Towards Better Understanding of the Opaque Phase in the Self-Assembly of PBd-PEO Polymers

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Abstract

Nano-carriers have the potential to be an enormous game-changer in medicinal drug delivery systems. The polymeric nano-carriers used in this study are a product of the self-assembly of amphiphilic block copolymers, a complicated process which must be understood completely to finely tune the desired morphology for drug delivery. The goal of this thesis is to gain a better understanding of the self-assembly process of amphiphilic block copolymers. Specifically, it will focus on the 'opaque phase' observed for poly(1,2-butadiene)-b-poly(ethylene oxide) (PBd-PEO) block copolymers, which seems to occur in the early stages of the self-assembly process.

A nano-precipitation method has been developed at the TU Delft, which induces self-assembly and brings forward the opaque phase. The used block copolymer has a hydrophobic PBd block and a hydrophilic PEO block. This block copolymer dissolves well in acetone, but upon water (H_2O) addition, it starts to self-assemble into spherical aggregates, useful for drug delivery. At small volumes of H_2O, the opaque phase appears and disappears as more H_2O is added. In this thesis, multiple samples have been prepared with the so-called Inverse Nanoprecipitation method and different experimental parameters among which the volume percentage of H_2O present in the sample, have been varied. The samples have been studied using Visual Inspection, Dynamic Light Scattering and Spin Echo Small Angle Neutron Scattering.

The experiments show that the time intervals between H_2O addition do not affect the formation of aggregates, but rather the 'when' of adding the H_2O. If this is added to the acetone before the block copolymer is dissolved, it affects the self-assembly process. A visual experiment showed that the opaque phase occurred 1.2±0.1 vol% H_2O earlier than in previous research, which might be a result of the lower room temperature during this thesis. Another significant result might be that the addition of acetone-D_6 or D_2O affects the self-assembly process, which must be considered for future SESANS measurements.

Lastly, during the opaque phase a strong temperature sensitivity is observed (which was already found in previous research at TU Delft, by E. Remmelts and further researched by R. Baaijens), high light scattering intensities are detected with DLS and for SESANS measurements the scattered neutron intensities were low. These observations all strongly point to a theory called 'pre-micellization', which gives a better understanding of the opaque phase.

Contents

Acronyms

ARI Applied Radiation and Isotopes. 1

CMC critical micelle concentration. 3, 4, 28

DLS Dynamic Light Scattering. ii, 1, 4, 8, 9, 11, 12, 14, 17, 20, 22, 27, 32–34

INP Inverse Nanoprecipation. 1, 4, 8–11, 14, 15

PBd-PEO poly(1,2-butadiene)-b-poly(ethylene oxide). 2

RID Reactor Institute Delft. 1

SESANS Spin Echo Small Angle Neutron Scattering. ii, 1, 6–9, 12, 20, 23, 24, 27, 32–34

1. Introduction

Cancer remains to be a growing health concern, as many types have increased in incidence in the last 50 years [1]. There are three main treatments, surgery, radiotherapy and chemotherapy, which all have shortcomings. Not all tumours can be removed surgically, radiotherapy damages surrounding tissue and chemotherapeutic drugs lack tumour-specific interactions causing them to also target healthy cells [2, 3]. There is however a promising solution to the problem of chemotherapy, in the form of nano-carriers. These are tiny packages carrying medicinal drugs inside them. They find the tumour via passive targeting, in which the nano-carriers are passively drawn into the tumour via its leaky vessels, using a technique called enhanced permeability and retention (EPR). When accumulated in the tumour, they cannot escape because of the dysfunctional lymphatic drainage of tumours. Via active targeting, in which the nano-carriers are manipulated to bind to the receptors from the target cells, higher retention can be achieved. These two targeting methods allow the nano-carriers to safely release the drugs inside the tumour and thus minimise the toxicity of chemotherapy [4].

Some of the structures used as nano-carriers are products of the self-assembly of amphiphilic block copolymers, consisting of a hydrophobic polymer chain linked to a hydrophilic polymer chain [5]. In a solution containing water they can assemble into a sphere with a hydrophobic core; a micelle, or into a hollow sphere with a bilayer membrane; a polymersome which can carry both hydrophobic and hydrophilic drugs [6]. These structures are also called aggregates. In this study, block copolymers with a hydrophobic PBd block and a hydrophilic PEO block are used. With the PEO block on the outside of the sphere, interaction with blood proteins is prevented, which decreases immune reactivity and hence increases tumour uptake [7].

All these characteristics of nano-carriers, especially the ones with a PEO block, sound very promising. However, there are some problems: most systems lack reproducibility, size control and tumour-specific interaction [8]. To gain better insight into these problems, a broad project has been set up on the development of nano-carriers for targeted delivery of medicinal drugs to tumour tissue with polymeric systems, at the research group Applied Radiation and Isotopes (ARI) from Reactor Institute Delft (RID) at the Delft University of Technology. In 2018 [9] and 2019 [10], research was already performed on this project and is continued in this thesis. From these studies, it was concluded that block copolymers dissolved in acetone form an opaque phase when water is added to the sample using a method called Inverse Nanoprecipation. This phase disappears when the sample is heated up and appears again when cooled down.

A lot is still unknown about the opaque phase, creating a knowledge gap in the self-assembly process. Without the understanding of this phase, it is not possible to manipulate the design of the nano-carriers, making it hard to use them as controlled drug delivery systems. The goal of this thesis is to fill a part of the knowledge gap. By changing experimental parameters, such as the volume percentage of H_2O and analysing them with DLS and SESANS it is determined how the size and concentration of the aggregates in the samples are affected by these changes.

Chapter 2 explains the necessary theories and measuring methods. This is followed by Chapter 3, which describes the performed experiments and needed materials. The results of the experiments are shown and discussed in Chapter 4. Chapter 5 gives a summary of the results and states the conclusion. Concluding this thesis, Chapter 6 offers recommendations for further research.

2. Theory

2.1. Poly(1,2-butadiene)-b-poly(ethylene oxide)

The polymer used is an amphiphilic block copolymer: a special kind of diblock copolymer. The best way to sketch what this type of block copolymer looks like is by imagining that one has blue building blocks, that love water (i.e. hydrophilic) and red building blocks that are afraid of water (i.e. hydrophobic). One building block is called a monomer, and a chain of multiple building blocks of the same colour is called a polymer. When a blue chain and a red chain are linked to each other this is called a diblock copolymer, but in the special case that one of these chains is hydrophilic and the other is hydrophobic, the total polymer is called an amphiphilic block copolymer. The polymer used in this study is poly(1,2-butadiene)-b-poly(ethylene oxide), with its chemical structure shown in Figure 2.1. The hydrophobic block is the polybutadiene block (hereafter referred to as the PBd block), and the hydrophilic part is the polyethylene oxide block (henceforth called the PEO block). The PBd block has molecular weight n and the PEO block has molecular weight m, denoted as $\mathrm{PBd}_n\mathrm{PEO}_m$ [9, 11].

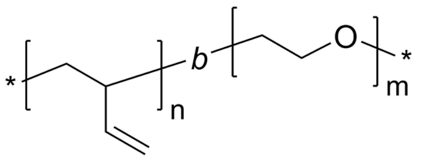

Figure 2.1 – The molecular structure of poly(1,2-butadiene)-b-poly(ethylene oxide). In the left brackets, the poly(1,2-butadiene) or PBd structure is shown and has molecular weight n. On the right, the poly(ethylene oxide) or PEO structure is displayed with molecular weight m [12].

Due to the combination of hydrophobic and hydrophilic parts, the block copolymer can self-assemble in solvents containing an organic solvent and water (H_2O). Self-assembly means that the block copolymer tends to spontaneously create a well-defined structure, also called an aggregate. [6]. This can result in various spatial organisations, depending on the chemical composition and length of the blocks. The system can be under thermodynamic or kinetic control. Under thermodynamic control, the block copolymer composition and the temperature regulate the aggregate's form. Under kinetic control, the chain dynamics in the hydrophobic region and hydrodynamic interaction between the block copolymers determine the rate of aggregation in the system. The self-assembled structures are held together by relatively weak forces, that can form and break without significant activation barriers. Because of this property, the thermodynamic equilibrium of the block copolymer system can be influenced in such a way that it will stay in a kinetically trapped state [9]. If the system is in thermodynamic equilibrium, one can predict the type of aggregate that will be formed using the packaging parameter $p = \frac{v}{a_0 l_c}$, with v the PEO block volume, a_0 the occupied area by the PBd headgroup, and l_c the length of the molecule. The three possible types are [8]:

- $p < \frac{1}{3}$, spherical micelles
- $\frac{1}{3} < p < \frac{1}{2}$, wormlike micelles
- $p > 1/2$, polymersomes or vesicles

In Figure 2.2 a sketch of the different forms is shown for visualisation purposes.

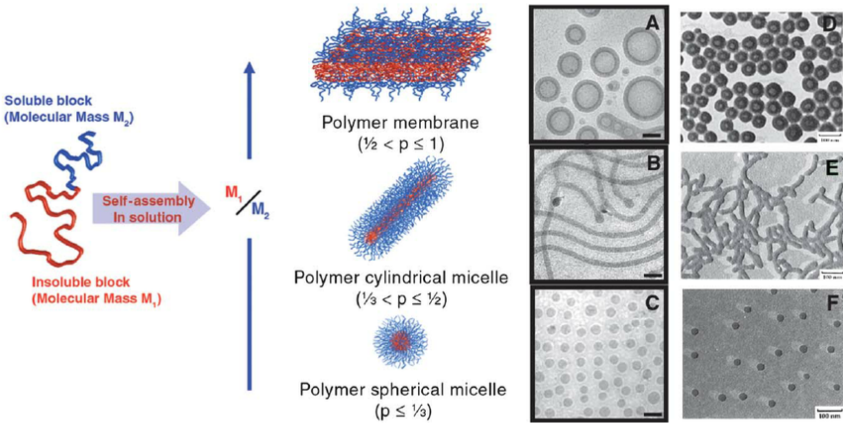

Figure 2.2 – On the left, a regular amphiphilic block copolymer is shown before self-assembly. After self-assembly three different structures can be formed. Sketches of these structures are shown in the middle and the figures A to F display how the structures look, seen under a microscope [13]

2.2. Critical Concentrations

Normally a very important property of polymeric solutions is the critical micelle concentration (CMC), which is the surfactant (an amphiphilic molecule) concentration above which the polymers tend to self-assemble into aggregates (micelles or polymersomes). To reach this concentration more surfactants need to be added to the solvent. However, in this study, it is not the surfactant level that is adjusted, but the H_2O levels are. A better way to characterise the samples, in this study, is by their ‘critical water concentration’. When H_2O is added to a solvent containing the PBd-PEO block copolymer, the quality of the solvent decreases for the PBd block. At the critical water concentration, the PBd blocks start to attract each other and link into chains to minimise interactions with H_2O, thus facing away from the H_2O. Meanwhile, the PEO block likes to interact with the H_2O and faces itself towards the H_2O. Thus forming either a micelle in which the PEO shell separates the PBd core and bulk water or a polymersome that encapsulates the H_2O present in the solvent [14, 15].

Around the CMC not a lot of research has been performed, because some of the system’s properties (like sensitivity to temperature change and instability of aggregate size) make it hard to characterise the system. A promising theory for what happens near the CMC

is pre-micellization. This is a molecular pre-aggregation stage before the polymers form aggregates. In this stage, there are many surfactant-accelerated reactions with surfactant concentrations below CMC [16]. Properties of this phase are the sensitiveness to temperature change, instability of aggregate size over time, high light scattering intensity peaks for DLS at low vol% H_2O and low intensity for neutron scattering measurements [17].
During the opaque phase observed when H_2O is added to a PBd-PEO sample, the system acts the same as a system close to the CMC. Therefore, the pre-micellization theory might also be valid for samples in which the H_2O volume is adjusted. The critical water concentration stage then coincides with the opaque phase [18].

2.3. Inverse Nanoprecipitation

A rapid way of preparing samples in which aggregates, like polymersomes or micelles, form is Inverse Nanoprecipitation (INP). To make the polymers self-assemble they are first dissolved in an organic solvent, a good solvent for both blocks. When a nonsolvent for the PBd block is pipetted to this solution, the quality of the solution decreases for the PBd block and when the CMC is reached the polymers start to form aggregates, as described in Chapter 2.2. Optionally, the structures can be 'frozen' (kinetically trapped state) by quenching the aggregated in an excess of aqueous solution [9, 19].
For this thesis, acetone is used as an organic solvent and H_2O as a nonsolvent for the PBd block.

2.4. Dynamic Light Scattering

The first characterisation method is Dynamic Light Scattering (DLS). This method provides a way to analyse the bulk of the sample and determine the mean radius, the hydrodynamic radius, of the aggregates in the samples. An example of an experimental setup is shown in Figure 2.3.

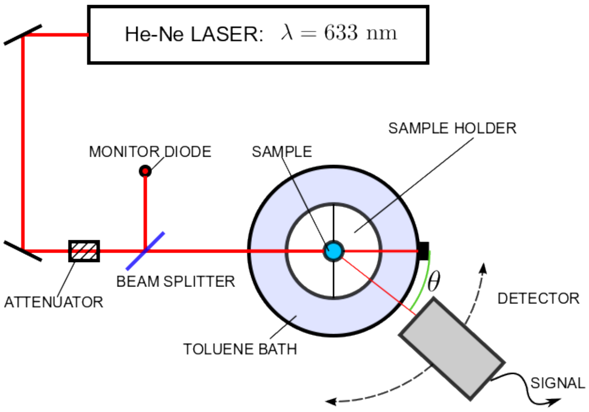

Figure 2.3 – A schematic overview of the experimental setup of the DLS. With mirrors, the laser is guided to the sample. When the laser reaches the sample, the light scatters in every direction. This is detected by the detector, placed at a certain angle, which sends the signal to the ALV-Correlator. [20].

A monochromatic beam is guided through a sample, causing the light to scatter in a certain pattern affected by the size and shape of the aggregates. The scattered intensity can be measured statically and dynamically, with the difference being that a dynamic measurement looks at the intensity fluctuations over time caused by the random motion of the particles (Brownian motion) whereas the static measurement only uses the scattered intensity at one moment. For a dynamic measurement, the detector records the intensity for some time, 30 seconds in this study, and then sends this to an autocorrelator. Here the intensity fluctuations are correlated to time and one can determine how rapidly the intensity fluctuates. The result is a second-order correlation function, which describes the motion of the aggregates and is expressed as

$$G_2(\tau) =< I(t)I(t+\tau) > . \tag{2.1}$$

Here t is the indicator for time, $(t+\tau)$ is the delayed time and τ is the lag time. A normalisation is needed to perform further calculations with this value, resulting in

$$g_2(\tau) = \frac{< I(t)I(t+\tau) >}{< I(t) >^2}. \tag{2.2}$$

Via the Siegert relation, a neat expression for $g_2(\tau)$ is obtained, from which we can almost calculate the hydrodynamic radius. The new function is

$$g_2(\tau) = 1 + \beta e^{-2D_\tau q^2 \tau}. \tag{2.3}$$

In which D_τ is the diffusion constant and q is the Bragg wave vector, written as

$$q = \frac{4\pi n}{\lambda} sin(\frac{\theta}{2}). \tag{2.4}$$

With the refractive index n, the wavelength λ and the angle at which the detector is placed as θ. By using the Stokes-Einstein equation, the hydrodynamic radius can be retrieved and is shown in equation 2.5.

$$R_h = \frac{k_B}{T} 6\pi\eta D_\tau. \tag{2.5}$$

Where k_B is the Boltzmann coefficient, T is the absolute temperature and η is the viscosity of the medium. [21, 22, 23]

2.4.1. Viscosity and Refractive Index

The samples are a mixture of acetone and H_2O, this changes the viscosity and refractive index of the system as seen in equations 2.4 and 2.5. For the viscosity the values stated in Dizechl et al [24] are used and for the refractive index, the values found in Kurtz et al [25] are used. These datasets do not contain the exact values for every ratio of acetone and H_2O, thus they are interpolated with the interpolate function from the scipy library [26] in Python. The viscosity depends on two variables, the acetone/H_2O ratio and the temperature, thus a 2D interpolation is needed. This is calculated with the scipy function 'interpolate.interp2d()', which automatically accounts for a two-variable dependency [26].

For the refractive index the temperature dependence is assumed to be very small (there is only a 0.002 decrease for a change from 0°C to 30°C) and is therefore not taken into account in the interpolation [27]. Thus a normal 1D interpolation is used for the refractive index, with the scipy function 'interpolate.interp1d()'. The results of these interpolations are shown in Figure 2.4.

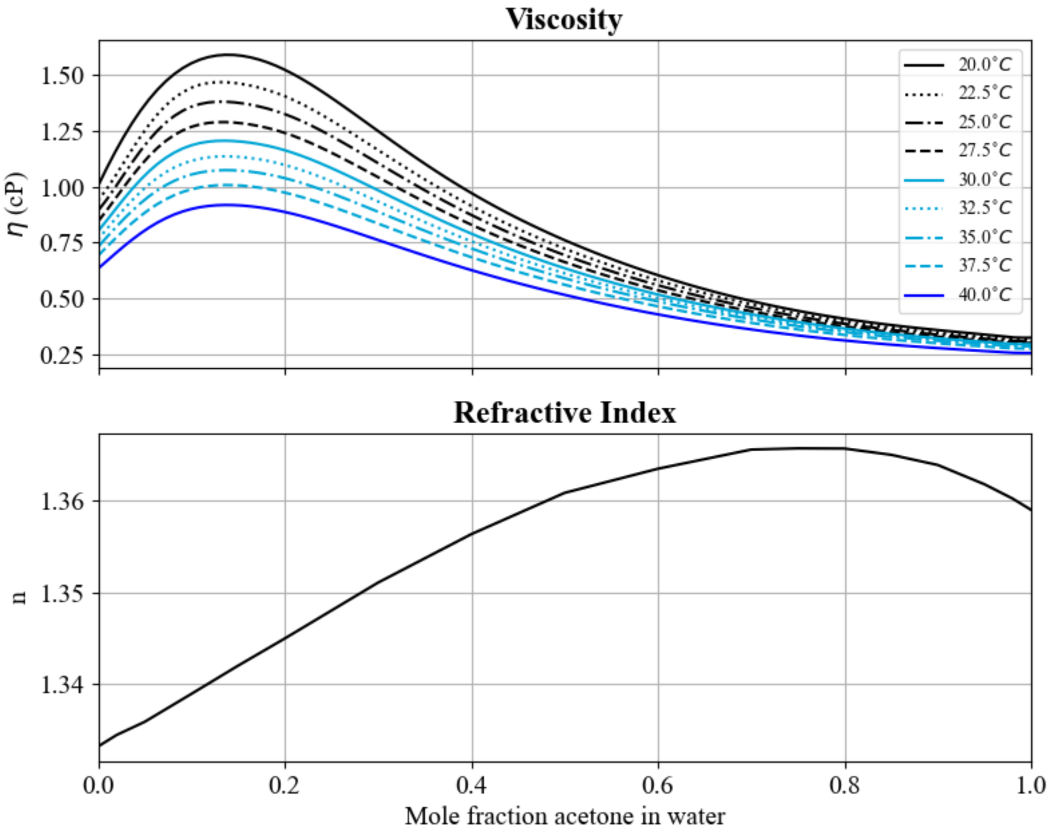

Figure 2.4 – The interpolations on the viscosity and refractive index, for an acetone-H_2O mixture.

2.5. Spin Echo Small Angle Neutron Scattering

Spin Echo Small Angle Neutron Scattering (SESANS) uses Larmor precession angle encoding to determine the size of particles in a solution. How a SESANS line-up schematically looks is displayed/shown in Figure 2.5.

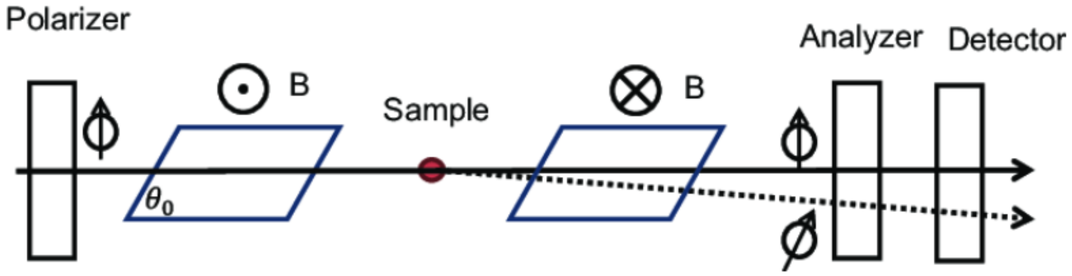

Figure 2.5 – The schematic setup of a SESANS measurement. The polariser prepares the neutron beam in a spin-up state. A perpendicular magnetic field introduces Larmor precession along the length of the first magnetic field. In the second magnetic field, with an opposite sign, the neutrons are rotated back and the field is tuned such that the remaining Larmor Phase is zero at the position of the analyser. When there is no sample the remaining phase is zero for all neutrons, but when there is a sample some of the neutrons are deflected and will change the phase and reduce the polarisation for the whole beam.

A neutron beam is transported through a polariser. This polariser forces the neutrons to choose between the 'spin-up' state or the 'spin-down' state, and only allows the neutrons in the spin-up state through. All neutrons left are now in a spin-up state and are guided through the first magnetic field that is orientated perpendicular to the magnetic moment of the neutrons. As a result of this field, a force starts to act on the neutrons. This causes the magnetic moment to spin, also called Larmor precession, in one direction for a certain number of times. This is the build-up of the precession phase, say for example 1000 full rotations. When there is no sample in the middle of the line-up, the spun neutrons pass through a second magnetic field. This field exerts a force in the opposite direction, like the field before, and spins them back the same number of times that they spun in the first magnetic field. When the setup is ideal, all neutrons are exactly back in the spin-up state. However, if one places a sample between the magnetic field, the scattered neutrons will slightly alter the path of the neutrons in the field and therefore pick up another precession phase.

This precession difference is indicated by ϕ and relates to the wave vector transfer Q_Z (equation 2.8) and the spin-echo length Z (equation 2.7) as shown in equation 2.6. It thus measures the influence of the sample on the neutrons' spin, also called the 'echo' of the spin. This results in a Spin-Echo measurement of neutrons scattered on a small angle.

$$\phi = \phi_1 - \phi_2 \approx c\lambda BL\theta_s < cot(\theta_0) >_\alpha \equiv ZQ_Z \tag{2.6}$$

$$Z = \frac{c\lambda^2 L < cot(\theta_0) >_\alpha}{2\pi} \tag{2.7}$$

$$Q_z = \frac{2\pi\theta_s}{\lambda} \tag{2.8}$$

Note that the setup is most likely not ideal, so a measurement without a sample needs to be made as a reference for the measurements with samples. With this reference point, the others need to be normalised by dividing them, which is done in equation 2.9.

$$\frac{P_Z(Z)}{P_0} = cos(\phi) = cos(ZQ_Z) \tag{2.9}$$

In the case of single scattering, the normalised polarisation is coupled to the SESANS correlation function $G(Z)$, which gives the probability of finding an equal scattering cross-section at points having a mutual distance Z in the samples. The obtained raw data from the SESANS measurement needs to be reduced via a Python script and can then be analysed with SasView, to obtain the radius of the aggregates.

2.6. Relevance

For a clear overview, this section will highlight the relevance of explained methods, such as INP, DLS and SESANS, for this thesis and thus for the sample characterisation.
INP serves as a rapid way to prepare the samples and let them form aggregates, but more importantly, this is the only sample preparation method in which the opaque phase occurs, making it an essential method for this study. The first characterisation method is DLS, with measures the bulk of the sample and thus gives information about the mean particle size in the samples. The second characterisation method is SESANS, which can analyse quite large structures ranging from 5 nm to 20 µm [28].
A possible theory, which can connect all obtained results at low vol% H_2O, is pre-micellization.

3. Methods and Materials

3.1. Materials

For a quick overview, the solvents and polymers are displayed in Table 3.1, with their specifications and manufacturer. The needed hardware is displayed in Table 3.2, which shows the materials needed per experiment along with their specifications and manufacturer. Some of the materials are marked with an '*', meaning that they are optional and only used in an experiment when mentioned.

Table 3.1 – An overview of the needed solvents and polymers, their manufacturers, their purity, and, if it applies, their rate of D-enrichment.

Materials	**Manufacturer**	**Purity**	**D-enrichment**
Milli-Q water (H_2O)	Merck Millipore	n.a.	n.a.
Deuterium oxide (D_2O)	Sigma Aldrich	99.9%	99.0%
Acetone (C_3H_6O)	Sigma Aldrich	99.9%	n.a.
Acetone-D_6 (C_3D_6O)	Sigma Aldrich	99.9%	99.0%
PB_{1700}d-PEO_{1000}	Polymer Source Inc.	$\frac{M_w}{M_n} = 1.04$	n.a.
PB_{1800}d-PEO_{900}	Polymer Source Inc.	$\frac{M_w}{M_n} = 1.04$	n.a.

Table 3.2 – The hardware used per experiment, and their most important parameters. The materials marked with an '*' are optional and are only used when explicitly stated.

Materials	**Experiment**	**Specification**	**Manufacturer**
Vial	INP	4 and 20ml	-
Magnetic stirrer and beads		300rpm	-
Syringe and needle*		2 to 5ml	-
Syringe filter*		0.20μm PTFE	-
Weighing scale		Error = 1mg	Mettler Toledo
Borosilicate tube	DLS	D = 10mm	Corning Inc
Quartz SUPRASIL® 300 cuvette	SESANS	Pathlength 2mm	Hellma Analytics
Pipette	all	2-20μl and 5-50μl	Thermo Fisher Scientific
Pipette		10-100μl	Gilson
Parafilm M		-	American National Can

3.2. INP

The method to prepare the samples for further experimentation was Inverse Nanoprecipation (INP). During this process, the acetone-block copolymer solution was made and the vol% of H_2O in the solution was chosen. All samples contained 20 mg/ml of the block copolymer. Stock solutions were prepared by weighing 20 mg of the block copolymer, times the number of ml acetone. After the acetone was added, the block copolymer dissolved within 5 minutes.

When the block copolymer was dissolved, 1 ml of the stock solvent was pipetted into a smaller vial of 4 ml. In the case that a syringe filter was used, 1 ml of stock solvent was sucked into a syringe with a needle. Then the syringe filter was put onto the syringe and it was emptied into a 4 ml vial.

A magnetic bead was put into the sample and the magnetic stirrer was set to 300 rpm. The H_2O was pipetted into the sample drop by drop under this magnetic stirring. In Table 3.3 an overview is given, that shows at which volume percentages different experiments were performed. The experiments 'Initial water' and 'Kinetics' were all prepared with the standard INP method, but for the 'Acetone-water solvent' experiment an acetone-H_2O solvent was made to dissolve the block copolymer. For the 'Increased water volume' experiment one sample was prepared at 3vol% H_2O. Hereafter the H_2O percentage in the sample was increased by 1vol%, corresponding to 10 µl H_2O.

Table 3.3 – An overview of the percentages at which the samples were prepared. A checkmark means that the sample was directly prepared with INP at the corresponding vol% H_2O. The up arrow indicates that the sample was made from its predecessor with an H_2O increase. A stripe indicates that no sample was prepared at that vol% H_2O.

Percentages %	**Initial water**	**Increased water volume**	**Acetone-water solvent**	**Kinetics**
0.01	-	-	✓	-
1	-	-	✓	-
3	✓	✓	✓	-
4	-	↑	-	✓
5	✓	↑	✓	✓
6	-	↑	-	✓
7	-	↑	-	-
8	✓	↑	✓	✓
9	-	↑	-	-
10	✓	↑	✓	✓
11	-	↑	-	-
12	-	↑	-	✓
>12	-	↑	-	-

The experiments described above all had acetone as organic solvent and H_2O as nonsolvent. For a D_2O/H_2O or acetone-D_6/acetone contrast series additional steps were taken during INP.

For the D_2O/H_2O contrast series, 0.5 ml of water mixture was prepared at the ratios D_2O/H_2O:100/0 to 0/100 with steps of 10. The block copolymers were dissolved in the organic solvent acetone-D_6 and then 6vol% of the water mixture was pipetted into the sample. A sample was prepared for each ratio, making 11 samples in total for this contrast series. In the acetone-D_6/acetone contrast series, the organic solvent was a mixture of acetone-D_6/acetone, at the ratios 0,100 10/90, 20/80, 30/70 and 40/60. The block copolymers were dissolved in the acetone mixture, after which 6vol% of H_2O was pipetted into the samples.

For all samples, the temperature at which they were prepared was room temperature (293±1 K).

3.3. Visual Inspection

With the INP method, the opaque phase was analysed by simply observing the sample. For this experiment 80mg of the block copolymer, was dissolved into 4ml of pure acetone. First, 2vol% H_2O was added. For an exact as possible determination of the opaque phase, an increment of 0.1vol% H_2O was used. When a change occurs in the sample, a photo was taken and the exact percentage was documented. This process was continued up to a value of 25vol% H_2O [9].

3.4. DLS

After the samples were prepared with INP, they were measured with DLS. At least 1ml sample was pipetted into a borosilicate tube used for DLS measurements. On the computer, the ALV-correlator software was started, and a background intensity was measured. If this value is below 14kHz, more toluene was pipetted into the toluene bath. Hereafter the samples were measured. To make sure that the diode does not break, the diode count was first lowered to 3000 and was then adjusted until a light scattering intensity around 100kHz was reached. The optimal value of the diode count was written down. After the measurement was done, the data was saved.

The saved data was then analysed via the Python script, found in Appendix A. This script automatically adjusts each experimental parameter to the right value in the ALV-Correlator Software, obtains the hydrodynamic radius by performing a CONTIN fit in the ALV-Correlator Software and saves the fitted data in the .xlsx format.

3.4.1. Equipment Specifications and Settings

Some of the specifications of this experiment cannot be changed, because they are determined by the hardware, or by how the hardware is placed. In the ALV-Correlator Software, a lot of parameters can be varied. Below an overview of these hardware specifications and the software settings is listed [9, 10].

Hardware and specifications

- Perking Elmer photon counter
- ALV-599/epp correlator
- Laser: JDS uniphase 633nm 35mW
- Goniometer: ALB sp 125 s/w 93
- Angle: 89.817°
- Temperature: room T (293K)

ALV-Correlator software settings

- Measurement duration: 30s
- Fit method: regularised $g_2(\tau)$ (CONTIN)
- Number of Gridpoints: 250
- X-axis fit: radius unweighted
- Refractive index: interpolation acetone/water
- Viscosity: interpolation acetone/water

3.4.2. Experiments

The initial water samples, the acetone-water solvent samples and the contrast series samples were all measured once with DLS. The increased water volume samples were measured after each increment.

The kinetics samples were measured once every 45 minutes over the course of 5 hours and 30 minutes.

For all samples, the time between the start of the sample preparation and the first measurement was around 30 minutes.

3.5. SESANS

When the samples were ready, they were pipetted into a Quartz SUPRASIL® 300 cuvette. The minimal height of the samples must be 10mm because this is the height of the neutron beam. Since the path length of the cuvette is 2mm, the minimal amount of solution needed is 0.4ml. Now the samples can be put into a holder, located in the neutron beam path of the SESANS apparatus. In the computer, the locations of the samples are programmed so that for each magnetic field strength, every sample and an empty location are measured. These measurements were done overnight because they took several hours to complete [9, 10].

SasView

The raw data obtained was first reduced with a Python script. This reduced data were then fitted with SasView (version 5.0.4). For the shape category and for the model a sphere was chosen. The fixed parameters are the sld (of the sphere), $\text{sld}_{solvent}$ and the background noise. The fit parameters were the scale and the radius.

The background was set to 0.0cm^{-1}. Both slds were calculated via the Python script found in Appendix B. In Table 3.4 the slds per D_2O/H_2O ratio are given. A very simple model is used, in which the total volume of the block copolymer and water mixture is found in the sphere and the total volume of the acetone-D_6 is found in the solution.

Table 3.4 – The calculated slds for the sphere and the solvent per D_2O/H_2O ratio. A model of a sphere is used in which a fraction of 0% acetone-D_6, 100% D_2O/H_2O and 100% block copolymer are present in the sphere. The remaining part of their volumes is present in the solvent.

D_2O/H_2O	sld_{sphere} (10^{-6}Å^{-2})	$\text{sld}_{solvent}$ (10^{-6}Å^{-2})
100/0	4.99	5.39
90/10	4.46	5.39
80/20	3.93	5.39
70/30	3.40	5.39
60/40	2.87	5.39
50/50	2.34	5.39

4. Results and Discussion

4.1. PBd_{1700}-PEO_{1000}

4.1.1. Visual Inspection

A new batch of the block copolymer was used for this thesis, compared to previous research performed by this research group [9, 10]. To see if the block copolymer reacts the same as in previous research a visual check was performed. The block copolymer was dissolved in 99.9% pure acetone and afterwards, H_2O was pipetted into the sample. A sample was prepared at 2vol% H_2O. With steps of 0.1vol%, the H_2O volume was increased and when a visible change occurred, a picture was taken (see Figure 4.1). At 2vol% the sample was still clear, but at 3.5vol% it started to become a little opaque. At 4.1vol% up to 6.7vol%, the sample reached its peak in opaqueness. From 6.7vol%, the opaqueness decreased until it became clear with a white hue at 10.0vol%. All found values have an error of ±0.1vol%. To summarise, four phases in opaqueness were observed: a somewhat cloudy phase between 3.5vol% and 4.1vol%, the opaque phase from 4.1vol% up to 6.7vol%, again a somewhat cloudy phase from 6.7vol% up to 10.0vol%, and from this point on until the end of the experiment (25vol%) there was a clear white phase, which is known to correspond to the self-assemblies.
Previous research has yielded different values. The first visual change and with it the opaque phase occurred at 5.2vol% and started to disappear again at 7.6vol%. At 11.3vol%, the solution became transparent [9]. When these values are compared, all changes seem to happen 1.2±0.1vol% later, the first cloudy phase observed in this experiment did not occur before, and the samples of previous research turned completely transparent, instead of transparent white like the samples of this experiment. There are two notable differences in sample preparation. The first is the usage of a syringe filter. However, the same experiments have been performed with and without the syringe filter and no significant differences were found. The second is the temperature. Previous research observed that the opaque phase disappears upon heating the sample [10].

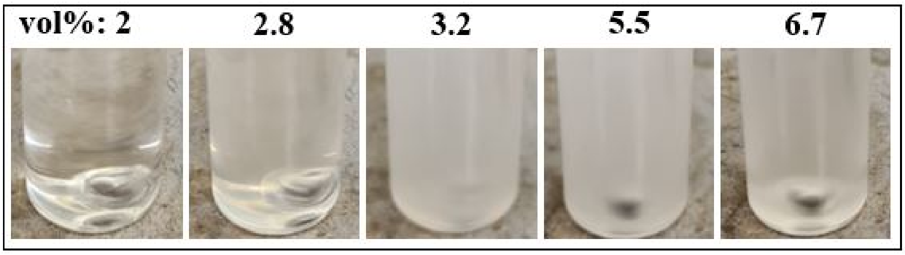

Figure 4.1 – An overview of the visual changes that occur to the PBd_{1700}-PEO_{1000} sample upon H_2O addition. Above the photos, their respective vol% of H_2O is given. They all have an error of ±0.1vol%. In all pictures, except the middle one, the magnetic bead used for magnetic stirring is visible.

4.1.2. DLS

To see how the samples react upon H_2O addition, an experiment was performed in which the vol% of H_2O was gradually increased and then the sample was measured with DLS. The samples were prepared with INP and the starting percentage was 3vol% H_2O. For these samples, a syringe filter was used after the block copolymer was dissolved into acetone before H_2O was added.
First, the hydrodynamic radius was obtained for the different H_2O concentrations. Two main different types of graphs were obtained. In Figure 4.2 A, a graph is shown in which one sharp peak is visible. This indicates that the system consists of structures of the same size and shape, and is thus monodisperse. In the second one, shown in Figure 4.2 B, two peaks are visible. One is very wide ($> 10^1$ nm) and there is a second small peak. This shows that the system is polydisperse, i.e. there are structures of different sizes, and they might have different shapes [22].

In the case of polydispersity, a plot with the corrected light scattering intensity is made, because this gives a better indication of whether the structures increased or decreased in size. A reference diode count was chosen and the ratio between reference diode count and actual diode count was determined. Since the light scattering intensity and diode count scale linearly, this factor can be used to correct the light scattering intensity for each sample. A higher light scattering intensity corresponds to larger particles and/or a larger concentration. Both of these graphs give information about the samples, however, the results become unreliable for radii above 1µm.

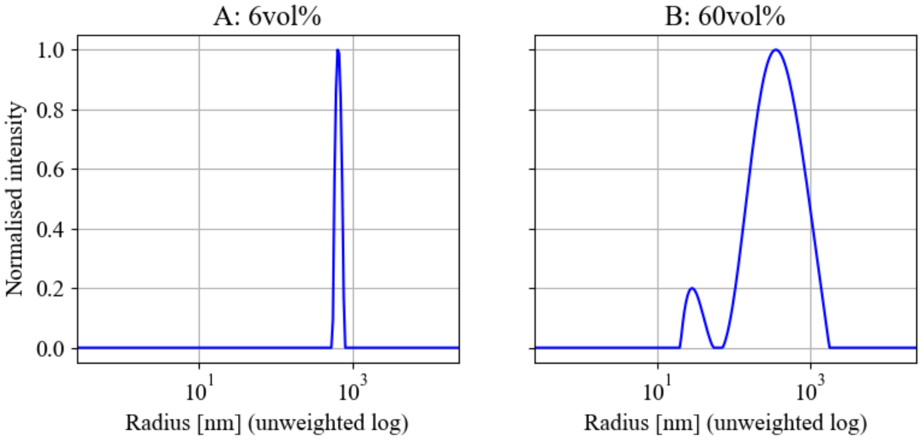

Figure 4.2 – An example of what the two most often obtained regularised fits from DLS look like. On the left (A) a graph is shown for a monodisperse system and on the right (B) a graph is shown for a polydisperse system.

Water Solution

For the $PBd_{1700}PEO_{1000}$ increased water volume samples, multiple peaks were observed for some percentages and because there are so many data sets only the graph of the corrected intensity was found useful and is shown in Figure 4.3. As expected from the theory [15] the intensity and thus the radius seems to stabilise above 19vol% H_2O. After each H_2O increment, the samples were shaken lightly for about 10 seconds. The first sample was measured 30 minutes after the start of the sample preparation.

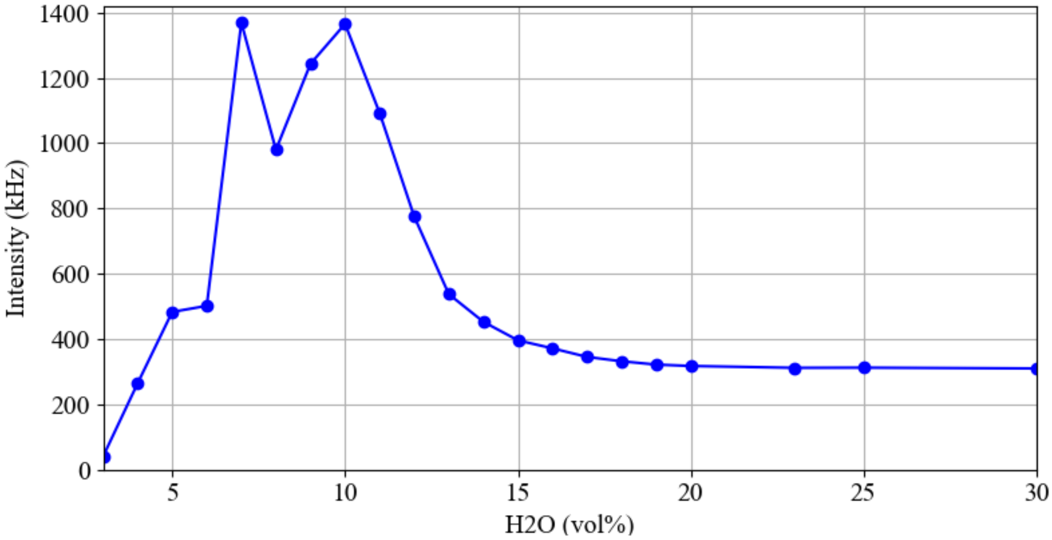

Figure 4.3 – The corrected light scattering intensity for a $PBd_{1700}PEO_{1000}$ sample prepared with INP, starting from 3vol% H_2O. For the first sample, the time between preparation and the measurement was around 30 minutes and after each H_2O volume increment, a measurement was performed after 10 seconds of lightly shaking the samples.

Acetone Water Solution

Normally the block copolymer is dissolved in acetone, in which both polymers are soluble. Thus the block copolymer dissolves quite fast and is ready for water addition. To see how the self-assembly is affected when H_2O is already in the solvent before the block copolymer is added, the acetone-water experiment was performed. Whilst preparing the samples with INP it became clear that the more H_2O already present in the solvent the harder it is for the block copolymer to dissolve or form aggregates. This seemed to happen from 5vol% H_2O and higher. For the 10vol% H_2O, it took somewhere between 30-45 minutes for the block copolymer to dissolve, with dissolve meaning that visually no block copolymer leftovers were seen. It was assumed that they either dissolved as unimers or formed aggregates. As a reference, the regularised fit is shown in Figure 4.4. For low volume percentages of H_2O, 3vol% or smaller, the block copolymer seems to dissolve into unimers and from 5vol% the block copolymer was able to form large aggregates. This is in agreement with the corrected intensity data, plotted in Figure 4.5. For the first three, the corrected intensity is almost zero, thus no aggregates are formed by the block copolymer. From 5vol% H_2O and on the intensity increases and there are aggregates to be found in the samples. Though these values seem to be much lower than observed when H_2O is added afterwards. A comparison between the water solution and acetone-water solution experiments will be discussed next.

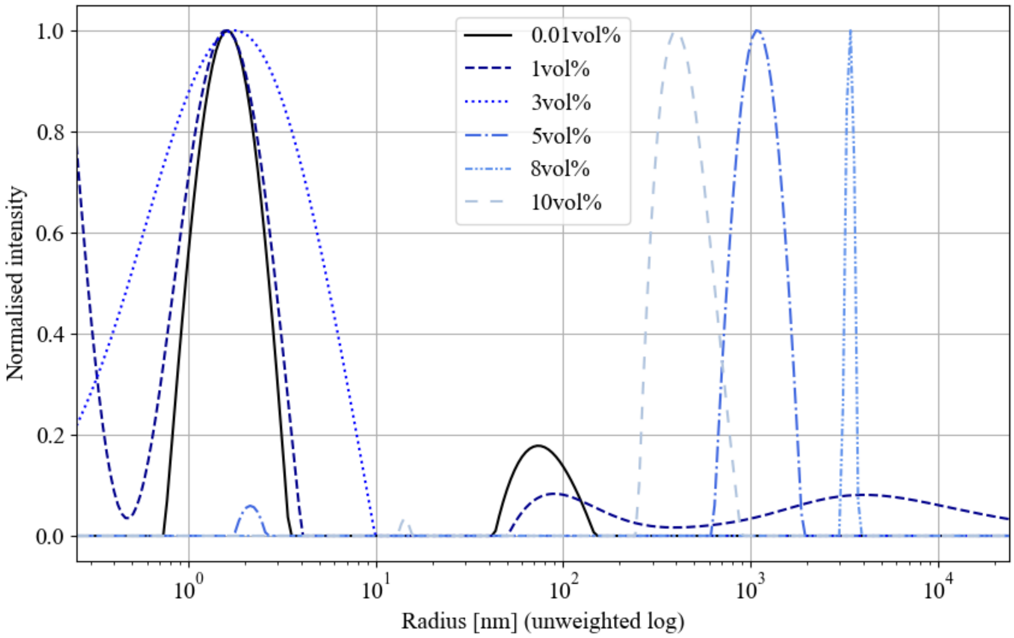

Figure 4.4 – A plot of the regularised fits for the $PBd_{1700}PEO_{1000}$ dissolved in a solution containing acetone and a varying volume of H_2O. The samples with an H_2O vol% below 3 do not seem to form aggregates or only very small ones. Large aggregates are observed for H_2O volume percentages >5.

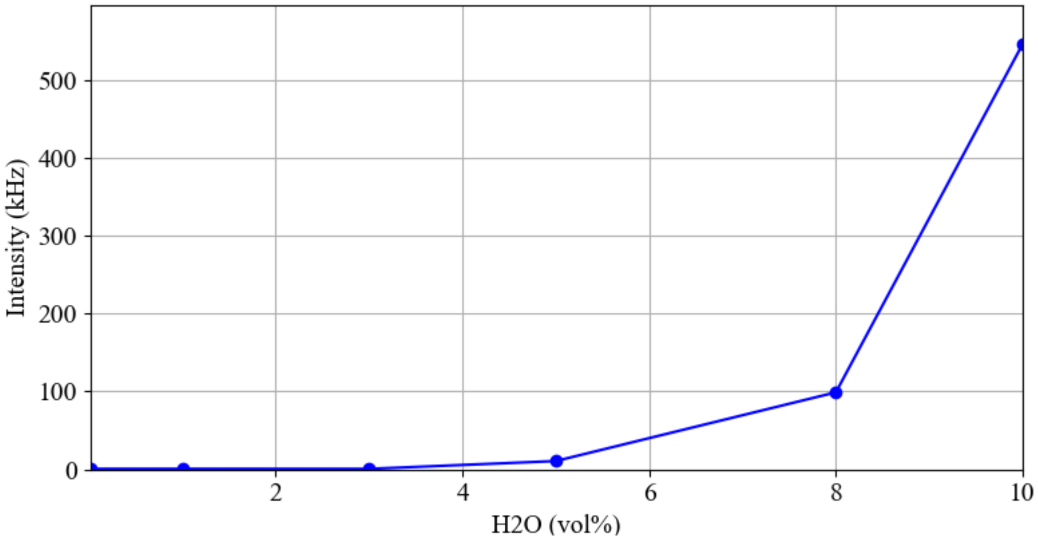

Figure 4.5 – The corrected intensity for every $PBd_{1700}PEO_{1000}$ sample dissolved in an acetone-water solvent. Since the intensity is close to zero for <5vol% no aggregates are formed yet, but hereafter the intensity increases which means that aggregates are formed in the samples.

Comparison

As seen in Table 3.3, some percentages of the acetone-water, initial water and increased water volume were chosen to be the same. These percentages are 3, 5, 8 and 10vol% H_2O. For clarity the difference between these experiments is stated again; in the acetone-water experiment, the H_2O is already added to the acetone before the block copolymer, in the initial water experiment the samples were directly prepared at the given volume percentages of H_2O and in the increased water volume the H_2O volume was increased step by step. Thus the water injection speed for the increased water volume experiment is much lower than for the initial water experiment. By comparing their obtained hydrodynamic radii (Figure 4.6) and corrected intensity (Figure 4.7), it can be made visible how the block copolymer reacts to different ways of H_2O addition. The labels in the legend stand for acetone-water (AW), initial water (WI) and increased water volume (WE).

The sample at 3vol% H_2O is the same for the initial water en increased water volume samples since the increased water volume series is started from the initial series. At this volume percentage, aggregates seem to already form for the regular water (WI and WE) samples, in contrast to the acetone-water sample. Hereafter, the peak of the acetone-water is constantly higher than those of the water samples. An interesting fact is that the peaks of the water samples are quite close to each other for every observed volume percentage. This might mean that the samples do not react very differently to different ways of water addition.

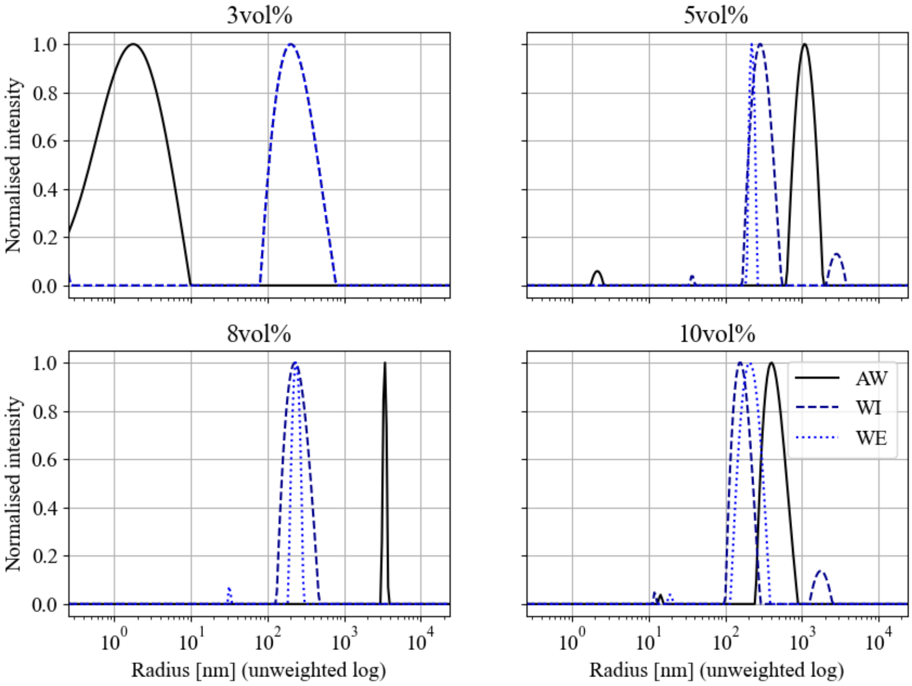

Figure 4.6 – An overview of the obtained hydrodynamic radius for the initial water (WI), increased water volume (WE) and acetone-water (AW) experiments. Each graph represents a DLS result at a different vol% of H_2O, as shown in their titles.

For further analysis, the corrected intensity plot is given in Figure 4.7. Two interesting things are seen in this graph. First, the observation that the water samples seem to react the same, seems to align with their corrected intensity plots. These two are quite close to each other, but the initial water samples seem to lag behind the increased water volume samples in aggregate size. Second, the corrected intensity of the acetone-water has a much lower value than those of the water samples. Since larger hydrodynamic radii were observed in the regularised fit, these results do not align with each other. A possible explanation might be that more aggregates are formed when H_2O is added after the block copolymer is dissolved into acetone, than in the acetone-water samples. When the concentration of aggregates is much higher, this might also result in higher intensity.

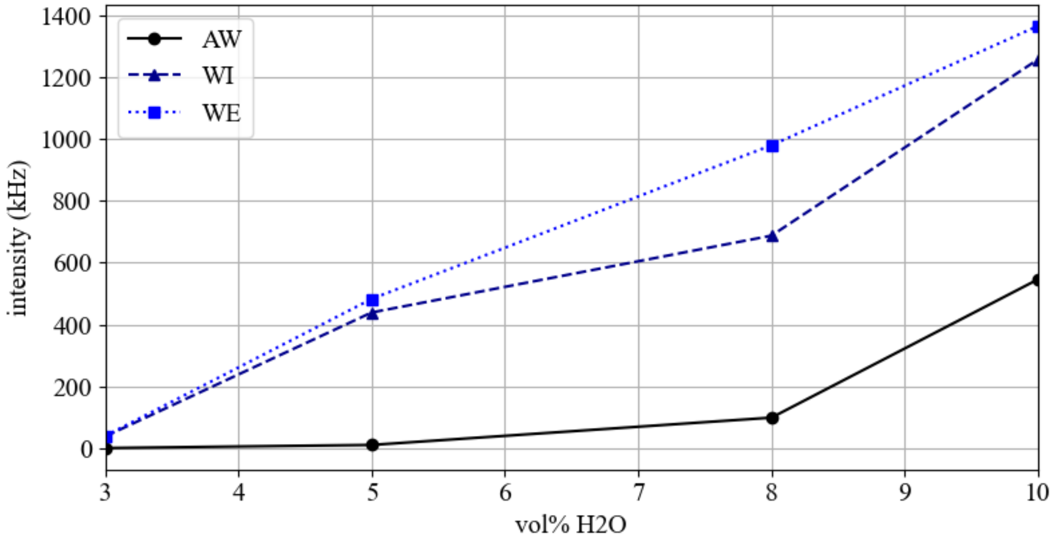

Figure 4.7 – The corrected intensities of the initial water, increased water volume and acetone-water experiments. These experiments were all performed at 3, 5, 8 and 10 vol% H_2O and are also compared at these points.

Kinetics

To see how stable the samples are at different vol% of H_2O multiple measurements over time were performed. Eight measurements were performed on six samples, with an interval of 45 minutes. Since the regularised fit plots all had one peak or only a small bump somewhere, the time could be plotted against the hydrodynamic radius for a clear overview as shown in Figure 4.8. The samples with 4 and 5 vol% H_2O were found to be the most unstable, with radii ranging from 600nm to 3µm over time. This is in contrast to the samples with 8, 10 and 12 vol% H_2O, which seem to be quite stable over time.

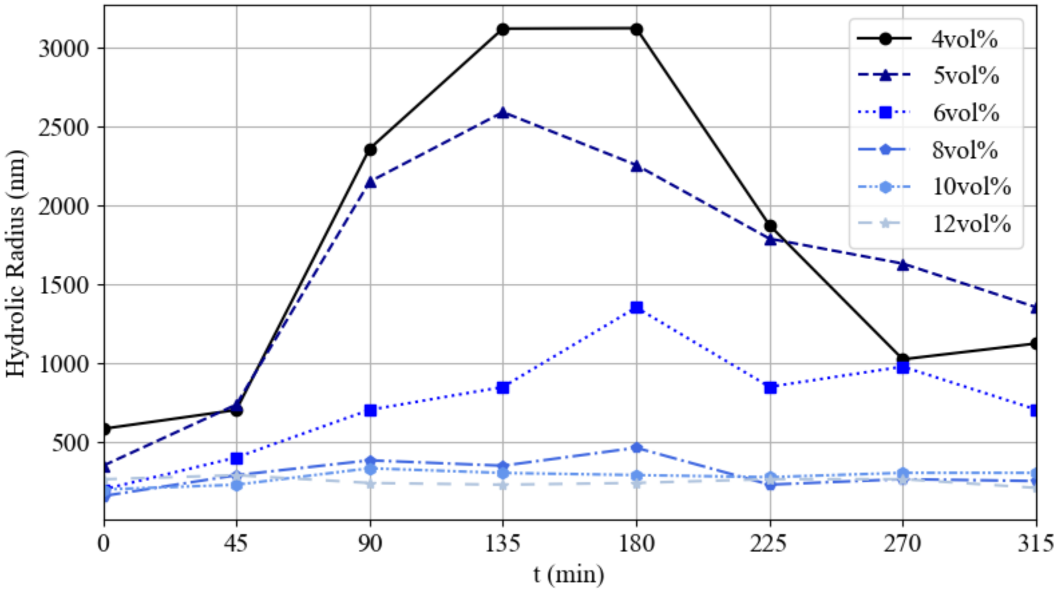

Figure 4.8 – The change in radius over time per sample. It looks like, the lower the vol% the more unstable the aggregate size becomes.

To check these observations a corrected intensity plot is shown in Figure 4.9. The results found before do not seem to align with the regularised fit results. The three samples with the lowest vol% H_2O had the most stable corrected intensity graph and the three highest vol% H_2O samples seem to have the most unstable intensity graphs. All light scattering intensities seem to be the most stable from 180 minutes.

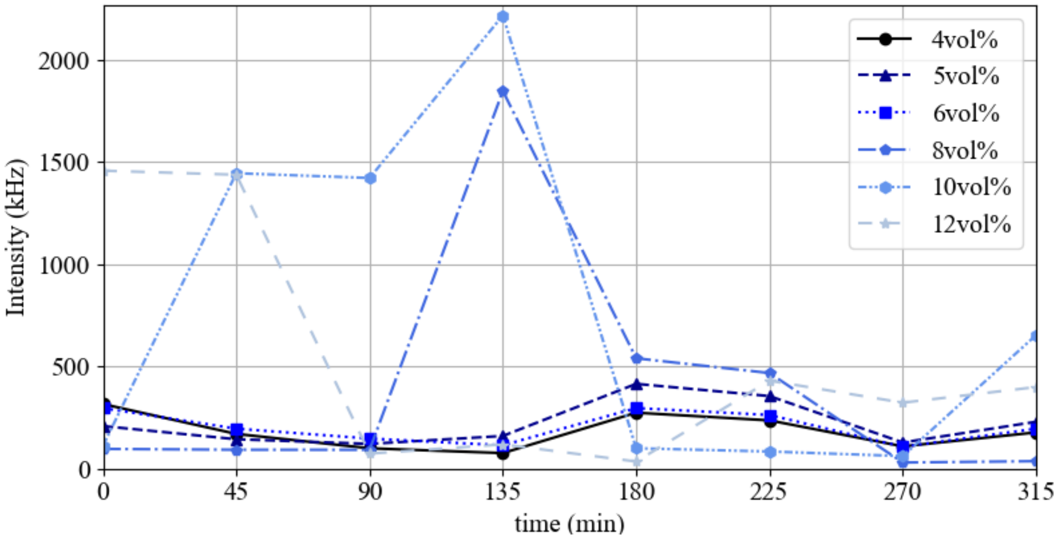

Figure 4.9 – A graph of the corrected intensity for the kinetics experiment on the $PBd_{1700}PEO_{1000}$ samples. The most unstable intensities are observed for the three highest vol% H_2O. After 180 minutes all light scattering intensities seem to stabilise.

4.1.3. SESANS Contrast Series

For the SESANS measurements, the solvents needed to be deuterated, to make 'contrast' for the neutrons. When a sample solely contains non-deuterated solvents the neutrons perceive the whole sample as a blank or 'white' space. By adding deuterated solvents to the system the 'colour' of the aggregates, or the background, changes to 'grey' and can now be seen by the neutrons. Because of this contrast, the neutrons scatter and their spin-echo length can be measured. Before measuring the samples with SESANS, a DLS measurement was performed to see if the samples react the same, regardless of D_2O or H_2O addition. In Figure 4.10, the D_2O percentage is plotted against the hydrodynamic radius. This could be done because all samples had one narrow peak in their regularised fit. One thing that stands out is that the aggregate size and concentration seem to be the most unstable for the ratios 50/50 to 10/90, having increased their size roughly 10 times.

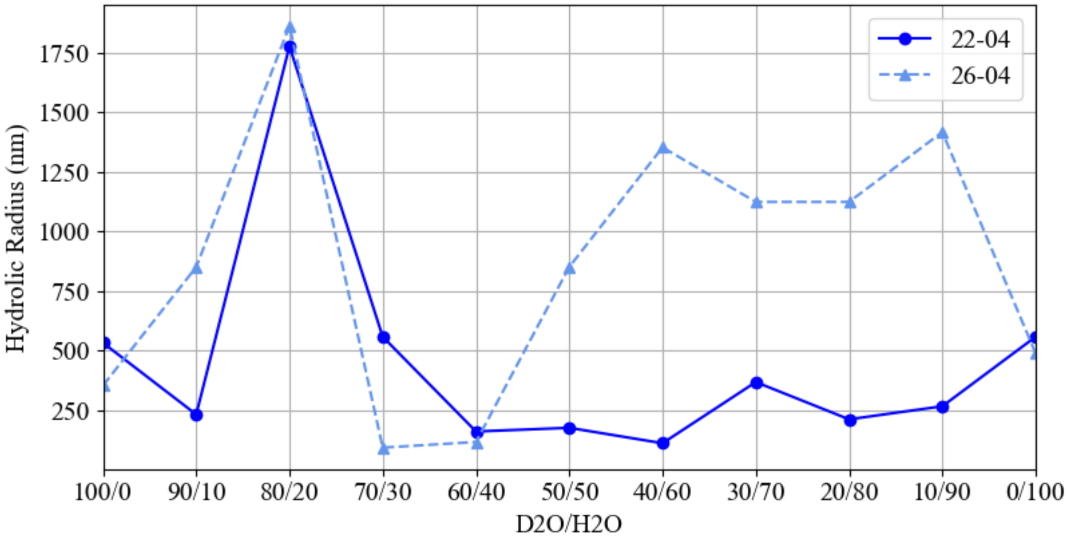

Figure 4.10 – The evolution of the hydrodynamic radius of samples with different D_2O percentages. It looks like the less D_2O is present in the solution, the more unstable its aggregate sizes become. Up to the point when there is no D_2O present in the samples.

In the corrected intensity plot (Figure 4.11) a lot of variation between intensity values is also observed. The intensities range from 110kHz up to 590kHz. This means that the self-assembly of the samples might be affected by the amount of D_2O present in the water solution.

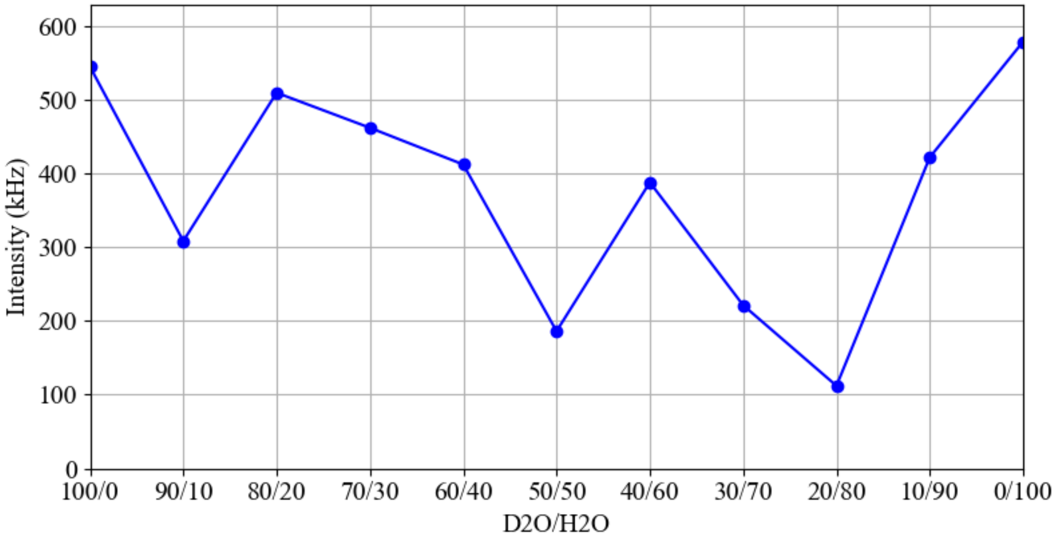

Figure 4.11 – The corrected intensity of the samples at 6vol% D_2O/H_2O mixture. On the x-axis, the ratios of D_2O/H_2O are given, with their respective corrected intensity on the y-axis.

A kinetics experiment was performed at D_2O/H_2O ratios 100/0, 80/20, 70/30, 60/40 and 0/100. There was a shortage of acetone-D_6, so not all ratios could be measured anymore. The total time taken for the measurement was 3 hours and the interval was 60 minutes. In Figure 4.12 one can see that almost all samples behave the same as observed in Figure 4.11. In both figures, the intensities lie between 50 and 600kHz. For the first measurement at 0 minutes, their similarity is the most, except for the 0/100 sample, which is a lot lower than before. For the 70/30 something curious is also observed. After 60 minutes the intensity doubles and does not lower that much after that. This observation was quite unexpected, since visually (Figure 4.13) the 70/30 sample was the least opaque. This could suggest that there is another correlation between visual opaqueness and the radii of the aggregates than thought before. Some of the other relations or possible causes of this observation are discussed next.

The light scattering intensity scales to the radii of the aggregates with a factor of 10^6 and the concentration, scales with a factor of 10^3. A sample with a high aggregate concentration, but with a lower aggregate size will thus yield a lower result than a sample with low aggregate concentration and high aggregate size. The visual opaqueness can thus be very deceiving when one wants to predict the outcome of the light scattering density.

Another option is that light scattering intensity is lost, due to the opaqueness of the samples. If the aggregate concentration in samples is high, an effect called multiple scattering can occur. This means that the light scattered on one aggregate does not go directly to the detector, but first scatters again on a second, third or even fourth aggregate. The light scattering angle cannot be fixed anymore and the value of q (equation 2.4) changes with it. As a result, the diffusion coefficient and particle size can no longer be determined unambiguously from the decay rate of the auto-correlation function, as found in equation 2.3. For concentrations below 10^{-4}, multiple scattering does not overshadow the light intensity of single scattering. For higher concentrations multiple scattering predominates over single scattering, the obtained radii are not reliable anymore and light scattering intensity can be lost [22].

For samples with a high concentration, it is also possible that particle interaction occurs. This affects the diffusion coefficient and thus the Stokes-Einstein relation (equation 2.5), with which the hydrodynamic radius is related to the diffusion coefficient [22].

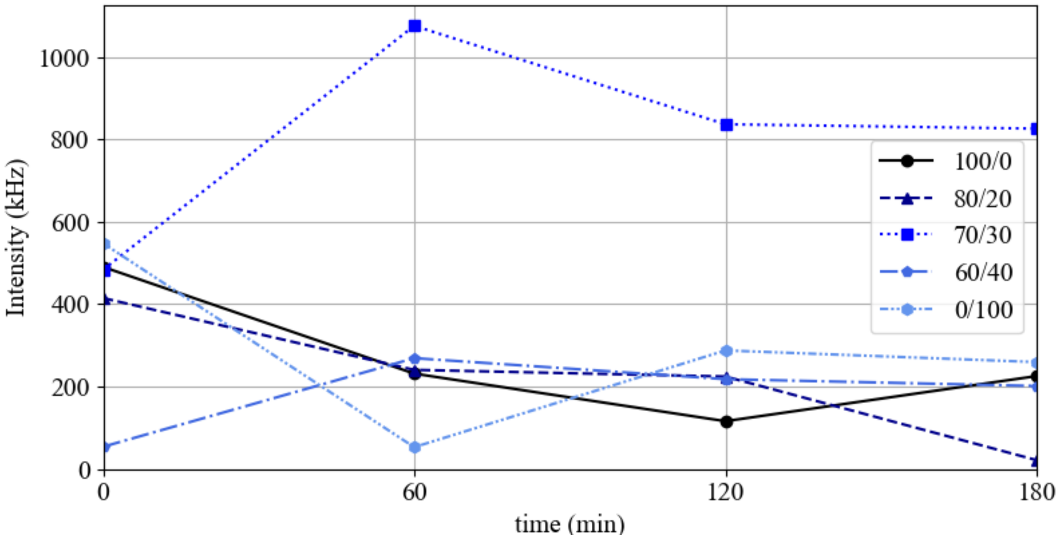

Figure 4.12 – The corrected intensity of a kinetics measurement on the D_2O/H_2O samples. Their respective ratios are shown in the legend.

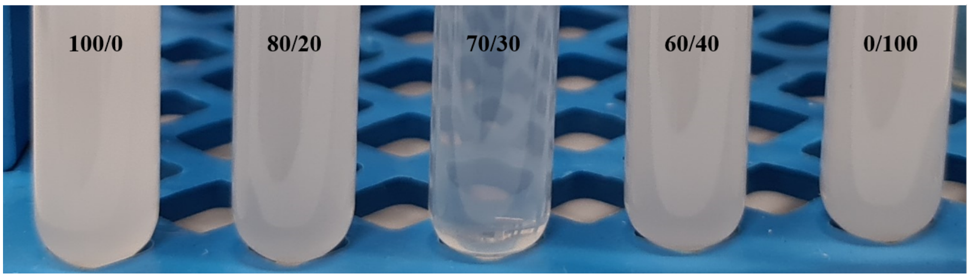

Figure 4.13 – A visual representation of the deuterated samples measured with DLS. All are quite opaque, except the D_2O/H_2O:70/30.

To see if it is the water mixture or the acetone that affects the system, an experiment was also performed in which the acetone solution was a mixture of acetone-D_6 and acetone. The H_2O was kept at 6vol% and measurements were taken each 60 minutes over the course of 3 hours. As shown in Figure 4.14 most samples are quite stable over time, but much difference in intensity is visible between the samples. The 60/40 sample seems to be more unstable, and its intensity decays twice as much as the others.

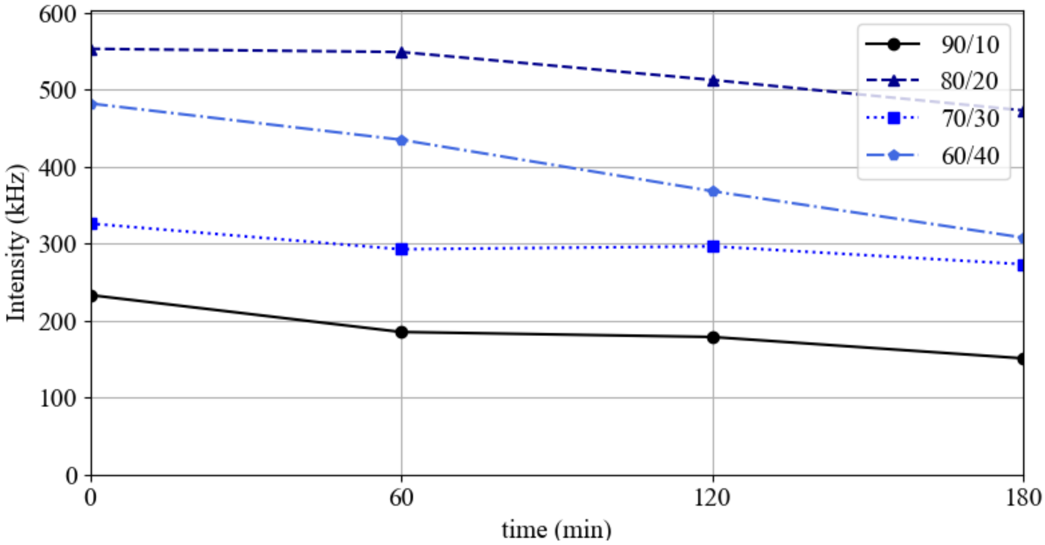

Figure 4.14 – The corrected intensity of samples prepared with a ratio of acetone/acetone-D$_6$. A measurement was performed every 60 minutes.

4.1.4. SESANS

To check the state of the SESANS apparatus for these measurements a plot of P_0 is made (Figure 4.15). Ideally, this measurement should be equal to 1 for all spin-echo lengths. For this setup, the polarisation becomes lower and lower for higher spin-echo lengths. To account for the non-ideal setup, the polarisation results for the samples are divided by this P_0.

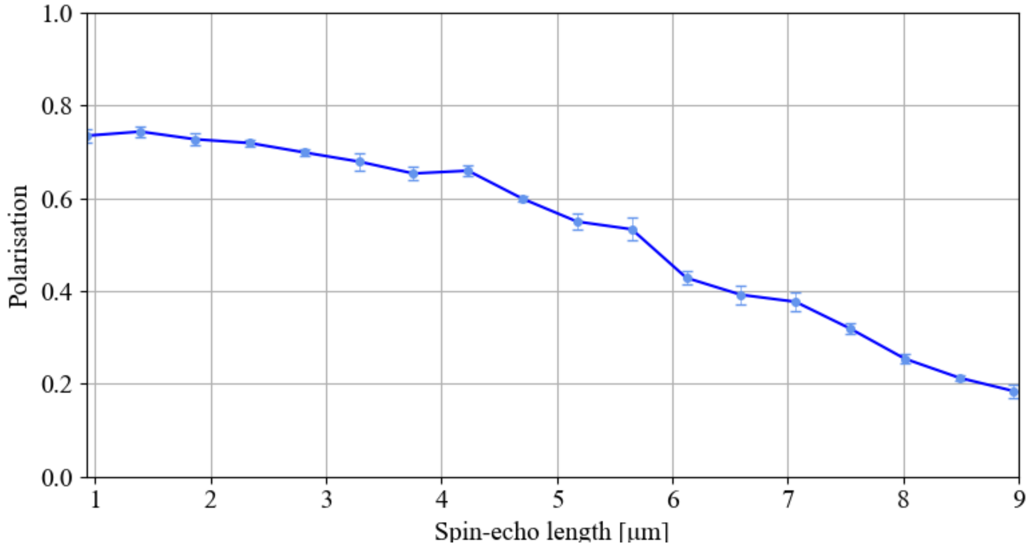

Figure 4.15 – A measurement of an ‘empty beam’, meaning that there is no sample. In an ideal setup, this value is equal to 1, but for this setup, it is not. This shows the state of the SESANS setup during the measurements.

First, a SESANS measurement was performed on D_2O/H_2O samples in a cuvette of pathlength 2.0mm. This was done for the ratios D_2O/H_2O: 100/0, 90/10, 80/20, 70/30, 60/40 and 50/50. The raw data were reduced with a Python script and then fitted in SasView. For the fit, a sphere model was used with the scale and radius as fit parameters and the scattering length densities were adjusted per D_2O/H_2O ratio, as described in Chapter 3.5. These results are shown in Figure 4.16. The polarisation did not even decrease by 20% (or down to -0.2 on the y-axis), thus the statistics were not optimal and the experiment was redone with cuvettes with a path length of 10mm. Additionally, an experiment was performed in which the acetone/acetone-D_6 was varied. Sadly the data retrieved from these samples yielded even worse statistics and could therefore not be used in this thesis.

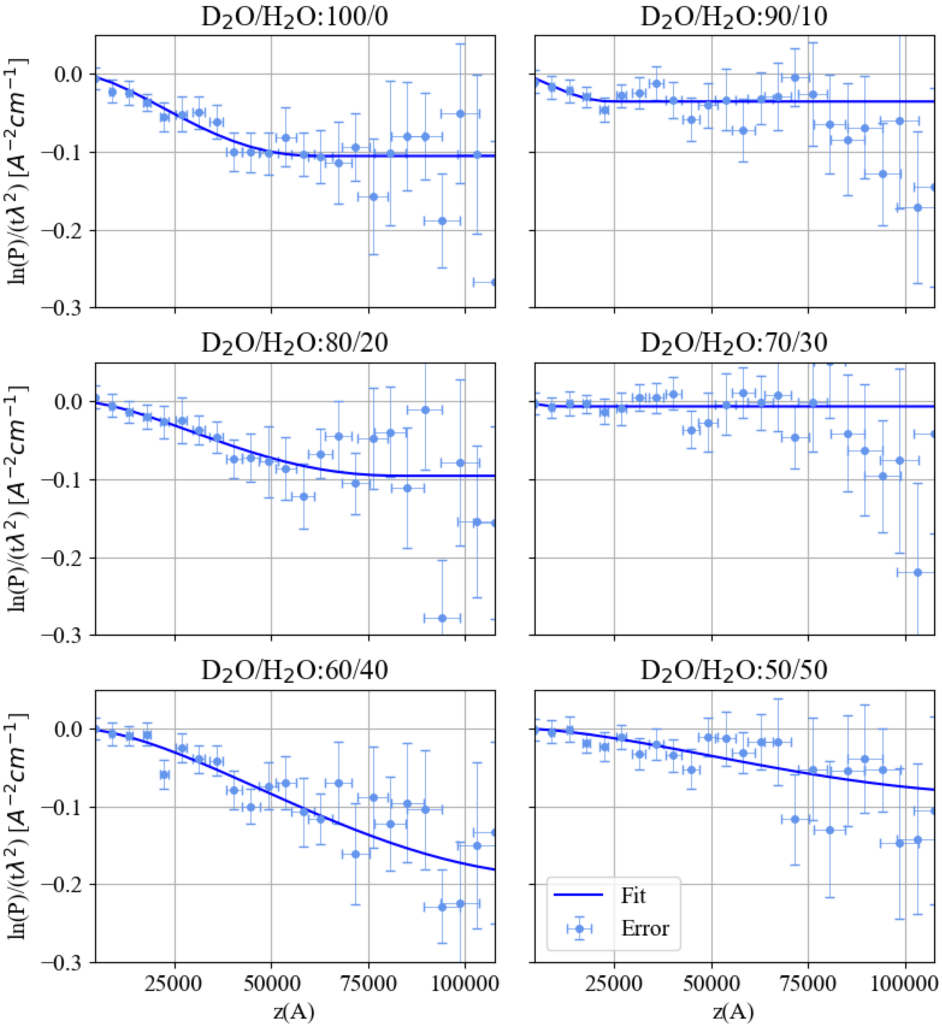

Figure 4.16 – The fitted results from SasView per sample. The point where the fitted line starts to stabilise corresponds to the found radius.

As said before, the decrease in polarisation is not much. However, some things can still be derived from these results. For the 70/30 sample contrast matching seems to occur, this tells us something about the consistency of the aggregates, which can help to form an accurate model for the aggregates. In the next paragraph, this phenomenon will be further discussed. Contrast matching at 70/30 was already observed in previous research [9] and is thus reproducible. An overview of the found radii for the different ratios is shown in Table 4.1.

Table 4.1 – The found radius per sample, retrieved from the fits made in SasView.

Ratio D_2O/H_2O	**Radius (µm)**
100/0	3.16 ± 0.53
90/10	1.25 ± 0.51
80/20	4.25 ± 1.10
70/30	0.529 ± 1.60
60/40	6.80 ± 2.05
50/50	7.11 ± 5.52

4.1.5. Contrast Matching

As stated before, contrast matching occurs when the neutrons cannot 'see' the difference between the solvent and the aggregates (spheres). For the D_2O/H_2O series, there are three components out of which the solvent and the sphere can exist, acetone-D_6, the D_2O/H_2O mixture and the block copolymer. Depending on the volume ratios present in the sphere or solution and the ratio of D_2O/H_2O, the spheres might become 'invisible' to the neutrons and they will not change their rotation anymore, due to contrast matching. At which ratio of D_2O/H_2O contrast matching can occur, can be predicted by calculating where the intersection of the sld_{sphere} and $\text{sld}_{solution}$ is. To make this calculation, one needs to know the amount of acetone-D_6, the D_2O/H_2O mixture and the block copolymer present in either the sphere or the solution. However, the consistency of the formed aggregates during the opaque phase is unknown.

In Figure 4.17 the slds of the used model for the SasView fit are plotted. Here the acetone-D_6 in the sphere was assumed to be 0% and the other particles were all in the sphere. There is no intersection between these lines, which means that no contrast matching should be observed if this model was right. However, as shown in 4.16, contrast matching does occur for these samples, around the ratio of D_2O/H_2O:70/30. One of the many possible options at which contrast matching occurs around 30% H_2O, is shown in Figure 4.18. The intersection point is at 34% H_2O and the sphere consists of 50% acetone-D_6, 70% of the D_2O/H_2O mixture and 40% of the block copolymer. The slds per component are calculated with the sld calculator of SasView. The sld_{sphere}, $\text{sld}_{solvent}$ and the intersection point are calculated via the script found in Appendix B.

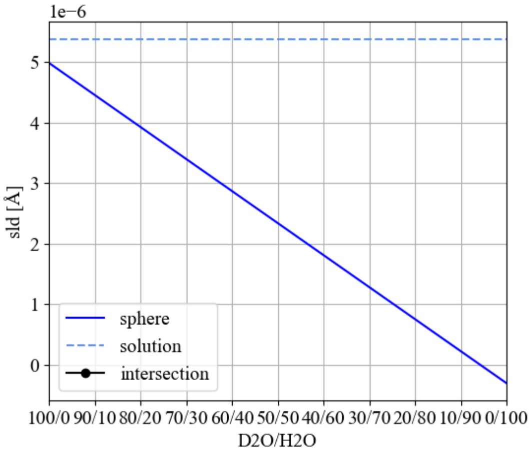

Figure 4.17 – The sld for the model of a simple sphere, in which all acetone is located in the solution and all water and block copolymer are located in the sphere.

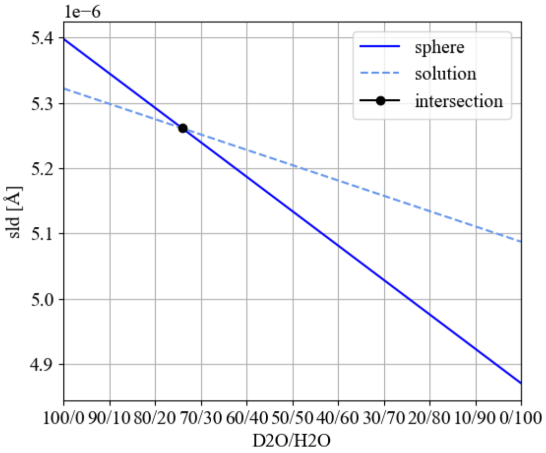

Figure 4.18 – A possible sld graph for another model. Here the lines cross each other, and at this intersection point, contrast matching occurs.

Since it is known that contrast matching occurs around D_2O/H_2O:70/30, a lot of the possible volume fractions for acetone-D_6, the D_2O/H_2O mixture and the block copolymer can be discarded. A script has been written which calculates all possible contrast matching points for all volumes, with a step size of 1% (thus resulting in $1 \cdot 10^6$ options). A condition was then set to the contrast matching point, it should be between 25% and 35% H_2O. The volume of the block copolymer present in the sphere was assumed to be at least 40%. This script is found in Appendix C. This resulted in a dataset of 32839 values, which is still a large dataset, but there is only 3.3% of the total options left. From this data, a contrast plot could be made of possible consistencies for the spherical aggregates formed in the opaque phase (Figure 4.19).

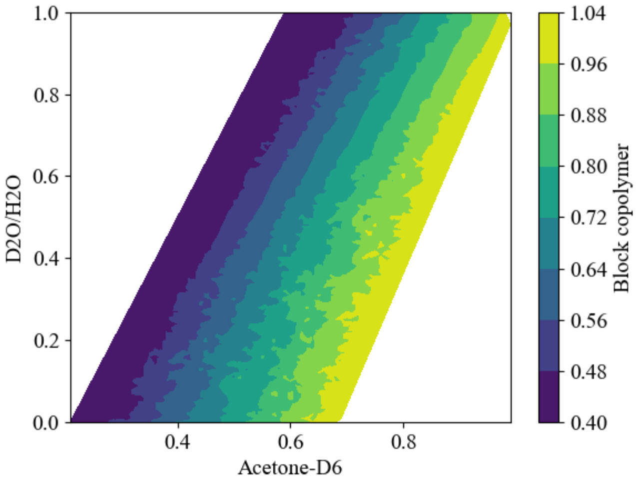

Figure 4.19 – A contour plot of the possible volume fractions acetone-D_6, the D_2O/H_2O mixture and the block copolymer present in the sphere. The corresponding contrast matching point to these values is set to be between 75/25 and 65/35 D_2O/H_2O ratio.

4.1.6. Overview

A lot of experiments with deuterated samples have been performed for the PBd_{1700}-PEO_{1000}. To summarise it in one picture an overview is shown in figure 4.20. Photos have been taken for a visual representation of the changes that occurred for every different ratio of D_2O/H_2O. Below these photos, their hydrodynamic radius is shown, retrieved from the regularised fit performed on two different days. The samples with ratios of 50/50 to 10/90 seem to be the most unstable and grew the most in size. As a reference, their SESANS results are also shown. One would expect that the most opaque samples would yield the highest radii, but this does not seem to be the case. There seems to be no trend between visual opaqueness and the size of the aggregates measured by DLS or SESANS.

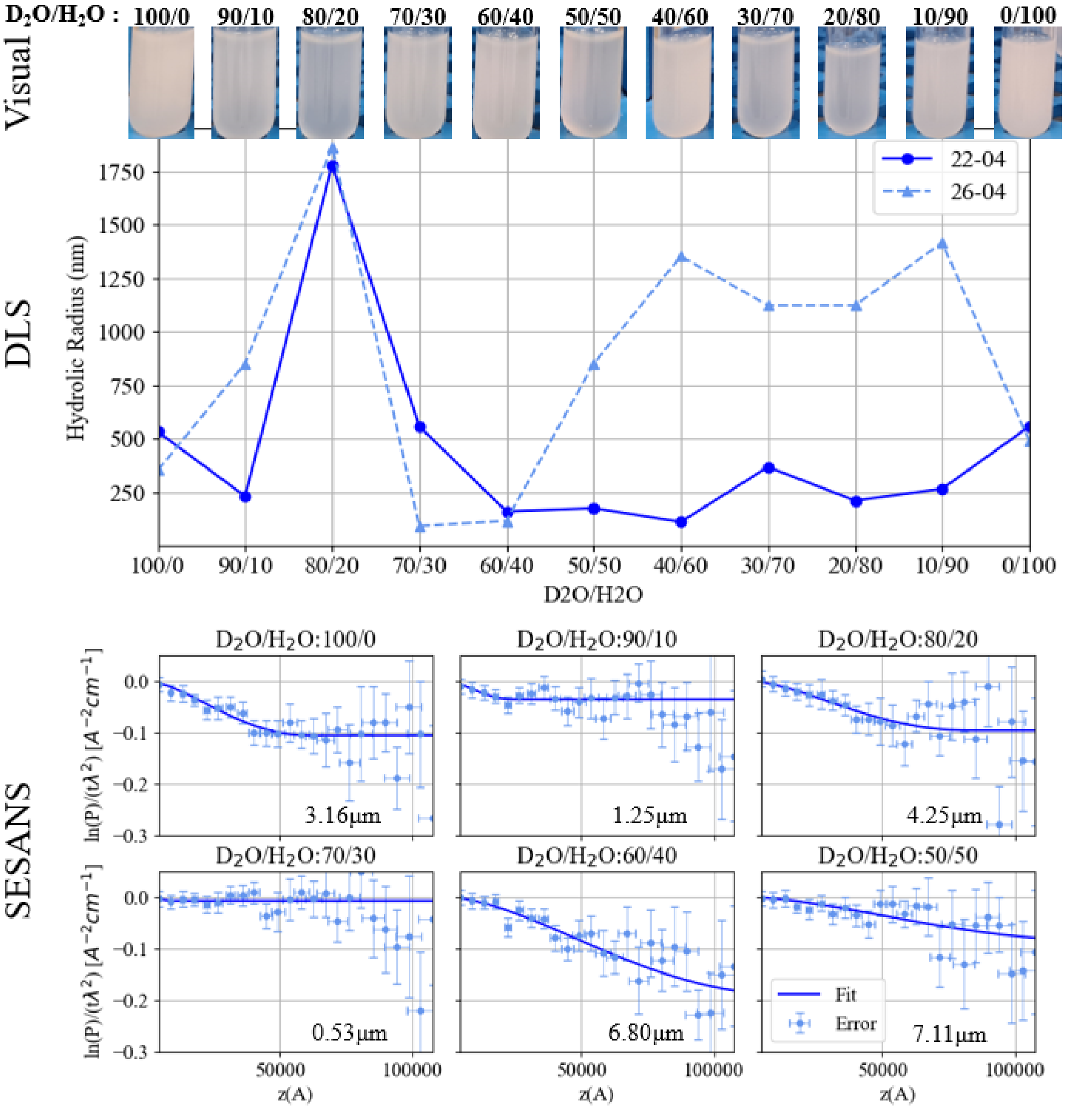

Figure 4.20 – An overview of the visual, DLS and SESANS results obtained from the D_2O samples.

4.2. PBd_{1800}-PEO_{900}

4.2.1. Visual

For this block copolymer, a new batch was used as well, with respect to previous research [9, 10]. Thus a similar visual experiment has been performed as with the PBd_{1700}-PEO_{1000}. Here three phases in opaqueness were observed. A very short first somewhat cloudy phase from 2.8vol% up to 3.2vol%, then the opaque phase started and lasted until 5.5vol%. From this point on the opaqueness decreases and at 6.5vol% it already almost reached its least opaqueness. Until the end of the experiment, at 25vol%, not much change was observed. All these values have an error of ±0.1vol% H_2O.

Again these results are very different from previous results [9]. In the beginning, the short cloudy phase was not observed and the opaque phase started at 4.2±0.65vol% H_2O. It lasted until 10.0±0.72vol% H_2O and then the sample became almost as clear as before any H_2O was added.

Again the only differences in sample preparation were the use of a syringe filter and the temperature. For the same reason as in 4.1.1, the temperature is the most plausible explanation for these differences.

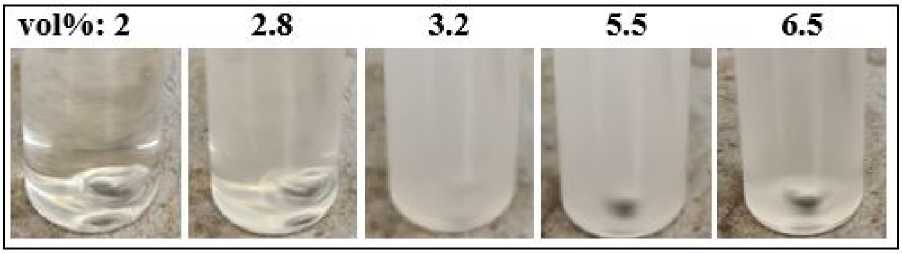

Figure 4.21 – The visual changes that occurred in the PBd_{1800}-PEO_{900} sample upon H_2O addition. Above the photos, their respective vol% of H_2O are given, which all have an error of ±0.1vol%. In all photos, the magnetic bead used for magnetic stirring is visible.

4.2.2. DLS

Water Solution

Similar water experiments have been performed as in Chapter 4.1.2. For these samples, a syringe filter was used as well. A plot of the corrected intensity for the increased water volume samples is shown in Figure 4.22. This graph is quite stable, with one high outlier at 4vol% H_2O. It might be possible that this is near the CMC resulting in a higher peak. The system also seems to stabilise at 14vol% H_2O, which is earlier than observed for the other block copolymer.

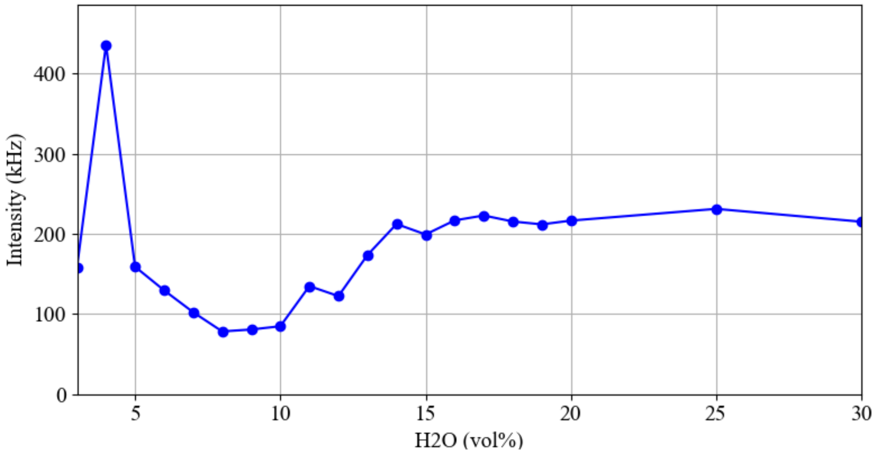

Figure 4.22 – The corrected intensity of the increased water volume experiment on the PB_{1800}d-PEO_{900}. At 14 vol% H_2O, the light scattering intensity stabilises.

Acetone Water Solution

To see how the self-assembly of this block copolymer reacts to an acetone-water mixture as solvent a similar experiment was performed as for the PBd_{1700}-PEO_{1000}. For reference, the plots of the regularised fit are shown in Figure 4.23. This graph aligns well with the corrected intensity graph from Figure 4.24. At 5vol% H_2O the highest intensity is observed and for this percentage, the highest hydrodynamic radius is also observed. However, all the corrected intensities are very small, which is quite strange. When the samples are opaque, they should scatter and yield a much higher intensity than retrieved from the measurement.

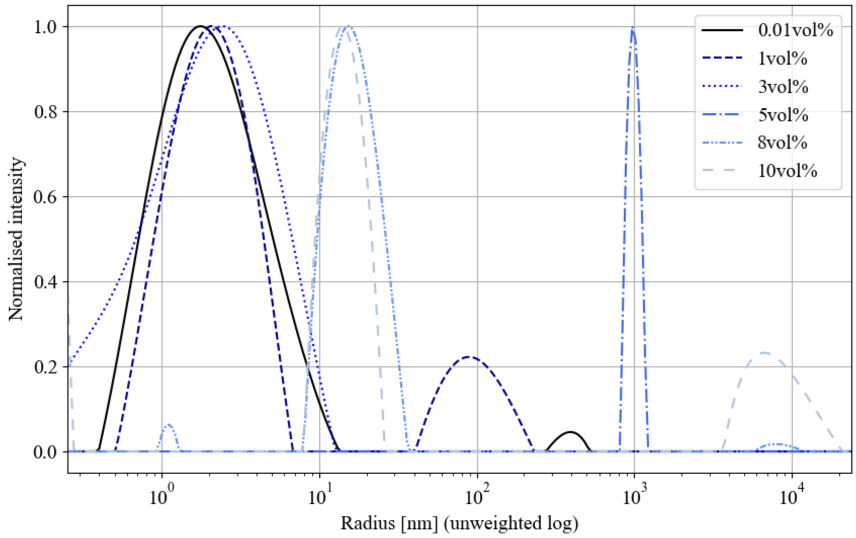

Figure 4.23 – The hydrodynamic radii of the acetone-water samples for PB_{1800}d-PEO_{900}. In the legend, their respective vol% H_2O are stated.

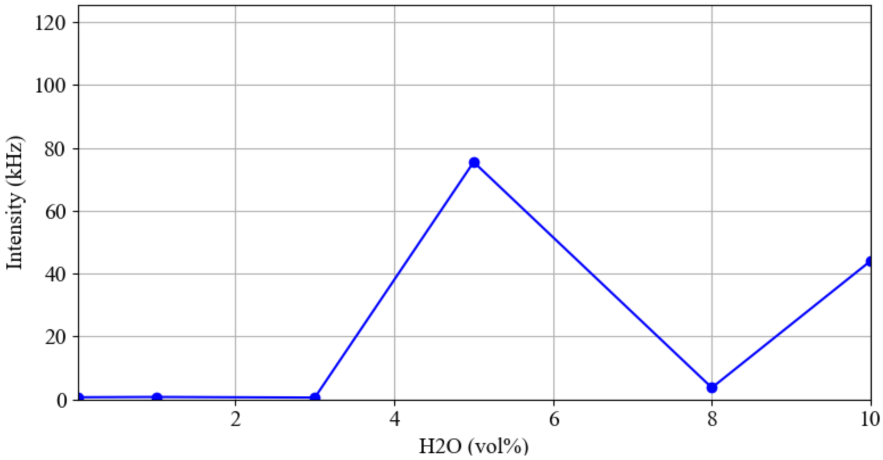

Figure 4.24 – The corrected intensity for the acetone-water experiment for PB_{1800}d-PEO_{900}. The calculated corrected intensities are very small.

Comparison

To see how the water-addition method affects the self-assembly of the block copolymer, a comparison is made between the previously found results. Their regularised plots are shown in Figure 4.25. For 3 and 5vol% H_2O, the samples are monodisperse and for 8 and 10 vol% H_2O, the samples become polydisperse. The observed peaks are quite close to each other, in contrast to the results from PBd_{1700}-PEO_{1000}.

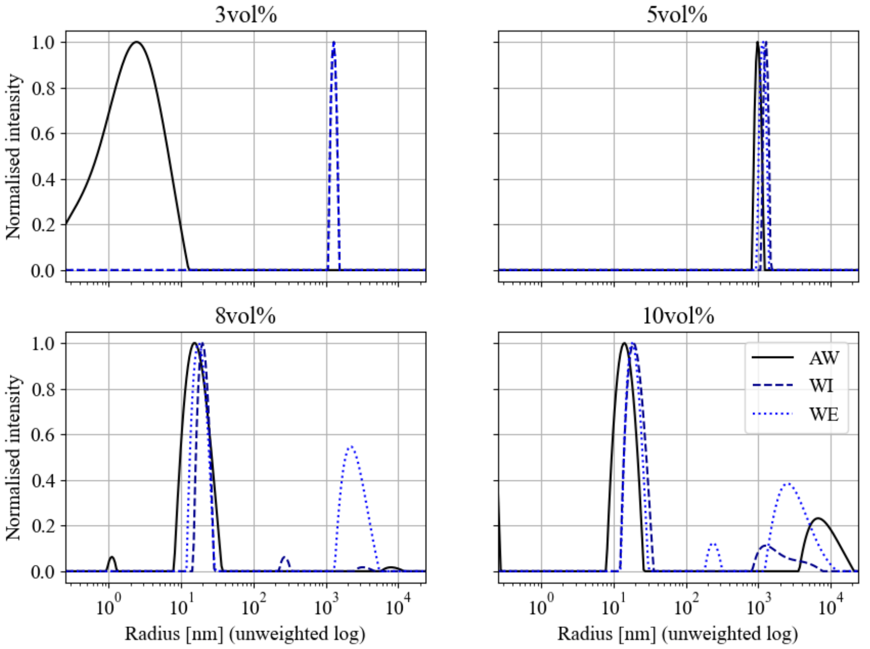

Figure 4.25 – An overview of the CONTIN fits for the compared samples. In the titles, their respective vol% H_2O is found and from left to right, they are 3,5,8 and 10 vol% H_2O.

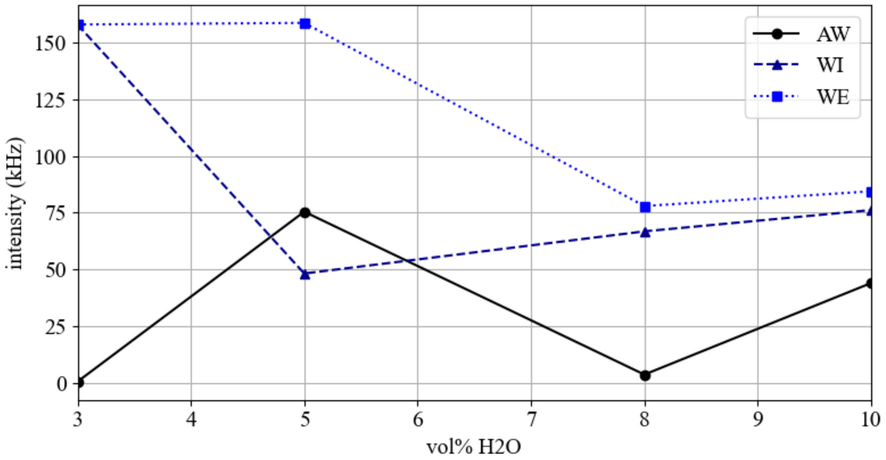

Figure 4.26 – The corrected intensity for the performed water experiments on the PBd_{1800}-PEO_{900}. The samples are compared at 3,5,8 and 10 vol% H_2O.

Kinetics

To test the stability of the system over time, a kinetics measurement was performed. A similar procedure was followed as described in 4.1.2. Since the regularised fits had multiple peaks the corrected intensity (Figure 4.27) is the only valuable data for this block copolymer. Again the intensity was found to be quite small. In contrast to the PBd_{1700}-PEO_{1000}, this block copolymer seems to be quite stable over time.

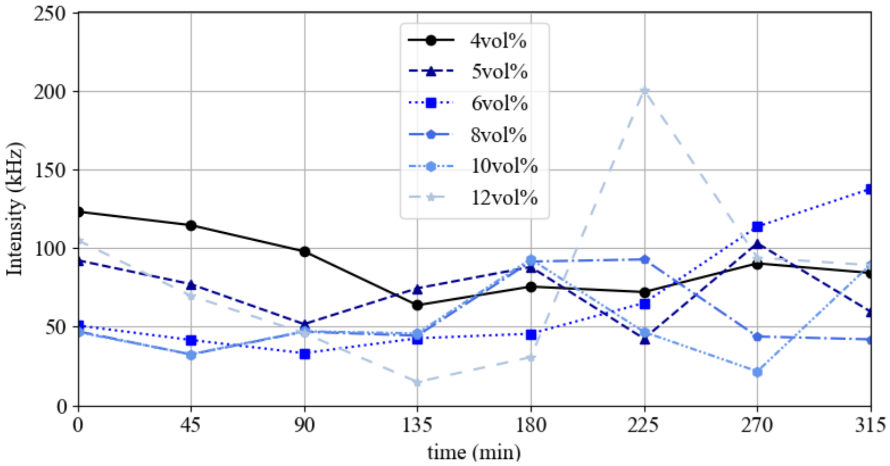

Figure 4.27 – The corrected intensity of the PBd_{1800}-PEO_{900}. Every 45 minutes a measurement was performed over the course of 5 hours and 15 minutes. The samples seem to be quite stable over time and have a low intensity overall.

4.3. Pre-Micellization

As mentioned in the theory a possible theory that can explain what happens during the opaque phase is pre-micellization. For these samples, this is the preaggregation stage around the critical water concentration, or also the opaque phase.

For the PBd_{1700}-PEO_{1000} a lot of results match the stated characteristics of pre-micellization. Previous research already determined that the opaque phase disappears when the sample is heated up. In this study, the opaque phase started earlier than observed in previous research. The temperature was the only notable difference between these experiments and thus it is most likely that the change in the opaque phase is caused by the sensitiveness to the temperature of formed aggregates during the pre-micellization stage. If pre-micellization is indeed true, high light scattering intensity peaks should be observed during the opaque phase, which is indeed the case as seen in Figure 4.3. The intensity peaks around the end of the opaque phase. With kinetics experiments, it was observed that during the opaque phase the radii of the samples prepared in the opaque phase are quite unstable (Figure 4.8), but when their corrected intensity (Figure 4.9) is examined they seem to be stable and the samples prepared at concentrations a little above the opaque phase become unstable. This might indicate that the aggregate size is unstable during the opaque phase and that shortly after it, the aggregate concentration becomes unstable. For SESANS the normalised polarisation is generally low, but for some cases, the polarisation is enough to obtain a radius of the aggregates.

In the visual experiment, the same sensitiveness for temperature change was observed for the PBd_{1800}-PEO_{900}. When the DLS results are observed one sees that during the opaque phase a relatively high-intensity peak is observed, see Figure 4.22, but when compared to the PBd_{1700}-PEO_{1000} this peak is roughly 3 times lower than the highest peak there. The corrected intensity from the kinetics block copolymer yielded a quite stable result, with an overall low light scattering intensity. Due to sedimentation, no SESANS experiments could be performed on this block copolymer.

To summarise, the PBd_{1700}-PEO_{1000} shows a lot of characteristics that match pre-micellization. However, the PBd_{1800}-PEO_{900} shows some hints of pre-micellization, but not as much as the PBd_{1700}-PEO_{1000} [21, 18].

5. Conclusion

Previous research has shown that an opaque phase occurs upon water addition to a PBd-PEO acetone solution. It was also discovered that this phase disappears when the sample is heated up and reappears when cooled down. This happens only in the early stages of the self-assembly process. To gain further insight into this early stage of the self-assembly process, several experiments have been performed in this thesis.

A visual experiment showed that both polymers, PBd_{1700}-PEO_{1000} and PBd_{1800}-PEO_{900}, had a cloudy phase before and after the opaque phase. The opaque phase lasted from 4.1±0.1 to 6.7±0.1 vol% H_2O and 3.2±0.1 to 5.5±0.1 vol% H_2O respectively. This is all 1.2±0.1vol% later than retrieved from earlier data. This is likely a result of the higher room temperature during the time of the previous research.
With DLS, it was established that the speed of water addition barely affects the self-assembly, but when an acetone-water solution was made before adding the block copolymer, the self-assembly process seemed to be slowed down. It was also found in the opaque phase, the samples are quite unstable over time. This phenomenon seems to be stronger in the PBd_{1700}-PEO_{1000} than in the PBd_{1800}-PEO_{900}.
For SESANS measurements, all samples were prepared at 6vol% D_2O/H_2O mixture with acetone-D_6 or an acetone-D_6/acetone mixture as solvent. Previously, it was assumed that the addition of deuterated solvents to the samples would not affect the self-assembly of the PBd-PEO, thus this variable was not taken into account in SESANS measurements. However, when DLS was performed on these samples, their corrected light intensity ranged from 100kHz to 600kHz. Thus the addition of deuterated solvents does affect the system and must from now on be taken into account for the future SESANS measurements.
At the D_2O/H_2O ratio of 70/30 contrast matching was observed. With this data and the assumption that at least 40% of the total block copolymer volume is present in the sphere, the possible options for the consistency of the formed aggregates were greatly reduced to 3.3% of all possible options.
The last observation is that the measured neutron intensities in SESANS were very low, sometimes resulting in unusable measurements. If all these observations are combined, they strongly point to the pre-micellization theory.

6. Recommendations

At lower temperatures, the opaque phase seems to occur earlier than at higher temperatures. How the opaque phase changes due to the temperature is not determined yet. For further experimentation, it is highly recommended to first find out how the occurrence of the opaque phase depends on the temperature. Hereafter all experiments should be performed at the same temperature, to minimise the effect of temperature change on the characterisation of the opaque phase.

Before any new SESANS measurements are performed, the way the self-assembly is affected by adding deuterated solvents to the PBd-PEO must first be properly researched. It could be interesting to measure samples at higher water vol%, with DLS and SESANS, maybe even in the self-assembly regime. This way the effect of deuterated solvents on the end product of the self-assembly could be found.

Hereafter a lot of experiments can still be done with SESANS. For the PBd_{1700}-PEO_{1000}, new measurements need to be done with an acetone/acetone-D_6 mixture as solvent. For better statistics, the D_2O/H_2O experiments also need to be redone with a 10 mm path-length cuvette.

For the PBd_{1800}-PEO_{900}, a good SESANS measurement was not possible due to sedimentation of the formed aggregates. Since this block copolymer also has an opaque phase, it can help to further understand this phase. Therefore it is recommended to find or create a setup in which this block copolymer can also be measured using SESANS.

To further reduce the possible options for the consistency of the aggregates formed, SESANS experiments with D_2O/H_2O ratios from 65/35 to 75/25 should be performed. Additional assumptions on the volume fractions of acetone-D_6, water mixture and block copolymer also reduce the possible options. From this dataset, realistic models can be chosen and further research can be performed to verify the consistency of the aggregates.

A strange observation made during this thesis is that there seems to be no correlation between the visual opaqueness of the samples and their DLS or SESANS results. Very opaque samples sometimes yielded lower intensities than clearer samples did. The correlation between visual opaqueness and aggregate size or concentration can also be researched to further determine the workings of the system during the opaque phase. The three stated options can be tested and in the case of multiple scattering or particle interaction, suitable corrections need to be made to the obtained DLS data.

One of the most important results of this thesis is that pre-micellization is a possible theory to explain the properties of the opaque phase. Therefore it is recommended that experiments are performed, specifically designed for the pre-micellization stage. This means taking into account all parameters that have a huge impact on this stage and trying to isolate them. For example, the before mentioned temperature dependency.

References

[1] Alyssa R. Scott, Kelsey C. Stoltzfus, Leila T. Tchelebi, Daniel M. Trifiletti, Eric J. Lehrer, Pooja Rao, Archie Bleyer, and Nicholas G. Zaorsky. Trends in cancer incidence in US adolescents and young adults, 1973-2015. *JAMA Network Open*, 3(12):e2027738, 2020-12-01. Retrieved June 2022.

[2] Manuel Arruebo, Nuria Vilaboa, Berta Sáez-Gutierrez, Julio Lambea, Alejandro Tres, Mónica Valladares, and Africa González-Fernández. Assessment of the evolution of cancer treatment therapies. *Cancers*, 3(3):3279–3330, 2011-08-12. Retrieved May 2022.

[3] Kevin Kelley, Jonathan Knisely, Marc Symons, and Rosamaria Ruggieri. Radioresistance of brain tumors. *Cancers*, 8(4):E42, 2016-03-30. Retrieved May 2022.

[4] Dan Peer, Jeffrey Karp, Seungpyo Hong, Omid Farokhzad, Rimona Margalit, and Robert Langer. Nanocarriers as an emerging platform for cancer therapy. *Nature nanotechnology*, 2:751–60, 2007-12-01. Retrieved May 2022.

[5] Corinna Fetsch, Jens Gaitzsch, Lea Messager, Giuseppe Battaglia, and Robert Luxenhofer. Self-assembly of amphiphilic block copolypeptoids – micelles, worms and polymersomes. *Scientific Reports*, 6(1):33491, 2016-09-26. Number: 1 Publisher: Nature Publishing Group Retrieved: May 2022.

[6] Xiao-ying Zhang and Pei-ying Zhang. Polymersomes in nanomedicine - a review. *Current Medicinal Chemistry*, 2017. Retrieved May 2022.

[7] Fenghua Meng, Gerard H. M. Engbers, Andrea Gessner, Reiner H. Müller, and Jan Feijen. Pegylated polystyrene particles as a model system for artificial cells. *Journal of Biomedical Materials Research Part A*, 70A(1):97–106, 2004. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jbm.a.30068 Retrieved: June 2022.

[8] Regina Bleul, Raphael Thiermann, and Michael Maskos. Techniques to control polymersome size. *Macromolecules*, 2015. Publisher: American Chemical Society Retrieved: May 2022.

[9] Emily Remmelts. On the self-assembly of polybutadiene-b-poly(ethylene oxide) block copolymers by inverse nanoprecipitation, 2018. Retrieved February 2022.

[10] Rebecca Baaijens. The influence of temperature on the initial phase of self-assembly of the block copolymer poly(1,2-butadiene)-b-poly(ethylene oxide, 2019. Retrieved February 2022.

[11] Monica Adams, Afsaneh Lavasanifar, and Glen Kwon. Amphiphilic block copolymers for drug delivery. *Journal of pharmaceutical sciences*, 92:1343–55, 2003-07-01. Retrieved May 2022.

[12] Poly(1,2-butadiene)-b-poly(ethylene oxide). `https://www.polymersource.ca/index.php?route=product/category&path=2_2190_17_129_2542_720&order=DESC&subtract=1&categorystart=.1-.2.3.3.1&serachproduct=`. Retrieved February 2022.

[13] S. Holder and N. Sommerdijk. New micellar morphologies from amphiphilic block copolymers: disks, toroids and bicontinuous micelles, 2011. Retrieved May 2022.

[14] Telmo J.V. Prazeres, Mariana Beija, Fábio V. Fernandes, Paulo G.A. Marcelino, José Paulo S. Farinha, and J.M.G. Martinho. Determination of the critical micelle concentration of surfactants and amphiphilic block copolymers using coumarin 153. *Inorganica Chimica Acta*, 381:181–187, 2012-02. Retrieved June 2022.

[15] Kevin Letchford and Helen Burt. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V*, 65(3):259–269, 2007-03. Retrieved May 2022.

[16] Yan Jiang, Hong Chen, Xiao-Hong Cui, Shi-Zhen Mao, Mai-Li Liu, Ping-Ya Luo, and You-Ru Du. 1h NMR study on pre-micellization of quaternary ammonium gemini surfactants. *Langmuir*, 24(7):3118–3121, 2008-04-01. Publisher: American Chemical Society Retrieved: June 2022.

[17] Ken Morishima and Takahiro Sato. Light scattering from hydrophobe-uptake spherical micelles near the critical micelle concentration. *Langmuir*, 30(39):11513–11519, 2014-10-07. Retrieved April 2022.

[18] Ludwik Leibler, Henri Orland, and John C. Wheeler. Theory of critical micelle concentration for solutions of block copolymers. *The Journal of Chemical Physics*, 79(7):3550–3557, 1983-10. Retrieved June 2022.

[19] Lifeng Zhang and Adi Eisenberg. Multiple morphologies and characteristics of "crew-cut" micelle-like aggregates of polystyrene- *b* -poly(acrylic acid) diblock copolymers in aqueous solutions. *Journal of the American Chemical Society*, 118(13):3168–3181, 1996-01-01. Retrieved June 2022.

[20] Prof. Dr. Frank Schreiber. Static and dynamic light scattering (sls/dls). Retrieved May 2022.

[21] Jörg Stetefeld, Sean A. McKenna, and Trushar R. Patel. Dynamic light scattering: a practical guide and applications in biomedical sciences. *Biophysical Reviews*, 8(4):409–427, 2016-12-01. Company: Springer Distributor: Springer Institution: Springer Label: Springer Number: 4 Publisher: Springer Berlin Heidelberg Retrieved: Retrieved March 2022.

[22] Robert Finsy. Particle sizing by quasi-eleastic light scattering, 1994. Retrieved April 2022.

[23] K. Schätzel. Correlation techniques in dynamic light scattering. *Institut für Angewandte Physik, Universität, D-2300 Kiel, Fed. Rep. Germany*, page 21, 1986. Retrieved May 2022.

[24] M. Dizechl and E. Marschall. Viscosity of some binary and ternary liquid mixtures. *J. Chem. Eng. Data; (United States)*, 27:3, 1982-07-01. Institution: Department of Mechanical and Environmental Engineering, University of California, Santa Barbara, California 93106 Retrieved: May 2022.

[25] S. S. Kurtz, A. E. Wikingsson, D. L. Camin, and A. R. Thompson. Refractive index and density of acetone-water solutions. *Journal of Chemical & Engineering Data*, 10(4):330–334, 1965-10. Retrieved May 2022.

[26] Interpolation (scipy.interpolate) — SciPy v1.8.1 manual. Retrieved June 2022.

[27] Alexey Bashkatov and Elina Genina. Water refractive index in dependence on temperature and wavelength: A simple approximation. *Proceedings of SPIE - The International Society for Optical Engineering*, 5068, 2003-10-14. Retrieved June 2022.

[28] M. Theo Rekveldt, Jeroen Plomp, Wim G. Bouwman, Wicher H. Kraan, Serguei Grigoriev, and Menno Blaauw. Spin-echo small angle neutron scattering in delft. *Review of Scientific Instruments*, 76(3):033901, 2005-03. Retrieved June 2022.

Appendix A: Data Processing DLS

```python
""" Automatizes the load and retrieval of data from A5ECORR.exe program
 In case of an error mid-calculaition. Move cursor to the TOP LEFT CORNER OF THE SCREEN.

 Before usage:
 - Adjust location of buttons according to your system's monitor resolution.
   The location of the buttons can be determined by making a screenshot and editing it in paint.
 - Adjust wait time according to your system's performance. Higher wait for slower computers

 ---------------------------------------------------------------
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 This program is free software: you can redistribute it and/or modify it under the terms of the
 GNU General Public License as published by the Free Software Foundation version 3.
 This program is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY;
 without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE.
 See the GNU General Public License for more details.

 You should have received a copy of the GNU General Public License along with this program.
 If not, see <http://www.gnu.org/licenses/>.
 """
#Import libraries
import time
import re
import pyautogui
from glob import glob
import pandas as pd
from scipy import interpolate

# Change according to your system's configuration
pyautogui.PAUSE = 0.27  # Seconds it waits between each command

# Location of all needed buttons, (0,0) is on TOP-LEFT
location_copydata_button = 1310, 615
location_temperature_button = 808, 990
location_viscosity_button = 671, 990
location_index_button = 555, 990
location_wave_button = 405, 990
location_angle_button = 265, 990
number_of_gridpoints = 1060, 475
reg_fit_ok = 670, 670
autosave_off = 120, 20

# ---------------------------------------------------------------
```

```
# Viscosity and refractive index interpolation
inter_visc = pd.read_csv('acetone_water.csv', header=1)
inter_index = pd.read_csv('acetone_water_refractive_index.csv', header
    =1)
getViscosity = interpolate.interp2d(inter_visc['temperature'],
    inter_visc['x_1a'], inter_visc['mu'], kind='quintic')
getRefIndex = interpolate.interp1d(inter_index['water_vol'],
    inter_index['index'], kind='linear')

#Needed to retrieve constants from ASC file
def isFloat(num):
    """
    Returns True if input is a number, otherwise False.
    """
    try:
        float(num)
        return True
    except (TypeError, ValueError):
        return False

#Retrieve constants from ASC file
def getConstVal(filename, Name):
    """
    Reads out constants from ALV autogenerated file.
    filename: Name of the file to read angle from.
    Name: The name of the constant the function should look for
    returns: constant value.
    """
    with open(filename, "r") as f:
        for line in f:
            if Name in line:
                lsplit = line.split()
                for el in lsplit:
                    if isFloat(el):
                        return float(el)
    print("Nothing found.")
    return

def get_files():
    """ Returns a list of files as a dictionary, based on the pattern:
    For water change samples
        New: <percentage>%<sample_indicator> '(\d+)%(\w+).ASC'
    For water & water-acetone samples
        New: <percentage>%<sample_indicator>_<number> '(\d+)%(\w+)_(\d)
    .ASC'
    For regular kinetics measurements (acetone and H2O)
        New: <percentage>%<time> '(\d+)%(\w+).ASC'
    For measurements with a mix of D2O/H2O or acetone-d6/acetone
        New: <percentage>%<time>_<D>_<H> '(\d+)%(\w+)_(\w+)_(\w+).ASC'
    For D2O/H2O '(\d+)_(\d+).ASC'
    For help using regular expressions, see: `https://regexr.com/`250
    """
    filenames = glob('*.ASC')
    files = []
    for name in filenames:
```

```
        #Regualar
        capture = re.split('(\d+)_(\d+).ASC', name)
        # #Deuterium
        # capture = re.split('(\d+)%(\w+)_(\w+)_(\w+).ASC', name)
        try:

            file = {
                'name': name,
                'D2O':capture[1],
                'H2O':capture[2],
                # #Decomment for regular
                # 'D': capture[3],
                # 'H': capture[4]
            }
            #Constants from file
            file['percentage'] = 6
            file['temp'] = getConstVal(name, 'Temperature')
            file['wave'] = getConstVal(name, 'Wavelength')
            file['angle'] = getConstVal(name, 'Angle')
            file['intensity'] = getConstVal(name, 'MeanCR0')

            #Calculate viscosity per sample
            concentration = 1-(int(file['percentage'])/100)
            celcius = file['temp']-272.15 #For the viscosity calculation the temperature must be in Celsius instead of Kelvin
            file['visc'] = float(round(getViscosity(celcius, concentration)[0],4))

            #Calculate refractive index per sample
            water_concentration = int(file['percentage'])
            file['index'] = float(round(float(getRefIndex(water_concentration)),4))

            files.append(file)
        except Exception as e:
            print(f"""\nERROR while reading `{name}`. Skipping file.\t\t{e}""")
    return files

def setup_a5ecorr():
    pyautogui.moveTo(location_copydata_button)
    pyautogui.click()
    pyautogui.hotkey('alt', 'w')
    pyautogui.press('3')
    pyautogui.hotkey('ctrl', 'f4')
    pyautogui.hotkey('alt', 'w')
    pyautogui.press('4')
    pyautogui.hotkey('ctrl', 'f4')
    pyautogui.hotkey('alt', 'w')
    pyautogui.press('1')
    pyautogui.hotkey('ctrl', 'f4')
    pyautogui.hotkey('alt', 'w')
    pyautogui.press('2')
    return None

```

```
def open_file_a5ecorr(filename):
    pyautogui.hotkey('alt', 'f')
    time.sleep(2)
    pyautogui.press('o')
    time.sleep(2)
    print(filename)
    pyautogui.typewrite(str(filename))
    pyautogui.press('enter')
    return None

def enter_properties(temp, visc):
    # Enter temperature
    pyautogui.moveTo(location_temperature_button)
    pyautogui.doubleClick()
    pyautogui.typewrite(str(temp))
    pyautogui.press('enter')
    # Enter viscosity
    pyautogui.moveTo(location_viscosity_button)
    pyautogui.doubleClick()
    pyautogui.typewrite(str(visc))
    pyautogui.press('enter')
    # Enter index
    pyautogui.moveTo(location_index_button)
    pyautogui.doubleClick()
    pyautogui.typewrite(str(f['index']))
    pyautogui.press('enter')
    # Enter wave
    pyautogui.moveTo(location_wave_button)
    pyautogui.doubleClick()
    pyautogui.typewrite(str(f['wave']))
    pyautogui.press('enter')
    # Enter angle
    pyautogui.moveTo(location_angle_button)
    pyautogui.doubleClick()
    pyautogui.typewrite(str(f['angle']))
    pyautogui.press('enter')

def generate_data():
    # Fit to regularized
    pyautogui.hotkey('alt', 'i')
    pyautogui.press('r')
    pyautogui.moveTo(number_of_gridpoints)
    pyautogui.doubleClick()
    pyautogui.typewrite(str(250))
    time.sleep(2)
    pyautogui.moveTo(reg_fit_ok)
    pyautogui.click()
    time.sleep(2)
    # Set as unweighted
    pyautogui.hotkey('alt', 'w')
    pyautogui.press('2')
    pyautogui.moveTo(location_copydata_button)
    pyautogui.rightClick()
    pyautogui.press('x')

```

```
    pyautogui.press(’x’)
    pyautogui.press(’enter’)
    pyautogui.press(’enter’)
    # Press ‘copy to clipboard‘ button and close the dataset to avoid clutter
    pyautogui.moveTo(location_copydata_button)
    pyautogui.click()
    pyautogui.hotkey(’alt’, ’w’)
    pyautogui.press(’5’)
    pyautogui.hotkey(’ctrl’, ’f4’)
    pyautogui.hotkey(’alt’, ’y’)


def paste_excel(file, filename):
    pyautogui.PAUSE = 0.6
    pyautogui.hotkey(’alt’, ’tab’)  # Move to Excel
    pyautogui.press(’f5’)
    pyautogui.typewrite(’A1’)
    pyautogui.press(’enter’)
    pyautogui.hotkey(’ctrl’, ’v’)  # Paste clipboard
    # Retrieve target column
    pyautogui.press(’f5’)
    pyautogui.typewrite(’B3:B303’)
    pyautogui.press(’enter’)
    time.sleep(1)
    pyautogui.hotkey(’ctrl’, ’pagedown’)
    # Change tab and paste summary
    for k, v in file.items():
        pyautogui.typewrite(str(k))
        pyautogui.press(’tab’)
        pyautogui.typewrite(str(v))
        pyautogui.press(’enter’)
    pyautogui.hotkey(’ctrl’, ’pageup’)
    pyautogui.hotkey(’ctrl’, ’c’)
    pyautogui.hotkey(’ctrl’, ’pagedown’)
    pyautogui.hotkey(’ctrl’, ’v’)
    pyautogui.hotkey(’ctrl’, ’up’)
    pyautogui.hotkey(’ctrl’, ’up’)
    pyautogui.hotkey(’ctrl’, ’up’)
    pyautogui.hotkey(’ctrl’, ’up’)
    pyautogui.hotkey(’ctrl’, ’pageup’)  # Back to original page
    # Save data
    pyautogui.hotkey(’alt’, ’f’)
    pyautogui.press(’a’)
    pyautogui.press(’y’)
    pyautogui.press(’3’)
    pyautogui.typewrite(filename)
    pyautogui.press(’enter’)
    # # Decomment when autosave switches ON automatically
    # time.sleep(3)
    # pyautogui.moveTo(autosave_off)
    # pyautogui.click()
    # Get ready for next file
    time.sleep(2)
    pyautogui.hotkey(’alt’, ’tab’)  # Back to A5ECORR.exe
```

```python
    return None

if __name__ == '__main__':
    # Validations before running
    pyautogui.moveTo(location_temperature_button)
    pyautogui.alert(text='Temperature button location',
                    title='Test', button='OK')
    pyautogui.moveTo(location_copydata_button)
    pyautogui.alert(text='Copy data button location',
                    title='Test', button='OK')
    pyautogui.alert(text='Ensure Excel with two empty sheets is opened on the nearest Alt-Tab',
                    title='Test', button='OK')
    time.sleep(3)

    # Start running
    files = get_files()
    pyautogui.hotkey('alt', 'w')
    pyautogui.press('t')
    setup_a5ecorr()
    for f in files:
        open_file_a5ecorr(f['name'])
        enter_properties(temp=f['temp'], visc=f['visc'])
        generate_data()
        paste_excel(f, f['name'])

    pyautogui.alert(text='Done!',
                title='Test', button='OK')
```

Listing 1 – Automated ALV-correlator data readout code.

Appendix B: sld Calculation

All constants needed to further calcualte the slds of the sphere and the solvent are given in the table below.

Table 6.1 – The needed constants to calculate the total slds of the sphere and solvent.

Chemical	**Density g/ml**	**sld** (10^{-6}Å^{-2})
H_2O	1.0	-5.60
D_2O	1.1	6.33
Acetone-D_6	0.87	5.39
PBd$_{1700}$-PEO$_{1000}$	1.1	5.45

For the block copolymer concentration is 20mg/ml and all samples were prepared at 6vol% water mixture.

The formulas used to calculate the sld$_{sphere}$ is

$$\begin{aligned}\text{sld}_{\text{ sphere}} =& \frac{V_{\text{PB-b-PEO,sphere}} \cdot \text{sld}_{\text{ PB-b-PEO}} + V_{\text{C}_3\text{D}_6\text{O, sphere}} \cdot \text{sld}_{\text{C}_3\text{D}_6\text{O}}}{(V_{\text{PB-b-PEO, sphere}} + V_{\text{C}_3\text{D}_6\text{O, sphere}} + V_{\text{H}_2\text{O, sphere}} + V_{\text{D}_2\text{O, sphere}})} \\ &+ \frac{V_{\text{H}_2\text{O, sphere}} \cdot \text{sld}_{\text{ H}_2\text{O}} + V_{\text{D}_2\text{O, sphere}} \cdot \text{sld}_{\text{D}_2\text{O}}}{(V_{\text{PB-b-PEO,sphere}} + V_{\text{C}_3\text{D}_6\text{o, sphere}} + V_{\text{H}_2\text{O,sphere}} + V_{\text{D}_2\text{O, sphere}})}.\end{aligned}$$

For the sld$_{solution}$

$$\begin{aligned}\text{sld}_{\text{ sphere}} =& \frac{V_{\text{PB-b-PEO,sphere}} \cdot \text{sld}_{\text{ PB-b-PEO}} + V_{\text{C}_3\text{D}_6\text{O, sphere}} \cdot \text{sld}_{\text{C}_3\text{D}_6\text{O}}}{(V_{\text{PB-b-PEO, sphere}} + V_{\text{C}_3\text{D}_6\text{O, sphere}} + V_{\text{H}_2\text{O, sphere}} + V_{\text{D}_2\text{O, sphere}})} \\ &+ \frac{V_{\text{H}_2\text{O, sphere}} \cdot \text{sld}_{\text{ H}_2\text{O}} + V_{\text{D}_2\text{O, sphere}} \cdot \text{sld}_{\text{D}_2\text{O}}}{(V_{\text{PB-b-PEO,sphere}} + V_{\text{C}_3\text{D}_6\text{o, sphere}} + V_{\text{H}_2\text{O,sphere}} + V_{\text{D}_2\text{O, sphere}})}\end{aligned}$$

was used.

The library made to calculate the slds for the samples:

```python
import numpy as np
import pandas as pd
from scipy import stats

#Readout excel file with standard sld data
def data_readout(file_name):
    #Extract data
    excel = pd.read_excel(file_name, engine='openpyxl', skiprows=0)
    data = np.array(excel) #Make np array for further calculations

    #Parameters from data
    DH = data[:,0] #Ratio D2O/H2O
    concentration = data[:,1] #Mass density D2O/H2O
    D2O_H2O_sld = data[:,2] #Calcluated slds D2O/H2O with sld calculator SasView
```

```
    DA_sld = data[:,3] #sld for acetone-D6
    PBd_PEO_sld = data[:,4] #sld for block copolymer

    return DH, concentration, D2O_H2O_sld, DA_sld, PBd_PEO_sld

def sld_and_intersect(DA_volume, polymer_volume, D2O_H2O_volume, D2O_H2O_sld, PBd_PEO_sld,
                        DA_ratio_sphere, DA_sld, D2O_H2O_ratio_sphere, polymer_ratio_sphere,
                        D2O, H2O):
    ###Variables
    #Calc D2O and H2O parameters
    D2O_volume = D2O*D2O_H2O_volume/100
    sld_D2O = D2O_H2O_sld[0]
    H2O_volume = H2O*D2O_H2O_volume/100
    sld_H2O = D2O_H2O_sld[-1]

    #Ratio particles present in solution
    DA_ratio_solution = 1-DA_ratio_sphere
    D2O_H2O_ratio_solution = 1-D2O_H2O_ratio_sphere
    polymer_ratio_solution = 1-polymer_ratio_sphere

    ###Sphere
    #Volume fractions present in sphere
    DA_sphere = DA_volume*DA_ratio_sphere
    D2O_H2O_sphere = D2O_H2O_volume*D2O_H2O_ratio_sphere
    H2O_sphere = H2O_volume*D2O_H2O_ratio_sphere
    D2O_sphere = D2O_volume*D2O_H2O_ratio_sphere
    polymer_sphere = polymer_volume*polymer_ratio_sphere

    #sld from formula
    sld_sphere = ((polymer_sphere*PBd_PEO_sld + DA_sphere*DA_sld)
                   / (polymer_sphere+DA_sphere+D2O_H2O_sphere)
                   + (H2O_sphere*sld_H2O + D2O_sphere*sld_D2O)
                   / (polymer_sphere+DA_sphere+D2O_H2O_sphere))

    ###Solution
    #Volume fractions present in solution
    DA_solution = DA_volume*DA_ratio_solution
    D2O_H2O_solution = D2O_H2O_volume*D2O_H2O_ratio_solution
    H2O_solution = H2O_volume*D2O_H2O_ratio_solution
    D2O_solution = D2O_volume*D2O_H2O_ratio_solution
    polymer_solution = polymer_volume*polymer_ratio_solution

    #sld from formula
    sld_solution = ((polymer_solution*PBd_PEO_sld + DA_solution*DA_sld)
                   / (polymer_solution+DA_solution+D2O_H2O_solution)
                   + (H2O_solution*sld_H2O + D2O_solution*sld_D2O)
                   / (polymer_solution+DA_solution+D2O_H2O_solution))

    ###Find contrast matching points by calculating the intersection of the two linear sld lines
    #The slds are not returned as floats, but should be floats
    sld_solution = sld_solution.astype(np.float)
    sld_sphere = sld_sphere.astype(np.float)
```

```
    #Fit lines to y=ax+b and extract a and b
    solution_slope, solution_start, r_value, p_value, std_err = stats.linregress(H2O,sld_solution)
    sphere_slope, sphere_start, r_value, p_value, std_err = stats.linregress(H2O,sld_sphere)
    #Intersection point at x = (b2-b1)/(a1-a2)
    H2O_intersect = (solution_start-sphere_start)/(sphere_slope-solution_slope)
    sld_intersect = solution_slope*H2O_intersect+solution_start

    return sld_sphere, sld_solution, H2O_intersect, sld_intersect
```

Listing 2 – Library to calculate slds.

The code used to plot the slds:

```
import numpy as np
import matplotlib.pyplot as plt
import xlsxwriter
import sld_calc as sld

''' This script can be used to make a plot for specific sphere/solution ratios. The found slds can then be filled in in SasView per sample. '''

#font
plt.rcParams['font.family'] = 'Times New Roman'
plt.rcParams.update({'font.size': 14})
linestyles = ['solid', 'dashed', 'dotted', 'dashdot', (0, (3, 1, 1, 1, 1, 1)), (0, (5, 5))]
markers = np.array(['o', '^','s', 'p', 'h', '*'])
colors = np.array(['black', 'darkblue', 'blue', 'royalblue', 'cornflowerblue', 'lightsteelblue'])

if __name__ == '__main__':
    #datareadout
    data = sld.data_readout("sld_data.xlsx")
    D2O_H2O_sld = data[2]
    DA_sld = data[3]
    PBd_PEO_sld = data[4]

    #Ratio D2O/H2O
    H2O = np.arange(0,101,10)
    D2O = 100-H2O
    #Make D2O/H2O labels for x-axis
    xlabel = []
    for i in range(len(D2O)):
        xlabel.append("{}/{}".format(D2O[i],H2O[i]))

    #Parameters
    DA_volume = 1 #ml

    concentration_water = 0.06 #vol%
    D2O_H2O_volume = concentration_water*DA_volume #ml

    polymer = 20 #mg
```

```
    polymer_density_g = 1.1 #g/cm3 = g/ml
    polymer_density = polymer_density_g*1000 #mg/ml-->[mg]/[mg/ml]=x ml
    polymer_volume = polymer/polymer_density

    #Ratio particles present in sphere
    DA_ratio_sphere = 0.5
    D2O_H2O_ratio_sphere = 0.7
    polymer_ratio_sphere = 0.4

    slds = sld.sld_and_intersect(DA_volume, polymer_volume, D2O_H2O_volume, D2O_H2O_sld, PBd_PEO_sld, DA_ratio_sphere, DA_sld, D2O_H2O_ratio_sphere, polymer_ratio_sphere,nD2O, H2O)
    sld_sphere = slds[0]
    sld_solution = slds[1]
    H2O_intersect = slds[2]
    sld_intersect = slds[3]

    # Make table
    table = xlsxwriter.Workbook('sld_calculated.xlsx')
    sheet = table.add_worksheet()
    sheet.write('A1', 'D2O/H2O')
    sheet.write('B1', 'sld_sphere')
    sheet.write('C1', 'sld_solution')
    sheet.write('D1', 'H2O_intersect')
    sheet.write('E1', 'sld_intersect')
    if (0<H2O_intersect<100):
        sheet.write('D2', H2O_intersect)
        sheet.write('E2', sld_intersect)

    row = 1
    for i in range(len(sld_sphere)):
        sheet.write(row, 0, xlabel[i])
        sheet.write(row, 1, sld_sphere[i])
        sheet.write(row, 2, sld_solution[i])
        row+=1
    table.close()

    #Plot to make intersection visual
    plt.figure(1, figsize=(6,5))
    #Sphere
    plt.plot(H2O, sld_sphere, color = colors[2], label = 'sphere')
    #Solvent
    plt.plot(H2O, sld_solution, color = colors[4], label = 'solution', linestyle = linestyles[1])
    #Intersection point
    plt.plot(H2O_intersect, sld_intersect, marker = markers[0], color = colors[0], label = 'intersection', linestyle = None)
    plt.xlim(xlabel[0], xlabel[-1])
    plt.xticks(ticks = H2O, labels = xlabel)
    plt.xlabel('D2O/H2O')
    plt.ylabel('sld [A]')
    plt.grid()
    plt.legend()
    plt.show()
```

Listing 3 – Code to plot the slds and find the intersection.

Appendix C: Contrast Matching

```python

import numpy as np
import xlsxwriter
import sld_calc as sld

if __name__ == '__main__':
    ###Data
    data = sld.data_readout("sld_data.xlsx")
    D2O_H2O_sld = data[2]
    DA_sld = data[3]
    PBd_PEO_sld = data[4]

    ###Parameters
    #Ratio D2O/H2O
    H2O = np.arange(0,101,10)
    D2O = 100-H2O

    #Make D2O/H2O labels for x-axis
    xlabel = []
    for i in range(len(D2O)):
        xlabel.append("{}/{}".format(D2O[i],H2O[i]))

    #Volumes
    DA_volume = 1 #ml

    concentration_water = 0.06 #vol%
    D2O_H2O_volume = concentration_water*DA_volume #ml

    polymer = 20 #mg
    polymer_density_g = 1.1 #g/cm3 = g/ml
    polymer_density = polymer_density_g*1000 #mg/ml --> [mg]/[mg/ml] = x ml
    polymer_volume = polymer/polymer_density #ml

    ###Variables
    #Define all volume fraction in sphere
    min_fraction = 0
    step_size_fraction = 0.01
    max_fraction = 1+step_size_fraction
    volumes = np.arange(min_fraction,max_fraction,step_size_fraction)

    #Only acceptable volume fractions (min in sphere, max in sphere, step size)
    volumes_DA = np.arange(0.01,1.01,0.01)
    volumes_D2O_H2O = np.arange(0,1.01,0.01)
    volumes_polymer = np.arange(0.4,1.01,0.01)

    #Contrast matching range
    min_contrast_match = 25
    max_contrast_match = 35

    ###Make and write to excel sheet
```

```
    table = xlsxwriter.Workbook('countour_plot_25_35.xlsx')
    sheet = table.add_worksheet()
    sheet.write('A1', 'Acetone in sphere')
    sheet.write('B1', 'D2O/H2O in sphere')
    sheet.write('C1', 'Polymer in sphere')
    sheet.write('D1', 'Contrast matching at (H2O)')
    sheet.write('E1', 'Contrast matching at sld')
    row = 1

    ### Find all possible ratios for which contrast matching occurs
    #Loop over al possible volume ratios, to find all contrast matching values for specified range
    for i in range(len(volumes_DA)):
        DA_ratio_sphere = volumes_DA[i]
        for k in range(len(volumes_D2O_H2O)):
            D2O_H2O_ratio_sphere = volumes_D2O_H2O[k]
            for l in range(len(volumes_polymer)):
                polymer_ratio_sphere = volumes_polymer[l]

                #Cannot divide by zero
                if (DA_ratio_sphere + D2O_H2O_ratio_sphere + polymer_ratio_sphere == 0):
                    continue
                if (DA_ratio_sphere + D2O_H2O_ratio_sphere + polymer_ratio_sphere == 3*volumes[-1]):
                    continue

                #Calculate the slds and extract the intersection points
                slds = sld.sld_and_intersect(DA_volume, polymer_volume, D2O_H2O_volume, D2O_H2O_sld, PBd_PEO_sld,
                        DA_ratio_sphere, DA_sld, D2O_H2O_ratio_sphere, polymer_ratio_sphere,
                        D2O, H2O)
                H2O_intersect = slds[2]
                sld_intersect = slds[3]

                if (H2O_intersect >= min_contrast_match and H2O_intersect <= max_contrast_match):
                    print('Contrast matching found at (ikl):[{},{},{}] for H2O={}'.format(DA_ratio_sphere, D2O_H2O_ratio_sphere, polymer_ratio_sphere, H2O_intersect))
                    sheet.write(row, 0, DA_ratio_sphere)
                    sheet.write(row, 1, D2O_H2O_ratio_sphere)
                    sheet.write(row, 2, polymer_ratio_sphere)
                    sheet.write(row, 3, H2O_intersect)
                    sheet.write(row, 4, sld_intersect)
                    row+=1
    table.close()
```

Listing 4 – Code to calculate the possible volume fractions which result in contrast matching.