparticles has been achieved by adding functional groups, such as polyethylene glycol (PEG), to create a hydrophilic and biocompatible shell and improve drug bioavailability²⁸. Biodegradable polymeric nanoparticles, such as chitosan, have often been evaluated in the sublingual route to enhance mucosal adhesion, protect against saliva washout, and improve drug residence time and systemic absorption after sublingual administration, as shown for ketorolac-loaded chitosan nanoparticles²⁹. In recent years, research has been focused on producing targeted, surface-engineered nanoparticles. In this regard, using MSNs as nanocarriers has been particularly attractive due to their high surface area and pore volume, stable and easily modifiable surface, and low toxicity^{30,31}.

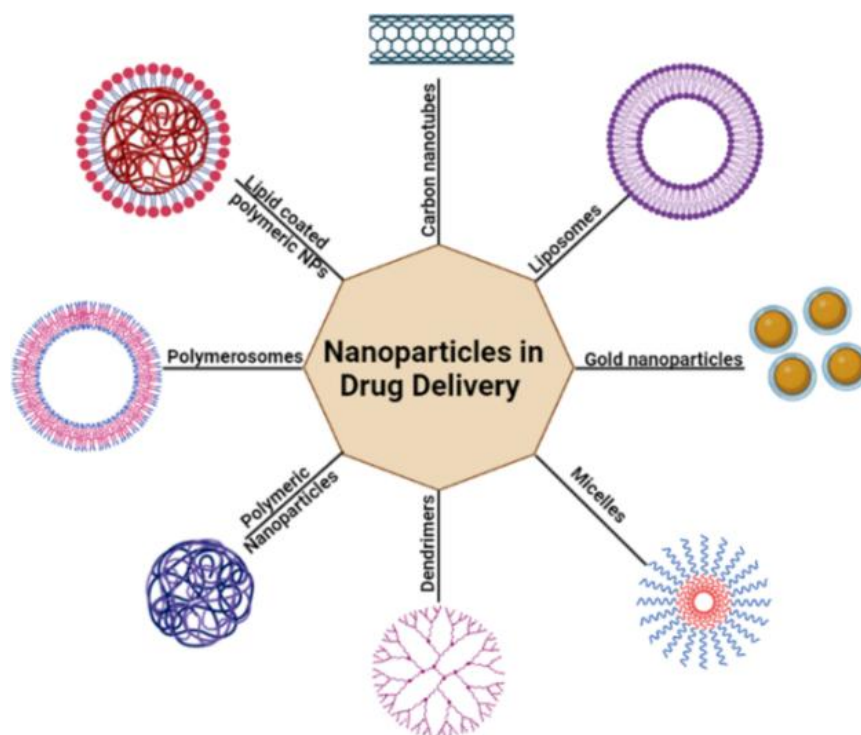


Figure 2. Nanoparticles used in drug delivery³². Reproduced from Molecular Biology Reports (2023), under CC BY 4.0.

1.2.2 MSNs for Efficient Drug Delivery

MSNs are composed of an inorganic silicon dioxide (SiO_2) skeleton with mesopores typically ranging 2–25 nm in diameter³³. Their high surface area (600–1000 m^2/g) and large pore volume of up to 1 cm^3/g enable high drug loading capacity³⁴. In MSNs systems, drug incorporation is determined by surface and pore accessibility. Therefore, high specific surface area values indicate a greater number of potential interaction sites within the pore network, which is generally associated with increased cargo-loading capacity. Furthermore, upon confinement, the drug exhibits an amorphous state within the silica network⁵, which improves its solubility. For example, *Florek et al.*³⁵ reported that drugs such as itraconazole and clotrimazole, known for their low water solubility, exhibited improved dissolution profiles and enhanced oral bioavailability when encapsulated in MSNs compared to their crystalline forms. MSNs are stable over a wide range of pH and temperature, in contrast to other polymeric particles³⁶. Moreover, the robust structure of MSNs serves as a shield for encapsulated molecules, providing excellent protection against enzymatic degradation of labile drugs, and making them attractive candidates for oral and sublingual formulations compared to unprotected drug-based alternatives³¹. Finally, it is worth noting that the silanol groups present in the silica structure provide a reactive surface that can be easily modified with various organic and inorganic functional groups, enabling controlled tuning of surface properties relevant to interactions with biological environments. For example, *Iriarte-Mesa et al.*³⁷ demonstrated that surface functionalization with phosphonate groups enhanced mucus penetration due to increased hydrophilicity and negative surface charge, whereas the so-called hybrid surface modifications, such as phosphonate groups combined with hydrophobic methyl or farnesyl moieties, altered epithelial interactions and promoted paracellular transport. This clear relationship between surface chemistry and barrier modulation is particularly relevant for sublingual delivery, where control over particle–mucosa interactions and retention time at the

absorption site is essential. Therefore, the versatile surface properties of silica, together with the ability to tune particle shape, morphology, size, and pore structure, make MSNs promising drug carriers to overcome the physiological challenges of the sublingual route.

1.2.3 Synthesis Strategies for MSNs

Several synthetic strategies have been developed to produce MSNs. The Stöber method was first established in the 1960s to deliver uniform, non-porous silica nanoparticles with a narrow size distribution. It involves adding a silica precursor (e.g., tetraethyl orthosilicate, TEOS) to an alcoholic aqueous solution containing ammonia at room temperature³⁸. Later, surfactant-templating strategies were introduced to create mesoporous materials using surfactant-templating agents such as Cetyltrimethylammonium chloride (CTAC)³⁹. Mesopore formation in soft-templated MSNs is achieved through a sol-gel process involving base-catalyzed hydrolysis and condensation of the silica precursor in the presence of an organic surfactant, which acts as a structure-directing agent. The amphiphilic surfactant undergoes self-assembly, forming micelles above the critical micelle concentration (Fig. 3). These micelles serve as templates around which hydrolyzed silica species condense. In this way, a hybrid organic-inorganic intermediate structure is formed, with the exterior composed of the silica frame and the interior filled with the organic surfactant. The hydrolysis and condensation of the silica precursor are catalyzed by a base. Using a mild organic base, such as Triethanolamine (TEA) or L-Lysine, favors slow growth, resulting in more uniform pores. After removal of the surfactant by calcination at 500°C or by solvent extraction, the remaining structure is a silica framework with a porous interior⁴⁰.

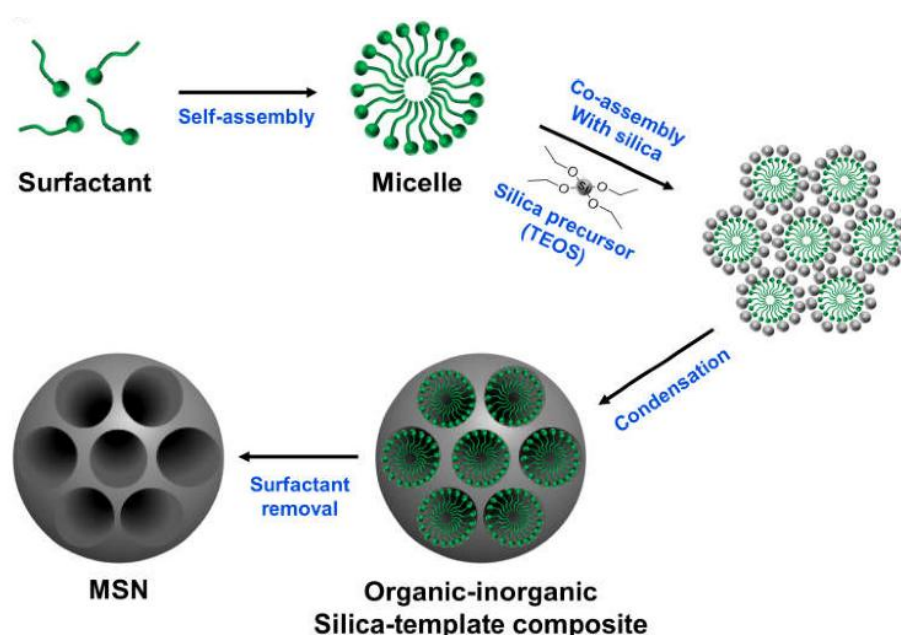


Figure 3. Schematic representation of MSNs synthesis via soft templating⁴¹. Adapted from Pharmaceutics (2023), under CC BY 4.0.

This synthetic pathway has enabled the development of a wide range of MSN architectures, such as spherical particles, dendritic structures, and materials with enlarged or hierarchical pore systems, which are particularly relevant for applications requiring high pore accessibility and

large cargo accommodation, including drug delivery and biomolecule confinement^{31,42}. Furthermore, by tuning reaction parameters, including surfactant concentration, silica precursor type, temperature, and solvent type, it is possible to control particle size, pore diameter, pore volume, and overall particle morphology, ranging from spherical or rod nanoparticles to more complex structures such as virus-like or dendritic^{6,7} (Fig. 4).

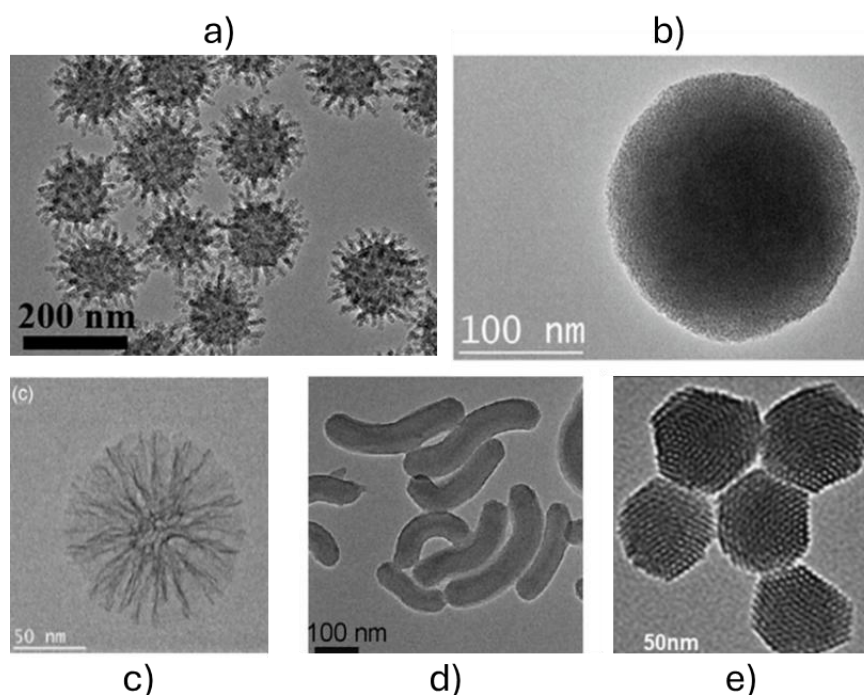


Figure 4. Representative transmission electron microscopy (TEM) of MSNs exhibiting several morphologies: a) Virus-like MSNs⁶, b) Spherical MSNs⁴³, c) Dendritic MSNs⁷, d) Rod-like silica nanoparticles, and ⁴⁴e) Hexagonal MSNs⁴⁵. Adapted with permission. Copyright © 2014, Tsinghua University Press and Springer-Verlag Berlin Heidelberg, Copyright © 2019 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Tailoring particle morphology is crucial for achieving the target biological performance required for a given application. For instance, *Yu et al.* reported that the cylindrical shape of MSNs nanorods facilitated the penetration through the mucosa in the gastrointestinal tract⁴⁶. This effect was attributed to their anisotropic shape, which promotes rotational motions under physiological fluid flow. This motion can reduce hydrodynamic resistance and ensure suitable orientation during interaction with the mucus surface, therefore facilitating deeper penetration through the mucus layer. Moreover, *Wang et al.* described the synthesis of virus-like particles to enhance cellular uptake due to their spiky, tubular form⁶.

In particular, the synthesis of dendritic MSNs (DMSNs) has attracted increased interest in the last few years due to their dendrimer-shaped, open pores. For example, *Juéré et al.* reported that, due to their large pores (6–20 nm), DMSNs can host large drug molecules such as insulin⁷, enabling uptake by intestinal cells and potentially reducing the dosing of therapeutics that would otherwise require parenteral delivery.

1.2.3.1 Synthesis of DMSNs

To obtain DMSNs with such central radial pore channels, modifications to the sol-gel method have been explored, including the use of the emulsion or biphasic stratification system, where a water-immiscible organic solvent (e.g., toluene, cyclohexane) is added to an aqueous solution⁴⁷. These solvents have a swelling effect on the micelles, resulting in larger, more open pores⁴⁸. Furthermore, using a co-surfactant, such as the nonionic Pluronic P123 or Pluronic F127 (Fig. 5), enables the formation of central radial pores⁴⁹. The use of these co-surfactants also allows the formation of DMSNs in aqueous solution using environmentally friendly methods, without adding any organic solvent. For example, *Liu et al.* reported the formation of dendritic structures using an anionic, aromatic sodium salicylate (NaSal) co-surfactant, therefore avoiding hazardous organic solvents and harsh reaction conditions⁵⁰. *Pan et al.* provided a comprehensive understanding of the synthesis and formation mechanism of DMSNs, concluding that spherical particles can be obtained under mild reaction conditions, without using organic solvents, thereby enabling low-toxicity synthesis routes without compromising particle morphology⁵¹. However, as reported in this review, an elevated temperature (up to 80°C) is necessary to boost the condensation rate of the silica precursor. This enables hydrolyzed silica to deposit quickly within the micellar structure before rearrangement occurs and the dendritic structure is lost⁵¹. A different strategy reported by *Wang et al.* is to use heptafluorobutyrate (FC4) to generate dendritic pores at room temperature⁵². As FC4 significantly reduces the surface tension at the water-surfactant interface, it acts as a micelle-swelling agent, eliminating the need to operate at elevated temperatures. The fluorocarbon additionally provides vesicle-like templating and multilayer shell formation, resulting in larger internal cavities than those of conventional MSNs derived from cationic surfactants⁵³.

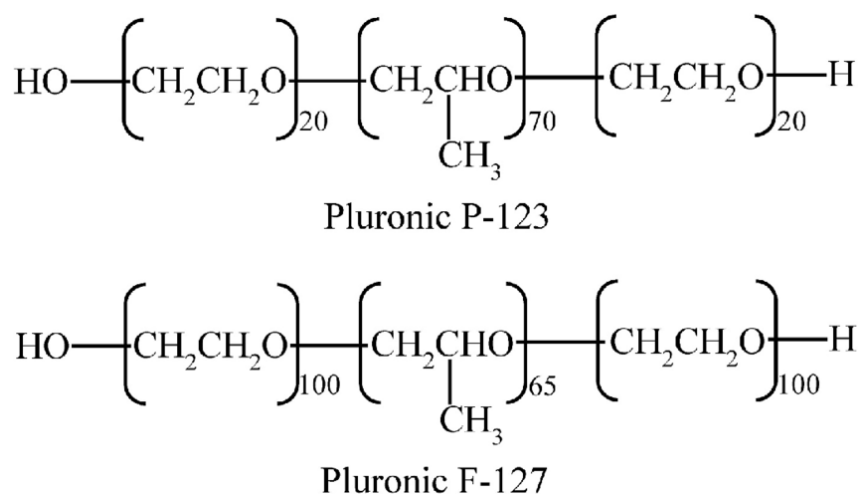


Figure 5. Pluronic co-surfactants used in the synthesis of DMSNs⁵⁴. Reproduced with permission. Copyright © 2022 Elsevier.

1.2.3.2 Tailoring MSNs' Properties

Tuning the synthesis parameters enables the obtaining of MSNs with unique properties for targeted drug delivery as well as other applications in catalysis, membranes, and other fields⁵⁵. The morphology of MSNs particles discussed in section 1.2.3 is not the only critical aspect for

their use in a particular application. For instance, particle size, pore structure, pore shape, and surface properties are all essential in determining how the nanoparticles perform across these applications⁵⁶.

Understanding the influence of physicochemical properties of MSNs on pharmacokinetic properties, including drug absorption, distribution, bioavailability, and clearance, is fundamental to selecting the optimal particle size of the carrier in drug delivery. For example, a small particle size (25–50 nm) has been associated with improved cell penetration⁵⁷. However, if the particles are too small (below 50 nm), they can increase the oxidative stress and inflammation, leading to cellular toxicity⁵⁸. Additionally, porosity is an important factor to consider for the drug loading capacity of MSNs and subsequent release profiles. For instance, in the work reported by *Juère et al.*, the larger pores of DMSNs (*i.e.*, 6 nm) facilitate the accommodation of insulin, enabling controlled release under gastrointestinal conditions⁷. Also, in a comparison of MSNs with small pores (3–6 nm) and large pores (11–16 nm) reported by *Huang et al.*, larger pores showed higher antigen binding across different conditions⁵⁹.

Numerous studies have focused on developing strategies to tailor MSNs' properties by tuning reactant ratios, synthesis temperature, and reaction time³⁵. For example, *Zainal et al.* demonstrated that raising the temperature from 30°C to 70°C increased particle size from 30 nm to 113 nm⁶⁰. This effect is attributed to the accelerated condensation of the silica precursor around the micelles. Furthermore, in a study by *Möller et al.*, the commonly used base catalyst, NaOH, was replaced with triethanolamine (TEA), yielding smaller particles (~100 nm). However, increasing the TEOS:TEA ratio from 1:1 to 1:4 led to particle agglomeration⁶¹. Thus, replacing the TEA base with a milder base catalyst, such as L-Lysine, decreased particle size while improving colloidal stability⁶². Particle size has also been controlled by adjusting experimental parameters such as stirring rate and pH. As reported by *Beltrán-Osuna et al.*, higher pH (increasing NaOH concentration) and stirring rates (700 rpm) produced smaller (~30–40 nm) and more uniform particles, while lower pH and stirring (250 rpm) yielded larger (~100 nm) particles⁶³.

The chain length of the precursor has also been shown to influence pore size, which increases linearly with increasing chain length⁶⁴. Thus, the precursor amount must be sufficient to achieve mesoporous structures; however, excessively high concentrations can result in disordered pores⁶⁵. Employing synthesis strategies for DMSN production, such as the use of co-surfactants or additives, inherently promotes pore expansion. For instance, the pore-expanding agent *N,N*-dimethylhexadecylamine (DMHA) increased the average pore size of MSNs to 5.6 nm⁶⁶, while trimethylbenzene (TMB) ensured the formation of dendritic particles, with pore size increasing from 8 nm to 12.6 nm, while particle size remained around 50 nm⁶⁷. In another study, *Kun-che et al.* investigated the use of alkanes such as hexane, octane, decane, and dodecane as pore-expanding agents⁶⁸.

1.2.3.3 Surface Functionalization

Beyond particle, porosity, and structural properties, surface functionalization is another crucial aspect to consider for enhancing the biological performance of mesoporous materials in drug delivery. Two primary approaches have been explored to introduce functional groups into MSNs, including post-grafting and co-condensation⁸. Post-grafting involves the attachment of functional groups to silica after MSN synthesis and can be performed after the calcination step or alternatively, before removing the surfactant template by extraction with HCl during particle synthesis⁶⁹. This method allows for the incorporation of a wide variety of functional moieties. However, it is limited by a potential non-uniform distribution, with organic functions tethered to

silica typically localized on the external particle surface, where silanol groups are more accessible. In contrast, co-condensation is a one-pot synthesis process in which functional silanes are incorporated simultaneously during MSNs formation. This approach ensures a more uniform distribution of functional groups, including within the pore interiors. Nevertheless, co-condensation offers less flexibility in choosing functional groups than post-grafting⁸.

One challenge in MSN-based formulations is the tendency of particles to aggregate in physiological solutions. This aggregation reduces circulation time and hinders cellular uptake in drug delivery systems⁹. In this regard, Polyethylene glycol (PEG) chains have proven effective as stabilizing groups to protect particles from aggregation in blood or physiological fluids, or to improve hemocompatibility⁷⁰. Moreover, stimuli-responsive functions have been incorporated into the silica network to induce reactions under specific conditions (pH, redox, etc.). For example, hollow MSNs have been functionalized with a hydrazone linker connecting hyaluronic acid (HA), which acts as both a cap and a targeting ligand. At low pH the hydrazone bonds cleave, releasing HA caps and freeing doxorubicin. This is highly significant for tumor therapy, where low pH levels are detected in tumor⁷¹. Another example is introducing functional groups for imaging purposes, where the MSNs particles can be functionalized by covalent bonding to fluorophores like Rhodamine B to track the cellular uptake⁷²⁻⁷⁴. On the other hand, small organic molecules, such as amines, have been used to increase the positive charge of MSNs, thereby improving drug loading via electrostatic interactions⁷⁵. On the other hand, thiol functions have been reported to improve mucoadhesion in oral delivery formulations⁷. Negatively charged species, such as phosphonate groups, have also improved drug release kinetics⁷⁶. Thus, tailoring MSNs with specific functional groups not only optimizes drug encapsulation and release but also extends the versatility of MSNs for multifunctional therapeutic and diagnostic applications.

MSNs can additionally be functionalized with biocompatible macromolecules. For example, *Meng et al.* reported coating MSNs to co-deliver Gemcitabine (hydrophilic) and Paclitaxel (hydrophobic) in pancreatic tumor cells, enhancing drug stability and systemic circulation and providing controlled release⁷⁷. Beyond improving stability and bioavailability, functionalization with biomolecules offers additional advantages with respect to small chemical functions. These biomolecules enable MSNs to interact more effectively with biological systems, allowing for improved targeted drug delivery⁷⁷. For example, in a study from *Martínez-Carmona et al.*, functionalization of MSNs with Concanavalin A (ConA), a lectin protein, resulted in targeted full release of the antibiotic levofloxacin in pathogen sites. In contrast, without the protein coating, the levofloxacin release only reached 30% after 48h. This was attributed to the ability of ConA to bind specifically to mannose or glucose residues on microbial cell walls. Moreover, the ConA formed a steric layer on the MSNs surface, avoiding a premature drug release⁷⁸.

Liposomes represent a particularly important class of biomolecules for the surface functionalization of MSNs, as they provide additional colloidal stability, controlled release, and enhanced biocompatibility of silica carriers^{9,79}. For example, *De Jesús Valle et al.* developed a mucoadhesive lyophilized liposome-based system for drug delivery through the sublingual mucosa, showing improved stability and enhanced mucosal interaction⁸⁰. Moreover, in sublingual drug delivery, the use of liposome vesicles instead of free lipids offers several advantages. While lipids may enhance mucosal permeability, liposomes provide a more organized bilayer structure, allowing efficient encapsulation of both hydrophilic and hydrophobic drugs and protection from salivary enzymes or pH variations⁸⁰.

1.3 Liposomes in MSN-Based Drug Delivery Systems

1.3.1 Liposome-coated DMSNs

Liposomes are spherical colloidal structures (vesicles) constituted by one or more lipid bilayers around an aqueous core (Fig.6). They can be prepared using the thin-film hydration method, which involves dissolving lipids like phosphatidylcholine, cholesterol, or PEGylated lipids in an organic solvent, followed by solvent evaporation until a dry, thin lipid film is created. When an aqueous solution, such as PBS, is added, the dry film hydrates, and the lipids spontaneously self-assemble into a bilayer, forming liposomes⁸¹.

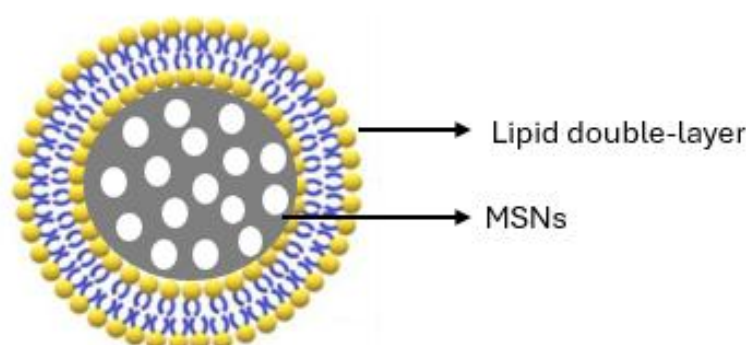


Figure 6. Lipid-bilayer encapsulated MSNs via Liposome fusion (L-MSNs).

Liposomes are widely used as drug carriers because they mimic natural cell membranes and can encapsulate both hydrophobic and hydrophilic drugs⁸¹. Liposomes can also be used to coat MSNs, providing biocompatible MSN-based hybrid materials⁹. There are several strategies to functionalize MSNs with lipid bilayers. One of the most common methods is liposome fusion, in which liposome vesicles are prepared separately and then mixed with MSNs. After sonication, the lipid structure is broken down into small unilamellar vesicles (SUVs). Due to electrostatic and hydrophobic interactions, SUVs are spread onto the MSNs' surface, forming a continuous supported lipid bilayer⁸². For example, *LaBauve et al.* used a mixture of phosphatidylcholine, phosphatidylserine, and cholesterol dissolved in chloroform to obtain a dry, thin film of liposomes, which was then combined with MSNs. The liposome fusion method enabled the formation of a supported bilayer, providing a biocompatible coating for MSNs that improved colloidal stability and reduced premature drug leakage⁹.

Another approach to coating MSNs with biomolecules, including lipids, is the layer-by-layer (LbL) technique. This method is based on the sequential adsorption of a complementary species onto the MSNs surface, allowing multilayer coatings to be formed one layer at a time⁸³. The interaction between the complementary molecules and the silica surface can be electrostatic or hydrophobic⁸³. For example, *Zhou et al.* described the deposition of phospholipids (1,2-dihexadecanoyl-sn-glycero-3-phosphocholine and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)]) on MSNs using the LbL technique. The surface of MSNs was made hydrophobic using an organosiloxane precursor, enabling hydrophobic interactions to drive the lipid coating⁸⁴. This strategy offered more precise MSN coating, preventing uneven or incomplete functionalization. However, only thin layers can be

deposited at a time by the LbL approach, which requires many cycles to achieve the desired coating thickness⁸⁵. In contrast, liposome fusion is a simple, one-step procedure that achieves high lipid coverage and mimics natural membranes, which is advantageous for drug delivery by enhancing release profiles and reducing immune responses⁸⁶.

Liposome-coated MSNs (L-MSNs) exhibit enhanced biocompatibility and improved drug encapsulation, as the liposomes provide an additional membrane barrier⁹. L-MSNs have also demonstrated slower drug release kinetics and enhanced cellular uptake. For example, *Liu et al.* showed that an L-MSN hybrid improved the delivery of Irinotecan by reducing drug leakage and controlling the release rate, thereby decreasing cellular toxicity in several target organs⁸⁷. In this work, liposomes were prepared using the thin-film hydration method with three lipids: phosphatidylcholine (CS-95) and cholesterol (CHO-HP) to improve MSNs biocompatibility⁸⁸, bilayer stability, and membrane fusion, as well as DSPE-PEG2000, a PEGylated lipid, to reduce particle aggregation and prevent mucus entrapment⁴⁶. The liposomes were fused on DMSNs, and the resulting hybrid system was stabilized using high-pressure homogenization (HOMO). HOMO is a technique in which the dispersion is forced through a narrow gap under high pressure (600 bar), resulting in shear forces that decrease particle size and produce a more homogeneous system. This method is commonly used in lipid-based nanocarriers to produce materials with improved colloidal stability and narrow particle-size distributions²⁷.

Evaluating the interaction of liposome-coated MSNs with biological systems, particularly their cytocompatibility and potential cellular uptake, is crucial to determine the effects of liposome coating on the biological performance of the functionalized MSNs. For this, *in vitro* cell studies provide important insight into how MSNs' size, surface chemistry, and composition influence cellular responses, measured by metabolic activity.

1.3.2 Cell Studies

A thorough evaluation of MSN–cell interactions is critical for drug delivery applications, as cellular stress responses can substantially affect therapeutic efficacy. Cell viability assays provide insight into whether the physicochemical properties of MSNs, including particle size, morphology, and surface chemistry, influence cytotoxicity, thereby supporting their suitability for biological use. Thus, cell viability assays have been used to evaluate the cellular tolerance of DMSNs of different sizes and surface chemistries under short- and prolonged-exposure conditions⁸⁹. This approach enables the identification of concentration- and time-dependent effects associated with MSNs when applied to cells, allowing direct comparison between bare and functionalized systems⁹⁰. For instance, the MTS assay 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium is a colorimetric method for assessing cell viability and metabolic activity. It is based on the reduction of the tetrazolium salt MTS by mitochondrial enzymes in metabolically active cells, resulting in the formation of a soluble formazan product. The amount of formazan produced is proportional to the number of viable cells and can be quantified by measuring its absorbance⁹¹.

Tetrazolium viability assays are used to evaluate cytotoxicity as a function of particle concentration and exposure time, thereby identifying potential dose-dependent effects and comparing the biocompatibility of nanoparticulate materials⁹². A commonly used method to assess cytotoxicity is the lactate dehydrogenase (LDH) assay. LDH is a stable cytosolic enzyme present in all cell types and is rapidly released into the culture supernatant when the plasma membrane is damaged, serving as an indicator of compromised membrane integrity and providing a more comprehensive assessment of nanoparticle-induced cytotoxicity⁹³.

On the other hand, cellular uptake is the process by which nanoparticles interact with the cell membrane, resulting in internalization. This can occur through various pathways, including endocytosis-mediated processes, and is strongly influenced by MSNs' size, surface charge, rigidity, and surface chemistry⁹⁴. For example, lipid-coated MSNs have been designed to mimic biological membranes, thereby altering their interactions with cells and potentially facilitating uptake⁷⁹. However, as demonstrated by temperature- and inhibitor-dependent uptake studies from *Rejman et al.*, MSN association with the cell surface does not necessarily imply intracellular uptake, and careful analysis is required to distinguish membrane-bound particles from truly internalized ones⁹⁵. Thus, fluorescence microscopy is widely used to characterize particle–cell interactions, enabling visualization of fluorescently labelled materials distributed throughout cell cultures. By labelling nanoparticles with fluorescent dyes such as Rhodamine, their localization can be analyzed relative to cellular components, such as the cytoskeleton and nucleus. Therefore, this technique provides information on particle–cell interactions and allows assessment of cellular morphology and stress responses associated with particle treatment⁹⁶.

1.4 Biocompatibility of MSNs

1.4.1 In Vivo Fate of MSNs: Distribution, Degradation, and Clearance

One of the key issues regarding MSN design for drug delivery applications is understanding their fate after administration. Many studies fail to address the in vivo behavior of MSNs, particularly their biodegradability and the elimination of their degradation byproducts⁹⁷. Although MSNs are promising carriers, it is essential to balance this with considerations of biodistribution, excretion, and toxicity to achieve clinical application and translation. Even though silica is generally considered safe and nontoxic by the Food and Drug Administration (FDA), a study review by *Croissant et al.* suggests that accumulation of nanoparticles in the body should be avoided to prevent side effects¹⁰.

In the sublingual delivery route, MSNs promote drug dissolution in saliva, thereby enhancing drug penetration through the mucosa. Particle size and surface functionalization play a critical role in MSNs penetration through the mucosa⁹⁸. So far, studies on MSNs ranging from 30 to 300 nm indicate that, after drug release, the MSNs carriers are washed out by saliva and enter the gastrointestinal tract⁹⁹. The silica framework is very rigid and stable, so a large part of it might be excreted unchanged, without undergoing hydrolysis¹⁰. MSNs may also undergo a slow degradation process, during which the Si–O–Si bonds break down, releasing orthosilicic acid [Si(OH)₄]. This compound is naturally biocompatible and is safely cleared by renal excretion³⁰.

Several factors influence the degradation kinetics of MSNs, including nanoparticle morphology, size, concentration, pore size, surface area, and other structural parameters¹⁰⁰. For instance, *Hao et al.* reported that pure MSNs particles in the range of 100–200 nm, and with spherical morphology, exhibited faster dissolution in Dulbecco's Modified Eagle Medium (DMEM) than their rod-shaped counterpart¹⁰¹. *Braun et al.* also investigated the degradation of MSNs in simulated body fluids. In this study, MSNs with different diameters (80, 200, and 1500 nm) were tested, showing dissolution profiles independent of particle size¹⁰². On the other hand, *Shen et al.* demonstrated, via Transmission Electron Microscopy (TEM) characterization, that DMSNs with 10 nm radial pores fully hydrolyzed after 24 h⁴².

Even though a portion of MSNs framework can be degraded after administration, biodistribution and the accumulation of byproducts are still open questions. For example, in a study by *Li et al.*, MSNs were shown to cause renal damage, including hemorrhage, vascular congestion, and renal

tubular necrosis, over a prolonged period of urinary excretion¹⁰³. Given the ongoing challenges in comprehending MSN toxicology, biodistribution, and clearance, the development of MSN carriers that degrade in a controlled, stimulus-triggered manner after drug release is a promising strategy for their clinical translation.

1.4.2 Strategies for Designing Biocompatible Nanocarriers

For safer and more efficient clinical translation of MSNs in drug delivery, one promising strategy is to design materials with stimuli-responsive behavior in physiological fluids. Thus, pH variations, as well as enzymatic and redox reactions, can be exploited to trigger controlled drug release and promote MSN biodegradation³¹. To achieve this stimulus-response in MSN-based formulations, several studies have described the incorporation of organic and inorganic moieties into the silica framework. These include methylene, ethylene, phenylene, as well as metal cations such as Ca, Fe, Mn, Al, and Zr^{104,105}.

Among these functionalities, the disulfide bond has been shown to be stable under mild oxidizing conditions (e.g., oxygen and blood) and at physiological pH¹⁰⁶, yet it remains sensitive to disulfide–thiol exchange reactions. Incorporating cleavable disulfide bonds within the silica framework (Fig.7) to generate mesoporous organosilica nanoparticles (MOSNs) is a promising strategy to enable intracellular redox-responsive biodegradation. This is especially important for targeting cancer cells, where glutathione (GSH) levels are elevated¹⁰⁷.

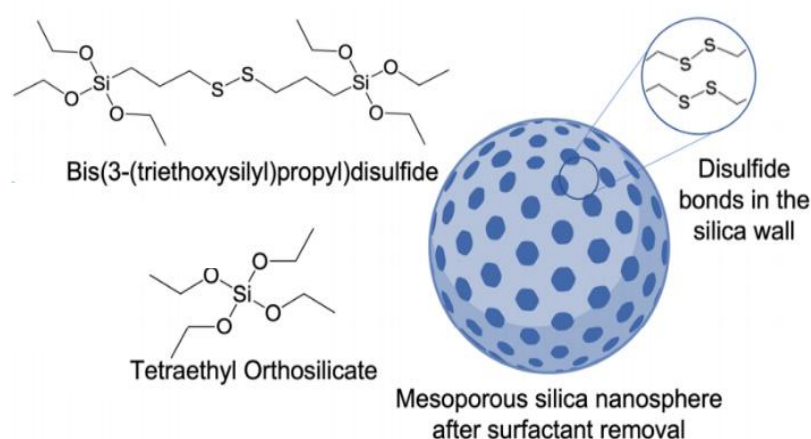


Figure 7. Modification of MSNs with disulfide-bridges¹⁰⁸. Adapted from Crystals (2023), under CC BY 4.0.

In the work conducted by *Wu et al.*, bis[3-(triethoxysilyl)propyl]tetrasulfide (BTES) was added simultaneously with the precursor (TEOS) in a one-pot synthesis at elevated temperatures (95°C). This also led to micelle expansion, significantly increasing the pore size of MOSNs¹⁰⁹. In a study from *Venedicto et al.*, MOSNs were synthesized via a co-condensation strategy, where a disulfide-containing silane, bis(3-(triethoxysilyl)propyl)disulfide (BTEPDS), was combined with the conventional silica precursor tetraethyl orthosilicate (TEOS) in the presence of the surfactant cetyltrimethylammonium bromide (CTAB) under alkaline conditions. During the process, BTEPDS and TEOS were hydrolyzed and condensed around the micelles, yielding spherical particles with

pore sizes of 2–4 nm¹⁰⁸. Furthermore, *Zhao et al.* used a two-step strategy to incorporate S-S bonds in the MSNs structure. In the first step, the thiol functionality (–SH) was introduced by mixing 3-mercaptopropyltrimethoxysilane (MPTMS) with the TEOS precursor, yielding spherical colloidal MSNs in the 50–80 nm range with a pore size of around 2 nm. In the second step, 2-carboxyethyl 2-pyridyl disulfide (Py–SS–COOH) was used as a crosslinker to react with thiol (–SH) groups, forming stable disulfide bonds¹¹⁰. This approach enabled control over the distribution of disulfide bonds into the silica framework. However, the one-step method described by *Venedicto et al.* and *Wu et al.* ensured the incorporation of disulfide functions in a single synthetic step, thereby providing a uniform distribution of these moieties throughout the silica matrix¹⁰⁸.

Disulfide moieties incorporated within the silica framework are highly stable under normal extracellular conditions, providing additional stability when carrying the drug and thereby preventing premature release during circulation^{10,106}. However, as previously mentioned, the disulfide functions are sensitive in reducing environments where glutathione (GSH) concentration is high. GSH is a small peptide composed of Glutamate, Cysteine, and Glycine (γ-Glu-Cys-Gly). It's synthesized in the cytosol of almost all cells. After sublingual drug absorption, in extracellular environment (saliva), GSH is present at a very low concentration at 2–20 μM¹¹¹, therefore the nanocarriers remain stable. However in intracellular environment the GSH concentration reaches 1–10 mM¹¹¹. When the MOSNs are delivered to cells in the presence of high levels of GSH, it (in its reduced form) reacts with S–S bonds in the MSN framework via thiol-disulfide exchange, breaking the S–S linkage. During the reduction of S–S functions, GSH oxidized to GSSG⁹⁷ (Fig.8)

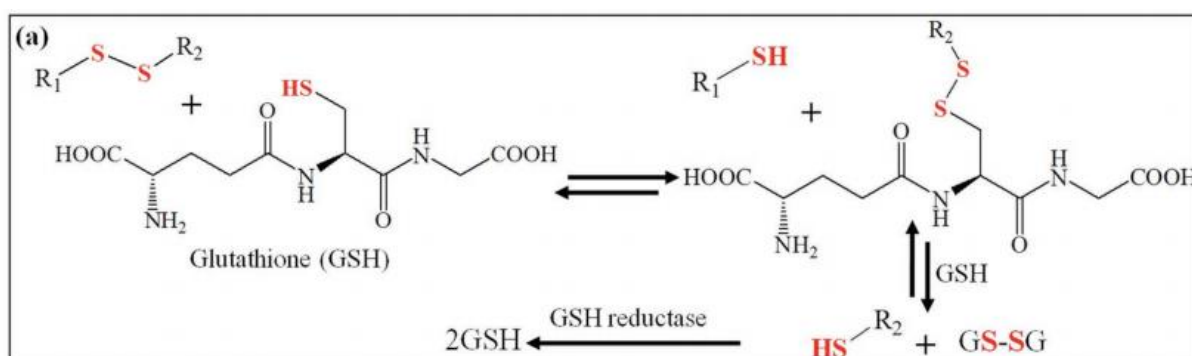


Figure 8. Chemical reaction of the disulfide bond reduction by GSH¹⁰⁶. Adapted from *Advanced Functional Materials* (2018), under CC BY 4.0.

One of the challenges that comes with incorporating S-S in the MSNs structure is reducing the accessible surface area and pore volume of the particles. For example, *Putz et al.* reported that incorporating methyltriethoxysilane (MTES) as a co-precursor decreased the average pore size of the MSNs from 2,6 nm to 0,8 nm¹¹². To date, only a limited number of studies have examined the incorporation of disulfide (S–S) linkages into MSNs while maintaining large pore architectures. Achieving this would combine the advantages of a dendritic, high-pore-volume structure with stimulus-responsive biodegradability provided by the S–S bonds. This design could further improve the particles' toxicological profile and potentially accelerate their translation into clinical trials.

2. Objectives

This work focuses on improving MSNs as carriers for sublingual drug delivery and is structured around two main objectives:

1. To engineer DMSNs suitable for sublingual drug delivery by tuning particle size, followed by surface functionalization with small functional groups (e.g., phosphonate moieties) or larger amphiphilic biomolecules (e.g., liposomes), thereby enhancing colloidal stability, cellular uptake, and potentially bioavailability.
2. To synthesize disulfide-bridged organosilica nanoparticles (DMOSNs) by introducing disulfide (S–S) linkages into the silica framework via co-condensation of functionalized silanes (BTES) with tetraethyl orthosilicate (TEOS), while preserving large pore sizes and enabling structural degradation under elevated glutathione (GSH) concentrations for redox-responsive biodegradability.

To accomplish these objectives, DMSNs with two different particle sizes (50 nm and 130 nm) were synthesized by adapting protocols reported in the literature^{37,52}. After optimizing the synthesis to achieve narrow particle-size distributions, the DMSNs were functionalized to further enhance their colloidal stability and interaction with buccal epithelial cells. Initially, DMSNs were modified via post-synthetic grafting with 3-(trihydroxysilyl)propyl methylphosphonate (THMP), enabling the incorporation of phosphonate (PO_3) moieties into the silica network and thereby improving colloidal stability by increasing the negative surface charge. To also enhance biocompatibility while maintaining accessible pores, the DMSNs were alternatively coated with liposomes composed of phosphatidylcholine (CS-95), DSPE-MPEG2000, and cholesterol (CHO-HP). The liposomes were prepared by thin-film hydration, rehydrated in the presence of DMSNs, and then subjected to high-pressure homogenization (HOMO) to achieve a uniform coating and particle size. Pure DMSNs (**DMSNs-50** and **DMSNs-130**), phosphonated DMSNs (**DMSNs-50- PO_3** and **DMSNs-130- PO_3**), and liposome-coated DMSNs (**L-DMSNs-50** and **L-DMSNs-130**) were evaluated for their interactions with the human TR146 cells. Cell viability following nanoparticle exposure was assessed using the MTS assay across different particle concentrations and incubation times, while fluorescence microscopy was used to qualitatively visualize particle–cell interactions and cellular localization.

The second part of this master's thesis focuses on the design of biodegradable MSNs to generate particles with the potential for safe excretion from the body, thereby minimizing silica accumulation and improving their toxicological profile. To achieve this, disulfide (S–S) bridges were introduced into the silica framework, enabling redox-responsive cleavage in the presence of GSH and subsequent degradation of the silica network. Initially, mesoporous organosilica nanoparticles (MOSNs) were synthesized using a previously reported one-step co-condensation method¹⁰⁹, in which bis[3-(triethoxysilyl)propyl] tetrasulfide (BTES) was co-condensed with tetraethyl orthosilicate (TEOS) in varying ratios under different temperature and stirring conditions. After tuning the synthesis parameters to obtain mesoporous spherical particles with diameters ranging from 50 to 150 nm, the work progressed toward the preparation of dendritic disulfide-bridged organosilica nanoparticles (DMOSNs) under the optimized conditions. The initial synthesis of S–S-bridged DMOSNs resulted in disorganized structures. Therefore, the protocol was further optimized by adjusting the solvent composition, reaction temperature, and stirring time, yielding well-defined spherical dendritic particles with uniform size distribution and large pore architecture. The resulting particles were subsequently evaluated for preliminary

biodegradability by incubation in GSH (28 mM) at 37°C. Changes in hydrodynamic diameter were monitored over time to assess structural degradation. In addition, small-angle X-ray scattering (SAXS) measurements were performed to further analyze potential alterations in the primary structure of the silica framework in the presence of GSH.

3. Methods

3.1 Reagents and Materials

Toluene (99%), tetraethoxysilane (TEOS, 98%), triethanolamine (TEA, 98%), *L*-lysine (98%), *p*-xylene (99%), toluene (99%, extra dry over molecular sieve), and sodium hydroxide solution (1.0 N) were obtained from Thermo Fisher Scientific (Germany). Hexane ($\geq 99\%$), (3-aminopropyl)-triethoxysilane ($\geq 98\%$), 3-(triethoxysilyl) propyl methylphosphonate (monosodium salt solution, 50 wt.% in H_2O), hexadecyltrimethylammonium bromide (CTAB, $\geq 98\%$), cetyltrimethylammonium chloride solution (CTAC, 25 wt.% in H_2O), *L*-glutathione ($\geq 98\%$), rhodamine B isothiocyanate ($>70\%$), and bis-(3-(triethoxysilyl)-propyl)-tetrasulfide (BTES) were obtained from Sigma-Aldrich (Germany). Sodium heptafluorobutyrate (FC4) and bis-(triethoxysilyl propyl) disulfide were purchased from Fluorochem (United Kingdom). Ethanol (96%) was purchased from Brenntag (Austria) and hydrochloric acid (≥ 37 wt.%) from VWR Chemicals (France). The lipids phosphatidylcholine (CS-95) and *N*-(carboxymethoxypolyethylenglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamin (DSPE-MPEG2000) were acquired from Lipoid GmbH (Germany). Cholesterol (CHO-HP) was purchased from Caesar & Loretz GmbH (Germany), dichloromethane from VWR Chemicals (Belgium), and anhydrous ethanol from Merck KGaA (Germany). Human buccal TR 146 cells were obtained from *Imperial Cancer Research Technology* (United Kingdom). Dulbecco's Modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), fetal bovine serum (FBS), penicillin-streptomycin (Pen Strep), Hank's Balanced Salt Solution (HBSS), and trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA, 0,25%) were obtained from Gibco, Life Technologies Corporation (United Kingdom). Formalin solution (10 % formalin, approx. 4 % formaldehyde), albumin from bovine serum, Triton X-100, and Nile Blue were acquired from Sigma Aldrich (Austria), and Alexa Fluor™ 488 Phalloidin and DAPI, from Thermo Fisher Scientific (Austria).

3.2 Material Synthesis

3.2.1 Synthesis of DMSNs at Room Temperature (**DMSN-50**)

To obtain DMSNs with a particle size of 50 nm, the protocol from *Wang et al.* was followed, with several adjustments⁵². Five batches of the product (**DMSN-50**) were prepared under identical conditions and combined in the end. In a 50 mL round-bottom flask, 68 mg of TEA was mixed with 25 mL of ultra-pure water (H_2O). Then, 380 mg of CTAB was added, and the mixture was sonicated until the CTAB was dissolved. Next, 74 mg of FC4 was added, and the mixture was stirred (150 rpm) at room temperature (25°C) for 1 h. Subsequently, 4 mL of TEOS was added dropwise (1 drop/s), and the reaction was kept under stirring (150 rpm) for 24 h. The particle suspension was then transferred to 25 mL Falcon tubes and centrifuged for 30 min at 10,000 rpm. The **DMSN-**

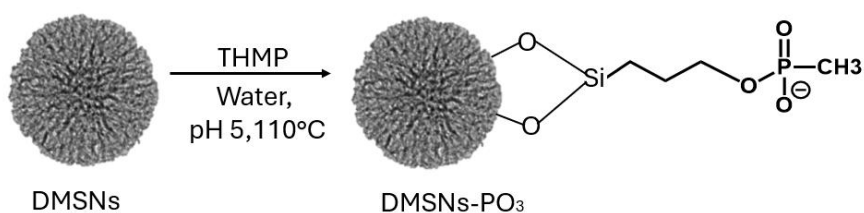
50 was washed 3 times with EtOH, and the CTAB was extracted by washing three times with EtOH containing HCl (12 M), following centrifugation (9000 rpm, 20 min). Afterward, the product was washed with water and with ethanol and centrifuged again (9000 rpm, 20 min). The particles were dried in an oven at 100°C overnight and then calcined for 5 h at 550°C.

3.2.2 Synthesis of DMSNs at 60°C (**DMSN-130**)

To obtain DMSNs with an average particle size of 130 nm (**DMSN-130**), the protocol reported by *Iriarte-Mesa et al.* was implemented³⁷. Four batches of the product were synthesized under identical conditions and combined at the end. In a 250 mL round-bottom flask, 360 mg of TEA was dissolved in 72 g of ultra-pure water (H₂O). Subsequently, 8 mL of CTAC was added, and the mixture was stirred at 150 rpm at 60°C for 1 h. A separate mixture containing 8 mL of TEOS and 32 mL of hexane was then prepared and added dropwise to the aqueous phase. The resulting biphasic system was maintained under stirring (150 rpm) at 60°C overnight to allow silica condensation. After completion of the reaction, the organic phase was removed, and the particles were collected by centrifugation (10,000 rpm, 30 min). The product was washed three times with ethanol, each followed by centrifugation (9000 rpm, 20 min), and subsequently dried overnight in an oven at 100°C. Finally, the dried **DMSN-130** particles were calcined at 550°C for 5 h to remove the surfactant template.

3.2.3 Functionalisation with THMP

In two separate round-bottom flasks, 200 mg of each **DMSN-50** and **DMSN-130** were combined with 25 mL of ultra-pure water, and the suspension was stirred (650 rpm) at room temperature (25°C) for 4 h. In parallel, 0,8 mL of THMP was added to 25 mL of water, and the pH of the aqueous solution was adjusted from an initial value of 10,5 to 5,3 by adding 3-4 mL of 0,1M HCl, following an already reported procedure³⁷. This THMP solution was added to the DMSNs suspensions, and the reaction was stirred (700 rpm) overnight at 110°C. The products (**DMSN-50-PO₃** and **DMSN-130-PO₃**) were isolated by centrifugation (9000 rpm, 20 min) and washed once with water and three times with ethanol (Scheme 1). The **DMSN-50-PO₃** and **DMSN-130-PO₃** were dried in an oven at 40°C overnight.



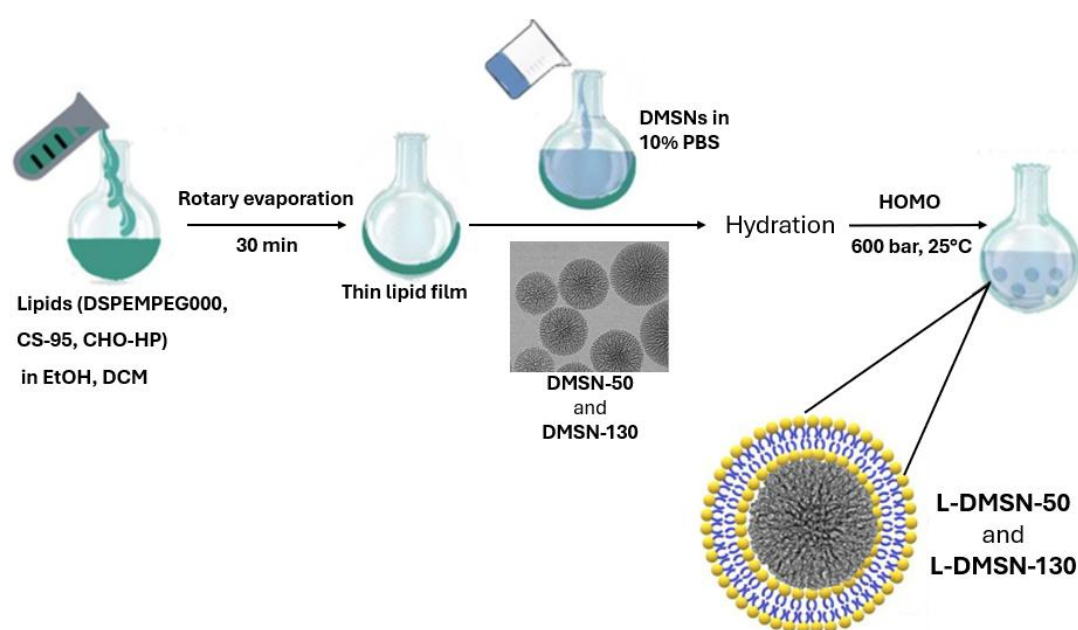
Scheme 1. Functionalization of DMSNs with PO₃ groups

3.2.3 Liposome Coating

The experimental part of the liposome coating was performed in collaboration with the Institute of Pharmaceutical Sciences at the University of Graz, under the supervision of Dr. Atida Selmani.

3.2.3.1 Liposome Coating of DMSNs via Co-hydration with Liposome Thin-film

The liposomes were prepared via the thin-film method (Scheme 2), followed by hydration in a DMSN-containing Phosphate Buffered Saline (PBS) suspension. First, 4,5 mg of DSPE-MPEG2000, 31,5 mg of CS-95, and 14 mg of CHO-HP were placed in a round-bottom flask, followed by the addition of 50 mL of anhydrous ethanol (EtOH) and 25 mL of dichloromethane (DCM). The lipids were dissolved by sonicating for 5 minutes. The mixture was rotoevaporated for 30 min to remove the solvents and obtain the thin film. From each particle (**DMSN-50** or **DMSN-130**), 25 mg was weighed and suspended in 75 mL of PBS solution. The particle suspensions were pre-heated and used to rehydrate the lipids at 50°C for 30 minutes. Liposome-coated particles (**L-DMSN-50** or **L-DMSN-130**) were then sonicated for 10 minutes at 30 % amplitude. Following sonication, HOMO was performed to achieve a uniform particle size. Homogenization performed for 15 cycles at 600 bars and room temperature (25°C). The materials obtained were stored at 4°C.



Scheme 2. Liposome preparation via *Thin-film* and coating of the DMSNs with liposomes

3.2.3.2 Cell Culture Studies

TR146 cells were cultured in DMEM supplemented with 10 % Fetal Bovine Serum (FBS), 1% Pen-Strep, and 1 % non-essential amino acid solution (NEAA) in a humidified atmosphere containing 5 % CO₂ at 37°C. The culture medium was replaced three times per week, and confluent cells were then cultured weekly using trypsin-Ethylenediaminetetraacetic acid (EDTA). For preparation of particle suspensions for cell treatment, serum-free DMEM without phenol red, supplemented with 1% Pen-Strep and 1% NEAA, was used. To evaluate the potential cytotoxicity of DMSNs, an MTS assay was performed following the protocol described by *Jeitler et al.*¹¹³. TR146 cells were seeded in 96-well plates at a density of 3×10^4 cells/well. After washing with PBS, cells were then

incubated with the following samples: Pure DMSNs (**DMSN-50** and **DMSN-130**), phosphonated DMSNs (**DMSN-50-PO₃** and **DMSN-130-PO₃**), liposome-coated DMSNs (**L-DMSN-50** and **L-DMSN-130**), and empty liposomes (control formulation). Particles were applied at concentrations of 10, 50, 100, 250, and 440 $\mu\text{g mL}^{-1}$. The concentrations of the control formulation (empty liposomes) were adjusted to match those in **L-DMSN-50** and **L-DMSN-130**. The cells were incubated with particle treatments for 4 h under standard culture conditions (37°C, 5% CO₂).

Cell viability was assessed using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay¹¹³. After nanoparticle incubation for 4 h, the treatment medium was removed, and cells were washed once with 100 μL Hank's Balanced Salt Solution (HBSS) to eliminate residual particles. Subsequently, 100 μL HBSS and 20 μL MTS reagent were added per well (final volume 120 μL per well). Plates were incubated for 3 h at 37°C to allow formation of the soluble formazan product. The absorbance at 490 nm was determined following a 3 h incubation period using a CLARIOstar Plus microplate reader. Untreated, blank-corrected wells served as the 100% viability control. Cell viability was expressed as a percentage relative to untreated control cells after blank correction.

3.2.3.3 Rhodamine Labelling and Cellular Uptake Studies

For cellular uptake studies, the DMSNs were first labeled with rhodamine B isothiocyanate following a previously reported protocol⁷, thereby enabling visualization of the particles upon interaction with TR146 cells. In four separate round-bottom flasks, 100 mg of DMSNs (**DMSN-50**, **DMSN-130**, **DMSN-50-PO₃**, or **DMSN-130-PO₃**) were prepared for rhodamine labelling by first outgassing them under vacuum at 80°C (300 rpm) overnight. In parallel, 21,38 mg of rhodamine was dissolved in 8 mL of absolute ethanol, followed by the addition of 3 μL of APTES. The mixture was stirred (1000 rpm) at room temperature under an argon atmosphere for 24 h. Subsequently, 30 mL of extra-dry toluene was added to each of the outgassed samples, and the suspensions were stirred (600 rpm) at 50°C. Then, 100 μL of the previously prepared rhodamine solution was added to each suspension, and the reaction was continued for 4 h under an argon-filled balloon. The products (**Rh-DMSN-50**, **Rh-DMSN-130**, **Rh-DMSN-50-PO₃**, and **Rh-DMSN-130-PO₃**) were isolated by centrifugation (9000 rpm, 20 min), washed once with toluene and six times with ethanol, and dried in an oven at 40°C overnight. The fluorescently labelled non-functionalized particles (**Rh-DMSN-50** and **Rh-DMSN-130**) were subsequently coated with liposomes as described in Section 3.2.3.1, resulting in **Rh-L-DMSN-50** and **Rh-L-DMSN-130**.

For intracellular localization studies, TR 146 cells were seeded at a density of 5×10^4 cells/well in 8-well chamber slides and incubated for 4 days. Before incubation for 4 h at 37°C with labelled materials (**Rh-DMSN-50**, **Rh-DMSN-130**, **Rh-DMSN-50-PO₃**, **Rh-DMSN-130-PO₃**, **Rh-L-DMSN-50**, and **Rh-L-DMSN-130**) at particle concentrations equilibrated to 250 $\mu\text{g mL}^{-1}$, the medium was removed, and the cells were washed with PBS. After 4 h, particles were removed, and cells were washed with PBS to remove unbound nanoparticles. For cell staining, cells were fixed in 4% formaldehyde, permeabilized with 0,1% Triton X-100, and blocked with 1% Bovine Serum Albumin (BSA). Then, 100 μL staining solution per well was added, consisting of 1% BSA in PBS supplemented with Alexa Fluor™ 488 Phalloidin for actin cytoskeleton staining and Hoechst/4',6-Diamidino-2-phenylindole (DAPI) for nuclear staining. Cells were incubated for 25 min at room temperature, protected from light, followed by two washing steps with PBS. Finally, samples were mounted with mounting medium and prepared for microscopy. Imaging was performed with a confocal laser scanning microscope (Leica Stellaris 5, Leica Microsystems, Germany), and image analysis was conducted with Fiji version 2.9.0.

3.2.4 Synthesis of MOSNs

Two types of MOSN particles were synthesized, following the protocol from *Wu et al.* with some modifications¹⁰⁹. By varying the molar amount of BTES (Fig.9) in relation to TEOS particles, **MOSN-T2.4** (BTES:TEOS = 0,5) and **MOSN-T1.2** (BTES:TEOS = 0,25) were obtained (Table 1). In two 50 mL round-bottom flasks, 2 g CTAC and 0,6 g TEA were mixed. Next, 20 mL of water was added to each flask, and the stirring (300 rpm) was kept at 95°C under reflux for 20 min. In the meantime, two mixtures containing TEOS and BTES were prepared. In the first mixture, 1 g TEOS and 1,3 g BTES were added in a molar ratio of 0,5 (**MOSN-T2.4**). In the second, 1 g of TEOS and 0,6 g of BTES were combined in a molar ratio of 0,25 (**MOSN-T1.2**). The reactions were stirred (300 rpm) at 95°C overnight (Scheme 3). The obtained particles were washed the following day according to the procedure described below. Initially, the particles were washed twice with water, each washing step followed by sonication for 10 min and centrifugation at 9000 rpm for 20 min. Subsequently, the particles were washed three times with ethanol, each cycle including sonication for 10 min and centrifugation under the same conditions. The washed particles were then dispersed in 20 mL of a mixture containing 10% (v/v) hydrochloric acid (12 M) and 90% (v/v) ethanol and stirred overnight at 78°C at 300 rpm to extract the CTAC template. After extraction, the particles were washed repeatedly with water, each cycle consisting of sonication for 10 min followed by centrifugation, until the supernatant reached a pH value of approximately 6 (six washing cycles). Finally, the products (**MOSN-T2.4** and **MOSN-T1.2**) were washed three times with ethanol, each washing step followed by sonication and centrifugation. The purified particles were dried overnight in an oven at 70°C.

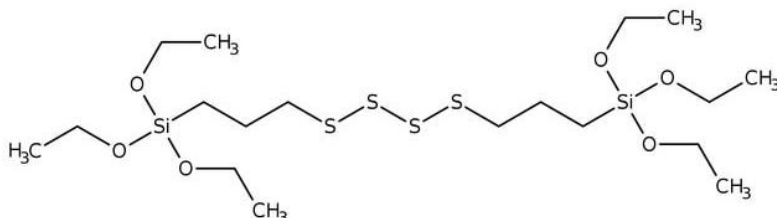
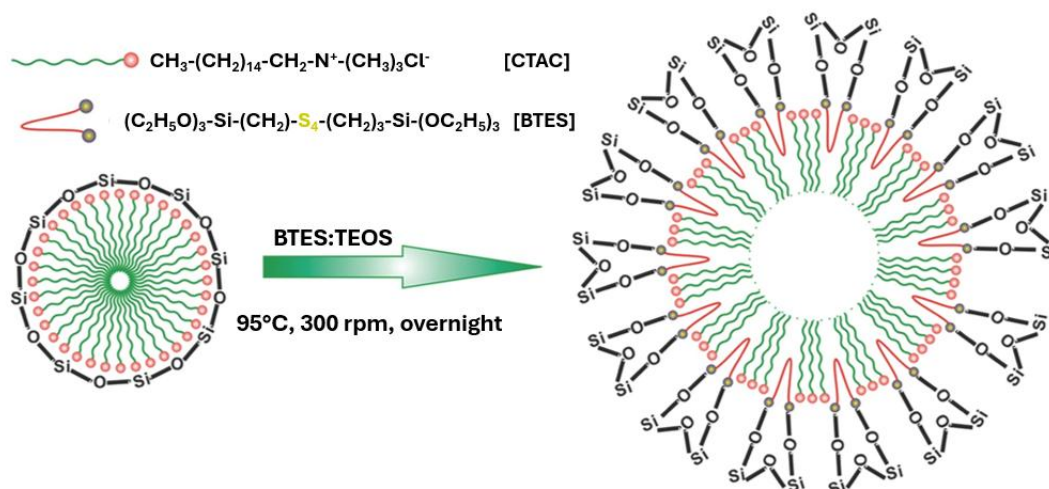
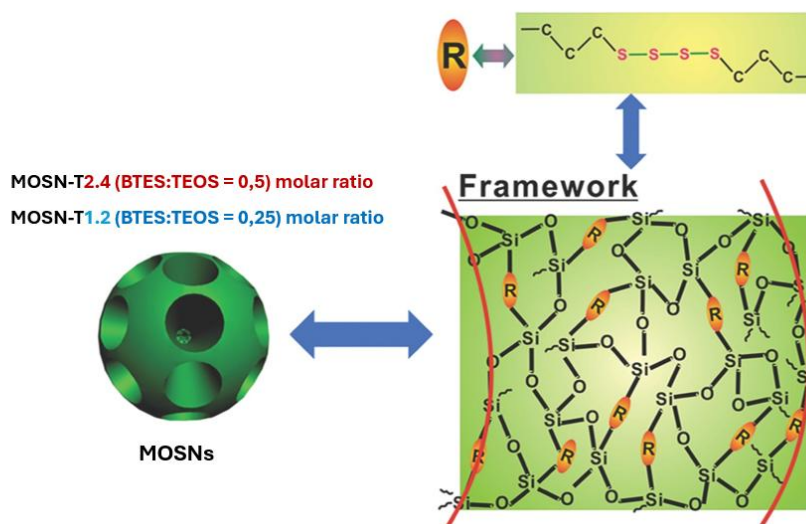


Figure 9. Chemical structure of Bis[3-(triethoxysilyl)propyl]tetrasulfide (BTES).

a)



b)



Scheme 3. Graphical representation of (a) the co-condensation of BTES into the silica framework and (b) tetrasulfide bridges inside the organosilica hybrid MOSNs. ¹⁰⁹ Adapted with permission. Copyright © 2014 WILEY-VCH

3.2.5 Synthesis of DMOSNs

The synthesis of DMOSNs reproduced that of the dendritic particles³⁷, while incorporating disulfide bridges by using BTES and increasing the reaction temperature up to 95°C. Specifically, this included adding 2,4 g TEA, 8 mL CTAC, and 80 mL water to 100 mL round-bottom flasks and stirring at 300 rpm at 95°C for 1h. A mixture of 4 g of TEOS and 32 mL of Toluene containing a variable amount of BTES was prepared. 5 g of BTES was added to obtain **DMOSN-TO1** particles, and 2,5 g of BTES was added to obtain **DMOSN-TO2** particles. The TEOS:BTES:Toluene mixture was then added dropwise to the corresponding round-bottom flasks, and the stirring was kept overnight at 95°C and 300 rpm. The organic phase was carefully removed using a syringe, and particle suspensions were then centrifuged (9000 rpm, 20 min). The resulting solids (**DMOSN-**

TO1 and **DMOSN-TO2**) were washed twice with water and three times with ethanol, with each washing step followed by sonication for 10 min and centrifugation at 9000 rpm for 20 min. Subsequently, the particles were extracted overnight at 78°C under stirring (300 rpm) using 20 mL of a mixture containing 10% (v/v) hydrochloric acid (12 M) and 90% (v/v) ethanol. After extraction, the particles were collected by centrifugation (9000 rpm, 30 min), washed with water, sonicated for 30 min, and centrifuged again. The washing cycle with water was repeated until the supernatant reached a pH value of approximately 6. Two final washing steps were performed using ethanol, each followed by sonication for 30 min and centrifugation. The particles were dried overnight at 70°C.

Following this synthesis, the first round of optimization was performed to obtain four additional DMOSNs (**DMOSN-OTO-3**, **DMOSN-OTO-4**, **DMOSN-OX-1**, and **DMOSN-OX-2**). The aim was to keep the same reaction conditions (95°C, 300 rpm) and change the base type, base amount, solvent type, and solvent amount. Table 1 summarizes the particles obtained and the synthesis conditions used. The amount of water (80 mL), CTAC (8 g), and the TEOS (4 g)/BTES (5 g) mixture was kept constant across all syntheses. The extraction, washing, and centrifugation steps were performed exactly as described previously for **DMOSN-TO1** and **DMOSN-TO2**.

A second round of optimization was conducted to obtain two additional DMOSNs (**DMOSN-OX-3** and **DMOSN-OX-4**). For this, the amount of water (80 mL), CTAC (8 g), L-Lysin (2,4 g), and the TEOS (4 g)/BTES (5 g) mixture was kept constant across all syntheses. The varying parameters are summarized in Table 1. The experimental conditions, including temperature (95°C) and stirring time (overnight), were also constant. However, the stirring speed was changed. After synthesis, the extraction, washing, and centrifugation steps were performed exactly as described for **DMOSN-TO1** and **DMOSN-TO2**.

Table 1. Variation of experimental conditions and resulting MOSNs and DMOSNs

	Base	Amount [g]	Co-solvent	Amount [ml]	Stirring speed	BTES:TEOS
MOSN-T2.4	TEA	0,6	-	-	300	0,5
MOSN-T1.2	TEA	0,6	-	-	300	0,25
DMOSN-OTO3	TEA	2,4	Toluene	16	300	0,5
DMOSN-OTO4	TEA	0,6	Toluene	32	300	0,5
DMOSN-OX1	L-Lysin	2,4	p-Xylene	16	300	0,5
DMOSN-OX2	L-Lysin	0,6	p-Xylene	32	300	0,5
DMOSN-OX3	L-Lysin	2,4	p-Xylene	16	150	0,5
DMOSN-OX4	L-Lysin	2,4	p-Xylene	8	300	0,5

3.2.6 Biodegradability Studies

To evaluate the potential structural degradation of DMOSNs in the presence of GSH, a preliminary biodegradability test was performed based on protocol from *Mekaru et al.* with some modifications⁹⁷. For this, 10 mg of DMOSNs (**DMOSN-OX-1**) and 10 mg of non-functionalized DMSNs (**DMSN-50** and **DMSN-130**), used as control materials, were dispersed in 4 mL of ultra-pure water. The particle suspensions were sonicated for 4 h. In the meantime, a 28 mM GSH solution was prepared by dissolving 0,43 g of L-glutathione in reduced form in 50 mL of water. Then, 10 mL of the GSH stock solution was added to each particle dispersion, ensuring a final GSH concentration of 20 mM. Simultaneously, the particles were prepared in control conditions,

consisting of 10 mg of the same materials, dispersed in 14 mL of ultrapure water instead of the GSH solution (20 mM). The hydrodynamic diameter of the colloidal suspensions, both in water (control) and in GSH (20 mM), was measured after 3h, 6h, and 24h of the addition of GSH. SAXS measurements were also performed for **DMOSN-OX-1**, **DMSN-50**, and **DMSN-130**, dispersed in both water and GSH (20 mM), 24 h after incubation at 37°C.

3.3 Material Characterization

3.3.1 Infrared (IR)- Spectrometry

Fourier-transform infrared (FTIR) spectra were recorded using a Bruker Vertex 70 FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. Spectra were collected over 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1} , averaging 32 scans per measurement. A background spectrum was acquired prior to each measurement and automatically subtracted from the sample spectrum.

3.3.2 Zeta Potential and Hydrodynamic diameter

DLS and zeta potential measurements were performed using a Nano Zetasizer Advance instrument (Malvern Panalytical, UK) operated with the Zetasizer software package. The measurements were performed at a 173° scattering angle and 25°C, with an equilibrium time of 120 s. Three measurements were performed for each sample. Samples were dispersed in water at a concentration of 0,7 mg/mL, followed by sonication (30 min for DMSNs and functionalized DMSNs, 1 h for organofunctionalized samples, and 3 h for the biodegradability studies). All measurements were carried out in disposable capillary cells (type DTS1070).

3.3.3 Nitrogen Physisorption Isotherms (77K)

N₂-physisorption isotherms were measured using an iQ3 gas sorption analyzer (Anton Paar QuantaTec Inc., USA) operated with ASiQwin software. Prior to analysis, samples (30–40 mg) were outgassed overnight in the instrument's degassing station until the temperature had stabilized and the pressure change (ΔP) was below 20 mTorr. The pure silica samples (**DMSNs-50** and **DMSNs-130**) were outgassed for 12 h at 150°C and the phosphonated particles (**DMSNs-50-PO₃** and **DMSNs-130-PO₃**) were at 80°C for 12h, and the disulfide-bridged silica samples were at 70°C for 12h. After cooling up to room temperature, the samples were transferred to the physisorption station and analyzed at liquid nitrogen temperature (77 K). Measurements were performed using 6 mm analysis cells. The specific surface area (S_{BET}) was derived from N₂-adsorption isotherms using the BET model within the relative pressure range of 0,05–0,30 (P/P_0). The total pore volume was calculated at $P/P_0 = 0,95$. Pore size distributions were determined by Non-local Density Functional Theory (NLDFT) analysis on the adsorption branch, assuming an amorphous SiO₂ surface with cylindrical pore geometry. Data evaluation was performed using ASiQwin 5.2 (Anton Paar Quantatech Inc.).

3.3.4 Thermogravimetric Analysis (TGA)

TGA was performed using a Netzsch STA 449 F3 Jupiter instrument (Selb, Germany). Samples were placed in crucibles and heated from 25°C to 800°C under a continuous flow of nitrogen (20 mL/min). The heating rate was set to 10°C/min, and the resulting mass loss (%) was recorded as a function of temperature. The mass losses were evaluated in the temperature range of 150–700°C.

3.3.5 Solid-state Magic Sngle Spinning Nuclear Magnetic Resonance Spectroscopy (MAS NMR)

Bruker Avance NEO 500 wide-bore system (Bruker BioSpin, Germany) was used to measure the MAS NMR of the samples at room temperature. For ^{29}Si NMR measurements, a resonance frequency of 99,38 MHz was used, with a MAS spinning rate of 8 kHz and a cross-polarization (CP) contact time of 5 ms. The ^{13}C NMR measurements were performed at a resonance frequency of 125,78 MHz, with a MAS spinning speed of 14 kHz and a CP contact time of 3 ms. The ^{31}P NMR experiments were done at 202.49 MHz, using a MAS spinning rate of 14 kHz and a CP contact time of 3 ms.

3.3.6 Transmission Electron Microscopy (TEM)

TEM images of the samples were acquired using a Philips CM200 microscope at 200 kV. For sample preparation, the particles were dispersed in water to obtain a suspension with a concentration of $0,7 \text{ mg}\cdot\text{mL}^{-1}$. Then, 2-3 drops of this suspension were placed in a carbon film-coated 300 mesh copper grid and allowed to dry prior to imaging.

3.3.7 Confocal Raman Microscopy

Raman spectroscopy was conducted with a WITec alpha300 A confocal Raman microscope (WITec, Germany) equipped with a UHTS300S GREEN NIR spectrograph (600 g mm^{-1} grating, blaze 500 nm) and an Andor DU401_BR-DD CCD detector cooled to -59°C . A 531 nm diode laser was used as the excitation source, and the beam was focused through a Zeiss EC 20 $\times$ /0,4 NA objective. The spectral information was collected over $100\text{--}1100 \text{ cm}^{-1}$, with an integration time of 6 s and 10 accumulations per measurement. The laser power used for functionalized silica and sulfide-bridged silica samples was 10 mW to avoid degradation of organic groups, and for the pure silica samples was 39,7 mW. Data were acquired and processed using WITec Control and Project FOCUS software.

3.3.8 Small-angle X-Ray Scattering (SAXS)

SAXS measurements were performed on selected samples (**DMOSN-OX-1**, **DMSN-50**, and **DMSN-130**) from the biodegradability studies (Section 3.2.6) using a Nano-inXider instrument (Xenocs, France) equipped with a Cu K α radiation source ($\lambda = 0,154 \text{ nm}$). SAXS data were collected to assess nanoscale structural changes following incubation in the presence of GSH (20 mM). Scattering profiles were analyzed to evaluate changes in particle organization and aggregation behavior.

4. Results and Discussion

4.1 Synthesis and Functionalization of DMSNs

Controlling the particle size of MSNs carriers is essential for drug delivery applications and begins with precise adjustment of synthesis parameters, such as temperature and stirring rate^{37,52}. In this work, two different DMSNs were synthesized: **DMSN-50** and **DMSN-130**, with average particle sizes of 50 nm and 130 nm, respectively.

4.1.1 Synthesis of Dendritic MSNs (DMSNs)

According to TEM images (Fig. 10), both materials exhibit spherical dendritic morphology. The smaller particles, **DMSN-50**, with a diameter of 50 ± 5 nm, and the bigger ones, **DMSN-130**, with a diameter of 130 ± 10 nm. Such differences in size were also confirmed through DLS measurements (Table 2), where the hydrodynamic diameters increased from 119 ± 1 nm (**DMSN-50**) to 162 ± 2 nm (**DMSN-130**), clearly demonstrating that the particle size can be tuned by adding hexane into the biphasic system and increasing the temperature to 60°C during the synthesis. DLS profiles for both **DMSN-50** and **DMSN-130** revealed a narrow particle size distribution (Fig. 11) with Polydispersity Index (PDI) values below 0,3 (Table 2)¹¹⁴.

The porosity of the DMSNs was characterized by N_2 -physisorption analysis. The isotherms shown in Fig. 12a have a typical type IV form, with capillary condensation observed in the relative pressure range $0,3 < P/P_0 < 0,8$, indicating the presence of mesopores. The isotherms also show a H1-type hysteresis loop, indicating uniform open radial pores, consistent with the dendritic, radially oriented pore channels observed in the TEM images (Fig. 10). The larger capillary condensation volume and wider hysteresis loop seen for **DMSN-130** reflect its larger pore diameter (7,3 nm) and significantly higher pore volume ($1,23 \text{ cm}^3 \cdot \text{g}^{-1}$), while the smaller **DMSN-50** particles show a flatter condensation step, matching its smaller pore size (2,5–4,8 nm) and lower pore volume ($0,48 \text{ cm}^3 \cdot \text{g}^{-1}$). Particle size and textural properties of **DMSN-50** and **DMSN-130** are summarized in Table 2.

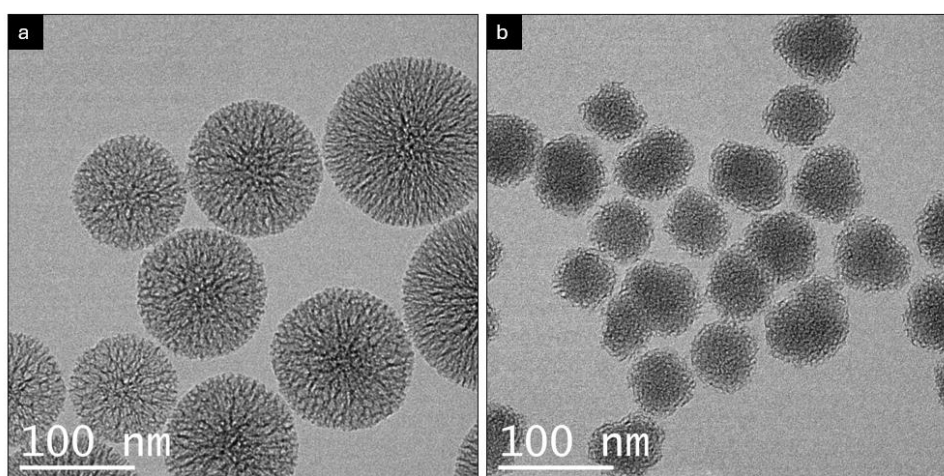


Figure 10. TEM images of non-functionalized dendritic particles: a) **DMSN-130**, b) **DMSN-50**.

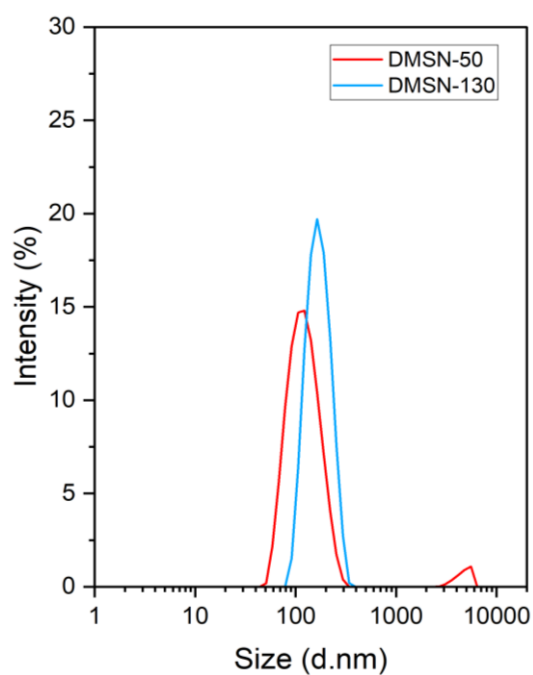


Figure 11. DLS profiles of **DMSN-50** and **DMSN-130**

Table 2. Hydrodynamic diameter, Polydispersity indexes (PDI), and Zeta Potential obtained from DLS analysis, and specific surface area (S_{BET}), pore volume, and pore size obtained from N_2 -physisorption analysis of **DMSN-50** and **DMSN-130**.

	Hydrodynamic diameter [nm]	PDI	Zeta Potential [mV]	S_{BET} [$\text{m}^2 \cdot \text{g}^{-1}$]	Pore size [nm]	Pore Volume [$\text{cm}^3 \cdot \text{g}^{-1}$]
DMSN-50	119 ± 1	0,186	$-29,3 \pm 1,1$	760	2,5 - 4,8	0,48
DMSN-130	162 ± 2	0,068	$-31,9 \pm 1,5$	826	7,3	1,23

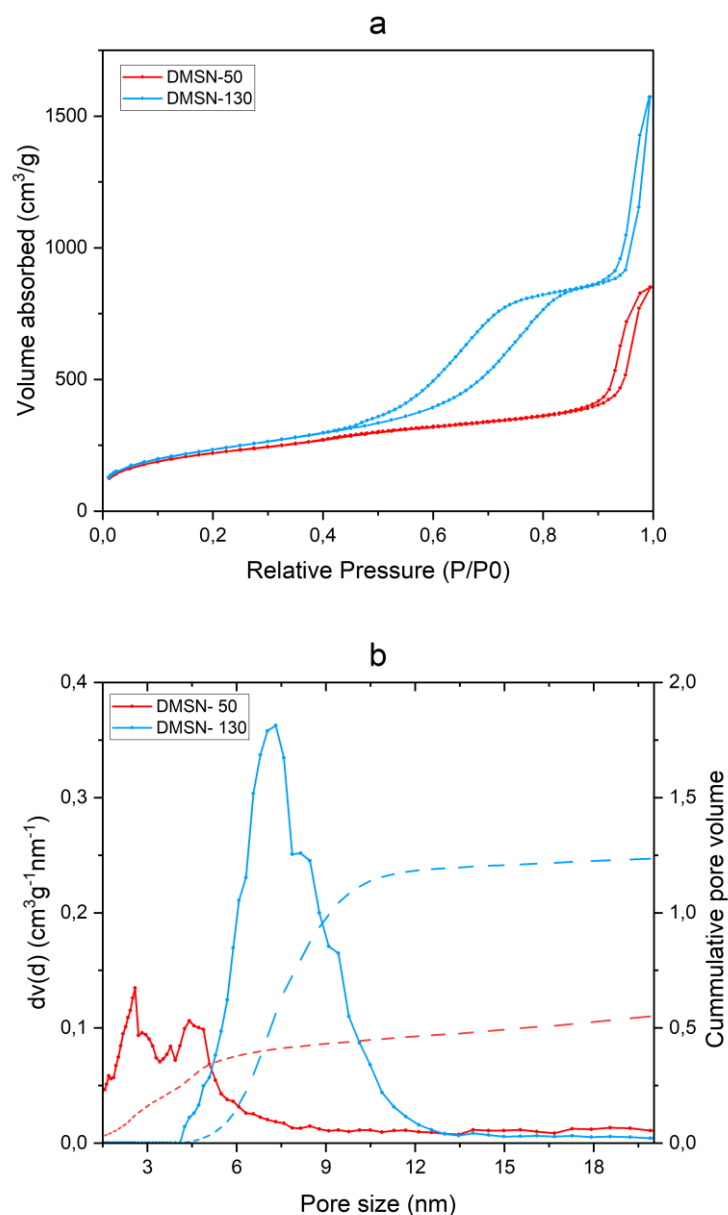


Figure 12. a) N_2 -adsorption isotherms (77K) and b) NLDFT pore size distributions of **DMSN-50** and **DMSN-130**.

4.1.2 Functionalization of DMSNs with THMP

The non-functionalized particles (**DMSN-50** and **DMSN-130**) were modified with THMP via a post-grafting strategy to insert phosphonate groups into the silica network, according to a reported protocol³⁷.

As shown in the DLS profiles (Fig. 13), the particle size distributions remain narrow for both functionalized materials (**DMSN-50-PO₃** and **DMSN-130-PO₃**), with no significant change in average particle size compared to the respective non-modified precursors, **DMSN-50** and **DMSN-**

130. Thus, the measured hydrodynamic diameters of **DMSN-50-PO₃** (128 ± 2 nm) and **DMSN-130-PO₃** (154 ± 1 nm) are very close to those of the calcined samples (119 ± 1 nm and 162 ± 2 nm, respectively). These small variations correlate with the modification of particle surface with the THMP silane and do not indicate any aggregation, as PDI values remain below 0,3. Zeta potential (Table 3) decreased after functionalization, which is to be expected after the inclusion of THMP, due to the negatively charged phosphonate functions grafted on the silica surface. This was corroborated by TGA analysis (Fig. 14), which showed 10% mass loss for **DMSN-50-PO₃** and **DMSN-130-PO₃** (Table 3), consistent with the thermal decomposition of the organic moieties in the grafted materials, thereby confirming successful silica functionalization.

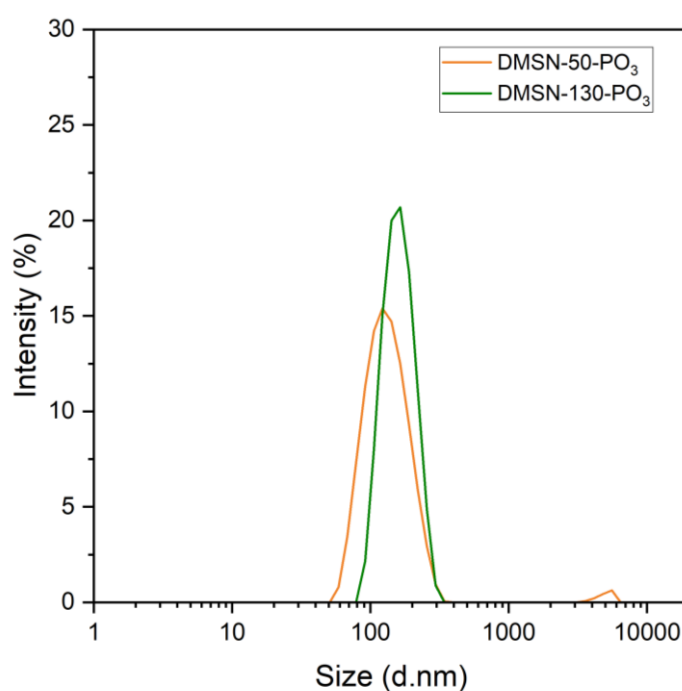


Figure 13. DLS profiles of **DMSN-50-PO₃** and **DMSN-130-PO₃**.

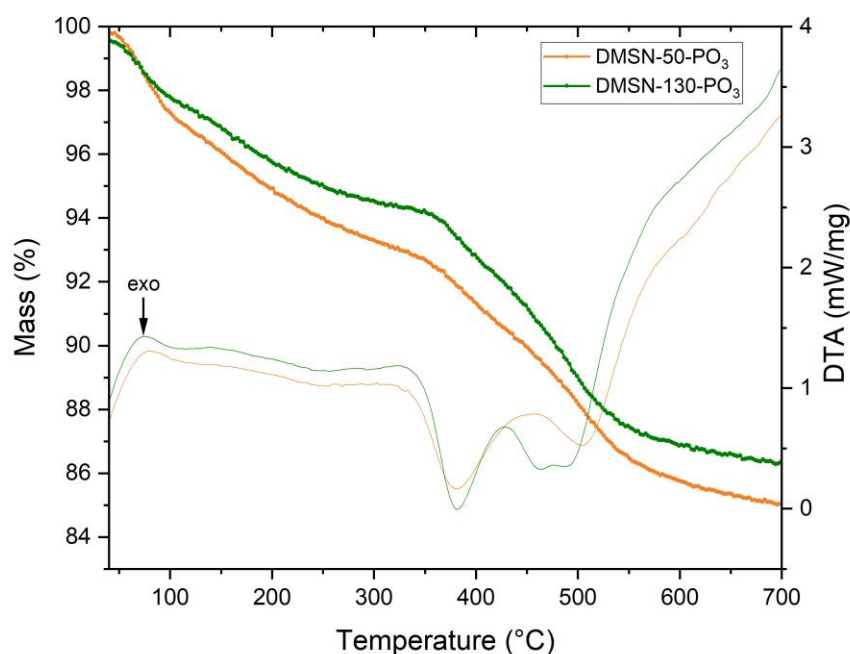


Figure 14. Mass loss (%) and DSC profiles of **DMSN-50-PO₃** and **DMSN-130-PO₃**

Table 3. Hydrodynamic diameter, Polydispersity indexes (PDI), and Zeta Potential obtained from DLS analysis, and mass loss [%] obtained from TGA analysis of functionalized samples **DMSN-50-PO₃** and **DMSN-130-PO₃**.

	Hydrodynamic diameter [nm]	PDI	Zeta Potential [mV]	Mass loss [%]
DMSN-50-PO₃	128 ± 2	0,126	-32,6 ± 0,2	10,3
DMSN-130-PO₃	154 ± 1	0,045	-37,7 ± 0,1	10,0

FTIR characterization (Fig. 15) further confirmed the successful grafting of the small organic molecules on the DMSNs. The main peak of Si-O-Si vibrations at around 1057–1059 cm⁻¹ was observed for both non-modified and functionalized materials. An additional peak at 1203 cm⁻¹ corresponding to the stretching vibrations of the phosphoryl (P=O) and P-O-Si bonds, was observed in both **DMSN-50-PO₃** and **DMSN-130-PO₃** spectra and confirmed the presence of phosphonated functions. Furthermore, the peaks at 2860–2978 cm⁻¹ (ν Csp³-H) and 1373 cm⁻¹ (δ C-H) aligned with the presence of aliphatic C-H bonds of the THMP structure.

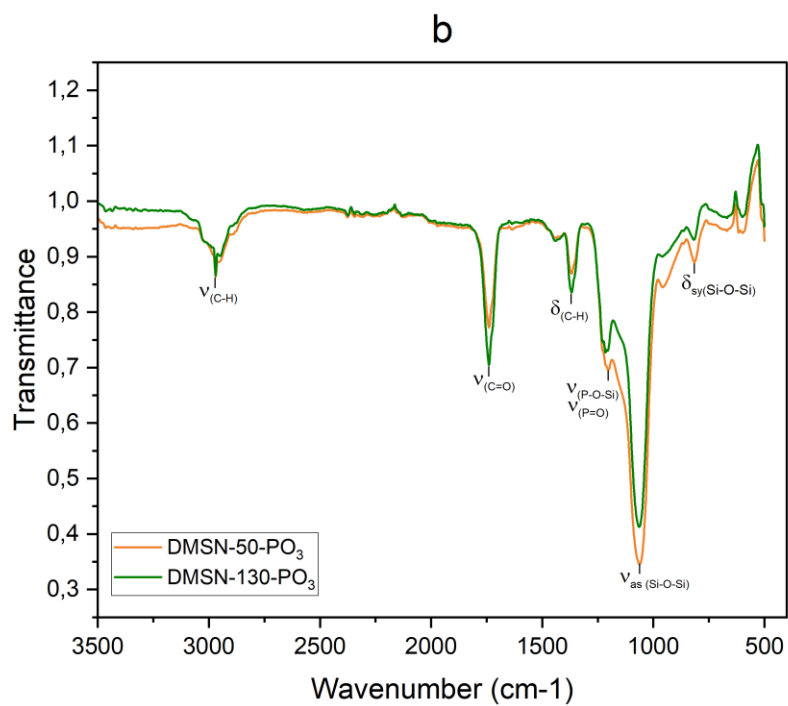
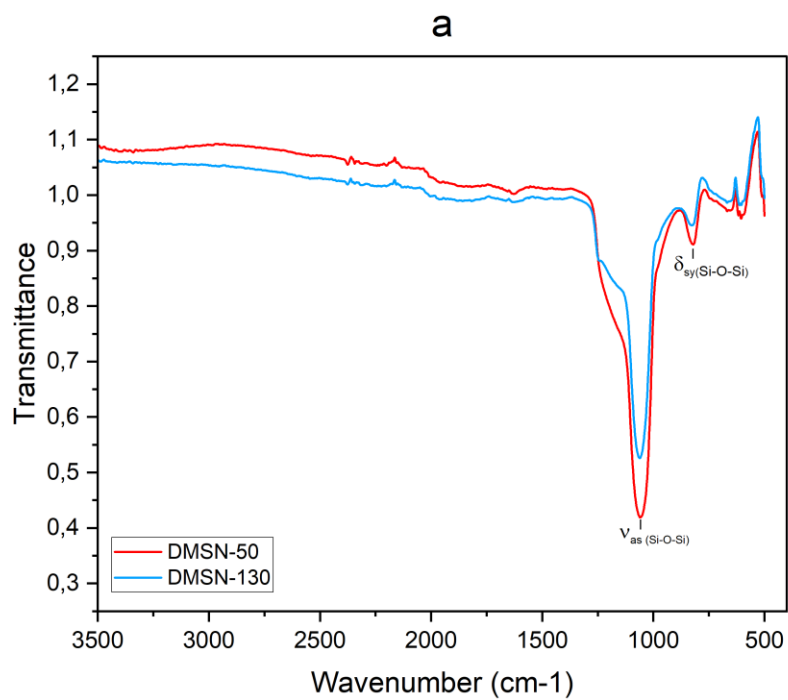


Figure 15. FTIR spectra of a) pure silica **DMSN-50** and **DMSN-130** particles and b) functionalized **DMSN-50-PO₃** and **DMSN-130-PO₃** particles.

The solid-state ^{29}Si CP/MAS NMR spectra of non-functionalized **DMSN-50** and **DMSN-130** (Fig. 16 a) reveal the typical Q^2 , Q^3 , and Q^4 signals of the silica framework (Scheme 4) responding to chemical shifts (δ) at -90 ppm, -100 ppm, and -110 ppm, respectively. These signals are also present in the functionalized particles (Fig. 16c), however, additional T^2 (-60 ppm) and T^3 (-67 ppm) peaks were observed, confirming surface modification in both functionalized samples, **DMSN-50-PO₃** and **DMSN-130-PO₃**. The absence of T^0 signals also indicated that the organic small molecules are not covalently attached to the silica. Analysis of the ^{13}C CP/MAS NMR spectra (Fig. 16b) revealed three distinct signals for both types of functionalized DMSNs. The peak at 7 ppm was attributed to the methyl group on the phosphonated species and to the α -carbon directly bonded to the silicon atom of THMP. Furthermore, the signal at 25 ppm was attributed to the terminal carbon directly attached to the PO_3 group of THMP, while the peak at 64 ppm was assigned to the β -carbon attached to the silicon. Furthermore, the presence of a strong signal at 25 ppm (Fig. 16d) in the ^{31}P MAS NMR spectra of the functionalized particles further confirmed the presence of the phosphonate species grafted onto both **DMSN-50-PO₃** and **DMSN-130-PO₃**.

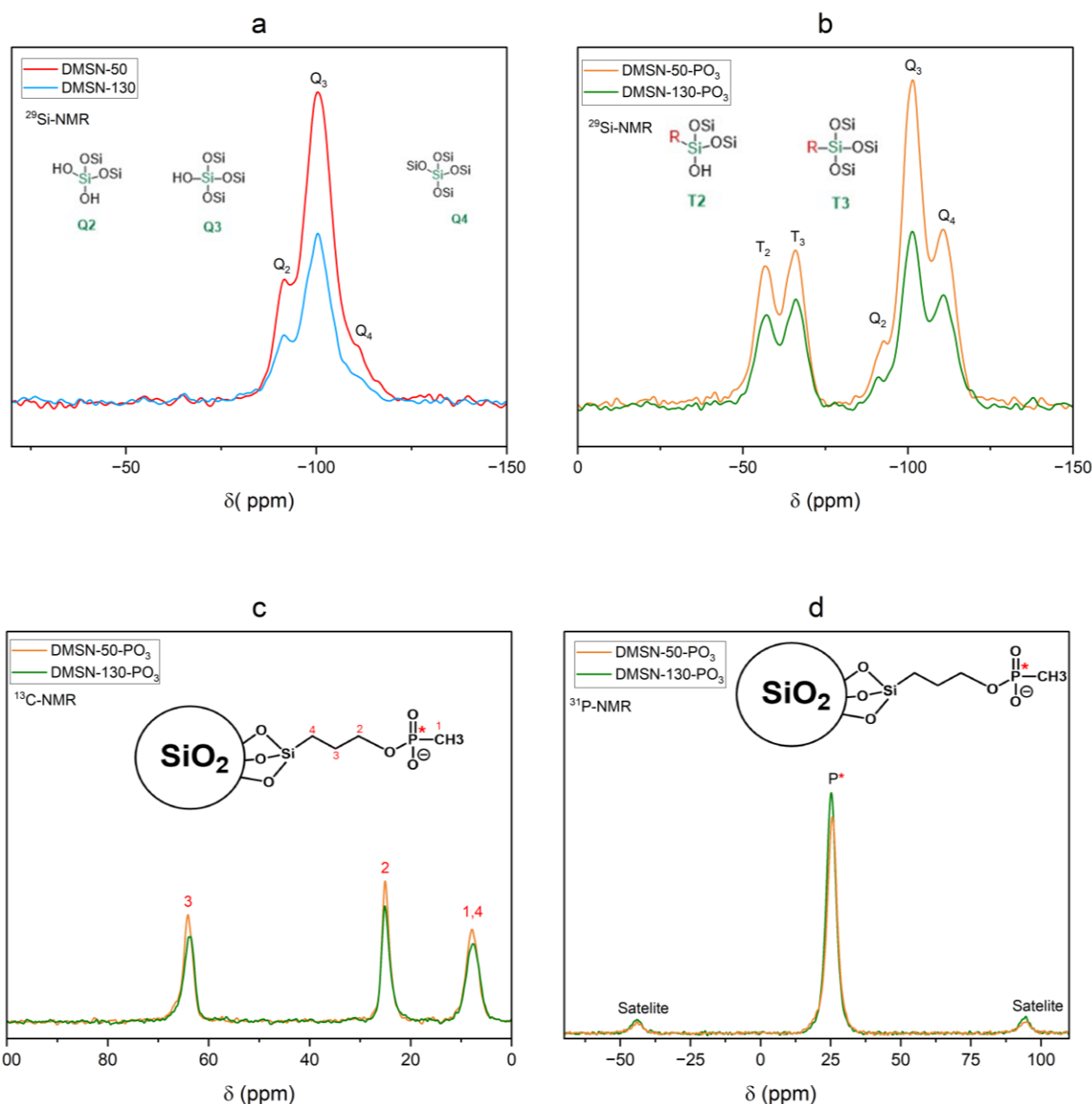


Figure 16. Solid-state a) ^{29}Si CP/MAS NMR spectra of **DMSN-50** and **DMSN-130** b) ^{29}Si CP/MAS NMR spectra of **DMSN-50-PO₃** and **DMSN-130-PO₃** c) ^{13}C CPNMR of **DMSN-50-PO₃** and **DMSN-130-PO₃** and d) ^{31}P MAS NMR of **DMSN-50-PO₃** and **DMSN-130-PO₃**.

The porosity of the DMSNs after functionalization was analyzed by N_2 -physisorption (Fig. 17). Both **DMSN-50-PO₃** and **DMSN-130-PO₃** exhibited a decrease in specific surface area after the post-grafting. For **DMSN-50-PO₃**, the surface area decreased from 760 to 421 $\text{m}^2\cdot\text{g}^{-1}$, and for **DMSN-130-PO₃** from 826 to 588 $\text{m}^2\cdot\text{g}^{-1}$. A similar reduction was observed in the pore volume where **DMSN-50-PO₃** decreased from 0,48 to 0,33 $\text{cm}^3\cdot\text{g}^{-1}$, and in **DMSN-130-PO₃** from 1,23 to 0,92 $\text{cm}^3\cdot\text{g}^{-1}$ (Table 4), as a result of the incorporation of organic molecules into the silica network. No significant change in pore size was observed compared to the corresponding precursors.

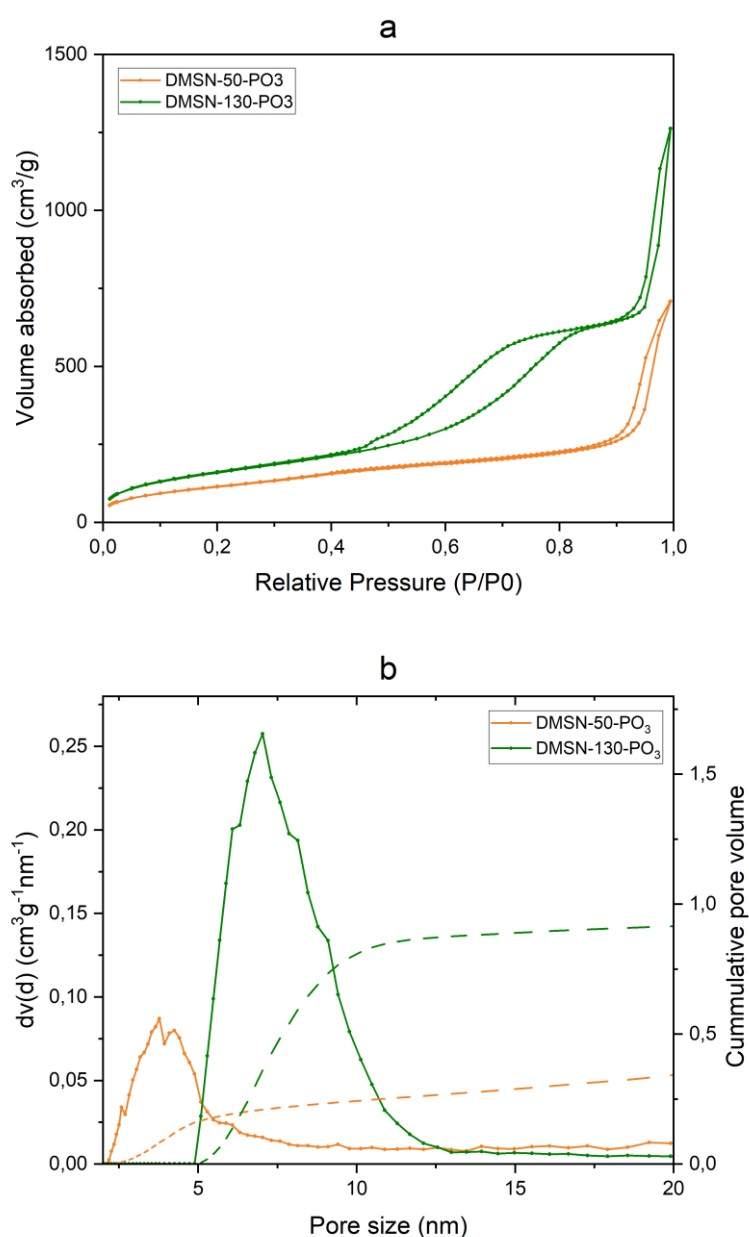


Figure 17. a) N₂-adsorption isotherms (77K) and b) NLDFT pore size distributions of **DMSN-50-PO₃** and **DMSN-130-PO₃**.

Table 4. Specific surface area (S_{BET}), pore volume, and pore size obtained from N₂-physisorption analysis of **DMSN-50-PO₃** and **DMSN-130-PO₃**.

	S_{BET} [m ² · g ⁻¹]	Pore size [nm]	Pore Volume [cm ³ · g ⁻¹]
DMSN-50-PO₃	421	3,8	0,33
DMSN-130-PO₃	588	7,0	0,93

4.1.3 Coating DMSNs with Liposomes

To obtain liposomes, three phospholipids N-(carbonyl-methoxypolyethylenglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamin (DSPE-MPEG2000), Phosphatidylcholine (CS-95), and cholesterol (CHO-HP), were selected to mimic the composition of the cell membrane. The structures of the lipids used for coating are shown in Figure 21. The liposomes were first prepared by thin- film hydration method. The resulting dry lipid film was rehydrated with the particle suspension (0,3 mg/ml in PBS) to obtain the liposome-coated DMSNs (**L-DMSN-50** and **L-DMSN-130**). From the TEM images of the liposome-coated particles (Fig.18), a more homogeneous coating appears to be achieved with **L-DMSN-130**, whereas in **L-DMSN-50** the particles appear embedded within the phospholipid-based matrix. From the DLS profiles of **L-DMSN-50** and **L-DMSN-130** (Fig.19), an increase in hydrodynamic diameter was observed with respect to the non-coated materials. Thus, compared with **DMSN-50** and **DMSN-130**, the particle size increased from 128 ± 2 nm to 271 ± 1 nm in **L-DMSN-50** and from 154 ± 1 nm to 430 ± 40 nm in **L-DMSN-130**, respectively, confirming the inclusion of the liposome layer. The zeta potential of the liposome-coated DMSNs showed no significant change with respect to the unmodified samples, remaining close to -32 mV for both coated **L-DMSN-50** and **L-DMSN-130**, while TGA (Fig.20) confirmed successful liposome incorporation, as evidenced by mass losses of 55% for **L-DMSN-50** and 54% for **L-DMSN-130** (Table 5). Such mass losses were attributed to the thermal decomposition of the lipid-derived organic material from the liposome layer (Fig. 21). Solid-state NMR further supported liposome coating by confirming the presence of lipid functions in the coated samples (Fig. 22b).

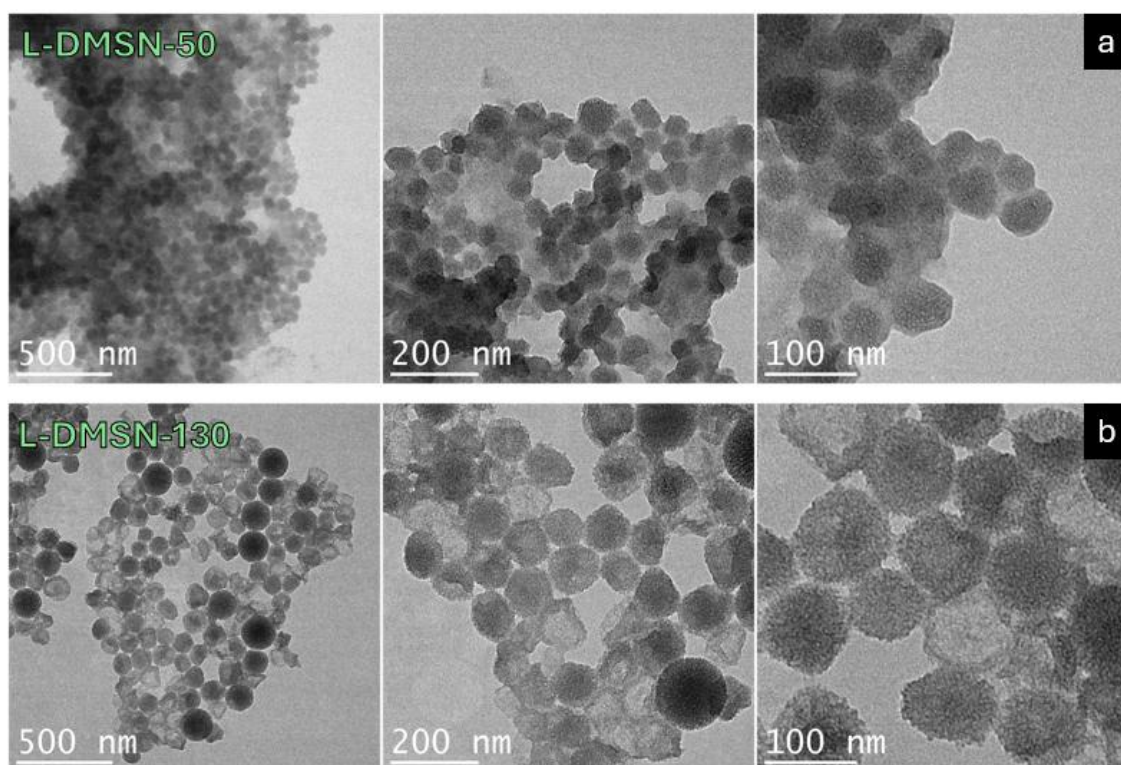


Figure 18. TEM images of liposome-coated particles a) **L-DMSN-50**, b) **L-DMSN-130**.

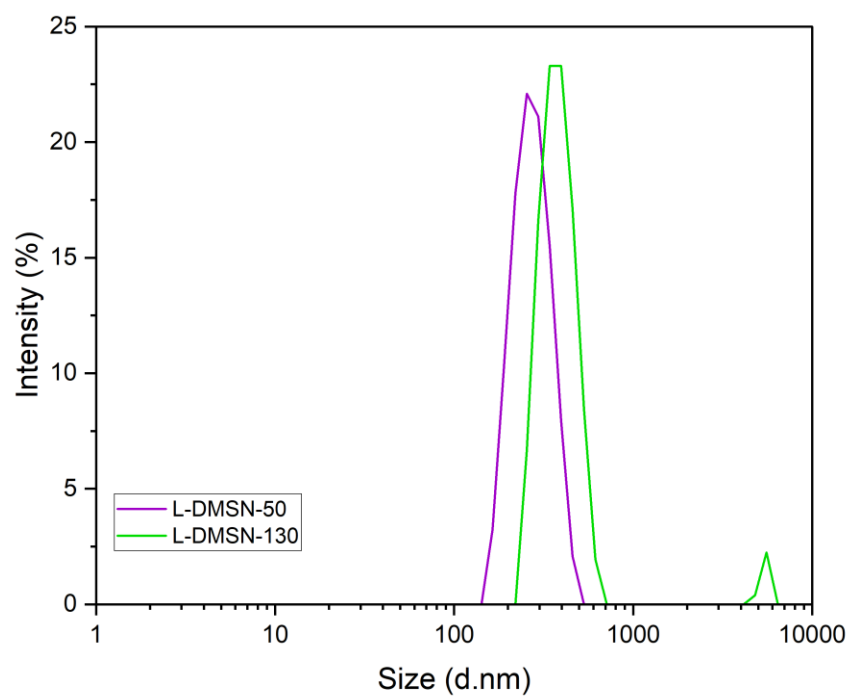


Figure 19. DLS-Profiles of the liposome-coated **L-DMSN-50** and **L-DMSN-130**

Table 5. Hydrodynamic diameter, Polydispersity indexes (PDI), and Zeta Potential obtained from DLS analysis, and mass loss [%] obtained from TGA analysis of the liposome-coated samples **L-DMSN-50** and **L-DMSN-130**.

	Hydrodynamic diameter [nm]	PDI	Zeta Potential [mV]	Mass loss [%]
L-DMSN-50	271 ± 1	0,28	-32,5 ± 0,6	55
L-DMSN-130	430 ± 40	0,34	-31,2 ± 0,2	54

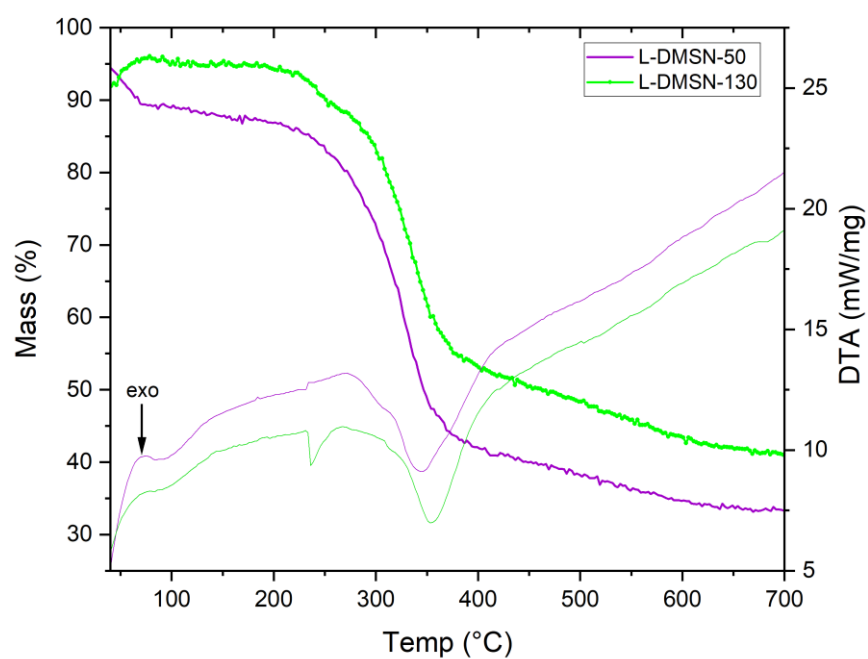


Figure 20. Mass loss (%) and DSC profiles of **L-DMSN-50** and **L-DMSN-130**.

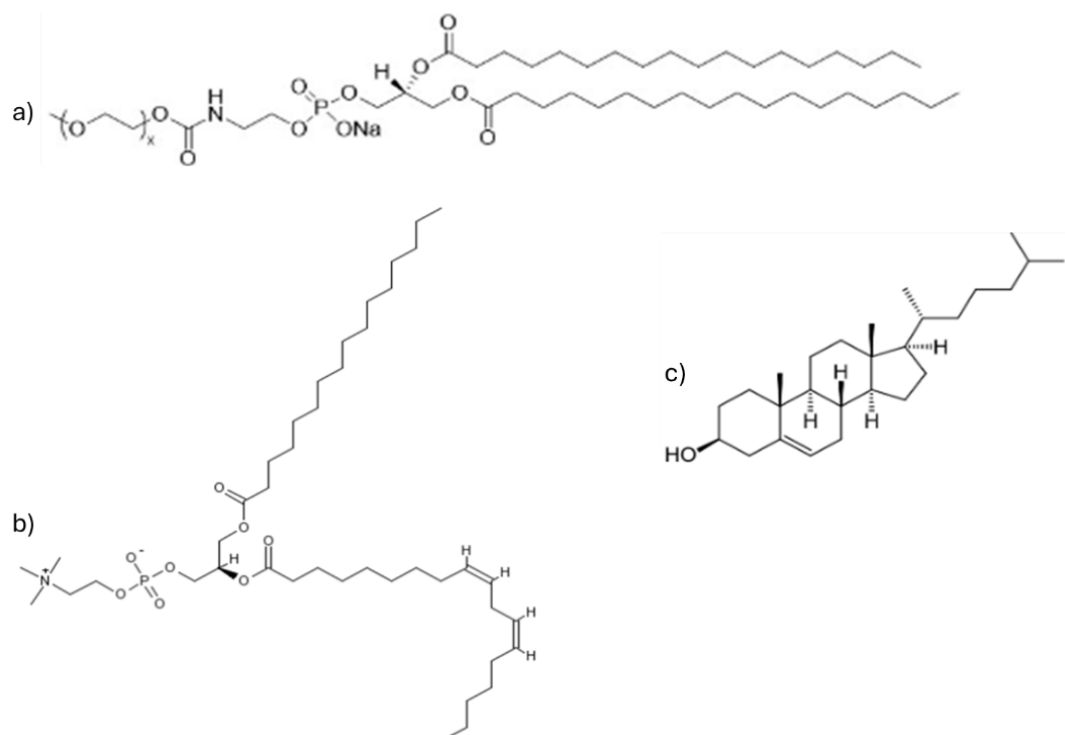
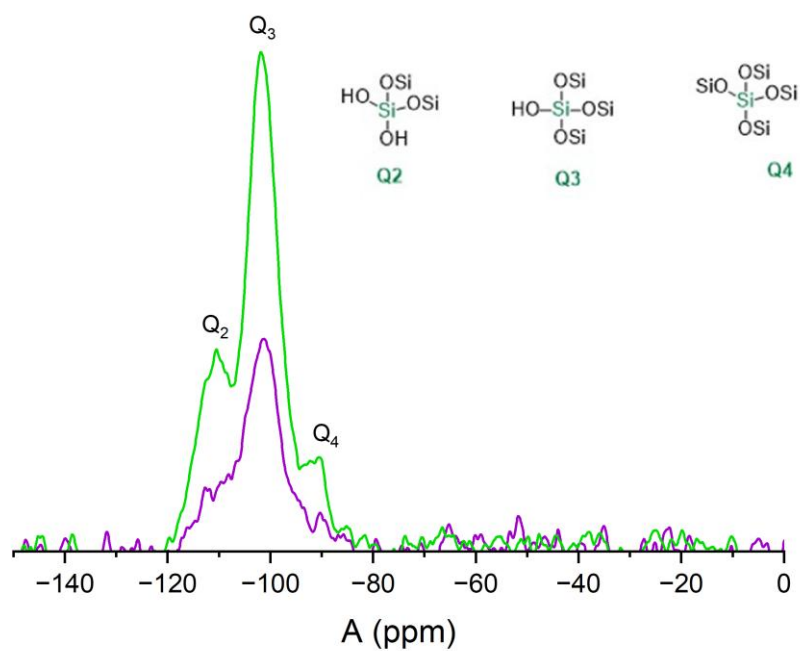


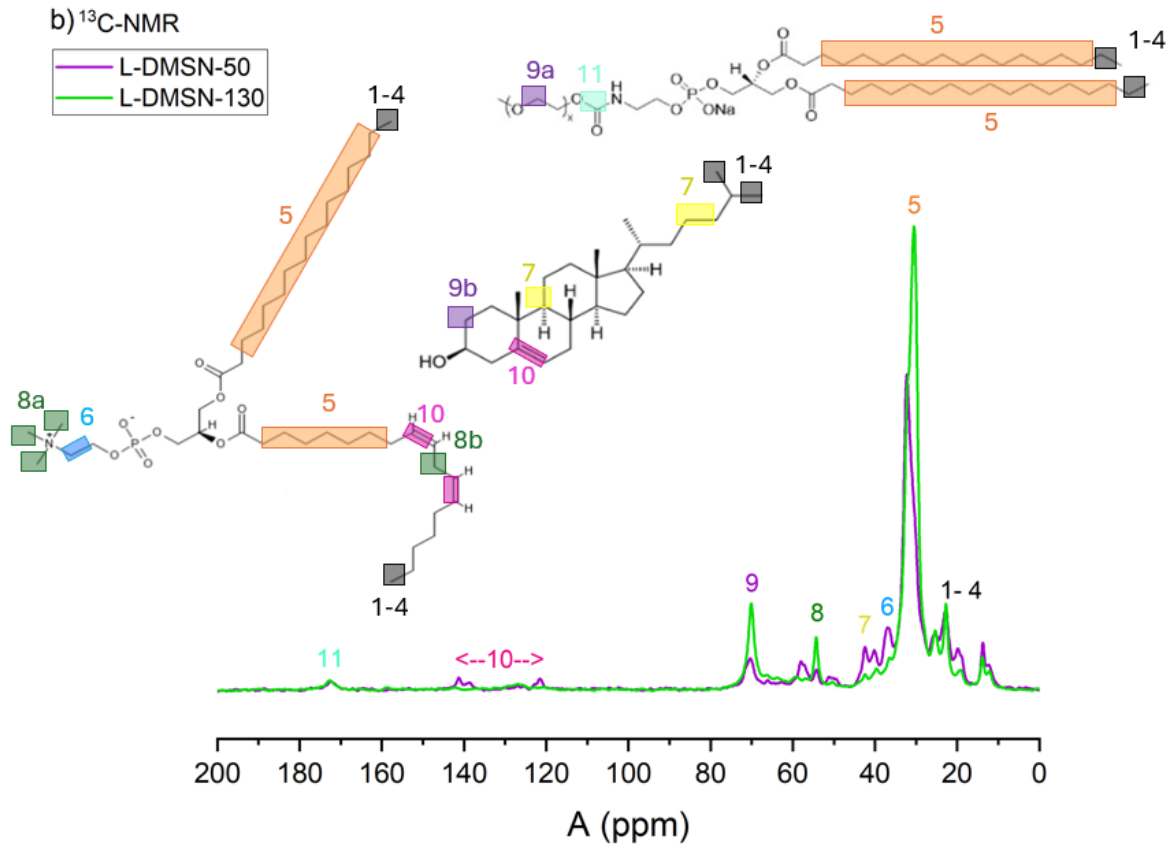
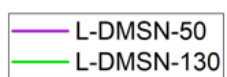
Figure 21. Structures of the lipids used to coat DMSNs particles: a) N-(carboxymethoxypolyethylenglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamin (DSPE-MPEG2000) b) Phosphatidylcholine (CS-95), and c) cholesterol (CHO-HP).

The solid-state ^{29}Si CP/MAS NMR (Fig. 22 a) data revealed the typical Q^2 , Q^3 , Q^4 signals for the silica framework responding to chemical shifts (δ) -90 ppm, -100 ppm, and -110 ppm, which indicate the retention of silica structure after the liposome coating. The absence of T signals was attributed to the lack of chemical modification of the silica network during liposome incorporation. ^{13}C CP/MAS NMR spectra (Fig. 22b) also provided information on the structure of the lipid moieties in silica coatings. The peaks observed in the 10–25 ppm region (signals 1–4) were attributed to the terminal CH_3 groups and short-chain CH_2 units of the phospholipid acyl tails (indicated in black). The signal at 30 ppm (5) corresponded to methylene segments of the lipid chains (orange). The signal (6) at 37 ppm aligned with the presence of $\text{N-CH}_2\text{-CH}_2$ groups from the phosphatidylcholine headgroup (blue), while signal (7) at 42 ppm aligned with carbon atoms in the steroid ring system of cholesterol (yellow). Signal (8), at 55 ppmppm originated from $\text{N}^+(\text{CH}_3)_3$ group of phosphatidylcholine (green). The peak observed at (9) at 70 ppm was attributed to the $\text{CH}_2\text{-O-}$ groups of the PEG chain in DSPE-MPEG2000, with possible contribution from cholesterol's C3 carbon (purple). Finally, signal (10) in the 120-140 ppm region (magenta) was assigned to the double bonds in phosphatidylcholine and the aromatic region of cholesterol. The further-shifted signal (11) at 172 ppm was attributed to the carbamate carbonyl-type carbon (turquoise) of DSPE-MPEG2000. ^{31}P MAS NMR (Fig. 22 c) additionally showed a characteristic signal at -1 ppm corresponding to the phosphonate functions in DSPE-MPEG2000 and phosphatidylcholine.

a) ^{29}Si -NMR



b) ^{13}C -NMR



c) ^{31}P -NMR

— L-DMSN-50
— L-DMSN-130

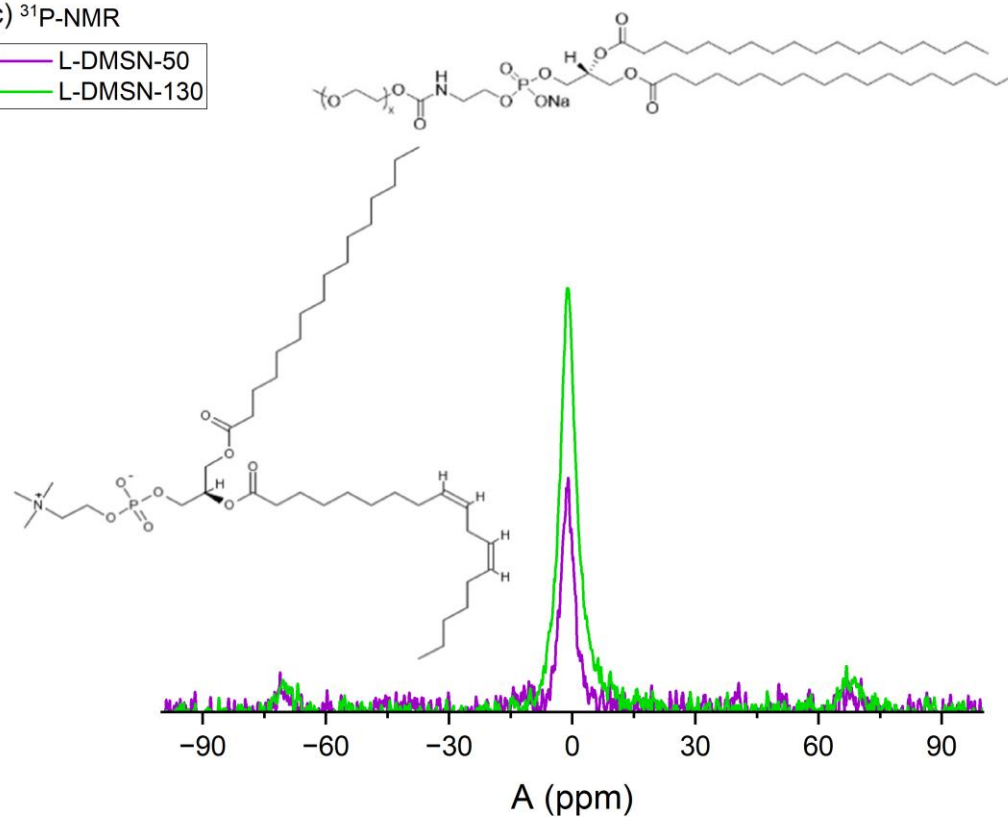


Figure 22. Solid-state a) ^{29}Si CP/MAS NMR, ^{13}C CP/MAS NMR and c) ^{31}P MAS NMR of **L-DMSN-50** and **L-DMSN-130**.

The N_2 -adsorption isotherms (Fig. 23) show a significant decrease in specific surface area (S_{BET}) compared to the uncoated DMSNs, further indicating the complete coating of the silica with organic functions.

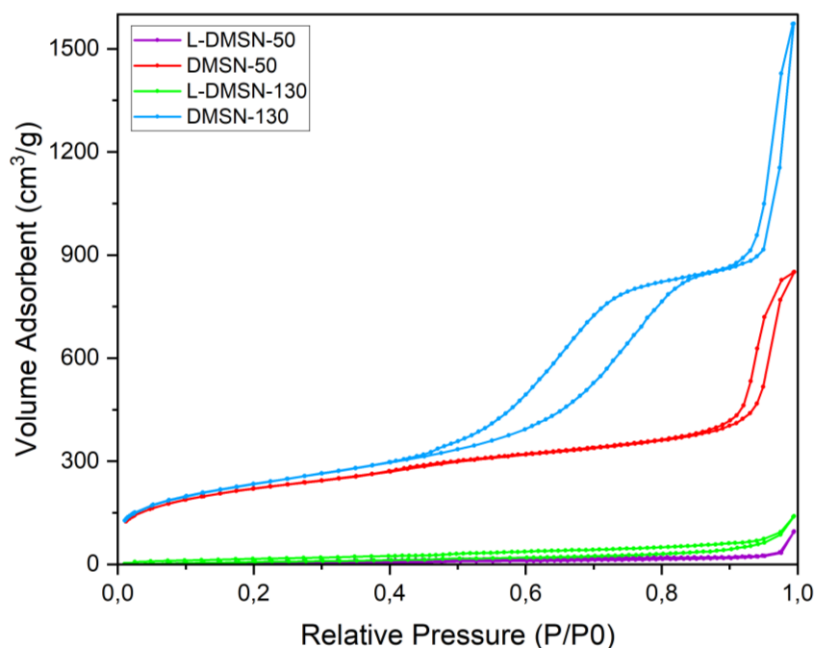


Figure 23. N₂-physisorption isotherms (77 K) of **L-DMSN-50**, **L-DMSN-130**, **DMSN-50** and **DMSN-130**.

4.1.4 Cell Studies of the Liposome-coated DMSNs

To evaluate the biocompatibility of the different DMSNs, cell studies were performed using TR146 human buccal epithelial cells as *in vitro* model. Cells were treated with pure silica nanoparticles (**DMSN-50** and **DMSN-130**), phosphonate-functionalized particles (**DMSN-50-PO₃** and **DMSN-130-PO₃**), and liposome-coated particles (**L-DMSN-50** and **L-DMSN-130**) at increasing concentrations ranging from 10 µg mL⁻¹ to 440 µg mL⁻¹. Cell viability was assessed using the MTS assay after 4 h of incubation with particle treatments, which measured cell metabolic activity as an indicator of viability. A safe concentration identified in the cell viability assays at 250 µg mL⁻¹, showing maintained cellular viability after up to 4 h of incubation with particle treatments, was selected to perform the cellular uptake studies under the same conditions as for the viability experiment, but using rhodamine-labelled materials (**Rh-DMSN-50**, **Rh-DMSN-130**, **Rh-DMSN-50-PO₃**, **Rh-DMSN-130-PO₃**, and liposome coted **L-DMSN-50-Rh** and **L-DMSN-130-Rh**). Fluorescence microscopy was then used to evaluate the interaction of silica particles with the buccal epithelial cells.

4.1.4.1 MTS cell viability

Cell viability after exposure to the different DMSN particle types was quantified using the MTS assay (Fig. 24). After 4 h of incubation, none of the particle treatments induced significant cytotoxicity at concentrations up to 250 µg mL⁻¹, with approximately 85–100% cell viability retained. However, at 440 µg mL⁻¹, a decrease in cell viability was observed, mainly in TR146 cells incubated with non-functionalized materials, particularly **DMSN-50**, which showed a 19% decrease compared to the non-treated cells (control). In contrast, phosphonate-functionalized DMSNs (**DMSN-50-PO₃** and **DMSN-130-PO₃**) maintained cell viability above 90% even at the highest concentration tested (440 µg mL⁻¹). On the other hand, liposome-coated DMSNs (**L-DMSN-50** and **L-DMSN-130**) induced a significant increase in cell viability at higher concentrations (250 and 440 µg mL⁻¹) compared to the control (non-treated TR146 cells), a

response similar to that observed in cells treated with empty liposomes tested at equivalent concentrations (125 and 220 $\mu\text{g mL}^{-1}$ correspondent to phospholipids in liposome-coated DMSNs) (Fig. 25). This suggested that both **L-DMSN-50** and **L-DMSN-130** promote metabolic stimulation in treated cells, likely due to increased intracellular lipid content. These findings supported the biological activity of the liposomal shell of the DMSNs and indicated effective interactions between the coated particles and the buccal epithelial cells, potentially facilitating cellular internalization.

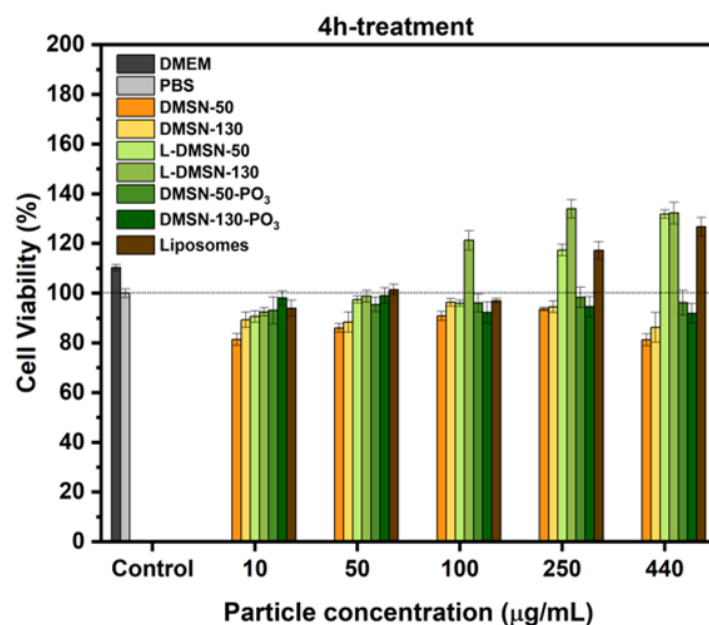


Figure 24. Cell viability of cells treated with DMSNs, functionalized DMSNs, and liposome-coated DMSNs following 4 h-exposure.

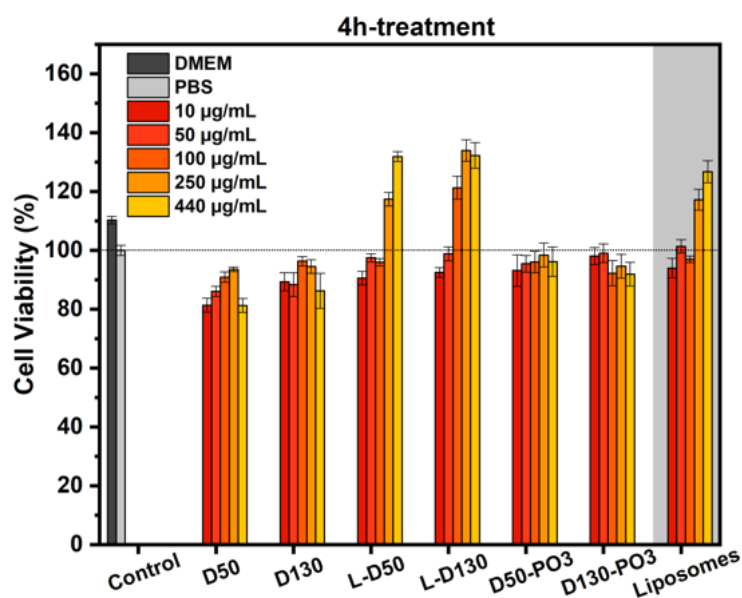


Figure 25. Dose-dependent effects of DMSNs, functionalized DMSNs, and liposome-coated DMSNs on cell viability after 4-h exposure

4.1.4.2 Rhodamine Labelling and Cellular Uptake

To further evaluate the interactions between silica particles and buccal epithelial cells, confirming cellular uptake and enabling a direct comparison of uptake efficiency among pure, phosphonate-functionalized, and liposome-coated DMSNs, the nanoparticles were fluorescently labeled with Rhodamine B isothiocyanate following a previously reported procedure⁷, which yielded the fluorescent materials **Rh-DMSN-50**, **Rh-DMSN-130**, **Rh-DMSN-50-PO₃**, and **Rh-DMSN-130-PO₃**. The labeled **Rh-DMSN-50** and **Rh-DMSN-130** particles were subsequently coated with liposomes via the thin-film hydration method to produce the hybrid systems **Rh-L-DMSN-50** and **Rh-L-DMSN-130**.

As observed from the DLS profiles (Fig. 27), the particle size remains narrow for rhodamine-labelled DMSNs, with no significant change in hydrodynamic diameters upon incorporation of the fluorophore. For example, the hydrodynamic size of **Rh-DMSN-50-PO₃** (114 ± 1 nm) and **Rh-DMSN-130-PO₃** (163 ± 1 nm) were similar to those of the precursors **DMSN-50-PO₃** (128 ± 2 nm) and **DMSN-130-PO₃** (154 ± 1 nm), respectively. Importantly, the PDI values remained below 0,3, confirming that fluorescent labelling does not produce significant particle aggregation. Zeta potential values of fluorescent materials (Table 6) remained negative after rhodamine labelling, similar to the respective precursors.

Similarly, **Rh-L-DMSN-50** and **Rh-L-DMSN-130** exhibited average hydrodynamic diameters of 390 ± 20 nm and 430 ± 60 nm, respectively, which are also similar to the non-labelled liposome-coated particles, **L-DMSN-50** (271 ± 1 nm) and **L-DMSN-130** (430 ± 40 nm). Broader PDI values (0,30–0,50) can be attributed to lipid coating and hydration layer rather than to rhodamine incorporation. Zeta potential measurements also remained similar for labelled samples in comparison to non-fluorescent liposome-coated particles, with values around -30 mV. TGA analysis (Fig. 26) revealed mass losses of 0,3%-2,9%, corresponding to the thermal decomposition of the incorporated rhodamine, thereby confirming successful fluorescent labeling. This low Rhodamine content correlated with the minimal alterations to particle size, colloidal stability, and surface chemistry, while still providing sufficient fluorescence intensity for reliable particle visualization.

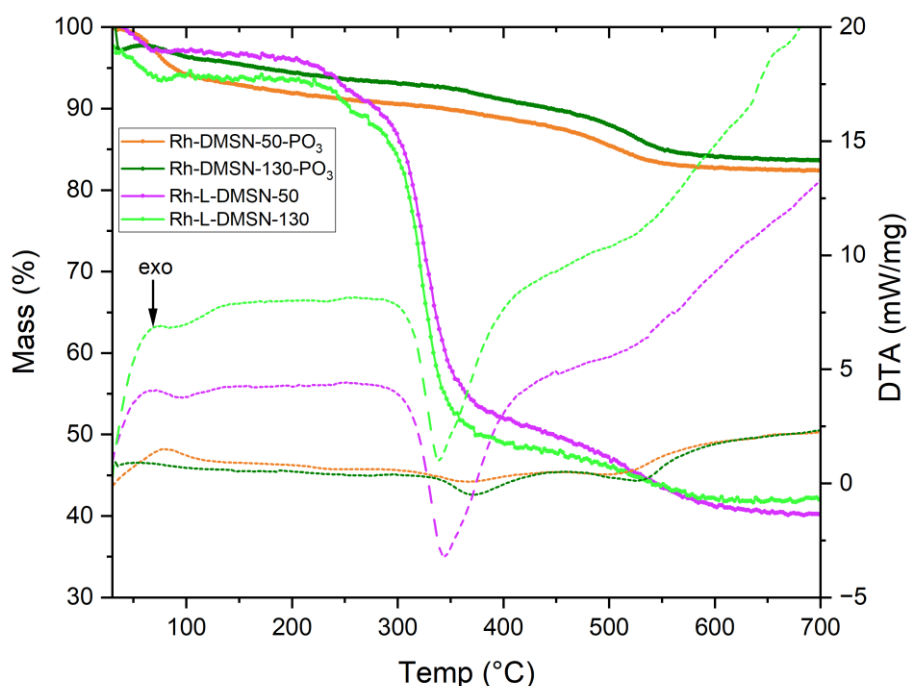


Figure 26. Mass loss (%) and DTA profiles of rhodamine-labeled samples **Rh-DMSN-50-PO₃**, **Rh-DMSN-130-PO₃**, **Rh-L-DMSN-50**, and **Rh-L-DMSN-130**.

The FTIR spectra of phosphonated functionalized DMSNs and the corresponding labelled samples showed no significant differences (Fig. 28), consistent with the low rhodamine content incorporated into the silica network determined by TGA. Thus, in the spectra of the rhodamine-labeled **Rh-DMSN-50-PO₃** and **Rh-DMSN-130-PO₃**, the characteristic silica bands, including the asymmetric Si–O–Si stretching vibration around 1080–1100 cm^{−1}, the symmetric stretching mode near 800 cm^{−1}, and the Si–O–Si bending vibration at approximately 460 cm^{−1} were observed as for the precursors **DMSN-50-PO₃** and **DMSN-130-PO₃**. The signal observed around 1200 cm^{−1} was attributed to the stretching vibrations of the phosphoryl (P=O) and P–O–Si bonds from THMP grafting, which remained unchanged after rhodamine labelling.

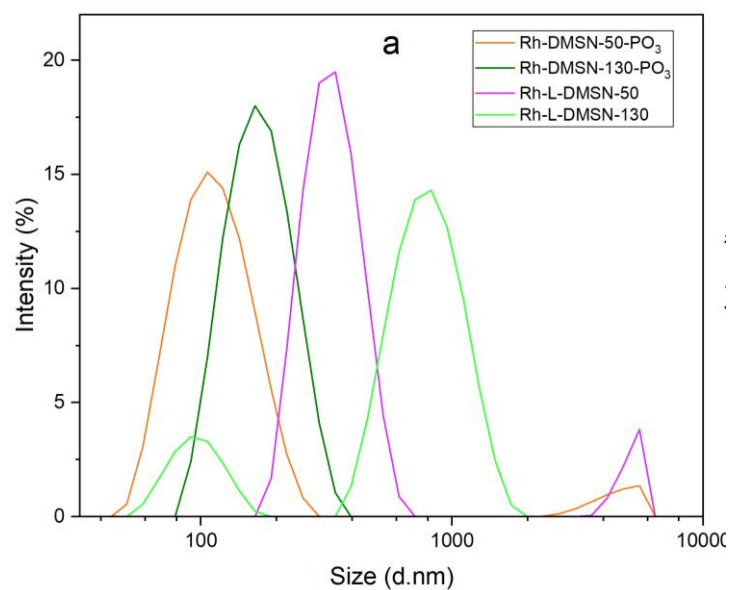


Figure 27. DLS profiles of rhodamine-labeled DMSNs phosphonated (**Rh-DMSN-50-PO₃** and **Rh-DMSN-130-PO₃**) and coated with liposomes (**Rh-L-DMSN-50** and **Rh-L-DMSN-130**)

Table 6. Hydrodynamic diameter, polydispersity indexes (PDI), and zeta potential obtained from DLS analysis, and mass loss [%] obtained from TGA analysis of the rhodamine-labelled samples **Rh-DMSN-50-PO₃**, **Rh-DMSN-130-PO₃**, **Rh-L-DMSN-50**, and **Rh-L-DMSN-130**.

	Hydrodynamic diameter [nm]	PDI	Zeta Potential [mV]	Mass loss [%]
Rh-DMSN-50- PO₃	114 ± 1	0,22	-21,3 ± 0,3	10,5 (1,6 % Rhod)
Rh-DMSN-130-PO₃	163 ±1	0,08	-33,8 ± 1,4	11,8 (0,3 % Rhod)
Rh-L-DMSN-50	389 ± 20	0,30	-33,1 ± 0,7	56,4 (1,3% Rhod)
Rh-L-DMSN-130	433 ± 58	0,50	-31,1 ± 0,8	51,7 (2,9% Rhod)

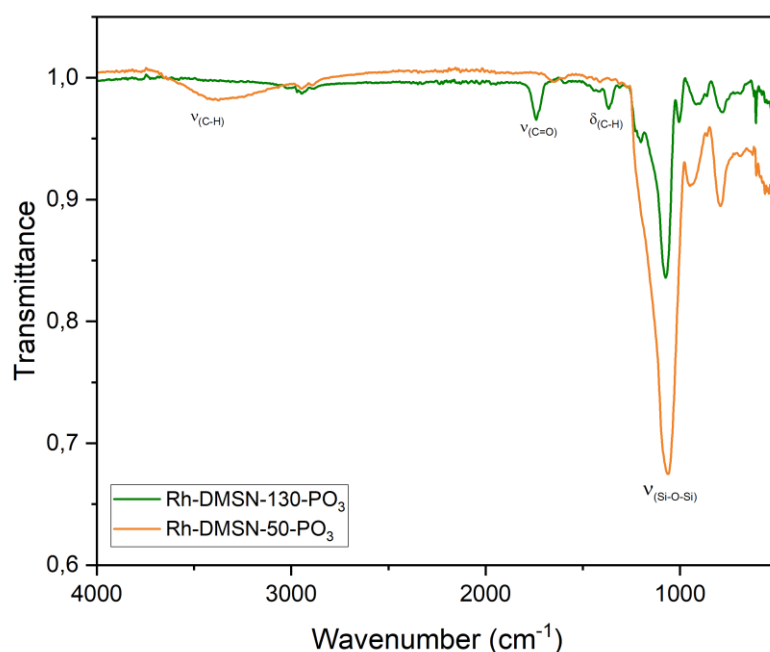


Figure 28. FTIR spectra of rhodamine labelled samples **Rh-DMSN-50-PO₃** and **Rh-DMSN-130-PO₃** particles

Figure 29 shows confocal fluorescence microscopy images of TR146 cells incubated with rhodamine-labeled nanoparticles at a concentration of $250 \mu\text{g}\cdot\text{mL}^{-1}$, which was previously confirmed to be non-cytotoxic in cell viability studies. The rhodamine fluorescence signal (red) was detected in all treated cells, even after several PBS washes following incubation for 4 h at 37°C . This confirmed successful fluorescent labeling and sustained interaction of the DMSNs with the buccal epithelial cells. **Rh-DMSN-50**, **Rh-DMSN-130**, **Rh-DMSN-50-PO₃**, and **Rh-DMSN-130-PO₃** were observed in association with the cell layer, predominantly at the outer cell membrane. However, clear evidence of intracellular internalization could not be conclusively demonstrated from single-plane (2D) images. On the other hand, Nile Blue-labeled empty liposomes (control treatment), applied at concentrations comparable to those used for **Rh-L-DMSN-50** and **Rh-L-DMSN-130**, produced diffuse cellular staining consistent with dye leakage, limiting their reliability for accurate uptake assessment. For the liposome-coated DMSNs (**Rh-L-DMSN-50** and **Rh-L-DMSN-130**), the fluorescence signal was weaker, and internalization could not be definitively evaluated based on 2D images alone. Therefore, further z-stack acquisition and 3D reconstruction would be required to distinguish true intracellular localization from surface association and to enable quantitative uptake analysis. Although preliminary, these findings confirm effective interaction of DMSNs with epithelial cells. Further optimization of the staining protocol and imaging analysis will be necessary to conclusively verify particle uptake.

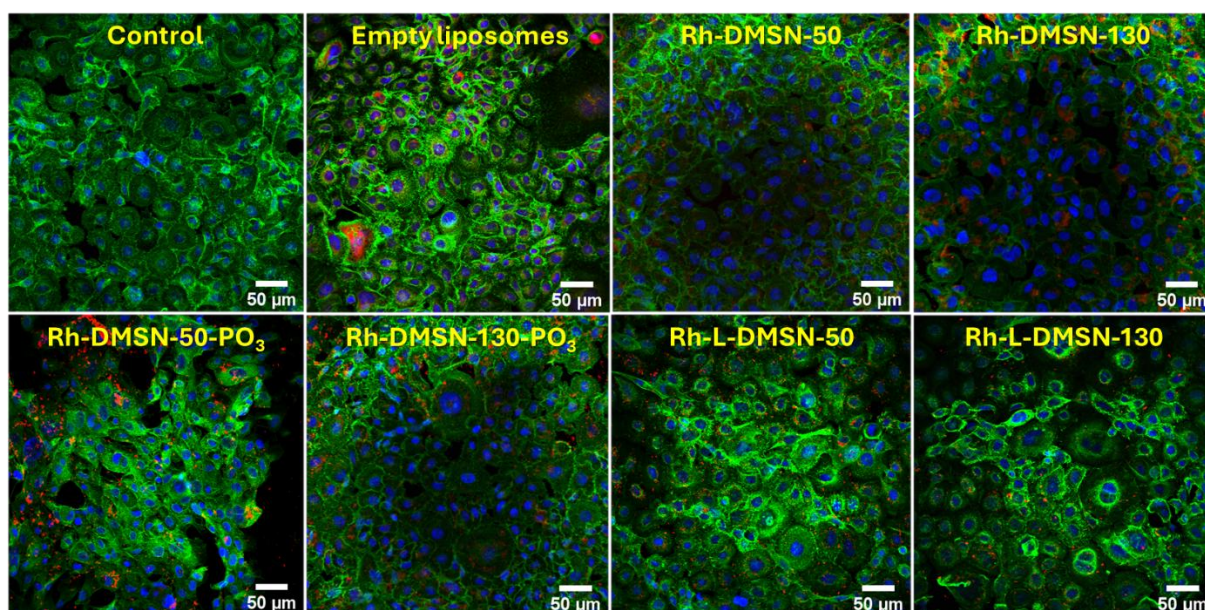


Figure 29. Representative confocal fluorescence microscopy images of TR146 cells incubated for 4 h at 37°C with rhodamine-labeled DMSNs ($250 \mu\text{g}\cdot\text{mL}^{-1}$, red). The actin cytoskeleton was stained with Alexa Fluor™ 488 Phalloidin (green), and the nuclei were stained with Hoechst/DAPI (blue). Control corresponds to non-treated TR146 cells. Nile Blue-labeled empty liposomes (control treatment) were applied to the cells at equivalent concentrations of phospholipids present in **Rh-L-DMSN-50** and **Rh-L-DMSN-130**. Scale bars stand for 50 μm .

4.2 Synthesis of Disulfide-bridged MSNs

4.2.1 Synthesis of MOSNs

Following a modified protocol based on *Wu et al.*, two mesoporous organosilica nanoparticles (MOSNs) were synthesized via a CTAC-templated, co-condensation approach, in which TEOS and BTES were mixed under basic conditions at 95°C¹⁰⁹. By varying the molar ratio of BTES to TEOS, two materials were obtained: **MOSNs-T2.4** (BTES:TEOS = 0,5) and **MOSNs-T1.2** (BTES:TEOS = 0,25). In both cases, the tetrasulfide bridges were incorporated directly into the silica framework via co-condensation reaction, yielding spherical particles, as observed in TEM images (Fig. 30). As shown by the DLS profiles of the MOSNs (Fig. 31), both **MOSN-T2.4** and **MOSN-T1.2** exhibited narrow particle size distributions, with hydrodynamic diameters of $171 \pm 1 \text{ nm}$ and $124 \pm 1 \text{ nm}$, respectively. The PDI values remained below 0,3 for both samples (0,275 for **MOSN-T2.4** and 0,198 for **MOSN-T1.2**), indicating minimal aggregation (Table 7). Furthermore, both materials showed negative zeta potentials (-21 mV for MOSN-T2.4 and -16 mV for MOSN-T1.2), similar to those of DMSNs (Table 7).

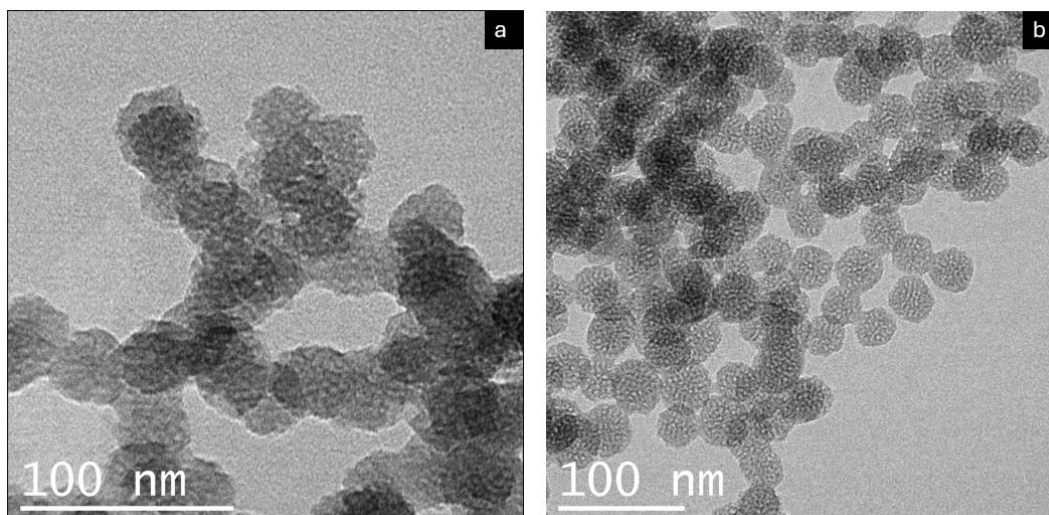


Figure 30. TEM images of tetrasulfide-bridged silica particles: a) **MOSN-T2.4** and b) **MOSN-T1.2**.

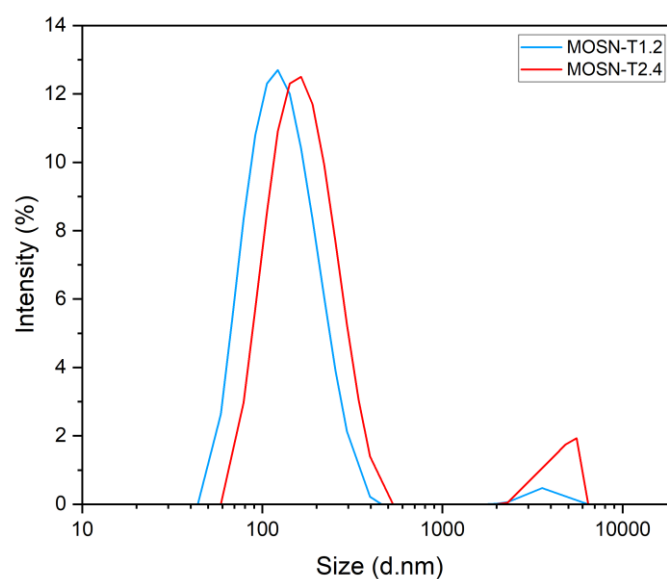


Figure 31. DLS profiles of **MOSN-T2.4** and **MOSN-T1.2**.

Table 7. Hydrodynamic diameter, polydispersity indexes (PDI), and zeta potential retained from DLS analysis, specific surface area (S_{BET}), pore volume, and pore size obtained from N_2 -physisorption analysis, and mass loss [%] obtained from TGA analysis of **MOSN-T2.4** and **MOSN-T1.2**.

	Hydrodynamic diameter [nm]	PDI	Zeta Potential [mV]	S_{BET} [$\text{m}^2 \cdot \text{g}^{-1}$]	Pore size [nm]	Pore Volume [$\text{cm}^3 \cdot \text{g}^{-1}$]	Mass loss [%]
MOSN-T2.4	171 ± 0	0,275	$-20,0 \pm 0,2$	399	2,6	0,27	36
MOSN-T1.2	124 ± 1	0,198	$-16,2 \pm 0,3$	855	2,6	0,46	33

N₂-physorption analysis (Fig. 32a) confirmed that both **MOSN-T2.4** and **MOSN-T1.2** exhibited type IV isotherms, characteristic of mesoporous materials. Furthermore, these materials displayed a narrow pore-size distribution with similar pore size of 2,6 nm (Fig. 32b). However, **MOSN-T1.2** showed a significantly higher specific surface area (855 m²·g⁻¹) and pore volume (0,46 cm³·g⁻¹) compared to **MOSN-T2.4** (399 m²·g⁻¹ and 0,27 cm³·g⁻¹, respectively) (Table 7).

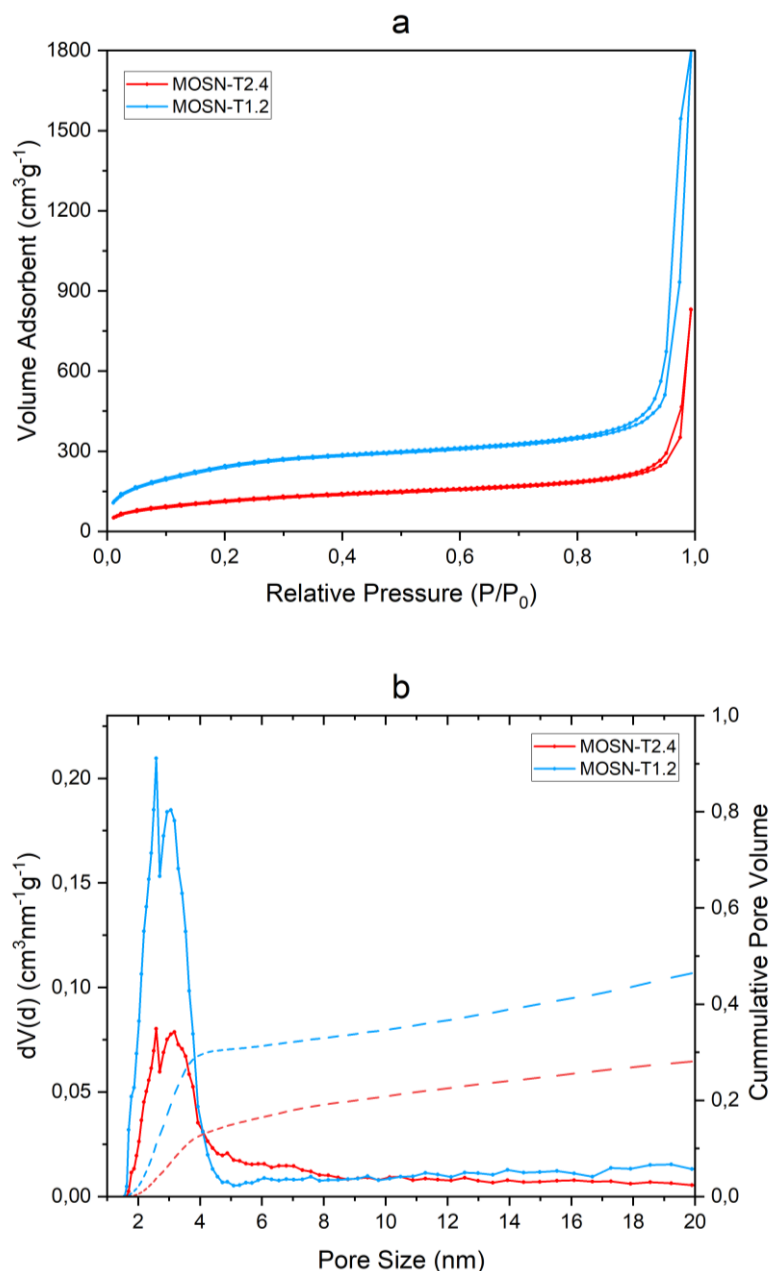


Figure 32. a) N₂-physorption isotherms (77 K) and b) NLDFT pore size distributions of **MOSN-T2.4** and **MOSN-T1.2**.

Moreover, thermograms obtained from TGA showed significant mass loss for both organosilica-based materials compared to the pure DMSN (**DMSN-50** and **DMSN-130**) (Fig. 33), corresponding to 36% for **MOSN-T2.4** and 33% for **MOSN-T1.2** (Table 7), due to the thermal

decomposition of the BTES incorporated into the silica framework. The slightly higher mass loss detected in **MOSN-T2.4** was consistent with the higher BTES content used during synthesis.

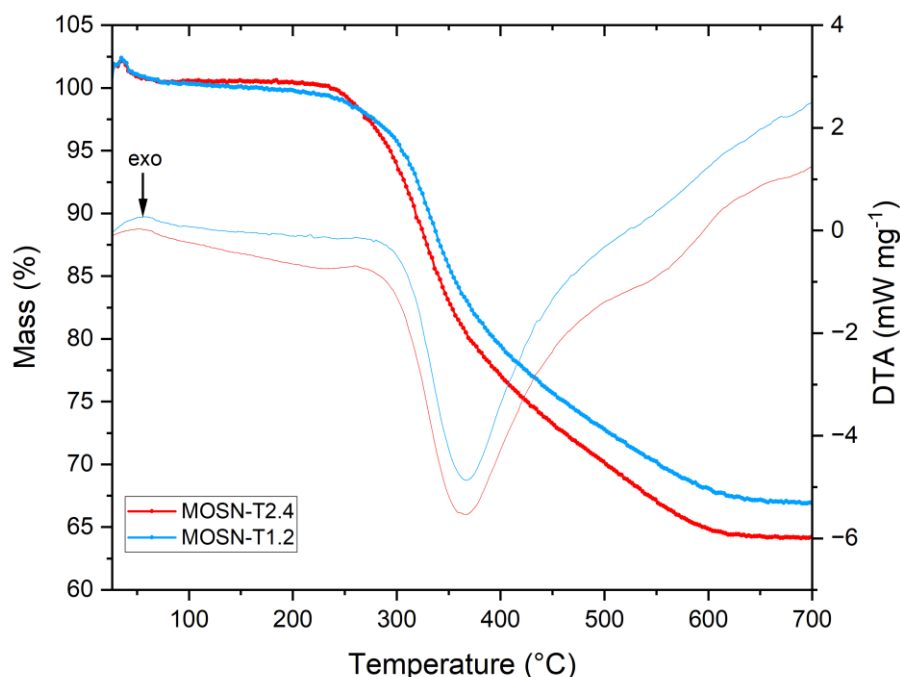


Figure 33. Mass loss (%) and DTA profiles of organosilica **MOSN-T1.2** and **MOSN-T2.4**.

This incorporation of BTES into the silica framework was supported by the solid-state NMR characterization. For instance, in the ^{29}Si -ssNMR spectra of **MOSN-T1.2** and **MOSN-T2.4** (Fig.34a), the presence of T-signals (T^2 at -58 ppm and T^3 at -67 ppm) confirmed that BTES moieties were covalently integrated into the silica structure, while Q-signals (Q^2 at -90 ppm, Q^3 at -101 ppm and Q^4 at -110 ppm), characteristic of the silica matrix were additionally observed. ^{13}C -ssNMR spectra (Fig.34 b) further exhibited the signals from carbon α of the BTES attached to the Si-framework (Si-CH_2) (10 ppm, signal 1), as well as from internal $-\text{CH}_2-$ functions (20–22 ppm, signal 2), and from the carbons connected to the polysulfide bridge (40 ppm, signal 4).

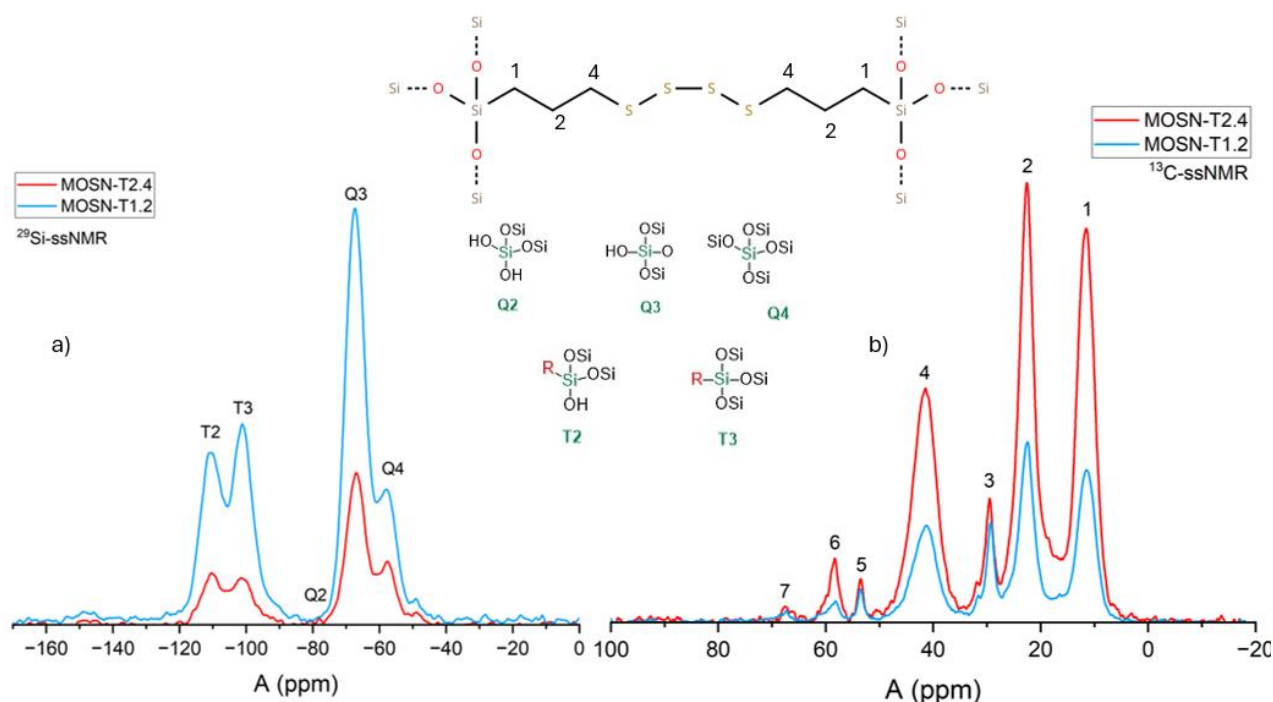


Figure 34. Solid-state a) ^{29}Si CP/MAS NMR and b) ^{13}C CP NMR spectra of **MOSN-T2.4** and **MOSN-T1.2**.

4.2.2 Synthesis of DMOSNs

Although the protocol implemented for the synthesis of MOSNs successfully yielded hybrid organosilica materials, obtaining materials with enlarged pores was of particular interest in order to broaden their potential applications as biodegradable carriers for the delivery of high-molecular-weight compounds, such as peptide drugs and biologics. For this reason, the synthesis of dendritic particles containing disulfide bridges within the silica network was further explored. The first approach to synthesizing dendritic mesoporous organosilica nanoparticles (DMOSNs) by co-condensation of BTES and TEOS was carried out using a modified protocol for the synthesis of pure DMSNs³⁷, yielding **DMOSN-TO-1** and **DMOSN-TO-2**. However, as shown in the TEM images (Fig. 35, a–b), these particles did not exhibit a well-defined dendritic structure. The synthesis protocol was therefore modified by varying the TEA amount and the toluene volume, resulting in the obtaining of **DMOSN-OTO-3** and **DMOSN-OTO-4**. The corresponding TEM images (Fig. 36, a–b) showed reduced particle aggregation with respect to **DMOSN-TO-1** and **DMOSN-TO-2**, and a more defined spherical morphology. Alternatively, TEA was replaced by *L*-lysine as the base catalyst, and *p*-xylene was used as the co-solvent instead of toluene, yielding **DMOSN-OX-1** and **DMOSN-OX-2** (Fig. 36, c, d). The DLS analyses of these materials (Fig. 37) confirmed that **DMOSN-OTO-4** exhibited the smallest average hydrodynamic diameter ($119 \pm 0,7$ nm) with a low PDI of 0,132, indicating a narrow size distribution. In contrast, **DMOSN-OTO-3** showed a slightly larger hydrodynamic diameter ($158 \pm 3,0$ nm) and a PDI of 0,298. Regarding the samples obtained with *L*-lysine, **DMOSN-OX-1** exhibited a hydrodynamic diameter of $133 \pm 1,0$ nm with the lowest PDI (0,101) among all samples, but **DMOSN-OX-2** presented a larger average size of $180 \pm 1,0$ nm and a PDI of 0,168 (Table 8). Overall, the DLS results are consistent with TEM images of the DMOSNs, showing small spherical particles with a narrow size distribution after the synthetic optimization procedure implemented.

The synthetic conditions adjusted for **DMOSN-OTO-3**, **DMOSN-OTO-4**, **DMOSN-OX-1**, and **DMOSN-OX-2** yielded smaller, irregularly shaped particles, which motivated a final round of optimization by reducing the stirring speed from 300 rpm to 150 rpm, which yielded **DMOSN-OX-3**, and by reducing the amount of co-solvent from 16 to 8 mL, which led to **DMOSN-OX-4**. These particles (Fig. 36, e–f) exhibited the most well-defined spherical morphology. Thus, the TEM characterization of **DMOSN-OX-3** and **DMOSN-OX-4** confirmed their uniform dendritic morphology, with a slight increase in particle size with respect to the other DMOSNs, and with clearly distinguishable radial pore channels. DLS analysis of these materials confirmed hydrodynamic diameters of 227 ± 1 nm and 137 ± 1 nm with PDI values of 0,136 and 0,135, for **DMOSN-OX-3** and **DMOSN-OX-4**, respectively. Zeta potential values of $-21 \pm 0,9$ mV (**DMOSN-OX-3**) and $-18 \pm 0,6$ mV (**DMOSN-OX-4**) further indicated negative surface charges, supporting particle colloidal stability (Table 8).

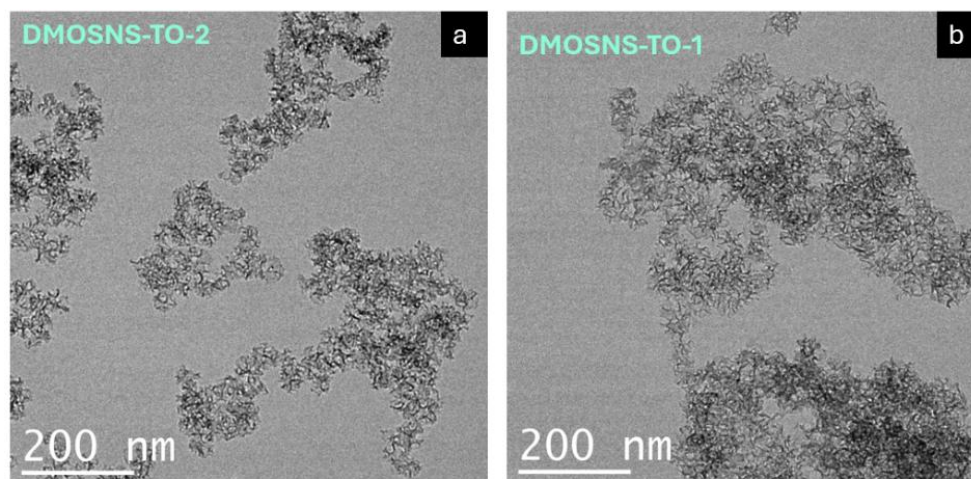


Figure 35. TEM images of tetrasulfide-bridged organosilica a) **DMOSN-TO-2** and b) **DMOSN-TO-1**.

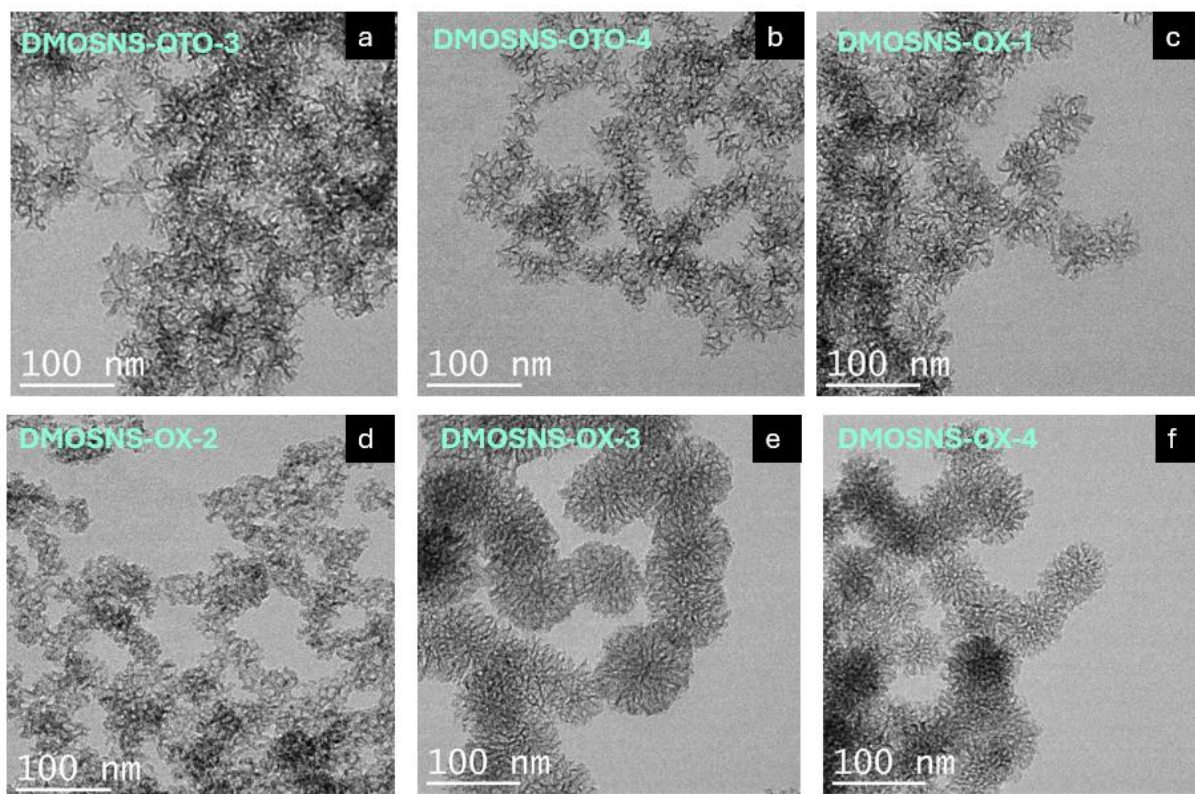


Figure 36. TEM images of tetrasulfide-bridged organosilica particles: a) **DMOSN-OTO-3**, b) **DMOSN-OTO-4**, c) **DMOSN-OX-1**, d) **DMOSN-OX-2**, e) **DMOSN-OX-3**, and f) **DMOSN-OX-4**.

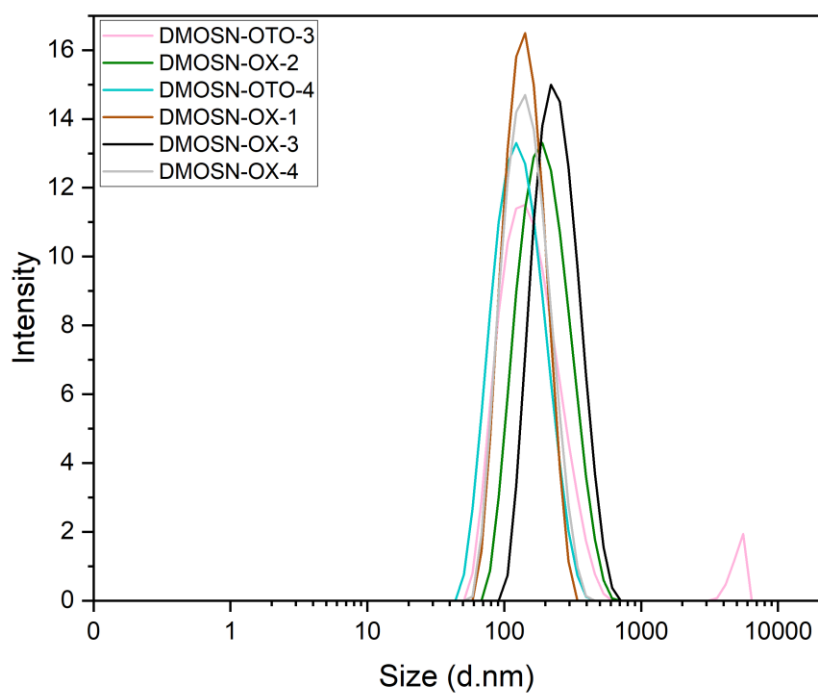


Figure 37. DLS profiles of tetrasulfide-bridged **DMOSN-OTO** and **DMOSN-OX** particles.

N₂-physisorption analysis (Fig.38a) confirmed the presence of mesopores in all DMOSNs materials. The materials obtained with TEA as base catalyst (**DMOSN-OTO-3** and **DMOSN-OTO-4**) exhibited specific surface areas of 577 m²·g⁻¹ and 623 m²·g⁻¹, respectively. Furthermore, these materials showed pore sizes of 7,0 nm (**DMOSN-OTO-3**) and 8,1 nm (**DMOSN-OTO-4**), and pore volumes of 0,98 cm³·g⁻¹ (**DMOSN-OTO-3**) and 0,96 cm³·g⁻¹ (**DMOSN-OTO-4**), indicating the formation of large, open dendritic pores. **DMOSN-OX-1** and **DMOSN-OX-2**, synthesized with L-Lysin and p-Xylene, exhibited similar high surface areas (641 m²·g⁻¹ and 577 m²·g⁻¹, respectively) to **DMOSN-OTO** materials. **DMOSN-OX-1**, synthesized with a lower amount of p-Xylene (16 mL) showed an average pore size of 7,0 nm and a pore volume of 0,96 cm³·g⁻¹, while **DMOSN-OX-2** exhibited a notably larger pore size of 9,1 nm and a pore volume of 0,86 cm³·g⁻¹, consistent with the inclusion of higher amount (32 mL) of p-Xylene. **DMOSN-OX-3** and **DMOSN-OX-4** exhibited similar surface area to the previous samples (619 m²·g⁻¹ and 565 m²·g⁻¹, respectively) but with a slight decrease in average pore size and a pore volume (6,5 nm and 0,88 cm³·g⁻¹ for **DMOSN-OX-3**, and 5,9 nm and 0,62 cm³·g⁻¹ for **DMOSN-OX-4**, respectively) (Table 8).

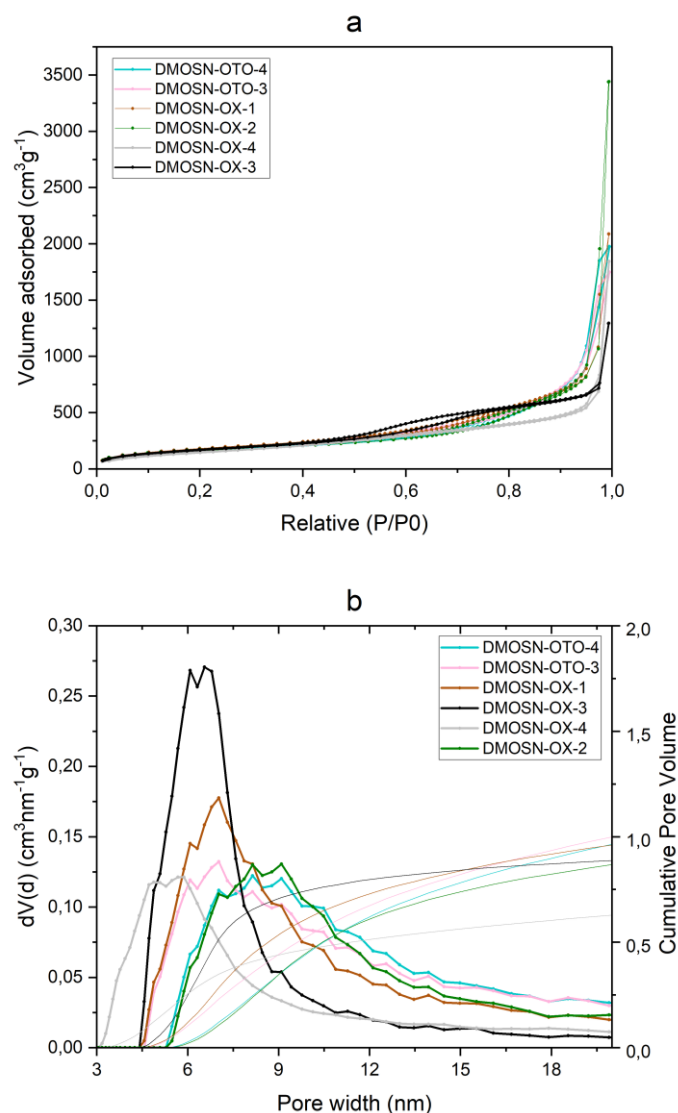


Figure 38. a) N₂-physisorption isotherms (77 K) and b) NLDFT pore size distributions of tetrasulfide-bridged organosilica particles **DMOSN-OTO** and **DMOSN-OX**.

Table 8. Hydrodynamic diameter, polydispersity indexes (PDI), and zeta potential obtained from DLS analysis; specific surface area (S_{BET}), pore volume, and pore size obtained from N_2 -physisorption analysis; and mass loss [%] obtained from TGA analysis of tetrasulfide-bridged organosilica particles **DMOSN-OTO** and **DMOSN-OX**.

	Hydrodynamic diameter [nm]	PDI	Zeta potential [mV]	S_{BET} [$\text{m}^2 \cdot \text{g}^{-1}$]	Pore size [nm]	Pore Volume [$\text{cm}^3 \cdot \text{g}^{-1}$]	Mass loss [%]
DMOSN-OTO-4	$119 \pm 0,7$	0,132	$-24,9 \pm 0,4$	577	8,1	0,96	28
DMOSN-OTO-3	$158 \pm 3,0$	0,298	$-22,4 \pm 0,2$	623	7,0	0,98	33
DMOSN-OX-1	$133 \pm 1,0$	0,101	$-22,1 \pm 0,7$	641	7,0	0,96	34
DMOSN-OX-2	$180 \pm 1,0$	0,168	$-31,2 \pm 0,6$	577	9,1	0,86	11
DMOSN-OX-3	$227 \pm 0,6$	0,136	$-21,3 \pm 0,9$	619	6,5	0,88	31
DMOSN-OX-4	$137 \pm 0,3$	0,135	$-17,6 \pm 0,6$	565	5,9	0,62	36

TGA analysis of the DMOSNs (Fig. 39) revealed mass losses for all the materials within the range of 11 to 36 %, as for the MOSNs samples (Table 8), which can be attributed to the decomposition of the organic functions from BTES incorporated into the silica framework. This was supported further by solid-state NMR. Thus, ^{29}Si -ssNMR spectra (Fig. 40a) showed the presence of T-signals (T^2 at -57 ppm and T^3 at -66 ppm), confirming that BTES was covalently integrated into the silica framework, while Q-signals (Q^2 at -91 ppm, Q^3 at -101 ppm, and Q^4 at -110 ppm) originated from the silica network. ^{13}C -ssNMR spectra (Fig. 40b) of the DMOSNs exhibited the characteristic signal of carbon α attached to the silicon atom in the BTES-silane ($\text{Si}-\text{CH}_2$, 10 ppm, signal 1), internal $-\text{CH}_2-$ (20–22 ppm, signal 2), and the carbon α attached to the polysulfide bridge (40 ppm, signal 4). Signals around 54–58 ppm (denoted as 5 and 6) were attributed to unhydrolyzed BTES ($-\text{OCH}_2\text{CH}_3$) present in the hybrid organosilica structure.

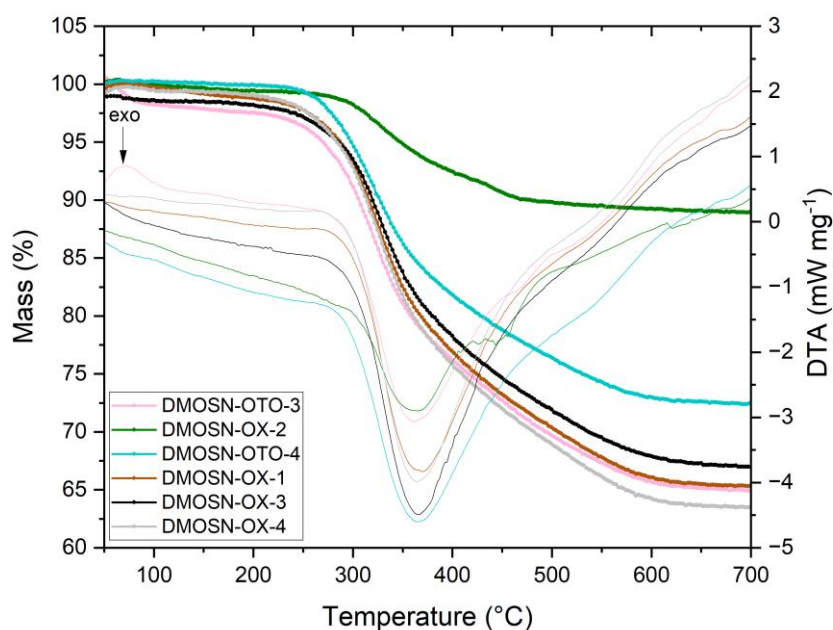


Figure 39. Mass loss (%) and DTA profile of tetrasulfide-bridged **DMOSN-OTO** and **DMOSN-OX** particles.

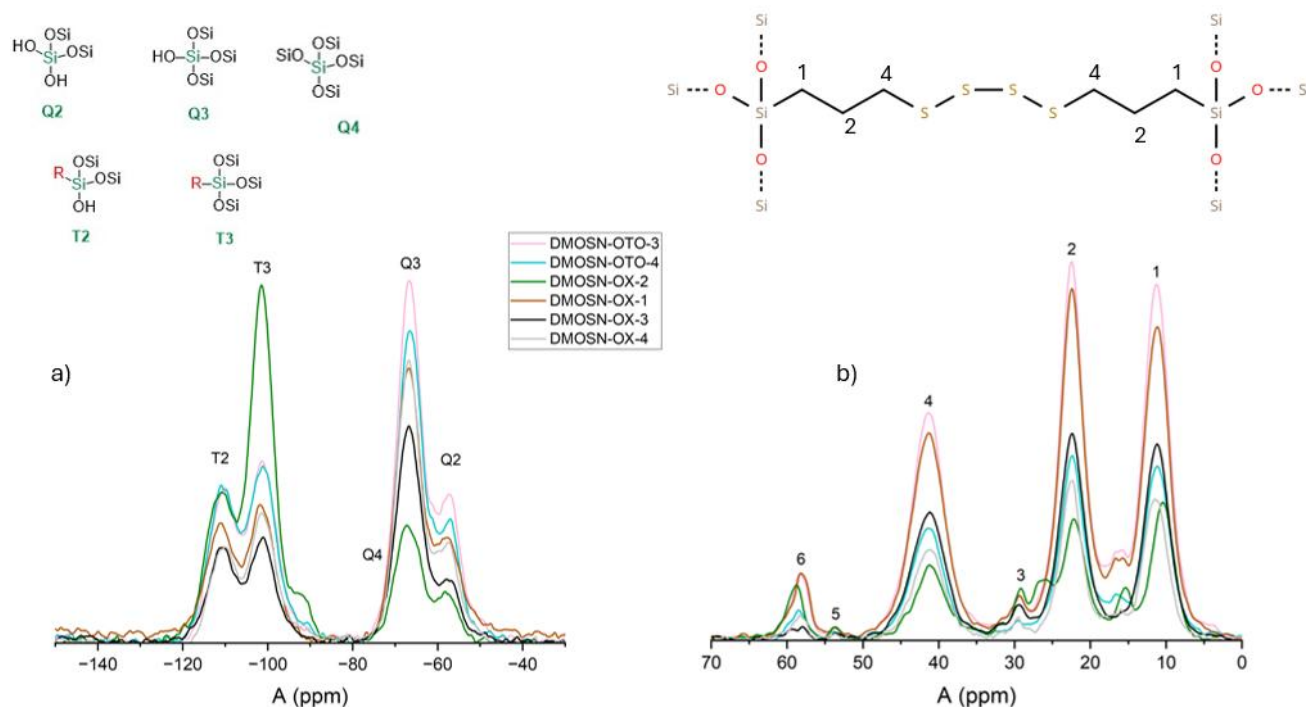


Figure 40. Solid-state a) ^{29}Si CP/MAS NMR and b) ^{13}C CP NMR spectra of tetrasulfide-bridged organosilica particles **DMOSN-OTO** and **DMOSN-OX**.

Raman spectra provided complementary information on the inorganic and organic composition of the tetrasulfide organosilane materials (Fig. 41). In high-frequency ($2800\text{--}3050\text{ cm}^{-1}$) region, pure BTES (control) exhibited strong characteristic CH_2 bands, whereas the pure DMSNs used as references (**DMSN-50** and **DMSN-130**) showed only very low intensity signals in this region. The spectra of the MOSNs and DMOSNs closely resembled that of pure BTES, displaying characteristic organic-related peaks, which confirmed the presence of organosilica moieties in these materials. This observation is consistent with the mass loss values obtained from TGA (Table 8). In contrast, the low-frequency region ($50\text{--}600\text{ cm}^{-1}$) exhibited intense signals from $\text{Si}\text{--}\text{O}\text{--}\text{Si}$ vibrational modes, which were more pronounced in the pure silica samples (**DMSN-50** and **DMSN-130**), as expected.

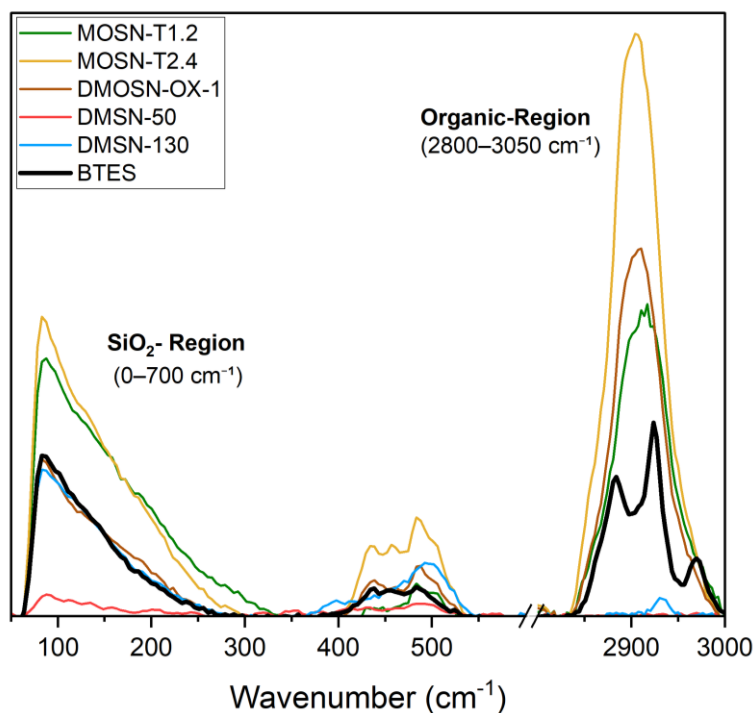


Figure 41. Raman spectra of tetrasulfide-bridged organosilica materials compared with pure DMSNs and free BTES.

In summary, Raman spectroscopy confirmed the successful incorporation of tetrasulfide bridges into the silica framework, aligning with TGA, and validating the presence of organic moieties within the DMOSNs. These materials exhibited well-defined spherical morphology with dendritic porosity, a narrow size distribution, and high surface areas, exhibiting mesopores, in a suitable range for biomolecule loading. The incorporation of tetrasulfide bridges introduced redox-responsive linkages into the silica network, enabling degradation under reductive conditions such as in the presence of glutathione (GSH). This feature makes the particles particularly attractive as biodegradable nanocarriers. Their degradation behavior and redox responsiveness were therefore further evaluated.

4.2.3 Biodegradability Studies

To achieve a more comprehensive understanding of the stability and redox response of tetrasulfide-bridged organosilica nanoparticles in aqueous environments and to compare them with the behavior of pure silica DMSNs, biodegradability studies were performed. Organosilica **DMOSN-OX-1**, as well as pure **DMSN-50** and **DMSN-130**, were incubated in water (control conditions) or in the presence of GSH (20 mM) at 37°C under stirring (80 rpm). The colloidal stability of particle suspensions was then monitored at regular intervals. Thus, DLS measurements (Fig. 42) allowed evaluation of changes in the nanoparticles' hydrodynamic size in the absence and presence of GSH. For example, **DMSN-50**, both in control conditions and in GSH, exhibited hydrodynamic diameters of 160–200 nm that remained stable over time. Similarly, **DMSN-130** was not affected by GSH addition, with no significant change in hydrodynamic size compared to the aqueous suspension (Table 9). These results indicated that pure DMSNs

(**DMSN-50** and **DMSN-130**) did not undergo significant aggregation upon exposure to GSH, as a result of the redox-stability of the silica framework. In contrast, **DMOSN-OX-1** showed a different behavior. In control conditions (aqueous suspension), the sample remained stable over time, with hydrodynamic sizes in the range of 140–150 nm; however, the presence of GSH led to significant particle aggregation up to 24 h of incubation at 37°C, attributed to cluster formation with hydrodynamic sizes in the order of $2 \cdot 10^4$ nm. This suggests that the tetrasulfide bridges incorporated into the organosilica framework interact with GSH, leading to particle destabilization and aggregation.

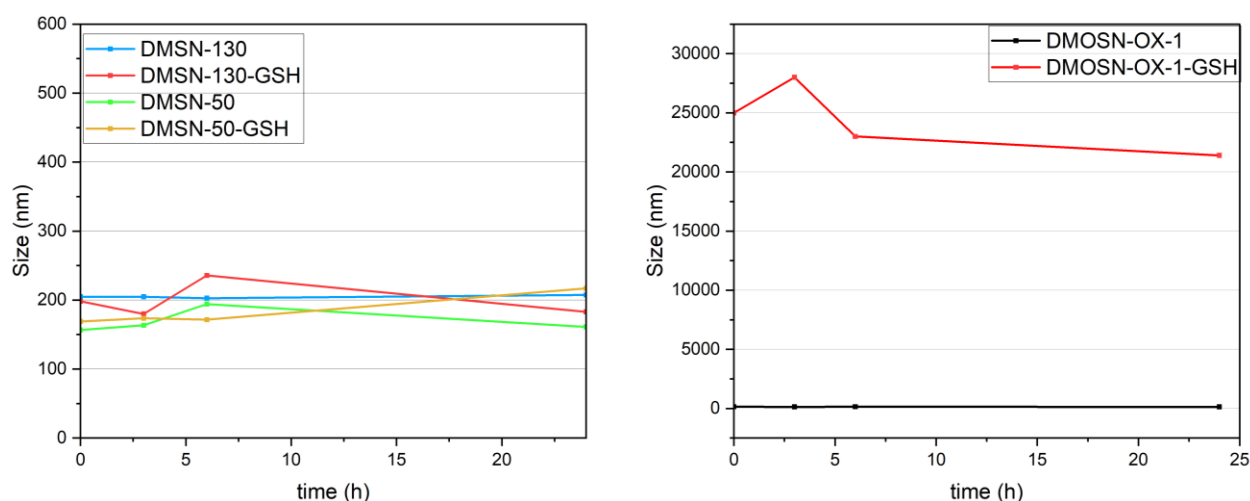


Figure 42. Hydrodynamic diameters of a) pure DMSNs (**DMSN-50** and **DMSN-130**), and b) **DMOSN-OX-1** measured by DLS over time after incubation at 37°C in water (control conditions) or in the presence of GSH (20 mM).

Table 9. Time-dependent hydrodynamic diameters of **DMSN-50**, **DMSN-130**, and **DMOSN-OX-1** in the absence and presence of GSH

	DMSN-50		DMSN-130		DMOSN-OX-1	
t (h)	Control (H₂O)	+GSH (28mM)	Control (H₂O)	+GSH (28mM)	Control (H₂O)	+GSH (28mM)
0	156	169	205	198	142	$2,5 \cdot 10^4$
3	163	173	204	180	140	$2,8 \cdot 10^4$
6	194	171	203	235	145	$2,3 \cdot 10^4$
24	161	217	207	183	137	$2,1 \cdot 10^4$

To further gain deeper insight into the particle aggregation behavior in the presence of GSH, Small-Angle X-ray Scattering (SAXS) measurements were performed to evaluate the structural stability of organosilica (DMOSNs) and pure silica (DMSNs) nanoparticles after 24 h incubation at 37°C, either in aqueous solution (control conditions) or in the presence of 20 mM GSH. The pure particles (**DMSN-50** and **DMSN-130**) displayed identical scattering profiles in water and in GSH-containing solution (Fig. 43, a-b), with no indication of aggregation, which confirmed their structural integrity and colloidal stability under reductive conditions. Similarly, **DMOSN-OX-1**

measured in water (Fig. 43c) showed overlapping scattering curves when recorded at three positions along the capillary (labeled 1, 2, and 3), indicating a homogeneous particle suspension and preservation of particle size, without signs of degradation. In contrast, **DMOSN-OX-1** incubated in GSH exhibited position-dependent scattering behavior, reflecting a non-homogeneous particle distribution within the capillary. This observation correlated with the formation of visible white precipitates on the capillary wall, attributed to particle aggregation and partial precipitation under reductive conditions.

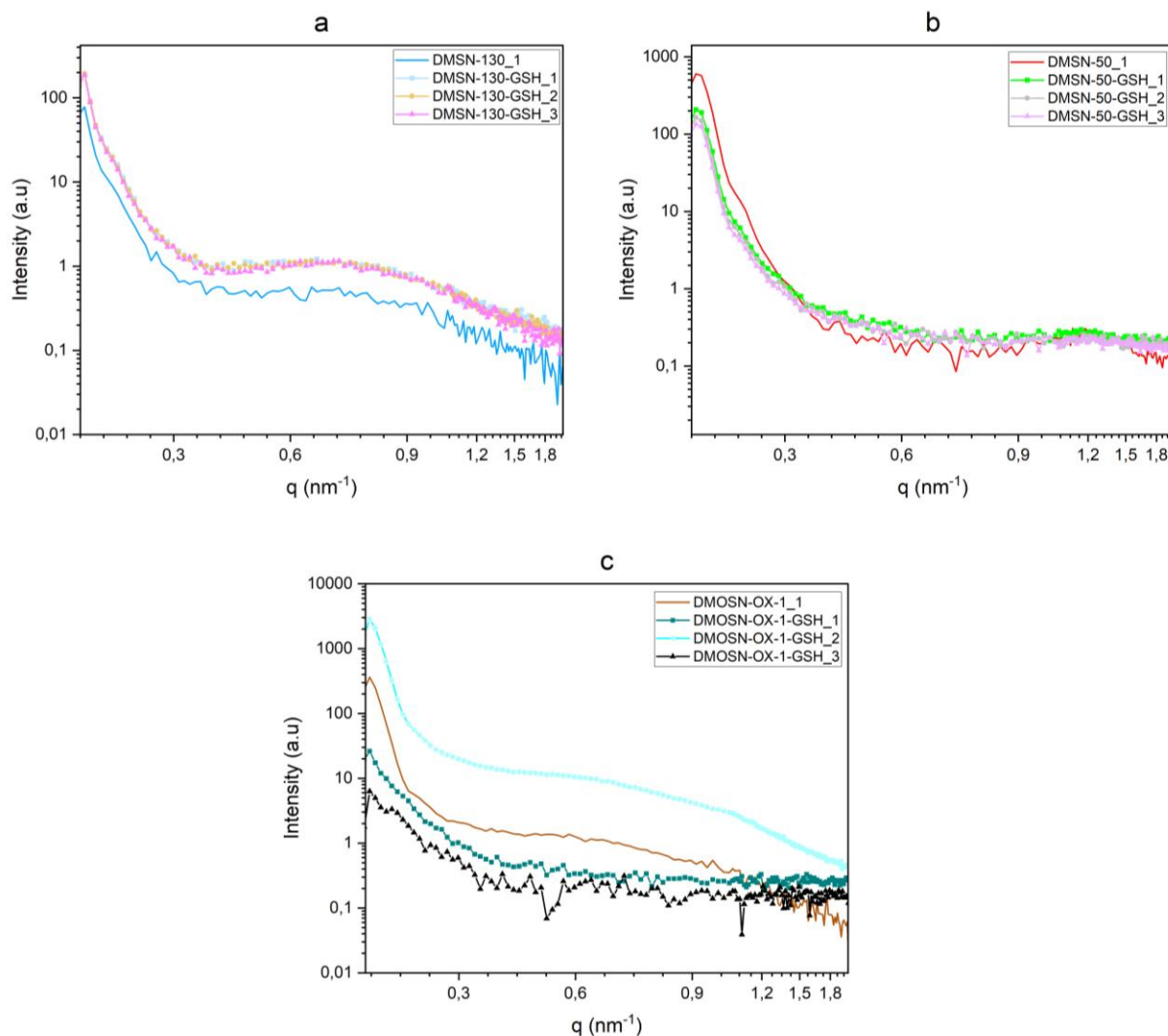


Figure 43. q -dependent scattering intensity profiles of a) **DMSN-130**, b) **DMSN-50**, and c) **DMOSN-OX-1** measured at three different capillary positions in the absence and presence of GSH (20 mM).

Normalization of the scattering curves confirmed that the nanostructured features were still present in the precipitated material. However, data fitting revealed a main particle size distribution centered at $45 \pm 11 \text{ nm}$, along with a secondary population corresponding to smaller structural features (in the higher q -range) with diameters of approximately $3.0 \pm 1.6 \text{ nm}$, suggesting partial particle deformation or disintegration. As previously reported, GSH-triggered degradation of disulfide-bridged organosilica frameworks is time-dependent and strongly influenced by experimental conditions, such as particle concentration, reducing-agent concentration, stirring rate, and temperature¹⁰⁶, and might not result in immediate structural

collapse. Although preliminary, these results indicated a GSH-responsive structural alteration of the tetrasulfide-bridged organosilica materials, consistent with the redox cleavage of sulfur bridges within the organosilica framework¹⁰⁸. This redox-triggered behavior highlights their potential as responsive delivery systems with tunable biodegradation properties.

5. Conclusion and Outlook

The first part of this Master's thesis focused on adjusting the physicochemical properties of DMSN nanocarriers for sublingual drug delivery applications, particularly particle size and surface chemistry. These parameters were tuned by modifying synthesis conditions, including reaction temperature, stirring rate, organic cosolvents, and pore swelling agents, as well as by applying post-grafting strategies for silica functionalization with hydrophilic and negatively charged moieties and liposome-based coatings. Additionally, the impact of silica modification on colloidal stability, cell viability, and particle–cell interactions was systematically investigated. The second part of the thesis addressed the biodegradability of MSNs, a key limitation hindering their clinical translation. To overcome this challenge, the synthesis of disulfide- and tetrasulfide-bridged organosilica particles was optimized to obtain spherical particles with narrow size distributions and mesopores suitable for therapeutic loading. Importantly, these materials exhibited redox-responsive behavior in the presence of GSH, enabling triggered structural deformation. This strategy offers a promising pathway to the development of biodegradable, stimuli-responsive nanocarriers with enhanced safety profiles for drug delivery applications.

5.1 Effect of Particle Size and Surface Chemistry on Particle-Cell Interactions

The results presented in this thesis demonstrate that surface chemistry plays a crucial role in improving the colloidal stability and cytocompatibility of MSNs and in modulating their interactions with buccal epithelial cells, whereas particle size appears to have a comparatively minor influence on the biological response of these materials. *In vitro* studies using TR146 cells revealed a dose-dependent decrease in cell viability upon treatment with non-functionalized particles, with a significant reduction in metabolic activity at higher concentrations. This confirmed that direct silica–cell interactions can impair cytocompatibility in the absence of chemical functionalities that can modulate them. Post-synthetic functionalization with THMP significantly improved the cellular tolerance of the silica particles, as phosphonate-modified DMSNs (**DMSN-50-PO₃** and **DMSN-130-PO₃**) maintained approximately 90–100% cell viability even at the highest concentration tested. On the other hand, liposome-coated DMSNs (**L-DMSN-50** and **L-DMSN-130**) increased metabolic activity, likely due to elevated intracellular phospholipid levels resulting from their enhanced interactions with buccal epithelial cells. This finding suggested improved cellular interactions with the coated DMSNs and potential lipid-shell-mediated uptake.

5.2 Development of Redox-Responsive Dendritic Organosilica Nanoparticles (DMOSNs)

In addition to surface engineering, this work addressed the limited biodegradability of MSNs, a key challenge that limits their clinical translation in drug-delivery applications. This was tackled by developing mesoporous organosilica nanoparticles (MOSNs) and dendritic mesoporous organosilica nanoparticles (DMOSNs) incorporating BTES via a co-condensation approach,

thereby introducing tetrasulfide bridges into the silica framework. Initial synthesis attempts did not yield well-defined dendritic morphologies, which required systematic optimization of reaction parameters, including base selection, organic co-solvent volumes, and stirring conditions. Replacing TEA with *L*-lysine and employing *p*-xylene as an organic cosolvent and pore-swelling agent significantly improved particle morphology and textural properties of the hybrid organosilica materials obtained. Thus, the optimized DMOSNs therefore exhibited a narrow size distribution with low polydispersity indices and negatively charged surfaces, supporting improved colloidal stability. Furthermore, the increase in pore size and preservation of dendritic mesoporosity resulted in highly open pore systems suitable for loading macromolecules of higher molecular weight. Preliminary biodegradability studies under reducing conditions (20 mM GSH) revealed colloidal destabilization and structural alterations in the organosilica particles, confirming the redox-responsive behavior of the incorporated tetrasulfide bridges. Although complete framework degradation could not be conclusively demonstrated, the observed redox-triggered response suggests that integrating cleavable tetrasulfide linkages into dendritic silica frameworks is a promising strategy for developing potentially biodegradable hybrid nanocarriers without compromising particle morphology or pore accessibility.

5.3 Future Directions in the Development of Safer and Functional Dendritic Silica Nanocarriers

The results presented in this thesis demonstrate that the rational design of functional nanocarriers requires integrating precise control over particle size and morphology, along with tailored surface modification and framework engineering. Dendritic architectures demonstrated exceptionally high surface areas (up to $826 \text{ m}^2 \cdot \text{g}^{-1}$ for DMSNs and $641 \text{ m}^2 \cdot \text{g}^{-1}$ for DMOSNs) and large pore volumes (up to $1,23 \text{ cm}^3 \cdot \text{g}^{-1}$ for DMSNs and $0,98 \text{ cm}^3 \cdot \text{g}^{-1}$ for DMOSNs), which might enable efficient drug loading and accommodation of biomacromolecules in future work. Surface functionalization with phosphonate groups improved colloidal stability and significantly reduced cytotoxic effects, while liposome coating further enhanced cytocompatibility and even stimulated metabolic activity in epithelial cells, favoring cellular interaction and potential uptake. These findings underscore the critical role of surface chemistry in modulating biological responses. At the same time, incorporating redox-responsive organosilica components introduced stimulus-sensitivity to the silica framework, offering a potential strategy to address long-term safety concerns associated with silica's persistence in biological systems. By establishing clear correlations among particle size, pore architecture, surface chemistry, and biological performance, this work provides a structured design framework for the development of biocompatible, potentially biodegradable dendritic nanocarriers. The insights gained are particularly relevant for future applications in sublingual and mucosal drug delivery, where epithelial compatibility, controlled nano-bio interactions, and long-term safety are critical requirements.

As further steps, the loading of therapeutic model compounds into the silica carriers developed in this thesis, followed by systematic evaluation of their release profiles and cellular responses, will be essential to fully assess the potential of the functionalized and liposome-coated materials for sublingual drug delivery applications. In parallel, more detailed investigations into the redox-responsive biodegradability of the organosilica-based materials should be conducted, including studies with cargo-loaded systems to evaluate stimulus-triggered release under physiologically relevant reducing conditions. This will enable a clearer understanding of the relationship between

framework degradation and controlled drug release behavior. The liposome-coating strategy could also be extended to the organosilica-based dendritic systems, thereby combining redox-responsive framework degradation with the enhanced membrane interaction provided by lipid coatings. Such a dual-functional approach may further enhance biocompatibility, promote buccal epithelial transport, enable triggered drug release, and ensure particle biosafety during administration. Finally, a comprehensive evaluation of long-term biodegradation behavior and *in vivo* particle biodistribution will be necessary to validate the translational potential of these nanocarriers. Altogether, these future investigations will broaden the application scope of dendritic silica and organosilica nanocarriers and further establish functional mesoporous materials as versatile and promising platforms for advanced drug delivery systems.

References

- (1) Pawar, P. P.; Ghorpade, H. S.; Kokane, B. A. Sublingual Route for Systemic Drug Delivery. *J. Drug Delivery Ther.* **2018**, 8 (6-s), 340–343. <https://doi.org/10.22270/jddt.v8i6-s.2097>.
- (2) Arav, Y. Advances in Modeling Approaches for Oral Drug Delivery: Artificial Intelligence, Physiologically-Based Pharmacokinetics, and First-Principles Models. *Pharmaceutics* **2024**, 16 (8), 978. <https://doi.org/10.3390/pharmaceutics16080978>.
- (3) Suharyani, I.; Fouad Abdelwahab Mohammed, A.; Muchtaridi, M.; Wathoni, N.; Abdassah, M. Evolution of Drug Delivery Systems for Recurrent Aphthous Stomatitis. *DDDT* **2021**, Volume 15, 4071–4089. <https://doi.org/10.2147/DDDT.S328371>.
- (4) Dan, N.; Samanta, K.; Almoazen, H. An Update on Pharmaceutical Strategies for Oral Delivery of Therapeutic Peptides and Proteins in Adults and Pediatrics. *Children* **2020**, 7 (12), 307. <https://doi.org/10.3390/children7120307>.
- (5) Maleki, A.; Kettiger, H.; Schoubben, A.; Rosenholm, J. M.; Ambroggi, V.; Hamidi, M. Mesoporous Silica Materials: From Physico-Chemical Properties to Enhanced Dissolution of Poorly Water-Soluble Drugs. *Journal of Controlled Release* **2017**, 262, 329–347. <https://doi.org/10.1016/j.jconrel.2017.07.047>.
- (6) Wang, W.; Wang, P.; Tang, X.; Elzatahry, A. A.; Wang, S.; Al-Dahyan, D.; Zhao, M.; Yao, C.; Hung, C.-T.; Zhu, X.; Zhao, T.; Li, X.; Zhang, F.; Zhao, D. Facile Synthesis of Uniform Virus-like Mesoporous Silica Nanoparticles for Enhanced Cellular Internalization. *ACS Cent. Sci.* **2017**, 3 (8), 839–846. <https://doi.org/10.1021/acscentsci.7b00257>.
- (7) Juère, E.; Caillard, R.; Marko, D.; Del Favero, G.; Kleitz, F. Smart Protein-Based Formulation of Dendritic Mesoporous Silica Nanoparticles: Toward Oral Delivery of Insulin. *Chemistry A European J* **2020**, 26 (23), 5195–5199. <https://doi.org/10.1002/chem.202000773>.
- (8) Von Baeckmann, C.; Guillet-Nicolas, R.; Renfer, D.; Kählig, H.; Kleitz, F. A Toolbox for the Synthesis of Multifunctionalized Mesoporous Silica Nanoparticles for Biomedical Applications. *ACS Omega* **2018**, 3 (12), 17496–17510. <https://doi.org/10.1021/acsomega.8b02784>.
- (9) LaBauve, A. E.; Rinker, T. E.; Nouredine, A.; Serda, R. E.; Howe, J. Y.; Sherman, M. B.; Rasley, A.; Brinker, C. J.; Sasaki, D. Y.; Negrete, O. A. Lipid-Coated Mesoporous Silica Nanoparticles for the Delivery of the ML336 Antiviral to Inhibit Encephalitic Alphavirus Infection. *Sci Rep* **2018**, 8 (1), 13990. <https://doi.org/10.1038/s41598-018-32033-w>.
- (10) Croissant, J. G.; Fatieiev, Y.; Khashab, N. M. Degradability and Clearance of Silicon, Organosilica, Silsesquioxane, Silica Mixed Oxide, and Mesoporous Silica Nanoparticles. *Advanced Materials* **2017**, 29 (9), 1604634. <https://doi.org/10.1002/adma.201604634>.
- (11) Li, Y.; Meng, Q.; Yang, M.; Liu, D.; Hou, X.; Tang, L.; Wang, X.; Lyu, Y.; Chen, X.; Liu, K.; Yu, A.-M.; Zuo, Z.; Bi, H. Current Trends in Drug Metabolism and Pharmacokinetics. *Acta Pharmaceutica Sinica B* **2019**, 9 (6), 1113–1144. <https://doi.org/10.1016/j.apsb.2019.10.001>.
- (12) Mathias, N. R.; Hussain, M. A. Non-Invasive Systemic Drug Delivery: Developability Considerations for Alternate Routes of Administration. *Journal of Pharmaceutical Sciences* **2010**, 99 (1), 1–20. <https://doi.org/10.1002/jps.21793>.

- (13) Alqahtani, M. S.; Kazi, M.; Alsenaidy, M. A.; Ahmad, M. Z. Advances in Oral Drug Delivery. *Front. Pharmacol.* **2021**, *12*, 618411. <https://doi.org/10.3389/fphar.2021.618411>.
- (14) Liu, L.; Liu, Y.; Zhou, X.; Xu, Z.; Zhang, Y.; Ji, L.; Hong, C.; Li, C. Analyzing the Metabolic Fate of Oral Administration Drugs: A Review and State-of-the-Art Roadmap. *Front. Pharmacol.* **2022**, *13*, 962718. <https://doi.org/10.3389/fphar.2022.962718>.
- (15) Stielow, M.; Witczyńska, A.; Kubryń, N.; Fijałkowski, Ł.; Nowaczyk, J.; Nowaczyk, A. The Bioavailability of Drugs—The Current State of Knowledge. *Molecules* **2023**, *28* (24), 8038. <https://doi.org/10.3390/molecules28248038>.
- (16) Jones, C. R.; Hatley, O. J. D.; Ungell, A.-L.; Hilgendorf, C.; Peters, S. A.; Rostami-Hodjegan, A. Gut Wall Metabolism. Application of Pre-Clinical Models for the Prediction of Human Drug Absorption and First-Pass Elimination. *AAPS J* **2016**, *18* (3), 589–604. <https://doi.org/10.1208/s12248-016-9889-y>.
- (17) Hua, S. Advances in Nanoparticulate Drug Delivery Approaches for Sublingual and Buccal Administration. *Front. Pharmacol.* **2019**, *10*, 1328. <https://doi.org/10.3389/fphar.2019.01328>.
- (18) Haddadzadegan, S.; Summonte, S.; Ricci, F.; Sandmeier, M.; Bernkop-Schnürch, A. Intraoral Drug Delivery: Bridging the Gap Between Academic Research and Industrial Innovations. *Adv Funct Materials* **2025**, *35* (35), 2500157. <https://doi.org/10.1002/adfm.202500157>.
- (19) Franz-Montan, M.; De Araújo, D. R.; De Moraes Ribeiro, L. N.; De Melo, N. F. S.; De Paula, E. Nanostructured Systems for Transbuccal Drug Delivery. In *Nanostructures for Oral Medicine*; Elsevier, 2017; pp 87–121. <https://doi.org/10.1016/B978-0-323-47720-8.00005-5>.
- (20) Wanasathop, A.; Patel, P. B.; Choi, H. A.; Li, S. K. Permeability of Buccal Mucosa. *Pharmaceutics* **2021**, *13* (11), 1814. <https://doi.org/10.3390/pharmaceutics13111814>.
- (21) Sogias, I. A.; Williams, A. C.; Khutoryanskiy, V. V. Why Is Chitosan Mucoadhesive? *Biomacromolecules* **2008**, *9* (7), 1837–1842. <https://doi.org/10.1021/bm800276d>.
- (22) Hansen, L. B.; Christrup, L. L.; Bundgaard, H. Saliva-Catalyzed Hydrolysis of a Ketobemidone Ester Prodrug: Factors Influencing Human Salivary Esterase Activity. *International Journal of Pharmaceutics* **1992**, *88* (1–3), 221–227. [https://doi.org/10.1016/0378-5173\(92\)90319-W](https://doi.org/10.1016/0378-5173(92)90319-W).
- (23) Bahraminejad, S.; Almoazen, H. Sublingual and Buccal Delivery: A Historical and Scientific Prescriptive. *Pharmaceutics* **2025**, *17* (8), 1073. <https://doi.org/10.3390/pharmaceutics17081073>.
- (24) Abdellatif, M. M.; Gebreel, R. M. Eprosartan Loaded Mesoporous Silica Nanoparticles Embedded in Mucoadhesive Buccal Films: A Strategy for Improved Bioavailability. *Journal of Drug Delivery Science and Technology* **2025**, *112*, 107248. <https://doi.org/10.1016/j.jddst.2025.107248>.
- (25) Mudshinge, S. R.; Deore, A. B.; Patil, S.; Bhalgat, C. M. Nanoparticles: Emerging Carriers for Drug Delivery. *Saudi Pharmaceutical Journal* **2011**, *19* (3), 129–141. <https://doi.org/10.1016/j.jsps.2011.04.001>.
- (26) Song, C. X.; Labhasetwar, V.; Murphy, H.; Qu, X.; Humphrey, W. R.; Shebuski, R. J.; Levy, R. J. Formulation and Characterization of Biodegradable Nanoparticles for Intravascular Local

Drug Delivery. *Journal of Controlled Release* **1997**, *43* (2–3), 197–212. [https://doi.org/10.1016/S0168-3659\(96\)01484-8](https://doi.org/10.1016/S0168-3659(96)01484-8).

(27) MÅller, R. Solid Lipid Nanoparticles (SLN) for Controlled Drug Delivery – a Review of the State of the Art. *European Journal of Pharmaceutics and Biopharmaceutics* **2000**, *50* (1), 161–177. [https://doi.org/10.1016/S0939-6411\(00\)00087-4](https://doi.org/10.1016/S0939-6411(00)00087-4).

(28) Park, J.; Fong, P. M.; Lu, J.; Russell, K. S.; Booth, C. J.; Saltzman, W. M.; Fahmy, T. M. PEGylated PLGA Nanoparticles for the Improved Delivery of Doxorubicin. *Nanomedicine: Nanotechnology, Biology and Medicine* **2009**, *5* (4), 410–418. <https://doi.org/10.1016/j.nano.2009.02.002>.

(29) Baltzley, S.; Malkawi, A. A.; Alsmadi, M.; Al-Ghananeem, A. M. Sublingual Spray Drug Delivery of Ketorolac-Loaded Chitosan Nanoparticles. *Drug Development and Industrial Pharmacy* **2018**, *44* (9), 1467–1472. <https://doi.org/10.1080/03639045.2018.1460378>.

(30) Vallet-Regí, M.; Colilla, M.; Izquierdo-Barba, I.; Manzano, M. Mesoporous Silica Nanoparticles for Drug Delivery: Current Insights. *Molecules* **2017**, *23* (1), 47. <https://doi.org/10.3390/molecules23010047>.

(31) Vallet-Regí, M.; Schüth, F.; Lozano, D.; Colilla, M.; Manzano, M. Engineering Mesoporous Silica Nanoparticles for Drug Delivery: Where Are We after Two Decades? *Chem. Soc. Rev.* **2022**, *51* (13), 5365–5451. <https://doi.org/10.1039/D1CS00659B>.

(32) Khafaga, D. S. R.; El-Khawaga, A. M.; Elfattah Mohammed, R. A.; Abdelhakim, H. K. Green Synthesis of Nano-Based Drug Delivery Systems Developed for Hepatocellular Carcinoma Treatment: A Review. *Mol Biol Rep* **2023**, *50* (12), 10351–10364. <https://doi.org/10.1007/s11033-023-08823-5>.

(33) Nouredine, A.; Maestas-Olguin, A.; Tang, L.; Corman-Hijar, J. I.; Olewine, M.; Krawchuck, J. A.; Tsala Ebode, J.; Edeh, C.; Dang, C.; Negrete, O. A.; Watt, J.; Howard, T.; Coker, E. N.; Guo, J.; Brinker, C. J. Future of Mesoporous Silica Nanoparticles in Nanomedicine: Protocol for Reproducible Synthesis, Characterization, Lipid Coating, and Loading of Therapeutics (Chemotherapeutic, Proteins, siRNA and mRNA). *ACS Nano* **2023**, *17* (17), 16308–16325. <https://doi.org/10.1021/acsnano.3c07621>.

(34) Paris, J. L.; Vallet-Regí, M. Mesoporous Silica Nanoparticles for Co-Delivery of Drugs and Nucleic Acids in Oncology: A Review. *Pharmaceutics* **2020**, *12* (6), 526. <https://doi.org/10.3390/pharmaceutics12060526>.

(35) Florek, J.; Guillet-Nicolas, R.; Kleitz, F. 4. Ordered Mesoporous Silica: Synthesis and Applications. In *Functional Materials*; Leclerc, M., Gauvin, R., Eds.; DE GRUYTER, 2014; pp 61–100. <https://doi.org/10.1515/9783110307825.61>.

(36) Rosenholm, J. M.; Sahlgren, C.; Lindén, M. Towards Multifunctional, Targeted Drug Delivery Systems Using Mesoporous Silica Nanoparticles – Opportunities & Challenges. *Nanoscale* **2010**, *2* (10), 1870. <https://doi.org/10.1039/c0nr00156b>.

(37) Iriarte-Mesa, C.; Bergen, J.; Danielyan, K.; Crudo, F.; Marko, D.; Kählig, H.; Del Favero, G.; Kleitz, F. Functionalization of Silica Nanoparticles for Tailored Interactions with Intestinal Cells and Chemical Modulation of Paracellular Permeability. *Small Science* **2025**, *5* (1), 2400112. <https://doi.org/10.1002/smssc.202400112>.

- (38) Stöber, W.; Fink, A.; Bohn, E. Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. *Journal of Colloid and Interface Science* **1968**, 26 (1), 62–69. [https://doi.org/10.1016/0021-9797\(68\)90272-5](https://doi.org/10.1016/0021-9797(68)90272-5).
- (39) Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S. Ordered Mesoporous Molecular Sieves Synthesized by a Liquid-Crystal Template Mechanism. *Nature* **1992**, 359 (6397), 710–712. <https://doi.org/10.1038/359710a0>.
- (40) Zhang, Q.; Ye, Z.; Wang, S.-T.; Yin, J. Facile One-Pot Synthesis of PEGylated Monodisperse Mesoporous Silica Nanoparticles with Controllable Particle Sizes. *Chinese Chemical Letters* **2014**, 25 (2), 257–260. <https://doi.org/10.1016/j.ccllet.2013.11.002>.
- (41) Khaliq, N. U.; Lee, J.; Kim, J.; Kim, Y.; Yu, S.; Kim, J.; Kim, S.; Sung, D.; Kim, H. Mesoporous Silica Nanoparticles as a Gene Delivery Platform for Cancer Therapy. *Pharmaceutics* **2023**, 15 (5), 1432. <https://doi.org/10.3390/pharmaceutics15051432>.
- (42) Shen, D.; Yang, J.; Li, X.; Zhou, L.; Zhang, R.; Li, W.; Chen, L.; Wang, R.; Zhang, F.; Zhao, D. Biphasic Stratification Approach to Three-Dimensional Dendritic Biodegradable Mesoporous Silica Nanospheres. *Nano Lett.* **2014**, 14 (2), 923–932. <https://doi.org/10.1021/nl404316v>.
- (43) Zang, H.; Fofana, J.; Xu, F.; Nodder, S. B.; Gummuluru, S.; Reinhard, B. M. Characterizing Lipid-Coated Mesoporous Silica Nanoparticles as CD169-Binding Delivery System for Rilpivirine and Cabotegravir. *Advanced NanoBiomed Research* **2022**, 2 (5), 2100157. <https://doi.org/10.1002/anbr.202100157>.
- (44) Yang, G.; Gong, H.; Qian, X.; Tan, P.; Li, Z.; Liu, T.; Liu, J.; Li, Y.; Liu, Z. Mesoporous Silica Nanorods Intrinsically Doped with Photosensitizers as a Multifunctional Drug Carrier for Combination Therapy of Cancer. *Nano Res.* **2015**, 8 (3), 751–764. <https://doi.org/10.1007/s12274-014-0558-0>.
- (45) Manzano, M.; Vallet-Regí, M. Mesoporous Silica Nanoparticles for Drug Delivery. *Adv Funct Materials* **2020**, 30 (2), 1902634. <https://doi.org/10.1002/adfm.201902634>.
- (46) Yu, M.; Wang, J.; Yang, Y.; Zhu, C.; Su, Q.; Guo, S.; Sun, J.; Gan, Y.; Shi, X.; Gao, H. Rotation-Facilitated Rapid Transport of Nanorods in Mucosal Tissues. *Nano Lett.* **2016**, 16 (11), 7176–7182. <https://doi.org/10.1021/acs.nanolett.6b03515>.
- (47) Zhang, S.; Bai, J.; Kong, W.; Song, H.; Liu, Y.; Liu, G.; Ma, L.; Zhou, L.; Jiang, Y. Dendritic Mesoporous Silica Nanoparticles for Enzyme Immobilization. *Green Chemical Engineering* **2024**, 5 (2), 173–186. <https://doi.org/10.1016/j.gce.2023.07.002>.
- (48) Zhang, C.; Wang, X.; Zhang, J.; Hou, S.; Tang, Q.; Duan, M.; Fang, S. Synthesis of Dendritic Mesoporous Silica Nanoparticles by Ultrasonic Assisted Microchannel Continuous Flow Reaction. *RSC Adv.* **2025**, 15 (22), 17230–17240. <https://doi.org/10.1039/D5RA00745C>.
- (49) Yu, Y.-J.; Xing, J.-L.; Pang, J.-L.; Jiang, S.-H.; Lam, K.-F.; Yang, T.-Q.; Xue, Q.-S.; Zhang, K.; Wu, P. Facile Synthesis of Size Controllable Dendritic Mesoporous Silica Nanoparticles. *ACS Appl. Mater. Interfaces* **2014**, 6 (24), 22655–22665. <https://doi.org/10.1021/am506653n>.
- (50) Liu, P.-C.; Yu, Y.-J.; Peng, B.; Ma, S.-Y.; Ning, T.-Y.; Shan, B.-Q.; Yang, T.-Q.; Xue, Q.-S.; Zhang, K.; Wu, P. A Dual-Templating Strategy for the Scale-up Synthesis of Dendritic Mesoporous Silica Nanospheres. *Green Chem.* **2017**, 19 (23), 5575–5581. <https://doi.org/10.1039/C7GC02139A>.

- (51) Hao, P.; Peng, B.; Shan, B.-Q.; Yang, T.-Q.; Zhang, K. Comprehensive Understanding of the Synthesis and Formation Mechanism of Dendritic Mesoporous Silica Nanospheres. *Nanoscale Adv.* **2020**, 2 (5), 1792–1810. <https://doi.org/10.1039/D0NA00219D>.
- (52) Wang, Y.; Song, H.; Yu, M.; Xu, C.; Liu, Y.; Tang, J.; Yang, Y.; Yu, C. Room Temperature Synthesis of Dendritic Mesoporous Silica Nanoparticles with Small Sizes and Enhanced mRNA Delivery Performance. *J. Mater. Chem. B* **2018**, 6 (24), 4089–4095. <https://doi.org/10.1039/C8TB00544C>.
- (53) Park, S. S.; Ameduri, B.; Ha, C.-S. One-Pot Synthesis of Alkylammonium-Functionalized Mesoporous Silica Hollow Spheres in Water and Films at the Air–Water Interface. *emergent mater.* **2019**, 2 (1), 45–58. <https://doi.org/10.1007/s42247-019-00028-6>.
- (54) Dash, S.; Rajesh, P.; Joshi, R. G.; Rajeswari, S.; Karunanithi, P. Pre-Irradiation of Surfactants to Enhance Their Capacity to Solubilise Drugs and Dyes. *Journal of the Indian Chemical Society* **2022**, 99 (11), 100751. <https://doi.org/10.1016/j.jics.2022.100751>.
- (55) Narayan, R.; Nayak, U. Y.; Raichur, A. M.; Garg, S. Mesoporous Silica Nanoparticles: A Comprehensive Review on Synthesis and Recent Advances. *Pharmaceutics* **2018**, 10 (3), 118. <https://doi.org/10.3390/pharmaceutics10030118>.
- (56) Peng, S.; Huang, B.; Lin, Y.; Pei, G.; Zhang, L. Effect of Surface Functionalization and Pore Structure Type on the Release Performance of Mesoporous Silica Nanoparticles. *Microporous and Mesoporous Materials* **2022**, 336, 111862. <https://doi.org/10.1016/j.micromeso.2022.111862>.
- (57) Pan, L.; He, Q.; Liu, J.; Chen, Y.; Ma, M.; Zhang, L.; Shi, J. Nuclear-Targeted Drug Delivery of TAT Peptide-Conjugated Monodisperse Mesoporous Silica Nanoparticles. *J. Am. Chem. Soc.* **2012**, 134 (13), 5722–5725. <https://doi.org/10.1021/ja211035w>.
- (58) Nel, A.; Xia, T.; Mädler, L.; Li, N. Toxic Potential of Materials at the Nanolevel. *Science* **2006**, 311 (5761), 622–627. <https://doi.org/10.1126/science.1114397>.
- (59) Huang, X.; Townley, H. E. An Assessment of Mesoporous Silica Nanoparticle Architectures as Antigen Carriers. *Pharmaceutics* **2020**, 12 (3), 294. <https://doi.org/10.3390/pharmaceutics12030294>.
- (60) Nor Ain Zainal; Syamsul Rizal Abdul Shukor; Harjatul Azwana Ab. Wab; Khairunisak Abdul Razak. Study on the Effect of Synthesis Parameters of Silica Nanoparticles Entrapped with Rifampicin. *Chemical Engineering Transactions* **2013**, 32, 2245–2250. <https://doi.org/10.3303/CET1332375>.
- (61) Möller, K.; Kobler, J.; Bein, T. Colloidal Suspensions of Nanometer-Sized Mesoporous Silica. *Adv Funct Materials* **2007**, 17 (4), 605–612. <https://doi.org/10.1002/adfm.200600578>.
- (62) Ma, K.; Werner-Zwanziger, U.; Zwanziger, J.; Wiesner, U. Controlling Growth of Ultrasmall Sub-10 Nm Fluorescent Mesoporous Silica Nanoparticles. *Chem. Mater.* **2013**, 25 (5), 677–691. <https://doi.org/10.1021/cm303242h>.
- (63) Beltrán-Osuna, Á. A.; Gómez Ribelles, J. L.; Perilla, J. E. A Study of Some Fundamental Physicochemical Variables on the Morphology of Mesoporous Silica Nanoparticles MCM-41 Type. *J Nanopart Res* **2017**, 19 (12), 381. <https://doi.org/10.1007/s11051-017-4077-2>.

- (64) Saha, A.; Mishra, P.; Biswas, G.; Bhakta, S. Greening the Pathways: A Comprehensive Review of Sustainable Synthesis Strategies for Silica Nanoparticles and Their Diverse Applications. *RSC Adv.* **2024**, *14* (16), 11197–11216. <https://doi.org/10.1039/D4RA01047G>.
- (65) Chiang, Y.-D.; Lian, H.-Y.; Leo, S.-Y.; Wang, S.-G.; Yamauchi, Y.; Wu, K. C.-W. Controlling Particle Size and Structural Properties of Mesoporous Silica Nanoparticles Using the Taguchi Method. *J. Phys. Chem. C* **2011**, *115* (27), 13158–13165. <https://doi.org/10.1021/jp201017e>.
- (66) Fuertes, A. B.; Valle-Vigón, P.; Sevilla, M. Synthesis of Colloidal Silica Nanoparticles of a Tunable Mesopore Size and Their Application to the Adsorption of Biomolecules. *Journal of Colloid and Interface Science* **2010**, *349* (1), 173–180. <https://doi.org/10.1016/j.jcis.2010.05.041>.
- (67) Godakhindi, V.; Yazdimamaghani, M.; Dam, S. K.; Ferdous, F.; Wang, A. Z.; Tarannum, M.; Serody, J.; Vivero-Escoto, J. L. Optimized Fabrication of Dendritic Mesoporous Silica Nanoparticles as Efficient Delivery System for Cancer Immunotherapy. *Small* **2024**, *20* (50), 2402802. <https://doi.org/10.1002/smll.202402802>.
- (68) Kao, K.-C.; Mou, C.-Y. Pore-Expanded Mesoporous Silica Nanoparticles with Alkanes/Ethanol as Pore Expanding Agent. *Microporous and Mesoporous Materials* **2013**, *169*, 7–15. <https://doi.org/10.1016/j.micromeso.2012.09.030>.
- (69) Vallet-Regí, M.; Balas, F.; Arcos, D. Mesoporous Materials for Drug Delivery. *Angew Chem Int Ed* **2007**, *46* (40), 7548–7558. <https://doi.org/10.1002/anie.200604488>.
- (70) Florek, J.; Caillard, R.; Kleitz, F. Evaluation of Mesoporous Silica Nanoparticles for Oral Drug Delivery – Current Status and Perspective of MSNs Drug Carriers. *Nanoscale* **2017**, *9* (40), 15252–15277. <https://doi.org/10.1039/C7NR05762H>.
- (71) Dai, L.; Zhang, Q.; Shen, X.; Sun, Q.; Mu, C.; Gu, H.; Cai, K. A pH-Responsive Nanocontainer Based on Hydrazone-Bearing Hollow Silica Nanoparticles for Targeted Tumor Therapy. *J. Mater. Chem. B* **2016**, *4* (26), 4594–4604. <https://doi.org/10.1039/C6TB01050D>.
- (72) Slowing, I. I.; Trewyn, B. G.; Giri, S.; Lin, V. S. -Y. Mesoporous Silica Nanoparticles for Drug Delivery and Biosensing Applications. *Adv Funct Materials* **2007**, *17* (8), 1225–1236. <https://doi.org/10.1002/adfm.200601191>.
- (73) He, Q.; Zhang, Z.; Gao, F.; Li, Y.; Shi, J. In Vivo Biodistribution and Urinary Excretion of Mesoporous Silica Nanoparticles: Effects of Particle Size and PEGylation. *Small* **2011**, *7* (2), 271–280. <https://doi.org/10.1002/smll.201001459>.
- (74) Lu, J.; Liong, M.; Sherman, S.; Xia, T.; Kovochich, M.; Nel, A. E.; Zink, J. I.; Tamanoi, F. Mesoporous Silica Nanoparticles for Cancer Therapy: Energy-Dependent Cellular Uptake and Delivery of Paclitaxel to Cancer Cells. *Nanobiotechnol* **2007**, *3* (2), 89–95. <https://doi.org/10.1007/s12030-008-9003-3>.
- (75) Slowing, I.; Viveroescoto, J.; Wu, C.; Lin, V. Mesoporous Silica Nanoparticles as Controlled Release Drug Delivery and Gene Transfection Carriers☆. *Advanced Drug Delivery Reviews* **2008**, *60* (11), 1278–1288. <https://doi.org/10.1016/j.addr.2008.03.012>.
- (76) Chaudhary, Z.; Subramaniam, S.; Khan, G. M.; Abeer, M. M.; Qu, Z.; Janjua, T.; Kumeria, T.; Batra, J.; Popat, A. Encapsulation and Controlled Release of Resveratrol Within Functionalized Mesoporous Silica Nanoparticles for Prostate Cancer Therapy. *Front. Bioeng. Biotechnol.* **2019**, *7*, 225. <https://doi.org/10.3389/fbioe.2019.00225>.

- (77) Meng, H.; Wang, M.; Liu, H.; Liu, X.; Situ, A.; Wu, B.; Ji, Z.; Chang, C. H.; Nel, A. E. Use of a Lipid-Coated Mesoporous Silica Nanoparticle Platform for Synergistic Gemcitabine and Paclitaxel Delivery to Human Pancreatic Cancer in Mice. *ACS Nano* **2015**, 9 (4), 3540–3557. <https://doi.org/10.1021/acsnano.5b00510>.
- (78) Martínez-Carmona, M.; Izquierdo-Barba, I.; Colilla, M.; Vallet-Regí, M. Concanavalin A-Targeted Mesoporous Silica Nanoparticles for Infection Treatment. *Acta Biomaterialia* **2019**, 96, 547–556. <https://doi.org/10.1016/j.actbio.2019.07.001>.
- (79) Durfee, P. N.; Lin, Y.-S.; Dunphy, D. R.; Muñiz, A. J.; Butler, K. S.; Humphrey, K. R.; Lokke, A. J.; Agola, J. O.; Chou, S. S.; Chen, I.-M.; Wharton, W.; Townson, J. L.; Willman, C. L.; Brinker, C. J. Mesoporous Silica Nanoparticle-Supported Lipid Bilayers (Protocells) for Active Targeting and Delivery to Individual Leukemia Cells. *ACS Nano* **2016**, 10 (9), 8325–8345. <https://doi.org/10.1021/acsnano.6b02819>.
- (80) De Jesús Valle, M. J.; Zarzuelo Castañeda, A.; Maderuelo, C.; Cencerrado Treviño, A.; Loureiro, J.; Coutinho, P.; Sánchez Navarro, A. Development of a Mucoadhesive Vehicle Based on Lyophilized Liposomes for Drug Delivery through the Sublingual Mucosa. *Pharmaceutics* **2022**, 14 (7), 1497. <https://doi.org/10.3390/pharmaceutics14071497>.
- (81) Zhang, H. Thin-Film Hydration Followed by Extrusion Method for Liposome Preparation. In *Liposomes*; D'Souza, G. G. M., Ed.; Methods in Molecular Biology; Springer New York: New York, NY, 2017; Vol. 1522, pp 17–22. https://doi.org/10.1007/978-1-4939-6591-5_2.
- (82) Tu, J.; Bussmann, J.; Du, G.; Gao, Y.; Bouwstra, J. A.; Kros, A. Lipid Bilayer-Coated Mesoporous Silica Nanoparticles Carrying Bovine Hemoglobin towards an Erythrocyte Mimic. *International Journal of Pharmaceutics* **2018**, 543 (1–2), 169–178. <https://doi.org/10.1016/j.ijpharm.2018.03.037>.
- (83) Zhang, S.; Xing, M.; Li, B. Biomimetic Layer-by-Layer Self-Assembly of Nanofilms, Nanocoatings, and 3D Scaffolds for Tissue Engineering. *IJMS* **2018**, 19 (6), 1641. <https://doi.org/10.3390/ijms19061641>.
- (84) Zhou, G.; Li, L.; Xing, J.; Cai, J.; Chen, J.; Liu, P.; Gu, N.; Ji, M. Layer-by-Layer Construction of Lipid Bilayer on Mesoporous Silica Nanoparticle to Improve Its Water Susceptibility and Hemocompatibility. *J Sol-Gel Sci Technol* **2017**, 82 (2), 490–499. <https://doi.org/10.1007/s10971-017-4330-2>.
- (85) Richardson, J. J.; Björnmalm, M.; Caruso, F. Technology-Driven Layer-by-Layer Assembly of Nanofilms. *Science* **2015**, 348 (6233), aaa2491. <https://doi.org/10.1126/science.aaa2491>.
- (86) Hammond, J.; Richards, C. J.; Ko, Y.; Jonker, T.; Åberg, C.; Roos, W. H.; Lira, R. B. Membrane Fusion-Based Drug Delivery Liposomes Transiently Modify the Material Properties of Synthetic and Biological Membranes. *Small* **2025**, 21 (12), 2408039. <https://doi.org/10.1002/sml.202408039>.
- (87) Liu, X.; Situ, A.; Kang, Y.; Villabroza, K. R.; Liao, Y.; Chang, C. H.; Donahue, T.; Nel, A. E.; Meng, H. Irinotecan Delivery by Lipid-Coated Mesoporous Silica Nanoparticles Shows Improved Efficacy and Safety over Liposomes for Pancreatic Cancer. *ACS Nano* **2016**, 10 (2), 2702–2715. <https://doi.org/10.1021/acsnano.5b07781>.

- (88) Liu, P.; Chen, G.; Zhang, J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. *Molecules* **2022**, *27* (4), 1372. <https://doi.org/10.3390/molecules27041372>.
- (89) Ahmadi, A.; Sokunbi, M.; Patel, T.; Chang, M.-W.; Ahmad, Z.; Singh, N. Influence of Critical Parameters on Cytotoxicity Induced by Mesoporous Silica Nanoparticles. *Nanomaterials* **2022**, *12* (12), 2016. <https://doi.org/10.3390/nano12122016>.
- (90) Wani, A.; Muthuswamy, E.; Savithra, G. H. L.; Mao, G.; Brock, S.; Oupický, D. Surface Functionalization of Mesoporous Silica Nanoparticles Controls Loading and Release Behavior of Mitoxantrone. *Pharm Res* **2012**, *29* (9), 2407–2418. <https://doi.org/10.1007/s11095-012-0766-9>.
- (91) Cory, A. H.; Owen, T. C.; Bartrop, J. A.; Cory, J. G. Use of an Aqueous Soluble Tetrazolium/Formazan Assay for Cell Growth Assays in Culture. *Cancer Communications* **1991**, *3* (7), 207–212. <https://doi.org/10.3727/095535491820873191>.
- (92) Kroll, A.; Pillukat, M. H.; Hahn, D.; Schnekenburger, J. Interference of Engineered Nanoparticles with in Vitro Toxicity Assays. *Arch Toxicol* **2012**, *86* (7), 1123–1136. <https://doi.org/10.1007/s00204-012-0837-z>.
- (93) Kumar, P.; Nagarajan, A.; Uchil, P. D. Analysis of Cell Viability by the Lactate Dehydrogenase Assay. *Cold Spring Harb Protoc* **2018**, *2018* (6), pdb.prot095497. <https://doi.org/10.1101/pdb.prot095497>.
- (94) Behzadi, S.; Serpooshan, V.; Tao, W.; Hamaly, M. A.; Alkawareek, M. Y.; Dreaden, E. C.; Brown, D.; Alkilany, A. M.; Farokhzad, O. C.; Mahmoudi, M. Cellular Uptake of Nanoparticles: Journey inside the Cell. *Chem. Soc. Rev.* **2017**, *46* (14), 4218–4244. <https://doi.org/10.1039/C6CS00636A>.
- (95) Rejman, J.; Oberle, V.; Zuhorn, I. S.; Hoekstra, D. Size-Dependent Internalization of Particles via the Pathways of Clathrin- and Caveolae-Mediated Endocytosis. *Biochemical Journal* **2004**, *377* (1), 159–169. <https://doi.org/10.1042/bj20031253>.
- (96) Senarath-Yapa, M. D.; Phimpivong, S.; Coym, J. W.; Wirth, M. J.; Aspinwall, C. A.; Saavedra, S. S. Preparation and Characterization of Poly(Lipid)-Coated, Fluorophore-Doped Silica Nanoparticles for Biolabeling and Cellular Imaging. *Langmuir* **2007**, *23* (25), 12624–12633. <https://doi.org/10.1021/la701917w>.
- (97) Mearu, H.; Yoshigoe, A.; Nakamura, M.; Doura, T.; Tamanoi, F. Biodegradability of Disulfide-Organosilica Nanoparticles Evaluated by Soft X-Ray Photoelectron Spectroscopy: Cancer Therapy Implications. *ACS Appl. Nano Mater.* **2019**, *2* (1), 479–488. <https://doi.org/10.1021/acsanm.8b02023>.
- (98) Şen Karaman, D.; Patrignani, G.; Rosqvist, E.; Smått, J.-H.; Orłowska, A.; Mustafa, R.; Preis, M.; Rosenholm, J. M. Mesoporous Silica Nanoparticles Facilitating the Dissolution of Poorly Soluble Drugs in Orodispersible Films. *European Journal of Pharmaceutical Sciences* **2018**, *122*, 152–159. <https://doi.org/10.1016/j.ejps.2018.06.027>.
- (99) M. Ways, T. M.; Ng, K. W.; Lau, W. M.; Khutoryanskiy, V. V. Silica Nanoparticles in Transmucosal Drug Delivery. *Pharmaceutics* **2020**, *12* (8), 751. <https://doi.org/10.3390/pharmaceutics12080751>.

- (100) Moya, S. E.; Hernández, R. R.; Angelomé, P. C. Degradation of Mesoporous Silica Materials in Biological Milieu: The Gateway for Therapeutic Applications. *Advanced NanoBiomed Research* **2024**, *4* (10), 2400005. <https://doi.org/10.1002/anbr.202400005>.
- (101) Hao, N.; Liu, H.; Li, L.; Chen, D.; Li, L.; Tang, F. *In Vitro* Degradation Behavior of Silica Nanoparticles Under Physiological Conditions. *J nanosci nanotechnol* **2012**, *12* (8), 6346–6354. <https://doi.org/10.1166/jnn.2012.6199>.
- (102) Braun, K.; Pochert, A.; Beck, M.; Fiedler, R.; Gruber, J.; Lindén, M. Dissolution Kinetics of Mesoporous Silica Nanoparticles in Different Simulated Body Fluids. *J Sol-Gel Sci Technol* **2016**, *79* (2), 319–327. <https://doi.org/10.1007/s10971-016-4053-9>.
- (103) Li, L.; Liu, T.; Fu, C.; Tan, L.; Meng, X.; Liu, H. Biodistribution, Excretion, and Toxicity of Mesoporous Silica Nanoparticles after Oral Administration Depend on Their Shape. *Nanomedicine: Nanotechnology, Biology and Medicine* **2015**, *11* (8), 1915–1924. <https://doi.org/10.1016/j.nano.2015.07.004>.
- (104) Croissant, J. G.; Cattoën, X.; Wong Chi Man, M.; Durand, J.-O.; Khashab, N. M. Syntheses and Applications of Periodic Mesoporous Organosilica Nanoparticles. *Nanoscale* **2015**, *7* (48), 20318–20334. <https://doi.org/10.1039/C5NR05649G>.
- (105) Paris, J. L.; Colilla, M.; Izquierdo-Barba, I.; Manzano, M.; Vallet-Regí, M. Tuning Mesoporous Silica Dissolution in Physiological Environments: A Review. *J Mater Sci* **2017**, *52* (15), 8761–8771. <https://doi.org/10.1007/s10853-017-0787-1>.
- (106) Du, X.; Kleitz, F.; Li, X.; Huang, H.; Zhang, X.; Qiao, S. Disulfide-Bridged Organosilica Frameworks: Designed, Synthesis, Redox-Triggered Biodegradation, and Nanobiomedical Applications. *Adv Funct Materials* **2018**, *28* (26), 1707325. <https://doi.org/10.1002/adfm.201707325>.
- (107) Gamcsik, M. P.; Kasibhatla, M. S.; Teeter, S. D.; Colvin, O. M. Glutathione Levels in Human Tumors. *Biomarkers* **2012**, *17* (8), 671–691. <https://doi.org/10.3109/1354750X.2012.715672>.
- (108) Venedicto, M.; Carrier, J.; Na, H.; Chang, C.-Y.; Radu, D. R.; Lai, C.-Y. Disulfide-Modified Mesoporous Silica Nanoparticles for Biomedical Applications. *Crystals* **2023**, *13* (7), 1067. <https://doi.org/10.3390/cryst13071067>.
- (109) Wu, M.; Meng, Q.; Chen, Y.; Du, Y.; Zhang, L.; Li, Y.; Zhang, L.; Shi, J. Large-Pore Ultrasmall Mesoporous Organosilica Nanoparticles: Micelle/Precursor Co-templating Assembly and Nuclear-Targeted Gene Delivery. *Advanced Materials* **2015**, *27* (2), 215–222. <https://doi.org/10.1002/adma.201404256>.
- (110) Zhao, Q.; Wang, C.; Liu, Y.; Wang, J.; Gao, Y.; Zhang, X.; Jiang, T.; Wang, S. PEGylated Mesoporous Silica as a Redox-Responsive Drug Delivery System for Loading Thiol-Containing Drugs. *International Journal of Pharmaceutics* **2014**, *477* (1–2), 613–622. <https://doi.org/10.1016/j.ijpharm.2014.10.056>.
- (111) Niu, B.; Liao, K.; Zhou, Y.; Wen, T.; Quan, G.; Pan, X.; Wu, C. Application of Glutathione Depletion in Cancer Therapy: Enhanced ROS-Based Therapy, Ferroptosis, and Chemotherapy. *Biomaterials* **2021**, *277*, 121110. <https://doi.org/10.1016/j.biomaterials.2021.121110>.
- (112) Putz, A.-M.; Wang, K.; Len, A.; Plocek, J.; Bezdzicka, P.; Kopitsa, G. P.; Khamova, T. V.; Ianăși, C.; Săcărescu, L.; Mitróová, Z.; Savii, C.; Yan, M.; Almásy, L. Mesoporous Silica Obtained with

Methyltriethoxysilane as Co-Precursor in Alkaline Medium. *Applied Surface Science* **2017**, 424, 275–281. <https://doi.org/10.1016/j.apsusc.2017.04.121>.

(113) Jeitler, R.; Glader, C.; Tetyczka, C.; Zeiringer, S.; Absenger-Novak, M.; Selmani, A.; Fröhlich, E.; Roblegg, E. Investigation of Cellular Interactions of Lipid-Structured Nanoparticles With Oral Mucosal Epithelial Cells. *Front. Mol. Biosci.* **2022**, 9, 917921. <https://doi.org/10.3389/fmolb.2022.917921>.

(114) Cunha, S.; Costa, C. P.; Loureiro, J. A.; Alves, J.; Peixoto, A. F.; Forbes, B.; Sousa Lobo, J. M.; Silva, A. C. Double Optimization of Rivastigmine-Loaded Nanostructured Lipid Carriers (NLC) for Nose-to-Brain Delivery Using the Quality by Design (QbD) Approach: Formulation Variables and Instrumental Parameters. *Pharmaceutics* **2020**, 12 (7), 599. <https://doi.org/10.3390/pharmaceutics12070599>.