

# Synthesis and study of SiO<sub>2</sub>-pNipam nanoparticles

---

Martí Balaguer, Gerard

Universitat Autònoma de Barcelona

Supervisor: Lara Frenzel

05/09/2018



In these two months at DESY I have been working with silica-pNipam nanogels. Here the procedure followed for the synthesis of those nanoparticles is presented in two independent steps. With regard to the study, the addition of ethanol as a cosolvent has resulted in a change of the hydrodynamic radius and the thermal response allows to see that the interactions change when modifying the percentage of volume of ethanol.

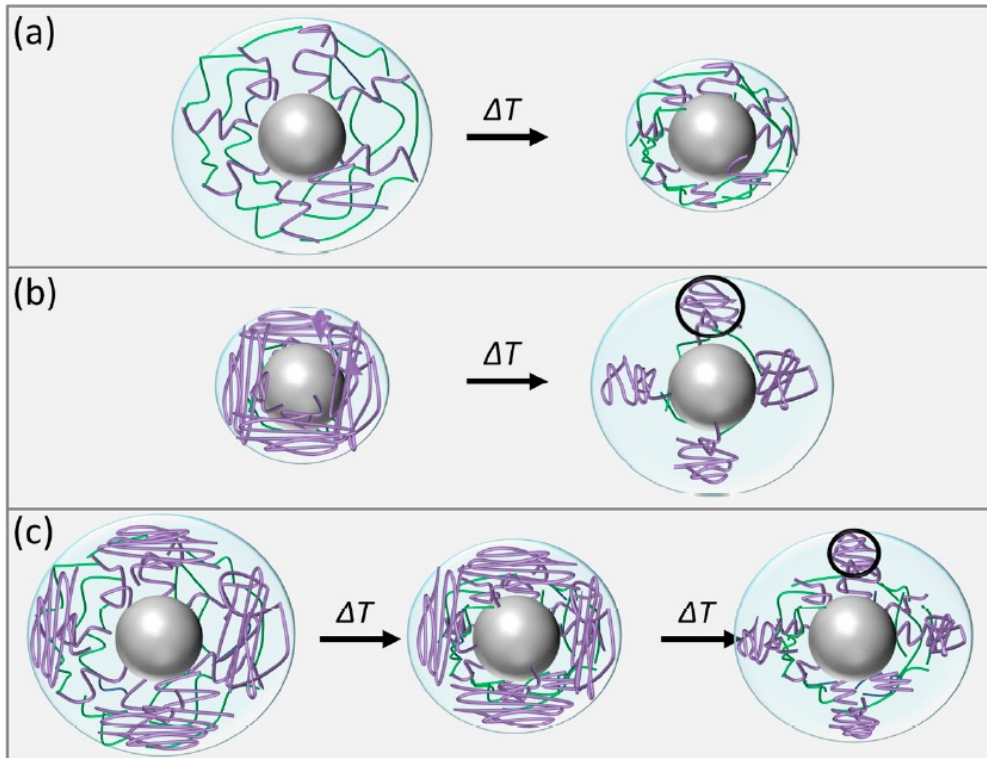
*Per aspera ad astra.*

## Index

|   |    |
|---|----|
| 1-.Introduction.....  | 2  |
| 2-.SiO <sub>2</sub> -pNipam synthesis.....                                | 3  |
| 2.1-.Silica cores.....  | 3  |
| 2.2-.Brushes.....   | 3  |
| 3-.Physical properties of water\ethanol mixtures.....                     | 5  |
| 4-.Preparation of the samples.....  | 6  |
| 5-.Dynamic Light Scattering (DLS).....                                    | 7  |
| 6-.Experimental setup and measurements.....                               | 9  |
| 7-.Results and discussion.....  | 9  |
| 7.1-.Variation of the hydrodynamic radius with ethanol concentration..... | 9  |
| 7.2-.Variation of the hydrodynamic radius with temperature.....           | 12 |
| 7.3-.A bit of dynamics.....   | 14 |
| 7.4-.About the synthesized nanoparticles.....                             | 14 |
| 8-.Acknowledgements.....  | 16 |
| 9-.References.....  | 17 |

## 1-Introduction

PNipam nanoparticles have been studied in a wide range of situations and it has been seen that their applications could be useful for the development of new technologies and the understanding of polymers and proteins behavior. This nanoparticles consist in cores made of silica with poly(N-isopropylacrylamide) in its surfaces. These polymers added can have various kinds of crosslinking and give different responses to stimuli such as temperature changes [1,2,19], as shown in figure 1.



**Figure 1.** PNipam nanoparticles with different crosslinking and their correspondent transitions.

When a shrinking or a swelling happens at a certain temperature it is said that there has been a volume phase transition. The temperature at which the transition happens is called lower critical solution temperature (LCST). Other things that can affect the size of the brush are the pH or the addition of cosolvents [1,2,11-19]. In my case I have studied the transitions of type a) shown in figure 1 and studied the effect of the addition of ethanol. In pure water is well known that the LCST is around 32-33°C. Then, the main objectives are to study the variation of the nanoparticles size changing the amount of ethanol at a given temperature and see after, for some samples, the behavior changing the temperature.

## 2.-SiO<sub>2</sub>-pNipam synthesis

The first thing I am going to explain is the synthesis of these nanoparticles. This procedure consists of two independent steps: making the silica cores and after a polymerization to obtain the brushes.

### 2.1-.Silica cores

This process is based on the Stöber synthesis [3], the aim is to obtain monodisperse silica cores. All the steps where the reagents are mixed or stirred are done in a fume hood. First, 420 mL of ethanol (>99.8%) were put into a 1L Erlenmeyer flask and closed with a glass stopper. Then, 20.0 mL of ammonium hydroxide 25% were added into the Erlenmeyer followed by a magnetic stirring bar and the flask was placed in a stirring plate at 500 rpm. The mixture was let to stir for a few minutes. Meanwhile, 30.0 mL of ethanol were put it in a 100 mL beaker. After, the beaker was placed in in high precision balance. A decrease of the mass was observed because of the evaporation of the ethanol, then, this step was done as quickly as possible. 7.0090 g of TEOS (tetraethyl orthosilicate) were weighted, probably it was a bit more due to that evaporation commented before. Next, the mixture of ethanol\TEOS was added to the Erlenmeyer and let all stir at 500 rpm for approximately 24 hours.

After the 24 hours, the solution changed from transparent to white, which was a sign that there were silica cores. Then, 2.000 mL of TPM (3-(trimethoxysilyl)propyl methacrylate) (95%) were added to the round bottom flask followed by 15 mL of ammonium hydroxide 25%. The mixture was let stirring for 24 hours.

When the reaction was finished the ammonia and part of the ethanol had to be evaporated until the remaining volume was around 50 mL. Due to technical reasons with the rotary evaporator the mixture was not able to be submitted to the process until five days after. In the meantime the sample was preserved in a 1L round bottom flask with a stopper inside the fume hood. Once the technical problem was solved the ammonia and ethanol were evaporated. The thermal bath was around 40-45°C and the pressure was set at 150 mbar with a  $\Delta p$  of 10 mbar, when the sample was more concentrated the pressure was lowered to 130 mbar. Once the ~50 mL were reached a dialysis tube was prepared, the sample was introduced and then the tube closed with a knot. Then, the tube was submerged in ethanol (~400mL) for a week, changing the ethanol every two days. It was important to take few drops of the product before putting it into the dialysis tube, so the size of the silica cores had to be checked. The measurements of the radius of the silica cores gave a value around 60 nm.

### 2.2-.Brushes

This second part is based on the method proposed by Nils Nun et al. [1], but with different amounts of reagents, so the aim is to get three kinds of SiO<sub>2</sub>-pNipam nanoparticles each with different size. The reactants and its amounts can be seen in

Table 1, where SDS is sodium dodecyl sulphate and BIS is N,N'-methylenebis(acrylamide). All the reagents are weighted with a high precision balance.

**Table 1. Amount of reagents for each brush.**

| Reagent   | Amount (g) |        |        |
|---|------------|--------|--------|
|   | #1         | #2     | #3     |
| SDS   | 0.1256     | 0.1253 | 0.1249 |
| NaSO <sub>3</sub>   | 0.1403     | 0.1400 | 0.1401 |
| (NH <sub>4</sub> ) <sub>2</sub> Fe(SO <sub>4</sub> ) <sub>2</sub> | 0.0018     | 0.0035 | 0.0209 |
| K <sub>2</sub> S <sub>2</sub> O <sub>8</sub>                      | 0.1360     | 0.1350 | 0.1347 |
| BIS   | 0.0227     | 0.0663 | 0.1983 |
| Nipam   | 0.5062     | 1.5003 | 3.5003 |

The procedure for this second part started diluting the silica cores with ethanol until a total volume of 60 mL, in order to make it easy to divide by three. After, 20 mL of the silica cores dilution were put in a round bottom flask with four openings, followed by 100 mL of ultrapure water to sweep along the silica cores in the inner lining of the flask<sup>1</sup>. The SDS and the sodium sulphite were diluted in a beaker with a bit of ultrapure water and it was added to the round bottom flask. More water was added afterwards; the total volume added counting the one used to dilute the salts was 450 mL. Then, the flask was placed in a bath with a stirring bar and different things were put in the apertures: a stopper, a tube to bubble nitrogen, a septum and a Dimroth condenser. The bath temperature was set to 60°C and nitrogen was bubbling for an hour. The stirring worked at 500 rpm. After, a few crystals of the ammonium iron(II) sulphate and the potassium peroxodisulphate, diluted in around 15 mL of water, were added. The BIS and Nipam diluted in 13 mL of ethanol were also put in through an opened syringe and a needle piercing the septum. Once the addition was finished, the syringe with the needle was taken out and the bubbling system was changed for an adaptor for the tube, just to maintain the nitrogen atmosphere. The mixture was let to react overnight.

The next day most of the solvent had to be removed, so the rotary evaporator was used decreasing slowly the pressure. At 100-85 mbar an intense bubbling was observed and then, when it ended. The pressure was lowered to 70 mbar and after, in little steps<sup>2</sup>, until around 50 mbar, in order to extract the water until only ~100 mL were remaining. The thermal bath was set around 40-45°C. After the evaporation the sample was filtered by gravity to eliminate any polymer rests and possible dust<sup>3</sup> and put to dialysis as done with the silica cores, but using water (~4 L) instead of ethanol.

<sup>1</sup> With #1 I added first the salts and after all the water, maybe it changes something in the mixture.

<sup>2</sup> I checked the evaporation every 20 minutes and each time I lowered the pressure as much as I was able to in steps of 1 mbar, always treating well the vacuum pump, not forcing it. The overall and ideal time for the evaporation is 4 hours.

<sup>3</sup> The filtration of the nanoparticles with the thinnest shell (#1) took me 30-40 minutes, and after there were white residues in the dialysis tube, maybe the reaction went wrong.

The water was changed twice every day, in the morning and in the afternoon, except Saturday and Sunday.

### 3-.Physical properties of water/ethanol mixtures

It is well known that properties such as the refractive index and the viscosity changes with temperature [4,5], and that the refractive index also has dependence with the wavelength [6]. These values have been reported for many people [4,5,7], but in order to make the measurements the values for intermediate temperatures were also needed. In this case polynomial approximations were made using five known values for the viscosity, corresponding to 20, 25, 30, 35 and 40°C. For the refractive index less data was available and only four points were used, corresponding to 20, 25, 30 and 35°C, which means that the values over 35°C were extrapolated, but the values were reasonable. In Table 2 and Table 3 the values that were used for the approximations can be seen. Due to the small difference between the wavelength used and the one for which the values have been found, the refractive indices were considered the same as found, so the error committed can be disregarded [6].

**Table 2.Viscosity values at different temperatures for different mixtures water/ethanol.**

| %vol. ethanol | Viscosity (cP) |        |        |        |        |
|---------------|----------------|--------|--------|--------|--------|
|               | 20°C           | 25°C   | 30°C   | 35°C   | 40°C   |
| 0             | 1.0030         | 0.8914 | 0.7982 | 0.7202 | 0.6531 |
| 10            | 1.2887         | 1.1703 | 1.0200 | 0.9015 | 0.7975 |
| 20            | 1.8106         | 1.5963 | 1.4539 | 1.2355 | 1.0857 |
| 30            | 2.3133         | 2.0140 | 1.7660 | 1.4731 | 1.3168 |
| 40            | 2.6163         | 2.2875 | 2.0492 | 1.7568 | 1.5674 |
| 50            | 3.0791         | 2.3869 | 2.2487 | 1.7896 | 1.5924 |
| 60            | 3.0705         | 2.4236 | 2.0666 | 1.6249 | 1.4442 |
| 70            | 2.7908         | 2.2249 | 1.8958 | 1.5197 | 1.3584 |
| 80            | 2.3723         | 2.0271 | 1.7968 | 1.4360 | 1.2748 |
| 90            | 1.9501         | 1.6594 | 1.5189 | 1.2371 | 1.1262 |
| 100           | 1.1890         | 1.0995 | 1.0606 | 0.9698 | 0.8661 |

**Table 3.Refractive index values at different temperatures for different mixtures water/ethanol.**

| %vol. ethanol | Refractive index |       |       |       |
|---------------|------------------|-------|-------|-------|
|               | 20°C             | 25°C  | 30°C  | 35°C  |
| 0             | 1.333            | 1.333 | 1.332 | 1.332 |
| 10            | 1.338            | 1.338 | 1.337 | 1.336 |
| 20            | 1.344            | 1.344 | 1.343 | 1.341 |
| 30            | 1.350            | 1.349 | 1.348 | 1.346 |

|            |       |       |       |       |
|------------|-------|-------|-------|-------|
| <b>40</b>  | 1.355 | 1.354 | 1.352 | 1.350 |
| <b>50</b>  | 1.359 | 1.358 | 1.356 | 1.354 |
| <b>60</b>  | 1.362 | 1.361 | 1.359 | 1.357 |
| <b>70</b>  | 1.364 | 1.363 | 1.361 | 1.359 |
| <b>80</b>  | 1.365 | 1.363 | 1.361 | 1.359 |
| <b>90</b>  | 1.364 | 1.363 | 1.360 | 1.358 |
| <b>100</b> | 1.361 | 1.360 | 1.358 | 1.356 |

It can be observed that the trend is not trivial and in this case formulas such as the Lorentz-Lorenz equation [8] or others [9] can't be applied, because we don't have a mixture of two organic solvents.

#### 4-.Preparation of the samples

To prepare the samples in order to do the measurements ultrapure water and ethanol (>99.8%) were used. The first step was preparing the tubes. To prevent any kind of interference due to other little particles such as dust, a nitrogen current was applied for a few seconds. Then, all the tubes with the correspondent percentages of ethanol were marked before filling them with the solvents and the pNipam samples. In Table 4 the amounts of each substance introduced in the tubes can be consulted. The additions were made using different micropipettes.

**Table 4.Volumes added in the tubes.**

| <b>%vol. ethanol</b> | <b>Vol. water (µL)</b> | <b>Vol. ethanol (µL)</b> | <b>Vol. pNipam in water (µL)</b> | <b>Vol. pNipam in ethanol(µL)</b> |
|----------------------|------------------------|--------------------------|----------------------------------|-----------------------------------|
| <b>0</b>             | 3000                   | 0                        | 50                               | 0                                 |
| <b>10</b>            | 2700                   | 300                      | 45                               | 5                                 |
| <b>20</b>            | 2400                   | 600                      | 40                               | 10                                |
| <b>30</b>            | 2100                   | 900                      | 35                               | 15                                |
| <b>40</b>            | 1800                   | 1200                     | 30                               | 20                                |
| <b>50</b>            | 1500                   | 1500                     | 25                               | 25                                |
| <b>60</b>            | 1200                   | 1800                     | 20                               | 30                                |
| <b>70</b>            | 900                    | 2100                     | 15                               | 35                                |
| <b>80</b>            | 600                    | 2400                     | 10                               | 40                                |
| <b>90</b>            | 300                    | 2700                     | 5                                | 45                                |
| <b>100</b>           | 0                      | 3000                     | 0                                | 50                                |

It is important to respect the proportions; little changes in the concentrations can drive to different values when measuring the characteristics of the samples, mainly when the amount of ethanol is small.

Another thing that has to be taken into account is the order in that one introduces the things into the tubes, because I observed some kind of a coagulation of the drops of the nanoparticles in few cases. The best method should be the following one: 1)

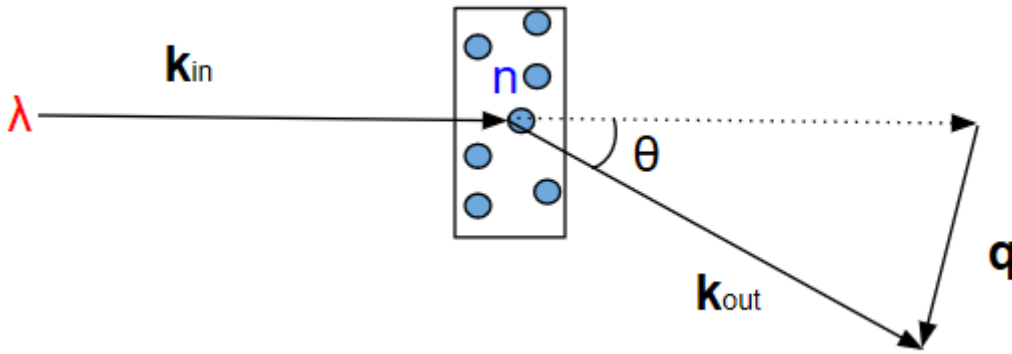
Major solvent. 2) SiO<sub>2</sub>-pNipam sample in the major solvent. 3) Mix if the volume allows it. 4) Minor solvent. 5) SiO<sub>2</sub>-pNipam sample in the minor solvent. In the case where the volumes of solvents are the same it does not matter which one you put first.

## 5-.Dynamic Light Scattering (DLS)

The dynamic light scattering is the technique I have used during the experiments to measure the hydrodynamic radius and other properties of the samples. As the name says the systems studied are dynamic systems, concretely we look at the speckle patterns generated when a sample is illuminated with a laser and its correlation through time [2,10]. The fluctuations of the intensity along time give us information about the colloidal suspension, which is contained in the normalized autocorrelation function

$$g_2(q, \tau) = \frac{\langle I(q,t)I(q,t+\tau) \rangle_t}{\langle I(q,t) \rangle_t^2} \quad (1)$$

where  $q = \frac{4\pi n}{\lambda} \sin(\theta/2)$  is the momentum transfer, being  $n$  the refractive index of the solvent,  $\theta$  the scattering angle at which the measure is done and  $\lambda$  the wavelength of the light used, as shown in figure 2, being  $|\mathbf{k}_{in}| = |\mathbf{k}_{out}|$ .



**Figure 2.** Scheme of the scattering process.

For Gaussian statistics we can use the normalized field correlation function

$$g_1(q, \tau) = \frac{\langle \vec{E}(q,t)\vec{E}^*(q,t+\tau) \rangle_t}{\langle I(q,t) \rangle_t^2} \quad (2)$$

where  $\vec{E}$  denotes the electric field. Then,  $g_2$  and  $g_1$  are related via the Siegert relation

$$g_2(q, \tau) = 1 + \beta |g_1(q, \tau)|^2 \quad (3)$$



where  $\beta$  is the coherent contrast, which typically has a value close to 1 for laser sources. The samples prepared were diluted in purpose, because in diluted suspensions the particles underlie Brownian motion. In these conditions  $g_1 = \exp[-\Gamma(q)t]$ , and thus

$$g_2(q, \tau) = 1 + \beta \exp[-2\Gamma(q)\tau] \quad (4)$$

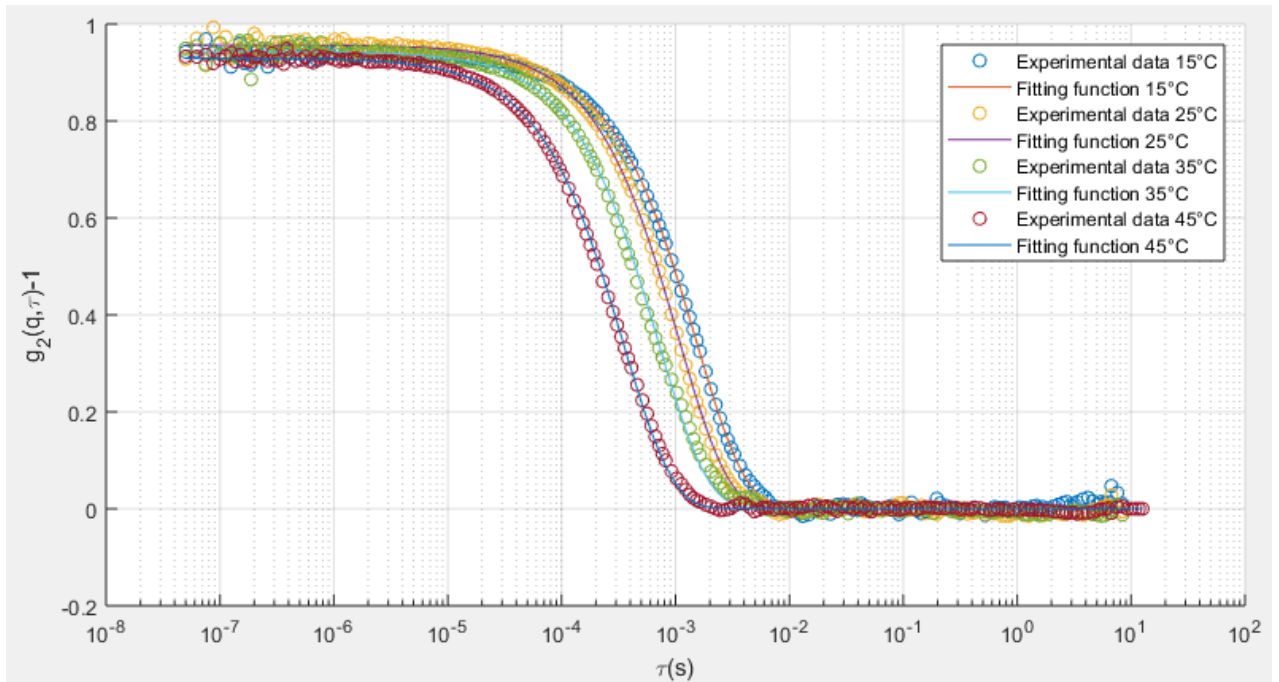
where  $\Gamma(q)$  denotes the relaxation rate that is given by

$$\Gamma(q) = Dq^2 \quad (5)$$

being  $D$  the translational diffusion coefficient. Moreover, the Stokes-Einstein relation allows us to calculate the hydrodynamic radius

$$R_H = \frac{k_B T}{6\pi\eta D} \quad (6)$$

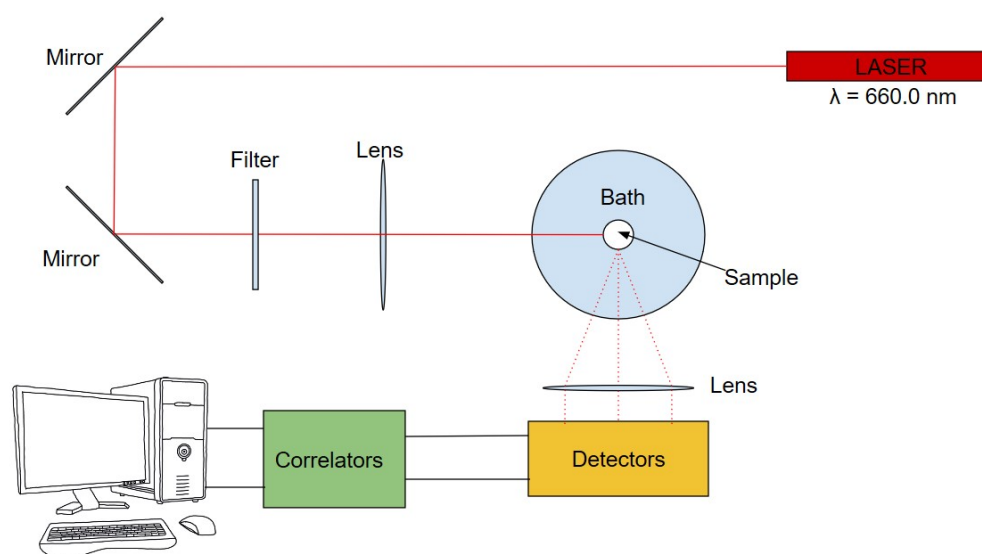
with the viscosity  $\eta$  of the solvent. Then, recording the intensity patterns at different angles, temperatures and compositions we can determine the value of the hydrodynamic radius and the diffusion coefficient in each case. In figure 3 it can be seen how the  $g_2$  function looks like with its correspondent fitting function.



**Figure 3.** Example of the  $g_2$  function and its fitting function for the diluted sample IL23-2 at different temperatures.

## 6-.Experimental setup and measurements

In my case the experimental set up was built I have only used the machine to measure. The set up consisted in the elements that can be seen in figure 4. In order to do the measurements I filled the options given in the DLS program. In figure 5 the window with the things I was able to modify can be seen, and the most important for my study are signaled. It is important to notice that the program has the values of viscosity and refractive index for some solvents, but in my case I had mixtures and I had to load a document with the values or to introduce them manually.



**Figure 4.** Experimental set up used for the measurements.

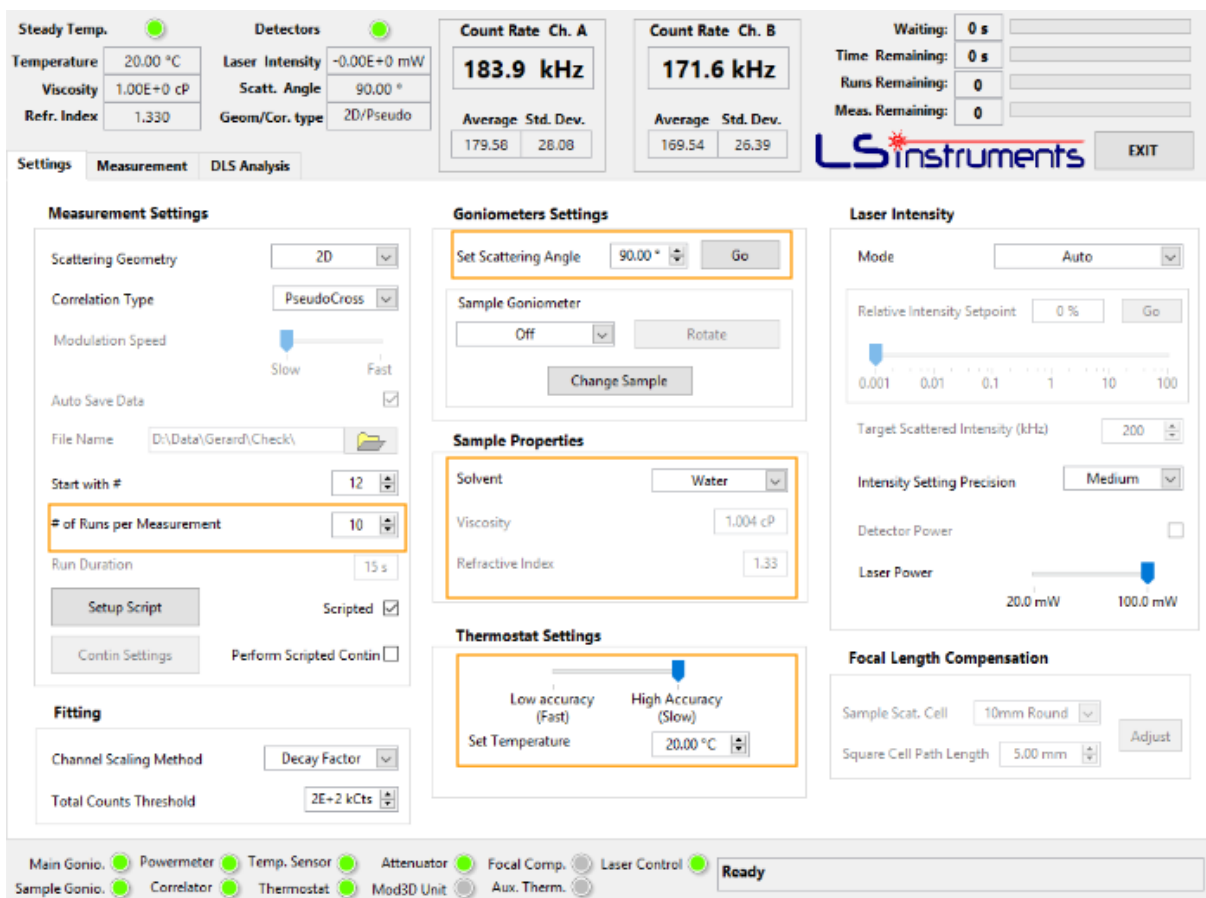
## 7-.Results and discussion

### 7.1-.Variation of the hydrodynamic radius with ethanol concentration

The first study consists on measuring the hydrodynamic radius of each sample prepared at 20°C and see how it changes. In order to do so, each sample has been in the bath for 15 minutes before the measurement. The results obtained are represented in figures 6 and 7, but to compare with the data found is easier to treat the things with %vol<sup>4</sup>. The trend looks like the one reported for Yunglon Yu et al. [11] and I. Bischofberger et al. [12] with the exception in the 10%vol. of ethanol, where I obtained an increase of  $R_H$  when the expected trend was a decrease in that point. It is clear that the minimum value is achieved at 30%vol. of ethanol, which agrees with the found results. The explanation to this kind of trend is quite complex and three main things have to be taken into account:

---

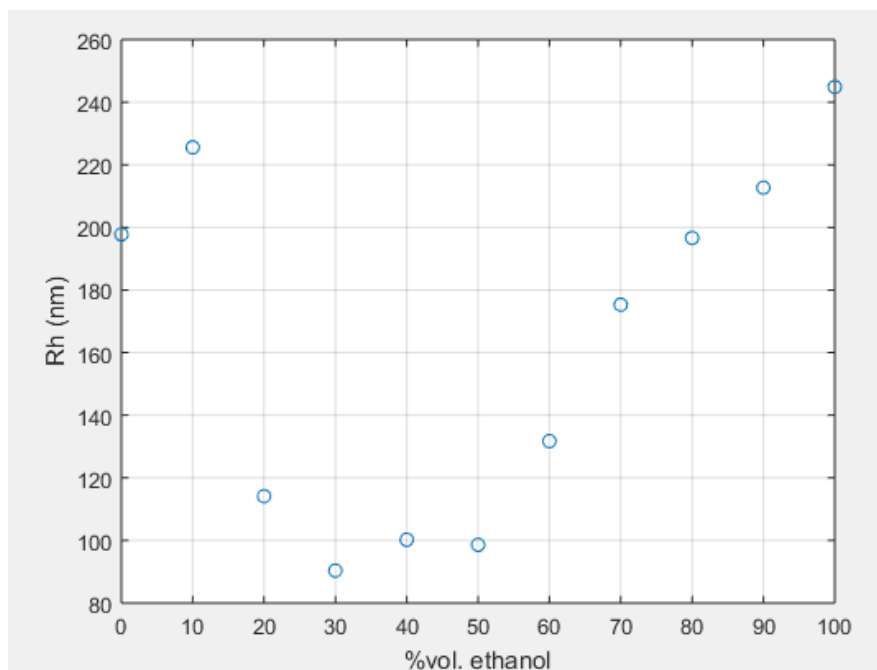
<sup>4</sup> The %volume is not strictly correct in this case, so we are not taking into account the contraction when mixing water and ethanol, in other words, the final volume is not the sum of the individual volumes mixed.



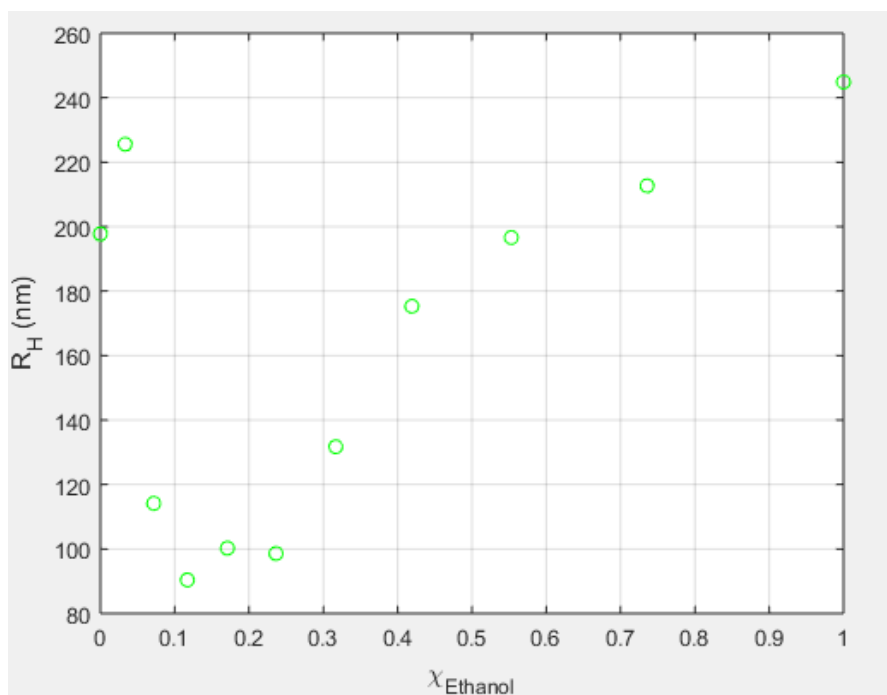
**Figure 5.** Window of the DLS program.

- There are nine possible kinds of hydrogen bonds that can be formed [13], so there are three donors (-NH, -OH<sub>water</sub>, -OH<sub>ethanol</sub>) and three acceptors (-C=O, -OH<sub>water</sub>, -OH<sub>ethanol</sub>).
- There are hydrophilic and hydrophobic parts in pNipam chains [14,15], which are schemed in figure 8.
- Clusters of water\ethanol ((H<sub>2</sub>O)<sub>m</sub>(EtOH)<sub>n</sub>) can be formed [16,17] due to a more favorable interaction than with pNipam.

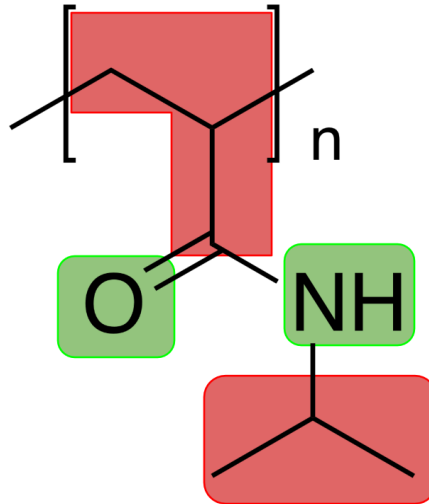
All three factors contribute in some way to the trend. It is clear that at low ethanol concentrations the interaction pNipam-water is weakened, but at higher concentrations of the alcohol the interaction pNipam-ethanol is the dominant one and causes bigger swelling than with pure water.



**Figure 6.** Hydrodynamic radius as a function of the %vol. of ethanol.



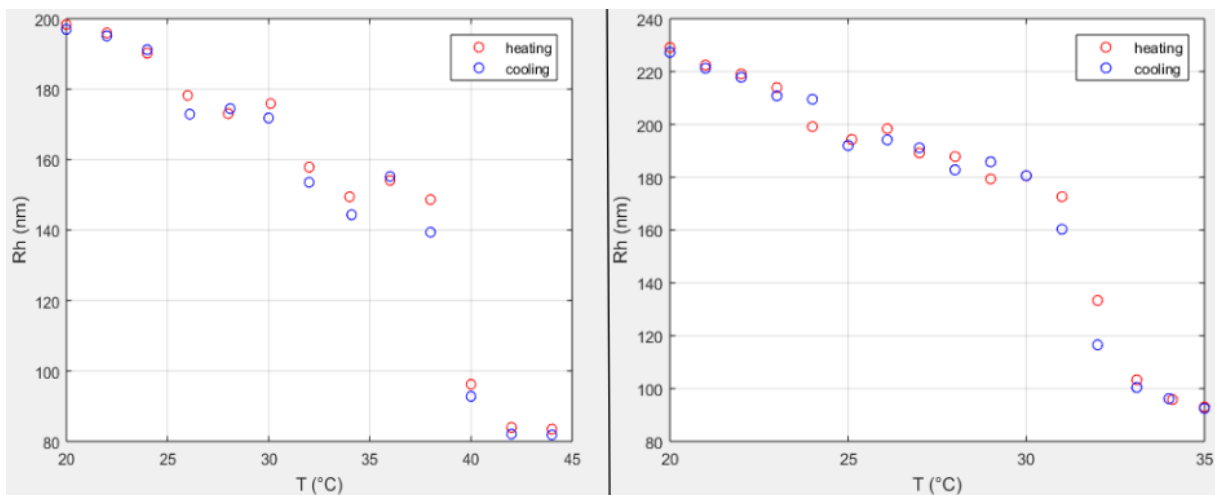
**Figure 7.** Hydrodynamic radius as a function of the molar fraction of ethanol.



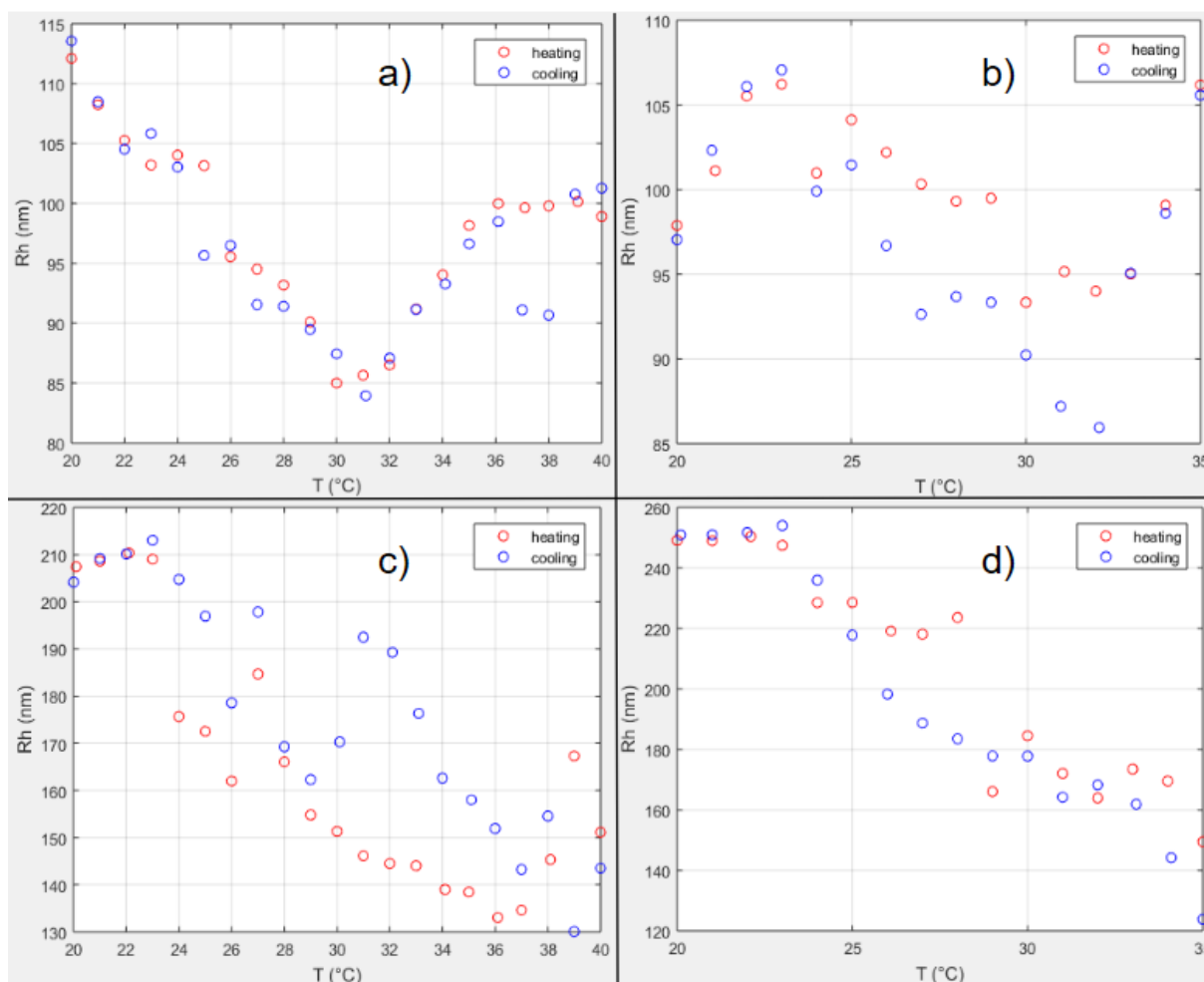
**Figure 8.** PNipam chemical structure with its hydrophilic (green) and hydrophobic (red) parts.

### 7.2-.Variation of the hydrodynamic radius with temperature

After varying the ethanol concentration the hydrodynamic radius of the samples was measured with 0, 10, 20, 50, 80 and 100%vol.of ethanol at different temperature ranges. The results obtained can be seen in figures 9 and 10.



**Figure 9.** Hydrodynamic radius as a function of temperature for the samples containing 0 (left) and 10% (right) vol. of ethanol.



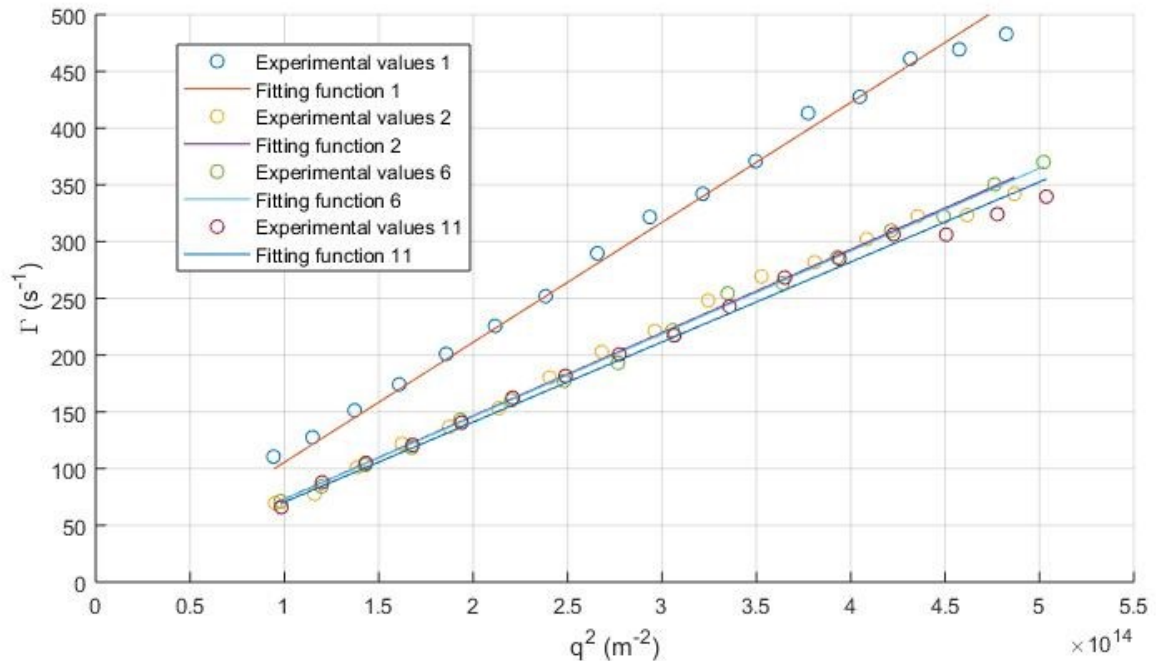
**Figure 10.** Hydrodynamic radius as a function of temperature for the samples containing a) 20, b) 50, c) 80 and d) 100%vol. of ethanol.

In figure 9 we can observe a transition for both samples, in pure water around 39.0-39.5°C and for the one containing 10%vol. of ethanol it is around 31.5-32.0°C, which means that there is a decrease of around 7-8°C in the LCST, which is in agreement with the data reported by Maria C.M. Costa et al. [18], but the values of the temperatures are different. I think there should be something wrong with my samples, because in pure water the transition should occur at 33°C approximately [19] and I obtained a value pretty far from this one.

For figure 10 no results have been found to compare with, but there is no appreciable trend in b) and c). In a) it seems that there is a deswelling and after the nanoparticles swell again around 30-31°C, but in less extent. It has been reported that no transition is observed in pure ethanol [18], but in the graphic a kind of trend can be appreciated, mainly when cooling. When heating there seems to be a sudden transition from 28 to 29°C, but I have found no explanations or reported data about that behavior. It seems that in cases a), b) and c) there is not a dominant interaction and there is a lot of competitiveness between the different hydrogen bonds and the swelling-deswelling produced because of the solvents mixtures and the temperature changes.

### 7.3-.A bit of dynamics

This last part is an extra I did to check that the samples were diluted enough to consider Brownian motion and that the problem of the point at 10%vol. ethanol in section 7.1 was not due to a high concentration of the nanoparticles. The results obtained are shown in figure 11.



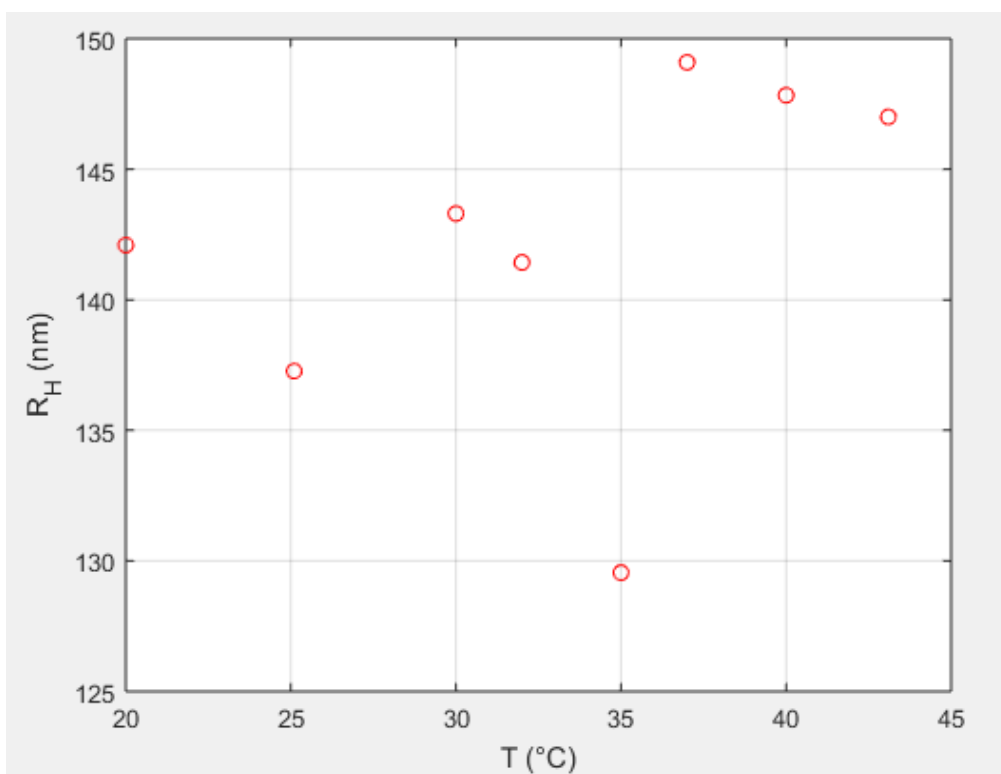
**Figure 11.** Relaxation rate as a function of  $q^2$  at 20°C for the samples containing 0 (1), 10 (2), 50 (6) and 100%vol. (11) of ethanol.

The linear fittings are good for all four cases, so the samples were diluted enough. The curious thing is that the diffusion coefficient, which we can obtain with eq. (5), decreases considerably from 0 to 10%vol. ethanol, which obtained values were  $10.565 \times 10^{-13}$  and  $7.325 \times 10^{-13}$   $m^2/s$  correspondingly, and then it remains more or less constant until the 100%, concretely  $7.293 \times 10^{-13}$   $m^2/s$  for sample 6 and  $7.051 \times 10^{-13}$   $m^2/s$  for 11. No clear explanations have been given, due to the complex interactions explained in subsection 7.1 [20].

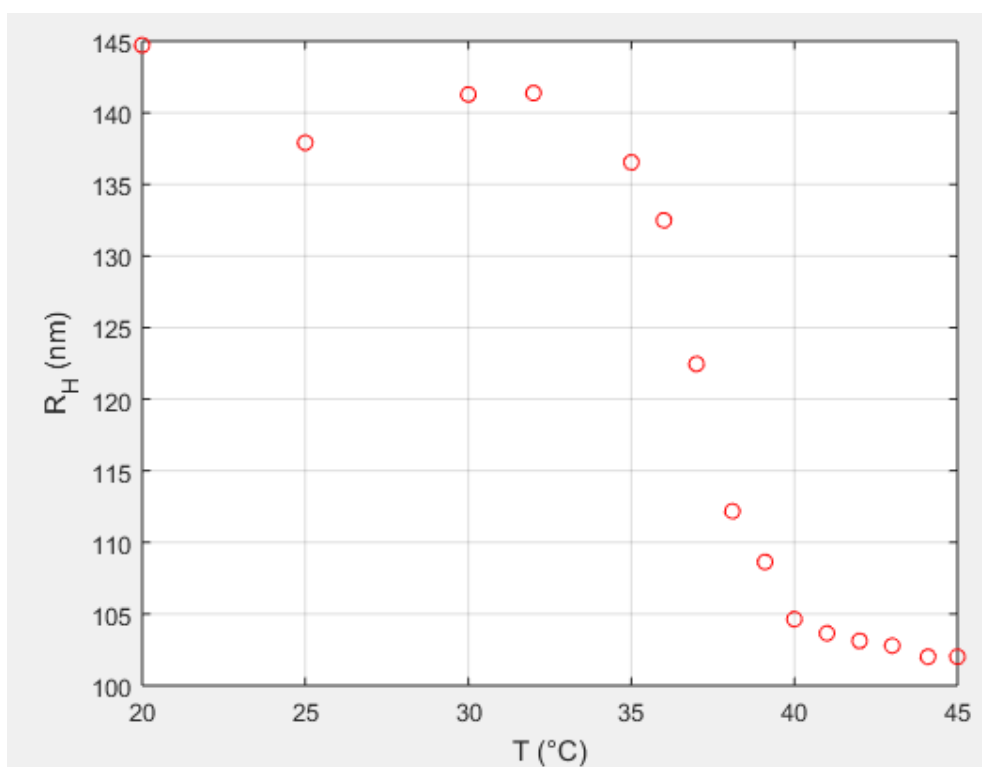
### 7.4-.About the synthesized nanoparticles

The final thing remaining was to check if the polymerization process in subsection 2.2 worked the right way. In order to do so, the thermal response is tested for few temperatures: 20, 25, 30, 32, 35°C and some other further temperatures, but only heating. In figure 12 the results for #1 are shown and it can be observed that there is no response, so something really went wrong during the synthesis in the end. Seeing no response for #2 in the expected range, further temperatures were tested until 45°C, and it can be seen in figure 13 that the collapse happened around 37°C, when

the expected was 32-33°C, but the nanogel characterization is a work that must be done afterwards.



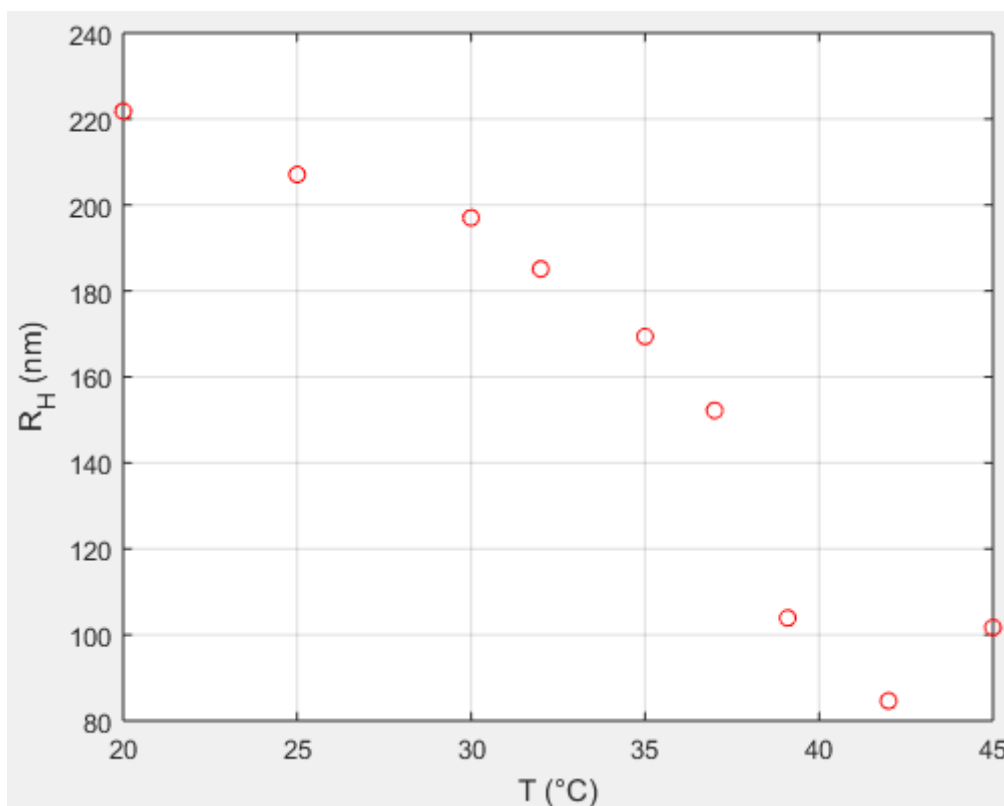
**Figure 12.** Hydrodynamic radius as a function of temperature for sample #1.



**Figure 13.** Hydrodynamic radius as a function of temperature for sample #2.



Finally, in figure 14 it can be seen that for #3 a collapse is observed around 38°C and the obtained brush is bigger at 20°C than #2 as wanted. It seems that after 42°C the nanoparticles could swell again.



**Figure 14.** Hydrodynamic radius as a function of temperature for sample #3.

## 8-.Acknowledgements

In these 52 days in Hamburg until this report has been sent I have lived many things I will remember the rest of my life and I have acquired experience in science inaccessible by other ways. First of all, thanks to Lara Frenzel, who has been supervising and helping me all along the programme and has taught me a lot of things related to her field of study. Thanks for believing that I was capable of carrying out the synthesis of the  $\text{SiO}_2$ -pNipam nanoparticles you need for your studies too. I also thank his help to Wojciech Roseker for the aid offered mainly when Lara was on vacation. In the chemical part I thank Michael Koof his assistance and tips to do the synthesis the better way possible and Irina Lokteva for the introduction to the laboratory. The funny part was the problem with the rotary evaporator, I know it was a problem, but trying to solve it with you was fun and interesting, in this part I also thank Michael Walther who helped us with this technical problem. I am glad to have been in the FS-CXS group and of had been given for the first time in my life the opportunity to do a work in which Physics and Chemistry were both required.

Finally, I thank all the organizers for the existence of this programme, because it is a really good chance, not only to work, but to know more about Germany, knowing people from other countries and to live different experiences in Hamburg and its surroundings.

## 9-References

[1]. Nils Nun , Stephan Hinrichs, Martin A. Schroer , Dina Sheyfer, Gerhard Grübel, Birgit Fischer. Tuning the Size of Thermoresponsive Poly(*N*-Isopropyl Acrylamide) Grafted Silica Microgels. *Gels*, **2017**, 3(3), 34.

[2] Martin A. Schroer, Julian Michalowsky, Birgit Fischer, Jens Smiatek and Gerhard Grübel. Stabilizing effect of TMAO on globular PNIPAM states: preferential attraction induces preferential hydration. *Phys.Chem.Chem.Phys.*, **2016**, 18, 31459.

[3]. Werner Stöber, Arthur Fink, Ernst Bohn. Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. *Journal of Colloid and Interface Science*, **1938**, 26, 62-69.

[4]. Nowakowska, Janina. The Refractive Indices of Ethyl Alcohol and Water Mixtures. *Master's Theses*, **1939**. Paper 668.

[5]. R.J. Jiménez Riobóo, M. Philipp, M.A. Ramos, J.K. Krüger. Concentration and temperature dependence of the refractive index of ethanol-water mixtures: Influence of intermolecular interactions. *Eur. Phys. J. E*, **2009**, 30, 19–26.

[6]. Alexey N.Bashkatov, Elina A. Genina. Water refractive index in dependence on temperature and wavelength: a simple approximation. Saratov Fall Meeting 2002: Optical Technologies in Biophysics and Medicine, **2003**.

[7]. Troy A. Scott Jr. Refractive Index of Ethanol-Water Mixtures and Density and Refractive Index of Ethanol-Water-Ethyl Ether Mixtures. *J. Phys. Chem.*, **1946**, 50 (5), 406–412.

[8]. K. P. Damor, S. P. Vyas & K. V. Goswami. Studies of Derived Specific Refractive Index Formula for Binary Organic Mixture and Compare with Lorentz & Lorenz Formula. *Journal of Applied Chemistry*, **2014**, 7(12), 48-51.

[9]. Sudhamsa B., Sarath Babu .M, Narendra K. Application of Refractive Index Mixing Rules to Binary System. *Journal of Applied Chemistry*, **2014**, 3(3), 1272-1276.

- [10]. Grübel G., Madsen A., Robert A. (2008) X-Ray Photon Correlation Spectroscopy (XPCS). In: Borsali R., Pecora R. (eds) *Soft Matter Characterization*. Springer, Dordrecht.
- [11]. Yunlong Yu, Marco Cirelli, Bernard D. Kieviet, E. Stefan Kooij, G.Julius Vancso, Sissi de Beer. Tunable friction by employment of co-non-solvency of PNIPAM brushes. *Polymer*, **2016**, 102, 372-378.
- [12]. I. Bischofberger, D. C. E. Calzolari, P. De Los Rios, I. Jelezarov, V. Trappe. Co-nonsolvency of PNIPAM at the transition between solvation mechanisms. *Soft Matter*, **2014**, 10, 8288.
- [13] A. K. Lele, S. K. Karode, M. V. Badiger, and R. A. Mashelkar. Prediction of re-entrant swelling behavior of poly(N-isopropyl acrylamide) gel in a mixture of ethanol–water using lattice fluid hydrogen bond theory. *The Journal of Chemical Physics*, **1997**, 107.
- [14] Elizaveta Burdukova, Haihong Li, Naoyuki Ishida, John-Paul O’Shea, George V. Franks. Temperature controlled surface hydrophobicity and interaction forces induced by poly (N-isopropylacrylamide). *Journal of Colloid and Interface Science*, **2010**, 342, 586-592.
- [15] Rio Kita, Gunnar Kircher, Simone Wiegand. Thermally induced sign change of Soret coefficient for dilute and semidilute solutions of poly( -isopropylacrylamide) in ethanol. *The Journal of Chemical Physics*, **2004**, 121(18).
- [16] Ying Liu, Xiaosen Luo, Zhonghua SHEN1, Jian Lu and Xiaowu Ni. Studies on Molecular Structure of Ethanol–Water Clusters by Fluorescence Spectroscopy. *Optical Review*, **2006**, 13, 303-307.
- [17] Wen-Yuan Chuang, Tai-Horng Young, Da-Ming Wang, Ruei-Liang Luo, Yi-Ming Sun. Swelling behavior of hydrophobic polymers in water/ethanol mixtures. *Polymer*, **2000**, 41, 8339-8347.
- [18] Maria C.M. Costa, Sérgio M.C. Silva, Filipe E. Antunes. Adjusting the low critical solution temperature of poly(N-isopropyl acrylamide) solutions by salts, ionic surfactants and solvents: A rheological study. *Journal of Molecular Liquids*, **2015**, 210, 113-118.
- [19] Klaus Tauer, Daniel Gau, Susanne Schulze, Antje Völkel, Rumiana Dimova. Thermal property changes of poly(N-isopropylacrylamide) microgel particles and block copolymers. *Colloid and Polymer Science* , **2009**, 287, 299-312.
- [20] Konstantinos Kyriakos, Martine Philipp, Luca Silvi, Wiebke Lohstroh, Winfried Petry, Peter Müller-Buschbaum, and Christine M. Papadakis. Solvent Dynamics in Solutions of PNIPAM in Water/Methanol Mixtures-A Quasi-Elastic Neutron Scattering Study. *The Journal of Physical Chemistry*, **2016**, 120, 4679-4688.