The rheometer as a tool to study a section of the Hofmeister Series

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Abstract

For more than a century, the Hofmeister Series has been an open problem in biophysics and colloid science. In this experiment, a standard viscometer has been used to test a section of the Series by comparing the viscosities of simulated egg white solutions after the addition of different salts ($KCl, NaCl, MgCl_2, CaCl_2, LiCl$ and $LiBr$). Considering the obtained data, we have that the trend of the $LiBr$ solution is clearly distinct from that of the other solutions, whose salts have Cl^- as an anion, and that it is quite complicated to rank the viscosity curves of the salts which have Cl^- as an anion. This agrees with what suggested by the theory, which says that the HS is dominated by anions.

Moreover, a small section of the Hofmeister Series for anions have been explained using qualitative arguments. As far as the author knows, a similar explanation have never been given before in literature.

Contents

> *"A deeper understanding of the Hofmeister series can be an extraordinarily valuable guide to designing experiments, including not only those probing the series per se, but also those designed to elucidate the adsorption, aggregation and stabilization phenomena which underlie so many biological events".*
>
> *M. G. Cacace et al. [4]*

1 Introduction and motivation

This work is the natural prosecution of my bachelor project [6], which I did during the spring semester 2008. In that work I used Fluorescence Correlation Spectroscopy (FCS) as a technique to test a section of the Hofmeister Series, by studying the diffusion of two fluorescent dyes (TMR and R6G[1]) in hens egg white with different salts in solution.

The results obtained, reported in Figure 1, were not very accurate, probably because the characteristics of the egg white solution considered were always different: the egg white composition changes both from egg to egg and both with the passing of the time, even in the range of two-three hours. A clear explanation for this phenomenon is not available in the literature; probably the changes in the egg white composition are connected with the fact that protein may aggregate as time passes.

It is interesting to notice that, as the egg white composition changes, also the pH changes (the pH value of the egg white changes with the passing of the time, growing from 7.6 up to 9.5 if long storage times are considered [6]).

For these reasons, the considered sample had to be changed frequently, and it was not useful to mix together the contents of different eggs to prepare a large egg white solution ($\sim 300ml$).

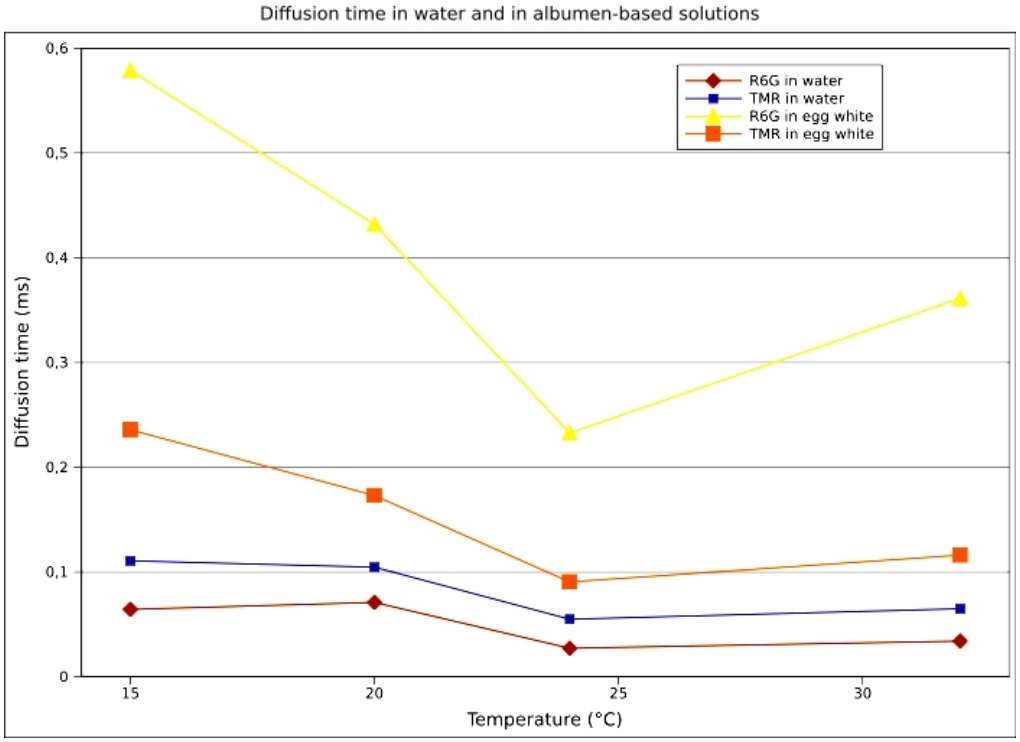

Figure 1: Diffusion time as a function of temperature. Picture taken from the previous work on the argument [6].

[1] Rhodamine 6G (R6G) and tetramethylrhodamine (TMR) are two rhodamine derivatives produced by Sigma-Aldrich (Sigma-Aldrich Pte Ltd., Singapore) . The main difference between them is that R6G has a permanent positive charge.

To have better results, in this work I investigated the Hofmeister Series using a different experimental technique.

First of all, I substituted the hens egg white with a simulated egg white solution, whose weight is 90% composed by ultrapure water and 10% composed by albumin, which is the first constituent of the albumen proteome (see Table 3). The composition of such a simulated egg white is a good approximation of the real egg white composition, reported in Table 2. Moreover, the simulated egg white solution used in the experiment has the same protein/water ratio as the solution considered by Franz Hofmeister and his team in their experiments[2].

Then I studied how the addition of different salts changes the viscosity of such a prepared artificial albumen. Thanks to the adoption of a simulated egg white solution, consider quasi-identical intracellular environments have been considered; moreover, it was possible to prepare the desired quantity of simulated egg white solution just before doing a measurement, avoiding problems connected with sample aging.

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strongly hydrated anions **weakly hydrated anions**

$$CH_3COO^- > SO_4^{2-} > HPO_4^{2-} > F^- > \mathbf{Cl^-} > \mathbf{Br^-} > I^- > NO_3^- > ClO_4^-$$

$$NH_4^+ > Cs^+ > Rb^+ > \mathbf{K^+} > \mathbf{Na^+} > H^+ > \mathbf{Ca^{2+}} > \mathbf{Mg^{2+}} > \mathbf{Li^+}$$

weakly hydrated cations **strongly hydrated cations**

kosmotropic, stabilizing ions	**chaotropic, destabilizing ions**
• ⇑ **surface tension**	• ⇓ **surface tension**
• ⇓**protein solubility**	• ⇑**protein solubility**
• ⇓**protein denaturation**	• ⇑**protein denaturation**
• ⇑**protein stability**	• ⇓**protein stability**

Table 1: Annotated version of the Hofmeister series, modified from [8] and [22]. Its most important features are presented in Subsection 2.1. The ions reported in bold are those considered in this work.

In the following subsections, the motivations for this work are presented, followed by a brief presentation of the key concepts involved.

1.1 Motivation

The expression "Hofmeister series" denotes a ranking of ions, which was first discovered considering the ability of ions to alter protein solubility. It consists in a succession of anions, which summarize the work made by Franz Hofmeister and his group in the 1880s-1890s, and a succession of cations, the *"lyotropic series"*, widely known during the end of the 19th century [27]. For a complete translation in English of the original Hofmeister's papers, originally published in archaic German in the *Archiv fuer experimentelle Pathologie und Pharmakologie*, see the work by Kunz et al. [24]. The first known publication of the lyotropic series is in *Kapillarchemie* by Herbert Freundlich [16], printed in 1909.

After more than a century from its birth, the molecular reactions at the base of the Hofmeister Series (later also called *HS*) are still unexplained. It has been found that the HS is involved in a great number of biological phenomena such as water retention by wool

[2] They prepared a solution combining egg whites from different eggs, which is then *"diluted with water until the protein concentration is 10 g in 100 ml"* [24].

[23], protein-protein interactions [15, 33], protein-DNA interactions [4], protein crystallization [13] and bacterial growth [28]. Thanks to advantages in computational and experimental techniques, in the last decade the HS and correlated phenomena attracted a growing number of scientists from different fields such as chemistry, biochemistry, biophysics, chemical physics and medicine, creating a fast-growing multidisciplinary literature.

At the moment the amount of articles on the argument is relatively limited (Google Scholar gives 4080 results[3] for "Hofmeister series"; as a reference two evergreen scientific expressions as "general relativity" and "Fourier series" respectively score about 217.000 and 415.000 results), and at present there are just three book containing the words "Hofmeister" and "series" in the title! This gives researchers the opportunity to leave a deep impact on the topic with a relatively small effort; the tax to pay is the absence of steady reference points.

In the present work and in the previous one [6] I studied the Hofmeister series considering egg white based solutions, like Franz Hofmeister and his team did in their experiments [24] in the 19th century.

1.2 The cell

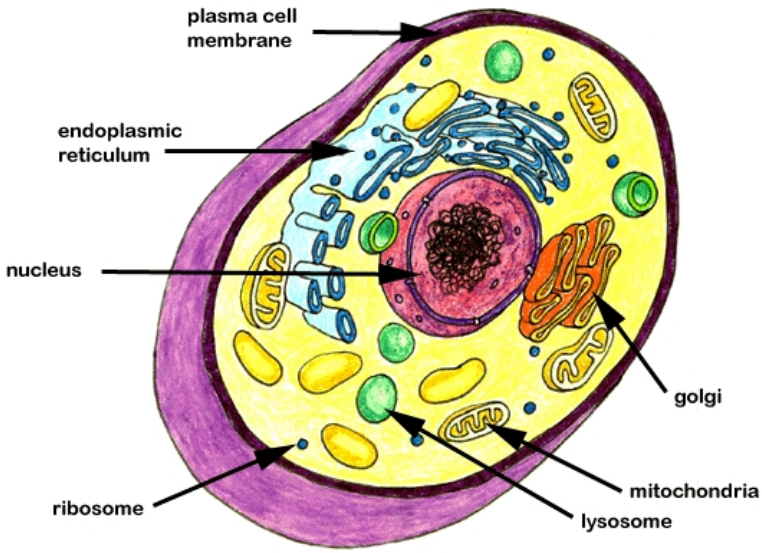

Figure 2: Biological cell, from [5].

The cell is the basic unit for all known living organisms. Even if there is a great variety of different cell types, the fundamental composition of all cells is the same: they consist of an aqueous solution of organic molecules enclosed by a lipid membrane. Prokaryotic cells have a stark structure, whereas eukaryotic cells contain many organelles, such as the nucleus, mitochondria, Golgi apparatus and other. Both in eukaryotic and in prokaryotic cells there are great quantities of proteins.

Eggs are giant eukaryotic cells.

1.3 The egg

The egg is an unicellular organism which contains the basic elements for life (water, proteins, lipids, DNA, vitamins and minerals), because its function is to give rise to a new living being. For this reason, and because of its low price, a very rich literature is

[3] Searches made on the 4th of June, 2010.

available on the egg and its parts. As a reference, I considered the books written by Burley and Vadehra [3], Yamamoto et al. [37], and the collective work "Bioactive Egg Compounds" [20].

Constituent	Percentage by weight of the whole albumen
Water	88.5÷88.0
Proteins	10.5÷11.0
Free carbohydrates	0.5÷0.9
Lipids	0.02÷0.2
Inorganic ions	0.5÷0.7

Table 2: Composition of the egg white. In the second column there is not a defined number but a gap, because two different sources have been considered: [32] and [36].

The egg is composed by three different constituents: the yolk, the albumen and the shell; as we can see from Table 2, the first component by weight of the egg white is water. The egg white protein composition is reported in Table 3.

1.4 Water

Water is a molecule with simple structure which is involved in all living mechanisms. About 70% of the human body consists of water and the standard amount of water in a cell stands between 55% and 90% [18].

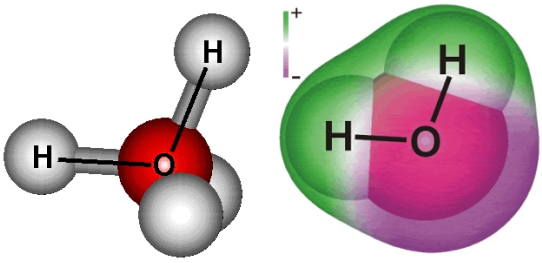

Figure 3: Left: oversimplified structure of a molecule of water. Right: more realistic representation, with shape and charge distribution. Figure taken from [9].

Considering a molecule of water, we have that since oxygen electronegativity is much bigger than hydrogen, thus forming a net positive charge on hydrogen atoms and a net

Protein	Percentage of total proteins
Ovalbumin	54
Ovotransferrin	12
Ovomucoid	11
Ovoglobulin G3	4
Ovoglobulin G4	4
Lysozime	3.4
Ovomucin	1.5
Ovoinhibitor	1.5
Ovoglycoprotein	1
Others	7.6

Table 3: Composition of the albumen proteome.

negative charge on oxygen atom. So molecules of water show a clear dipolar structure.

Taking into account a set of water molecules at a certain temperature T, we have that these are interconnected with hydrogen bonds forming a quasi regular network, whose regularity depends on temperature. Low-temperature ice shows a perfect hydrogen bonding network; with ice melting 13% of hydrogen bonds are broken, and 8% more are broken upon heating water up to 100 °C. All of the other hydrogen bonds are broken up upon vaporization [18].

1.4.1 Water structure and behaviour

The *anomalous properties* of water are those where the behavior of water is quite different from what is found with other liquids [9]. Only a number of these, like its high melting and boiling point, can be explained considering hydrogen bonds only. To explain the others, a theory about the *two state water clustering* has been purposed [10]. According to this theory, water molecules organize their mutual position maximizing or Van der Waals interactions or the strength of ionic bonds.

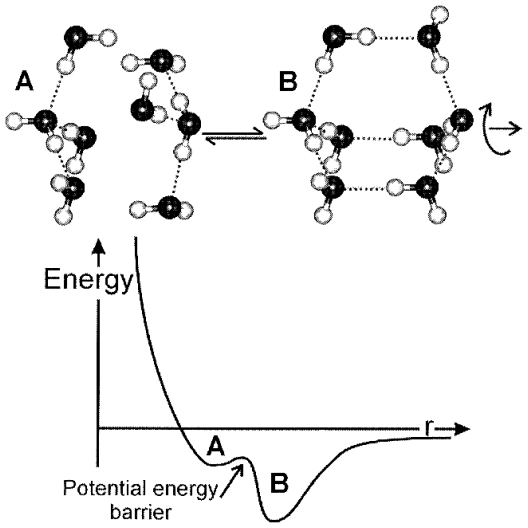

Figure 4: Above: water clusters behaviour. Below: energy of a water cluster, with minima corresponding to particular molecule configurations. Figure modified from [11].

At the top of Figure 4 there are the two possible water clusters. We have structure A when van der Waals force is maximized, and structure B when the intensity of hydrogen bonds is maximized. Structure A, which is similar to ice III, has higher density than structure B, which instead is similar to ice I, and it shows weaker but more numerous water-water bonds. Because of the potential energy barrier a group of water molecules prefer structure A or structure B, with little time spent dwelling in intermediate processes.

Using this simple two state models, the volumetric properties of a solution can be explained [7].

1.4.2 Intracellular water

In this section we will consider how water molecules behave in the intracellular environment. Intracellular water shows very different characteristics when compared to pure water, mainly because there are a great number of particles and charges to be considered. To qualitatively describe the structuring of intracellular water we will rely on the *polarized multilayer theory* made by Gilbert Ling [26] and on the *gel sol transition theory* by Gerald Pollack [35].

First of all, we have to consider all the different egg white proteins. Proteins are composed by amino acids, and each amino acid presents several charges. These are more likely to be situated on the protein surface rather than in its interior [35]. Water molecules are attracted by charges on the surface of the proteins, and they arrange themselves in various layers around the surface of the protein (for an oversimplified view, see Figure 5). To explain this phenomenon, we can consider what happens when a protein is added to a watery solution. At first (part 1 of Figure 5) there are interactions only between charges on the surface of the proteins amino acids (colored in brown) and a few water molecules, directly attracted. Then (part 2), every water molecule bond to the surface of the protein attracts other water molecules, because of the dipolar nature of water. This happens both horizontally and vertically around the amino acid surface. Moreover, water molecules adjacent to the surface induce new charges on the surface of the amino acid, which in turn attract new water molecules. This process finishes when the entire surface of the protein is covered by water layers (part 3). This structuring of water around a solute is called *hydration*, and it's strongly dependent upon the kind of solute considered.

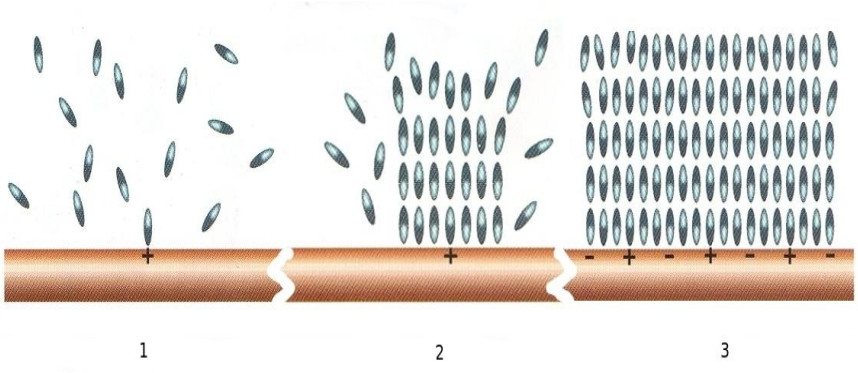

Figure 5: Interaction between water dipoles and surface amino acid charges, with gradual formation of layers of water molecules. Modified figure taken from [35].

A quite realistic description of how water behaves inside the cell is more complicated, and a general theory comprehending all the various phenomena is still lacking. We will solve this problem by following Chaplin's qualitative approach [11].

1.4.3 Comparison between intracellular and pure water

The main difference between pure[4] and intracellular water is density, which is lower inside the cell than outside. This is mainly due to the extensive surface effect of membranes[5].

2 Theoretical background

2.1 The Hofmeister series

In the 1880s-1890 Franz Hofmeister published a couple of articles describing what happens when different salts are added to an egg white solution [24]. They performed a number of experiments, in each of whom protein precipitation is studied by adding a particular salt to an egg white solution: *"In a series of experiments it was determined at which addition of salts first clouding of globulin became visible*[6]*. An uncertainty occurred because some salts developed their precipitation power only after a certain time. It was*

[4] With "pure water" we denote a liquid composed by H_2O molecules only.
[5] As an example, the liver cell contains about 100,000 μm^2 membranes surface [35].
[6] Yes, the Hofmeister Series has been discovered through eye-based observations.

then necessary to wait for some minutes or even hours before it was possible to indicate the concentration below which no clouding occurred, even after several days (evaporation was excluded)" [24].

Considering the usual representation of the Hofmeister Series (see Table 4), we have that it is composed by two series of ions, one on the top of each other. The series on the top represents the results of Hofmeister's work as a ranking of anions, ordered following their ability to precipitate proteins, while the series on the bottom is a series of cations (the *lythotropic series*, widely known at the end of the 19th century), which has been added after the publication of Franz Hofmeister's papers.

$$CH_3COO^- > SO_4^{2-} > HPO_4^{2-} > F^- > \boldsymbol{Cl^-} > \boldsymbol{Br^-} > I^- > NO_3^- > ClO_4^-$$
$$NH_4^+ > Cs^+ > Rb^+ > \boldsymbol{K^+} > \boldsymbol{Na^+} > H^+ > \boldsymbol{Ca^{2+}} > \boldsymbol{Mg^{2+}} > \boldsymbol{Li^+}$$

Table 4: The Hofmeister series, modified from [8] and [22]. Anions are reported above and cations below; the ions reported in bold are those considered in this work.

These are the most important characteristics of the series reported in Figure 4:

- considering the series of anions, we have that the hydration strength decreases from the left to the right, while for the series of cations it decreases in the opposite direction;

- the ions on the left of Na^+ are usually known as *kosmotropic*, while those on the right are denoted as *chaotropic*. In the literature, ions are called kosmotropic if they improve the quality of water network structures and stabilize proteins. In reverse, ions are called *chaotropic* if they destroy water structures and destabilize proteins. Even if it has been recently shown that the classical definition of kosmotropic and chaotropic ions reported before is not completely correct[7] [1, 31], these two concepts are very useful to understand more than a century of attempts to explain the Hofmeister Series. When kosmotropic ions are added to a certain solution, they increase the surface tension and the stability of the proteins in solution, decreasing the solubility and the risk of denaturation. On the opposite, when chaotropic ions are added they decrease the surface tension and the stability of the proteins, increasing both the solubility and the risk of denaturation.

All these observations are included in the annotated version of the Hofmeister Series, reported in Table 5.

2.1.1 Three observations

Collins and Washabaugh first noticed that when a salt, formed by an ion and a cation both taken form the HS, is added to an aqueous solution, the resulting solution has the following characteristics [12]:

1. the effects of the HS are present at every salt concentration, and become important when the molarity stands between 10 mM and 1 M, mainly because these effects are hardly measurable at lower salt concentrations;

2. the HS is dominated by anions. As a matter of fact, for the same ionic radius the anion-water bond is considerably stronger than the cation-water one, because water hydrogen atoms can approach more closely than the water oxygen atoms. Moreover, salting out effect strongly depends on the kind of anion, whereas it weakly depends on the kind of cation, as described in [34];

[7] Recently, it has been demonstrated that the addition of ions to a water based solution affects only the stability of proteins, and not water structures. A more detailed explanation is available in Subsection 2.1.2.

3. for all the salts, the effects of the HS are additive: if two chaotropic ions are added to a certain solution, proteins stability decreases more than if a single chaotropic ion is added.

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strongly hydrated anions **weakly hydrated anions**

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kosmotropic, stabilizing ions	**chaotropic, destabilizing ions**
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Table 5: Annotated version of the Hofmeister series, modified from [8] and [22]. The ions reported in bold are those considered in this work.

2.1.2 Possible theoretical explanations

In his article, Hofmeister explained qualitatively the phenomena he observed saying: *"To explain the globulin precipitating effect itself, and the existing correspondence, nothing is more appropriate than the assumption that the globulin precipitation is caused by the added salts, absorbing the solvent from the globulin. Further, the strength of this water absorbing effect varies from salt to salt"* [24]. Notably, this can be considered correct also nowadays.

To physically explain Hofmeister's words, two theories can be used:

1. a qualitative theory, supported mainly by biologists, according whom solutes act by altering water structures [14, 30, 38, 41];

2. a modified version of the DLVO[8] theory, which is more physically consistent than the previous one. According to this theory, described in details in the next subsection, what solutes do is basically alter protein hydration shells.

Using the notation presented in Figure 4, the first theory predicts that when a salt is added to a certain solute, the percentage of water organized in clusters of the kind A (or B) changes. Recently performed experiments [1, 17, 40] showed that this theory is not correct (outside the direct vicinity of the salt particles, the amount of clusters of kind A (or B) is not affected by the addition of chaotopic/kosmotropic salts [31]), so at present the only plausible molecular-level explanation of the Hofmeister Series is based on the DLVO theory, recently modified by Ninham et al. [2, 29] so as to consider also dissipation forces. Interestingly, this hypothesis has been examined much more theoretically rather than experimentally [17].

2.1.3 DLVO theory and dissipation forces

Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of interparticle interactions treats colloid stability in terms of a balance of attractive van der Waals forces and repulsive

[8] Derjaguin-Landau-Verwey-Overbeek.

electrical double-layer forces [40]. One of the most important characteristics of this theory is that it describes electrostatic interactions between molecules in ionic solution using the Poisson-Boltzmann equation (1) to describe electrostatic interactions; in this way, ions in solution are considered as point charges, and ions specificity is lost.

This is tragic when one is working with the Hofmeister Series, which is a classical example of ion specificity (when an ion is added to a solute, the effect changes from ion to ion), or with high ion concentrations ($M > 0.1$), because in this case it is not enough to consider electrostatic forces only.

To solve these two problems, Ninham et al. [2, 29] improved the the DLVO theory by introducing a *dispersion potential*, which enables to consider short range interactions and ion specificity. Using the DLVO theory with a dispersion potential, it has been calculated that what solutes do is basically alter protein hydration shells [40].

2.1.4 Poisson-Boltzmann equation and Debye Hückel equation

With MKS units, the Poisson-Boltzmann equation is

$$\vec{\nabla} \cdot \left[\epsilon(\vec{r})\vec{\nabla}\Psi(\vec{r})\right] = -\rho^f(\vec{r}) - \sum_i c_i^\infty z_i q \lambda(\vec{r}) \cdot \exp\left[\frac{-z_i q \Psi(\vec{r})}{k_B T}\right] \tag{1}$$

where $\vec{\nabla}\cdot$ is the divergence operator, $\epsilon(\vec{r})$ represents the position-dependent dielectric, $\vec{\nabla}\Psi(\vec{r})$ is the gradient of the electrostatic potential, $\rho^f(\vec{r})$ is represents the charge density for the solute, c_i^∞ is the concentration of the ion i at an infinite distance from the solute, z_i is the charge of the ion i, q is the charge of a proton, k_B is the Boltzmann constant, T is the temperature of the solution, and $\lambda(\vec{r})$ quantifies the accessibility of position r for the ions in solution.

If the potential is not large compared to kT, the equation can be linearized to be solved more efficiently, leading to the Debye Hückel equation, which can be used to calculate the activity coefficient of an ion in a dilute solution of known ionic strength:

$$\log(\gamma_i) = -\frac{z_i^2 q^2 \kappa}{8\pi\varepsilon_r\varepsilon_0 k_B T} = -\frac{z_i^2 q^3}{4\pi(\varepsilon_r\varepsilon_0 k_B T)^{3/2}}\sqrt{\frac{I}{2}} = -A z_i^2 \sqrt{I} \tag{2}$$

where $\kappa = \lambda_D = \left(\frac{\epsilon_r\epsilon_0 k_B T}{q^2 N_0}\right)^{1/2}$ is the Debye lenght, ε_r is the relative permittivity of the solvent, ε_0 is the permittivity of free space, I is the ionic strength of the solution and A is a constant that depends on the solvent.

3 Materials and methods

3.1 Sample preparation

This is the procedure followed to collect the data:

1. Before starting a new set of measurements, prepare a solution of ultrapure water[9] (90% of the total weight) and albumin[10] (10% of the weight). Approx. 2 μl of solution per measure are needed.

2. Prepare the simulated egg white solution, adding 150mM of the considered salt (if this is formed by univalent ions, or 50 mM if the ions are divalent) and 10mM of Hepes to the water-based solution prepared before. The salt concentration reported above have been used because they noticeably change the viscosity of the egg white solution without radically altering its properties.

3. Sonicate the simulated egg white solution for 20÷30 sec.

[9] Distilled using using *The EasyPure RF Water Purification System* (Barnstead International, Iowa, USA).

[10] *Albumin from chicken egg white, Grade III* (Sigma-Aldrich, Missouri, USA).

Figure 6: Simulated egg white solution with macroscopic protein aggregate circled in red. To avoid the formation of similar aggregates, which noticeably alter viscosity values, a new simulated egg white solution has been prepared before every set of measurements.

4. Put approx. 2 μl of the simulated egg white solution in a clean sample holder.
5. Store the rest of the simulated egg white solution in the fridge.
6. Calibrate the rehometer and perform the viscosity measurement.
7. After every measurement, wash the sample holder and put a new simulated egg white solution in it.

As reported in Table 6, in all of the cases considered the pH stands around 9, which is the pH of the egg white. For this reason, it was not necessary to adjust the pH of the simulated egg white solution.

Salt added to the egg white solution	pH
$MgCl_2$	9.09
$CaCl_2$	9.32
KCl	9.32
$LiCl$	9.34
$NaCl$	9.42
$LiBr$	9.62

Table 6: pH values corresponding to different salts in solution. The salt concentration used is 150 mM if the salt is formed by univalent ions, and 50 mM if it is formed by divalent ions.

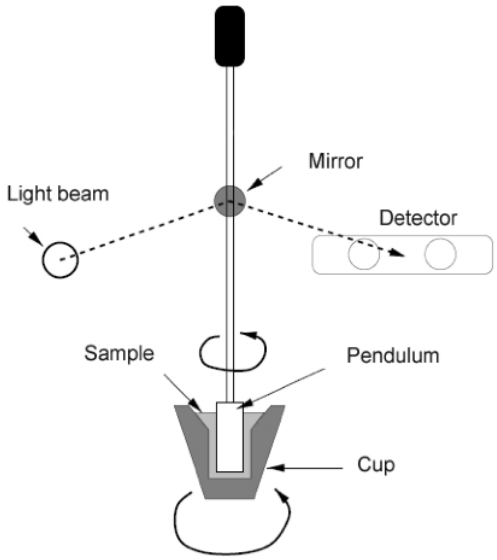

Figure 7: Scemating drawing of the viscometer used [39].

3.2 The viscosimeter

Viscosity is a measure of the resistance of a fluid to being deformed by shear stress. In this experiment, the viscosity was measured with a rotation viscosimeter[11]. The rotation viscosimeter, shown in Figure 3.2, works as follows: a small metal cup filled with the sample is rotated with a constant velocity. A small pendulum is hanging down into the cup so that the weight is covered by the sample. To prevent large fluctuations in the viscosity because of variations in pendulum to wall distance, during the calibration it has been checked, by using the two screws underneath the apparatus, that the pendulum is exactly centered in the cup.

Figure 3.2 shows the principle behind the viscosimeter. A light beam is reflected in a mirror attached to the pendulum, to two photodetectors. The rotation viscosimeter uses the fact that a rotational force is required to turn an object in a fluid. In this case an electronic device exerts just enough opposite torque on the pendulum so the photodetectors measure a light intensity equal to the light intensity measured when the cup is not rotating. The voltage required to exert this torque is the value displayed by the machine as a measure proportional to the viscosity. The temperature of the sample is regulated by sending water from a large water tank under the metal cup and concealing the apparatus with a plexiglas cage and a flamingo cage. Temperature measurements are done by a thermometer situated underneath the sample cup. To prevent the sample from drying out there is a water-filled container inside.

3.3 Data selection

Following the procedure presented in subsection 3.1, for every salt a set of $6 \div 8$ measurements have been collected. In the considered experiment, the total amount of time necessary to measure once the viscosity values of one solution is approximately 2 hours, considering also the time required to cool the rheometer after a measurements. For a certain solution, at least a day is required to obtain a complete set of measurements: several data sets are needed because it is necessary to check that

a) the trend of viscosity is similar among the data sets;

[11] *Low Shear 30 Sinus* (Contraves, Switzerland).

b) in each data set the trend of viscosity is “clear”, with limited fluctuation and no steps.

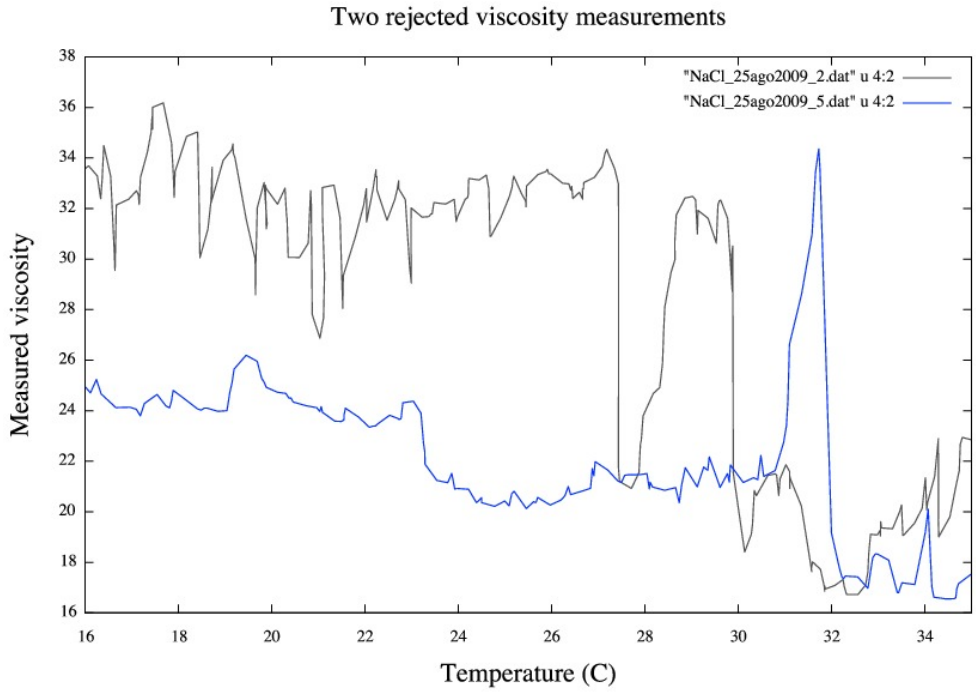

Figure 8: Two examples of datasets ignored because of their “bad” shape.

Many data sets had to be rejected because they didn’t satisfy one or both of the listed properties; moreover, for every solution, there are noticeable differences between the different data sets, probably because the temperature is changing too fast[12], and so the solutions are never in a state of thermal equilibrium. This problem can’t be solved changing the acquisition time: a reduction of it would cause causes greater fluctuations, whereas an increasing of it would let proteins aggregate. So for every set of measurements it has been chosen as representative the less noisy, more regular curve.

To decide what is the best dataset to consider, the procedure presented above has been applied only because the datasets were very noisy; in case of better data the minimum chi squared estimation and similar mathematical methods should have been used.

4 Results

4.1 What to expect from the data, and explanation of a section of the HS

In the present experiment, the addition of six salts to a simulated egg white solution has been studied. The salts considered are reported in Figure 4.1; five of them are formed by the combination of the anion Cl^- with different cations, while $LiBr$ is the only salt which has Br^- as an anion. Considering Table 4.1, which reports the main characteristics of the anions of the Hofmeister Series standing between F^- and I^-, we can notice three trends, proceeding from the left to the right, from ”neutral” anions to weakly hydrated anions: the ionic weight and Pauling electronegativity decrease while

[12]The temperature change is 20 °C in $\sim$ 50 min.

Anion	F^-	Cl^-	Br^-	I^-
Weight $\Leftarrow$	19.00 g/mol	35.45 g/mol	79.90 g/mol	126.90 g/mol
Electronegativity* $\Leftarrow$	3.98	3.20	2.96	2.60
Covalent radius $\Rightarrow$	57 ± 3 pm	102 ± 4 pm	120 ± 3 pm	139 ± 3pm
Protein instability $\Rightarrow$				

Table 7: Main characteristics of a section of the Hofmeister Series for the anions, including the two anions considered in the experiment (Cl^- and Br^-); data taken from [25]. *: to quantify electronegativity, the Pauling scale have been considered. As indicated by the arrows, the covalent radius is the only quantity increasing from the left to the right.

the covalent radius increases. In the present analysis, we are considering anions only because it's them which dominate the Hofmeister Series [34].

Even if the trends regarding weight, electronegativity and covalent radius can be noticed only locally in the considered region, and not globally in the entire HS, we can use them to figure out what to expect from the results. These are the considerations we can do:

1. The position of $LiBr$ and $LiCl$ in the Hofmeister Series can be explained by considering their properties, reported in Table 4.1:

   - the covalent radius quantifies the size of an ion involved in a covalent bond. Considering how the charge is spread in a molecule, we have that a salt has the structure of a dipole, with the positive pole on the anion. As the size of the anion increases its surface charge density decreases, and it is therefore harder for the anion to attract water molecules.

     Considering for example $LiBr$ and $LiCl$, we have that since the anions Br^- attract less water molecules than the anions Cl^-, it is reasonable to suppose that a protein will be surrounded by more water molecules when Br^- is in solution rather than in the other case: as recently shown in the literature [1, 31], the influence of salts on the structure of hydrogen bond networks can be ignored, so if the "less bounded" water molecules are not attracted by the ions, then they can move towards the proteins surface, increasing its instability;
   - electronegativity is the ability of an ion to attract electrons, and negative charges in general. In Table 4.1 electronegativity decreases from the left to the right, increasing protein instability; following the reasoning presented in the previous point, this could be another reason why $Cl^- > Br^-$ in the Hofmeister Series.

2. Extending the previous considerations to the whole section of the Hofmeister Series $F^- > Cl^- > Br^- > I^-$, we can explain why the anions are ordered in this way. As far as the author knows, a similar explanation have never been given before in literature.

By basing on the characteristics of the anions considered, it is reasonable to expect that, in a graph representing viscosity as a function of temperature, the spacing between the viscosity of the ions which have Cl^- as an anion is smaller than the spacing between the viscosity of one of them and $LiBr$. Below we will see that the obtained results are in good agreement with this hypothesis, while in the matter of salts composed by the same anion and different cations the data collected don't show any clear confirmation of the series $KCl > NaCl > MgCl_2 > CaCl_2 > LiCl$, expected from theory.

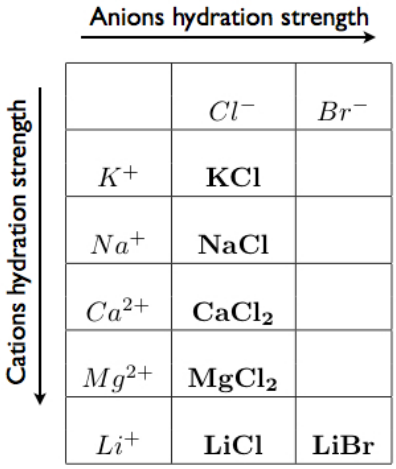

Figure 9: Salts considered in the present work. In the first row there are the anions used, and in the first column there are the cations. From the theory, we expect the viscosity to be similar among the elements of a column, and to change noticeably from one column to another. In other words, in the table above viscosity changes little horizontally, and greatly vertically.

4.2 Viscosity measurements

4.2.1 Water viscosity

To fit the data relative to the viscosity of ultrapure water, I used an equation of the form $f(x) = a + bx + cx^2$. As a result of the fit, we have $a = 14.3090 \pm 0.0991$, $b = -0.5134 \pm 0.00882$, $c = 0.0082 \pm 0.0002$, with $\chi^2 = 0.26$.

4.2.2 Different ions viscosities

In the viscosity measurements, I considered salts composed by univalent or divalent ions. Before to compare the viscosity measurements relative to the different salts, the viscosity values of $CaCl_2$ and $MgCl_2$ have been multiplied by 1.5. This multiplication is necessary to consider the same amount of charges per salt: the considered concentration of salts composed by univalent ions used in the experiment is 150 mM, and in this case there are 2 charges per molecule; the considered concentration of salts composed by divalent ions is 50 mM, and there are 4 charges per molecule. To have the same amount of charges in every solution, we have to multiply the viscosity measurements obtained working with divalent ions by x, where $x = 1.5$ is derived from $150 \cdot 2 = 50 \cdot 4 \cdot x$.

When the different viscosity datasets are represented together (see figure 4.2.2), we can notice that

- the viscosity values for the solution with $LiBr$ as a salt are greater than the values recorded for the other solutions;
- while it is easy to compare the trend of the $LiBr$ solution with that of the other solutions, whose salts have Cl^- as an anion, it is quite complicated to rank the other viscosity curves. This agrees with what suggested by the theory, which says that the HS is dominated by anions [12, 34], so it is reasonable to expect a bigger difference between solutions whose salts are formed by the same cation combined with different anions, rather than between solutions whose salts are formed by the same anion combined with different cations (see also Figure 4.1);
- considering the various viscosity curves, bigger fluctuations can be noticed as the temperature exceeds 24 °C, probably because of degradation phenomena which take place after a certain temperature, or after a certain period of time.

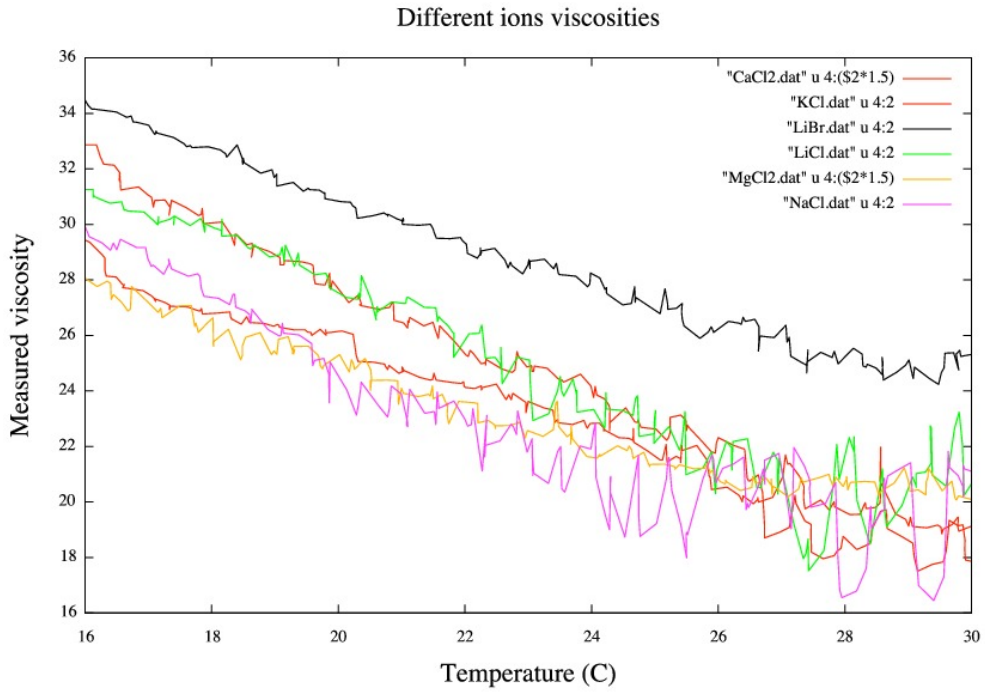

Figure 10: Viscosity of the considered solutions as a function of temperature.

To compare the viscosity curves relative to solutions whose salts have Cl^- as an anion, I fitted them with an equation like $f(x) = a + bx + cx^2$, which has the same form of the function best fitting pure water viscosity. The result is show in Figure 4.2.2; to study it, I divided the figure in eight sections, each delimited by the intersection of two fitting curves, and for every section I considered how the fitting curves are ranked. In none of the sections the ranking of the solution viscosities follows what expected from the Hofmeister Series.

Another attempt to better compare the different ion viscosities have been done dividing them by the function $f(x) = 14,3090 - 0,5134 \cdot x + 0,0083 \cdot x^2$, derived fitting the measured viscosity of ultrapure water. In principle, it should be easier to compare the different viscosity curves after this procedure.

Results are shown in Figure 4.2.2; as in the previous case, in none of the sections the viscosities ranking follows what expected from the Hofmeister Series.

Salt	χ^2
$CaCl_2$	0.68
KCl	1.51
$LiBr$	0.36
$LiCl$	3.96
$MgCl_2$	0.48
$NaCl$	4.35

Table 8: χ^2 relative to the fits of the viscosities of the different solutions, divided by the fitting function of water, $f(x) = 14,3090 - 0,5134 \cdot x + 0,0083 \cdot x^2$.

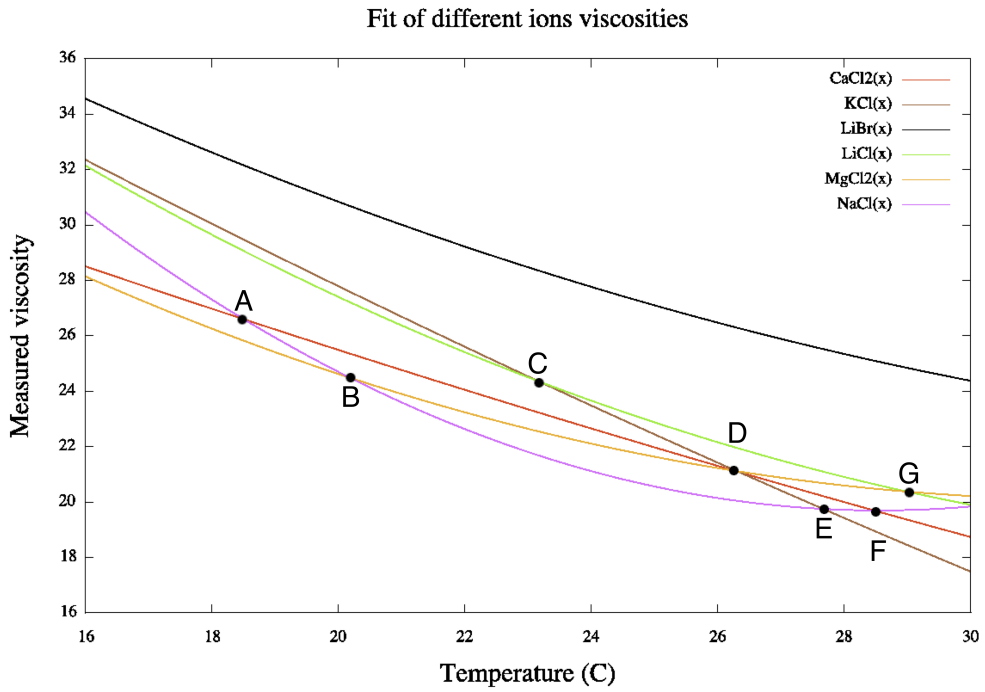

Figure 11: Viscosity of the considered solutions as a function of temperature. Each of the sections considered is delimited by capital letters.

5 Conclusions and perspectives

With the experimental technique used in this experiment, it has been possible to observe how the viscosity changes when a salt with a different cation is added to an egg-white based solution, while the results are not clear to interprete when a salt with a different cation is added instead.

Moreover, considering the physical properties of four anions, a reasonable explanation for a section of the Hofmeister Series has been provided.

Considering the obtained results, we have that the current experiment could be redone in three different ways:

1. repeating the performed experiment with greater salt concentrations, it should be possible to separate the viscosity curves relative to different cations;

2. considering solutions which salts are formed by a "neutral" cation (like Na^+ and K^+, which are in the middle of the Hofmeister Series), and studying how the viscosity changes when the cation is combined with different anions. With such an experiment, it should be possible to see if viscosity increases or decreases as we move to one side of the Hofmeister Series to the other;

3. using a "neutral" anion, like Cl^-, which is placed in the middle of the Hofmeister Series, it should be possible to measure how the viscosity changes when cations placed at opposite sides of the Hofmeister Series (like NH_4^+ and Li^+) are combined with this "neutral" anion.

Considering what reported in the literature, these are two other experiments which could be easily performed:

1. as underlined by Zhang and Cremer in their overview of the Hofmeister series [40], kosmotropic ions have stabilizing and salting-out effects both on proteins and

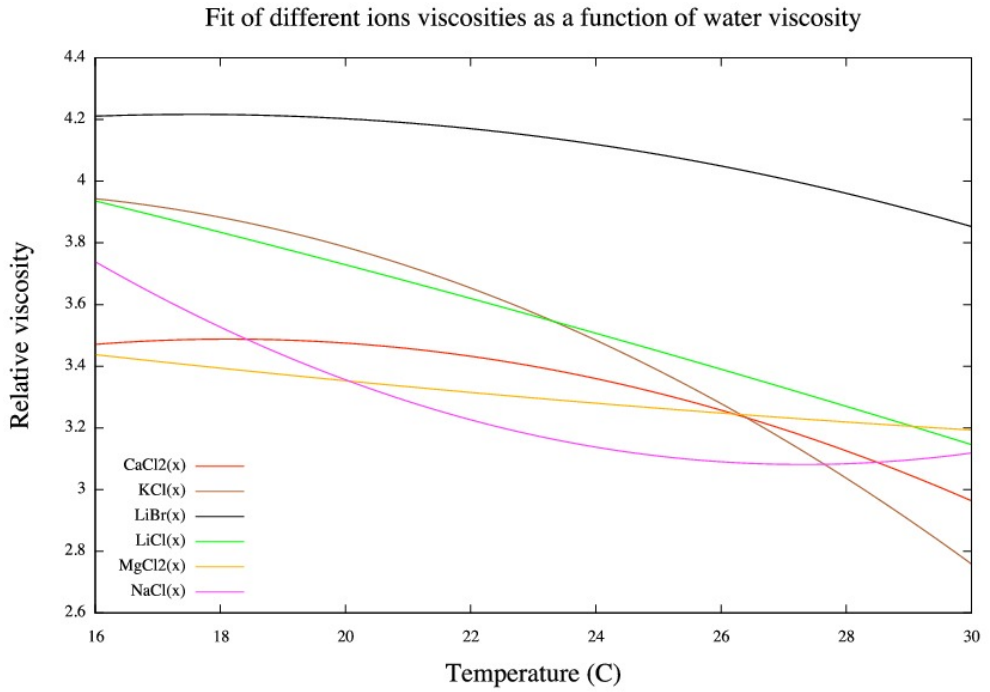

Figure 12: Viscosity of the considered solutions as a function of temperature, after being divided by the fitting function of water, $f(x) = 14,3090 - 0,5134 \cdot x + 0,0083 \cdot x^2$.

on macromolecules. In this work and in the previous one [6] I used proteins to investigate the HS; it would be interesting to continue this series of studies focusing also on different macromolecules;

2. in one of their papers, Hochachka and Somero [19] showed that a number of methylamine solutes, like sarcosine and betaine, stabilize the proteins structure, as done by part of the salts from the Hofmeister Series. Taking inspiration from this, it would be interesting to repeat the viscosity measurements done in this project mixing the simulated egg white with methylamine solutes, instead of with salts.

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