Well defined nanoparticles through controlled non-equilibrium micellization of block copolymers

Line Trosterud Resvold

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Faculty of Mathematics and Natural Sciences
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Well defined nanoparticles through controlled non-equilibrium micellization of block copolymers

by
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Abstract

The aim of this work is to investigate if size and morphology of nanoparticles can be controlled by changing the hydrophilic ratio of the amphiphilic polymers, changing polymer concentration or simply by changing the preparation method of the particles. An amphiphilic diblock, methoxy poly(ethylene oxide)-block-polycaprolactone (PEO-PCL), with various lengths of the hydrophobic block was utilized. For accurate control of the preparation, a stopped flow apparatus (SFA) was used. The controlled co-solvent method was used for preparation of the final micelles with THF as the organic solvent. Therefore it is also natural to investigate how remaining solvent, specifically in the core, could affect the micelle, and to develop a good method for gently and effective removal of the organic solvent.

Small angle X-ray scattering (SAXS) was used to measure size, aggregation number, amount of solvent in the core and radius of the micelles. The SAXS analysis also required complementary measurements of density, size, hydrophilic ratio, molecular weights, polydispersity and crystallinity. In order to obtain a complement and facilitate a thorough SAXS analysis, other measurements were performed using nuclear magnetic resonance (NMR), dynamic light scattering (DLS), densitometry, differential scanning calorimetry (DSC), and gel permeation chromatography (GPC).

For the difference in polymer concentrations, 1wt% and 0.5wt%, no structural differences are observed, but by changing the block lengths of PCL from 2kDa to 4kDa an increased size and aggregation number were found. No crystallization was detected by DSC or density measurement; hence the core of the micelle in this work is amorphous.

For the polymer with 2kDa PCL length, no structural effects were seen as the mixing rate was increased, hence the micelle is dynamic and in equilibrium. However, for the polymer with 4kDa PCL length, a structural change was observed for increasing mixing rate. The length of the hydrophobic block is casing the energy barrier for unimer release so high that the micelles will not gain equilibrium through unimer exchange. The micelles are in a frozen, non-equilibrium state.
Acknowledgement

Firstly I would like to thank my supervisor, Bo Nyström, for his patience and for sharing his knowledge. For all the interesting discussions we had and for your kind support. I also would like to express my deep gratitude and respect to my co-supervisor Dr. Reidar Lund, whose guide and support has been essential for the completion of this thesis. Thank you for all your help with the SAXS analyses, encouraging me through hard periods, for the fantastic, inspiring visits to ESRF in Grenoble and so much more.

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Thank you Bente Amalie Breiby for running my samples on DSC even in stressful periods, and thanks to Antje Hofgaard for the training and guiding during TEM analysis. I am also very grateful for being in this research group with all the interesting, inspiring and nice people. During my time of studies here at UiO I have meet some really fantastic people, thank you for all the good laughs and talks, and for your friendships that will last long after finishing my times of studying here at UiO.

I want to thank my parents for all your support and encouragement, and also for stepping in as babysitters. Also I would like to thank my sister for always listening patiently to my frustrations and joys during my time of studying. A special thanks to all of my friends that have cheered on me, you are fantastic.

But my deepest gratitude goes to the people in the word that I love the most; my husband and my son. My lovely son helped me through this stressful period of time in his way, nothing cheers you up like a big hug and the words “I love you mommy”. And my biggest thanks are for my husband, Joakim, for helping me so much, being so patient and for being the best dad in the world. I love you.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>A(Q)</td>
<td>scattering amplitude</td>
</tr>
<tr>
<td>a₀</td>
<td>area of the hydrophilic block on the interface</td>
</tr>
<tr>
<td>A₂</td>
<td>second virial coefficient</td>
</tr>
<tr>
<td>Aₕ</td>
<td>amplitude of the fast mode</td>
</tr>
<tr>
<td>Aₛ</td>
<td>amplitude of the slow mode</td>
</tr>
<tr>
<td>bᵢ</td>
<td>scattering length</td>
</tr>
<tr>
<td>C</td>
<td>polymer concentration</td>
</tr>
<tr>
<td>Cᵣ</td>
<td>heat capacity</td>
</tr>
<tr>
<td>Cₛ</td>
<td>cluster scale factor</td>
</tr>
<tr>
<td>cₛolute</td>
<td>weight fraction of polymer in the solvent</td>
</tr>
<tr>
<td>CWC</td>
<td>critical water content</td>
</tr>
<tr>
<td>D</td>
<td>diffusion coefficient</td>
</tr>
<tr>
<td>d</td>
<td>density</td>
</tr>
<tr>
<td>dᵥ</td>
<td>fractal dimension</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO-d6</td>
<td>deuterated dimethyl sulfoxide</td>
</tr>
<tr>
<td>dn/dc</td>
<td>refractive index increment</td>
</tr>
<tr>
<td>dR</td>
<td>corona thickness</td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimetry</td>
</tr>
<tr>
<td>dΣ/dΩ</td>
<td>differential scattering cross-section</td>
</tr>
<tr>
<td>F(Q)_blob</td>
<td>free energy, blob scattering</td>
</tr>
<tr>
<td>fₙ</td>
<td>hydrophobic fraction</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>f_PEO</td>
<td>fraction of hydrophilic (PEO) block</td>
</tr>
<tr>
<td>g¹(t)</td>
<td>first order electric field autocorrelation</td>
</tr>
<tr>
<td>g²(Q,t)</td>
<td>second order intensity autocorrelation</td>
</tr>
<tr>
<td>GPC</td>
<td>gel permeation chromatography</td>
</tr>
<tr>
<td>I(Q)</td>
<td>scattering intensity</td>
</tr>
<tr>
<td>I_b</td>
<td>NMR integral from ethyl group from PEO</td>
</tr>
<tr>
<td>I_c</td>
<td>NMR integral from methyl group from PCL</td>
</tr>
<tr>
<td>k</td>
<td>numerical constant, 1.06</td>
</tr>
<tr>
<td>K⁺</td>
<td>optical constant</td>
</tr>
<tr>
<td>kₘ</td>
<td>Boltzmann constant</td>
</tr>
<tr>
<td>kᵢ</td>
<td>incident wave vector</td>
</tr>
<tr>
<td>kₛ</td>
<td>scattered wave vector</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>$l_0$</td>
<td>hydrophobic length</td>
</tr>
<tr>
<td>$l_{solvent}$</td>
<td>solvent length</td>
</tr>
<tr>
<td>$M_n$</td>
<td>number average molecular weight</td>
</tr>
<tr>
<td>$M_w$</td>
<td>weight average molecular weight</td>
</tr>
<tr>
<td>MWCO</td>
<td>molecular weight cut of</td>
</tr>
<tr>
<td>$n$</td>
<td>refractive index</td>
</tr>
<tr>
<td>$n(r)$</td>
<td>density profile</td>
</tr>
<tr>
<td>$N_A$</td>
<td>Avogadro’s number</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NP</td>
<td>nanoparticle</td>
</tr>
<tr>
<td>$N_p$</td>
<td>aggregation number</td>
</tr>
<tr>
<td>$p$</td>
<td>packing parameter</td>
</tr>
<tr>
<td>$P$</td>
<td>aggregation number</td>
</tr>
<tr>
<td>$P(Q)$</td>
<td>formfactor</td>
</tr>
<tr>
<td>PCL</td>
<td>poly(caprolactone)</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>PEO</td>
<td>poly(ethylene oxide)</td>
</tr>
<tr>
<td>$p_{m}$</td>
<td>density of the sample</td>
</tr>
<tr>
<td>$p_{solvent}$</td>
<td>density of the solvent</td>
</tr>
<tr>
<td>$Q$</td>
<td>scattering vector</td>
</tr>
<tr>
<td>$r$</td>
<td>distance between scattering centers</td>
</tr>
<tr>
<td>$R$</td>
<td>the vector from center of mass</td>
</tr>
<tr>
<td>$R$</td>
<td>gasconstant</td>
</tr>
<tr>
<td>$R(t)$</td>
<td>rate of chain expulsion</td>
</tr>
<tr>
<td>$R(\theta)$</td>
<td>rayleigh ratio</td>
</tr>
<tr>
<td>$R_c$</td>
<td>radius of the core</td>
</tr>
<tr>
<td>$R_g$</td>
<td>radius of gyration</td>
</tr>
<tr>
<td>$R_H$</td>
<td>hydrodynamic radius</td>
</tr>
<tr>
<td>$R_m$</td>
<td>radius micelle</td>
</tr>
<tr>
<td>ROP</td>
<td>ring opening polymerization</td>
</tr>
<tr>
<td>$S(Q)$</td>
<td>structure factor</td>
</tr>
<tr>
<td>SAXS</td>
<td>small angle X-ray scattering</td>
</tr>
<tr>
<td>SFA</td>
<td>stopped flow apparatus</td>
</tr>
<tr>
<td>$t$</td>
<td>time</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature in kelvin</td>
</tr>
<tr>
<td>$T_c$</td>
<td>crystalline temperature</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>$T_g$</td>
<td>glass transition temperature</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>$T_m$</td>
<td>melting temperature</td>
</tr>
<tr>
<td>$V_0$</td>
<td>Hydrophobic volume</td>
</tr>
</tbody>
</table>
\( V_{\text{core}} \) volume of the core
\( V_P \) total volume of the polymer
\( V_{\text{PEO}} \) volume of the corona
\( V_s \) scattering volume
\( V_{\text{sample}} \) sample volume in DSC
\( V_{\text{solvent}} \) molar volume, solvent
\( \text{wt\%} \) weight percent
\( \beta_f \) the width of relaxation times, fast mode
\( \beta_s \) the width of relaxation times, slow mode
\( \gamma \) surface tension
\( \Gamma \) gamma function
\( \delta \) Hildebrand's solubility parameter
\( \delta_s \) chemical shift
\( \Delta T/\delta t \) temperature scan rate
\( \Delta W \) heat flow
\( \Delta \rho_{\text{core}} \) scattering length density difference core-solvent
\( \Delta \rho_{\text{PEO}} \) scattering length density difference PEO-solvent
\( \varepsilon \) ellipse parameter
\( \eta \) viscosity of the medium
\( \lambda \) wavelength
\( \nu \) Flory exponent
\( \nu_{\text{solute}} \) partial specific volume
\( \xi \) correlation length
\( \rho_0 \) scattering length density for the solvent
\( \rho_{0}(r) \) normalized density distribution
\( \rho_p \) scattering length density (SLD) for the particle
\( \rho_{\text{solvent}} \) scattering length density for the solvent
\( \sigma \) smearing parameter of corona into solvent
\( \sigma_{\text{int}} \) Gaussian width of core-corona interface
\( \sigma_{p,\text{gauss}} \) Gaussian width of aggregation number
\( \tau_f \) relaxation times, fast mode
\( \tau_s \) relaxation times, slow mode
\( \phi \) volume fraction polymer
\( \chi \) Flory-Huggins parameter
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1 Introduction

There were 14 million new cancer cases and 8.2 million cancer deaths reported worldwide in 2012. Based on World Health organization’s predictions these numbers are increasing. Such high numbers of cancer cases give a high motivation for finding good, safe and effective treatments. Today treatment of cancer could be by surgery, radiotherapy, chemotherapy or combination of these methods [1]. In traditional chemotherapy different drugs are used to destroy cancer cells, but a challenge with this treatment is that these drugs also could harm the healthy cells[2]. Therefore the dosage of the therapeutic agent could be difficult to determine because a too low dose would be ineffective against the tumor, but at higher dosages, the toxicity could be intolerable for the patient. It is desirable with a treatment where the drug passes through the body, without affecting anything, until it arrives at its destination; the tumor tissue.

Drug delivery systems are methods to safely and controllably transport drugs to achieve a therapeutic effect in humans or animals. Many different drug delivery systems are being researched upon including liposomes, microspheres, gels among others [3, 4]. A promising candidate for drug delivery is nanoparticles/micelles composed of biodegradable polymers due to their stability, biocompatibility, possibility of targeting, controllable release of drugs and safe degradation [3, 5].

These nano-carriers could deliver drugs to various tissues due to their small size, and by tuning their size, their destination tissue could be chosen. For usage in drug delivery systems, nano-carriers in the size range from 10-100nm is desirable. For sizes smaller than this the nanoparticles would be eliminated by the kidney, and for sizes larger than 100 nm, it can be problematic to enter the tumor[6]. Tumors grow fast, and as a result to this, the vasculature in tumors has bigger passages than healthy tissue and is said to be leaky to macromolecules. The macromolecules could leak from the blood vessels and accumulate in the tumor if they exhibit the right sizes, this is a phenomenon known as enhanced permeability and retention (EPR) effect[6].See Figure 1. Size control is hence essential.

By changing the chemical composition of polymers, total molecular weight and block length ratios, the size of these nanoparticles can be tuned in a controlled manner [3, 7]. It is also showed that preparation methods of the micelles will affect the final sizes and morphologies[8]. The ability to control the structural characteristics of the particles opens
large opportunities. However, it is also introducing some challenges, because the preparation methods have to be accurately controlled to be able to reproduce the nanoparticles with the same structural characteristics.

![Figure 1: Enhanced permeability and retention (EPR) effect, accumulation of nano-carriers in the tumor][6]

Nanoparticles made of biodegradable polymers are of great interest for use in drug delivery systems[9]. They can accumulate in the tumor, degrade and release the therapeutic agent; and the degradable products are safely transported out of the body by the body’s own mechanisms.

**Aim of project**

Methoxy Poly(ethylene oxide)-block-Polycaprolactone (PEO-PCL) is a well-known copolymer for nanostructures used in research of drug delivery systems. However not so many studies give a detailed description on size and morphology variations, due to controllable changes in the micelle-preparation methods, for this polymer. In this study this will be investigated by using a stopped flow apparatus (SFA) for an accurate control of mixing. The PEO-PCL micelles will further be characterized primarily by small angle X-ray scattering (SAXS) and dynamic light scattering (DLS).

The preparation method used requires mixing of organic solvent with water, where the organic solvent has to be removed afterwards. Very few studies describe this removal of organic solvent in detail[10], but this will be done in this study by nuclear magnetic resonance (NMR) and SAXS.
2 Theoretical background and methodology

In this chapter a description of the preparation method, and the motivation of the choice of preparation method, will be given. A description of the theoretical aspects, and methods used, are also described to give a deeper understanding.

2.1 Methoxy Poly(ethylene oxide)-block-Polycaprolactone (PEO-PCL)

A main motivation for the selection of the polymer used in this project is that it is approved by the U.S. Food and Drug Administration (FDA). This is a big advantage both economically and timewise, because the process for FDA approval is a long and costly process. Methoxy Poly(ethylene oxide)-block-Polycaprolactone (PEO-PCL) is a well-known block copolymer in research for drug delivery systems[10-16].

![Figure 2: Metoxy poly (ethylene oxide)-b-polycaprolactone](image)

This polymer is an amphiphilic block-polymer built up from poly(ethylene oxide) (PEO) and polycaprolactone (PCL). PEO is an uncharged, hydrophilic and linear polyether which is commercially available\(^1\). Polycaprolactone is polyester with a low melting temperature of 60 °C. It has semi-crystalline structure, dependent on the molecular weight.

In this thesis this amphiphilic blockcopolymer will be referred to as PEO-PCL, and the lengths (m and n, see Figure 2) would be noted using the number average molecular weight, \(M_n\), measured with nuclear magnetic resonance (NMR).

---

\(^1\) [Link to Sigma-Aldrich](http://www.sigmaaldrich.com/catalog/product/sigma/t5267?lang=en&region=NO)
2.1 Self-assembly of amphiphilic block copolymers

Amphiphilic block copolymers contain both hydrophobic and hydrophilic parts/blocks covalently linked together. In a selective solvent, a good solvent for only one of the blocks, these amphiphilic block copolymers may self-assembly into micelles; this process is called micellization. The main driving force is lowering of interfacial energy by letting the soluble part face the solvent and the insoluble parts be hidden within the core of the micelle. The thermodynamic favored morphology is decided by the degree of stretching of the core-forming block, the steric crowding of chains in the corona and the quality of core-solvent interaction [11]. A sketch of the polymer, micelle and core-shell model is shown in Figure 3.

![Figure 3: A sketch of amphiphilic block copolymers self-assembly in water making up a core of the hydrophobic block and shell or a corona of the hydrophilic block.](image)

Thermodynamics predict an ideal size and morphology for the micelles in equilibrium, but for polymeric micelles the system could be in a non-equilibrium state due to slow kinetic. Therefore it is an increasing amount of research of these nanostructures in non-equilibrium state, where the structural characteristics could be dependent, and tuned, by the preparation method.
## 2.2 Micelle preparation methods

There are three main approaches used to prepare micelles; i) the direct dissolution method, ii) the co-solvent method iii) the controlled co-solvent method

1. **The direct dissolution method** is the simplest technique where the polymer self assembles into nanostructures purely by adding water. To use this method the polymer has to be soluble in water.

2. **The co-solvent method** is a common method used to prepare nanostructures. The method involves dissolving the polymer in an organic solvent (for drug-loading the drug should also be soluble in this solvent) and mixing in a solvent which is insoluble for one of the polymer blocks. This solvent is usually water. Then the organic solvent is removed; often by dialysis and this is probably why many articles describe this as the dialysis method.[4] Another name for this is the solvent displacement method [12] because it is based on a change in the interfacial tension when the organic solvent is displaced by water. Many articles also refer to this method as the solvent switch method and nanoprecipitation method [5, 12, 13]

3. **The controlled co-solvent method** is similar to the previous mentioned cosolvent method except that the organic solvent, with the dissolved polymer, is mixed in a controlled manner. By changing mixing speed of the organic solvent and water, nanoparticles structural characteristics may change.[14] Volume and mixing speed can be controlled very accurately by using a stopped flow apparatus (SFA).

All three methods can be used for drug-loading of nanostructures.[5, 15-17]

The easiest method would be direct dissolution, but this method is not always possible to use. This is often the case if the hydrophobic block is relatively long causing high hydrophobicity [15, 16]. On the other side, longer hydrophobic block lengths are attractive due to a higher drug loading capacity for hydrophobic drugs. Direct dissolution of PEO-PCL, with similar block lengths to the polymers used in this project, has been tested by Vangeyte P. et al to be not successful, it creates huge, polydisperse, unreproducible aggregates [8].

When using the co-solvent and controlled co-solvent methods, the choice of solvent and selected mixing ratios are very important. Vangeyte P. et al also tested different organic
solvents to dissolve PEO-PCL and found that different solvents could affect the sizes when using co-solvent method. Dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) resulted in larger sizes than when they used DMF, probably because of high viscosity for the DMSO and lower miscibility of water and THF, resulting in a slower mixing. A difference could also be seen if water was mixed with the organic solvent or the opposite. For THF, addition of water to the organic solution resulted in almost a doubling of micelle size.

To make a good choice of preparation method and used solvent, the work done by Borsali et al was conferred. They tested the direct dissolution method and the co-solvent (nanoprecipitation method) for PEO2-PCL2. They found that direct dissolution was a simple, but not well working method, not showing a narrow size distribution, but large and broadly dispersed macromolecular aggregates due to the low solubility of PCL in water. For the co-solvent (nanoprecipitation) method, two organic solvents were tested, acetone and THF. When using acetone they obtained a broad size distribution and irreproducible results for the nanostructured sizes, whereas with THF they obtained narrow size-distribution and reproducible sizes. [16] This gives motivation to use the co-solvent method with THF as the organic solvent to gain control over the structural characteristics of the nanoparticles.

Traces of organic solvent in the solution may lead to more unstable nanoparticles[18], therefore the ability to remove the solvent is important. Previously we tested different solvents for polymer solubility, and the amount of the organic solvent that could be removed by rotary evaporation from the mixed solution\(^2\). Ethanol and acetone showed poor solubility for the polymers and acetonitrile was especially difficult to remove from water as it forms an azeotrope with water. THF showed the best solubility of the polymers and also the best ability to be removed by rotary evaporation, and therefore THF will be used as the organic solvent in this work.

For the choice of mixing ratios, the work done by Jette K., et al was consulted. They tested the critical water content (CWC), the water content needed to induce self-assembly, for PEO5-PCL2.5 and PEO5-PCL4 by adding water to the polymer solution in acetonitrile (ACN). They found that for PEO5-PCL4 at least 40 percent water was needed to induce self-assembly and that increasing the water content up to 90 percent did not change the nanostructural size. Moreover they showed that shorter PCL lengths have a higher CWC, measured to 50 percent for PEO5-PCL2.5[15]. Having water content above CWC is

\(^2\) Performed by Jakob Stensgaard Diget
obviously important for choosing the THF-water ratio. Also the concentration of polymer in the total mixture will be diluted when mixing with water, therefore the water content should not be too high to avoid this dilution to cause complications reaching the desired end concentration of the polymer. There is also an upper limit on concentration of polymer solubility in the organic solvent.

On the other hand, it is important to not have too high content of the organic solvent, here THF, in the solution, as we want to remove all of the THF after mixing. Higher content of THF in solution would also make the nanostructures more unstable which is very unfavorable for controlling the structural characteristics.

2.1 Controlled co-solvent method

Mixing by hand is a rather slow and unreliable method. It is very complicated to have a controlled continuous flow and comparable mixing speed for all the mixes.

For accurately controlled mixing a stopped flow apparatus (SFA) was used. The earliest work using SFA and light scattering was performed by Bednar et al. in 1988[19]. Inside the SFA used in this work there are three reservoirs with stamps that are independently controlled by a computer, which allow us to control volumes and mixing rates for the different syringes. By using SFA we can achieve very well reproducible preparation methods, very fast turbulent flow and homogeneous mixing.[11, 20]

2.2 Micellar morphology

Amphiphilic block copolymers will not necessary self-assemble into spherical structures, but can have cylindrical or ellipsoidal, or even lamellar or vesicles structures, based on the hydrophilic fraction of the block copolymer, solvent quality and temperature. This is due to stretching of the core forming polymer block that is more dominating for the spherical structure, but a crystalline core could also affect the morphology [4, 11].
By varying the length of the two blocks the properties of the polymer can change drastically. It is shown that decreasing the fraction, $f_{PEO}$, could change the morphology of the nanostructures from spherical, cylindrical to vesicles, see Figure 4 [17].

The morphology could be described by the packing parameter, $p$:

$$p = \frac{V_0}{a_0 l_0}$$

(1)

where $V_0$ is the hydrophobic volume, $l_0$ is the hydrophobic length and $a_0$ is the area of the hydrophilic block at the interface [21, 22].

For our system a more simplified description of the molecular to nanostructure geometries is to use the fraction of the hydrophilic block[23]:

$$f_{PEO} = \frac{M_W(PEO)}{M_W(PCL)}$$

(2)

where $M_w$ is the weight average molecular weights, here for the two blocks, PEO and PCL. This gives a rough estimate of which structures to expect for the self-assembled structures.

![Figure 4: A rough description of packing parameters and hydrophilic fractions, affection on morphology [23]](image)

<table>
<thead>
<tr>
<th>fraction of hydrophilic block, PEO</th>
<th>nanostructure morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-40% polymersomes</td>
<td>Polymer membrane ($\frac{1}{2} &lt; p \leq 1$)</td>
</tr>
<tr>
<td>40-50% cylinders</td>
<td>Polymer cylindrical micelle ($\frac{1}{3} &lt; p \leq \frac{1}{2}$)</td>
</tr>
<tr>
<td>&gt;50% spheres</td>
<td>Polymer spherical micelle ($p \leq \frac{1}{3}$)</td>
</tr>
</tbody>
</table>
2.3 Removal of solvent

There are several methods for removal of different solvents. The methods used in this study are rotary evaporation and dialysis. Higher content of organic solvent will lead to faster kinetics [18], hence an unwanted instability for the particles. The organic solvent that is used is THF.

**Rotary evaporation**

Rotary evaporation take advantage of different evaporation temperatures between the two solvents. THF exhibits an evaporation temperature of approximately 66 °C while water has 100 °C. Only by heating we could evaporate most of the THF

The problem is that not all samples can stand higher temperatures so by increasing the vacuum, evaporation will occur at lower temperatures and the solvent will be removed gentler. Our system includes polycaprolactone which is sensitive for higher temperatures due to hydrolysis[9]. By increasing the temperature the kinetics of the hydrolyzing of PCL will increase. This is an unwanted effect and therefor it is favorable to use lower temperatures for removal of THF to avoid hydrolysis as much as possible.

The advantage of this method is that it is quick and easy to control, but the disadvantage is that it is hard to remove the last remaining of THF without removing also the other solvent, water, and then changing the concentration. A sketch on how the micelle is formed, how the solvent is trapped in the micellar core and the effect of removal of the solvent is shown in Figure 5.

![Figure 5: A sketch showing self-assembly and the effect of removing of solvent](image-url)
Dialysis uses the properties of diffusion and osmosis. Random movement of molecules in the solution will have a net movement of molecules from a volume of higher concentration to a volume of lower concentration until equilibrium – randomly distribution- is reached. In dialysis the sample is put into a closed dialyzing tube and put into a bath of solvent, usually water. The dialyzing tube consist of a semi-permeable membrane with a chosen pore size (Molecular weight cut off = MWCO) which allow solvent molecules to pass, but the solute, here nanostructures, will be trapped inside the tube. If the water bath is changed several times with clean water the equilibrium is controlled in such a way that the unwanted solvent, here THF, can be driven out of the dialyzing-tube. To make this process faster, a larger container of water and magnetic stirring are used. The advantage of this method is that it is easy. The disadvantages are that this is a slow process and that the concentration can change due to water molecules also diffusing through the membrane.

2.4 Control of nanostructure via the preparation-method

Different preparation methods of micelles can lead to changes in drug loading and structure of the final nanostructures, because of kinetic effects, leading effectively to non-ergodicity [14, 17].

2.4.1 Kinetic processes in micellar systems

Thermodynamics predict an ideal size and morphology for the system in equilibrium. To gain this equilibrium state the micelles need to redistribute their chains either by unimer exchange or by fusion and fission of micelles, see Figure 6. For polymeric micelles the fission-fusion process is not likely to happen because of repulsive interactions of the corona forming block[11, 24] Instead, the redistribution process is dominated by single unimer exchange. This has been found to be the case even under non-equilibrium conditions, when micelles are formed [11].
When the polymers are arranged in a micelle the core forming hydrophobic block would not prefer to go out in the solution because it requires exposure of the hydrophobic block against water, leading to an increase in the interfacial energy. The rate of chain expulsion is extremely dependent on the surface tension and chain length in a manner that can be expressed as a double exponential [11, 24]:

\[ R(t) \sim \exp(-t \cdot \exp(M_{PCL} \cdot \gamma)) \]  

were \( \gamma \) is the interfacial tension between PCL block and water. Hence as the length of this hydrophobic block increases, bringing unimers into solution becomes more energetically unfavored leading to very slow unimer release kinetics. At some point this process becomes so slow that we can say that the process does not happen within practical time scales of days, weeks or even years, and the micelles can be considered as frozen [4, 11, 17, 18, 25]. Frozen micelles will be referred to as nanoparticles throughout this thesis.
Through this project the name, micelles, will be used if the system is "living", meaning that there is unimer kinetics in between the micelles. Nanoparticles will be used if the micelles are in a frozen state.

2.5 Polymer and nanoparticle characterization

2.5.1 Basic theory of Scattering

Scattering methods are powerful approaches to characterize different material properties such as the thermodynamic, dynamic and structural properties. By changing the scattering source of light, X-rays and neutrons different size regions can be explored and also giving different contrasts. Often a combination of the different scattering techniques is needed to give a strong analysis of the system, but in this project only light scattering and X-ray scattering are used. An easy overview of the scattering events is shown in Figure 8.

Figure 8: An overview of scattering event, beam from the source with modulus $k_i$ is hitting the sample and is scattered with modulus $k_s$ before hitting the detector.
The beam passes through the sample, scatters from the sample and the scattering angles and intensities are being measured at the detector. By assuming monochromatic planar waves, the modulus of the scattered wave can be expressed as:

\[ k_s = |k_s| = k_i = \frac{2\pi}{\lambda} \]  

(4)

and the modulus of momentum transfer can be expressed by:

\[ |Q| = |k_s - k_i| = Q = 4\pi n \frac{\sin(\theta)}{\lambda} \]  

(5)

where \(2\theta\) is the scattering angle, \(k_s\) is the scattered wave and \(k_i\) is the incident wave and \(n\) is the refractive index. For X-rays \(n\) is close to 1.

### 2.5.2 Dynamic Light scattering (DLS)

DLS is also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS). It is based on detecting fluctuations due to e.g. Brownian motions. In a dilute solution the measured diffusion time can be related to the hydrodynamic size assuming spherical symmetry by using the Stokes-Einstein equation[26]:

\[ R_H = \frac{k_BT}{6\pi \eta D} \]  

(6)

where \(R_H\) is the hydrodynamic radius, \(D\) is the translational diffusion coefficient, \(k_B\) is the Boltzmann constant, \(T\) is the absolute temperature and \(\eta\) is the viscosity of the medium.

From DLS measurement we experimentally obtain the second intensity autocorrelation \(g^2(Q,t) - 1\) which can be converted to the first-order electric field autocorrelation by the Siegert relation[27]:

\[ g^{(2)}(Q, t) = 1 + B|g^{(1)}(t)|^2 \]  

(7)
where $B$ is an instrumental parameter. The first order electric field autocorrelation is described by the Kolrausch-Williams-Watts (KWW) function:

$$g^{(1)}(t) = \exp \left[ - \left( \frac{t}{\tau_{fe}} \right)^{\beta_f} \right]$$

(8)

For a bimodal distribution of particle sizes, e.g. micelles and larger clusters the correlation functions can be described by the sum of two stretched exponentials (two relaxation modes):

$$g^{(1)}(t) = A_f \exp \left[ - \left( \frac{t}{\tau_{fe}} \right)^{\beta_f} \right] + A_s \exp \left[ - \left( \frac{t}{\tau_{se}} \right)^{\beta_s} \right]$$

(9)

This equation describes both “fast” and “slow” relaxation modes. $A_f$ and $A_s$ are the amplitudes for the fast and the slow relaxation mode, respectively, $\tau_{fe}$ and $\tau_{se}$ are the fast and slow effective relaxation times and $\beta_f$ and $\beta_s$ are the width of distribution of relaxation times. The mean relaxation times are given by:

$$\tau_f = \frac{\tau_{fe}}{\beta_f} \Gamma \left( \frac{1}{\beta_f} \right)$$

(10)

$$\tau_s = \frac{\tau_{se}}{\beta_s} \Gamma \left( \frac{1}{\beta_s} \right)$$

(11)

Where $\Gamma(\beta_f^{-1})$ and $\Gamma(\beta_s^{-1})$ are the gamma functions of $\beta_f^{-1}$ and $\beta_s^{-1}$. The fast mode is usually diffusive meaning that we have a $q^2$ dependence to the diffusion coefficient, $D$:

$$\tau_f^{-1} = Dq^2$$

(12)

The diffusion coefficient combined with Stokes-Einstein equation will give the hydrodynamic radius of the particles in the solution[28].

Often the second intensity field autocorrelation $g^{(2)}$ is converted to the first electric field autocorrelation $g^{(1)}$ before further analysis. Since the baseline for the autocorrelation is
fluctuating around zero, giving both negative and positive numbers, this conversion leads to elimination of the negative numbers due to problems taking the square root of these negative values. Because of this the correlation function will not go completely to zero, but create an artificial baseline. A solution to this is to analyze the second intensity field autocorrelation \( g^{(2)} \) directly using this equation:

\[
g^{(2)}(t) = \left( A_f \exp \left[ -\left( \frac{t}{\tau_{fe}} \right)^{\beta_f} \right] + A_s \exp \left[ -\left( \frac{t}{\tau_{se}} \right)^{\beta_s} \right] \right)^2
\]

(13)

This method is preferred if a second mode is present but not very dominating, making it possible to determine the second mode without problems with overlapping with the artificial baseline.

### 2.5.3 Small Angle X-Ray Scattering (SAXS)

SAXS is an analytical method measuring the average size and shape of particles like polymers, colloids and more. The size range which could be analyzed is approximately 1-100nm and concentration range from 0.1wt% to 99.9wt% depending on the atomic numbers of the observed particle, hence the number of electrons.[29]

For SAXS the X-rays is elastically scattered because of the high energy of the radiation, hence it remains energy throughout the scattering event, also called Rayleigh or Thomson scattering. This is giving a scattering pattern due to interference effects, which holds information about size and shape of the particles. For a dilute solution with random distribution of the particles we can consider the scattering of each particle individually. The phase factors are described by their positions: \( \exp(i\mathbf{Q} \cdot \mathbf{r}_i) \).[30] To get the full scattering amplitude for the particle all scattering events has to be summed to give the total amplitude [11, 30, 31]:

\[
A(\mathbf{Q}) = \sum_i^N b_i e^{i\mathbf{Q} \cdot \mathbf{r}_i}
\]

(14)
where $r$ is the distance between scattering centers and $b_i$ is the scattering length which for x-rays is linearly dependent of the number of electrons. At the detector the intensity, which is the squared of the amplitudes, are measured[29]:

$$ I(Q) = |A(Q)|^2 $$  \hspace{1cm} (15)

Taking into account coherent scattering from particles dispersed in a solvent, and that the scattering coordinates can be regarded as continuous. We can be expressed the macroscopic differential scattering cross-section as[11]:

$$ \frac{d\Sigma}{d\Omega}(Q) = \frac{I(Q)}{V_s} = \frac{N_p}{V_s} (\rho_p - \rho_o)^2 \cdot V_p^2 \cdot P(Q) \cdot S(Q) $$  \hspace{1cm} (16)

where $V_s$ is the scattering volume, $\rho_p$ is the scattering length density (SLD) for the particle, $\rho_o$ is the SLD for the solvent defined as $\rho = \Sigma b_i/V_p$, $V_p$ is the volume of particle, $N_p$ is the aggregation number, $P(Q)$ is the form factor and $S(Q)$ is the structure factor. The expression can be divided into two parts; the form factor that describes the intraparticle correlations as and size and shape, and the structure factor that describes the interparticle correlations as interaction between particles. For diluted systems these interparticle interactions can be negligible. The structure factor is [11, 29, 31]:

$$ S(Q) = \frac{1}{N_p} \sum_{i=1}^{N_p} \sum_{j=1}^{N_p} \exp(iQ \cdot (R_i - R_j)) \approx 1 $$  \hspace{1cm} (17)

and the form factor is given by:

$$ P(Q) = \langle |A(Q)|^2 \rangle $$  \hspace{1cm} (18)

where the amplitude is given by:

$$ A(Q) = \int_{V_p} n(r)(r) \exp(iQ \cdot r) dV_p $$  \hspace{1cm} (19)
where \( \mathbf{R} \) is the vector to the center of mass of, \( \rho_n(\mathbf{r}) \) is the normalized density distribution of the particle and \( \mathbf{r} \) is the vector from the center of mass to a point within the particle.

To obtain a simple picture of how different shapes would influence the scattering curves, a theoretical image is presented in Figure 9. Here the form factor of a polymer, a sphere, a cylinder and shell is described. A rough estimation of the shape could be made based on these features and by estimations of the slope in intermediate \( Q \) regime. For high \( Q \) values where the slope is given by the fractal dimension, \( d_f \):

\[
Q^{-d_f} \approx \begin{cases} 
1 & \text{rod like} \\
1.7 \text{ to } 2 & \text{random coil} \\
3 & \text{globular structure}
\end{cases}
\]

For the scattering curves where the slope in the intermediate regime gets steeper, goes towards -4, a more complex structure is observed.

From Figure 9 it is only the theoretical part of the form factor scattering that is shown. When other factors like polydispersity or instrumental resolution is considered, this scattering would be “smeared out”, and the characteristic “bumps” would not be as distinct as shown here.

![Figure 9: Theoretical scattering curves from different shapes: polymer, cylinder, sphere and shell described by Lund et al.[11]](image-url)
Modeling of SAXS data

The data were analyzed using a modified spherical core-shell model based on a previously reported model[32, 33]:

\[
I(Q, P) = (V_{core}^2 \Delta \rho_{core}^2 A(Q)_{core}^2 + P^2 \cdot V_{PEO}^2 \cdot \Delta \rho_{PEO} \cdot A(Q)_{shell}^2
+ 2(P - F(0)) \cdot V_{core} V_{PEO} \Delta \rho_{shell} \Delta \rho_{core} A(Q)_{core} A(Q)_{shell}
+ V_{PEO}^2 \cdot \Delta \rho_{PEO}^2 F(Q)_{chain}
\]  

where \( P \) is aggregation number, \( \Delta \rho \) is scattering contrast; \( V_{core} \) is the volume of the core:

\[
V_{core} = \frac{4\pi Rc^3}{3}
\]

and \( R_c \) is the core radius given by:

\[
R_c = \left( \frac{3PM_{PCL}}{d_{PCL} 4\pi f_c} \right)^{1/3}
\]

For a core swollen by solvent the scattering length density of the core would change and would be defined as:

\[
\rho_{core} = f_c \cdot \rho_{PCL} + (1-f_c) \cdot \rho_{solvent}
\]

where \( \rho_{solvent} \) is the scattering length density for the solvent, here THF. Scattering length densities used are \( \rho_{THF}=8.36 \cdot 10^{11}\text{cm}^{-2}, \rho_{PCL}=1.08 \cdot 10^{11}\text{cm}^{-2} \) and \( \rho_{PEO}=1.11 \cdot 10^{11}\text{cm}^{-2} \).

The scattering amplitude of the core is given by:

\[
A(Q)_{core} = \frac{3(\sin(QR_c) - QR_c \cos(QR_c))}{(QR_c)^3}
\]

and the scattering from the shell is given by:

\[\text{\footnote{The fitting with this model were performed in collaboration with Reidar Lund}}\]
\[
A(Q)_{\text{shell}} = \int_{R_c}^{\infty} 4\pi r^2 n(r) \cdot \frac{\sin(Qr)}{Qr} dr
\]

where the density profile is given by:

\[
n(r) = \frac{1}{1 + \exp\left(\frac{r - R_m}{\sigma \cdot R_m}\right)}
\]

The scattering of the shell polymer chains was included according to Pedersen et al[34]:

\[
F_{\text{blob}}(Q) = \frac{P(Q)_{\text{chain}}}{1 + \nu \cdot P(Q)_{\text{chain}}}
\]

where \(P(Q)_{\text{chain}}\) is the form factor for a polymer chain, \(\nu\) is a parameter related to chain-chain interaction within the corona and scales with the effective concentration of corona chains. The form factor of a polymer chain could be expressed by Beaucage equation [35]:

\[
P(Q)_{\text{chain}} = \exp\left(-\frac{Q^2 R_g^2}{3}\right) + \left(\frac{d_f}{R_g^{d_f}}\right) \Gamma\left(\frac{d_f}{2}\right) \left(\frac{\text{erf}\left(\frac{QkR_g}{\sqrt{6}}\right)}{Q}\right)^{d_f}
\]

where \(d_f\) is the fractal dimension which could be in the range 1-3, but for polymers in a good solvent it is usually 1.7, \(k\) is a numerical constant equal 1.06 and \(R_g\) is the radius of gyration of a single chain and could roughly be estimated for PEO from the equation[36]:

\[
R_g = 0.215 \cdot M_w^{0.583}
\]

In order to take into account a distribution of, we included a Gaussian distribution of \(P[11]:\)

\[
\langle I(Q) \rangle = \frac{\varphi}{\langle P \rangle V_{\text{PEO-PCL}}} \cdot \frac{1}{\sqrt{2\pi}\sigma_p^2} \int_0^{\infty} I(Q,P) \exp\left(-\frac{(P - \langle P \rangle)^2}{2\sigma_p^2}\right) dP
\]

where \(V\) is the total volume of the diblock polymer, \(<P>\) is the mean aggregation number; \(\varphi\) is the total amphiphilic volume fraction and \(\sigma_p\) is the Gaussian width.
Modeling of diblock polymers\textsuperscript{4}

For analyzing the polymers dissolved in THF the diblock-Beaucage-model were used

\[ I = \frac{C_{sf}}{(1 + Q^2 \cdot \xi^2)^2} + \frac{\varphi \cdot V_p \cdot \Delta \rho \cdot P_{beau}(Q)}{N_{AVO} \cdot 1 + 2 \cdot A_2 \cdot C \cdot P_{beau}(Q)} \]  \hspace{1cm} (31)

where $C_{sf}$ is the cluster scale factor, $\varphi$ is the volume fraction, $\xi$ is the correlation length, $N_{AVO}$ is Avogadro’s number, and $\Delta \rho$ is the contrast.

Model for the nanostructures with ellipsoidal model\textsuperscript{5}

The ellipsoidal model used in this project is described elsewhere by Js Pedersen [37], but with adjustment to include excluded volume effects by R. Lund [38]

\textbf{2.5.4 Nuclear Magnetic Resonance (NMR)}

Nuclear magnetic resonance (NMR) spectroscopy is a method to determine the chemical structure of the compounds. It uses nuclear spins to map a spectrum of the different nuclei in the molecule. There are other types of NMR, but in this research only proton-NMR was used.

An external magnetic field is applied to the sample and the spins align either with or against this field. Spins aligned with the field have a lower energy; hence more spins are aligned with the field. By applying electromagnetic radiation of a specific frequency, energy absorption occurs and the spin “flips” to the higher energy state.

The electrons near nuclei will set up a tiny local magnetic field of their own, which acts against the applied external field. Nuclei that have more electrons surrounding them, more shielded and holds a lower effective field, need a higher applied field to spin ”flip” if the radiation frequency is held constant. A highly shielded nuclei is said to be upfield (right) in the NMR spectra and opposite, deshielded nuclei is said to be downfield (left). [39]

To position the absorptions, the NMR chart is calibrated using a reference point, often tetramethylsilane (TMS). The peak from TMS is set to zero and usually other absorptions

\textsuperscript{4} The fitting with this model was performed by Reidar Lund
\textsuperscript{5} The fitting with this model was performed in collaboration with Reidar Lund
occur downfield from TMS. Describing the position we use delta ($\delta_s$) scale which is defined by one part per million (1ppm) of the spectrometer operating frequency[39].

$$\delta_s = \frac{Chemical\,\,shift\,\,(number\,\,of\,\,Hz\,\,downfield\,\,from\,\,TMS)}{spectrometer\,\,frequency\,\,in\,\,MHz} \quad (32)$$

Highly shielded nuclei have a lower chemical shift than the less shielded. To get a better resolution of the spectra we use a higher spectrometer frequency. In this study we used spectrometers with 300, 400 and 600 MHz.

Some other important information, helpful for analyzing the spectra, are proton counting and spin-spin splitting:

Spin-spin splitting is that a single peak is split into multiple peaks, called a multiplet. This is caused by interaction, or coupling, of the spins of nearby nuclei. An easy way to predict the splitting is given by the general rule called the n+1 rule. Protons that have n equivalent neighboring protons show n + 1 peaks in their NMR spectrum.[39] This is a very helpful tool to understand the structure from the spectra.

For proton counting we use the property that each of the protons with the same shift contributes to the total peak for that specific shift. By integrating the peaks it is possible to measure the relative numbers of the different kinds of protons in a molecule.[39] This is a helpful tool for calculating the $M_n$ and fraction of blocks in a copolymer.

In this study, proton counting is also used to calculate the amount of THF left in the solution. NMR measurements are done before and after removal of THF.

For each measurement the ratio between NMR-solvent, DMSO, and the mixed solution consisting of water, which contain a small amount of THF, is held constant. As the amount of THF in water decreases, this can be observed as a decrease in the THF peak when the DMSO peak is held constant. By comparing the integrals for the different measurements, the removal of THF could be followed percentage. Knowing the amount of THF in the original solution allow us to calculate the THF content also for the other solutions.
2.5.5 Gel Permeation Chromatography (GPC)

GPC is a powerful technique for fractionating polymer and determine the weight average molecular weights, Mw, and polydispersity (PDI) of the molecular weights. The chromatography material within the columns as shown in Figure 10, consists of a stable, cross-linked polymer gel with different pore-sizes. Larger particles will be excluded by the smaller pore-sizes and will pass more quickly through the channel than the smaller particles, which may diffuse into the smaller pores, hence giving them a longer path length. [40, 41]

![Figure 10: Describing the function of GPC. To the left: smaller particles can go in to the smaller pore-sizes, giving them a longer path and hence a slower retention time[42]. To the right: A rough description of the function of GPC: A) all particles flows into the column, B) throughout the column the different sizes are being separated, C) the bigger sizes exits the column and are being detected, D) the smaller sizes exits the column and are being detected [43]](image)

When calculating molecular weights with GPC, it is usually necessary to use a standard, but a GPC could also be coupled to a multi angle light scattering detector (MALS). A GPC normally uses an Mw calibration curve obtained by analyzing a specific polymer e.g. PEG, dextran or polystyrene of different sizes. This curve is then used to calculate the Mw of the polymer being analyzed. This gives an error because the chemical nature of the polymer used to obtain the calibration curve is often different from the polymer being analyzed. The GPC used in this study is equipped with a MALS which gives more precise and trustable calculations of the molecular weights.
The $M_w$ is calculated using Zimm equation[40]:

$$\frac{K^*}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2c \quad (33)$$

where $R(\theta)$ is the Rayleigh ratio describing the ratio in between scattered and incident light taking into account the angle, distance from the detector to scattered volume, incident light intensity and volume of sample illuminated (measured by the instrument), $M_w$ is the weight average molecular weight, $P(\theta)$ is the form factor which is a function of size, shape and structure, $A_2$ is the second virial coefficient (mol·mL/g$^2$) giving the solute-solvent interaction and $K^*$ is the optical constant:

$$K^* = \frac{4\pi^2 n_0^2}{\lambda_0 N_A} \left(\frac{dn}{dc}\right)^2 \quad (34)$$

where $n_0$ is the solvent refractive index, $N_A$ is Avogadro’s number, $\lambda_0$ is wavelength of incident beam. For diluted samples $A_2$ is often set to 0.

From GPC we obtain $M_w$, $M_n$ and the polydispersity. A short description of the definitions of these can be found in the appendix E.

### 2.5.6 Differential Scanning calorimetry (DSC)

Differential scanning calorimetry is a method that can be used to describe the degree of crystallinity in a polymeric system. Often polymer are said to be amorphous or crystalline, but usually it is not so simple, often it can exhibit both amorphous and crystalline parts at the same time giving a degree of crystallinity, called semi-crystalline polymers, see Figure 9.
Figure 11: Polymer semi-crystallinity[44], showing both crystalline and amorphous regions.

Amorphous polymers have a glass transition temperature, $T_g$. Below this temperature the polymer is randomly orientated, but exhibits very slow dynamics, and is said to be in the glassy state. Heating above this temperature allows molecular motion in the polymer. This transition will take place over a temperature range.

Crystalline polymers exhibit a melting temperature, $T_m$, when polymers “fall out” of their crystal structures and become disordered liquids giving an endothermic peak, in a calorimetric experiment. But crystalline polymers also have a crystalline temperature, $T_c$, where the polymer has enough energy to go into a more ordered state in an exothermic process.

Since polymer could be both crystalline and amorphous it is possible to observe all transition temperatures for the same polymer. Some common methods to investigate crystallinity are densitometry and differential scanning calorimetry. [40, 45]

**Differential scanning calorimetry (DSC), function:**

Figure 12: Function of the DSC: one pan/chamber is filled with sample containing particles; the other is filled only with the solvent. It requires different amount of applied energy to heat up these pans at the same temperature rate, and this difference is measured and calculated.
In the DSC-instrument there are two pans, one with the sample and the other with the pure solvent. Underneath these pans there are two heaters that are working independently to keep the temperatures in the two pans exactly the same. Since one of the pans also contains polymer the two heaters will require different amount of power to keep the same temperatures for the two pans and it is this power that is being measured by a computer and transferred as heat capacity.

### 2.5.7 Densitometry

Densitometry was used to investigate crystallinity and to find the density for PCL required for SAXS modeling. It measures the period of oscillation of a U-formed, hollow tube filled with the sample and uses the relation between oscillation and density. The apparent partial specific volume of the solute is determined from the density measurements for the sample and the pure solvent[46]:

\[
\nu_{\text{solute}} = \left( \frac{1}{c_{\text{solute}}} \right) \left( \frac{1}{\rho_m} \right) - \left( \frac{1-c_{\text{solute}}}{c_{\text{solute}}} \right) \left( \frac{1}{\rho_{\text{solvent}}} \right)
\]  

(35)

where \(\nu_{\text{solute}}\) is partial specific volume, \(c_{\text{solute}}\) is the weight fraction of polymer in the solvent, \(\rho_m\) is the density of the sample, and \(\rho_{\text{solvent}}\) is measured density of the solvent. Solvents used for density measurements in this study were water, DMF and THF.

The specific density, \(d\), is given by:

\[
d = \frac{1}{\nu_{\text{solute}}}
\]  

(36)

Since the polymer used in this study is a diblock copolymer and we are interested in the density for PCL and PEG separately; we assume that the densities for each of the polymer blocks are additive:

\[
d_{\text{total}} = m_{\text{PCL}} \rho_{\text{PCL}} + m_{\text{PEG}} \rho_{\text{PEG}}
\]  

(37)
where $m_{\text{PCL}}$ and $m_{\text{PEG}}$ are mass fractions of the two blocks PEO and PCL, respectively, $d_{\text{PCL}}$ and $d_{\text{PEG}}$ are the densities of the two blocks and $d_{\text{total}}$ is the density of the whole diblock which we get from the density measurement.

To check for crystallinity in the sample we take use of the properties of higher densities for crystalline polymers. This should give a change in the density for a temperature range when heated to $T_m$. 
3 Experimental

3.1 Sample preparation

3.1.1 Stock solution
PEO5-PCL4 was dissolved in THF, heated to approximately 40 °C and stirred at 50-80rpm until the solution became homogenized. Stock solutions with polymer concentrations of 2.5, 5 and 10 wt% were prepared.

For PEO5-PCL2 the preparation method was the same as for PEO5-PCL. A stock solution with polymer concentration 5wt%, was made.

For both PEO5-PCL4 and PEO5-PCL2 THF with 99% purity was filtrated with 0.45µm PTFE filter (Millex-LH 4mm PTFE membrane, Merck Millipore) before use to avoid possible dust.

3.1.2 “Hand” mixing method
The stock solution was mixed with milliQ water filtrated with 0.1µm filter (Millex-VV 33mm, PVDF membran, Merck Millipore) at volume concentration 1:10 using a pipette. We distinguish between hand mix 0.5mL/s and hand mix 0.01mL/s. In both situations, the solution was stirred at 400-500rpm while adding polymer solution and the final solution was stirred for 10 min in total before removing THF by a rotary evaporator which will be described in paragraph 3.1.4.
3.1.3 Controlled mixing by using a stopped flow apparatus (SFA)

Controlled mixing was done with a SFM3000 stopped flow apparatus from BioLogic. It uses three syringes, one with the stock solution and two filled with water. The mixing consists of three phases:

1) Stock solution from syringe 1 and water from syringe 2 are mixed turbulently at a mixing ratio of 1:1.

2) Syringe 2 provides water to the capillary to “push” the solution further to the incoming point for syringe 3. The amount of water corresponds to the dead volume between the mixing reservoir and incoming point for syringe 3, and the water flow is kept constant at 8mL/s.

3) Syringe 3 provides water to the sample to obtain volume concentration stock solution to water 1:10 at a constant flow of 8mL/s. The time between the different phases is 2ms.

Figure 13: The stopped flow apparatus (SFA) and an easy sketch of the inside of SFA is shown.

We used 3-4 different total mixing rates for phase 1: 2mL/s, 4mL/s, 8mL/s, and 11mL/s. 5-7 min after mixing, the process of removing THF on the rotary evaporator was started. This time correspond to the time it takes to transfer the solution and to connect it to the rotary evaporator.
3.1.4 Rotary evaporator

To remove THF a rotary evaporator was used. The solution was added to a round bottom flask (or made directly in this flask for the hand-mixed solutions). The water-bath was set to 35 °C. First the solution was rotating in the bath without vacuum for 5 min at 280rpm, then the vacuum was turned on and slowly lowered to 40mBar over 15-20min. This routine was carefully repeated every time to ensure removal of approximately the same amount of THF for each sample. Samples were measured with NMR before and after rotary-evaporation to check the content of THF in solution as described in paragraph 3.2.1.

3.1.5 Dialysis

To completely remove THF from the samples they were dialyzed with float-A-Lyzers provided by SpectrumLabs. For PEG5-PCL4 we used a 5 mL dialysis tube with a molecular weight cut-off of 3.5-5 kDa and for PEG5-PCL2 we used 500-1000Da. Samples were dialyzed over 24-48 hours with 3-5 changes of water as the last step of preparation. Again the sample was measured with NMR to check the THF content, and for some of the samples extra NMR measurements were conducted before and after dialysis to have the polymer concentration under control.
3.1.6 Beta-carotene as model drug

To check for the particles ability for drug loading, a model drug, betacarotene, was used. Many drugs used for chemotherapy are hydrophobic, therefore betacarotene, which is also hydrophobic, but not toxic, serves as a good and safe model drug. Beta-carotene was dissolved in THF and mixed with the polymer solution to end concentration 5 wt% of beta-carotene and 5 wt% PEO5-PCL4 in stock solution. The process of mixing with water and removing THF was performed the same way as without beta-carotene, and after THF was removed the final solution was filtrated through 5.0 micrometer filter to remove the excess of beta-carotene. These samples were not dialyzed.

3.2 Characterization

3.2.1 Dynamic Light scattering (DLS)

DLS measurements were performed with an ALV/CGS-8F multidetector goniometer system, with eight (fiber-optical) detectors from ALV-GmbH, Langen, Germany. The intensity correlation functions were measured at eight scattering angles simultaneously in the range from 22° to 124°. The beam is supplied by a HeNe-laser with the wavelength 632.8nm and vertically polarized light.

To avoid dust, samples were filtrated through a 5μm filter (Millex-SV 25mm PVDF membrane, Merck Millipore) inside a glovebox into a pre-cleaned 10mm NMR tube. Then the tube is put into the sample holder, which consists of a bath of cis-decalin to match the refractive index of the tube. All samples were measured at 25°C with 0.25wt% polymer for 5-10min. Data from 6-7 detectors was used in the analyses of correlation functions.

3.2.1 Small Angle X-Ray Scattering (SAXS)

The SAXS instrument used for this project is located at RECX (Resource Center of X-rays) national laboratory at the University of Oslo. The apparatus is a NanoSTAR from Bruker. The Cu Ka radiation is monochromatic with wavelength λ=0.154nm and is “optimized” to give a good flux and a low background by passing through two pin-holes: first a Pt slit with
diameter 750µm and then a scatterless slit of 550µm. Samples are injected into a
temperature-controlled quartz capillary located in the beam in the vacuum chamber. The two-
dimensional data sets are recorded by a two-dimensional position-sensitive VANTEC
detector.

Samples were measured for 1 hour at approximately 25°C. Polymer concentrations were 1,
0.5 and 0.25wt% and the background scattering from the solvent (water) was subtracted.

For the measurements used to investigate the effect of temperature; 0.5wt% were used, and
temperatures of 25, 35, 45 and 55 °C were chosen.

For the measurements of polymers dissolved in THF, 10, 5, 2.5 and 0.5 wt% concentrations
were used. Background subtracted was THF and measurement time was 900-1800s.

### 3.2.1 Nuclear Magnetic Resonance (NMR)

NMR spectra were obtained from a Bruker AVII 400, Bruker AVIII HD 400 and Bruker
AVII 600MHz NMR spectrometer using BACS automatic sample changer. To follow the
amount of THF 24µL sample was added to 576µL DMSO-d6 to obtain a good signal to noise
ratio.

To investigate the concentration-changes of the polymer during the dialyzing process 0.8mL
sample was taken out from the solution before and after dialyzing. These solutions were dried
and the remaining polymer was dissolved in 600µL deuterated chloroform and measured by
NMR. The amounts were carefully exanimated by weighing in the solutions.

When we measured the number-average molecular weight, Mn, and the fraction of PCL,
10mg polymer was dissolved in 675µL chloroform and slowly heated to 40 degrees. This was
measured on AVII600MHz to ensure good resolution.

The data was processed using the Bruker TopSpin (version 3.2) software and using solvent
peak, 2.49ppm for DMSO-d6 and 7.24ppm for chloroform-d, as references for the chemical
shifts.

### 3.2.2 Gel Permeation Spectroscopy (GPC)

Gel Permeation Chromatography (GPC) was performed on a Tosoh EcoSEC dual detection
GPC system coupled to an external Wyatt Technologies miniDAWN Treos multi-angle light
scattering (MALS) detector. Samples were measured at 35 °C with HPLC grade THF as the mobile phase at a flow rate of 0.5mL/min. Injections volumes of 10μL were made at 2.5mg/mL sample concentration. The column used was 4.6x300mm, linear 5μm from MZ-Analysentechnik. Refractive index increment (dn/dc) values were calculated using dn/dc values from Wyatt’s database for the homopolymers and Astra 6 software package from Wyatt Technologies was used to analyze the data recorded.

3.2.1 Differential Scanning Calorimetry (DSC)

DSC measurements were performed at the Department of Pharmacy by Bente Amalie Breiby on a nano-DSC from TA-Instruments. All collected DSC thermograms were taken from 0.5wt% PEGPCL2 in a temperature range from 5-80 °C and 20-80 °C with a heating rate of 2 °C/min. These data were analyzed by NanoAnalyze and further studied in Origin where the water was subtracted and the measured heat flows were converted into specific heat capacity[48]:

\[ C_p = \Delta W \cdot \left( \frac{\Delta T}{\delta t} \right)^{-1} \left( V_s \cdot f_c \cdot C \right)^{-1} \text{(J/g/K)} \]  

(38)

where \( \Delta W \) is the heat flow (J/s) after subtraction of water signal, \( \Delta T/\delta t \) is the temperature scan rate (K/s), \( f_c \) is the weight fraction of the core forming block PCL and \( C \) is the polymer concentration (g/mL), \( V_s \) is the sample volume (300μl for nano-DSC).

3.2.2 Densitometry

Measurements were performed on a DMA5000 densitometer from Anton Paar, Graz using the oscillating tube technique to determine the density.

To check for crystallinity, samples were measured from 5 to 55°C. These samples were prepared as described under subchapter 27, except that they were not dialyzed.

To measure the densities of PCL at 25°C, the polymer was dried in vacuo at 55°C for 24 hours. This was done to get away possible water content in the polymer. This polymer was then dissolved in DMF with 1wt% concentration, heated to approximately 40°C and stirred to obtain a homogenous solution. Samples were measured at 25 °C to match the temperatures used for SAXS and DLS measurements.
3.1 Chemicals

The chemicals used in this project are given in Table 1

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Short name</th>
<th>Formula</th>
<th>Purity [%]</th>
<th>purchaser</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrahydrofuran</td>
<td>THF</td>
<td>(CH$_2$)$_4$O</td>
<td>99.99</td>
<td>Merck</td>
<td>Sample preparation GPC</td>
</tr>
<tr>
<td>MilliQ Type I</td>
<td>-</td>
<td>H$_2$O</td>
<td>-</td>
<td>-</td>
<td>Dialyse Sample preparation</td>
</tr>
<tr>
<td>Dimethyl Sulfoxide</td>
<td>DMSO-d$_6$</td>
<td>C$_2$H$_6$OS</td>
<td>99.9</td>
<td>Cambridge Isotope Laboratory</td>
<td>NMR solvent</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>(CD$_3$)$_2$S(O)</td>
<td>99.96</td>
<td>Euriso-top</td>
<td>NMR solvent</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>DMF</td>
<td>C$_3$H$_7$NO</td>
<td>99.9</td>
<td>Sigma-Aldrich</td>
<td>Solvent used for density measurements</td>
</tr>
<tr>
<td>Phosphorus pentoxide</td>
<td>-</td>
<td>P$_2$O$_5$</td>
<td>99</td>
<td>Sigma-Aldrich</td>
<td>In vacuum oven to remove water from polymer</td>
</tr>
</tbody>
</table>

3.2 Synthesis of the polymers

The polymers used in this study were synthesized and purified by PhD fellow Jakob Stensgaard Diget. The polymers were synthesized using ring opening polymerization (ROP), see Figure 16. The resulting solid polymer was dissolved in dichloromethane and precipitated in diethyl ether. This procedure was repeated twice, resulting in pure polymer that was dried in vacuo at 60 °C for 48 hrs.

![Figure 16: Schematics of the ring opening polymerization](image-url)
4 Results and Discussion

To analyze the data obtained from the SAXS measurements some knowledge of the system is needed. A good starting point would be to determine the ratios of the different blocks, the number average molecular weight, $M_n$, and the weight average molecular weight, $M_w$. The approach used to determine these properties of the polymers, combines measurements by NMR and GPC.

4.1 Characterization of Polymers: Molecular weight determination

To calculate the total number average molecular weight of the diblock polymer the hydrophilic ratio measured from NMR and the $M_n$ of PEO given from Sigma is combined:

$$M_n(\text{total}) = \frac{M_n(\text{PEO, Sigma}) - M(OH)}{f_{\text{hydrophilic}}} + M(OH) + M(CH_3) \quad (39)$$

To find the $f_{\text{hydrophilic}}$ from NMR we first have to calculate the repeating units. The repeating units could be calculated using different approaches, which take use of different groups of the polymer. In our calculations the groups chosen are shown in Figure 17 and assigned in the H-NMR spectra in Figure 18.

![Figure 17: showing the functional groups used for characterization from NMR](image)

The methoxy group at the end is chosen as the reference to have integration value 1, the ethyl group from PEO, and the methylene group next to the ester group from PCL, (see Figure 17) are chosen as references for the two blocks of the polymer. The hydrophilic ratio is calculated using this method:
\[ f_{\text{hydrophitic}} = \frac{\left( \frac{I_B}{4} \cdot \frac{3}{4} \right) \cdot M_{\text{PEO,monomer}}}{\left( \frac{I_B}{4} \cdot \frac{3}{4} \right) \cdot M_{\text{PEO,monomer}} + \left( \frac{I_C}{2} \right) \cdot M_{\text{PCL,monomer}}} \]  

where \( I_B \) is the integral from ethyl group from PEO (B), \( I_C \) is the methylene group from PCL (C) and \( M_{\text{PEO,monomer}} \) is 44g/mol and \( M_{\text{PCL,monomer}} \) is 116g/mol. Here the methoxy end group (A) is set to one, the equation also corrects for different amounts of hydrogen contributions for the different NMR signals.

![Figure 18: PEO5-PCL2 in chloroform, \textsuperscript{1}H-NMR](image)

Some “undefined” peaks appear at 1.25ppm and around 0.85ppm which probably comes from the silica grease which was used to seal the coupling between the round bottom flask and the rotary evaporator setup. Also another peak shows at 1.8 to 1.53ppm. This peak is probably a water peak. See explanation in appendix A.
To calculate the weight average molecular weight, $M_w$, we use the number average molecular weight, $M_n$, obtain from NMR measurements and the Polydispersity (PDI) from the GPC measurements:

$$M_w = M_n \cdot PDI$$ (41)

Figure 20: Elution time for GPC measurements for PEO5-PCL2 and PEO5-PCL4. Fluctuation in the refractive index signal after the elution peaks is an instrumental artifact which would not be discussed. More information in the appendix E.
To calculate molecular weights from GPC we needed to find the Refractive index increment. Tabulated values were used to calculate the dn/dc value for our system:

\[
\frac{dn}{dc}(PEOPCL) = \frac{dn}{dc}(PEO) \cdot f(PEG) + \frac{dn}{dc}(PCL) \cdot f(PCL)
\]

(42)

where dn/dc value used for PEO is 0.0668mL/g and dn/dc value used for PCL is 0.07925mL/g[49, 50].

To fit the SAXS data we need to know the \(M_w\) of PEO and PCL. For PEO we use the value given by Sigma Aldrich.

To calculate the weight \(M_w\) for PCL this approach was used:

- \(M_w(PCL) = (PDI(GPC) \times M_n(tot,NMR)) - M_w(PEO, sigma)\)

(43)

- \(M_n(PCL) = M_n(tot,NMR) - M_n(PEO, sigma)\)

(44)

Table 2: Molecular weights obtained by NMR and GPC

<table>
<thead>
<tr>
<th></th>
<th>PEO5-PCL2</th>
<th>PEO5-PCL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M_w) (GPC) [g/mol]</td>
<td>10.690</td>
<td>10.500</td>
</tr>
<tr>
<td>(M_n) total (GPC) [g/mol]</td>
<td>8360</td>
<td>8860</td>
</tr>
<tr>
<td>PDI (Mw/Mn)</td>
<td>1.28</td>
<td>1.19</td>
</tr>
<tr>
<td>(M_n) total (NMR) [g/mol]</td>
<td>7250</td>
<td>8980</td>
</tr>
<tr>
<td>(M_n) PEG (Sigma Aldrich) [g/mol]</td>
<td>4866</td>
<td>4866</td>
</tr>
<tr>
<td>(M_n) PCL (NMR) [g/mol]</td>
<td>2380</td>
<td>4120</td>
</tr>
<tr>
<td>(M_w) total [g/mol]</td>
<td>9270</td>
<td>10.650</td>
</tr>
<tr>
<td>(f_{hydrophilic}) (NMR)</td>
<td>0.671</td>
<td>0.542</td>
</tr>
<tr>
<td>(M_w) PEO (Sigma Aldrich) [g/mol]</td>
<td>5039</td>
<td>5039</td>
</tr>
<tr>
<td>(M_w) PCL [g/mol]</td>
<td>4230</td>
<td>5610</td>
</tr>
</tbody>
</table>

From Table 2 there are some deviations between the molecular weights obtained from NMR and GPC. NMR shows a clear difference in the weights for PCL, while from GPC the
polymers show approximately the same weights. In praxis it should be possible to calculate the $M_n$ obtained from NMR, to the $M_w$ obtained by GPC, by using the PDI:

$$PDI = \frac{M_w}{M_n}$$

(45)

For the polymers used in this project this was not possible. Many articles describing this problem concerning this mismatching between NMR and GPC data for PEO-PCL diblock copolymers, but most of them describe the differences due to their use of different standards [7, 12, 51, 52]. In our studies standards are not used because the GPC is connected with a MALS detector. A proper explanation of these deviations is still lacking, but it may be due to small differences in refractive index between PEO and the solvent or interactions between the polymer and the column. Because of this we chose an approach to calculate the weights using equation 26, 30 and 31 where NMR data is weighted / trusted more. We chose not to go further into this discussion. But more insight could be obtained by carrying out GPC measurements in DMF, or the weight average molecular weights could be determined from SAXS measurements.

To determine the $M_w$ from SAXS, new samples of both PEO5-PCL2 and PEO5-PCL4 in THF were made at a concentration of 10wt% with dilution series of 5, 2.5 and 0.5wt%. The samples were measured with SAXS to determine the $M_w$ of the polymers. See Figure 21.

![Figure 21](image-url)

Figure 21: PEO5-PCL2 and PEO5-PCL4 dissolved in THF measured on SAXS in different concentrations for finding the $M_w$. 

39
The $M_w$ were tested with measurements from SAXS. Some misfit between the model fitting and the scattered curve are seen for the highest concentration of polymer, especially for PEO5-PCL2. This could be due to the high concentrations and the fact that we use only one second virial coefficient, $A_2$, in the model. In reality, for such high concentrations, the system may not be perfectly described by only using the second virial coefficient, but virial coefficients of higher orders may be needed. Describing the system by using higher orders of virial coefficient could be complicated [53] and will not be done in this project. For obtained data from the SAXS fitting, see Table 3.

<table>
<thead>
<tr>
<th>PEO5-PCL4</th>
<th>0.5wt%</th>
<th>2.5wt%</th>
<th>5wt%</th>
<th>10wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_w$ (PEO) [g/mol]</td>
<td>5040</td>
<td>5040</td>
<td>5040</td>
<td>5040</td>
</tr>
<tr>
<td>$M_w$ (PCL) [g/mol]</td>
<td>5600</td>
<td>5600</td>
<td>5600</td>
<td>5600</td>
</tr>
<tr>
<td>$R_g$ [Å]</td>
<td>46</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>$d_f$</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$A_2$</td>
<td>0.0098</td>
<td>0.0098</td>
<td>0.0098</td>
<td>0.0098</td>
</tr>
<tr>
<td>$C_{sf}$</td>
<td>13</td>
<td>3.0</td>
<td>1.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PEO5–PCL2</th>
<th>0.5wt%</th>
<th>2.5wt%</th>
<th>5wt%</th>
<th>10wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_w$ (PEO) [g/mol]</td>
<td>5040</td>
<td>5040</td>
<td>5040</td>
<td>5040</td>
</tr>
<tr>
<td>$M_w$ (PCL) [g/mol]</td>
<td>4200</td>
<td>4200</td>
<td>4200</td>
<td>4200</td>
</tr>
<tr>
<td>$R_g$ [Å]</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>$d_f$</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$A_2$</td>
<td>0.019</td>
<td>0.019</td>
<td>0.019</td>
<td>0.019</td>
</tr>
<tr>
<td>$C_{sf}$</td>
<td>256</td>
<td>51</td>
<td>33</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 3: Results of the fitting of PEO5-PCL2 and PEO5-PCL4 dissolved in THF confirm the molecular weights calculated from NMR and GPC.

The polymer scattering were fitted with the diblock-Beaucage-model described in chapter 2.5.3 with the $M_w$ obtained from GPC and NMR measurement as input parameters. A decrease of the $R_g$ is seen for the shorter polymer, as expected, but also some clusters are seen from the cluster scale factors, $C_{sf}$. The relatively well fitted scattering curves, give an indication, of a good weight determination from GPC and NMR.
4.2 Formation of nanoparticles: Processing and removal of THF

After hand mixing, and removal of THF, the scattering curves from SAXS measurements changed. The polymer in THF shows a typical simple polymer curve, but in water they show a more typical scattering from more complex nanostructures. See Figure 22. This can be observed from the more curved shape and a steeper slope, of approximately -4, in the intermediate Q regime.

Figure 22: SAXS data showing the scattering data from polymer in THF and after mixing with water in ratio 1:10 at approximately 0.5mL/s.

To follow the amount of THF in the final sample, NMR was used. Most of the THF is removed by using rotary evaporation. With some calculations using the integrals from the NMR-spectra, the amount of removed THF is found to be approximately 98% of the original THF. This is rather good, but for drug delivery purposes, the solutions should be completely free of THF since THF is considered potentially carcinogenic [54]. Another reason is that THF in solution would make the final nanostructures in solution less stable, which would be much undesirable for this study. Some of the THF will gather in the core of the nanostructures, making the core less dense, which will affect the results from the SAXS. This will result in a more challenging fitting procedure by introducing more parameters.

To avoid this problem, dialysis was also used to remove the remaining THF after rotary evaporation. After using rotary evaporator, less than 0.15wt% of the solution consist of THF and after dialysis less than 0.03wt%. For some of the solutions no THF were detected, see Figure 23, and Table 11 in appendix H, for more detailed information.
It is also important to mention that there is some insecurity from the NMR spectra. Some of the peaks had small overlap with other peaks, giving higher integrals. To avoid this problem as much as possible, the integrals were taken without the satellites. The measurements are also very sensitive for ratio of sample and DMSO, and also the deuturation degree of the DMSO. Therefore all volumes were weighted in carefully and calculations were done if some aberrations were seen, to correct for this errors. For the samples that were compared, the same bottle of DMSO was used.

4.3 Local structure of nanostructures:
Crystalline Cores?

PCL is a semi crystalline polymer, where the degree of crystallization is determined by the length of the polymer. Therefore measurements had to be done to find the degree of crystallization of the polymer, with the specific PCL length, used in this work. Samples were
measured both with a temperature range for density and with DSC to search for indications of a crystalline PCL-core.

He C. et al found the melting temperature, \( T_m \), and the crystallization temperature, \( T_c \), for PEO2-PCL6 to be 58,5 and 35,4 °C respectively[55]. For our system of PEO5-PCL4 we expect these temperatures to be close to these values, but we also have to consider a small deviation because the melting point also is affected by the size of the nanostructured core, as described by Zinn T. et al[56]. This deviation arises from the Laplace pressure, which will decrease with increasing curvature, hence a lowering in the melting temperature is expected.

From Figure 24 no peaks are showing for PEO5-PCL4 0.5wt% indicating that there is no crystallinity in the nanostructure. It is also important to have in mind that this sample was measured after removing THF only by rotary evaporator, giving 0.093wt% THF in the solution and 30wt% THF in the core, if we assume that all the THF is gathered in the core. A new sample was made where THF was removed both by rotary evaporator and dialysis. No peaks are showing for PEO5-PCL4 0.5wt%. Also for this sample there was some remaining of THF, much less than for the previous sample, but still it was 0.029wt% THF in the solution and 11wt% of the core consist of THF, if we again assume that all the THF is gathered in the core.

The data obtained from the DSC measurement show that there are no crystalline parts in the micelle, hence an amorphous core. Some densitometry measurements were also performed to confirm these results.
Density measurements

Within the temperature range from 5-55°C there is no breakpoint in the slope for the densities (Figure 26), which also points to an amorphous core. Higher temperatures would have been desirable, but unfortunately higher temperatures could not be reached for this system without creating bobbles.

![Figure 26: PEGPCL 2 hand mix 1wt% measured density from 5 to 55 degrees.](image)

The density for PCL is needed for SAXS analysis. Ideally the density could be found from the previous density measurements. Unfortunately there were some difficulties with measuring this density of the prepared nanostructures. In many of the cases it could be due to low concentration of the prepared nanostructures or uncertainty in the final nanostructure concentration, after many preparation steps of the solution, before measuring the density. It could also have been a problem that some of the measurements still contained a small amount of THF in the solution. Density measurements are very sensitive for concentration and background subtraction.

To compensate for this problem, we decided to dissolve the polymer directly in DMF. To directly dissolve the polymer in THF could also be a solution, but because of its volatile nature, DMF was chosen to keep the concentration fully under control by avoiding of solvent-evaporation. Five independent samples were made and measured by the density instrument to get an idea of error margins on our density value.
The density for PCL was calculated using the total measured density, density for PEO and the fractions of PEO and PCL:

\[ d_{\text{PCL}} = \frac{d_{\text{total}} - (f_{\text{PEO}} \cdot d_{\text{PEO}})}{f_{\text{PCL}}} \]  

(46)

The density was found to be 1.03 g/mL with standard deviation of 0.03 g/mL. This measured density is close to reported density for amorphous PCL of 1.08 g/mL [57], and is used in the models for SAXS fitting. Density used for PEO is 1.2 g/mL [58], which is the same density as previously measured by the research group for PEO2.

### 4.4 Structural control of micelles

From dynamic light scattering experiments the hydrodynamic radiuses were calculated by using the method described in chapter 2.6.2, where the second intensity autocorrelation \(g^2-1\) directly are being analyzed. For the hand-mixed samples the fitting of these data are shown in Figure 27 together with the calculated sizes. The width of the relaxation time for the fast mode, \(\beta_f\), is 1 for all of the fittings, which indicate no polydispersity. The correlation functions, the fitting curves and the resulting sizes are shown in Figure 27.

The differences in between the nanoparticle-sizes are small, only differing by less than 2 nm for the nanostructures. This is considered within the range of error from the measurements, hence from these measurements no clear difference in the sizes is observed, either for the concentration changes or for the two polymers with different PCL lengths. It is important to mention that these fittings were performed with a double stretched exponential, hence two sizes were calculated. The slow relaxation times could indicate some aggregation in our sample. These sizes were calculated to 50-200nm with a very broad size distribution. These data will not be considered further here, for more detailed information see appendix D.
It is important to remember that the radius obtained by DLS is the hydrodynamic radius which not includes the morphologies of the particles, but just transfer all motions, diffusive and rotational, to a spherical size. Furthermore the hydrodynamic radius in praxis also consists of a thin layer of water-molecules giving a larger size.

In order to fit the data obtained with SAXS some knowledge of the morphology of the particles is very helpful. Lacking this information, different fitting models had to be tested. First a simple spherical core-shell model was tested without any success, and also a cylindrical model was tested. By using an ellipsoidal fitting model and including a fraction of solvent in the core, we were able to fit the SAXS data. However it is well known that the scattering curve from an ellipsoidal shape is hard to distinguish from a spherical shape of high polydispersity. A new trial of the spherical model was done, both including polydispersity and fraction of THF in the core. The model is described in section 4.2.4. This model was also successful. From Figure 28 and Figure 29 the scattering curve for PEO5-
PCL2 is shown with fitting of the ellipsoidal model with a fraction of THF in the core, and the spherical model with a fraction of THF in the core and polydispersity.

**Ellipsoidal model fit**

**Spherical model fit**

Figure 28: PEO5-PCL2 hand mixed sample measured on SAXS and fitted with ellipsoidal model with weight fraction of THF in the core.

Figure 29: PEO5-PCL2 hand mixed sample measured on SAXS and fitted with spherical model with polydispersity and a weight fraction of THF in the core.

<table>
<thead>
<tr>
<th>PEO5-PCL2</th>
<th>0.5mL/s before dialysis</th>
<th>0.5mL/s after dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>ε</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>R [Å]</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td>dR [Å]</td>
<td>54</td>
<td>62</td>
</tr>
<tr>
<td>f_c</td>
<td>0.67</td>
<td>0.72</td>
</tr>
<tr>
<td>ν</td>
<td>1.3</td>
<td>5.5</td>
</tr>
<tr>
<td>d_f</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>R_g [Å]</td>
<td>23</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PEO5-PCL2</th>
<th>0.5mL/s before dialysis</th>
<th>0.5mL/s after dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;P&gt;</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>dR[Å]</td>
<td>62</td>
<td>68</td>
</tr>
<tr>
<td>R_c [Å]</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>σ</td>
<td>0.072</td>
<td>0.072</td>
</tr>
<tr>
<td>R_g [Å]</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>ν</td>
<td>1.6</td>
<td>5.0</td>
</tr>
<tr>
<td>d_f</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>f_c</td>
<td>0.62</td>
<td>0.67</td>
</tr>
<tr>
<td>σ_int [Å]</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>σ_p,gauss</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The first assumption of monodisperse nanostructures came from fitting of the DLS data. These data were not very well fitted if polydispersity was included for the fast mode. But DLS has a lower resolution than SAXS, meaning that DLS is not as precise as SAXS analysis for size determination; because within this resolution, the polydispersity calculated from SAXS could be hidden, giving rise to the idea of spherical nanostructures with polydispersity.
The polydispersity is calculated from the SAXS fitting by the Gaussian width of aggregation, \( P \). This is connected to the polydispersity of the micellar size by:

\[
\sigma_{Rm, gauss} \approx \frac{1}{3} \sigma_{p, gauss}
\]  

(47)

Hence the polydispersity of the micellar sizes are not as pronounced and may not be visible from the DLS measurements.

From the information we have obtained from experiments, an exact decision of spherical or ellipsoidal particles cannot be made. But by carefully calculating the hydrophilic fractions for the polymers, the nanostructured morphology could be predicted theoretically. See equation 2. The fraction was 0.67 for PEO5-PCL2 and 0.54 for PEO5-PCL4. Both of these polymers would theoretically indicate spherical shapes.

Our conclusion was to use the spherical model with polydispersity for micelles of both PEO5-PCL2 and PEO5-PCL4. Sizes and morphology for PEO5-PCL4 was also measured on transmission electron microscope (TEM). The TEM pictures were not fully successful, but showing a lump of many aggregated nanoparticles varying in shape and size. Because of the drying process needed, the TEM data is unreliable. All other measurements where performed in liquid medium, giving other conditions for the system. Therefore the TEM results are not discussed in more detail here. Some of the TEM pictures are shown in the appendix B.

### 4.4.1 Hydrophobic block length and concentration effects

From Figure 30 it can be seen a large difference in between the scattering curve for PEO5-PCL2 and PEO5-PCL4. This was expected due to the difference in the length of PCL, where shorter hydrophobic lengths usually give smaller nanostructures[59]. Results from the fitting of these SAXS data with the spherical model are shown in Table 5.

The polymer with a shorter PCL length show almost half the aggregation number than for the polymers with longer PCL length. The thickness of the core and the radius of the corona are also smaller. This is in correspondence to the literature, where the size is dependent mostly of the hydrophobic block length and not so much affected by the hydrophilic block[45, 60]. There is not much difference in between 1 and 0.5wt% concentrations for PEO5-PCL4.
These results correspond to what Riely et al. found in their study of PEG-PLA. For shorter length of PLA, between 2 and 30kDa, they found that the size and aggregation number of the nanoparticles were only dependent of the block length and not of the concentrations[61].

In the beginning of the project we were interested to investigate how the chain lengths of PCL would affect the structural characteristics of the nanostructures prepared by controlled co-solvent method. To do this it would have been preferable to have three or more polymers with different lengths of PCL to compare. It is worth mentioning that in the beginning also a third polymer with PCL length of 13kDa was chosen. This polymer was fully dissolved in THF, but after mixing with water the polymer precipitated out of solution at the wanted concentration of 0.5wt%. Due to this we chose not to do more experiments with this polymer, but the measurements that were performed can be found in the appendix C.

### 4.4.2 Removal of solvent

The step of removal of solvent is very important. Remaining of organic solvent in the solution during solvent removal could increase the unimer kinetics. To avoid this problem the THF was removed rather quickly by a rotary evaporator before removing more of the organic solvent using dialysis. An example of the difference in the scattering curves for the particles
are seen in Figure 31 with the fitting curves, and the parameters from the fitting is shown in Table 5. The aggregation numbers for the nanostructures of PEO5-PCL4 would not change during the further organic solvent removal by dialysis, but a small deviation is seen for the dynamic micelles of PEO5-PCL2. The differences are very small but the trend is the same. After solvent removal by dialysis the core decreases in size, the corona is usually enlarged and the overall micelle size will increase. The dynamic micelle would correspond to this by unimer exchange, hence a change in the aggregation number.

![Scattering curve from SAXS for PEO5-PCL4 fitted with spherical core shell model](image)

Figure 31: Scattering curve from SAXS for PEO5-PCL4 fitted with spherical core shell model

The nanostructures are often described as nice spheres, but for polymeric micelles they are in reality more “fuzzy”. This surface roughness is described by the smearing coefficient, $\sigma$, which gives the fraction of the core block that is in interference with the solvent, hence the fraction where water is penetrating the PEO shell. In our system the smearing coefficient is between 0.05 and 0.07 and in good agreement with what is typical for polymeric micelles. The interface between the core and corona could not be described by a smooth transition, but more overlapping between the core and the corona forming block is present in the transition region. This is described by the overlapping distance, here found to be approximately 5\AA.
Table 5: Results from fitting of 0.5wt% PEO5-PCL2, 0.5wt% PEO5-PCL4 and 1wt% PEO5-PCL4 by the spherical model. Showing values for aggregation number (P), thickness of the shell (dR), radius of the core (Rc), smearing of the corona block into the solvent (σ), the radius of gyration (Rg), fractal dimention df, the fraction of hydrophilic block in the core, the overlapping of cor-corana (σ_{int}) and the width of distribution of P (σ_{gauss}).

<table>
<thead>
<tr>
<th>compare hand mix</th>
<th>1wt% PEO5-PCL4 before dialysis</th>
<th>1wt% PEO5-PCL4 after dialysis</th>
<th>0.5wt% PEO5-PCL4 before dialysis</th>
<th>0.5wt% PEO5-PCL4 after dialysis</th>
<th>0.5wt% PEO5-PCL2 before dialysis</th>
<th>0.5wt% PEO5-PCL2 after dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>66</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>dR [Å]</td>
<td>95</td>
<td>100</td>
<td>104</td>
<td>104</td>
<td>62</td>
<td>68</td>
</tr>
<tr>
<td>Rc [Å]</td>
<td>59</td>
<td>58</td>
<td>58</td>
<td>57</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>R_m [Å]</td>
<td>160</td>
<td>164</td>
<td>167</td>
<td>166</td>
<td>109</td>
<td>114</td>
</tr>
<tr>
<td>σ</td>
<td>0.065</td>
<td>0.064</td>
<td>0.050</td>
<td>0.050</td>
<td>0.072</td>
<td>0.072</td>
</tr>
<tr>
<td>R_g [Å]</td>
<td>17</td>
<td>17</td>
<td>15</td>
<td>16</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>f_core</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>σ_{int} [Å]</td>
<td>5.5</td>
<td>5.0</td>
<td>1.9</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>σ_{gauss}</td>
<td>0.85</td>
<td>0.85</td>
<td>0.99</td>
<td>0.99</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

The radius of gyration, R_g of the polymers, and the fractal dimension d_f is very sensitive to background subtraction and will hence not be evaluated.

The fraction of the solvent in the core, here THF, is approximately 70%, hence 30% of the corona consists of THF, and approximately 6% is removed during dialysis. Comparing these results to the NMR results where only 0-10wt% THF is remaining in the core could give an indication that something else could be in the core as e.g. water.

### 4.4.3 Water in the micellar core?

To investigate this hypothesis of water in the micellar core further more knowledge of the surface tension between PCL-water and PCL-THF is needed. Hildebrand’s solubility parameter, δ, combined with Flory Huggins parameter, χ, will give an estimation of these interfacial tensions[62]:

\[
χ = χ_h + χ_s = χ_h + \frac{V_{solvent}}{RT} (δ_1 - δ_0)^2
\]

Where δ_1 and δ_0 are Hildebrand’s solubility parameters for the polymer and the solvent, χ_s is the entropic Flory Huggins parameter and χ_h is the enthalpy Flory Huggins parameter usually
set to 0.34 for neutral polymers. From the total Flory Huggins parameter the interfacial
tension could be estimated:

\[ \gamma = \frac{k_b T}{l_{\text{solvent}}^2} \left( \frac{\chi}{6} \right)^{1/2} \]  \hspace{1cm} (49)

Where \( k_b \) is the Boltzmann constant, \( T \) is the temperature and \( l_{\text{solvent}} \) is the solvent length estimated from:

\[ l_{\text{solvent}} \approx (V_0)^{1/3} \]  \hspace{1cm} (50)

The calculated Flory Huggins parameters and the interfacial energies is shown in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Molar volume, ( V_s ) [cm(^3)/mol]</th>
<th>Total solubility parameter, ( \delta ) [63] [MPa(^{1/2})]</th>
<th>Flory Huggins parameter, ( \chi )</th>
<th>Interfacial energy, ( \gamma ) [mN/m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER</td>
<td>18</td>
<td>47.8</td>
<td>5.8</td>
<td>42.0</td>
</tr>
<tr>
<td>THF</td>
<td>81.7</td>
<td>19.46</td>
<td>0.34</td>
<td>3.7</td>
</tr>
</tbody>
</table>

A high interfacial tension between water and PCL shows that water prefers not to be in the
micelle core. Then it is more likely that most of the THF is accumulated in the micellar core
due to low interfacial tension between PCL and THF. Swollen micellar cores has previously
been observed for \( h \)-polystyrene-\( d \)-polybutadiene (PS-PB) micelles[64]. This was explained
as a result of the low interfacial tension between the core forming block and the organic
solvent, which correspond to what is found in this project.

From the NMR, many of the measurements contained a small amount of THF. See appendix
H. Calculations of the amount of THF left in the solution are based on the integrals of the
NMR peaks. When these integrals are very small, the signal to noise decreases, increasing the
error. The calculations are very sensitive to the ratios of sample to NMR-solvent, therefore
these volumes/masses were controlled both by use of Finnpipette and weight, and could be
corrected if needed. The integrals are also very sensitive to fluctuation in the baseline;
therefore some samples were measured over days to check for instrumental and human
analysis error. The standard deviations were found to be approximately 1\%, 4\% and 6\% for
the integral of THF measured before rotary evaporation, before dialysis and after dialysis respectively. At the same time the evaporation of THF in DMSO-d6 was followed over three days, and no trend of decreasing amount of THF was seen within this timeframe. These results confirm that this is a useful method to follow the amount of THF during the different procedures.

Still a higher fraction of solvent seems to be found in the core from the SAXS measurements than observed from the NMR measurements. This result has not been fully understood, but it could be due to some error margins, either from the NMR measurements underestimates the amount of THF in solution or that SAXS overestimates the fraction of THF in core. From SAXS it could be due to insecurity in the parameters put in to the model used for fitting. Some deviations were found for PCL density, but in the model this were set to a constant at the average value of density that were found. Error in the density would have a large impact in the model.

Using another SAXS model would not influence much on the difference in solvent fraction in the core, considering both the spherical and the ellipsoidal model giving a similar fraction. However if the solution consists of a mixture of spherical and ellipsoidal structures, while the analysis is done with the spherical model, this could have an influence on the hydrophobic fraction in the core.

4.5 Kinetic control of nanoparticle formation: Effect of Mixing rate

The samples made at different mixing rates for the two polymers, and for different concentrations were measured by dynamic light scattering (DLS). From Figure 32 the sizes (hydrodynamic radius) are plotted for the different mixing rates. There are some small fluctuations for the PEO5-PCL4, but these fluctuations are small and within the calculated standard deviations. No clear size-difference could be seen for the different mixing rates by using DLS. If the average sizes is calculated we obtain, 14 nm for 1wt% PEO5-PCL4, 13 nm for 0.5wt% PEO5-PCL4 and 11nm for 0.5wt% PEO5-PCL2. These differences are small, and within the error margin of the measurements.
Figure 32: Mean size of nanostructures, standard deviation calculated from two measurements to give a rough estimation of the error for PEO5-PCL4 0.5 and 1 wt%.

In addition to DLS measurements, SAXS measurements were performed for the same samples. This gives more detailed information, where internal information of the nanostructures could be found. See Figure 33, Figure 34 and Figure 35 for the scattering curves for 0.5wt% PEO5-PCL2, 0.5wt% PEO5-PCL4 and 1wt% PEO5-PCL4 respectively for the nanostructures after removed THF by dialysis, with corresponding fits.

Figure 33: Scattering curve from PEO5-PCL2 show no difference in the curves for the different mixing rates. The micelles are in equilibrium due to the shorter PCL chain.
From the scattering curves for PEO5-PCL2 no difference is seen for the different mixing rates, while for PEO5-PCL4 a lowering in the scattering intensity for the low Q-values is seen. It is interesting to find such differences in between the polymers with 2 and 4 kDa of PCL. This indicates that the polymer with PCL length of 4kDa is kinetically frozen in a non-equilibrium state, while the polymer of 2kDa PCL is dynamic and in equilibrium. Others have reported frozen micelles for core-formic blocks of only 1kDa for poly-(ethylene-propylene) PEP-PEO [65]. The reason why they observe frozen micelles for shorter blocks could be due to hydrophobicity and that PEP are considered as more hydrophobic than PCL, making the release of unimers into water-solution even more energetically unfavorable. More investigation can be done by comparing the interfacial tension for PEP and PCL. The surface tension of PEP was calculated with the same procedure as previously reported in this project from Hildebrand’s solubility parameter, $\delta_{PEP} \approx 8.5\text{MPa}^{1/2}$[66] to $\gamma=58\text{mN/m}$. Compared to previously calculated surface tension for PCL of 42mN/m (see Table 6) a higher surface tension is found for PEP, hence PEP is more hydrophobic than PCL. From equation 3 it is shown that the rate of unimer chains has a double exponential dependency of the surface tension and the molecular weight. Therefor in our system a longer PCL, hence a higher molecular weight of the hydrophobic block is needed to obtain the same unimer change rate as for the polymer consisting of PB.
The interfacial energies reported here are only rough estimates. Usually the measured interfacial tension is lower than the calculated values, hence lower interfacial energies can be found in literature. As an example the measured interfacial energy between water and PEP is measured to 46 mN/m [65] which is much lower than the calculated interfacial tension.

![Scattering curve from 1wt% PEO5-PCL4](image)

**Figure 35:** Scattering curve from 1wt% PEO5-PCL4 shows a difference in the curves for the different mixing rates. The nanoparticles are in non-equilibrium state due to the longer PCL chain.

The results from the fitting, using the spherical model, are plotted in Figure 36 to Figure 39 to show the effects of increasing mixing rates. The aggregation numbers for different mixing rates are shown in Figure 36. A clear trend of decreasing aggregation numbers vs increasing mixing rate is seen for PEO5-PCL4, while for PEO5-PCL2 no change is seen for the mixing rates.
Figure 36: A decrease in the aggregation number is seen for 1 and 0.5wt% PEO5-PCL4 for increasing mixing rates. For PEO5-PCL2 the aggregation number is approximately constant through all mixing rates.

From the SAXS measurements a corresponding decrease in both the size of the core and the corona was seen, shown in Figure 37, for increasing mixing rates. Johnson and Prud’homme investigated micellization kinetics by using an analytical confined impinging (CIJ) mixer for testing the effect of mixing speeds on particle size and also the effect of concentration of poly(butyl acrylate)-poly(acrylic acid) (PBA-PAA) [60]. They found that increasing the mixing rate lead to a decrease of the particle sizes. A limitation of the mixing speed was found, where further increasing of mixing speed would not have an effect on the sizes. In this mixing regime no effect of the concentration is seen. For the slower mixes, an increase of the polymer concentration leads to larger particles.

In our study, the nanoparticle sizes is decreasing with increasing mixing rate, as seen in the study by Johnson and Prud’homme[60], but no sign of the breakpoint for the mixing rate are seen for the data analysed here. Neither did we observe any clear size differences for the two concentrations. In the work of Johnson and Prud’homme they used mostly lower concentrations. It would have been interesting to analyse the 0.25wt% PEO5-PCL4 nanoparticles to compare, but due to irreproducible data this was not done. The scattering curves for these data can be found in the appendix F.
The SAXS data were fitted with a spherical core shell model also including polydispersity. In Figure 38 the polydispersity’s are plotted as a function of mixing rate, but no clear trend is seen for increased mixing rates.

The polydispersity is shown as the Gaussian width of aggregation numbers, and less polydispersity is seen for the dynamic micelles. The obtained nanostructural polydispersity’s from the SAXS fitting would be affected by the polydispersity of the polymers itself. The PDI for both polymers are calculated, and given in Table 2, where PEO5-PCL2 has a PDI of 1.28 and PEO5-PCL4 has a PDI of 1.19. (The linkage between PDI and Gaussian width is $PDI = 1 + \sigma^2$). But even though the polymer of the dynamic micelles has the highest polydispersity index, the micelles show a less polydisperse system than for the frozen micelles.
Another feature needed to be taken into consideration when talking about polydispersity is the SAXS model itself. Even though a conclusion of spheres was taken, the reality is that the hydrophilic fraction is close to the transition ratio of cylindrical micelles. Therefore it is not so unlikely that the nanoparticles could exhibit an ellipsoidal shape, which is a transition between the spherical and cylindrical form or that a mixture of spherical and ellipsoidal structures could be present.

From the investigation of the effect of organic solvent removal by dialysis, it can be seen that the size of the core is decreasing while the coronal size is increasing, showed in Figure 39. This observation may be due to that THF in PCL core will swell the core, and by removal of the solvent, the core will be more compact and shrink in size. The corresponding increase in size for the corona is due to an increase in steric repulsion between the PEO chains, due to less available surface area for each block on the core-corona interface, inducing stretching of the PEO chains.

The area per chain could be calculated:

\[ a_0 = \frac{4\pi R_c^2}{P} \quad (51) \]

where \( R_c \) is the radius of the core, and \( P \) is the aggregation number. A decrease in \( a_0 \) will cause steric hindering and hence stretching of the PEO chains giving a thicker PEO-corona. From Table 7 an increase of the chain area is seen for all samples during THF removal, but
also an increase is seen for the faster mixes. These results correspond with the increase seen for the corona thickness after THF removal, and also the decrease of the corona thickness for the faster mixes.

Table 7: Surface area per chain decreases with increasing mixing rates and is decreased after dialyzing for 0.5wt% PEO5-PCL4.

<table>
<thead>
<tr>
<th>Mix rate [mL/s]</th>
<th>$a_0$ before dialysis [Å$^2$]</th>
<th>$a_0$ after dialysis [Å$^2$]</th>
<th>decrease in $a_0$ during THF removal [Å$^2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>667</td>
<td>637</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>689</td>
<td>657</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>708</td>
<td>682</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>799</td>
<td>781</td>
<td>19</td>
</tr>
</tbody>
</table>

4.6 Stability

To be able to control the particles sizes is the first step of the procedure, but it would further be preferable if these particles were stable over a period of time. Therefore some of the samples were followed over a period of three days to search for structural changes in the scattering curves. The removal of THF is shown in Figure 40 for fast mix in A and hand-mixed sample in B, followed by scattering curves for fast mix and hand-mixed sample, with measurements done by three days time interval in between, respectively shown in C and D in the figure.
Removal of organic solvent leads to stability over time

**Figure 40:** A and B show how removal of solvent affects the scattering curve. C and D show that after removal of solvent no time effect on the structure could be seen for days. A and C describes the fastest mixing and B and C show the slow handmixing.

For the samples where THF was removed only by rotary evaporator, measured with a time interval of three days in between, some changes in the scattering curve could be seen. This is probably due to evaporation of THF, which was hard to prevent. For scattering curves, see appendix G. Some of the THF gathered in the core could swell the core. THF in the solvent could lead to instability for the nanoparticles by lowering the barrier for micellar unimer exchange. Hence the barrier for release of unimer into solution could be lowered due to lower interfacial energy between PCL and THF than for PCL and water.

For sample where the THF was further removed with dialysis, no structural changes were seen form the scattering curves within the time interval. The structures were stable both for
the fastest and the slow hand mixed sample, indicating a strong stability for these nanoparticles. Preferable these nanoparticles should be measured over a longer time interval. These samples were stored in a refrigerator at approximately 4°C, except during the measurements, where it was stored in room temperature.

**Temperature dependence**

The nanoparticles were tested if they were sensitive to temperature changes. PEO5-PCL4 was measured at different temperatures, but no changes in the scattering curves were detected, see Figure 41. They are stable up to 55°C. Higher temperatures were not chosen to avoid complications due to bobbles.

A slight increase in the shell thickness could be observed for the higher temperatures. This is due to lowering in interfacial energy equation between the water and PEO, provoking the chains to stretch out into the solution. This will not be very clear from the scattering curves since the effect is so small.

By heating the solution, the interfacial tension between PCL and water is also lowered. A question is if the interfacial tension is lowered so much that the frozen micelles will become dynamic. Since no structural changes are seen for higher temperatures this will not happen within the chosen temperature rage.

![Figure 41: No temperature effects for PEO5-PCL4 mixed at 8mL/s](image-url)
4.7 Reproducibility

One important feature when dealing with accurate control of mixing is reproducibility. It would be favorable if the structural characteristics could be controlled and be reproduced accurately, instead of nanoparticle structures which could be influenced by the person making it. By use of SFA we avoid these human errors, when dealing with mixing speeds. To investigate the reproducibility, several samples were made from the same polymer, the same concentration and by the same mixing rate on SFA for comparison. See for an example. The scattering curves are overlapping; hence no structural changes are seen.

Early in the project some samples were measured at ESRF in Grenoble, see Figure 43. Unfortunately these samples were not analyzed due to an unknown fraction of solvent in the core. These samples were not dialyzed and the remaining of THF was not followed by NMR. These data will only be used as an illustrative picture of the reproducibility for the samples mixed by SFA.

Comparing the data from measurements done at ESRF to the data measured at RECX lab at UiO they show good reproducibility for PEO5-PCL4, see Figure 43. There is approximately one year in- between the samples were made and measured at Uio and at ESRF.

The scattering curve for the fastest mixing rate, of 11mL/s, is similar for measurement performed at ESRF, to the measurement performed at RECX. The hand mixed samples,
approximately 0.5mL/s and 0.01mL/s show a small difference in their scattering curves. More data can be found in appendix G.

### 4.8 Loading of beta-carotene

An interesting ability to test for these particles was if they were able to encapsulate drugs. Theoretically it serves as a good nano-carrier for hydrophobic drugs like doxorubicin, which is a hydrophobic cancer drug. This drug is very toxic and careful attention has to be taken if working with this drug. To avoid this problem, a model drug can be used for testing [67], here beta-carotene was used in the starting face of testing the drug loading possibilities. The scattering curves for PEO5-PCL4 at different mixing rates are shown in Figure 44 for the nanoparticle itself and also for the samples containing beta-carotene.

![Graphs showing scattering curves for different mixing rates](image)

Figure 44: loading of beta carotene in PEO5-PCL4 at different mixing rates
For the samples mixed with SFA the scattering curves for loaded particles seems to be shifted a small step towards higher q-values. This indicates a larger particle, meaning that the beta-carotene may be gathered in the particle and causing it to swell. A difference between the slow hand mixed sample, and the fast mixed SFA samples, can be seen, which could indicate higher drug loading ability for the nanoparticles prepared by rapid mixing. This could indicate better drug loading for the faster mixes. Dashtimoghadam E. et al obtained, in their study of microfluidic mixing of chitosan, a higher drug loading efficiently for faster mixing[68]. A similarity to these findings may be what is observed also in this study, but more investigations have to be done to be able to confirm this. It is also important to mention that they used a different system, were laminar flow was used, so comparison of the results should be done with caution. In our study the amount of THF was not followed by NMR measurements and therefore not fitted. Ideally these measurements should be repeated with corresponding NMR measurements and fitted with the correct model.
5 Conclusions

In this work the molecular weights of PCL and PEO, with polydispersity were carefully calculated for the two polymers using NMR and GPC. The weights were in addition controlled by SAXS measurements that seemed to be in agreement with the calculated weights obtained from NMR and GPC. The hydrophilic ratios of the PEO-PCL were found to be 0.67 and 0.54, which indicates a spherical micelle shape. Hence the SAXS model used in this work was a spherical core-shell model including polydispersity and a fraction of solvent in the core.

The concentration effect was tested for PEO5-PCL4 with polymer concentrations of 0.5wt% and 1.0wt% in the mixed solution. No effects on structure were seen. However an increased size and aggregation number were observed when increasing the hydrophobic block length. From the SAXS analysis the sizes were found to increase from 11.2nm to 16.4nm and the aggregation number increased from 30 to 60. From the DLS measurements no clear difference was seen.

For PEO5-PCL2 the preparation method is not affecting the micelle structure because of the short PCL chain that allows for unimer changes between micelles, hence the system reach equilibrium and the structure is determined by thermodynamics. However, for PEO5-PCL4, the hydrophobic block length is sufficiently long enough to slow down the rate of unimer exchange to such an extent that we can say that the aggregation number is kept constant; hence a non-equilibrium frozen micelle is obtained. For these polymers the structure is dependent of the preparation method. For PEO5-PCL4 a decrease in the aggregation number from 65 to 40, and a decrease in $R_m$ from 16.5nm to 14.2nm, were seen.

By removing THF with both the rotary evaporator and dialysis, a faster and more careful removal of THF was obtained. The THF removal was followed by using NMR. Some remaining of THF could be seen for some of the samples, but the amount of THF in solution calculated from NMR is not in agreement with the amount of solvent found from the SAXS measurements. It is believed that this could be due to an underestimation of THF from the NMR measurements or because the assumption of spherical micelles is not fully correct. It could be that we have a mixture of spherical and ellipsoidal micelles. This has to be further investigated.
In the project we also confirmed that the nanoparticles formed were stable over a period of three days, and not much affected by increase of temperature up to 55°C. The prepared micelles were also well reproducible.

A small test of drug loading with beta-carotene as a model drug was performed. An interesting difference in the structure is seen for the micelles, when beta-carotene is dissolved in the organic solvent together with the polymer in prior to the mixing. This could indicate drug loading of the micelles and was mostly observed for the rapid mixes. These data are not fitted, therefore these results only give a vague indication, and more analysis has to be done before confirming this.
6 Perspectives

During this study we have focused on spherical, polydisperse nanostructures. To verify the morphology it would be very useful to do cryo-TEM. This method may also be a helpful tool to get an idea of the polydispersity or if larger aggregates are present. To get a more precise $M_w$ and PDI of the polymer used in this project, GPC should be done using DMF as the solvent.

The procedure of THF removal, combining rotary evaporator with dialysis, showed good results for THF removal. However, for many of the samples a small amount of THF was still in the solution after the dialysis. Here it is still room for improvement.

But the importance of a quick solvent removal was never investigated. For verifying this, some samples should also be made without using the rotary evaporation, only dialysis, to compare. Other solvent removal techniques could also be tested.

A slow relaxation time, $\tau_s$, was seen from the DLS measurements, but this was not described in detail. Often this slow mode was not very distinct and could be hard to distinguish from the fast relaxation time, $\tau_f$. More investigations of the slow mode should be done.

It would also be very interesting to test polymers with a longer PCL chain. In our study a polymer with a PCL block length of 13kDa was tested, but it precipitated out of solution. Preferably a polymer with a block length between 4kDa and 13kDa could be tested.

Further investigation of drug loading should be done. It would be interesting to see if the drug loading could be increased by using faster mixing.

A very interesting thing to do is to follow the kinetics of micelle-formation by directly connect SFA to SAXS. The kinetics of micelle-formation is very fast, in the order of microseconds to milliseconds, and need to be tested with more powerful X-ray sources.
References


44. http://www.materials.unsw.edu.au/tutorials/online-tutorials/5-crystalline-or-amorphous (08.05.2016).


Appendix:

Appendix A: NMR specifications

Some peaks were found in the NMR spectra that needed further investigation to give a determination of the origin for these peaks.

Figure 45: Some of the peaks near to 1.8ppm moves with the different PEO-PCL that are measured

From Figure 46 the undefined peaks are described from water and silicon grease. The water peak is moving most likely caused by temperature changes. The peaks from the grease are located at approximately 1.25ppm and between 0.84 to 0.87ppm. The intensity for the peaks is different between the two spectrums, the concentrations were not considered to be important, only the shift and the appearance of the peaks are important. From the water peak we get a singlet both in spectrum A and B, but the position of the peak is changing probably due to the unstable temperatures which were not controllable or detected for the instruments. But for the polymer spectrum, A, we can see a more broad peak, this can be due to hydrogen-bonds to PEO giving a more broad peak because water is in different environments, and this can have a higher shift because of less shielding of the protons in hydrogen bonding. For the peaks from the grease the position is similar for both spectrum A and B and also the multiplicity of the splitting looks similar. But within the peak located at 1.25ppm the peak for the hydroxyl group also is located and probably this is why we can see a more prong peak for the polymer spectrum.
Figure 46: The «undefined» peaks in the spectrums for pegpcl1 to pegpcl5 were believed to come from the high vacuum grease (silicon grease) and from water. To check our hypothesis we also measured a sample only containing grease and water showed in the lowermost spectra (The uppermost spectrum is from pegpcl2). The red marked peaks is the peaks comes from water and is moving probably due to temperature changes, the blue marked peaks is from the grease.
Appendix B: TEM images

Figure 47: TEM pictures, most small spheres of approximately 30 nm that is aggregated

Figure 48: TEM pictures of aggregates of spheres/elongated spheres of approximately 40 nm (NP12)

Figure 49: TEM pictures of aggregated spheres off approximately 40 nm, here it seems like there is more polydispersity (NP12)
Approach and instrumental for TEM imaging:
The suspensions of particles were absorbed on hexagonal formvar grids for about 2-3 min. Then they were washed four times with MQ-water, stained with 4% uranyl acetate and then dried. The imaging was performed with a Phillips transmission electron microscope CM100 (Philips, Eindhoven, Netherlands). The images were recorded digitally with a Quemsa TEM CCD camera (Olympus Soft Imaging Solutions, Germany) and iTEM software (Olympus Soft Imaging Solutions, Germany).
Appendix C: NMR, GPC and DSC measurements of PEO5-PCL13

The concept was to compare three polymers with different length of the hydrophobic block, PCL, to see if there was a trend. Unfortunately the polymer with the longest PCL chain precipitated out of solution in wanted concentration and could not be used. Some polymer analysis were performed in prior to this discovery and are shown here. Micelles were made with 0.2wt% for DSC measurement.

Figure 50: NMR spectra, PEO5-PCL13, $^1$H-NMR
Table 8: GPC and NMR results for PEO5-PCL13

<table>
<thead>
<tr>
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<tr>
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<tr>
<td>Mn (directly from GPC</td>
<td>13100</td>
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<tr>
<td>measurements) [g/mol]</td>
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</tr>
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<td>PDI (Mw/Mn)</td>
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<tr>
<td>Mn_{tot} (NMR) [g/mol]</td>
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<td>Mn_{PEG} (Sigma) [g/mol]</td>
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<tr>
<td>Mn_{PCL} (NMR) [g/mol]</td>
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<td>Mw (total, calculated) [g/mol]</td>
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<td>f(hydrophilic) from NMR</td>
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<td>Mw (PEG, sigma)</td>
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<tr>
<td>Mw (PCL)</td>
<td>18500</td>
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</table>

Figure 51: Heat capacity for PEO5-PCL13 show no peaks after removed THF only with rotary evaporator
Appendix D: DLS measurements

Many samples were measured by DLS; here are some of the representative samples for 0.5wt% PEO5-PCL2, 0.5wt% PEO5-PCL4 and 1wt% PEO5-PCL4

![Graphs showing DLS measurements for PEO5-PCL2 and PEO5-PCL4 with various flow rates and concentrations.](image-url)

Figure 52: DLS measurements PEO5-PCL2
Figure 53: DLS measurements 0.5wt% PEO5-PCL4
Figure 54: DLS measurements 1wt% PEO-PCL4
Slow relaxation modes

Samples were analyzed by two modes, but only one of the modes is used in the project. The slow mode was not very clear and for most of the measurements it was only shown for a couple of the lowest angles. For the higher angles very few points of the correlation function are used to determine the second mode, hence the sizes were fluctuating and not reliable. A couple of examples are shown here.

![Graph showing slow relaxation modes](image)

**Figure 55: Slow mode from DLS fitting, PEO5-PCL4**

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<tr>
<th>Q</th>
<th>A</th>
<th>tau1</th>
<th>beta1</th>
<th>tau2</th>
<th>beta 2</th>
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<td>1</td>
<td>12.971</td>
<td>0.722</td>
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<td>0.86</td>
<td>0.271</td>
<td>1</td>
<td>4.340</td>
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<td>0.92</td>
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<td>1</td>
<td>0.903</td>
<td>0.974</td>
</tr>
<tr>
<td>0.00187</td>
<td>0.89</td>
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<td>1</td>
<td>0.751</td>
<td>1</td>
</tr>
<tr>
<td>0.00212</td>
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<td>0.105</td>
<td>1</td>
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<td>0.960</td>
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<tr>
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<td>0.00249</td>
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<td>0.083</td>
<td>1</td>
<td>0.340</td>
<td>0.765</td>
</tr>
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</table>
The amplitude, $A$, was usually lower for the first angles, and increasing towards 1 for higher angles. For the fast relaxation mode $\beta$ was set to 1, changes of this value would not give better fitting. Beta 2 had a variety of values, but when $A$ is close to 1, these values contain a lot of error and are not so trustable.
Appendix E: GPC specification and determination of $M_w$, $M_n$ and PDI

From light scattering measurements the Molar mass for each “slice” in the chromatogram is calculated from the intensity and concentration giving us the total weight average molecular weight. The definition of the weight average molecular weight is given by:

$$
\bar{M}_w = \frac{\sum c_i M_i}{\sum c_i}
$$

And the number average molar mass is given by:

$$
\bar{M}_n = \frac{\sum c_i}{\sum c_i/M_i}
$$

The concentration is measured from the refractive index detector. $M_n$ is basically the total weight of all polymer in the sample divided by the numbers of particles, but for calculating the $M_w$, also the fact that larger particles contain more of the mass of the polymers than the smaller particles do, is taken into account.

The Polydispersity index, PDI, can be calculated:

$$
PDI = \frac{M_w}{M_n}
$$

There are other ways to calculate PDI using other molecular weights from GPC, but these methods will not be included as it is not used in this project. For perfectly monodisperse samples all the molar mass averages have the same value giving a PDI of 1.000.
Figure 57: Elution time for GPC measurements of pure PEO and a blank sample to see what caused the fluctuation in refractive index signal
Appendix F: SAXS measurements on 0.25wt% PEO5-PCL4

Micelles were prepared also with 0.25wt% PEO5-PCL4, but these data were not so reproducible and will not be given more attention. The reproducibility problems are believed to come from poor contrasts for the SAXS measurements. Still the same trend can be seen from the scattering curves, as for the higher concentration mixes.

Because of problems with reproducibility for the lowest concentration, these results were chosen not to be further analyzed. The reproducibility problems are believed to come from poor contrasts for the SAXS measurements. Still the trend can be seen from the scattering curves.
Appendix G: Reproducibility

The particles show good reproducibility, but a small deviation could be seen for the hand mix. This deviation can come from difference in THF removal, the difference in mixing speed or simply because hand mixes are harder to reproduce accurately.

Figure 58: testing of reproducibility. Different samples were made with different stem solutions to check for reproducibility.
Figure 59: Change in the particle structure is seen for the samples where the THF were not removed by dialysis.
## Appendix H: Polymer and THF concentrations

### Table 11: Polymer concentration and calculations on THF remaining in solution.

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<tr>
<th>nanoparticle number</th>
<th>mix rate [mL/s]</th>
<th>polymer concentration in stock solution [wt%]</th>
<th>polymer concentration [mg/mL]</th>
<th>polymer concentration wt%</th>
<th>THF in solution wt%</th>
<th>THF in core wt%</th>
<th>after dialysis</th>
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<td><strong>0.5wt% PEO5-PCL4 with betacarotene</strong></td>
<td>np69b 4</td>
<td>4.20</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>0.47</td>
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<td>-</td>
<td>-</td>
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<td></td>
<td>np74b hand 0.5</td>
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<td>0.33</td>
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<td>-</td>
<td>-</td>
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<td>np99 4</td>
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<td>0.085</td>
<td>26.40</td>
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<td>1.34</td>
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<td>1.15</td>
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</tbody>
</table>
## Appendix I: SAXS fitting parameters: spherical model

### Table 12: Spherical fitting for 0.5wt% PEO5-PCL2, $M_W$ (PEO) = 5040g/mol, $M_W$ (PCL) = 4200g/mol, $d_f$=1.7

<table>
<thead>
<tr>
<th>0.5wt% PEO5-PCL2</th>
<th>0.5mL/s before dialysis</th>
<th>0.5mL/s after dialysis</th>
<th>4mL/s before dialysis</th>
<th>4mL/s after dialysis</th>
<th>8mL/s before dialysis</th>
<th>8mL/s after dialysis</th>
<th>11mL/s before dialysis</th>
<th>11mL/s after dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;P&gt;</td>
<td>29</td>
<td>32</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>dR</td>
<td>62</td>
<td>68</td>
<td>62</td>
<td>61</td>
<td>65</td>
<td>67</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td>$R_c$</td>
<td>43</td>
<td>42</td>
<td>43</td>
<td>42</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.072</td>
<td>0.072</td>
<td>0.092</td>
<td>0.092</td>
<td>0.072</td>
<td>0.071</td>
<td>0.072</td>
<td>0.072</td>
</tr>
<tr>
<td>$R_g$</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>$\nu$</td>
<td>1.6</td>
<td>5.0</td>
<td>6.5</td>
<td>6.5</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>$f_{core}$</td>
<td>0.61</td>
<td>0.67</td>
<td>0.62</td>
<td>0.65</td>
<td>0.61</td>
<td>0.63</td>
<td>0.61</td>
<td>0.63</td>
</tr>
<tr>
<td>$\sigma_{int}$</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>$\sigma_{P,gauss}$</td>
<td>0.50</td>
<td>0.50</td>
<td>0.35</td>
<td>0.35</td>
<td>0.39</td>
<td>0.47</td>
<td>0.35</td>
<td>0.56</td>
</tr>
</tbody>
</table>

### Table 13: Spherical fitting 0.5wt% PEO5-PCL4, $M_W$ (PEO) = 5040g/mol, $M_W$ (PCL) = 5600g/mol, $d_f$=2

<table>
<thead>
<tr>
<th>0.5wt% PEO5-PCL4</th>
<th>0.5mL/s before dialysis</th>
<th>0.5mL/s after dialysis</th>
<th>2mL/s before dialysis</th>
<th>2mL/s after dialysis</th>
<th>4mL/s before dialysis</th>
<th>4mL/s after dialysis</th>
<th>11mL/s before dialysis</th>
<th>11mL/s after dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;P&gt;</td>
<td>64</td>
<td>64</td>
<td>59</td>
<td>59</td>
<td>55</td>
<td>55</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>dR</td>
<td>104</td>
<td>104</td>
<td>96</td>
<td>96</td>
<td>91</td>
<td>96</td>
<td>83</td>
<td>88</td>
</tr>
<tr>
<td>$R_c$</td>
<td>58</td>
<td>57</td>
<td>57</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>$R_g$</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>17</td>
<td>16</td>
<td>19</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>$\nu$</td>
<td>0.0032</td>
<td>3.3·10^{-6}</td>
<td>4.4·10^{-4}</td>
<td>2.0·10^{-4}</td>
<td>1.5·10^{-4}</td>
<td>0.0023</td>
<td>0.051</td>
<td>0.0016</td>
</tr>
<tr>
<td>$f_{core}$</td>
<td>0.70</td>
<td>0.75</td>
<td>0.69</td>
<td>0.73</td>
<td>0.69</td>
<td>0.73</td>
<td>0.69</td>
<td>0.71</td>
</tr>
<tr>
<td>$\sigma_{int}$</td>
<td>1.9</td>
<td>5</td>
<td>4.3</td>
<td>5.6</td>
<td>4.6</td>
<td>2.2</td>
<td>7.4</td>
<td>5.7</td>
</tr>
<tr>
<td>$\sigma_{P,gauss}$</td>
<td>0.99</td>
<td>0.99</td>
<td>0.83</td>
<td>0.83</td>
<td>0.7</td>
<td>0.7</td>
<td>0.74</td>
<td>0.74</td>
</tr>
</tbody>
</table>
Table 14: Spherical fitting for 0.5wt% PEO5-PCL4, \( M_W (PEO) = 5040 \text{g/mol}, M_W (PCL) = 5600 \text{g/mol}, d_f=2 \)

<table>
<thead>
<tr>
<th>1wt% PEO5-PCL4</th>
<th>0.5mL/s before dialysis</th>
<th>0.5mL/s after dialysis</th>
<th>2mL/s before dialysis</th>
<th>2mL/s after dialysis</th>
<th>4mL/s before dialysis</th>
<th>4mL/s after dialysis</th>
<th>8mL/s before dialysis</th>
<th>8mL/s after dialysis</th>
<th>11mL/s before dialysis</th>
<th>11mL/s after dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;P&gt;)</td>
<td>66</td>
<td>66</td>
<td>61</td>
<td>61</td>
<td>50</td>
<td>50</td>
<td>51</td>
<td>51</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>dR</td>
<td>95</td>
<td>99</td>
<td>90</td>
<td>96</td>
<td>88</td>
<td>93</td>
<td>85</td>
<td>87</td>
<td>81</td>
<td>85</td>
</tr>
<tr>
<td>(R_c)</td>
<td>59</td>
<td>58</td>
<td>58</td>
<td>56</td>
<td>54</td>
<td>53</td>
<td>54</td>
<td>54</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td>(\sigma)</td>
<td>0.065</td>
<td>0.064</td>
<td>0.067</td>
<td>0.067</td>
<td>0.064</td>
<td>0.064</td>
<td>0.065</td>
<td>0.065</td>
<td>0.085</td>
<td>0.085</td>
</tr>
<tr>
<td>(R_g)</td>
<td>17</td>
<td>16</td>
<td>19</td>
<td>18</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>(v)</td>
<td>0.0015</td>
<td>9.6 \times 10^{-4}</td>
<td>6.2 \times 10^{-4}</td>
<td>4.8 \times 10^{-4}</td>
<td>9.6 \times 10^{-4}</td>
<td>9.6 \times 10^{-4}</td>
<td>8.1 \times 10^{-4}</td>
<td>5.2 \times 10^{-4}</td>
<td>0.0041</td>
<td>2.9 \times 10^{-4}</td>
</tr>
<tr>
<td>(f_{core})</td>
<td>0.69</td>
<td>0.75</td>
<td>0.67</td>
<td>0.72</td>
<td>0.66</td>
<td>0.72</td>
<td>0.68</td>
<td>0.70</td>
<td>0.66</td>
<td>0.69</td>
</tr>
<tr>
<td>(\sigma_{int})</td>
<td>5.5</td>
<td>5.0</td>
<td>3.3</td>
<td>0.53</td>
<td>0.80</td>
<td>0.0027</td>
<td>11</td>
<td>4.4</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td>(\sigma_{P,\text{gauss}})</td>
<td>0.85</td>
<td>0.85</td>
<td>0.77</td>
<td>0.77</td>
<td>0.82</td>
<td>0.82</td>
<td>0.60</td>
<td>0.60</td>
<td>0.82</td>
<td>0.82</td>
</tr>
</tbody>
</table>