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„Developing of manufacturing processes for micro-nanoplastics from polypropylene and polyethylene terephthalate“

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Abstract

Kunststoffe sind eine der Hauptquellen der Umweltverschmutzung, da sie in verschiedenen Formen allgegenwärtig in der Umwelt nachgewiesen wurden. Mikro- und Nanokunststoffe (MNP) entstehen durch den langsamen Abbau von Kunststoffpolymeren und treten auf drei Hauptwegen mit dem menschlichen Körper in Kontakt: Absorption durch die Haut, Einatmen und Verschlucken. Bislang gibt es noch nicht genügend Daten über die Verbreitung von MNP in der Umwelt und ihre gesundheitlichen Auswirkungen auf Organismen.

In dieser Arbeit wird die Entwicklung und Optimierung von Produktionsmethoden für MNP als Testmaterialien diskutiert. Es wurden zwei Polymertypen untersucht: Polypropylen (PP) und Polyethylenterephthalat (PET). Diese beiden Polymere wurden aufgrund der Vielfalt ihrer chemischen und physikalischen Eigenschaften ausgewählt und repräsentieren somit ein breites Spektrum von MNP in der Umwelt. Die NP wurden mit der Technik der Nanofällung hergestellt, die eine kurze Solubilisierung, Ausfällung, Wäsche und Resuspension umfasst, um eine Partikelgröße zwischen 0,1 und 1 µm zu erreichen. Für die PP-Nanopräzipitation wurden die Vorbehandlung (Schmelzen bei 180 °C für 3 Stunden), die Solubilisierungstemperatur (185 °C), die Fällungstechnik (doppeltes Pipettieren in einem Eisbad) und die Fällungszeit (wenige Minuten) optimiert. Auch für die PET-Nanofällung wurden die Löslichkeitstemperatur (220 °C), die Löslichkeitsdauer (45 Minuten) und die Fällungsdauer (30 Minuten) optimiert. Diese Methode wurde aufgrund ihrer Einfachheit, Effizienz und Schnelligkeit eingesetzt. Die resultierende Partikelgrößenverteilung (PSD) wurde mittels Laserdiffraktometrie (LD) gemessen. Um die Stabilität zu erhöhen, wurde das Medium von Ethanol zu Glycerin mit einer Endkonzentration von 40 mg/g ausgetauscht. Darüber hinaus wurden fluoreszenzmarkierte Partikel hergestellt, indem der Farbstoff Poly(9,9-dioctylfluoren-alt-benzothiadiazol) (F8BT) während des Solubilisierungsprozesses zugegeben wurde und mit dem zu untersuchenden Polymer co-präzipitiert.

Eine andere Methode zur Herstellung von PP in Mikrongröße als Testmaterial wurde verwendet. Das optimierte Protokoll umfasst: Vorbehandlung von PP (Schmelzen für 1 Stunde bei 180°C), Versprödung (Einfrieren über Nacht bei -70°C) und mechanisches Nassmahlen, gefolgt von Sieben und Sammeln der angestrebten Größenbereiche. Diese Methode wurde gewählt, da sie den natürlichen Abbau von Kunststoffen in der Umwelt simuliert.

Durch die Optimierung der Nanofällungsmethode wurden PP- und PET-NP-Chargen mit PSD im Bereich von 0,1-1 µm erfolgreich hergestellt und in 40 mg/g Glycerin redispergiert. Durch Kopräzipitation mit dem Fluoreszenzfarbstoff F8BT wurden außerdem einige markierte NPs mit einer PSD im Bereich von 0,1-1 µm erfolgreich hergestellt. Darüber hinaus wurden auch PP-Chargen in Mikrongröße (38-50, 50-100, 100-180 und 180-250 µm) durch mechanisches Nassmahlen erfolgreich hergestellt.

In dieser Arbeit wurden reproduzierbare Methoden zur Herstellung von MNPs-Testmaterialien aus PP und PET entwickelt und die hergestellten Testmaterialien erfolgreich an unsere Projektpartner für weitere Untersuchungen geschickt.

Plastics are one of the major sources of environmental pollution, as they have been ubiquitously detected in several forms in the environment. Micro- and nanoplastics (MNPs) result from the slow degradation of plastic polymers and interact with the human body by three main exposure routes: absorption through skin, inhalation and ingestion. To date, there is still not enough data on the prevalence of MNPs in the environment and their health impacts on organisms.

In this thesis, the development and optimization of production methods for MNPs as test materials will be discussed. Two polymer types were studied: Polypropylene (PP) and polyethylene terephthalate (PET). These two polymers were chosen due to the diversity in their chemical and physical properties, thus representing a wide range of MNPs in the environment. NPs were produced by the nanoprecipitation technique which briefly involves solubilisation, precipitation, washing and resuspension to achieve a particle size range between 0.1-1 µm. For PP nanoprecipitation the pretreatment (melting at 180 °C for 3 hours), solubilization temperature (185 °C), precipitation technique (double pipetting in an ice bathe) and precipitation time (few minutes) were optimized. Also, for PET nanoprecipitation the solubilization temperature (220 °C), solubilization time (45 minutes) and precipitation time (30 minutes) were optimized. This method was employed because of its easy, efficient and fast nature. The resulting particle size distribution (PSD) was measured via laser diffraction (LD). To increase stability the medium was exchanged from ethanol to glycerol with a final concentration of 40 mg/g. Moreover, fluorescently labelled particles were produced by adding the dye Poly(9,9-dioctylfluorene-alt-benzothiadiazole) (F8BT) during the solubilisation process and co-precipitate with the polymer of interest.

Another method for the preparation of micron sized PP as test materials was used. The optimized protocol includes: Pretreatment of PP (melting for 1 hour at 180°C), embrittling (freezing overnight at -70°C) and mechanical wet grinding, followed by sieving and collecting the targeted size ranges. This method was chosen, since it was found to simulate the natural degradation of plastics in the environment.

Through optimization of nano-precipitation method, PP and PET-NPs batches with PSD within the range of 0.1-1 µm were successfully produced and redispersed into 40 mg/g glycerol. Also, through co-precipitation with the fluorescent dye F8BT, some labelled NPs batches with PSD within the range of 0.1-1 µm were successfully produces. Moreover, micron sized PP batches (38-50, 50-100, 100-180 and 180-250 µm) were also successfully produced through mechanical wet grinding.

In this Thesis, reproducible methods were successfully developed to produce MNPs test materials from PP and PET and the produced test materials were successfully sent to our project partners for further investigations.

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List of Abbreviations

HDPE	high-density polyethylene
LDPE	Low-density polyethylene
LD	Laser diffraction
MNPs	Micro-and nanoplastics
MPs	Microplastics
NPs	Nanoplastics
PP	Polypropylene
PET	Polyethylene terephthalate
PS	Polystyrol
PSD	Particle size distribution
PVC	Polyvinylchlorid
WWTPs	Waste water treatment plants

1. Introduction

Plastic particles have been recently ubiquitously detected in the environment, since they are used in almost all aspects of life. Nowadays they are considered one of the main environmental pollutants. The scope of this thesis would be on producing MNPs test materials that simulate those available in the environment to investigate their impact on human health.

1.1. Plastics

1.1.1. What are plastics

Plastics are synthetic polymers that are made by covalent binding of hundreds or thousands of organic monomers [1]. The word plastic is derived from the Greek word “Plasticos” which means moulded. This refers to one of the most important properties of plastics which is their capability to be reshaped [2].This allows various applications of these materials summarized in Table 1 Polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polyvinyl chloride (PVC) and polystyrene (PS) are widely produced. Together, these types of plastic account for about 90% of total global plastic production [3]. Due to many beneficial characteristics such as low price, durability, low weight and good ductility, Plastics are widely used in the production of lots of essentials used in everyday life [4]. Subsequently, due to the high annual global plastic production, that has exceeded 300 million tons and their slow degradation, its accumulation has gradually increased(Figure 1) [5].

Plastic class	Application
PET	Soft drink, water, juice, and beer bottles, functional/waterproof/ synthetic clothing/textile, containers for foods and liquid, glass fiber for engineering resins.
HDPE	Milk jugs, juice bottles, bleach, detergent and household cleaner bottles, butter and yogurt containers.
LDPE	Plastic bags, six-pack rings, netting, drinking straws, wire cables.
PP	Rope, bottle caps, netting, car bumpers, flowerpot, folders.
PS	Disposable plates and cup, meat trays, egg cartons, carry-out containers, aspirin bottles.
PVC	Window cleaner and detergent bottles, shampoo bottles, cooking oil bottles, clear food packaging, medical equipment, boots, clothing.

Table 1 Common applications of plastic found in environment.[5].

Plastics could be classified into several groups according to their physical and chemical properties for example according to shape and morphology plastics could be classified into pellets, fibers, sheets, fragments or films. According to the chemical structure plastics could be classified into plastics with carbon-carbon backbone as PVC and PS that are susceptible to photo and thermal degradation and are used for the production of one-use products or plastics that contain heteroatoms such as PET which are not susceptible to normal degradation conditions. It is noted that physicochemical properties of MNPs can also be altered by their chemical additives (e.g., diisoheptyl phthalate, dibutyl phthalate, butylated hydroxytoluene). Generally, PVC, nylon and PET are prone sinking, while PP, PE and PS are more likely to float or being suspended in aqueous environments. Plastics could be also classified according to size and origin which will be discussed in details in the following paragraphs.[6]

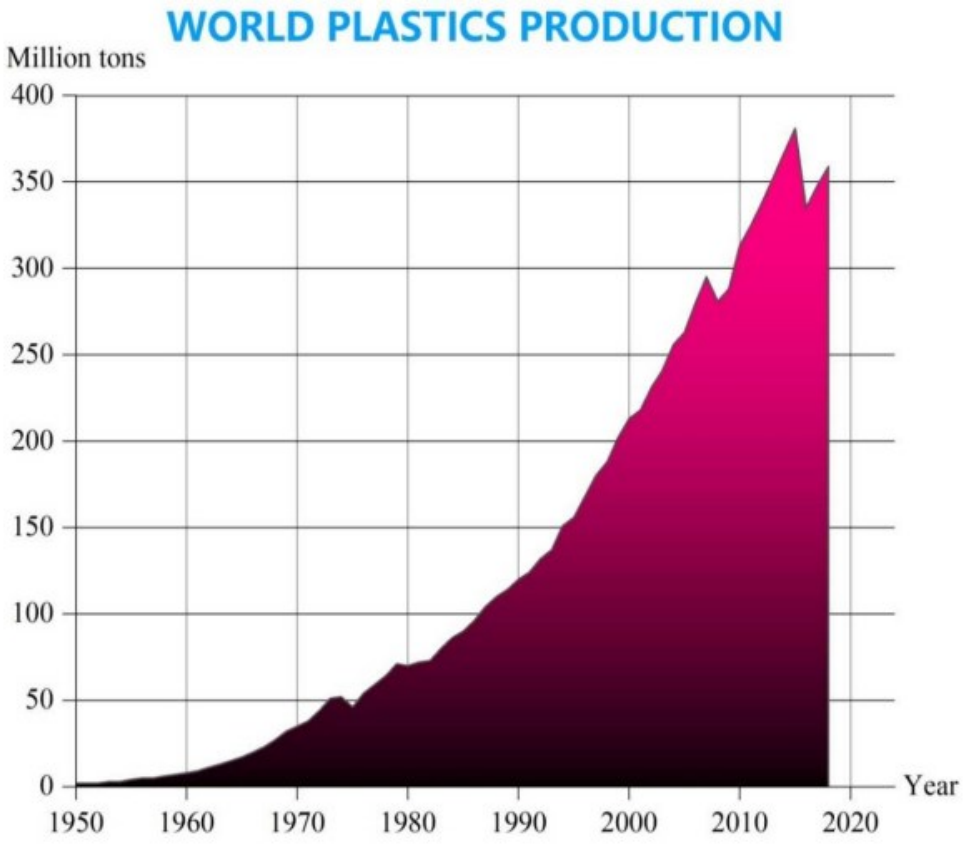

Figure 1 Global plastic production [7]

1.1.2. What are MNPs

MNPs are plastic particles that originate either from international manufacturing or as a result of degradation of bigger plastic particles [8][9]. Plastics that are manufactured to be of a microscopic size are defined as primary MNPs. The sources of primary MNPs can be

generally divided into several categories, mainly facial-cleansers and cosmetics, air-blasting media, vectors for drugs, and virgin plastic production pellets [10]. Secondary MNPs are eventually formed from larger plastic particles after breaking down into smaller particles through physical, chemical and/or biological processes. Thus, the sources of secondary MNPs are not only numerous, but also diverse [11].

Plastic particles are divided into three main size classes which are macro-, micro- and nanoplastics [12]. Macroplastics are Plastic particles with particle size more than 5 mm [13]. MPs are plastic particles with a size less than 5 mm, while NPs are plastic particles with a size less than 0.001 mm [14]. The micro and nano sized plastics can result in the environment through different degradation mechanisms of bigger sized plastics.

1.2. Degradation mechanisms of plastics

When plastics are exposed to the environment, they undergo various degradation processes. This affects the physicochemical properties of these plastics, such as colour, surface morphology, crystallinity, particle size, density, reactivity, surface functionality, and hydrophobicity [15]. Environmental factors and the properties of plastic materials (e.g., size and density) will affect the degradation rate of plastics[10]. Weathering is considered to be the primary process for plastic degradation [16]. Another important process is sunlight-induced photodecomposition, which can lead to bond breakage, thus causing degradation and oxidation of plastics [9]. Plastic fragments are also susceptible to breakage by mechanical forces, such as abrasion, fluctuation and turbulence [17]. In addition, changes of the external environment such as very deep ocean depths with low oxygen and saline conditions in the low-energy marine environment of the benthic zone, the degradation rate of microplastics slows down significantly [18]. After degradation of plastics, the resulting MNPs could be transported between environmental compartments.

1.3. Transport, and fate of MNPs

MNPs could be transported to and from different environmental compartments such as air, freshwater, soil and ocean.

The main sources of MNPs in air are erosion of synthetic rubber tires, synthetic textiles and dust [19]. Other sources may include building materials, industrial wastes, plastic fragments from furniture, particle resuspension, landfills, traffic particles, tumble dryer exhaust, and sewage sludge used as fertilizer [20]. MNPs from these various sources are continuously transported through the air and settle in soil or sediment after being discharged into the air, which may play an important role in human respiratory exposure. The fate of MNPs in outdoor atmospheric environments is affected by many other factors, such as wind direction and speed, vertical pollution concentration gradient, precipitation, and temperature [19].

MNPs that are not retained in sewage sludge or that have not been filtered out during the sewage treatment process will be released into freshwater [11]. Freshwater bodies are the main drinking water sources for human consumption, which are thus considered as potential exposure sources of MNPs to humans [21]. The main sources of MNPs in freshwater are the ones with industrial origins in plastic resin powder, microbeads present in personal care products, pellet spillage from air blasting machines, as well as raw materials used to produce plastic products [22]. Many factors affect the transport of MNPs in freshwater, such as the water body size, wind, currents, and particle density [23]. Additionally, the waste water treatment plants (WWTPs) could be a potential source of MNPs in the environment [24].

Soil acts as an important reservoir for MNPs [25]. Soil plastics can originate from the weathering and disintegration of plastic fragments on farmland, fragmentation of plastic waste, littering, atmospheric deposition and surface runoff [26]. Once released into soil, MNPs may persist and accumulate, which affects the growth and reproduction of soil organisms and their biodiversity [27]. MNPs in topsoil may also move to deeper soils through several processes such as agricultural cultivation, soil cracks, or the disturbance of soil organisms [28]. Thus, MNPs can inhibit the growth of crops, which in turn may reduce food production [29].

The ocean is the largest sink for plastic waste which undergoes many several pathways to get into the marine environment [30]. Land-based plastic debris reaches up to 80% of plastic waste in the ocean [31]. Indirectly through beach littering, atmospheric transport and rivers or directly *via* fishing activities, shipping and aquaculture.

As a result of that wide distribution of MNPs in all environmental compartments, MNPs pollution became a serious issue to consider.

1.4. MNPs as environmental pollutants

Recently, MNPs have been considered one of the most widely spread environmental pollutants. Through the properties and distribution mechanisms described above, plastics are ubiquitously found. Moreover, Due to their hydrophobic surface, small size and large surface area, MNPs can act as a carrier for various toxic pollutants that are adsorbed on their surface[32], including heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), and other contaminants such as polyfluoroalkyl substances (PFAS). These compounds are not soluble in water but soluble in fats, thus they can penetrate cells and accumulate in fatty tissues of living organisms.[33] Large amounts of MNPs will have toxic effects on organisms, causing severe ecological risks. Furthermore, they could also be transferred through food chains or be inhaled, causing severe health problems such as cancer and lung diseases [34].

1.5. Human exposure to MNPs in the environment.

Humans are exposed to MNPs mainly through ingestion, inhalation and dermal absorption [35] (**Error! Reference source not found.**) MNPs are detected in people's daily diet including seafood [36], sea salt [37], honey, sugar and drinking water [38]. Recent studies also revealed that MNPs with a size of 0.2 and 2 µm can penetrate the roots of wheat and lettuce and enter the leaves [39]. In these studies, it was also indicated that MNPs (especially NPs) may enter the seeds/fruits of crops and likely enter the human body through food consumption.

Studies on airborne MNPs reported that humans may inhale up to 272 plastic particles per day [40]. The penetration depth of MNPs into the lung depends on their size [41].Particles below 2.5 µm will primarily remain in the lungs and can pass through the respiratory barrier. Therefore, NPs are expected to exhibit more adverse effects than MPs [42].

Dermal contact is considered a less significant pathway[43]. Since particle absorption through the skin requires penetration of the *stratum corneum*, only particles with sizes below 100 nm will be absorbed[20]. Thus, only NPs could eventually penetrate through the skin barrier [44].

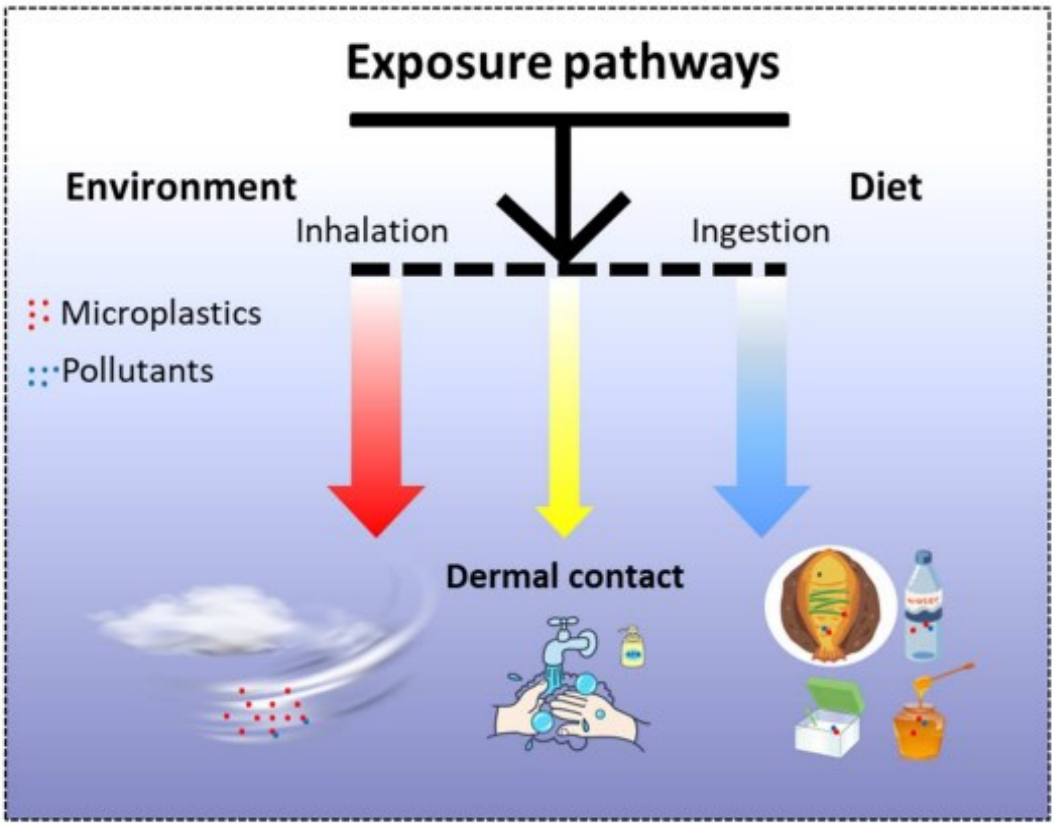

Figure 2 Main exposure pathways of Microplastics. [5]

1.6. Possible health implications of MNPs on human

When MNPs enter the body, they are resistant to chemical degradation *in vivo*. Therefore, their biological persistence and dosage are considered important factors that lead to health risks [45]. Plastics were found to increase the risk of impaired brain and neurological functions, cancer, chemotherapy delay, adult-onset diabetes, chromosomal and reproductive system abnormalities, obesity, early puberty, drug resistance and cardiovascular damage. A study by Centre for Disease Control and Prevention (CDC) says that 93% of the examined subjects have bisphenol A (a dangerous plastic additive) in their urine [46]. Bisphenol A is considered as an endocrine disrupting compound which interacts with oestrogen and androgen causing sex-related problems[33]

It was confirmed In previous studies that MPs can generate reactive oxygen species, increase glutathione S-transferase activity and activate antioxidant-related enzymes and mitogen-activated proteins kinase signalling pathways [47]. MPs could also cause neurotoxicity by acetylcholinesterase inhibition [48]. MPs could also reduce lipid digestion through the formation of MPs-oil droplets and inhibition of digestive enzymatic activities based on a simulated digestion investigation [49].

However, there is very few research available on the exact health impacts of MNPs and very few commercially available test materials for further research. That's why we are aiming to develop reliable methods to produce MNPs test materials. Our focus was on PP and PET, since they are two of the most widely used plastics and have different properties, thus the results could be applicable on wide range of plastics.

MNPs can be produced by two main methods which are either top down and bottom up. The top down procedure is the transformation of large sized particles into smaller sized particles. Although this method is simple to use, it is ineffective for manufacturing extremely small particles. The bottom up method is a constructive method to produce MNPs through the growth and self-assembly of molecules. In contrast to the top down method, the bottom up approach is capable of producing NPs. [50]

1.7. Imptox project

Imptox is a research project funded by the EU Horizon 2020 funding program that aims to develop analytical methods to characterize and measure MNPs in the environment to determine their effects on human health. In this project the effect of ingestion and inhalation of MNPs carrying different pollutants such as heavy metals, bacteria, allergens and toxins will be evaluated by examining MNPs in seafood, seawater and freshwater spray aerosols. Is also within the scope of the project evaluate the effect of MNPs on asthma and allergic diseases specially in children.

1.7.1. Study Hypotheses

It is hypothesized that due to the hydrophobic surface of MNPs, different contaminants could be adsorbed on their surface. The type of these contaminants would depend on the MNPs composition that would act as a vector to transport these contaminants into the body. It is hypothesized that particularly the proteins from the environment or food that are adsorbed on the MNPs surface plays a role in the hypersensitivity disorders.

1.7.2. Project objectives

Based on the previous hypotheses, the scope of Imptox is to investigate the ability of MNPs to bind and attract molecules and to understand their role in allergic disorders through interaction with human immune system.[51]

1.8. Aims of the Thesis.

The aim of this thesis is to develop and optimize a reproducible manufacturing process to prepare test materials from PP and PET with particle size ranges between 0.1-1 µm and micron sized PP.

2. Materials and methods.

2.1. Materials and Instruments

PP and PET (bottle grade) pellets were provided by PlasticsEurope. Ethanol 96% (C_2H_60, d: 0.78945 g/ cm^3, M= 46.07 g/mol) was purchased from Brenntag (Brenntag Austria GmbH, Vienna, Austria) The fluorescent dye F8BT (698687, $(C_{35}H_{42}N_2S)_n$, Mn ≤ 25000), Tween® 80 (P1754, $C_{64}H_{124}O_{26}$, M= 1.310 g/mol, d: 1.064 g/cm^3), Glycerol (8187091000, $C_3H_8O_3$, M= 92.09 g/mol, 1 L=1.261 kg), Benzyl alcohol (108006, C_7H_8O, d: 1.045 g/mL, M=108.14 g/mol) and Xylol (1.08297, C_8H_{10}, M= 106.17 g/mol, 1L=0.87g) were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Mastersizer 3000 (Malvern Panalytical GmbH, Kassel, Germany) was used for PSD measurement. Knife mill GM 200 (Retsch GmbH, Haan, Germany) was used for mechanical wet grinding. AS 200 sieve shaker, five stainless steel sieves with different mesh sizes (38 µm, 50 µm, 100 µm, 180 µm and 250 µm) and a pan (Retsch GmbH, Haan, Germany) were uses foe MPs fraction separation. Elmasonic P ultrasound bath (Elma Schmidbauer GmbH, Singen, Germany) was used for sonication. Diaphragm vacuum pump (Vacuubrand GmbH, Wertheim, Germany) was used for filtration and washing. Heidolph VV2011 rotary evaporator (Heidolph instruments GmbH, Schwabach, Germany) was used for medium change and drying powders. Vortex mixer Reax 2000 (Heidolph instruments GmbH, Schwabach, Germany). Neylon membrane filters (0.22, 0.8 &10 µm) were used for filtration.

2.2. Methods

2.2.1. PP pretreatment.

Before further processing, PP pellets were melted into equally sized cuboids. Silicon moulds with 160 chambers (1x1 cm) were used and each one was filled with 8 pellets, as shown in Figure 3 The prepared moulds were then heated in an oven at 180 °C for one (when grinded) to three (for nanoprecipitation) hours, followed by a cool down phase until room temperature was reached. Obtained PP cuboids for grinding, were subsequently frozen overnight at -70 °C.

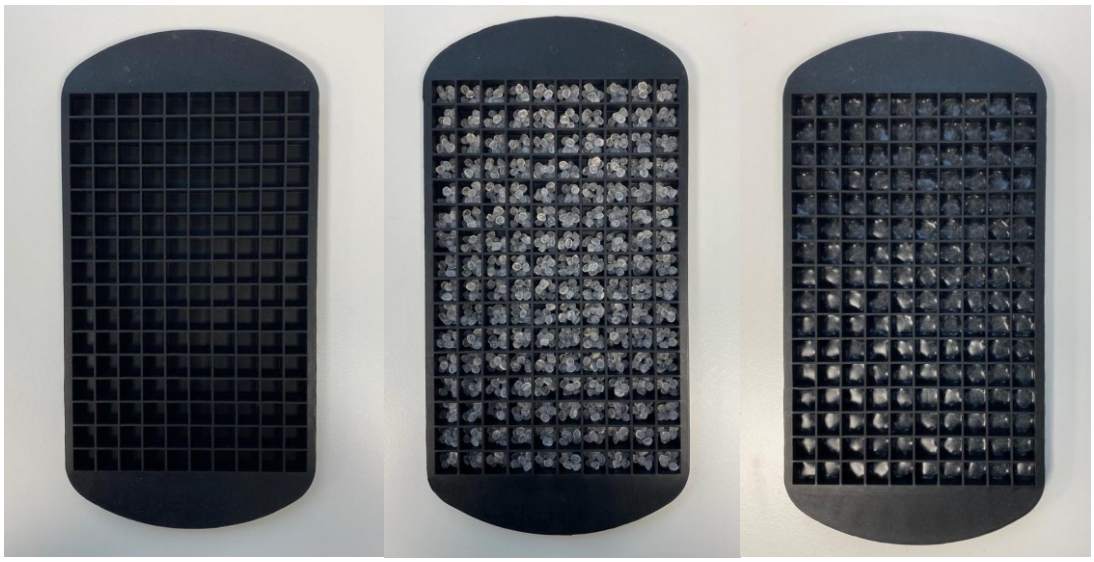

Figure 3 PP pretreatment by melting the pellets in silicon moulds into cuboids at 180 °C

2.2.2. PP nanoprecipitation

This method was developed and optimized to produce PP NPs with a particle size range between 0.1-1 µm. It can be summarized in 4 main steps: solubilisation, precipitation, washing and resuspension.

For solubilisation, a heating plate with magnetic stirring function was employed. A silicon bath was heated to 185 °C and stirred with 250 rpm. The pretreated PP cuboids (260 mg) were weighed into a round bottom flask and a magnetic stir bar and xylol (40 mL) were added. The flask was then connected to a reflux condenser and heated for 30 minutes until the cuboids were completely solubilised. (Figure 4)

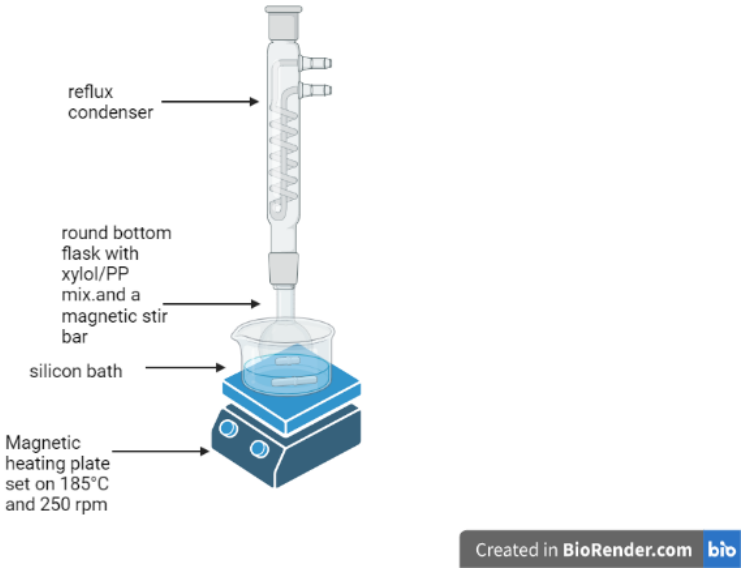

Figure 4 PP solubilisation

Precipitation of the PP/xylol solution was performed as follows. A beaker with filtered (through a 0.22 µm filter using a vacuum pump) ethanol (120 mL, 96%, v/v) was cooled with an ice bath and simultaneously stirred at 250 rpm. The whole amount of the hot PP/xylol mixture was transferred with two 25 mL pipets into the beaker at the same time (double pipetting). The suspension was then stirred until completely cooled down (five minutes) Figure 5 shows the instrumental setup.

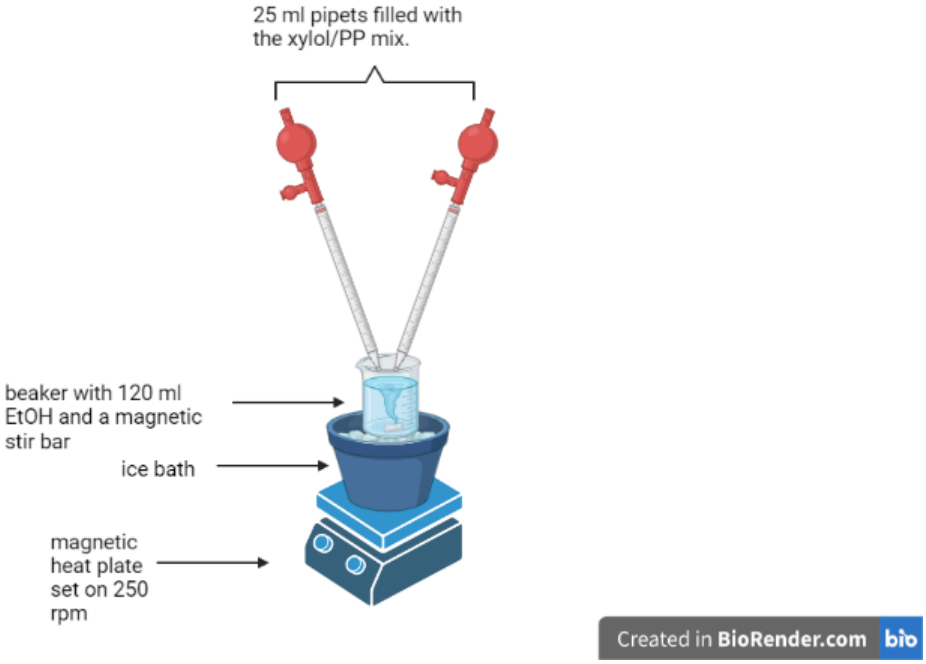

Figure 5 PP precipitation by double pipetting

The following washing step was crucial to remove xylol. The mixture was first filtered with a nylon membrane filter (0.8 µm) using a vacuum pump. Subsequently, the NPs were washed five times with filtered ethanol (100 mL, 96%, v/v). NPs were occasionally scrapped off the filter gently and resuspended by pressing them against the wall, so that enclosed xylol was also removed.

The last step was the resuspension of the washed NPs. Filtered Ethanol (26 mL, 96%, v/v) was added to an Erlenmeyer flask (50 mL), to obtain a concentration of approximately 10 mg/mL. The filter with the produced NPs was then transferred into the flask and sonicated until all particles disattached from the membrane. The flask with the NPs suspension was then sonicated and vortexed until no more clumps were visible (**Figure 6**)

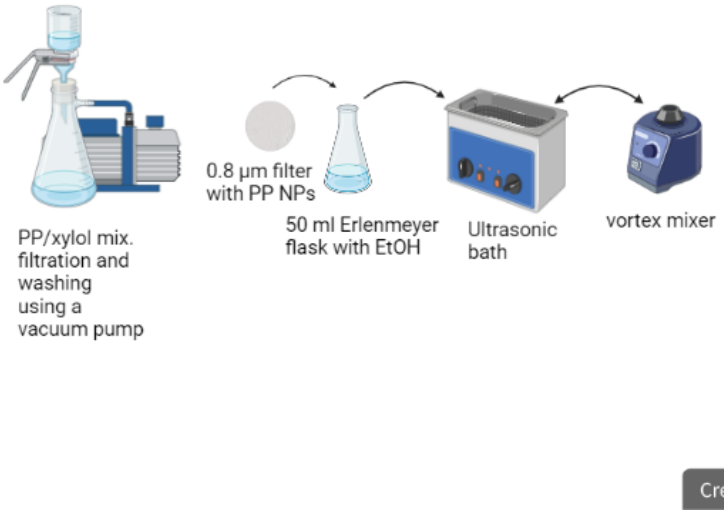

Figure 6 PP filtration, washing and resuspension

2.2.3. PET nanoprecipitation

Similarly, to PP, a PET nanoprecipitation process was developed and optimized to produce PET NPs with a particle size range between 0.1-1 µm. The four main steps are the same but with slight differences such as the solubilisation temperature and the precipitation technique.

For solubilisation, the procedures were similar to PP except that, PET pellets (260 mg) were solubilised in benzyl alcohol, the solubilisation temperature was 220 °C and the mixture was left stirring for 45 minutes

For PET precipitation, the solubilised PET/benzyl alcohol mixture (10 mL) was then taken into a pipet (10 mL) and transferred to the beaker with the pipet tip touching the glass wall and moving in a half circle motion. This step was repeated until the whole mixture was transferred to the beaker. The mixture was then left stirring for 30 minutes in room temperature. (Figure 7)

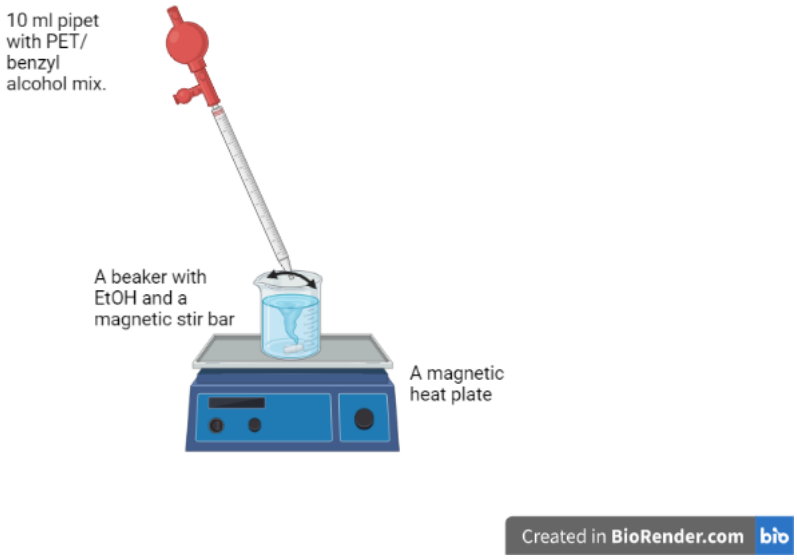

Figure 7 PET precipitation

The washing and resuspension steps were exactly the same as in PP nanoprecipitation (section 2.2.2. PP nanoprecipitation

2.2.4. Fluorescent labelling

Precipitation also allows the use of fluorescent labels, which can be used to tag and track the produced NPs. In this study the polymeric fluorophore Poly(9,9-dioctylfluorene-alt-benzothiadiazole) (F8BT) was used. The dye (2mg) was first solubilised in xylol or benzyl alcohol (about 2 mL) and then it was added to the PP/xylol or PET/benzyl alcohol mixture after complete solubilisation of the PP or PET pellets. The mixture was then connected to the reflux condenser and heated for 15 more minutes. All other steps were carried out the same way as described in sections 2.2.2. PP nanoprecipitationand 2.2.3. PET **nanoprecipitation**) Because of the light sensitivity of the label, the production needs to be performed under light protection.

2.2.5. Medium change from ethanol to glycerol

Until this point, ethanol was used in all the stages of production to ensure easy handling and sterility. However, ethanol is not suitable for cell culture experiments due to its cytotoxicity. The suspension medium had to be exchanged. Glycerol was found to give the suspension the required colloidal stability and extend shelf-life.

A final concentration of 40 mg solids per g suspension was targeted. Therefore the concentration of the ethanol suspension had to be determined. An Eppendorf tube (2 mL)

was weighed, suspension (1 mL) was transferred to the tube and then the tube was weighed again. The cover of the epi was then removed and the epi was connected to the rotary evaporator. The temperature was set on 40 °C and the pressure on 250 mbar. The pressure was then gradually decreased until the ethanol was completely evaporated leaving only the dried NPs powder in the epi. The tube was then weighed again and the concentration was calculated as follows;

Equation 1

$$conc\ (X)(mg/g) = \frac{(wt\ tube + powder) - wt\ tube}{(wt\ tube + susp) - wt\ tube} x1000$$

The suspension was then transferred into a pre-weighed round bottom flask (Z) and the total weight of the suspension (Y) was determined. The amount of NPs in the suspension (N) was then calculated using the following equation:

Equation 2

$$\boldsymbol{N(mg) = Y \times X}$$

The amount of glycerol (Glyc) needed to achieve a concentration of 40 mg/g was then calculated:

Equation 3

$$Glyc(g) = \frac{\mathrm{N}}{40}$$

Glycerol was warmed up to 70 °C for easier mixing and subsequently added to the flask. It was then sonicated and vortexed until glycerol was evenly mixed with the ethanolic suspension. Ethanol was then removed using a rotary evaporator. The water bath temperature was set to 40 °C and the pressure was initially reduced to 300 mbar and then gradually decreased. The theoretical final weight was calculated, to define the actual endpoint:

Equation 4

$$Supposed\ final\ weight\ (g) = wt.flask + Glyc + N$$

The flask was then weighed every 15 minutes and when the relative standard deviation (RSD) between the last three measurements was less than 1% it was assumed that the evaporation process was completed.

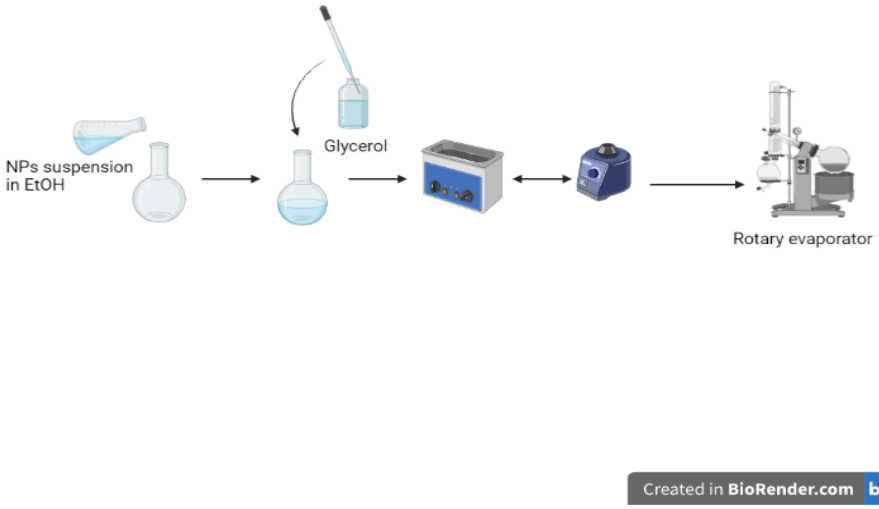

Figure 8 Medium change from ethanol to glycerol

2.2.6 Mechanical wet grinding of PP

A wet grinding procedure was developed and optimized to produce PP MPs with multiple different size ranges from 1-800 µm. The grinding process was carried out by the Knife mill GM 200 (Retsch GmbH, Haan, Germany). First, the PP pellets were pretreated as discussed in (section 2.2.1. PP pretreatment.). The obtained cuboids (20-25 g) and ethanol (40 mL) were transferred into the milling stainless steel container. Grinding was carried out in 18 cycles (**Table 2**). So the process started with 2 Hit cycles where the blades come in contact with the cuboids with the blunt side that aimed to apply high impact on the cuboids the rest of the cycles were on the cut mode where the blades come in contact with the cuboids with the sharp side with the aim of cutting the cuboids into smaller pieces. Starting from cycle 5 volume reduction was applied by using the volume reduction lid, which reduces the chamber volume, thus more contact of particles with the blade and more efficient grinding. After each grinding cycle, a pause of 2-3 minutes was necessary to avoid overheating. The walls of the container were scrapped with a spatula in between the cycles to increase contact time with the blades. Every fourth cycle the walls were also rinsed with as little ethanol as possible to avoid overfilling of the container.

To increase efficiency and reduce waste, already ground PP particles (> 250 µm) were reused. Similar to the cuboids, the MPs were frozen at -70 °C overnight and regrinded. The same procedure was applied, but the process started directly at cycle number 5 (volume reduction lid). The obtained MPs suspension was then size fractionated.

Cycle No.	Rpms	Grinding mode
1	4000	Hit
2	4000	Hit
3	5000	Cut
4	5000	Cut

5	5000	Cut
6	5000	Cut
7	6500	Cut
8	6500	Cut
9	7500	Cut
10	7500	Cut
11	8500	Cut
12	8500	Cut
13	10000	Cut
14	10000	Cut
15	10000	Cut
16	10000	Cut
17	10000	Cut
18	10000	Cut

Table 2 PP grinding cycles

Figure 9 Knife mill GM 200 (Retsch GmbH, Haan, Germany)

2.2.7. Sieving

To separate the targeted size fractions, sieving was carried out using the AS 200 sieve shaker (Retsch GmbH, Haan, Germany). A sieve tower consisting of five stainless steel sieves with different mesh sizes (38 µm, 50 µm, 100 µm, 180 µm and 250 µm) were assembled ascendingly. At the very bottom a collecting pan was used. The sieve shaker was operated in interval mode with an amplitude of 1.5 mm. The PP/ethanol suspension was transferred to the 250 µm sieve and sieved for 15 minutes. The 250 µm and the 180 µm sieves were then dried in an oven at 50 °C for 15 minutes. An additional sieving step was carried out for 15 more minutes of dry sieving. MPs of the different size ranges were collected separately. The bigger fractions (> 250 µm and 180-250 µm) were directly collected in dry state into vials. The smaller fractions (100-180 µm, 50-100 µm, 38-50 µm and < 38 µm) had to be collected with ethanol into a beaker, to overcome electro statical forces. Subsequently, the

suspensions were filtered through a 10 µm nylon mesh filter by vacuum filtration. The MPs and the filter were transferred into a vial and dried using a rotary evaporator.

Figure 10 AS 200 sieve shaker (Retsch GmbH, Haan, Germany)

2.2.8. PSD measurement

The final production step was the determination of the PSD by laser diffraction The final step in the production process was to measure the PSD of the produced MNPs and to make sure that they are within the targeted particle size range. The PSD was measured by laser diffraction using the " Malvern Mastersizer 3000". A sample of each batch was prepared using a Tween® 80 solution (5%) as a surfactant. The Tween® 80 solution (5%) was prepared by adding distilled water (20 mL) to Tween® 80 (1mL) into a flask with a magnetic stir bar and stirring at 250 rpm then vortexing until Tween® 80 was completely dissolved. To avoid particle aggregation, a sample was prepared by adding (40 µL) of the suspension to Tween® 80 (400 µL) with the ratio 1:10 suspension: Tween® 80 . For NP suspensions in glycerol, a much higher dilution was required (up to 1:40, suspension: Tween®). Dry powders of MPs were prepared by adding about 200 µL of ethanol (96%, v/v) to a spatula tip of powder, followed by 800 µL of a Tween® 80 solution (5%). The samples were then sonicated and vortexed to ensure a well dispersed sample. The measurement cell of the device (HydroSV) was then filled with particle free water (prepared by passing water through a membrane filter (0.1-1 µm) to trap all the impurities in the water) and 400 µL Tween 80® 5%. Measurement settings are described in **Table 3** .After the background measurement, sample was added until an obscuration of 4-6% in case of NPs and 10-12% in case of MPs was reached. The PSD was then measured 10 times and the average PSD was reported.

Sample	Particle type	Material	Dispersant
PP-NP	spherical	PP	water
PET-NP	spherical	PET	water
Milled PP	Non-spherical	PP	water
Milled PET	Non-spherical	PET	water

Table 3 Mastersizer® 3000 adjustments for PP and PET MNPs measurement.

3. Results and discussion

The scope of this thesis was the development and optimization of the production procedures of MNPs, to achieve specific particle size ranges. Two polymer types (PP and PET) with vast differences in chemical and physical properties were chosen. Each procedure and polymer needed optimization at various points, to achieve the required quality and reproducibility.

3.1. Optimization of procedures

A first protocol was developed based on the work of Achilias et al. [52] and Lee et al. [53]. Neither of these methods alone was suitable to produce PP and PET NPs with a particle size range between 0.1-1 µm. Particles produced according to the original protocols resulted in larger particle sizes, hence optimization was needed. Furthermore, the lack of reproducibility and stability studies needed to be addressed.

At first, critical parameters were identified. It was hypothesized that the solubilisation temperature and time, the precipitation technique and temperature, as well as the ratios of solvent to nonsolvent and solvent to polymer mass may have the greatest influence on the PSD. Because studying all these factors would go beyond the time scale of a masters project, five of those factors were considered and optimized for PP (Table 4) and three for PET (**Table 5**).

3.1.1. Optimization of PP nanoprecipitation

PP is a hydrophobic polymer, since it is nonpolar with high log P value(the logarithmic function of the partition coefficient of octanol in water, that is used to measure the degree of hydrophobicity of substances) and since its chemical structure has only carbon and hydrogen atoms with no oxygen atoms($(C_3H_6)_n$) which makes it highly hydrophobic in comparison to for example ethanol(C_2H_6O) or PET($(C_{10}H_8O_4)_n$), therefore the described pretreatment (section 2.2.1. PP pretreatment.) plays a crucial role in obtaining nano sized particles. Other factors as shown in **Error! Reference source not found.** were also optimized in previous work made by our team to obtain the required results. [52], [53] .

	Literature	Optimization
Starting material	PP pellets	Pretreated PP cuboids
Solubilisation temperature	110-140 °C	185 °C
Precipitation technique	Single pipetting	Double pipetting
Precipitation temperature	Room temperature	Ice bath
Stirring time	3 hours	Few minutes

Table 4 Comparison between PP nanoprecipitation method in literature and after optimization

Figure 11 PSD of two PP NPs batches before and after optimization of procedures Shows the difference between two PP batches before and after optimization. The PSD in the nano range of the unoptimized batch was much lower (smaller curve) than that of the optimized batch, while the PSD in the micro range was much higher. Also as shown in **Table 5** The Dx 50 of the unoptimized batch was out of the required particle size range while that of the optimized batch was within the range and Dx 90 in the unoptimized batch showed much bigger particle sizes than that of the optimized

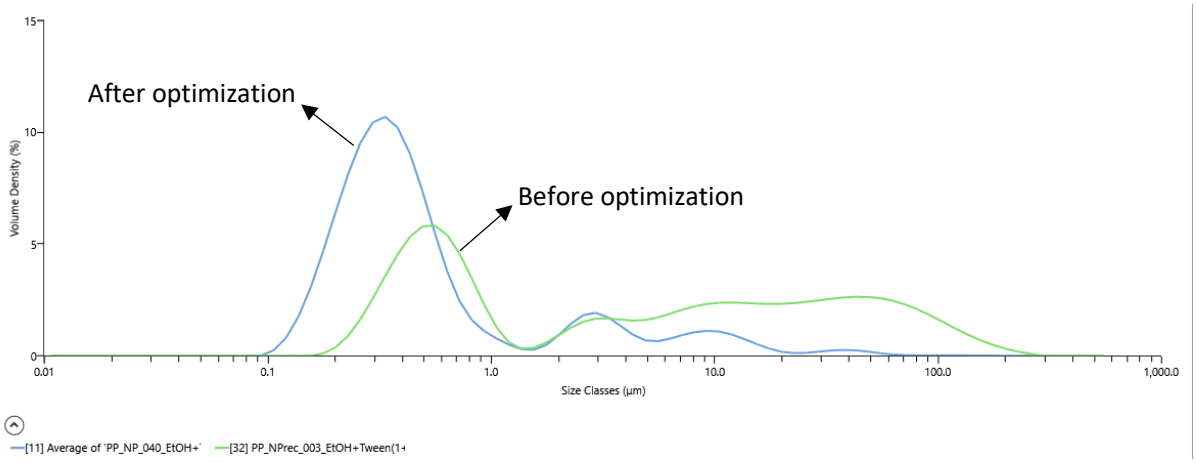

Figure 11 PSD of two PP NPs batches before and after optimization of procedures

	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
Before optimization	0.387	4.70	65.9
After optimization	0.194	0.375	3.87

Table 5 PSD of two PP NPs batches before and after optimization of procedures

Based on previous findings, a trial was set up to produce different PP NP batches with different solubilisation temperatures and pretreatment conditions. So, a batch with pellets solubilised at 135°C, a batch with cuboids pretreated for 3 hours and solubilised at 185 °C, a batch with cuboids pretreated for only one hour and solubilised at 135 °C and a batch with pellets solubilised at 185 °C were prepared and measured. As shown in Figure 12 only the batch produced by the optimized solubilisation temperature (185°C) and pretreated PP cuboids (3 hours) gave a large peak in the nano fraction, while the other batches gave a reduced nano fraction and wide peaks in the micro meter range. Also as shown in **Table 6** only the Dx 50 of the non-optimized procedures was obviously out of the required particle size range. This could be explained through increasing the hydrophilicity of PP by thermal oxidation, thus better resuspension of the produced NPs in ethanol or water [54][55].

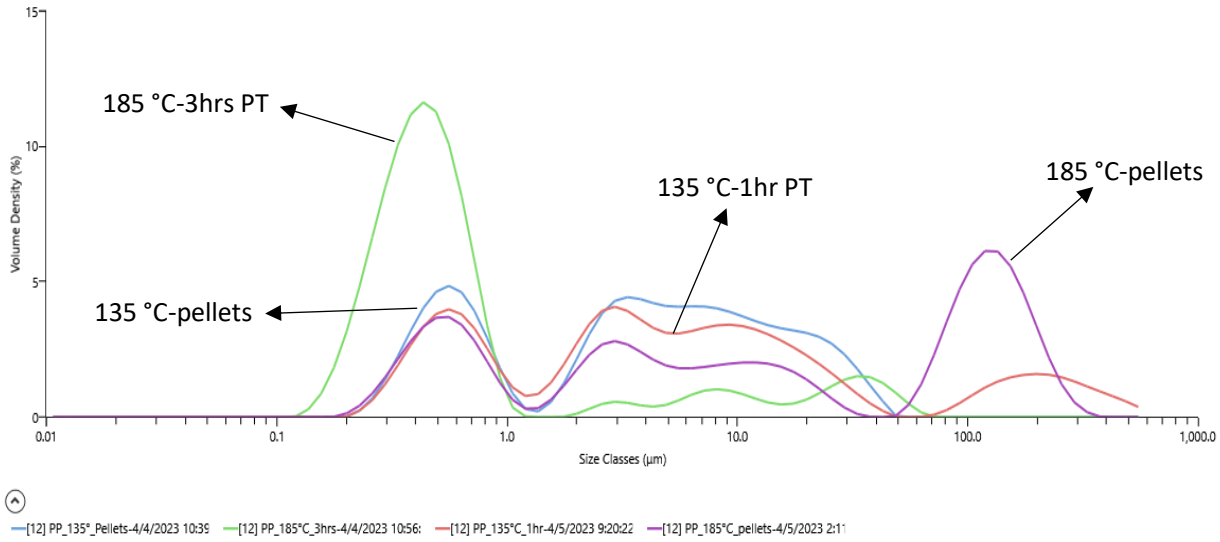

Figure 12 PSD of PP NPs produced by nanoprecipitation at different pretreatment conditions and solubilisation temperature.

Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
PP_135°C_1hr	0.493	4.59	154
PP_185°C_3hrs	0.249	0.466	11.2
PP_135°_Pellets	0.464	4.06	20.8
PP_185°C_pellets	0.474	10.4	164

Table 6 PSD of PP NPs produced by nanoprecipitation at different pretreatment conditions and solubilisation temperature.

3.1.2. PET nanoprecipitation

Similar to PP, the protocol developed for PET nanoprecipitation also had to be optimized.[52], [53]

	Literature	Optimization
Solubilisation temperature	180 °C	220 °C
Solubilisation time	30 minutes	45 minutes
Stirring time	3 hours	30 minutes

Table 7 Comparison between PET nanoprecipitation method in literature and after optimization.

Figure 13 shows the difference between two PET batches before and after optimization. The PSD in the nano range of the unoptimized batch was much lower than that of the optimized batch, while the PSD in the micro range was much higher. Also as shown in Table 8 The Dx 50 of both batches was within the required range but the Dx 90 of the unoptimized batch was much higher than the required range.

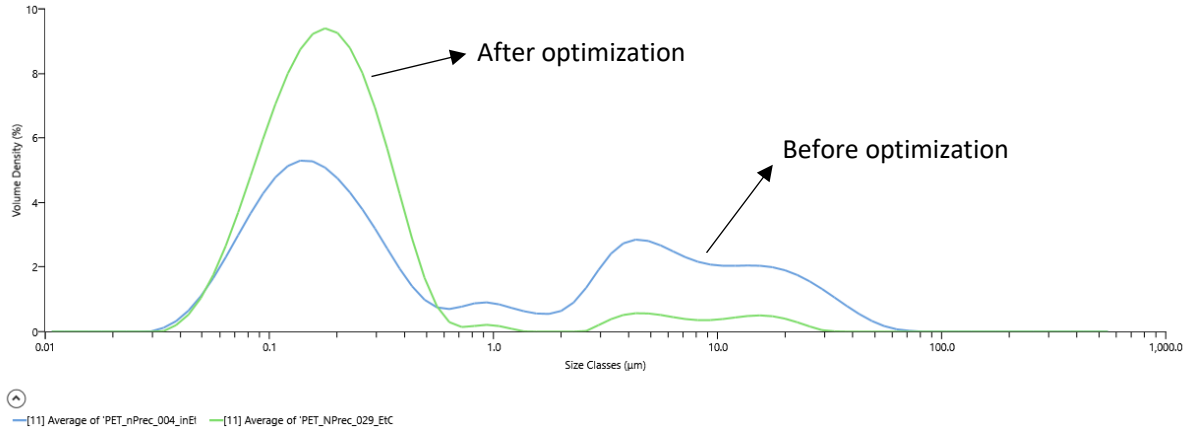

Figure 13 PSD of two PET NPs batches before and after optimization of procedures

Sample	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
Before optimization	0.0853	0.334	15.9
After optimization	0.0817	0.181	0.440

Table 8 PSD of two PET NPs batches before and after optimization of procedures

3.1.3. PSD measurement

Validation of the PSD is an integral part of material production to assure that the targeted values are reached. Experimenters often do not have the instrumentation and rely on the given specifications. Sample preparation is a key step during the validation process. Measurements via laser diffraction cannot differentiate between aggregates and the actual size of particles. Therefore, the preparation technique was revised.

It was found that the ratio of suspension to tween was an important point, especially when preparing NP samples. Figure 14 shows two measurements of the same sample. When a ratio of 1:5 was used (green curve), a reduced nano fraction and multiple peaks in the micro meter range could be observed. However, by preparing the sample again with increased Tween® 80 concentration (1:10) the peaks above one micro meter disappeared and the submicron peak elevated. This indicated that larger peaks were aggregated particles and the production was successful. A ratio of tween to suspension between 10:1 up to 40:1 was required.

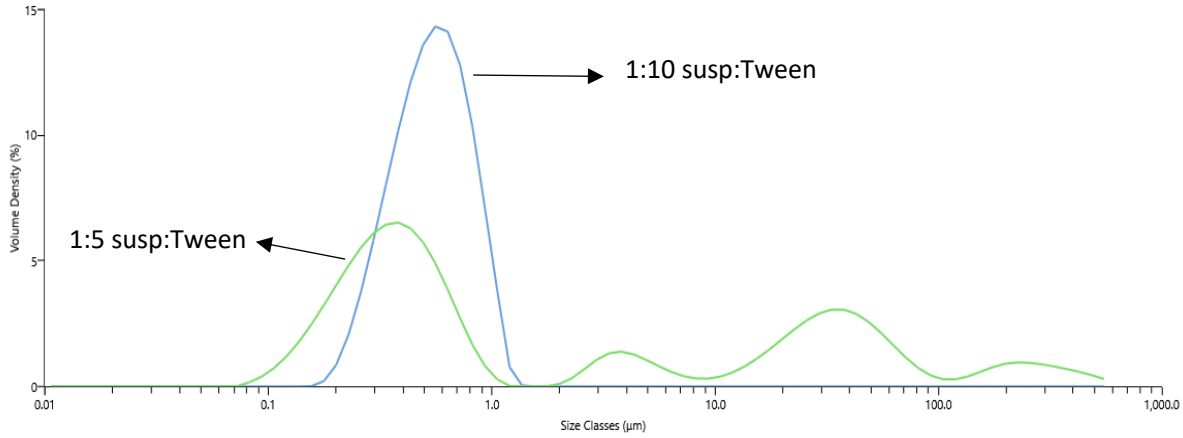

Figure 14 PSD of two PET NPs samples of the same batch prepared by different suspension :tween ratios.

It was also observed that freshly prepared Tween 80® solution led to better dispersions. Figure 15 compares two measurements of the same sample. For the PSD of the sample prepared with a tween solution prepared a week before (blue curve), a large PSD was observed in the micro meter range, however when the sample was prepared with fresh tween solution (green curve) the bigger curve was observed in the nano range and the PSD in the micro range was remarkably minimised.

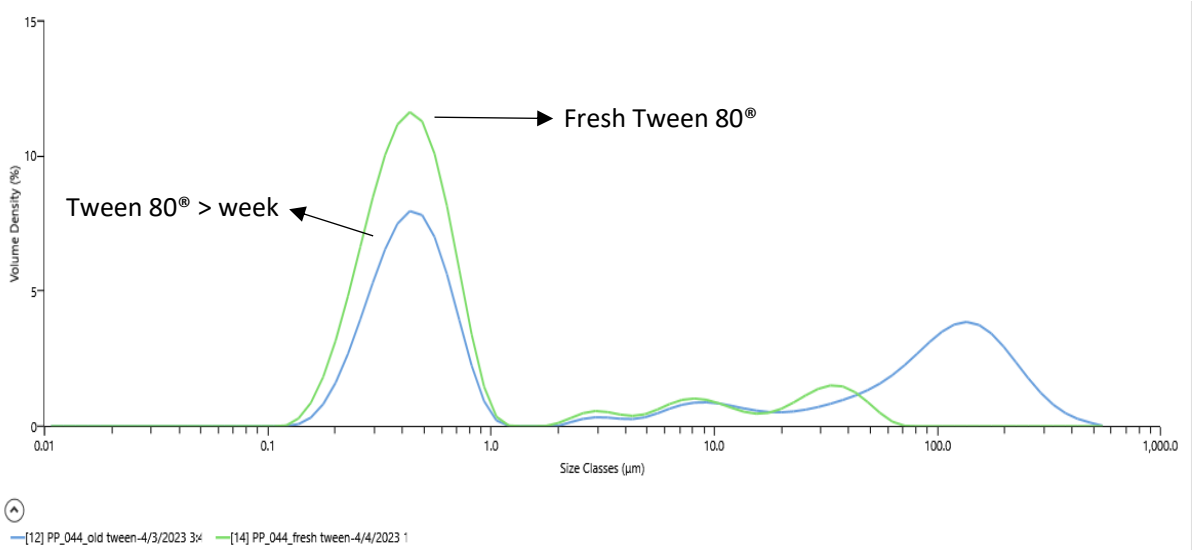

Figure 15 PSD of two PP samples of the same batch prepared by old and fresh tween

Another crucial point that had a great influence on the obtained results was the sample sonication. For sonication an ultrasound water bath sonicator with frequency 37 kHz was used (Elmasonic P ultrasound bath (Elma Schmidbauer GmbH, Singen, Germany)). Since NPs can't be seen by naked eyes, the samples should be sonicated until no more aggregates could be seen in the vial. As shown in Figure 16, the sample prepared by just leaving the sample in the ultrasound bath for 10 minutes (green curve) gave almost no PSD in the nano

range, however when the sample was held in the ultrasound bath with an angle that ensured that maximum energy is coming through the solution until no more particles could be seen (blue curve),a large peak appeared in the nano range and the curve in the micro range almost disappeared.

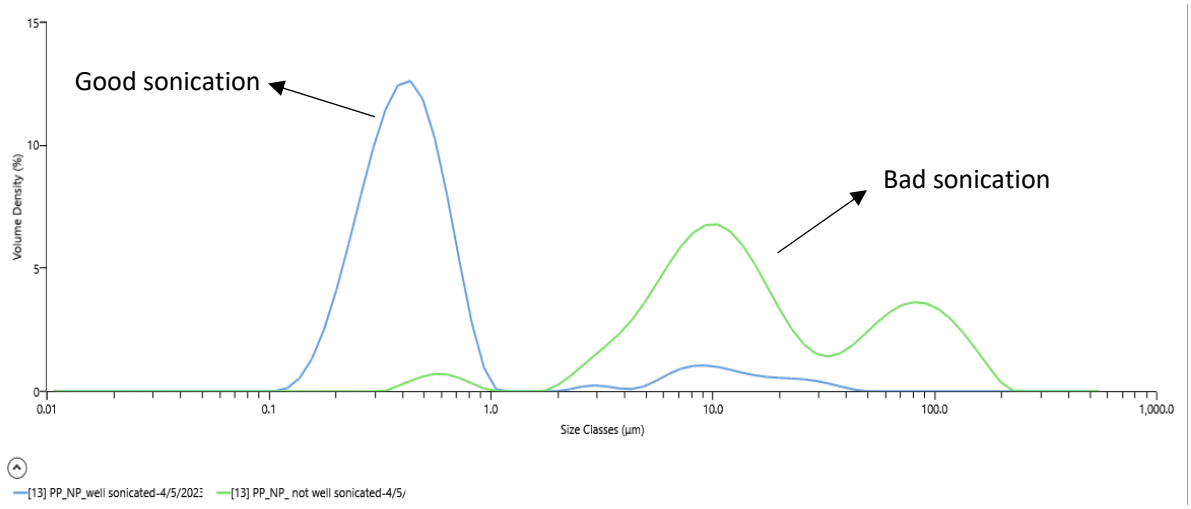

Figure 16 PSD of two PP NPs samples of the same batch with different sonication levels

3.2. Reproducibility

Reproducibility is the ability of the procedures to produce the same results when carried out under the same conditions. A method is considered to be reproducible if at least 3 similar results were obtained by carrying out the same procedures under similar conditions. So, after procedures optimization the reproducibility was assessed by repeating the optimized procedures and measuring the results to approve the reliability of methods.

3.2.1. Nanoprecipitation

Reproducibility of nanoprecipitation was tested by measuring and comparing PSDs of the produced batches. Figure 17 shows the the PSD of 8 batches of PP-NPs and all batches showed a large peak in the nano range (0.1-1 µm) with minimal PSD in the micro meter range. **Figure** 18 shows the the PSD of 5 batches of PET-NPs and also all batches showed a large peak in the nano range (0.1-1 µm) with minimal PSD in the micro meter range. Accordingly, reproducibility was successfully achieved in both PP and PET nanoprecipitation.

However, having a look at the RSD (relative standard deviation) in **Table** 9 and **Table** 10 , it appears to be high specially in PET and Dx 90 in PP. This shows low precision of the obtained results, although they are within the required range. Further optimization could be done in future work to improve the precision of results.

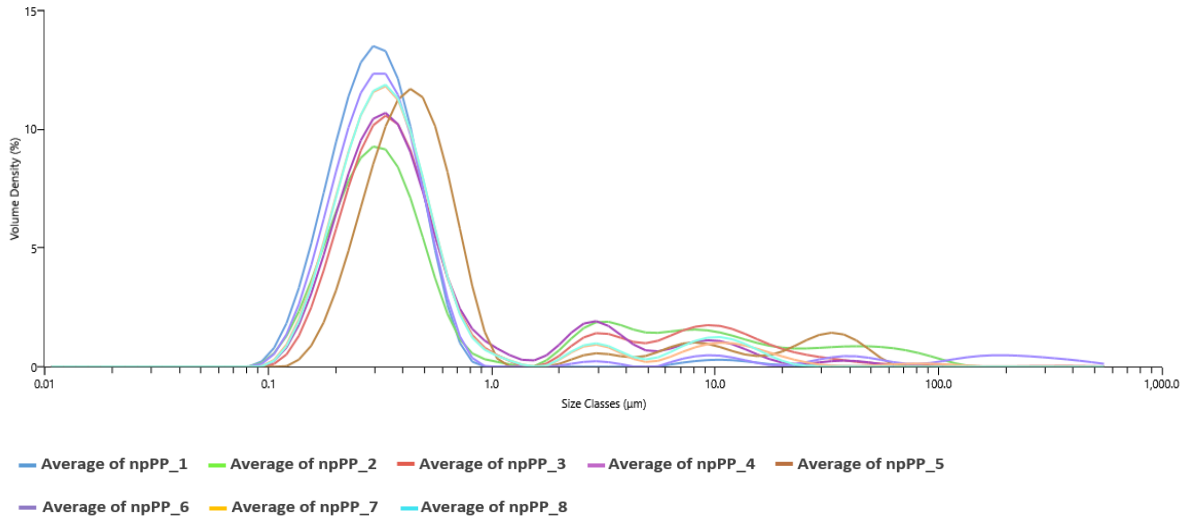

Figure 17 Average PSD of different PP NPs batches produced by the optimized nanoprecipitation method.

	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	Average of npPP_1	0.168	0.297	0.512
	Average of npPP_2	0.184	0.386	14.9
	Average of npPP_3	0.204	0.395	9.08
	Average of npPP_4	0.194	0.375	3.87
	Average of npPP_5	0.248	0.463	9.89
	Average of npPP_6	0.177	0.323	0.847
	Average of npPP_7	0.189	0.348	2.81
	Average of npPP_8	0.19	0.349	2.9
Mean		0.194	0.367	5.6
1xStd Dev		0.0242	0.0507	5.12
1RSD (%)		12.4	13.8	91.4

Table 9 Mean, SD and RSD of the average PSD of different PP NPs batches produced by the optimized nanoprecipitation method.

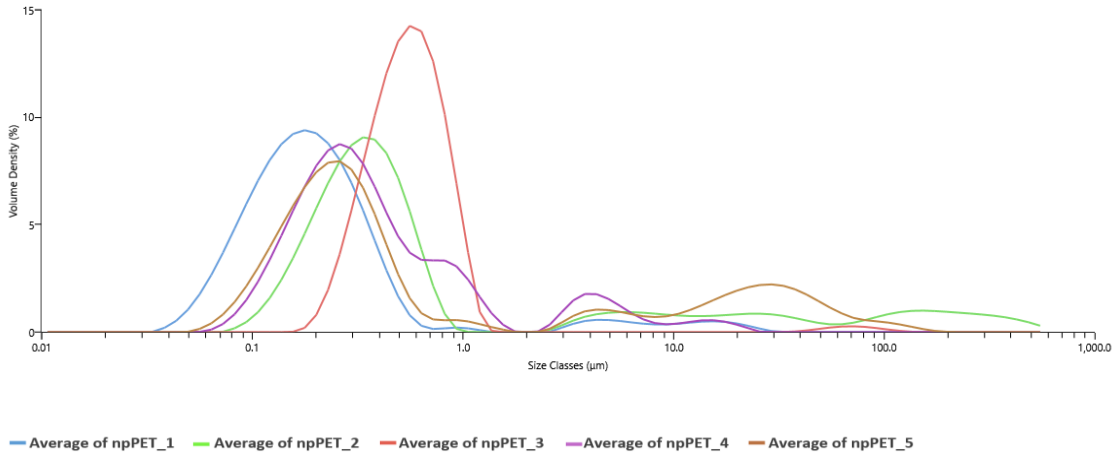

Figure 18 Average PSD of different PP NPs batches produced by the optimized nanoprecipitation method.

	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	Average of npPET_1	0.0817	0.181	0.44
	Average of npPET_2	0.181	0.399	82.3
	Average of npPET_3	0.314	0.544	0.887
	Average of npPET_4	0.142	0.319	3.42
	Average of npPET_5	0.129	0.312	31.6
Mean		0.169	0.351	23.7
1xStd Dev		0.0879	0.133	35.3
1RSD (%)		51.9	37.9	149

Table 10 Mean, SD and RSD of the average PSD of different PP NPs batches produced by the optimized nanoprecipitation method.

3.2.2. Fluorescent labelling

Besides npPP and npPET using native granules, fluorescently labelled particles were produced employing co-precipitation with the fluorescent dye F8BT. The aim of labelling NPs is to make them trackable. For example, labelled NPs will be used in investigations on cells to see if they can penetrate the cell membrane and to find the subsequent possible health impacts.

F8BT was chosen because of its high molecular weight (9-255 kg/mol) and therefore high stability through minimizing the leakage of dye from the NPs [56]. Furthermore, it has a high degree of polymerisation that ranges between ~6 to ~163 repeated units per chain, which leads to a higher fluorescent power. Also, its stokes shift (79nm), excitation wavelength (466 nm) and emission wavelength (576 nm) [57]are within the range most instruments can detect. F8BT is also soluble in the 2 solvents used for nanoprecipitation (xylol and benzyl alcohol) which makes it easier to be mixed with the solubilised NPs. These properties make this fluorophore a promising candidate for labelling polymers like PP and PET.

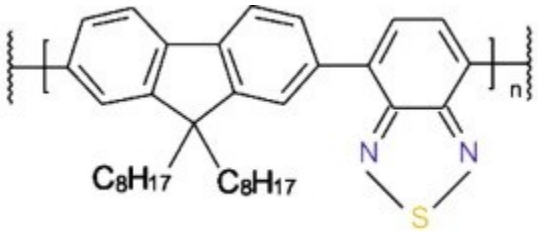

Figure 19 F8BT polymer molecule

Another step was added to produce fluorescently labelled NPs by adding the fluorescent dye F8BT. As shown in Figure 20, the PSD of 4 batches of PP-NPs prepared by co-precipitation with F8BT was measured and all batches gave a large peak in the nano range with minimal PSD in the micro range, thus reproducibility of fluorescent labelling was also achieved.

Similar to unlabelled batches the RSD shown in Table 11 was so high which means the results show big deviation from each other.

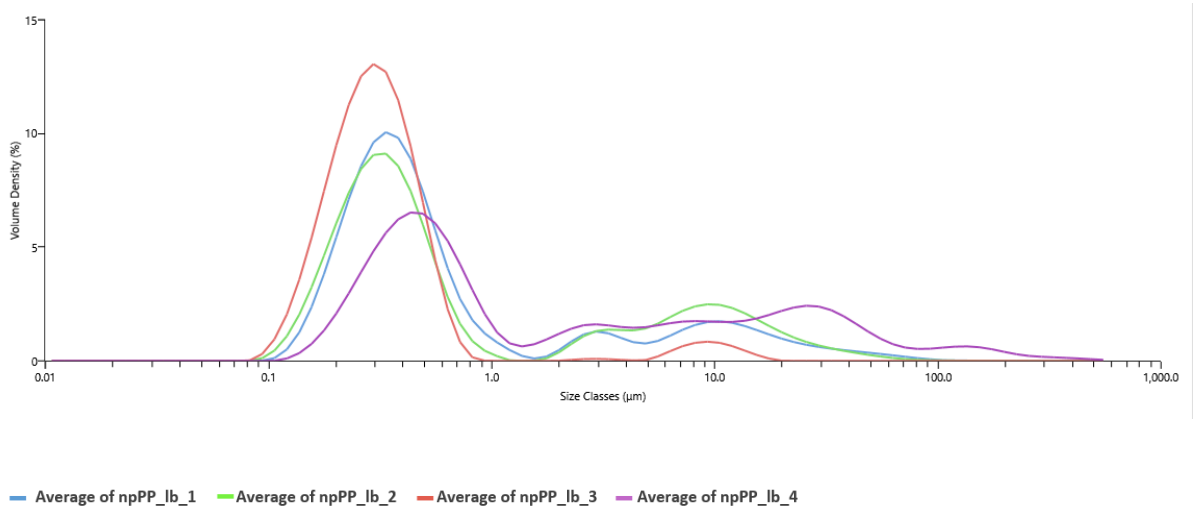

Figure 20 Average PSD of different PP NPs fluorescently labelled batches produced by the optimized nanoprecipitation method.

	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	Average of npPP_lb_1	0.191	0.403	12.3
	Average of npPP_lb_2	0.207	0.412	10.9
	Average of npPP_lb_3	0.165	0.296	0.54
	Average of npPP_lb_4	0.281	0.873	37.2
Mean		0.207	0.478	14.6
1xStd Dev		0.0439	0.226	13.5
1RSD (%)		21.2	47.4	92.4

Table 11 Mean, SD and RSD of the average PSD of different PP NPs fluorescently labelled batches produced by the optimized nanoprecipitation method.

3.2.3. Medium change to glycerol

Exchange of the dispersion medium from ethanol to glycerol was the final step. This was necessary to achieve colloidal stability, ensure redispersability and to provide test materials suitable for cell culture experiments. As shown in Figure 21 and Figure 22 The PSD of 4 PP-NPs batches and 4 PET-NPs batches redispersed in 40 mg/g glycerol was measured and all batches gave a large peak in the nano range with minimal PSD in the micro range. Accordingly, reproducibility of medium change was also successfully achieved for both PP and PET.

Also, here the RSD is high specially by PET and Dx 90 in PP (Table 12 and Table 13), which may require further optimization of procedures to be improved.

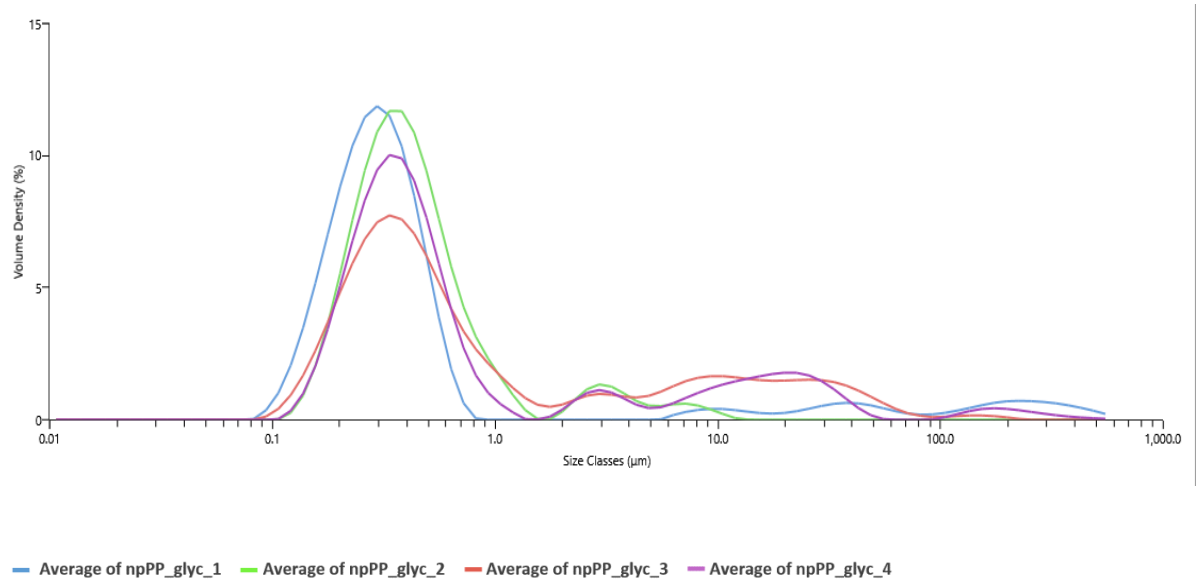

Figure 21 Average PSD of different PP NPs batches produced by the optimized nanoprecipitation method after medium change to 40mg/g glycerol.

	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	Average of npPP_glyc_1	0.166	0.308	23.6
	Average of npPP_glyc_2	0.213	0.389	1.06
	Average of npPP_glyc_3	0.202	0.49	20.4
	Average of npPP_glyc_4	0.215	0.426	19.4
Mean		0.199	0.403	16.1
1xStd Dev		0.0228	0.0759	10.2
1RSD (%)		11.4	18.8	63.3

Table 12 Mean, SD and RSD of the average PSD of different PP NPs batches produced by the optimized nanoprecipitation method after medium change to 40mg/g glycerol.

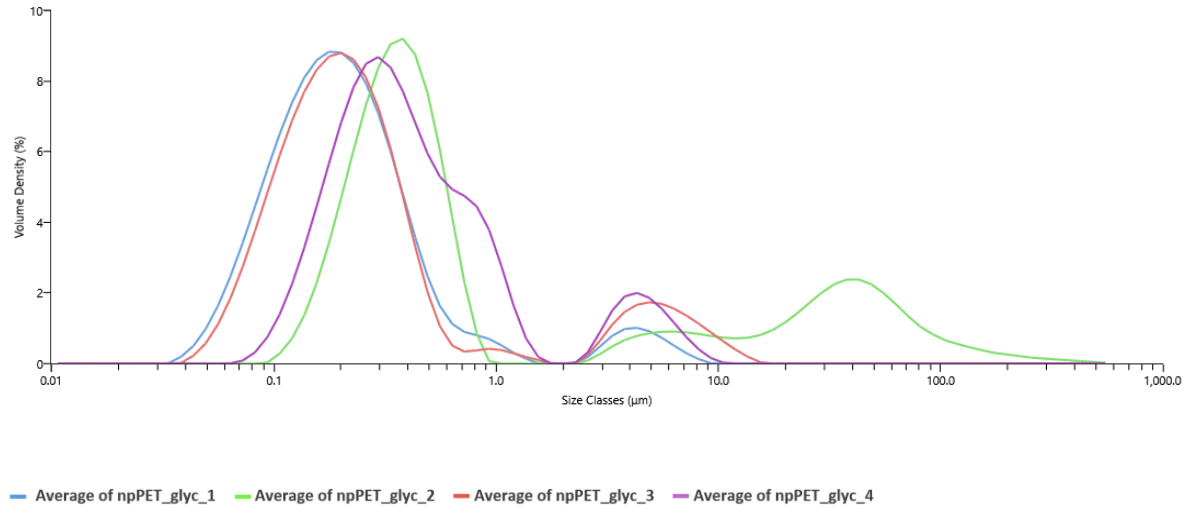

Figure 22 Average PSD of different PET NPs batches produced by the optimized nanoprecipitation method after medium change to 40mg/g glycerol.

	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	Average of npPET_glyc_1	0.0837	0.192	0.529
	Average of npPET_glyc_2	0.21	0.45	47.3
	Average of npPET_glyc_3	0.0919	0.21	3.8
	Average of npPET_glyc_4	0.164	0.366	2.87
Mean		0.137	0.305	13.6
1xStd Dev		0.0605	0.124	22.5
1RSD (%)		44	40.9	165

Table 13 Mean, SD and RSD of the average PSD of different PET NPs batches produced by the optimized nanoprecipitation method after medium change to 40mg/g glycerol.

3.3. Mechanical wet grinding of PP

For the production of MPs an alternative process had to be employed. The challenge was to obtain enough of the targeted size ranges. Furthermore, preserving the chemical and physical properties by avoiding thermal degradation during the grinding process. As a top down method, mechanical wet grinding simulates the natural degradation of plastics in the environment [58]. Wet grinding was preferred to dry grinding, since ethanol acts as a dispersant that prevents agglomeration and enables grinding to fine powder which can't be achieved through dry grinding. [59]

For optimal results, PP pellets were pretreated as described in section 2.2.1. PP pretreatment. This resulted in equally sized cuboids for grinding uniformity and increased contact time with the blades. Cryogenic treatment of the cuboids was found to increase its brittleness and breakability, thus more efficient grinding and higher yield. [60].

Heat leads to changes in the chemical and physical properties of polymers and is known as thermal degradation. Processes like oxidation and decrease in crystallinity effect [61] and also leads to, which doesn't act in favour of the further investigations made by our project partners,

It was observed that the amount of ethanol added between the cycles for rinsing strongly influenced the produced heat during milling. Accordingly, the frequency and amount of ethanol used was reduced to a minimum.

Additionally, refreezing the preground MPs (>250 µm) and regrinding them using the protocol described in section 2.2.6 Mechanical wet grinding of PP led to increased yield compared to using cuboids (this was based on only personal observation, but unfortunately obtaining solid data was beyond the You scope of this thesis). Testing the reproducibility of this process was beyond the scope of this thesis. Further future studies are needed to improve economics, yield and reduce the production time.

MPs test materials were successfully produced through mechanical wet grinding and after isolating the targeted size ranges (section 2.2.7. Sieving, PSDs were measured by laser diffraction and microscopic imaging. We targeted 4 size ranges; 38-50, 50-100, 100-180, 180-250 µm and as shown in **Figure** 23, **Figure** 24, **Figure** 25 and **Figure** 26 and **Table** 14, the PSD of

the isolated size fractions 38-50 and 50-100 µm was within the targeted size range. For the PSD of the fraction 100-180 µm, Dx 50 was lower than the targeted range. The particles 180-250 showed a slightly higher Dx 50 than the required range. However, the microscopic images of the isolated Particle sizes in **Figure** 28, Figure 27 and **Figure** 29 matched the targeted size ranges.

So further optimization of grinding procedures could be done in the future to achieve more accurate PSD for the required particle size ranges.

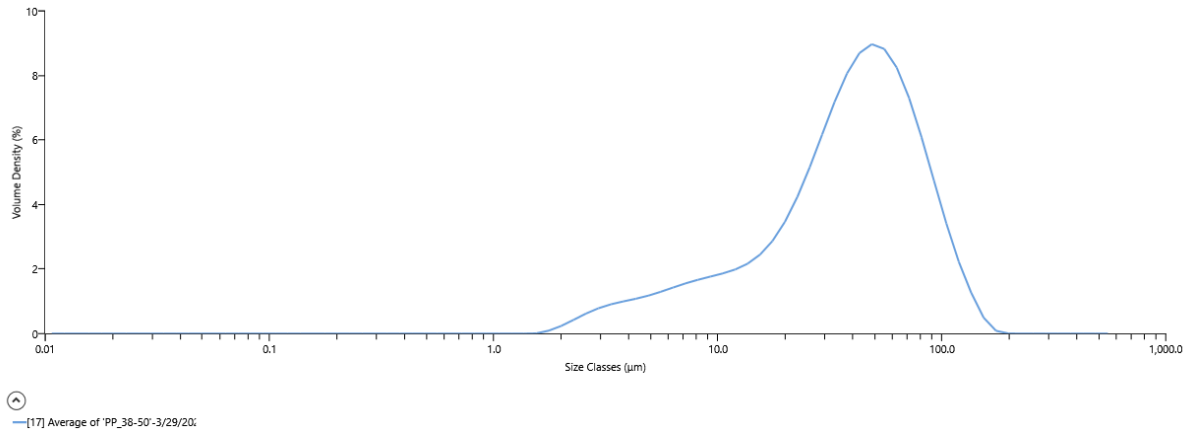

Figure 23 Average of PSD of PP MPs with particle size range between 38-50 µm produced by mechanical wet grinding

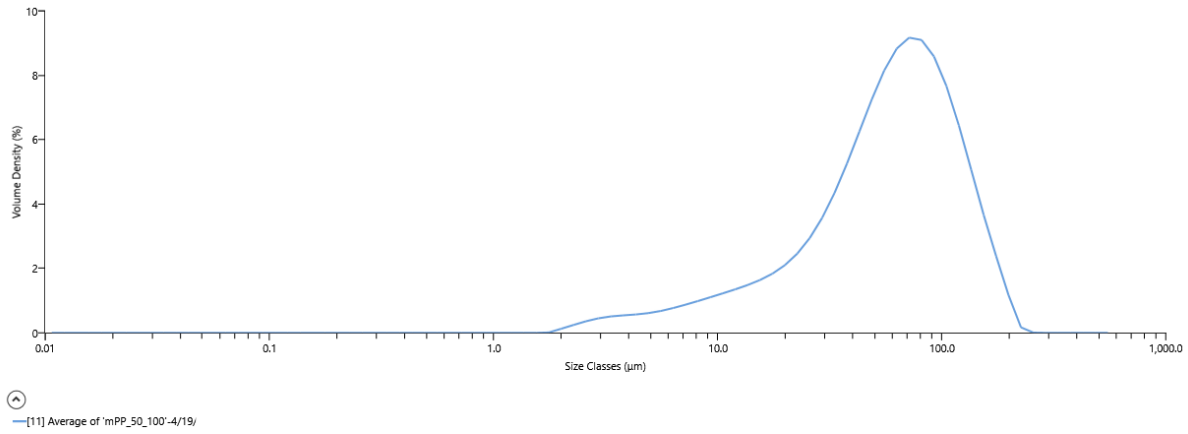

Figure 24 Average of PSD of PP MPs with particle size range between 50-100 µm produced by mechanical wet grinding

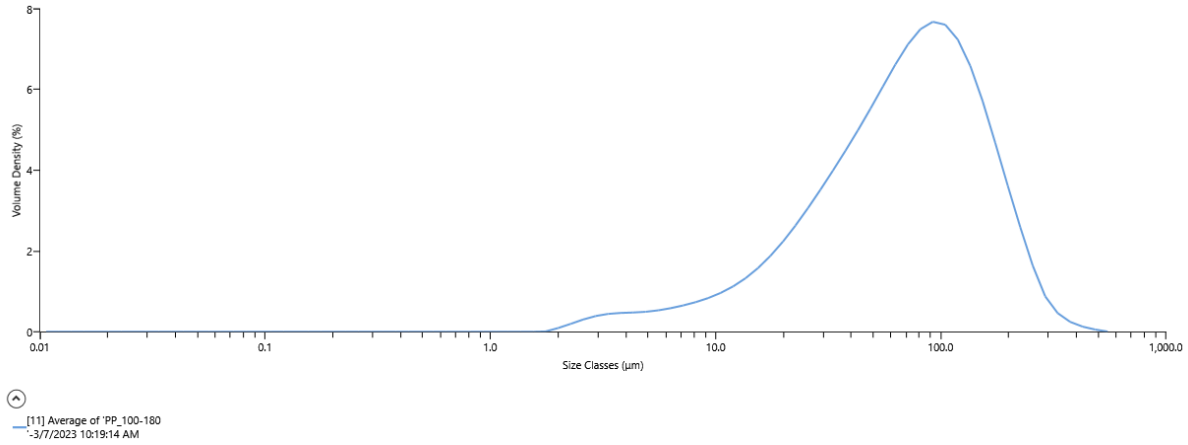

Figure 25 Average of PSD of PP MPs with particle size range between 100-180 µm produced by mechanical wet grinding

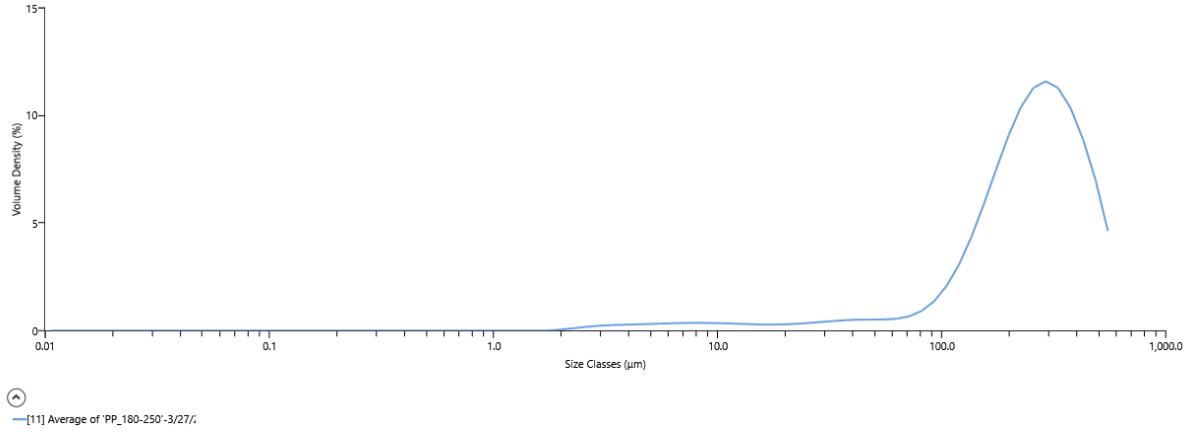

Figure 26 Average of PSD of PP MPs with particle size range between 180-250 µm produced by mechanical wet grinding

Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
Average of 'PP_38-50'	8.49	40.4	86.6
Average of 'PP_50_100'	14.6	60.9	128
Average of 'PP_100-180	17.4	71.6	174
Average of 'PP_180-250'	95.7	255	453

Table 14 Average of PSD of PP MPs with different particle size ranges produced by mechanical wet grinding

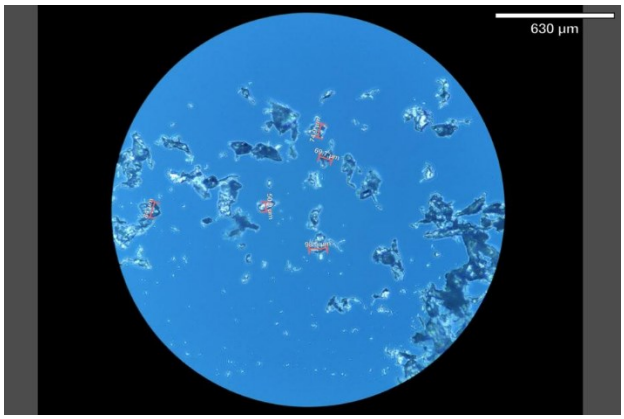

Figure 28 Microscope image of grinded PP MPs with particle size range 50-100 µm

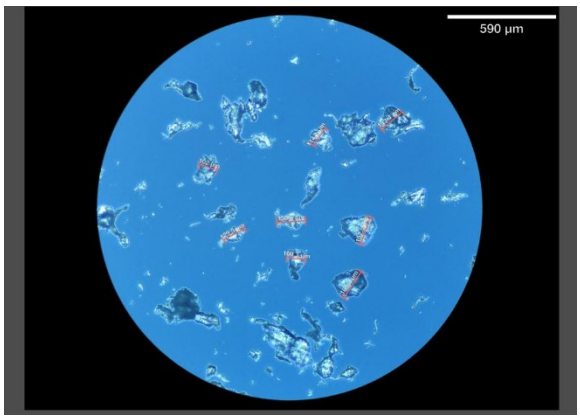

Figure 27 Microscope image of grinded PP MPs with particle size range 100-180 µm

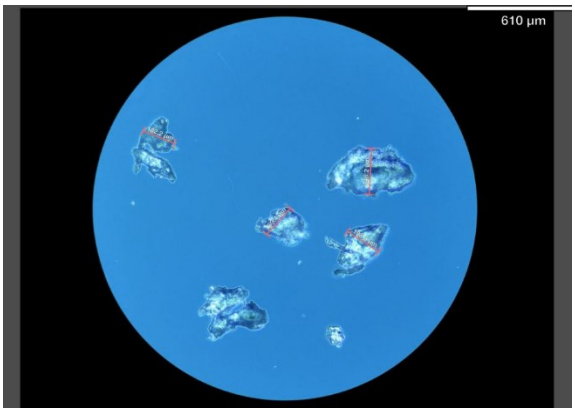

Figure 29 Microscope image of grinded PP MPs with particle size range 180-250 µm

3.4. Medium change from ethanol to glycerol

As NPs are unstable and always tend to aggregate to increase their particle size and reduce their surface energy, the suspension medium had to provide adequate colloidal stability and prevent NPs reaggregation in the first line [62]. Glycerol was found to be the most suitable substitute to ethanol due to its high viscosity (15 poise) compared to ethanol(0.011 poise) and water(0.01002 poise), thus providing the required colloidal stability and also due to its suitability for cell culture. Also, an advantage of glycerol is the big difference in boiling points between ethanol (78 °C) and glycerol (290 °C), which makes it easier to evaporate ethanol from the NPs/glycerol/ethanol mixture by the rotary evaporator during the medium change process leaving the NPs suspended only in glycerol. Another important advantage of glycerol is, that it preserves the PSD after the medium change. As shown if **Figure** 30, the PSD of a PP-NPs batch before (blue curve) and after (green curve) medium change is almost the same with a large peak in the nano range (0.1-1 µm) and minimal PSD in the micro range.

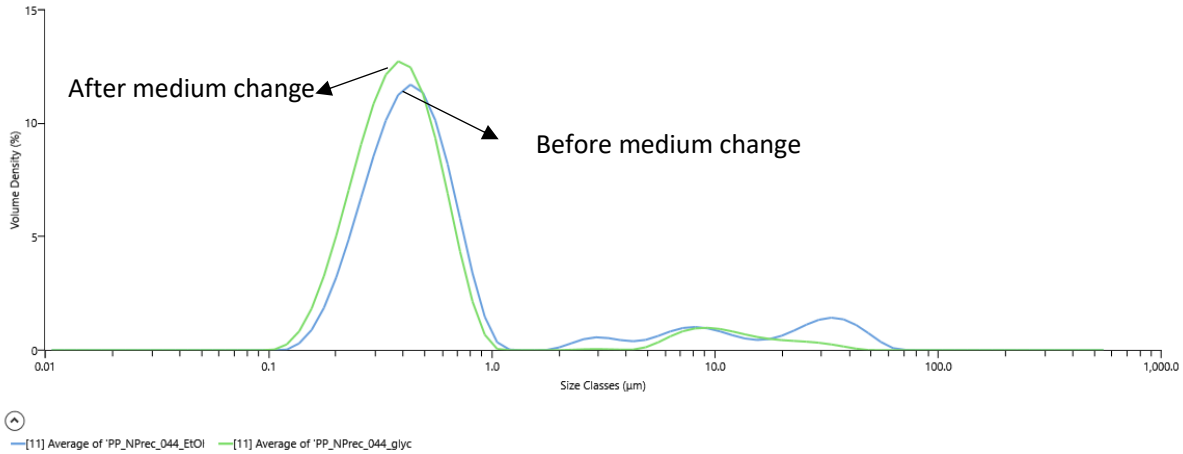

Figure 30 PSD of a PP-NPs batch before and after medium change to 40 mg/g glycerol

4. Conclusion

MNPs are considered as one of the most widely spread environmental pollutants found in almost all environmental compartments. Unfortunately, there is a very few commercially available MNPs test materials. This is why one part of the Imptox project aims to produce MNPs as test materials to be used for toxicological examination.

In this thesis two MNPs manufacturing methods to Produce MNPs with particle sizes ranging between 0.1-1 µm and 1-800 µm were discussed.

Through optimization of manufacturing procedures, it was achieve to reproducibly produce PP and PET NPs with particle size range of 0.1-1 µm through nanoprecipitation. For best results, PP had to be pretreated by melting at 180 °C for 3 hours. This improved its hydrophilicity through oxidation [63]. Ethanol was used in all stages of production not only because is provides a good dispersion medium and helps avoiding aggregation, but also due to its preservative and antimicrobial function. However, to provide a cell culture suitable medium and to achieve colloidal stability, ethanol was substituted by glycerol as a dispersant for the final product.

Furthermore, PP in the micron size range were successfully produced through mechanical wet grinding. Optimal results were obtained by pretreatment of the PP pellets by melting at 180 °C for one hour. Subsequent overnight freezing at -70 °C increased the brittleness and breakability of the particles. Sieving was performed to separate the targeted particle size ranges. Additionally, based on only personal observations, batches produced by refreezing and regrinding of preground but too big MPs (> 250 µm), which was found to increase yields and reduce waste. Testing the reproducibility of this method was beyond the scope this thesis.

Although some questions could be answered within this thesis, there is still a lot to be investigated in further studies. Development and optimization of a manufacturing method for the production of MPs with particle size range between 1-10 µm is still an unsolved challenge.

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References

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