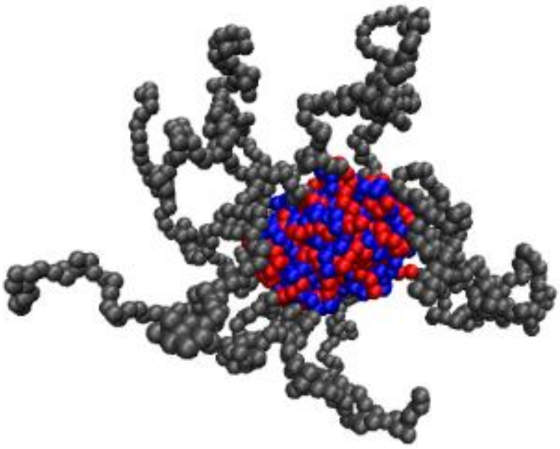
The Effect of the Stoichiometry on the 3D-structure of C3Ms



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Introduction

Complex Coacervate Core Micelles (C3Ms) are molecular structures with a prosperous future in many applications. They can play a role in purifying of water[1], antifouling [2] and encapsulating processes[3]. C3Ms are structures made of polyelectrolytes. They consist of two main parts, a complex coacervate core and a neutral corona(Figure 1). The complex core allows encapsulation of polar or charged molecules (bio)molecules like medicines, DNA and nanoparticles[3]. Due to the neutral corona, these (bio)molecules can be transported safely through hostile environments like the human body. The neutral corona is formed by a neutral polymer block which is attached to at least one of the polyelectrolytes. As said before, this encapsulation process is useful in drug delivery and purifying of water and has antifouling properties. To control these processes, a good understanding of the stability and formation of these micelles is essential. Yet, little is known about the influence of conditions like the stoichiometry of the polyelectrolytes while this could possibly greatly influence the 3D structure of the micelles. A connection between the stoichiometry and the size of the C3M has been found[4]. The stoichiometry is described by the f+ ratio:

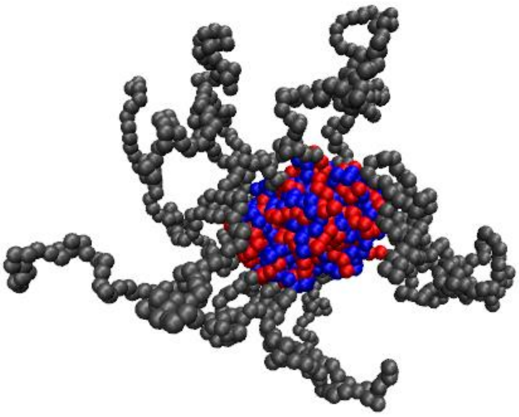

Figure 1: A Complex Coacervate Core Micelle. The grey strands create the neutral corona, the complex coacervate core is formed by the positive and negative polymers displayed in red and blue.

Equation 1

$$f+=\frac{N_{pos}}{N_{pos}+N_{neg}}$$

Where N_{pos} is the amount of positive monomers and N_{neg} is the amount of negative monomers. As is visible in Equation 1, at a complete equilibrium, the f+ ratio will be 0.5. At an excess of negative monomers, the f+ ratio will be below 0.5 and at an excess of positive monomers, the f+ ratio will be higher than 0.5. At a f+ ratio of 0.4, it is expected that the complex will form a worm-like structure instead of the regular spherical shape which is found at f+ = 0.5[4]. However, this is not confirmed yet.

In this thesis, the effect of the stoichiometry of the polyelectrolytes on the 3D structure and shape of the C3Ms will be studied. This will be done using Langevin Dynamics simulations. Langevin Dynamics are a branch of Molecular Dynamics in simulations. Molecules are created in a three dimensional space and are followed through time. At every time step, all forces on the molecule are calculated. This way, an accurate molecular system can be simulated. Langevin Dynamics are unique because friction is also accounted for[5]. It causes a damping effect which can be used to simulate an even more realistic model to mimic the C3Ms. Random collisions are implemented to mimic the solvent molecules without making the model heavy to run for a computer. They are partly determined by the collision parameter. The magnitude of this collision parameter regulate the strength of the inertial forces relative to the random (external) forces [6].

To measure the size and shape of the complexes, multiple tools can be used. In polymer physics, the radius of gyration, R_g, is a common tool to calculate the size of the polymer chain[7]. The radius of gyration can be calculated by the following equation:

Equation 2

$$R_g^2 = \frac{1}{N}\sum_{k=1}^{N}(\boldsymbol{r_k} - \boldsymbol{r_{mean}})^2$$

Where N is the amount of monomers in the complex and r is the position of the monomer. This way, the size of the polymer complex can be measured accurately. A micelle can grow due to multiple reasons. It can fuse together with another micelle or it can absorb an extra polymer. It can also shrink thanks to the opposite reasons, it can split up into two micelles or it can release a polymer. However, the size of the complex is not the only thing of interest. The shape of the complex is also important to calculate. This can be done by using R_g and N. These two variables are related to each other with the following factor D_f:

Equation 3

$$N \propto R_g^{D_f}$$

Where D is the fractal dimension. The fractal dimension is a variable which indicates how the object will grow. When the complex grows in mass, or in our case monomers, the radius of gyration will grow the same with the fractal dimension. The fractal dimension of a perfectly round shape is 3, the fractal dimension of a perfectly straight line is 1. When the fractal dimension of the micelles is calculated, it is possible to say something about how round or straight that micelle is. This way, we know more about its shape.

It is suspected that the micelles will take a worm-like shape below a f+ ratio of 0.5 and at a high N_{cneg}[4]. N_{cneg} is the length of the negative polymer chain.

As said before, the shape and size of the micelle can have a big impact on the stability and the exchange kinetics of the C3M. Because of this, the influence of the f+ ratio of a system on the shape and size of the micelles formed will be studied in this thesis. We will study the formation process of a C3M and how the stoichiometry influences this.

Methods

As said before, Langevin Dynamics simulations were used to obtain the data. The HOOMD-blue package was used for these simulations. A model was created to accurately mimic the conditions of a real molecular system. Different interaction potentials have been used to mimic these conditions. One of them is the Coulomb potential:

Equation 4

$$U_{coul} = \frac{1}{4\pi\varepsilon_0\varepsilon_r}\frac{q_1q_2}{r_{12}}$$

Where U_{coul} is the Coulomb potential, q is the charge of the monomers and r is the distance in between monomers. The Coulomb potential creates a force between the different polymers which pulls opposing charges towards each other and pushes like charges apart. This attracting force is counteracted by the Lennard Jones potential:

Equation 5

$$U_{LJ} = 4\varepsilon\left[\left(\frac{\sigma}{r_{12}}\right)^{12} - \left(\frac{\sigma}{r_{12}}\right)^{6}\right]$$

Where σ is the distance at which the potential between the monomers is 0. ε is the depth of the potential wall. The Lennard Jones potential describes the repelling and attracting effects of neutral and charged effective monomers . For neutral monomers and ions, the following variables were used:

$$r_{cut} = 2^{\frac{1}{6}}\sigma, \quad \varepsilon = 1\ k_BT$$

For the weakly attractive polyelectrolytes, the following variables were used:

$$r_{cut} = 2.5\sigma, \quad \varepsilon = 0.15\ k_BT$$

Another interaction between the polymers are finitely extensible nonlinear elastic(FENE) bonds:

Equation 6

$$U_{FENE} = -\frac{1}{2}kR_0^2 \ln\left[1 - \left(\frac{r_{12}}{R_0}\right)^2\right]$$

With:

$$R_0 = 1.5\sigma, \;\; k = 30k_BT/\sigma^2$$

These variables are based on a model for coacervate nanostructures[8].

In our own model, diblock copolymers were used with a positive block of 20 monomers and a neutral block of 50 monomers. Negative polymers with 20, 60 and 100 monomers and counter ions to all of these polymers have also been used.

To measure the size and shape, a box counting method was used. 10 boxes with increasing volume where used to accurately measure the amount of monomers in the complex and the radius of gyration of the complex(Figure 2). With the data of all these boxes and using Equation 3, the fractal dimension can be calculated. The neutral monomers have not been included in these calculations. Only the polyelectrolytes have been accounted for.

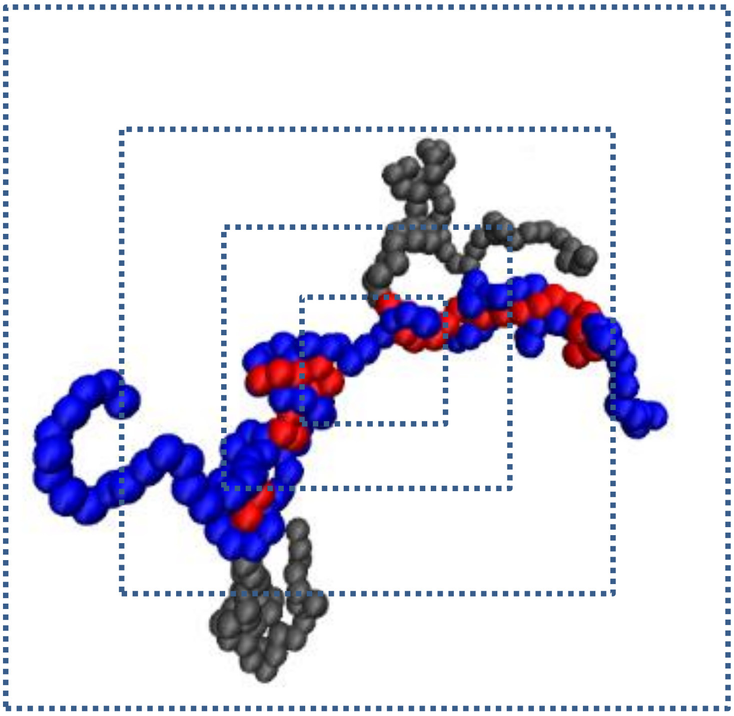

Figure 2: A visualisation of the box method used for measuring.

Results and Discussion

To be able to study the exchange dynamics and encapsulating abilities of C3Ms, the physical properties of the complexes has to be known. To study the shape of the complexes, the radius of gyration can be measured. It can be calculated by using Equation 2.

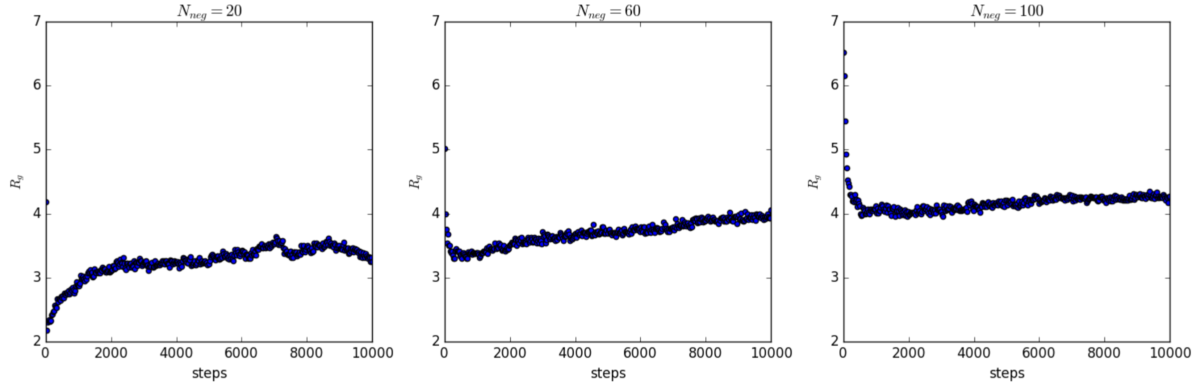

Figure 3: The radius of gyration at different steps through time with a negative polymer length of respectively 20, 60, 100 blocks. The f+ ratio is 0.5.

R_g rapidly increases in the system with N_{cneg} = 20(Figure 3). After around 3000 steps the R_g becomes stable, with some fluctuations. In the system with N_{neg} =60, the R_g drops a little bit, after which the R_g starts to increase. With N_{neg} = 100, R_g drops initially even more. It also has a higher/larger initial R_g than the systems with N_{neg} = 20 and N_{neg} = 60. The system with N_{neg} = 60 has a higher/larger R_g than the system with N_{neg} = 20.

The fast increase of R_g for N_{cneg} can be explained by the fusing of the complexes trough time into bigger complexes. At the start of the simulation, this effect is bigger because there are a lot more small complexes which leads to more fusing of these complexes. However, R_g of N_{cneg} = 60 and N_{cneg} = 100 initially show a decrease in size while their size is increasing in the rest of the simulation. This can be caused by another effect acting on the R_g of the structures. The R_g is also dependent on the shape of the structure. When the complex contains much more monomers of a certain charge than other, the like charges will repel each other turning the complex into a worm-like complex as is visible in Figure 4.

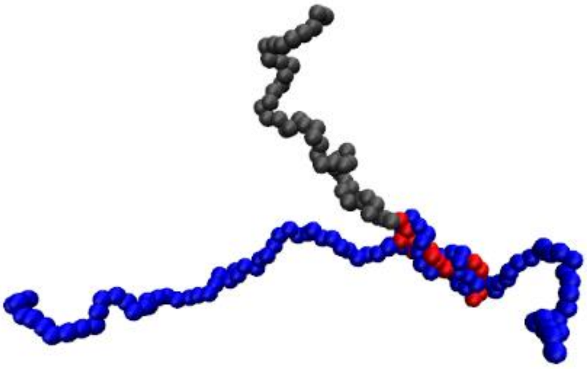

Figure 4: A complex with N_{neg} = 100 with one negative polymer and 1 positive polymer.

This is why you see a small peak at the beginning of the N_{cneg} = 60 and N_{cneg} = 100 simulations. At this point in the simulation, the complexes contain much more negative monomers than positive monomers. This is due to the lack of time for the complexes to fuse together with other positive polymers. Due to the lack of positive monomers, the electrostatic repulsion between the negative monomers is much less damped. This is why the negative monomers repel each other and the structure becomes more stretched out. The peak is higher at N_{cneg} = 100 because the negative polymers are longer and so repulsion is even bigger. When a complex contains many positive and negative monomers, the complex is much better able to fold up into an energy favourable shape(Figure 5).

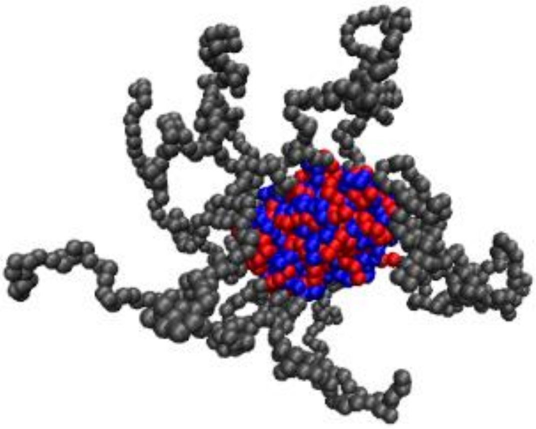

Figure 5: A complex with N_{cneg} =100 with many positive and negative monomers.

The R_g at the end of the simulations is higher in the system with N_{cneg} = 100 than the simulation with N_{cneg} = 20(Figure 3). With these data and other simulations, the mean R_g at the end of the simulation can be calculated for many different f+ ratios and negative homopolymer lengths N_{cneg}.

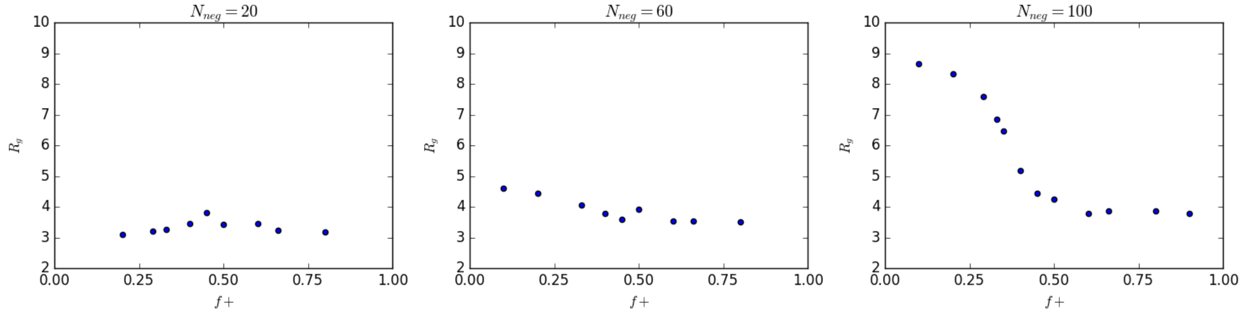

Figure 6: Radius of gyration of the complexes at different f+ ratios with N_{neg} of respectively 20, 60 and 100. All R_g are calculated at the end of the simulation.

A clear distinction is visible between the different N_{neg} graphs(Figure 6). At higher N_{neg} values, the difference between the R_g at f+ ratios above 0.5 and the R_g at f+ ratios below 0.5 becomes much bigger. At a lower f+ ratio, the R_g is much bigger than at a high f+ ratio with N_{neg} =100.

As said earlier, the radius of gyration depends on the shape and on the amount of polymers in the complex. Because of this, it is important to look at the amount of polymers in the complex.

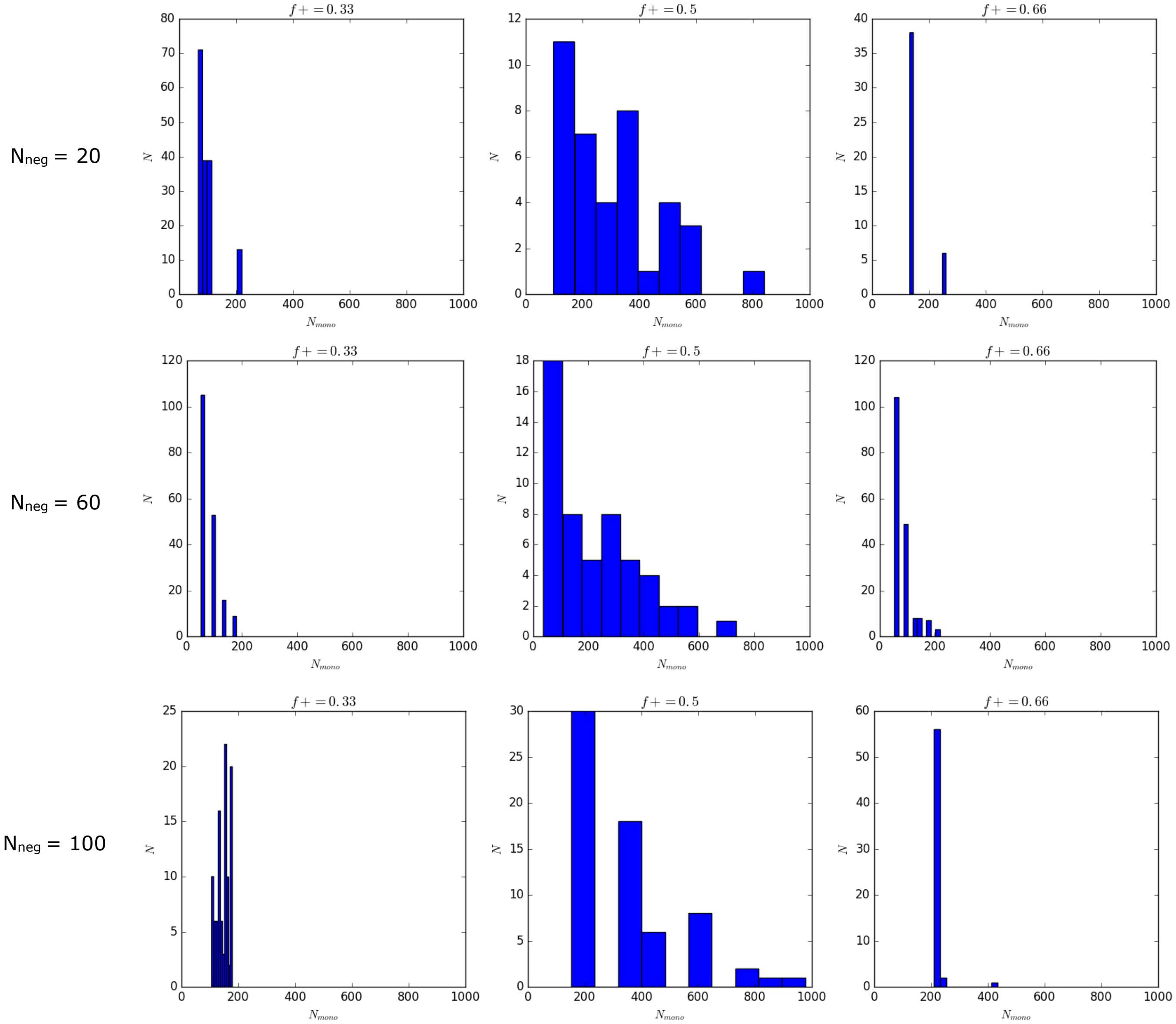

Figure 7: N_{mono} of the complexes at different N_{neg} and f+ ratios.

There is not much different between the different negative polymer block lengths(Figure 7). They all follow a similar pattern, with small and similar complexes at the extreme f+ ratios and big and many different sizes at a f+ ratio of 0.5. The size of the complex for N_{cneg} = 100 is a bit higher than the size of the complexes for N_{cneg} = 20. The shape of the complexes is possibly responsibly for this difference. The fractal dimension, D_f may give information about the shape.

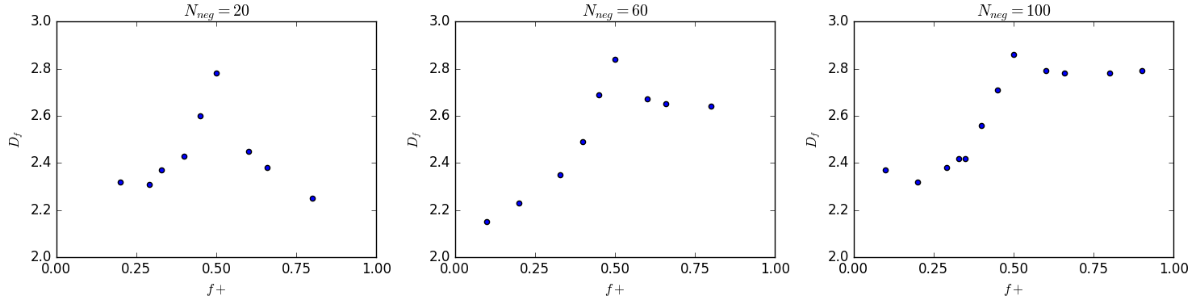

Figure 8: Fractal dimension D_f of the complexes at different f+ ratios with N_{neg} of respectively 20, 60 and 100.

At f+ = 0.5, the complexes have the highest D_f(Figure 8). A D_f of 2.8 shows that the complexes have a spherical shape. At this point C3Ms will be formed. At a small N_{neg}, above and below f+ = 0.5, the fractal dimension decreases. At a high/large polyanion lengths however, the D_f stays relatively high above f+ =0.5. At a certain polyanion length and a low f+ ratio, the complex contains few positive monomers, resulting in a situation like in Figure 4. This is the same at all N_{neg} resulting in a lower D_f with a f+ below 0.5. At N_{neg} = 100 however, the situation changes at a high f+. At this high f+, there is an excess of positive monomers. Meanwhile, every negative polymer still has 100 negative monomers. This means that a lot positive monomers are able to bind to it and make a big complex which can fold up very well. This results in a relatively round micelle which has a high D_f. At N_{neg}=20, this doesn't occur because

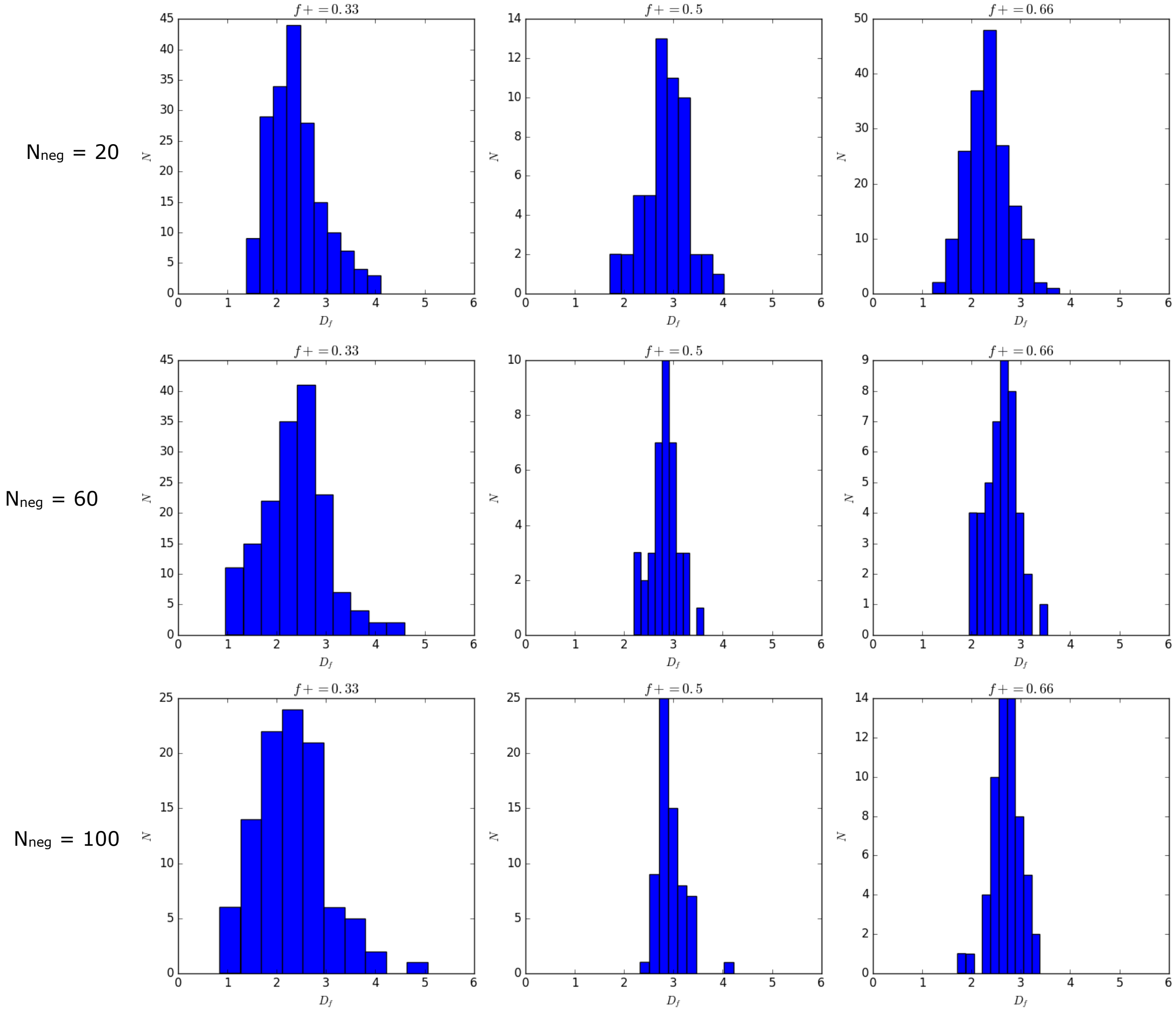

Figure 9: D_f at different N_{neg} and f+ ratios.

In Figure 9, it becomes clear that the simulations with average high D_f values in Figure 8 have more precise values/ have a smaller fractal dimension distribution . This could be due to the method of measuring D_f. The box counting method is used and because of this, it is possible to have these weird data sets. When a polymer is shaped like a worm, it is possible that the polymer curls up into a U or C shape like in Figure 10. In the smallest box, it could be possible that there are no/barely any monomers. Meanwhile, the next box could contain a normal amount of monomers. This leads to the possibility that the amount of monomers can increase a lot, while the radius of gyration changes very little. This leads to these unrealistically high D_f values. The opposite also occurs. The R_g can change by a lot, while almost no monomers appear to be in the box. This could lead to these low D_f values. These effects are also an indication that the complexes with a high f+ ratio and high N_{neg} values are more spherical, using the data from Figure 9. Because the data is more spread out and more extreme, we can say the polymers are more worm-like shaped.

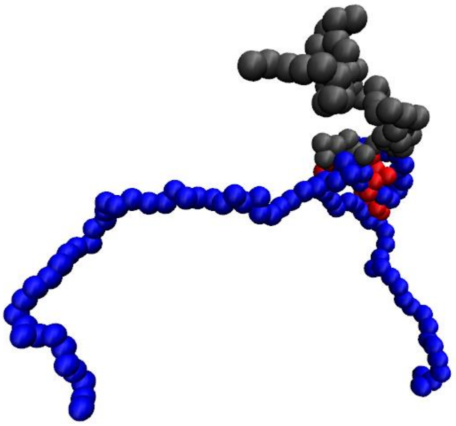

Figure 10: a C shaped polymer.

In Figure 4 and Figure 5, two processes have been observed. The folding process and the aggregation process. To have a better look at the difference between the folding process which makes the complex shrink and the aggregation process which enlarges the complex, the amount of monomers and radius of gyration of individual complexes can be measured.

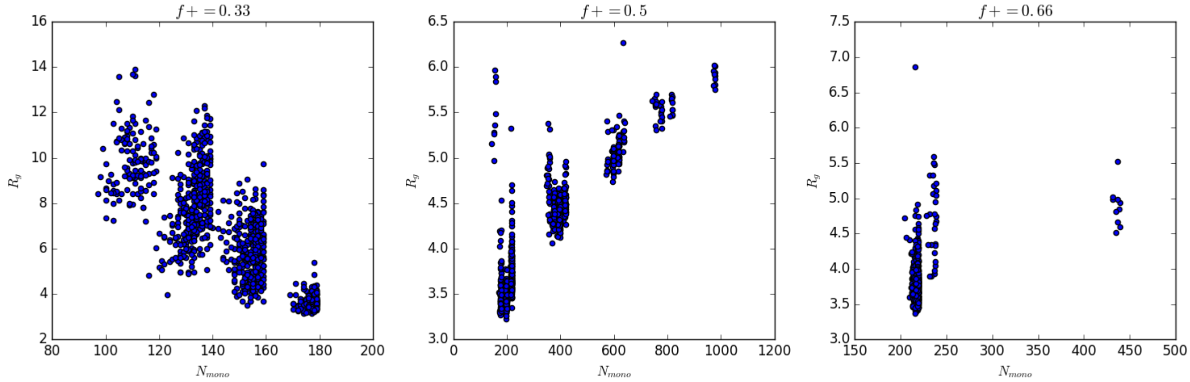

Figure 11: R_g relative to N_{mono} of individual complexes with N_{neg} = 100.

The amount of monomers has a big effect on the radius of gyration(Figure 11). At a low f+ ratio, the addition of a positive monomer will lead to a decrease of the size of the complex. This is caused by the folding effect. At a high f+ ratio, the addition of a positive monomer will lead to an increase of the size of the complex. This is due to the complex being saturated with positive monomers because of the high f+ ratio. When a positive monomer is added, the negative monomer will be stretched more because of the repelling effect the positive monomers have on each other. When two complexes merge together, the size of the complex will increase, this is the aggregation effect. This effect is visible at f+ = 0.5(Figure 11).Figure 11: R_g relative to N_{mono} of individual complexes with N_{neg} = 100.

There is a lot of difference in R_g between complexes with the same number of monomers(Figure 11). The reason for this can be the place where the positive monomers bind to the negative polymer or it can be that the polymers are not very rigid and tend to move and change of shape a lot. In Figure 12 a single complex is measured through time, to see if the complex stays the same or if the size changes much.

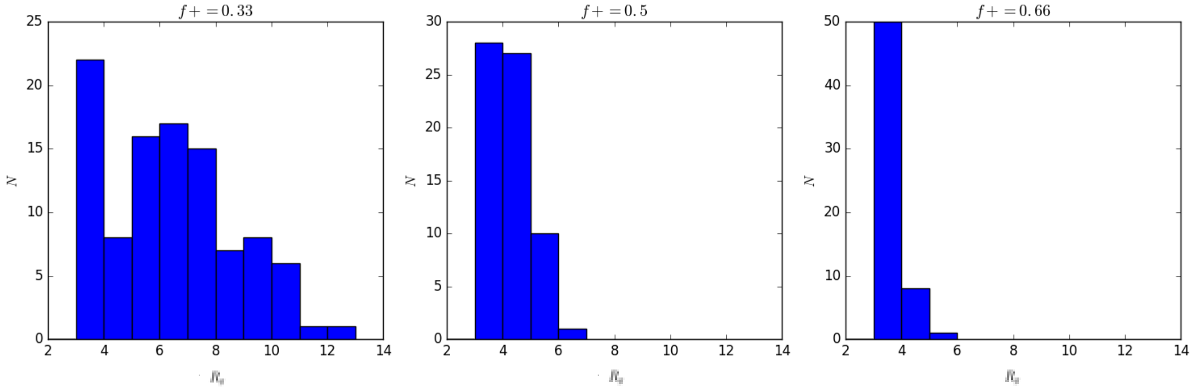

Figure 12: R_g values of a single complex trough time with N_{neg} = 100 and differing f+ ratios.

As is visible in Figure 12, the size of a single complex changes a lot. This means the complex is not very rigid and will change shape easily. Especially at a low f+ ratio, when the complex is in a worm-like shape, the size changes a lot.

Conclusion and Outlook

From the results, it becomes clear that the complexes are effected a lot by the stoichiometry. At a high f+ ratio, the structures formed are not very big. They don't have a high radius of gyration(Figure 6). This is the same for structures with a low f+ ratio and a low negative polymer block length. At a low f+ ratio and a high N_{cneg} however, this changes. The complexes formed are much bigger. This is due to the low fractal dimension (Figure 8) and the relatively big amount of monomers in the complex (Figure 7). The size of these structures is dynamic, they constantly twist and turn around in the solvent (Figure 11). Also through time, they are constantly growing and shrinking. This is caused by two processes. The folding of the structure on itself and the aggregation of other polymers to the complex. The folding process is especially relevant at the start of the simulation (Figure 3), at this point the complexes are still very stretched out and mostly only have one negative and positive polymer. At a high N_{cneg}, this creates a low local f+ ratio which turns out into a big complex as seen before in Figure 6. At this point, the complexes formed have a low fractal dimension as is visible in Figure 8. This is due to the negative polymers repelling each other resulting into a more stretched out structure. This is conform with literature findings[4]. At a higher f+ and a low N_{cneg} the same effect occurs, but now due to the excess of positive polymers. At a high f+ and a high N_{cneg}, the complexes still have at least 100 negative monomers. This results in a lower local f+ ratio which leads to a lower D_f as is visible in Figure 8.

In this thesis, a local connected dimension box counting method was used to measure D_f. This resulted in some interesting results as is visible in Figure 9. This is due to weird shaped complexes like in figure Figure 10. Because of the unusual shape, the steps didn't create a smooth transition between the different boxes. At a next step, the complex could "aggregate" many monomers while the R_g didn't have to change as much with it. This results in these unexpected D_f values. This effect also works the other way around so some complexes seem to have a lower D_f than in reality. In this thesis, it is assumed that these two effects cancel each other out but the method of measuring has much room for improvement in the future.

Lab experiments to complement these theories are also useful for future research. Some DLS and SLS measurements were made during this thesis but due to some technical difficulties, these have not been used in the final results. Unrealistically big complexes where found. These difficulties where probably due to the type of polymers used. Longer and bigger simulations could also be useful to be better able to cancel out the folding effect which still occurs at extreme f+ ratios. Also, more research about the effect of the shape and size of the complexes on the exchange kinetics will be essential to use the complexes for actual purposes like drug delivery.

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