Professor

Dr. Tommy Nylander

Department of Physical Chemistry

Kemicentrum

LUNDS UNIVERSITET

Adsorption of lysozyme on Silica Surfaces and Specific ion effects

Chemistry: Project Work – Physical Chemistry – KEMP37

June, 2019

LUNDS UNIVERSITET

ABSTRACT

Biomedical application of nanoparticles is largely associated to their fate in biological media which, in fact, is related to their surface properties. Surface functionalitzation plays a key role in determining biodegradation, cytotoxicity and biodistribution through interactions which may be meditated by the macromolecules occurring in biological media. This project is focused on the effect of different buffers on lysozyme adsorption on flat silica surfaces and hydrophobized silica surfaces as well as mesoporous silica nanoparticles functionalized with amino groups (MSN-NH_2). In order to know the adsorption amount of lysozyme we used ellipsometry, where each buffer has been studied independently at two different buffer concentrations at the same pH. The effect of different buffers can be related to specific ion effect described interaction of buffers and salts induces relevant effects on the charged interfaces (Hofmeister effect), and thus lysozyme loading.

Lysozyme adsorption on silica and hydrophobized silica as well mesoporous silica at pH 7.15 was found to be buffer specific. The BES buffer seems to present a unique characteristic behavior which does not allow desorption. TRIS buffer shows the highest lysozyme adsorption amounts, while citrate and phosphate buffer usually show quite similar results.

The sequential addition of MSN-NH_2 causes more extensive desorption of lysozyme from the flat surface as observed with ellipsometry. Large effects of the buffer are also observed. The competitive adsorption to the particles, i. e. lysozyme protein corona formation, is likely to promote detachment from the silica surface.

Keywords: mesoporous silica nanoparticles, ellispometry, surfaces, protein corona.

''Be gentle to people when you go up; you will find them all when you go down'' – Eduard Punset.

First of all, I would like to express my deep gratitude to Professor Tommy Nylander, my project supervisor, for his guidance and opinions of this research work which perhaps it will result in a paper publication.

Secondly, I would also like to thank for their advice and assistance to all personnel from the Physical Chemistry department. My grateful thanks are also extended the other PhD-students who helped me out and trained at some point.

I am also deeply appreciated for receiving the MSNs samples to Professor Andrea Salis and Professor Maura Monduzzi from Università degli studi di Cagliari, Sardinia.

I would also like to give recognition to my home university, Universitat de Barcelona (UB), for simplifying all the Erasmus paper work system.

I wish to thank my parents for their support and encouragement throughout my study.

Last but not least, I am deeply satisfied that I have finished this project with fruitful results although these were interrupted by my severe leg injury during this Erasmus.

It has been a pleasure to study at the Physical Chemistry Department in Lund University.

CONTENTS

1. INTRODUCTION

Cancer is treated with chemotherapeutic drugs which are able to restrain tumor's growth, but they are also toxic for healthy cells, thus resulting in adverse side effects. The use of improved pharmaceutical formulations, which release the drug at the target cancerous tissue only, would reduce undesired side effects of the chemotherapeutics. In this context, researchers are focusing on nanomaterials as smart carriers for targeted drug delivery and stimuli-responsive systems, useful for the treatment of cancer. [2]

Ordered mesoporous silicates, synthesized for the first time in the early 1990s, are nanostructured materials with a big potential in nanomedicine applications. Among them mesoporous silica nanoparticles (MSNs) are promising drug carrier candidates for innovative pharmaceutical formulations. This thanks, as a result of their characteristic textural and structural features. Indeed, their high surface area (up to 1400 m^2/g), the narrow distribution of pore size (1–2 Å), and the high pore volume (1–3 cm^3/g), allow to load high amounts of drugs which can then be released at a sustained rate. [2]

Moreover, the external surface of MSNs can easily be functionalized with target biomolecules, i.e. proteins (lysozyme in this study)/antibodies, peptides or saccharides, that can be recognized by receptors that are overexpressed in tumor cells. [2]

Alternatively, these macromolecules can modify their conformation, as a response to a change of environmental conditions (i.e. pH), thus acting as a stimuliresponsive system. In general, the external functionalization affects MSNs biocompatibility, biodistribution, pharmacokinetics, particle stability, circulation time, tumor accumulation, cellular uptake, and therapeutic efficacy. [2]

When dispersed in a biological medium, nanoparticles (NPs) seek to lower their interficial energy by adsorbing biomolecules such as proteins, peptides, or glycolipids. This surface layer of biomolecules, known as the "protein corona" (PC), is due to the physico-chemical interactions established between the NPs and the biomolecules occurring in the biological fluid. The nature of the PC depends on several NPs properties, such as, their chemical composition, size, shape, surface charge, and hydrophilic/hydrophobic character. PC also depends on the composition of the biological medium, and on proteins concentration in blood plasma, temperature, administration route as well as composition of the cell membrane. [2]

Here the interactions between lysozyme (LYZ) with amino-functionalized mesoporous silica nanoparticles (MSN-NH_2) as well as flat silica and hydrophobized silica are investigated. The aim of the work is to reveal how protein absorbed amount and layers thickness is effect of ionic composition of the buffers.

For this purpose, the interaction between LYZ and different silica surfaces were studied using the following buffers salts: tris(hydroxymethyl)aminomethane (TRIS), citrate, phosphate and N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid sodium salt (BES). In all cases, the pH was kept constant at 7.15 and both 10mM and 50mM of buffers were investigated.

In addition, the bulk properties of MSN-NH_2 and LYZ were investigated in the different buffer systems using light scattering and zeta potential measurements.

2.0 THEORY

2.1. ELLIPSOMETRY

2.1.1. ELLIPSOMETRY TECHNIQUE:

Ellipsometry is based on the measurement of changes in ellipticity of polarized light upon reflection. Ellipticity could be described as the ratio and phase difference of two plane polarized light waves, one oscillating in parallel with the plane of incidence and the other perpendicular to it. It is used to accurately determine thickness and refractive indices of films. Spectroscopic ellipsometry also gives information on the absorption of the sample at different wave lengths. [3]

Ellipsometry has a wide spectrum of application for the study of processes taking place at surfaces. Here I have used ellipsometry as a tool for the study of adsorption of proteins (lysozyme) at interfaces. [3]

Often, the main interest concerning adsorption is the amount adsorbed per unit area.

The adsorbed amount (ɣ) can be calculated as [3]:

$$\gamma = \frac{d_f \cdot (n_f - n_0)}{dn/_{dc}}$$

Where d_f are the thickness values measured, n_f are the refractive index of the buffers measured by the ellipsometer, while n_0 are the refractive index of the solution and dn/dc is the index increment of the substance in the film (around 0.140).

2.1.2 THE ELLIPSOMETER:

The ellipsometer used was a Rudolph thin film type 43603-200E (Rudolph Research, Fairfield, N.J., USA) has been used. The light source is a xenon arc lamp light source filtered to select a wavelength of 401.5 nm. The light beam passes through a sandwich consisting of two optical components, a plane polarizer and a quarter wave plate. By rotating this unit circularly polarized light with varying intensity can be obtained. [3]

The light then passes through a polarizer and then elliptically polarized light is generated by means of a retardation plate, a compensator. The ellipticity of the light is then varied by means of rotating the polarizer. For a null ellipsometer, as used in the present study, the elliptically polarized light beam is adjusted in such a way that it after reflected surface emerges as a linearly polarized light. [3] The rotation, azimuth, of the linearly polarized light after reflection is detected by using another polarized, i.e. the analyzer.

The polarizer and analyzer prisms are provided with scales which allow the reading of the azimuth within a hundredth of a degree. The compensator is a first order quarter-wave plate set for a wavelength of 401.5 nm. [3]

The light intensity after the analyzer is measured by a photomultiplier. [3]

The stepping motor that rotates the polarizer and analyzer, as well as the compensator, are connected to an interface which, in turn, is connected to a personal computer. [3]

The positions of the polarizer and analyzer giving minimum light intensity are obtained by using the so-called ''method of swings''. While keeping the position of the analyzer fixed, the position of the polarizer is swept from its approximate minimum position, thus increasing the light intensity from 0,4 to 1,0 V. The direction of movement is then reversed until the same intensity is reached at the other side of the minimum. By assuming symmetry, the exact position of the minimum is obtained as the mean of the two values. The polarizer is now positioned at the value obtained, and the procedure is repeated for the analyzer. [3]

The computer simultaneously performs calculations of the index of refraction for the bare surface or the thickness, and the refractive index of the adsorbed layer, from which the amounts adsorbed for an adsorbed layer. Data are also stored for further evaluation. [3]

The sample is immersed in a medium contained in a trapezoid cuvette (7mL volume) made of fused quartz (Hellma, Germany), allowing light to pass perpendicularly to the cuvette walls prior to and after reflection. The medium in the cuvette is agitated with a magnetic stirrer. The temperature is controlled at 25ºC by circulating thermostated water through the metal block that serves as the cuvette holder. [3]

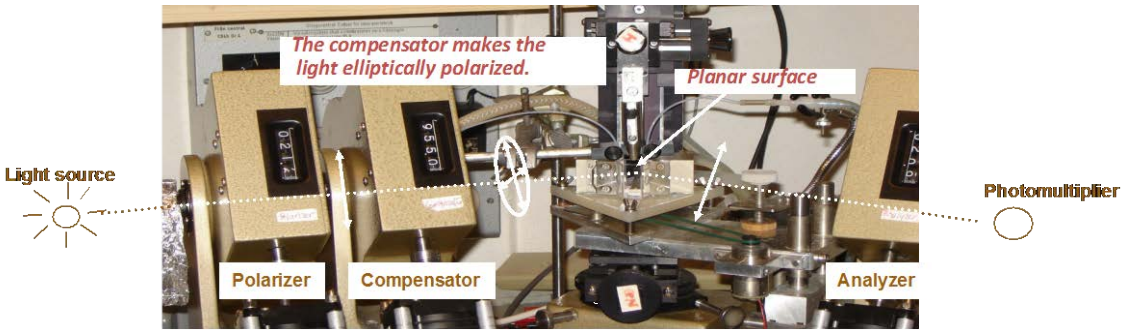


Figure 1. Rudolph ellipsometer used. [Source 1]

2.2 Dynamic light scattering

2.2.1 The DLS and Zeta Potential technique

The dynamic light scattering (*DLS)*, is a scattering technique that can be used to determine the size average of suspended nanoparticles, including proteins, through fluctuation distribution. In this study, it will be used to determine some physical properties on several solutions.

Zeta potential is a physical property which is exhibited by any particle in suspension. It can be used to optimize the formulations of suspensions and emulsions. Knowledge of the zeta potential can be useful to predict long term suspensions' stability. Nevertheless, it was not useful in this study.

2.2.2 The DLS instrument

Light scattering. *DLS - Malvern Zetasizer S. Serial MAL1019687.*

For convenient DLS and SLS measurements and determination of eletrophoretic mobility (or zeta potential), a Zetasizer Nano ZS from Malvern Instruments Ltd, Worshestershire, UK is used.

The instruments measures DLS and SLS at a set angle of 173° using the NIBS technology. The zeta potential (or electrophoretic mobility) measurements using M3-PALS technology are performed at 20°C. The laser used is a 4 mW He-Ne laser (632.8 nm) and the detection unit comprises an avalanche photodiode. The temperature range of the instrument is 2-90 °C.

Disposable plastic cuvettes were used for the Zetasizer.

2.3. MESOPOROUS SILICA NANOPARTICLES (MSN)

The aim of this project is to reveal the effect of different buffers on lysozyme protein adsorption on different surfaces. Protein adsorption in different types of electrolytes was measured in ellipsometry on silica wafers.

A MSNs sample functionalized with amino groups has been used (MSN-NH2). These have a porous hexagonal ordered structure (2 nm, monodemal) with a distribuition of 1-3 cm3/g, compraising a high surface area (from 1000 to 1400 m2/g). The particles size are about 100-120 nm with a zeta potential of + 30 mV, although it has not been yet measured for our samples. [2]

This study changes systematically the electrolytes composition and buffer solutions in order to see the Hofmeister effect on the adsorbed amount, which was studied though UV measurements in previous researches.

The MSNs functionalized with amino groups (aminopropyl groups) were received from Professor Andrea Salis and Maura Monduzzi, Università degli studi di Cagliari, Sardinia.

2.4. FUNCTIONALITZATION

When dispersed in a biological medium, nanoparticles seek to lower their surface energy by adsorbing –on their surface- biomolecules such as proteins, peptides, or glycolipids. That process is known as functionalitzation. This surface layer of biomolecules, known as the "protein corona" (PC), is due to the physico-chemical interactions established between the NPs and the biomolecules occurring in the biological fluid. The nature of the PC depends on several NPs features, such as, their chemical composition, size, shape, surface charge, and hydrophilic/hydrophobic character. [1]

2.5. EFFECT OF BUFFERS AND SALTS

Lysozyme adsorption on mesoporous silica at pH 7,15 is buffer specific. The synergistic action of buffers and salts induces relevant effects on the charged interfaces, and thus on lysozyme loading. These findings, suggest the occurrence of Hofmeister phenomena also for buffers. [2]

$$pH = pKa + log\frac{[A^-]}{[HA]}$$

Hofmeister (ion specific) effects are phenomena related to the chemical nature of electrolytes. Although they are ubiquitous in all chemical, colloidal, and biological systems,1–5 they cannot be quantified in terms of the conventional physico-chemical theories (i.e. Debye–Huckel, DLVO, etc.). These are limit theories, based on electrostatics, and valid at infinite dilution only. The gap between theories and

Hofmeister related experiments is usually very large since the ion specific effects are generally observed at high concentrations in the presence of strong electrolytes 0.3–3 M.6 This is very far from the validity's domain of both limit laws, and their extensions (valid up to 10-3 to 10-2 M).3 Nevertheless, it has been demonstrated that high concentrations of strong electrolytes are not strictly necessary to observe ion dependent phenomena. Several experiments showed that ion specificity occurs at physiological salt concentrations (0.1–0.15 M), and even below. [2]

According to HH equation, it is necessary to fix the pH of the experiment for weak electrolytes in order to give Hofmeister effects. Typical buffer concentrations are in the range 10–100 mM for the experiments I will carry out (specifically, 10 and 50mM). The implicit assumption is that, due to their low concentration, the buffer ions should not display any specific effect. This is not so. Indeed it has recently been observed that, even at the same nominal pH, lysozyme electrophoretic mobility was buffer dependent.

The following buffer salts formulas were used:

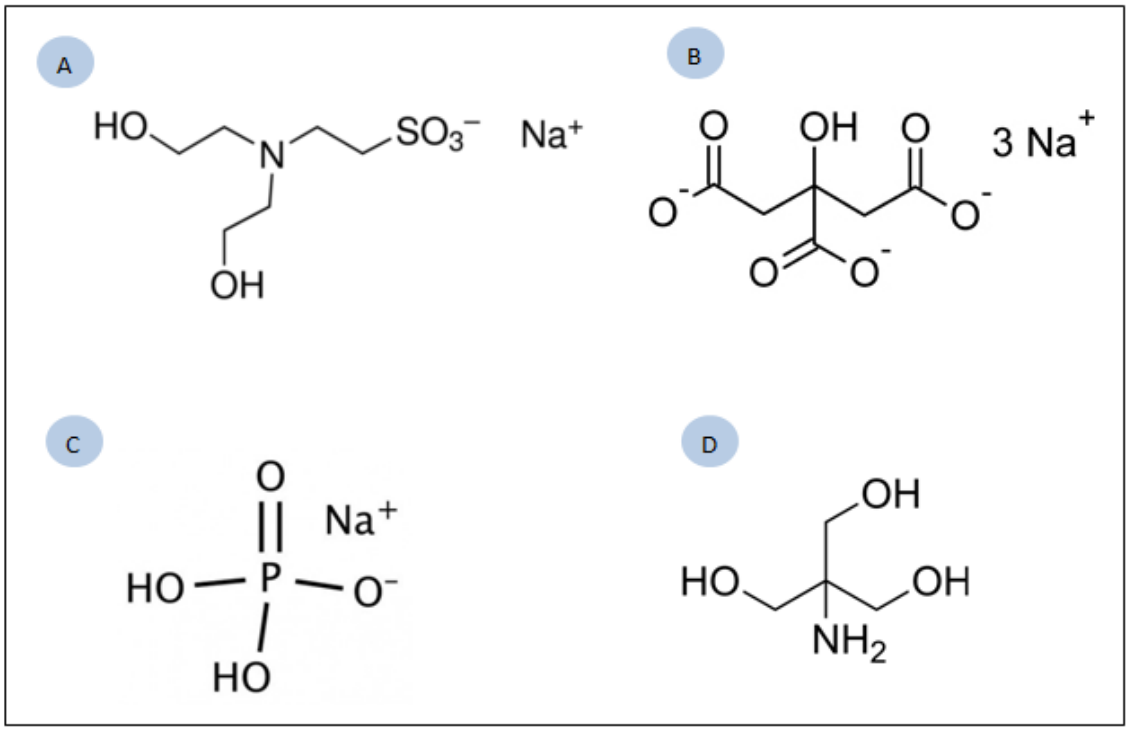


Figure 2. Molecular formulas of the buffer salts used at pH= 7.15: A is monosodium tris(hydroxymethyl)aminomethane (TRIS). B is trisodium citrate. C is monosodium dihydrogen phosphate. D is 2-Amino-2-(hydroxymethyl)propane-1,3-diol (BES).

Buffer salt	pK_a (25ºC)	Charge at pH=7.15
BES	7.1	1 negative
Citrate	3.13; 4.76; 6.40	3 negative
Phosphate	2.12; 7.21; 12.67	1 negative
TRIS	8.07	Positive

2.6. FORMATION OF A LAYER PROTEIN (CORONA) ON MODIFIED MSN SURFACES

The surface charge of functionalized MSNs modulates the interactions with LYZ though Van der Waals forces. A protein corona is due to interactions established between the nanoparticles and the bimolecules occurring (MSN-NH_2) in the buffered fluid (BES, citrate, phosphate and TRIS).

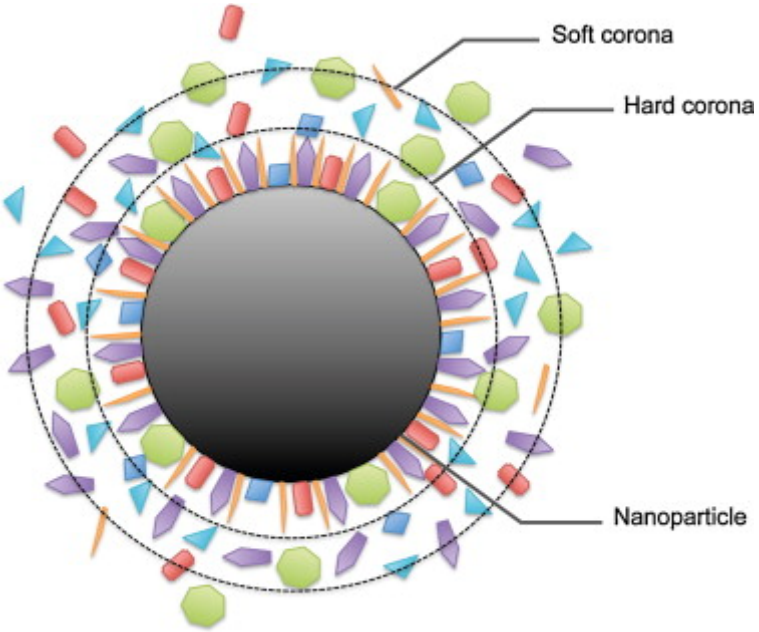


Figure 3. Representation of a protein corona. NPs are attached to the surface provoking a two layers protein corona. [Source 2]

After administration of nanoparticle (NP) into biological fluids, an NP–protein complex is formed, which represents the "true identity" of NP in our body. Hence, protein–NP interaction should be carefully investigated to predict and control the fate of NPs or drug-loaded NPs, including systemic circulation, biodistribution, and bioavailability. [5]

2.7. HOFMEISTER EFFECT

The Hofmeister series is a classification of ions in order of their ability to salt out or salt in proteins. [6] The effects of these changes were first worked out by Franz Hofmeister, who studied the effects of cations and anions on the solubility of proteins. [7]

Hofmeister discovered a series of salts that have consistent effects on the solubility of proteins and (it was discovered later) on the stability of their secondary and tertiary structure. Anions appear to have a larger effect than cations [8], and are usually ordered.

The mechanism of the Hofmeister series is not entirely clear, but does not seem to result from changes in general water structure, instead more specific interactions between ions and proteins and ions and the water molecules directly contacting the proteins may be more important. [9] Recent simulation studies have shown that the variation in solvation energy between the ions and the surrounding water molecules underlies the mechanism of the Hofmeister series. [10-11]

Early members of the series increase solvent surface tension and decrease the solubility of nonpolar molecules ("salting out"); in effect, they *strengthen* the hydrophobic interaction. By contrast, later salts in the series increase the solubility of nonpolar molecules ("salting in") and decrease the order in water; in effect, they *weaken* the hydrophobic effect. The salting out effect is commonly exploited in protein purification through the use of ammonium sulfate precipitation. [12]

In our low concentration ranges where ionic strengths are low, the salting in effect is produced. This refers to the effect where increasing the ionic strength of a solution increases the solubility of some solute (such as a lysozyme protein). [13]

3. MATERIALS AND METHODS

3.1 MATERIALS

- Lysozyme powder from chicken egg white (Sigma-Aldrich). Muramidase or N-acetylmuramide glycanhydrolase**.** 0,5 g were dissolved at 50mL of MiliQ water. The solution (10mg/mL) has been stored at the lab fridge a 4ºC and renewed every six weeks.
- BES 99+% for biochemistry (ACROS ORGANICS). N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, N,N-Bis(2-hydroxyethyl)taurine. CAS 10191-18-1. The BES solutions were prepared dissolving the powder into MiliQ Water, then the pH was adjusted to 7,15 with NaOH or HCl prepared also in MiliQ Water.
- TRIS(HYDROXYMETHYL) AMINOMETHANE (Sigma-Aldrich). The TRIS solutions were prepared dissolving the powder into MiliQ Water, then the pH was adjusted to 7,15 with NaOH or HCl prepared also in MiliQ Water.
- Sodium citrate tribasic dihydrate. ACS reagent >99,0% (Sigma-Aldrich). $C_6H_5Na_3O_7 \cdot H_2O$. The citrate solutions were prepared dissolving the powder into MiliQ Water, then the pH was adjusted to 7,15 with NaOH or HCl prepared also in MiliQ Water.
- Di-Sodium hydrogen phosphate anhydrous (AnalaR NORMAPUR). Na_2HPO_4. The phosphate solutions were prepared dissolving the powder into MiliQ

Water, then the pH was adjusted to 7,15 with NaOH or HCl prepared also in MiliQ Water.

- Mesoporous silica nanoparticles functionalized with amino grouos (MSN-NH2). These have a porous ordered (hexagonal) structure with monomodal pore size (about 2 nm) distribution and high surface area (around 1000 m2/g). Usually the particle size is about 100-120 nm and zeta potential around +30 mV.
- Silica wafers. The surface was cut in a 1,2x2cm surface. The hydrophilic surfaces were cleaned and positively charged. The hydrophobic ones were silanized with a O layer

3.2 METHODS

Mainly, only these three techniques were used:

- Ellipsometry for surface characterization. *Ellipsometer Rudolf model 43603-200E*.

In order to study the adsorption of the lysozyme on a silica surface depending on the buffer, some ellipsometric measurements have been carried out. A sample of 0.5 mL of the previous lysozyme solution was added to a 5 mL cuvette containing each one of the buffers.

The substrates were cleaned first in a base mixture of 25% NH_4OH, 30% H_2O_2 and H_2O (1/1/5, by volume) at 80ºC for 5 min, and rinsed with water, followed by an acid mixture of 32% HCl, 30% H_2O_2, and water (1/1/5, by volume) at 80ºC for 5 min. The substrates were thoroughly rinsed with water and then ethanol, to be stored in 99,7% ethanol and then plasma cleaned (except for silanized surfaces). [3]

The hydrophobic silica surfaces were prepared by a gas-phase silanization. The surfaces were placed in a desiccator with 1 mL of dimethyloctylchlorosilane (DMOCS) with a vacuum pump and left overnight at room temperature. [3]

The ellispometry equipment needed to be calibrated before each measurement. The calibration process included steps such as setting the compensator micrometer at 19.519, cuvette alignment, surface alignment and finding the minima and zone.

In pursuance of studying the interaction effects, lysozyme adsorption was measured for in 10 and 50mM blank buffers on both kinds of surfaces, and with the MSN-NH_2 interactions.

At first, desorption was measured while rinsing the cuvette with buffer at a pace of 10mL/min for 5 minutes. Afterwards, the measurements had to stop temporally

when the nanoparticles were added for 30 min, as they were too opaque and blocked the light stray.

- Light scattering. *DLS - Malvern Zetasizer S. Serial MAL1019687.*
- Refractive Index. B+S Abbe 60/ED refractometer:

For the ellipsometry, the RI at a certain wave length was necessary as it was a parameter imputed into the softeware.

This technique permits the refractive indices (RI) at multiple wave lenghts, with a high precision. This allows to extrapolate or interpolate the measured values and determine the RI of a sample at a particular wavelength, e.g. 632.8 nm (the one of a red He-Ne laser.) The accessible range is from 1.30 to 1.74. 2. A spectral bulb, that has several well-known strong peaks of the emission, is used to illuminate the sample. An OSRAM mercury-vapor bulb mounted inside an extemal holder was used.

The refractometer is calibrated in a way that the position of a refracted spectral line can be recorded as a scale reading. The value of the scale reading is unambiguously converted into the refractive index with a free software or with calibration tables.

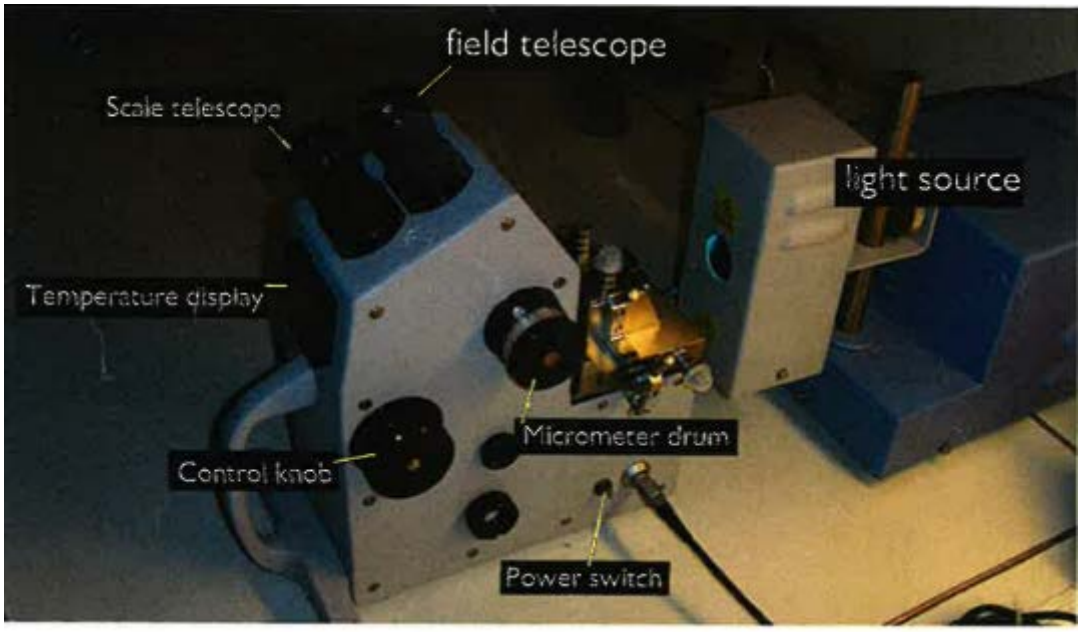


Figure 4. B+S Abbe 60/ED refractometer parts.

In the optical sight three referenced wave lenghts are represented in three ranges of colours are represented. The optical cross has to point the field's border in order to calibrate it. Distilled MiliQ water was used as a test sample:

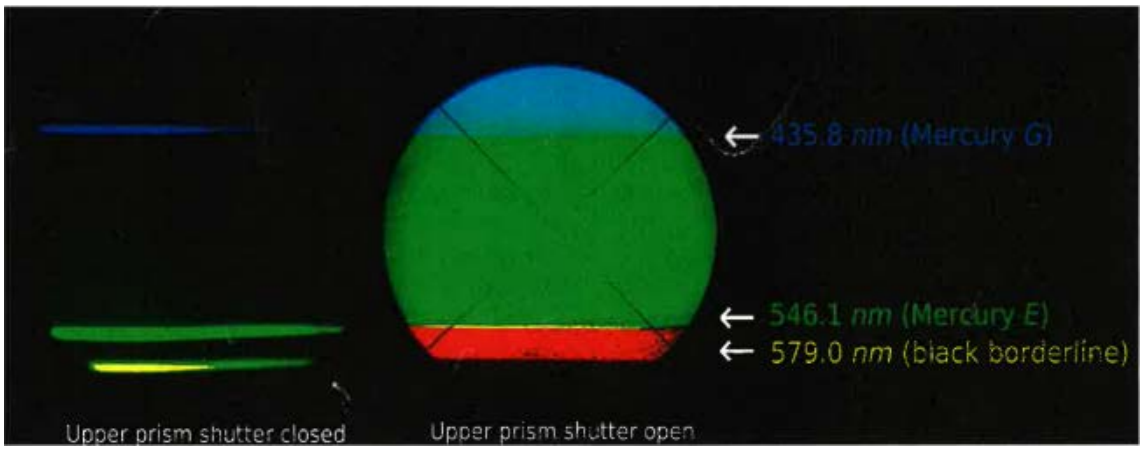


Figure 5. Wave lengths color fields though the optical scale telescope.

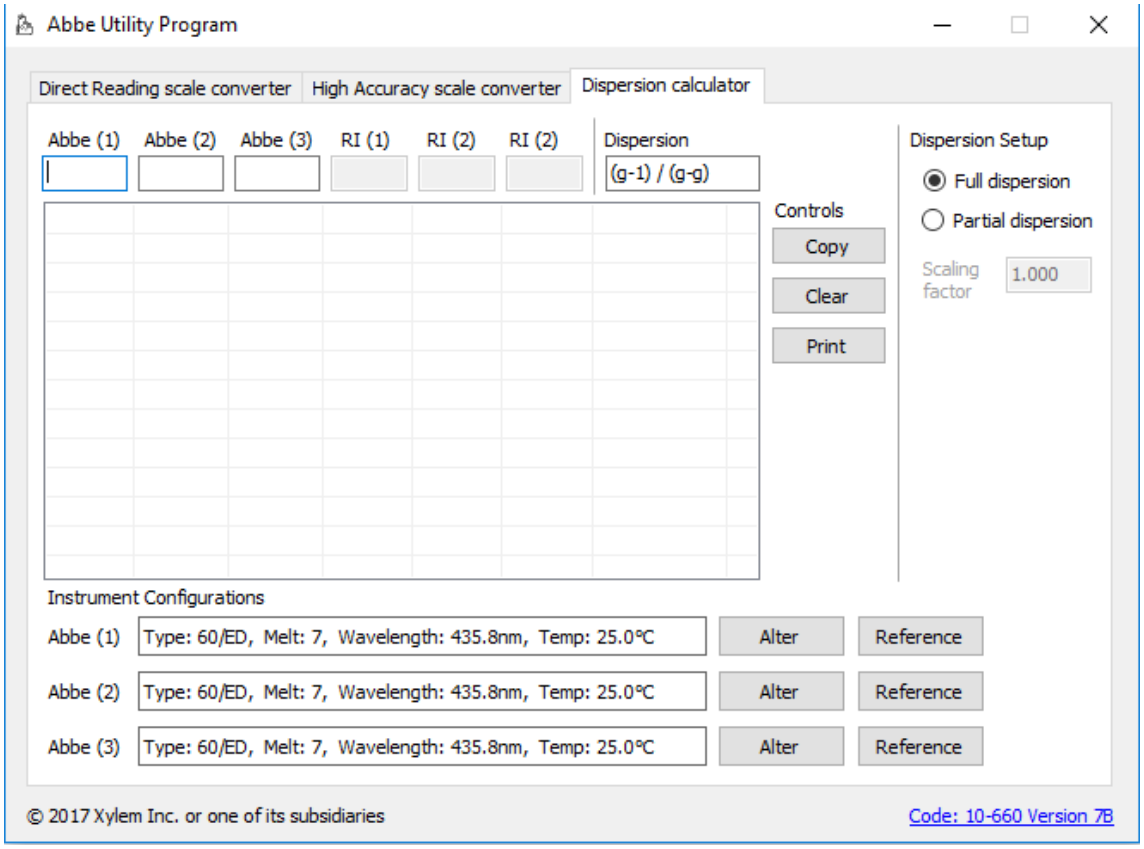


Figure 6. Abbe Utility Program. Software calculator.

4.0 RESULTS:

CHEMICAL COMPOUNDS USED:

All following materials and have been prepared with MiliQ water:

- 10 mg/mL Lysozyme solution (renewed every 6 weeks)
- 10, 50 mM BES buffer solution at pH=7.15
- 10, 50 mM Citrate buffer solution at pH=7.15
- 10, 50 mM Phosphate buffer solution at pH=7.15
- 10, 50 mM TRIS buffer solution at pH=7.15
- Solid amino-functionalized mesoporous silica nanoparticles

4.1 REFRACTIVE INDEX CALCULATION

The refractive index of the buffer solutions has been measured with the refractometer B+*S Abbe 60/ED* at multiples wavelengths. An OSRAM mercury-vapor bulb mounted inside an external holder was used. The software used to treat the data is called *Abbe Unility*.

Buffer solution	435.8 nm	546.1 nm	579.0 nm
50 mM BES	1.37057	1.34136	1.36629
50 mM Citrate	1.34224	1.36721	1.37111
50 mM Phosphate	1.34117	1.36610	1.37038
50 mM TRIS	1.34139	1.36617	1.37010
10 mM BES	1.34056	1.36512	1.36942
10 mM Citrate	1.34047	1.36536	1.36960
10 mM Phosphate	1.34045	1.36508	1.36947
10 mM TRIS	1.34028	1.36612	1.36927

The values that the ellipsometer takes into as the buffer refractive index are at 435.8 nm, the closest measured to 401.5 nm on which the ellipsometer works.

$$\gamma = \frac{df \cdot (Nf - No)}{dn/dc}$$

In order to readjust the values and data given:

Symbol	Concept	Data
γ	Amount adsorbed (mg/m^2)	Measured
d_f	Thickness	Measured
n_f	RI values from the ellisometer	Buffer
n_o	Buffer RI	MiliQ water
Dn/de	RI increment of the lysozyme in the film	Depending on the buffer (circa. 0.18)

4.2 LIGHT SCATTERING:

In order to know the size of the nanoparticles, DLS measurements were taken. 0,1mL of lysozyme were added with a syringe into 1mL of the correspondent buffer in a plastic disposable cuvette:

10 mM buffer + LYZ	Size average (nm)	Deviation (nm)	Result (nm)
BES	1024.2	96.6	1000 (100)
Citrate	431.0	44.9	400 (40)
Phosphate	443.6	75.3	400 (80)
TRIS	97.34	4.71	100 (5)

10 mM buffer + LYZ	Zeta Potential (mV)	Deviation (mV)
BES	7.91	2.17
Citrate	0	0
Phosphate	0.829	0.266
TRIS	2.52	0.53

All values have to be around 0 mV.

As the deviation values were that high, the same samples were left overnight to see if aggregation in suspended particles was a key factor which did not allow to take proper measurements.

Aggregation 10 mM buffer + LYZ	Size Average (nm)	Deviation (nm)	Result (nm)
BES	845.5	706.2	Inconclusive
Citrate	1250	360.8	1300 (400)
Phosphate	1119	996.6	Inconclusive
TRIS	141.1	19.2	140 (20)

50 mM buffer + LYZ	Size Average (nm)	Deviation (nm)
BES	798.3	134.7
Citrate	166.4	127.9
Phosphate	200.2	136.9
TRIS	253.4	40.4

These last two table results are inconclusive due to the random aggregation and high deviation.

4.3 ELLISPOMETRY

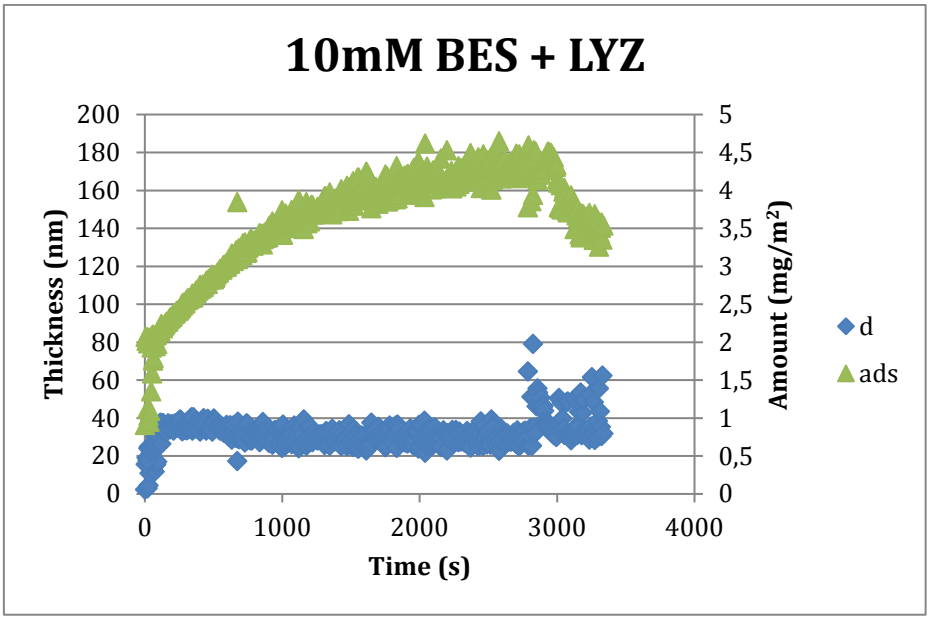
All surfaces are cut in a 2x1.2cm size. (Note: that hydrophilic surfaces are not specified in the subtitles, only the hydrophobic ones)

The first half of the following graphs are for the lysozyme adsorption in a buffer (without MSNs). Thickness (nm) will be represented on the left axis in blue. The amount of lysozyme adsorbed (mg/m^2) will be represented on the right axis in green.

When MSNs are being used, adsorption will be represented in red instead of green.

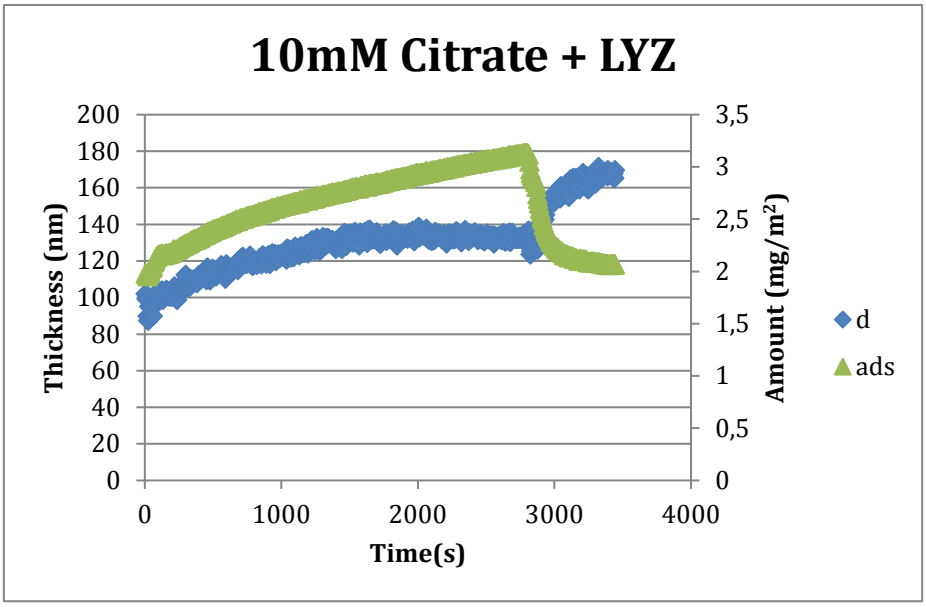
Knowing that at T= 298K and desorption rate was over a pace of 10mM/min. The following results have been gathered:

ADSORPTION FROM 10MM BUFFERS



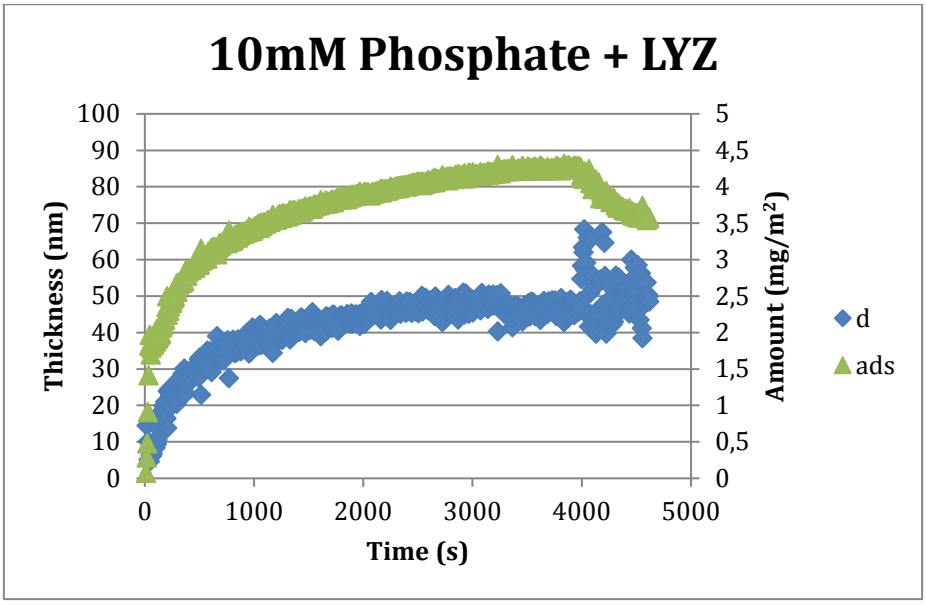
Graph 1. A 4.5 mL of 10mM BES solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry.

The adsorbed amount is then desorbed from around 4,25 to 3,25 mg/m^2. Results are noisy as it was the first ellipsometric ever taken.



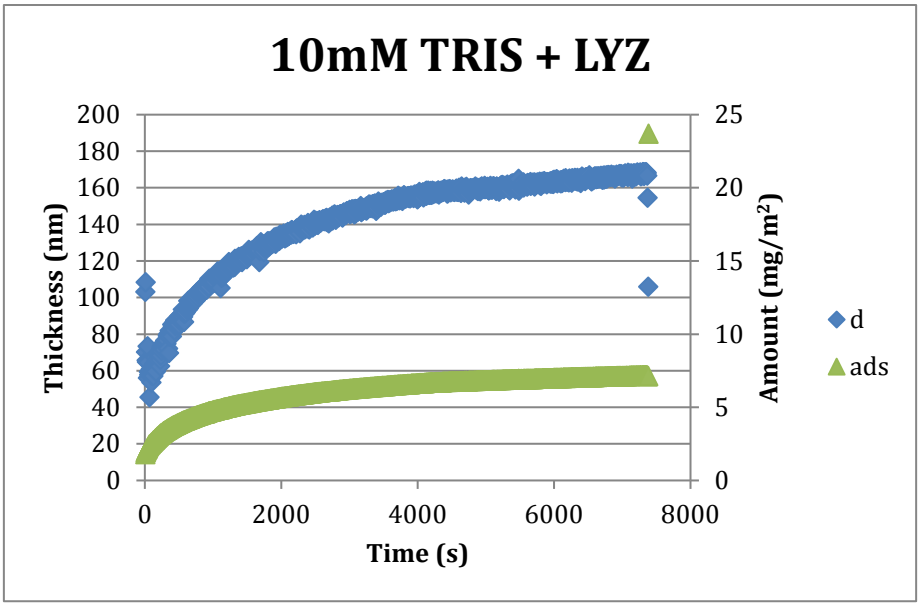
Graph 2. A 4.5 mL of 10mM citrate solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry.

The adsorbed amount is then desorbed from around 3.25 to 1.9 mg/m^2.



Graph 3. A 4.5 mL of 10mM phosphate solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry.

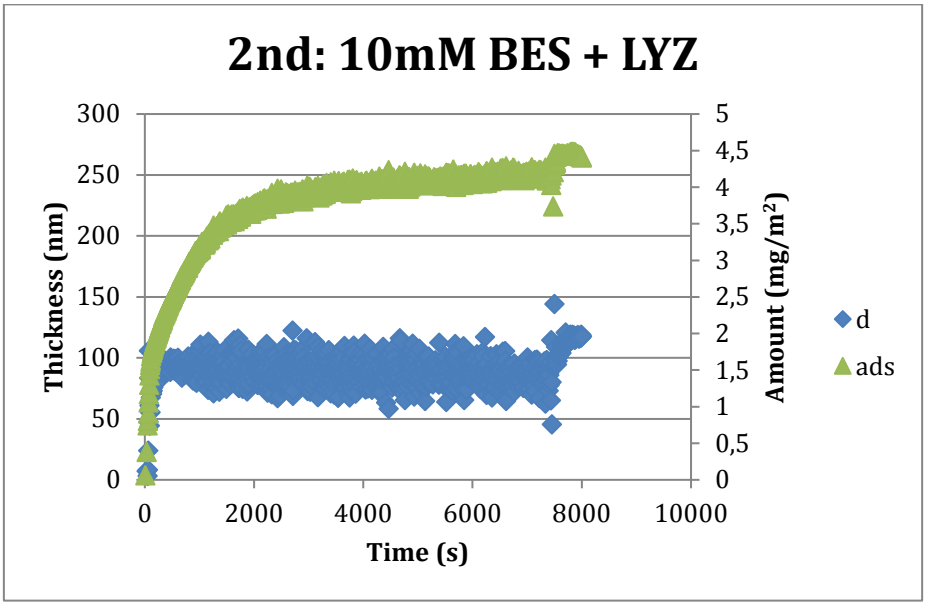
The adsorbed amount is then desorbed from around 4.25 to 3.5 mg/m^2.



Graph 4. A 4.5 mL of 10mM TRIS solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry.

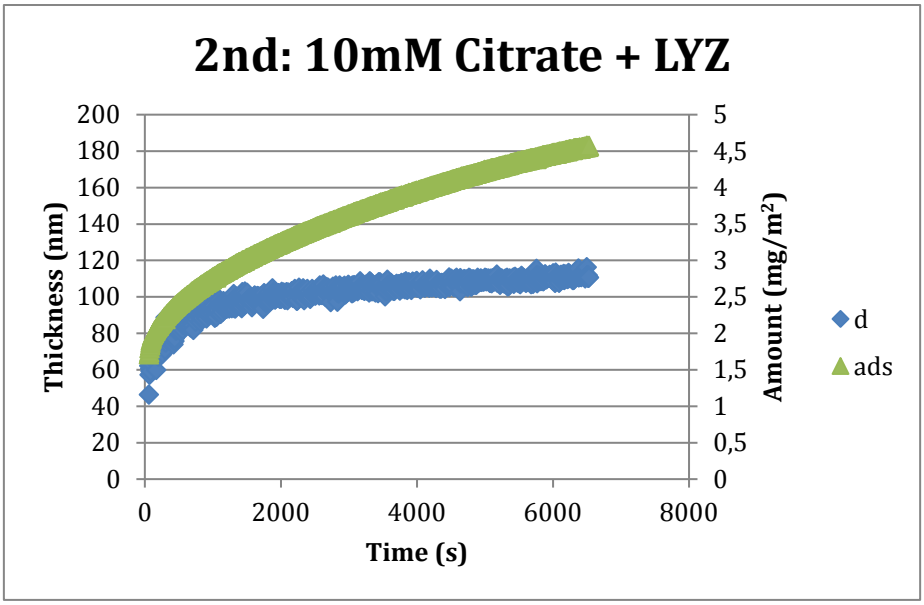
Desorption has not been measured due to stirring problems.

The same kind of measurements was measured again in order to have more proper results and compare the reproducibility:



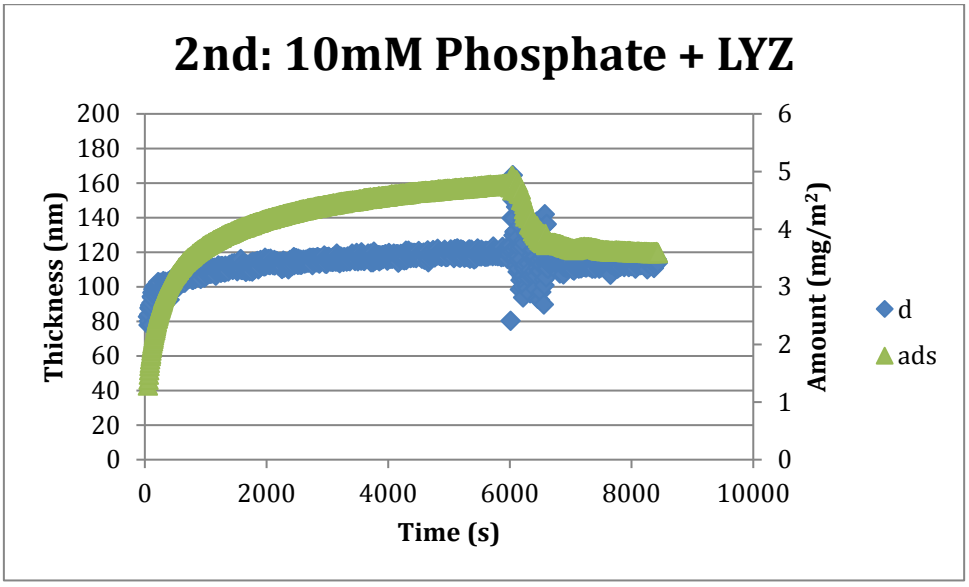
Graph 5. A 4.5 mL of 10mM BES solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry for a second time.

The adsorbed amount increases after rinsing the cuvette.



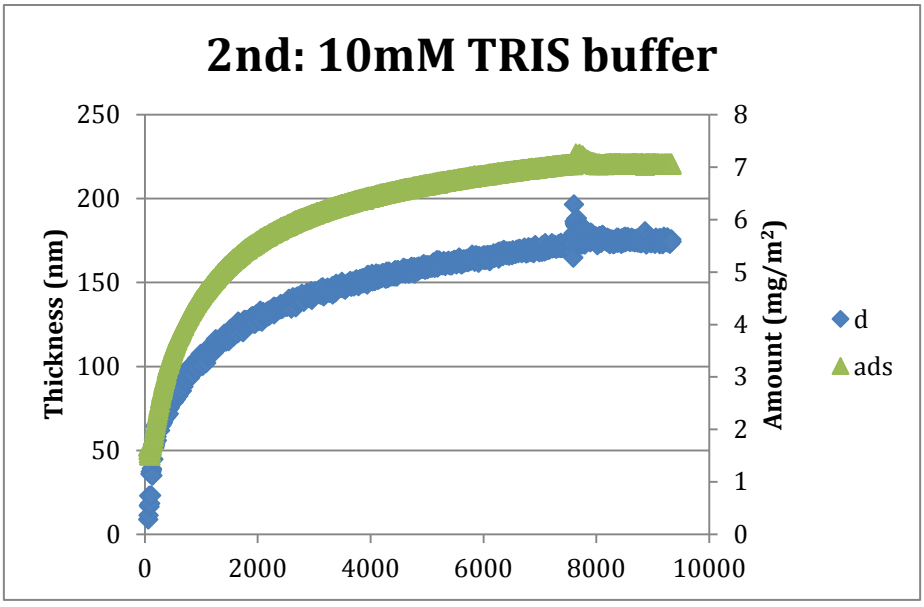
Graph 6. A 4.5 mL of 10mM citrate solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry for a second time.

Desorption was not measured.



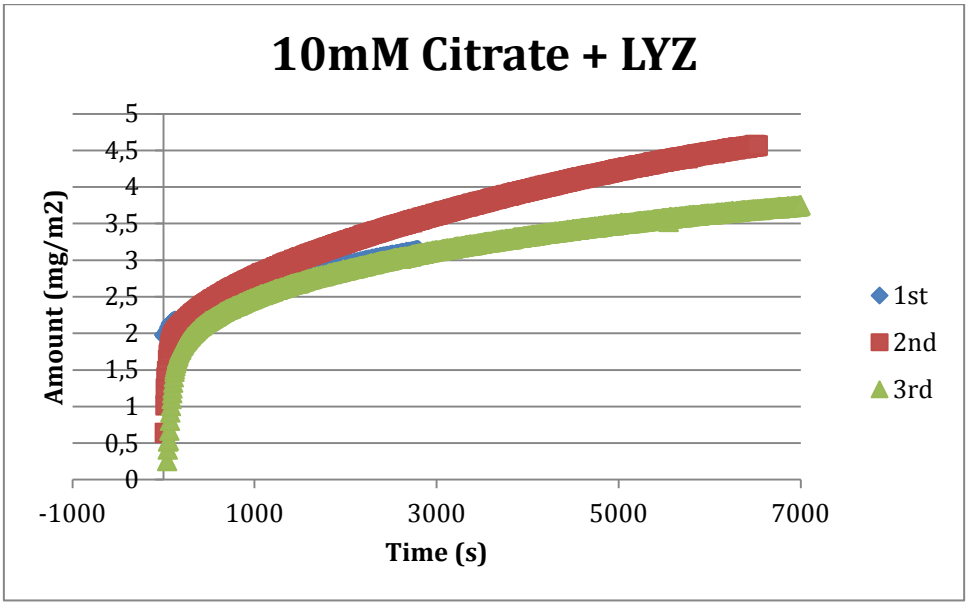
Graph 7. A 4.5 mL of 10mM phosphate solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry for a second time.

The adsorbed amount is then desorbed from around 5.0 to 3.4 mg/m^2.



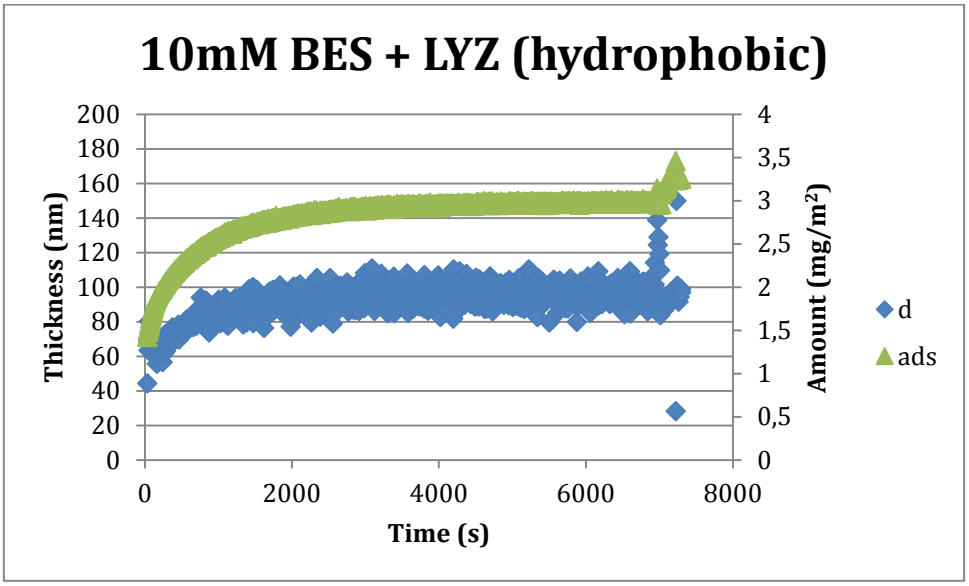
Graph 8. A 4.5 mL of 10mM TRIS solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry for a second time.

The rinsed amount is barely desorbed.



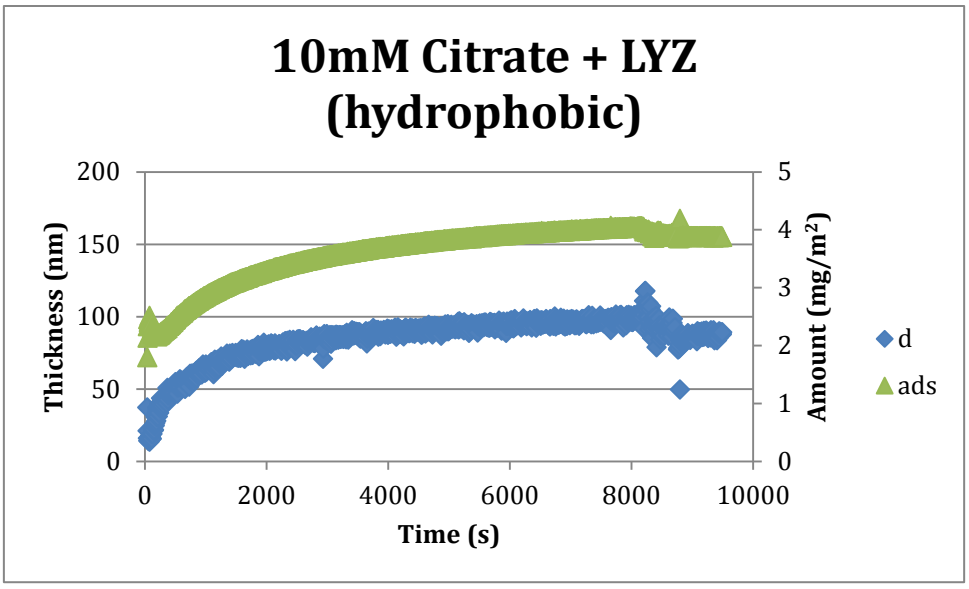
Graph 9. The 4.5 mL of 10mM citrate solution and 0.5 mL of lysozyme ellipsometric measurements were compared in order to rule out the different result.

First and third measurements are identical, thus the second value was ruled out. A miscalibration could have been the origin of the mistake.



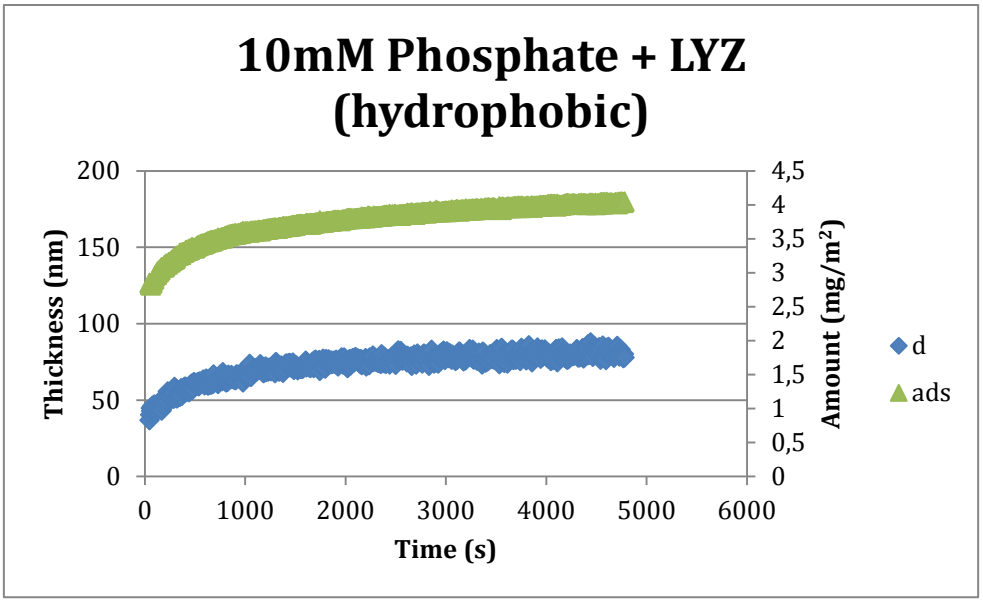
Graph 10. A 4.5 mL of 10mM BES solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry on a silanized silica surface.

Adsorption increases after rinsing the cuvette.



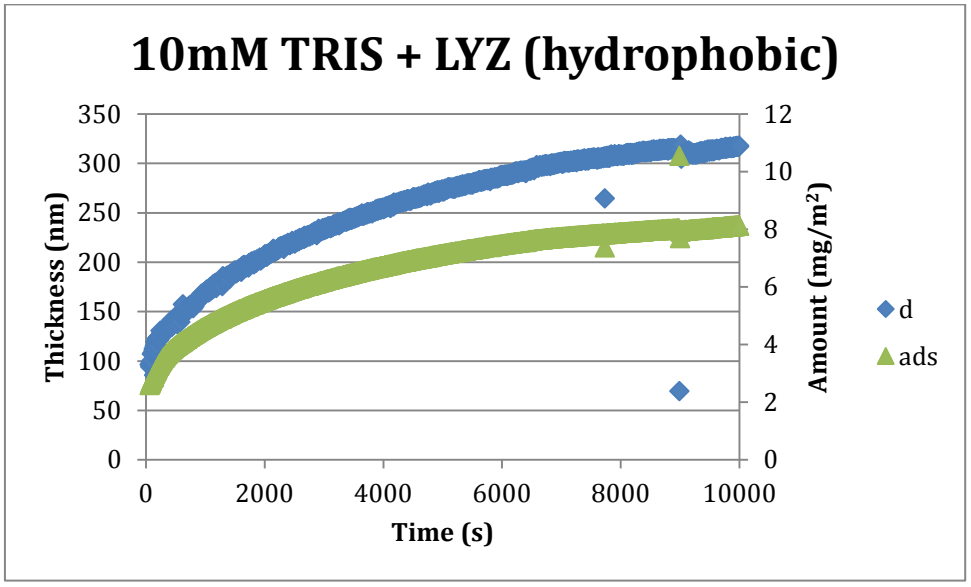
Graph 11. A 4.5 mL of 10mM citrate solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry on a silanized silica surface.

The rinsed amount is barely desorbed.



Graph 12. A 4.5 mL of 10mM phosphate solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry on a silanized silica surface.

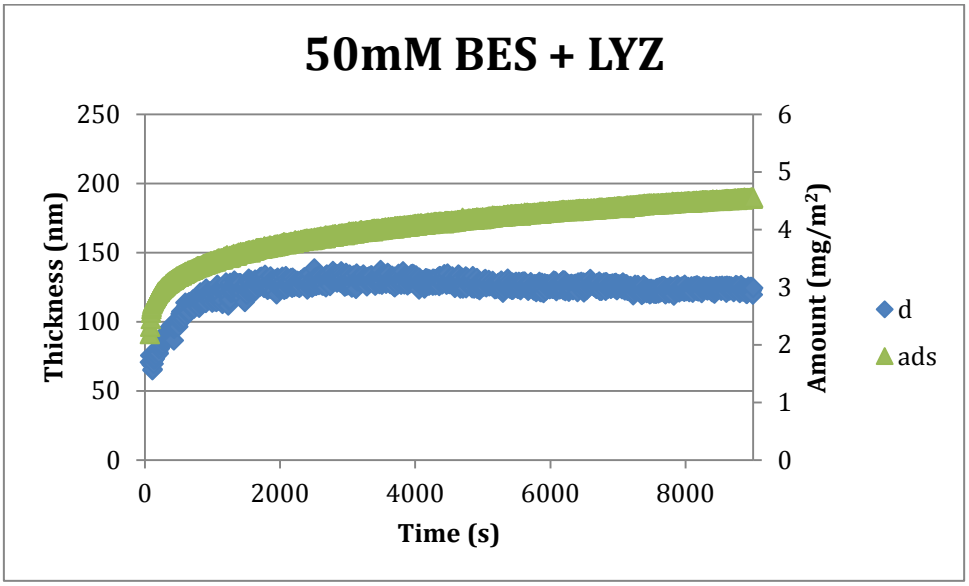
Desorption was not measured.



Graph 13. A 4.5 mL of 10mM TRIS solution and 0.5 mL of lysozyme were measured with Rudolph ellipsometry on a silanized silica surface.

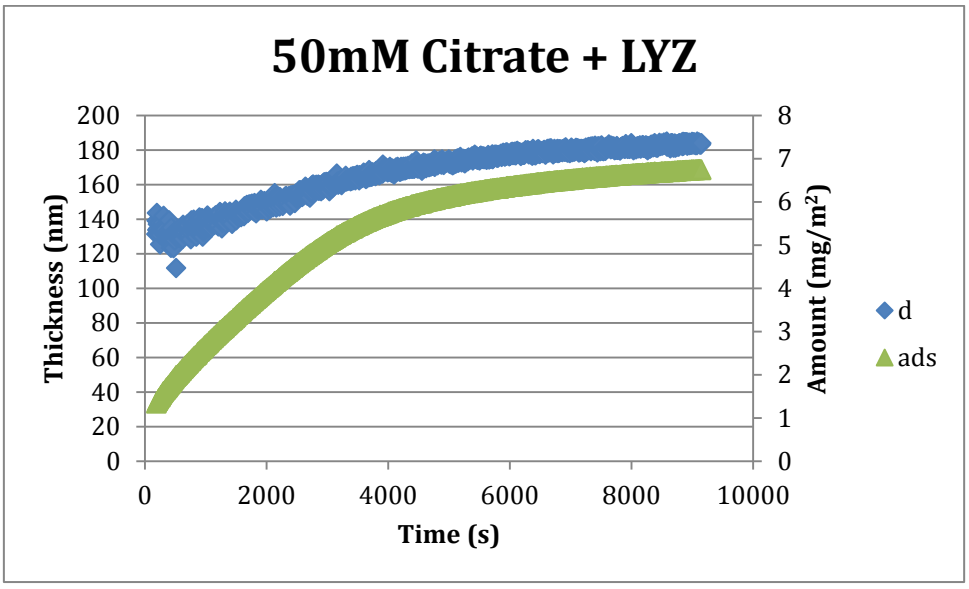
The rinsed amount is barely desorbed.

50mM BUFFER ON A HYDROPHILIC SURFACE



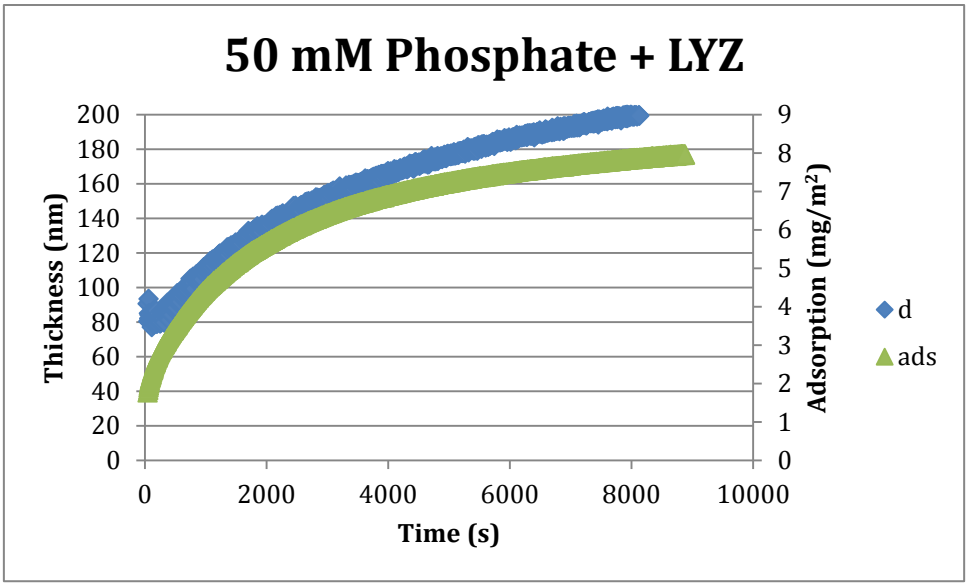
Graph 14. A 4.5 mL of 50mM BES solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

Desorption was not measured.



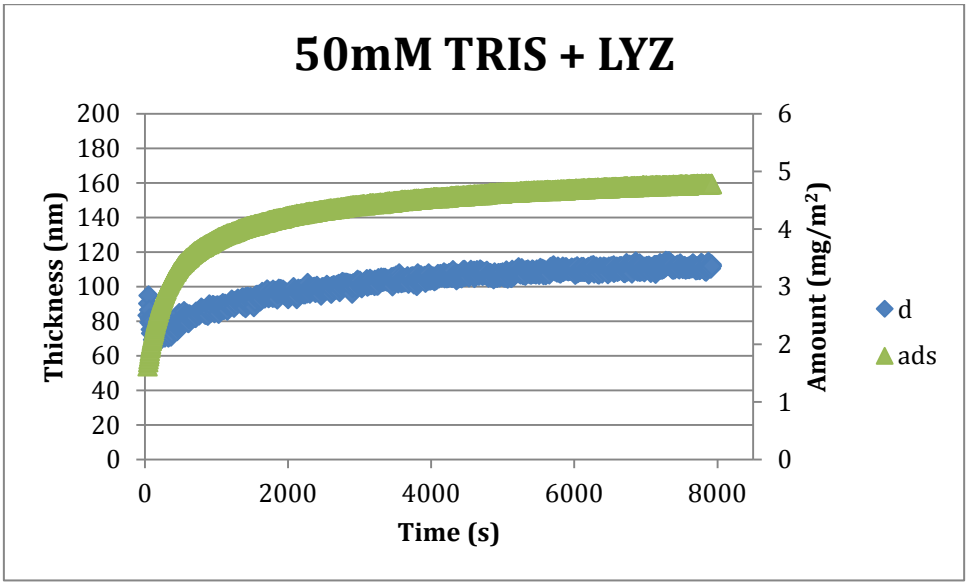
Graph 15. A 4.5 mL of 50mM citrate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

Desorption was not measured.



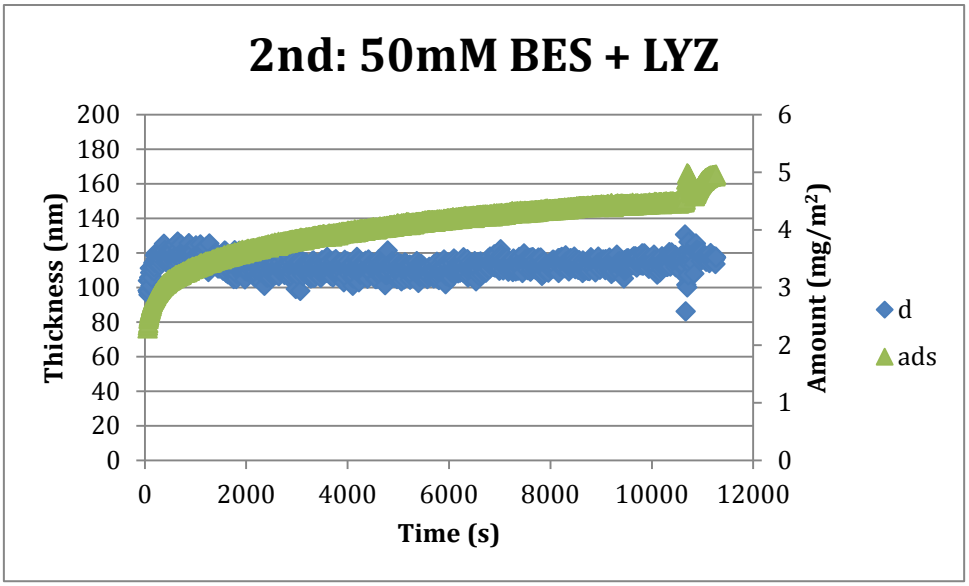
Graph 16. A 4.5 mL of 50mM phosphate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

Desorption was not measured.



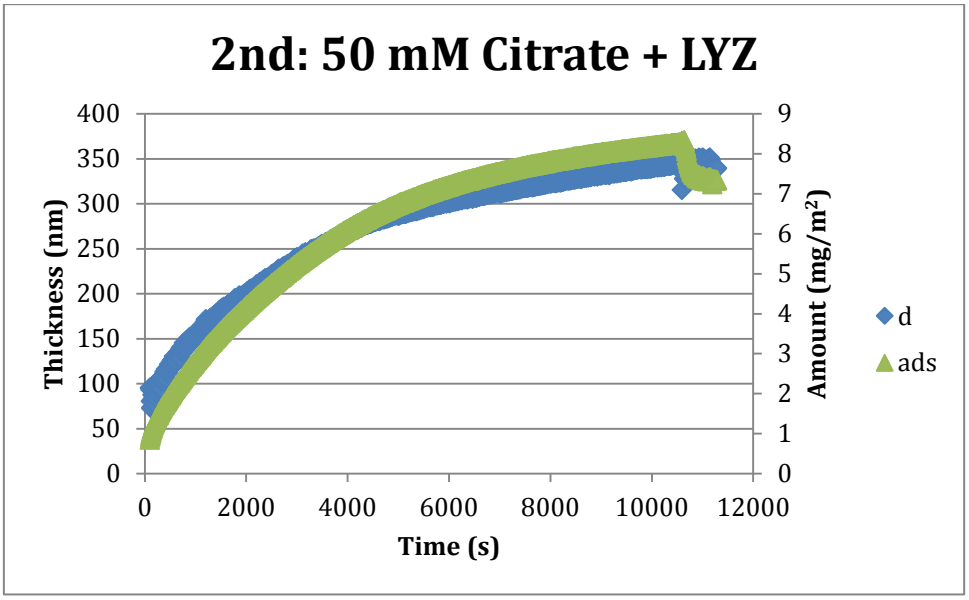
Graph 17. A 4.5 mL of 50mM TRIS solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

Desorption was not measured.



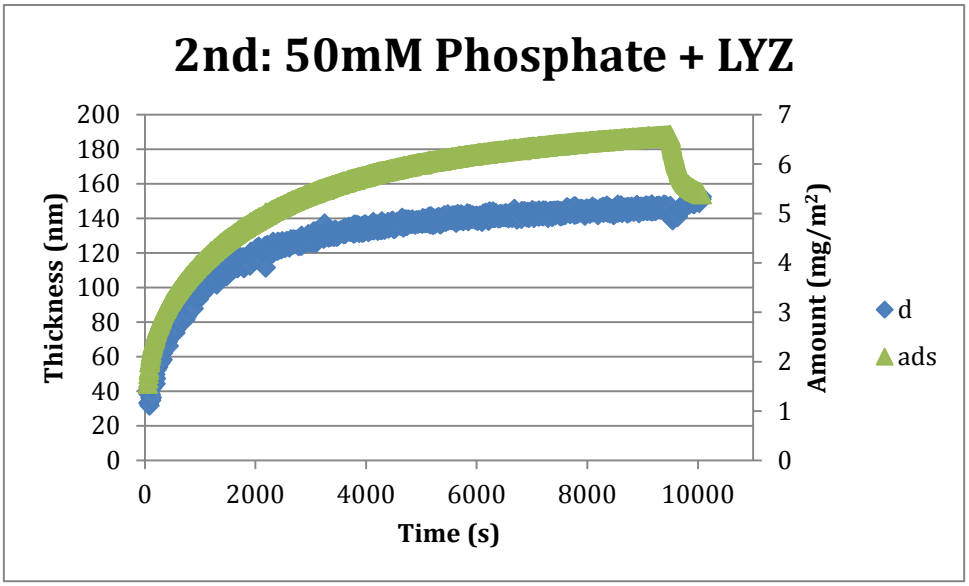
Graph 18.- A 4.5 mL of 50mM BES solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

Note that adsorption increases while rinsing.



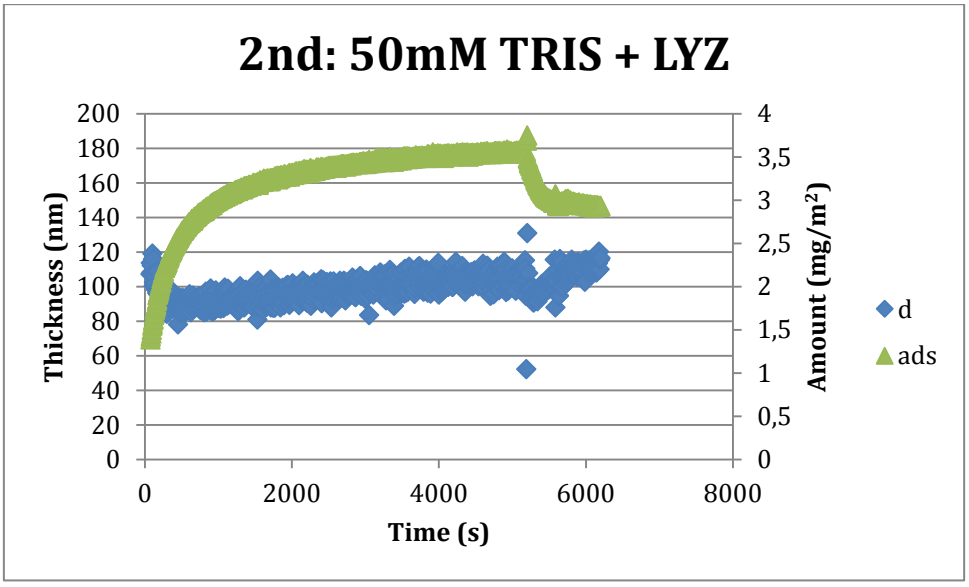
Graph 19. A 4.5 mL of 50mM citrate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 8.25 to 7.25 mg/m2.



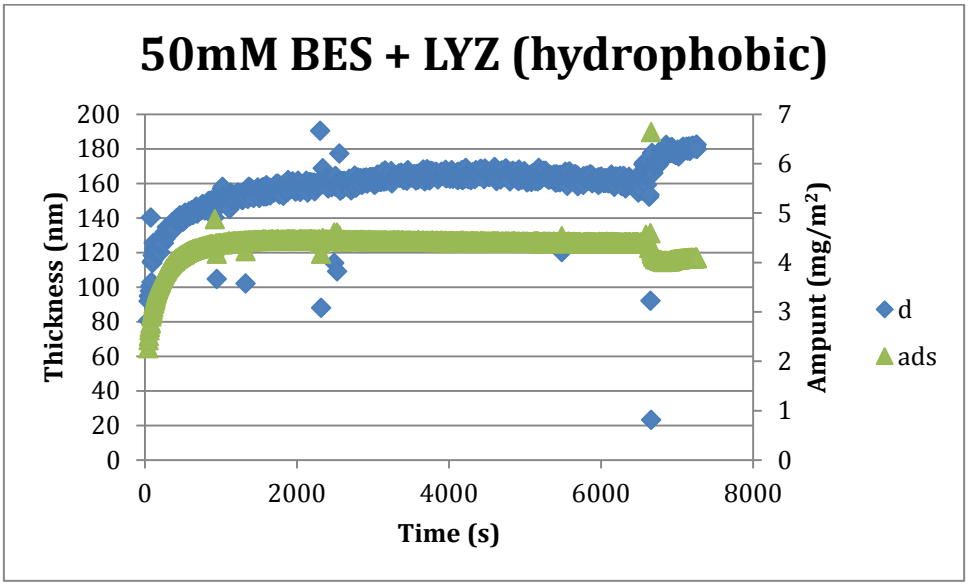
Graph 20. A 4.5 mL of 50mM phosphate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 6.75 to 5.5 mg/m2.



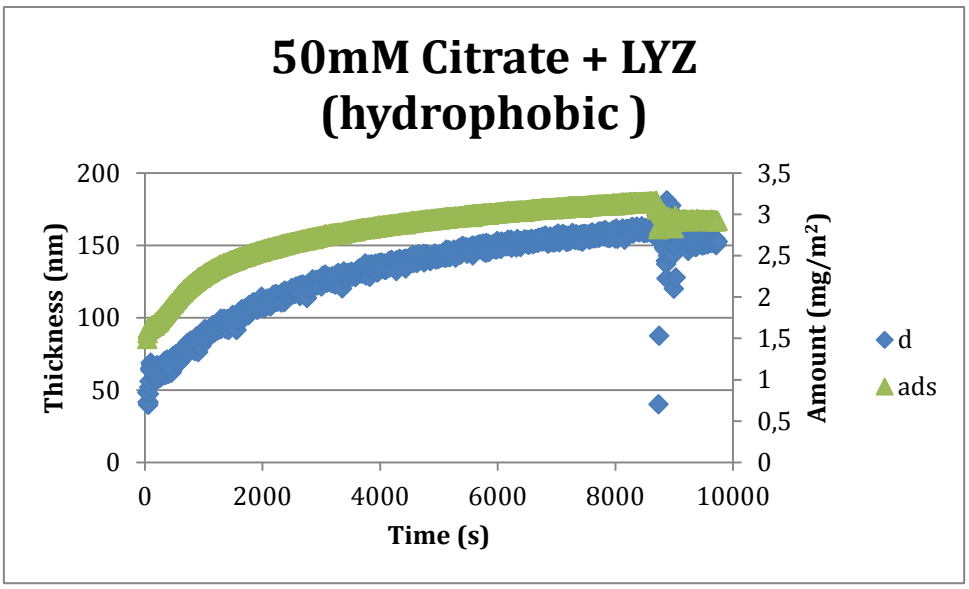
Graph 21. A 4.5 mL of 50mM TRIS solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 3.5 to 2.9 mg/m2.



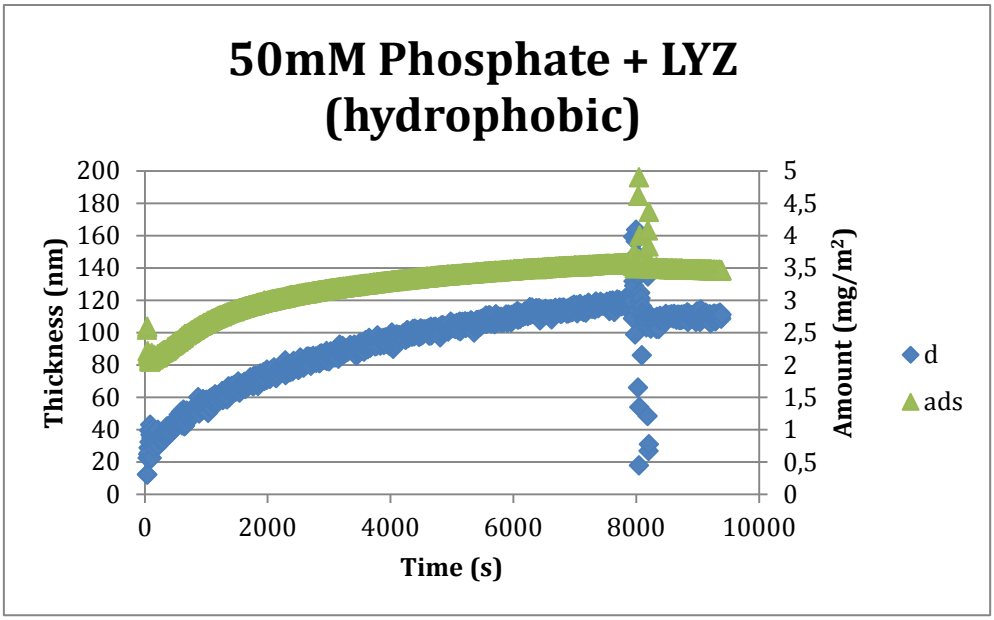
Graph 22. A 4.5 mL of 50mM BES solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized surface.

The adsorbed amount slightly decreases after rinsing the cuvette although thickness increases.



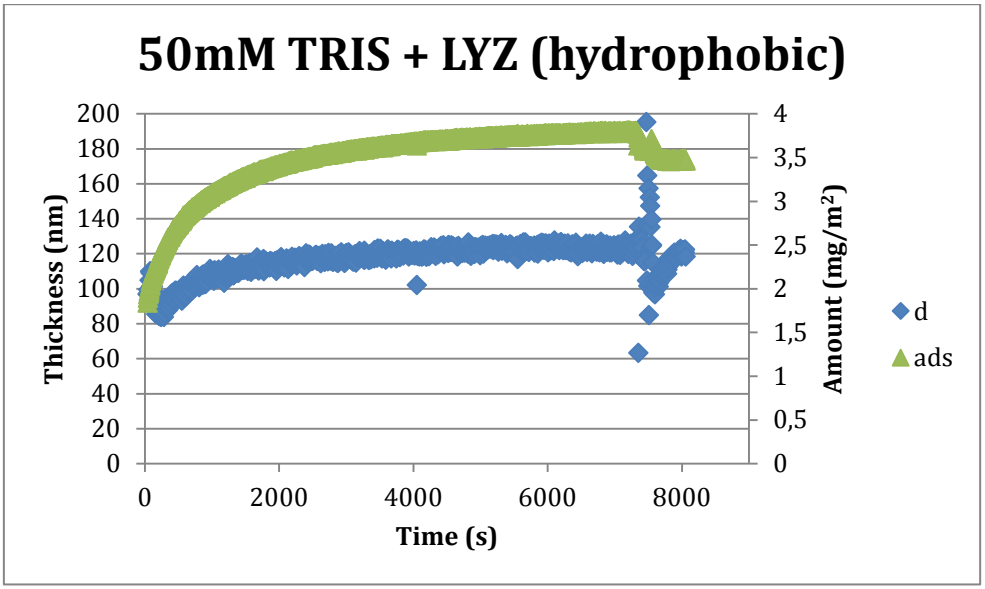
Graph 23. A 4.5 mL of 50mM citrate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized surface.

The adsorbed amount slightly decreases after rinsing the cuvette, so does the thickness.



Graph 24. A 4.5 mL of 50mM phosphate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized surface.

The amount adsorbed does not changes after rinsing, although thickness shrikes.

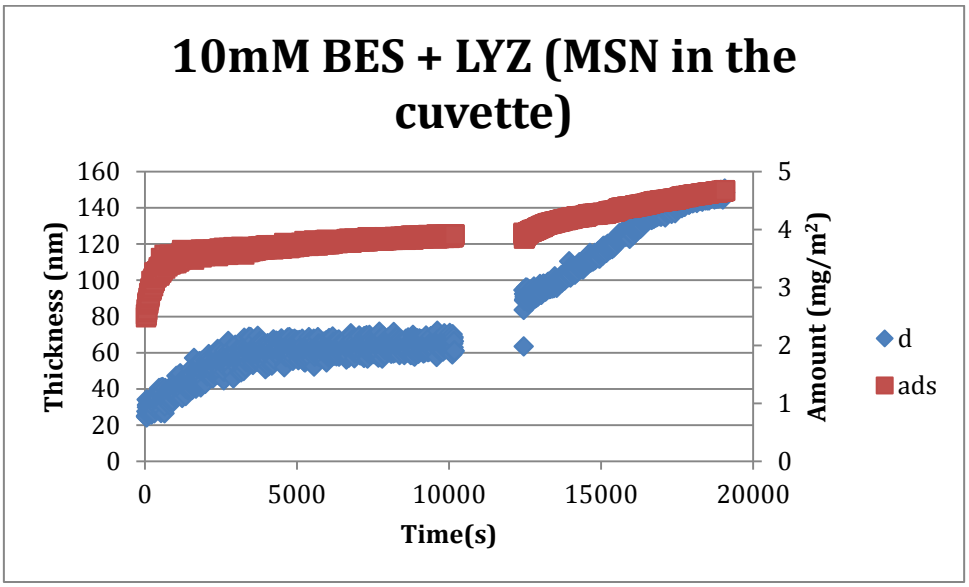


Graph 25. A 4.5 mL of 50mM TRIS solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized surface.

The amount adsorbed decreases from around 3.75 to 3.4 mg/m^2.

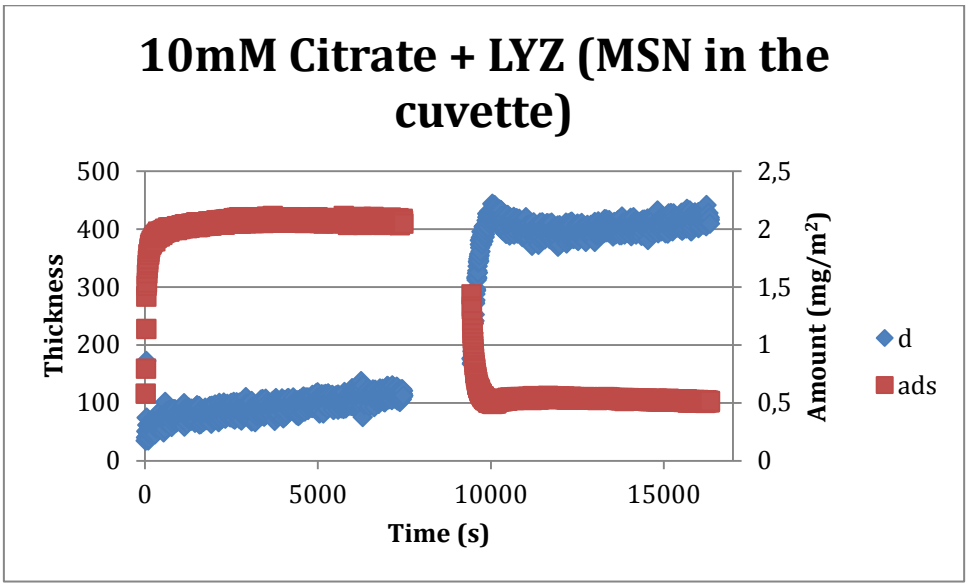
ADSORPTION IN THE PRESENCE OF MSNS IN 10MM BUFFERS:

After the normal lysozyme adsorption, 0.010g of MSN-NH_2 were added and left in the cuvette for 30 minutes and then they were rinsed out for all the following measurements.



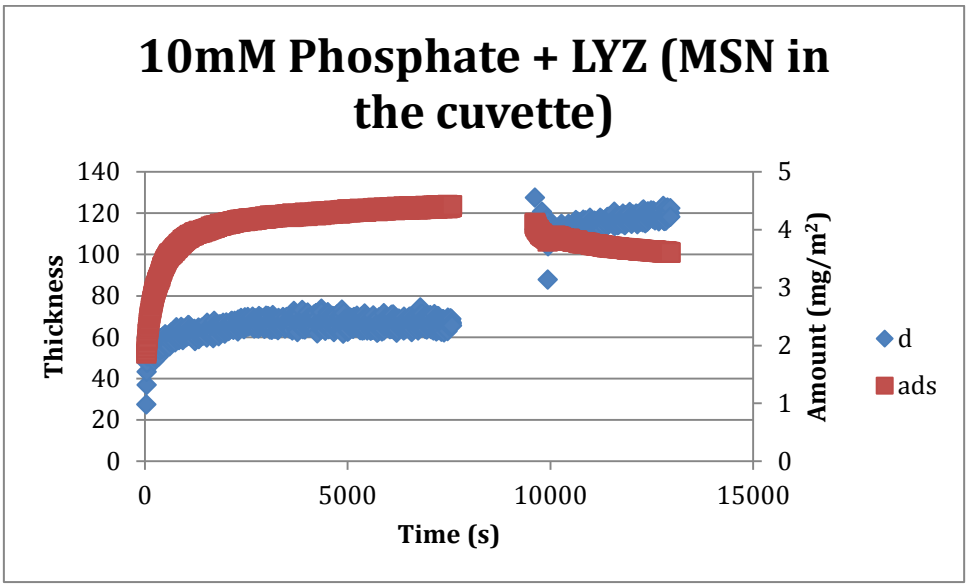
Graph 26. A 4.5 mL of 10mM BES solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The adsorbed amount increases from around 4.0 to 4.75 mg/m^2 after rinsing the cuvette while thickness is significantly increased.



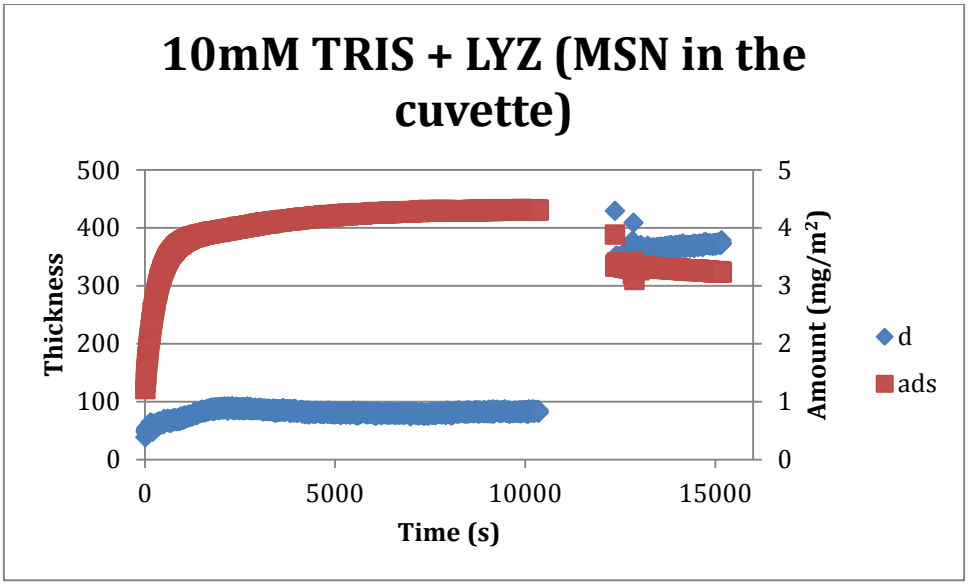
Graph 27. A 4.5 mL of 10mM citrate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 2.1 to 0.5 mg/m^2.



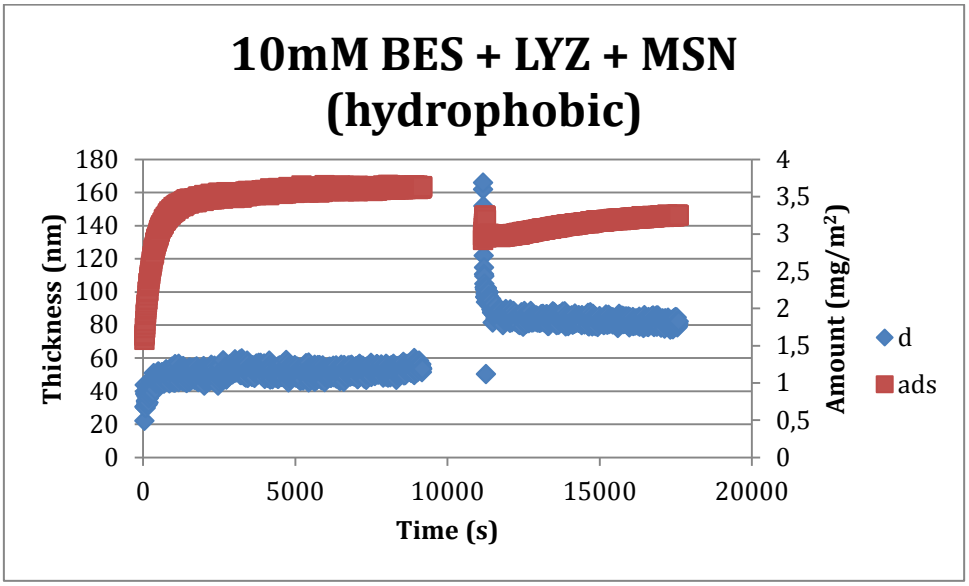
Graph 28. A 4.5 mL of 10mM phosphate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 4.5 to 3.5 mg/m^2.



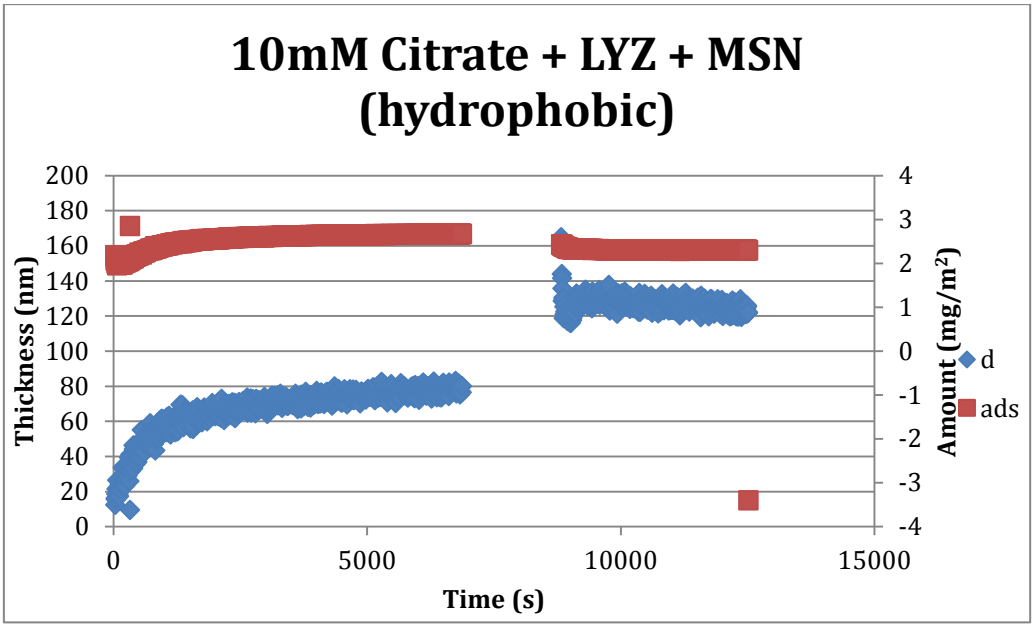
Graph 29. A 4.5 mL of 10mM TRIS solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 4.3 to 3.3 mg/m^2.



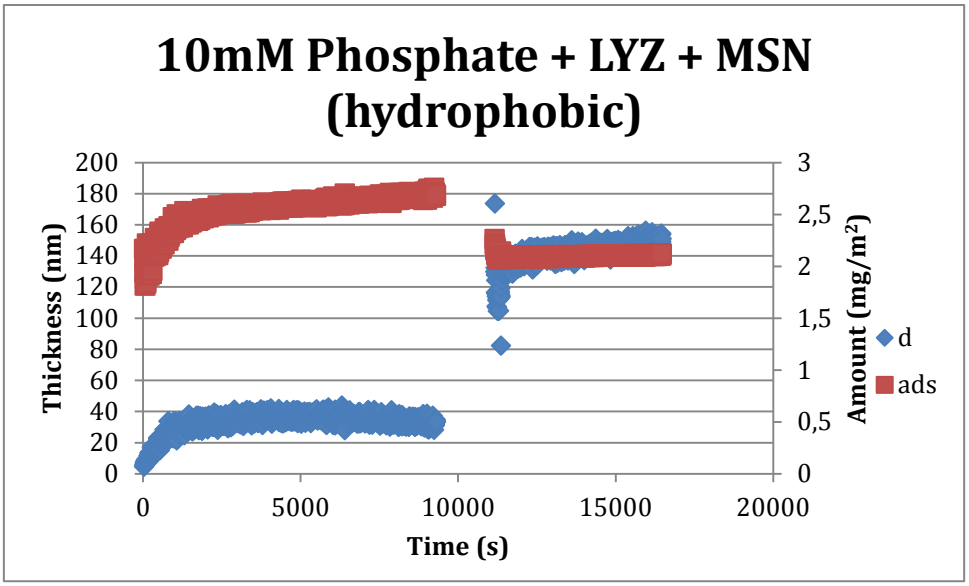
Graph 30. A 4.5 mL of 10mM BES solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface.

The amount is desorbed from around 3.6 to 2.8 mg/m² but then partially readsorbed over time.



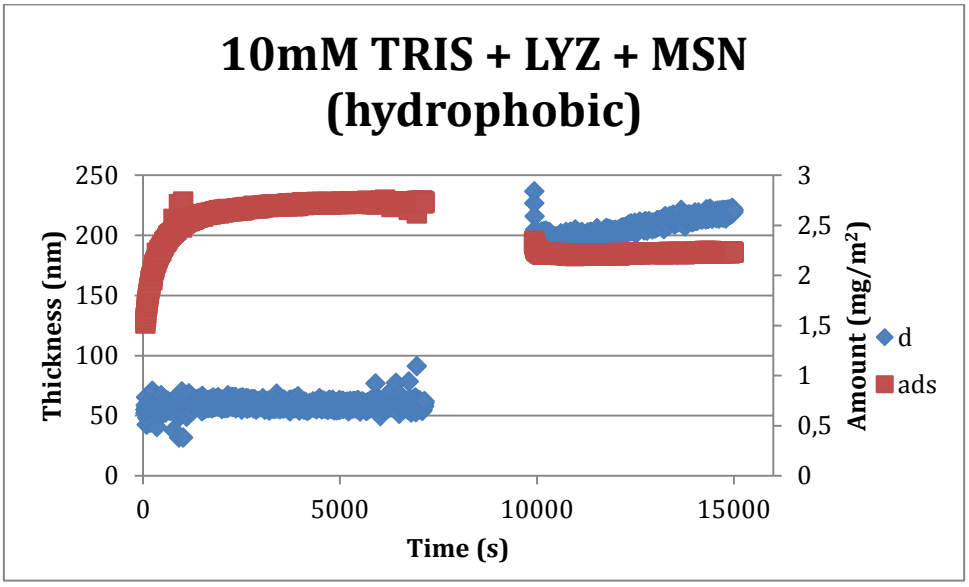
Graph 31. A 4.5 mL of 10mM citrate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface.

The amount is slightly desorbed although thickness increases significantly.



Graph 32. A 4.5 mL of 10mM phosphate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface.

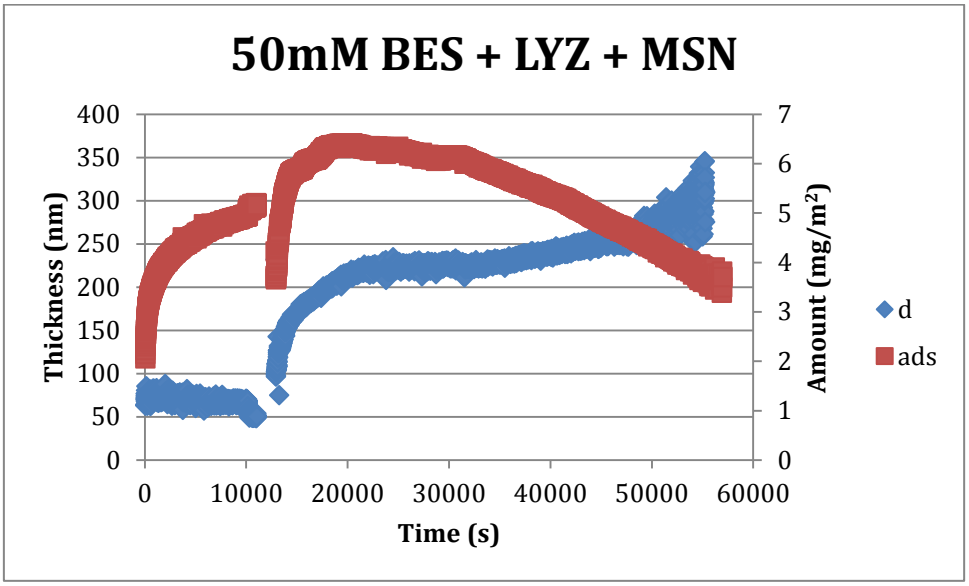
The amount is desorbed from around 2.75 to 2.2 mg/m^2. Thickness increases significantly.



Graph 33. A 4.5 mL of 10mM TRIS solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface.

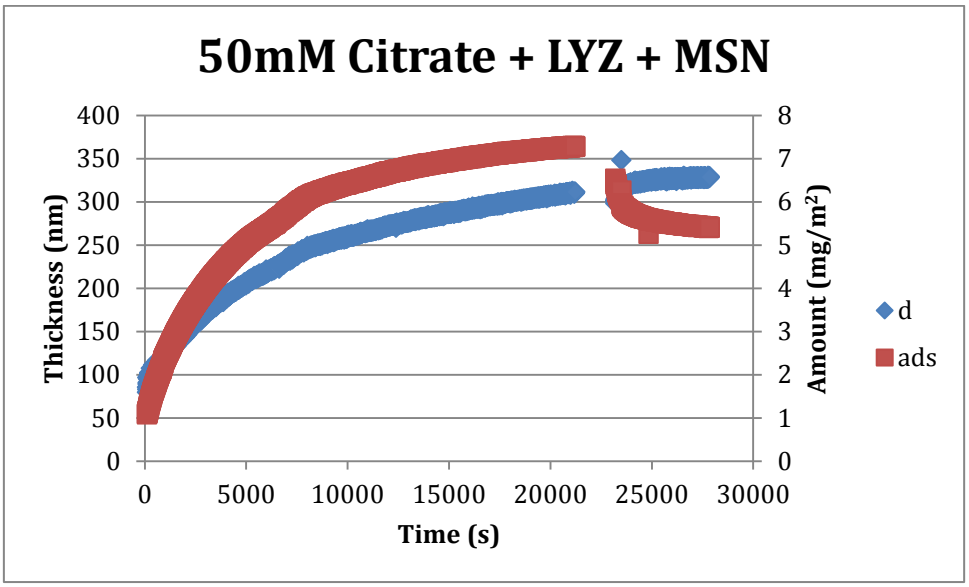
The amount is slightly desorbed from around 2.75 to 2.3 mg/m^2. Thickness increases significantly

ADSORPTION IN THE PRESENCE OF MSNS IN 50MM BUFFERS



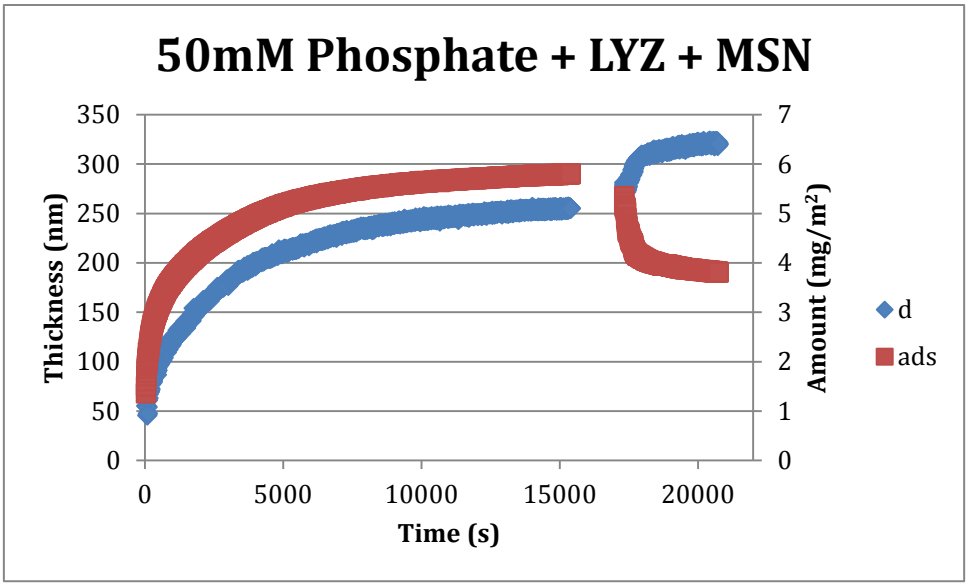
Graph 34. A 4.5 mL of 50mM BES solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The adsorbed amount increases significantly and then it is desorbed over time.



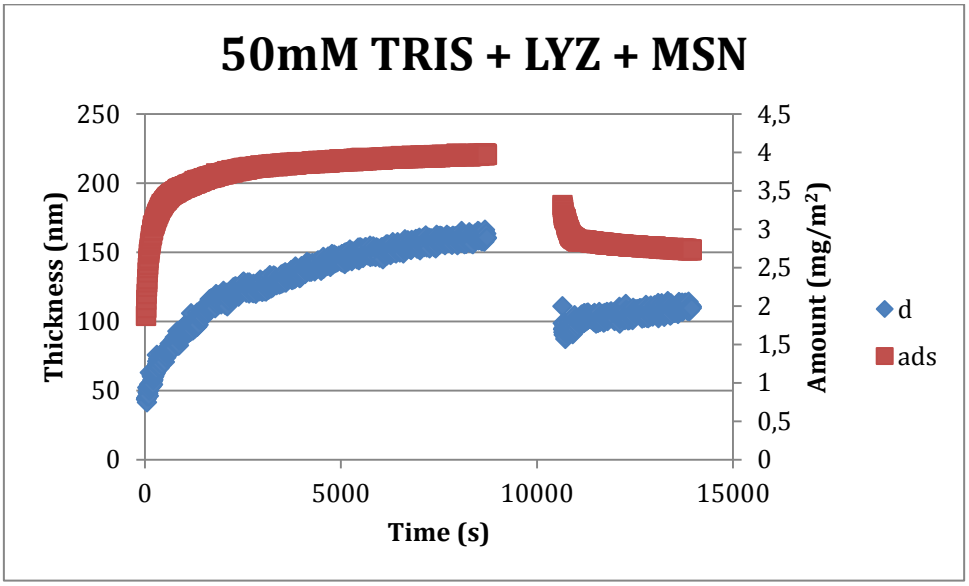
Graph 35. A 4.5 mL of 50mM citrate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 7.0 to 5.5 mg/m^2. Thickness slightly increases.



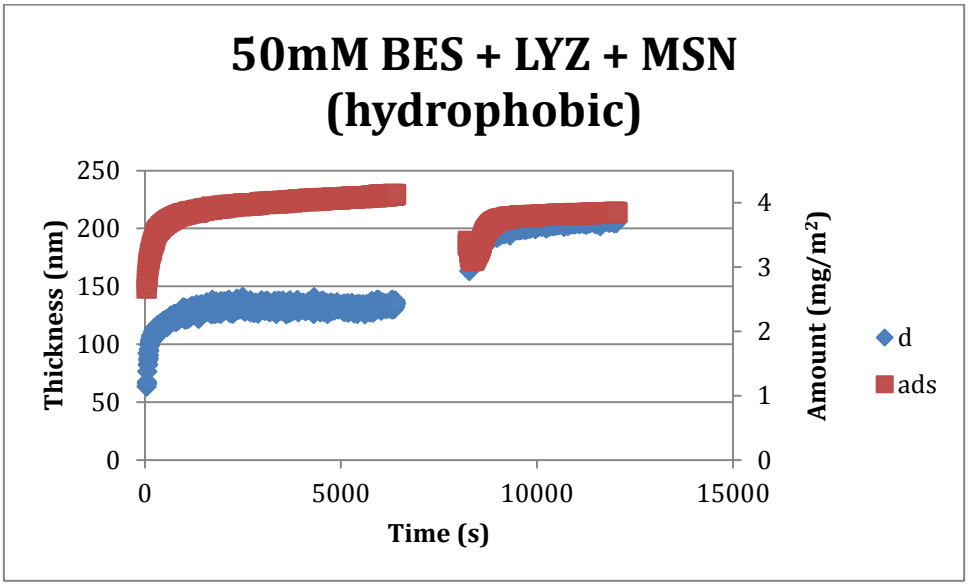
Graph 36. A 4.5 mL of 50mM phosphate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 6.0 to 4.0 mg/m^2. Thickness increases.



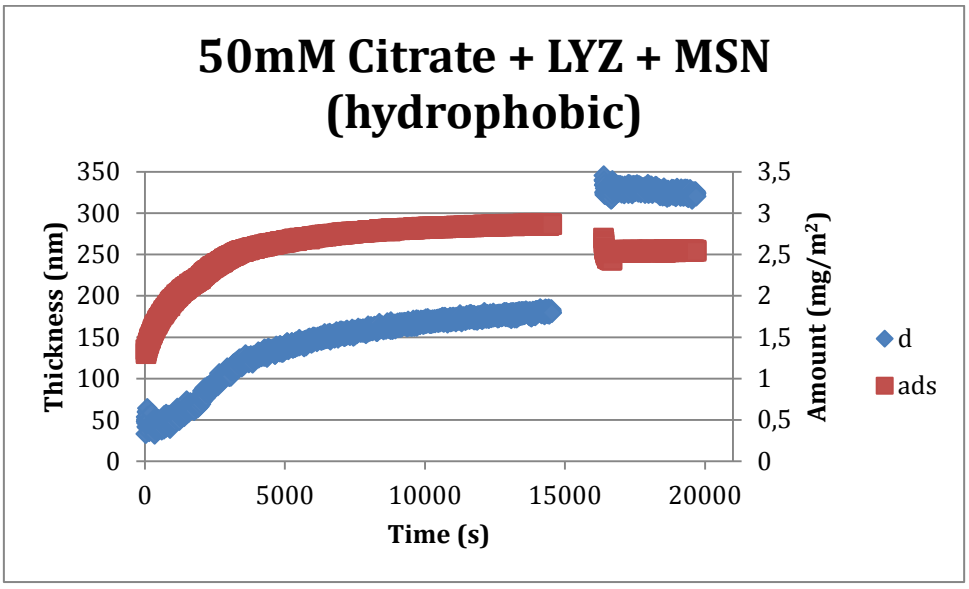
Graph 37. A 4.5 mL of 50mM TRIS solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry.

The amount is desorbed from around 4.0 to 2.6 mg/m^2. Thickness decreases significantly.



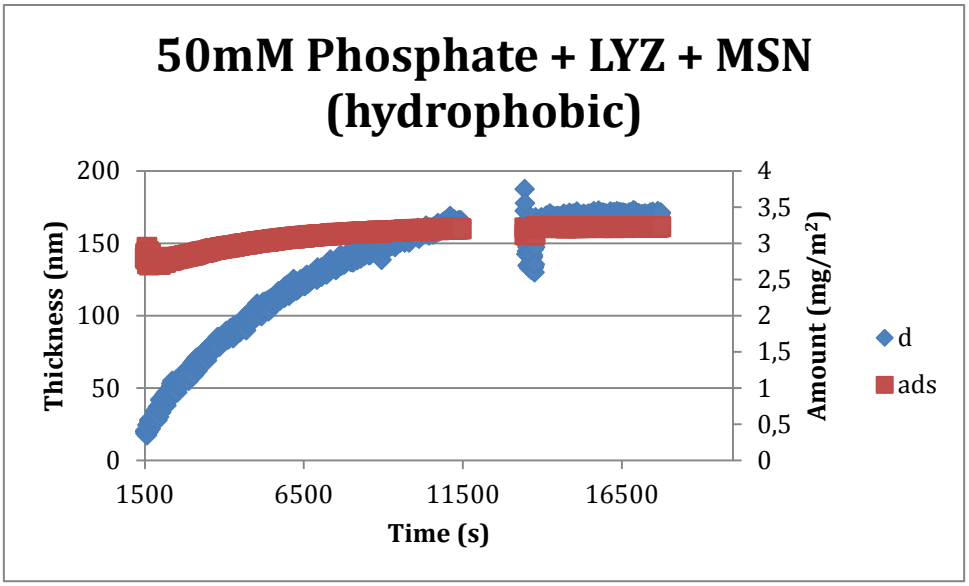
Graph 38. A 4.5 mL of 50mM BES solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface.

The amount is slightly desorbed. Thickness decreases significantly.



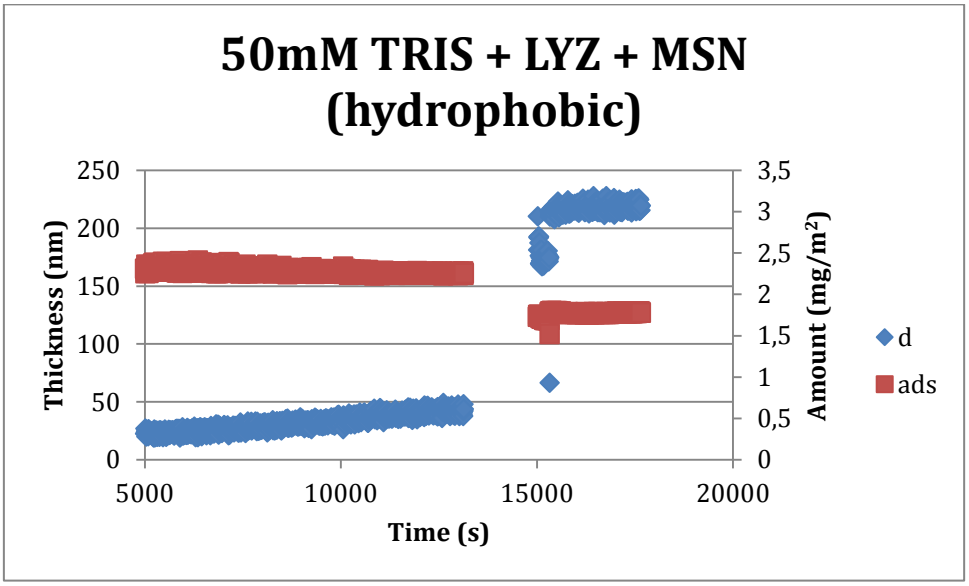
Graph 39. A 4.5 mL of 50mM citrate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface

The amount is slightly desorbed. Thickness decreases significantly.



Graph 40. A 4.5 mL of 50mM phosphate solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface.

The amount and thickness are barely desorbed.



Graph 41. A 4.5 mL of 50mM TRIS solution and 0.5 mL of 10mg/mL lysozyme were measured through ellipsometry on a silanized silica surface.

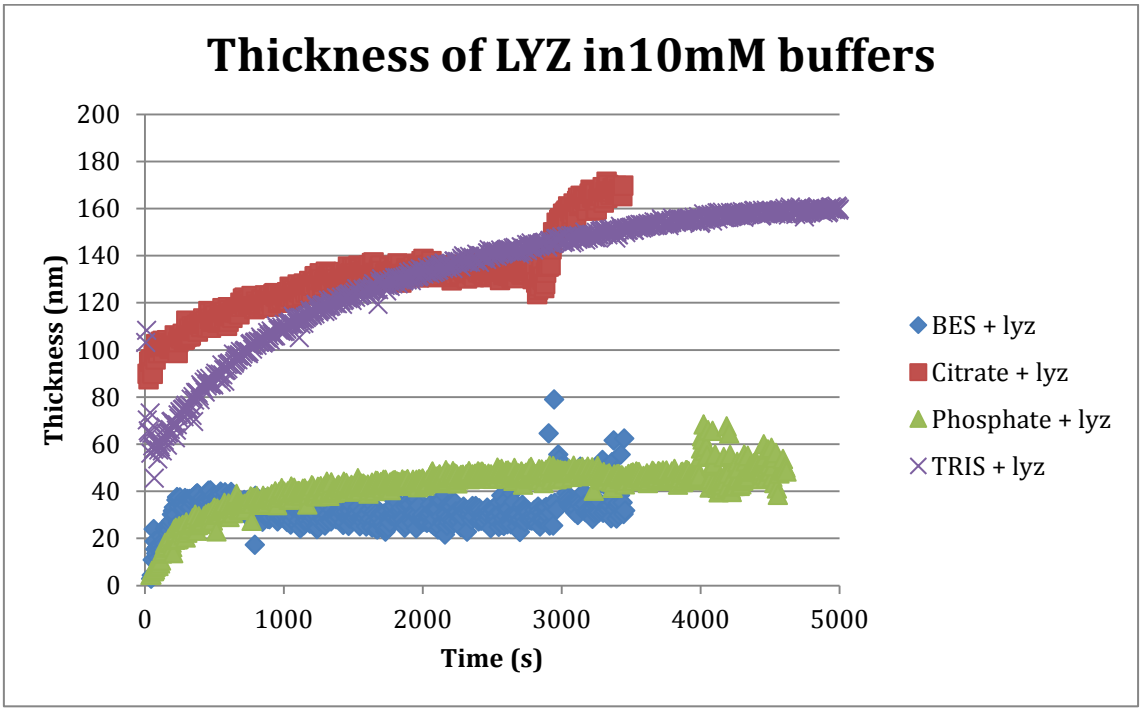
Thickness layer increased significantly and the amount was desorbed from around 2.1 to 1.8 mg/m^2.

6.0 Discussion

In this section, the previous results are compared between the same buffer's characteristics.

BES buffer is represented in blue, citrate in red, phosphate in green and TRIS in purple.

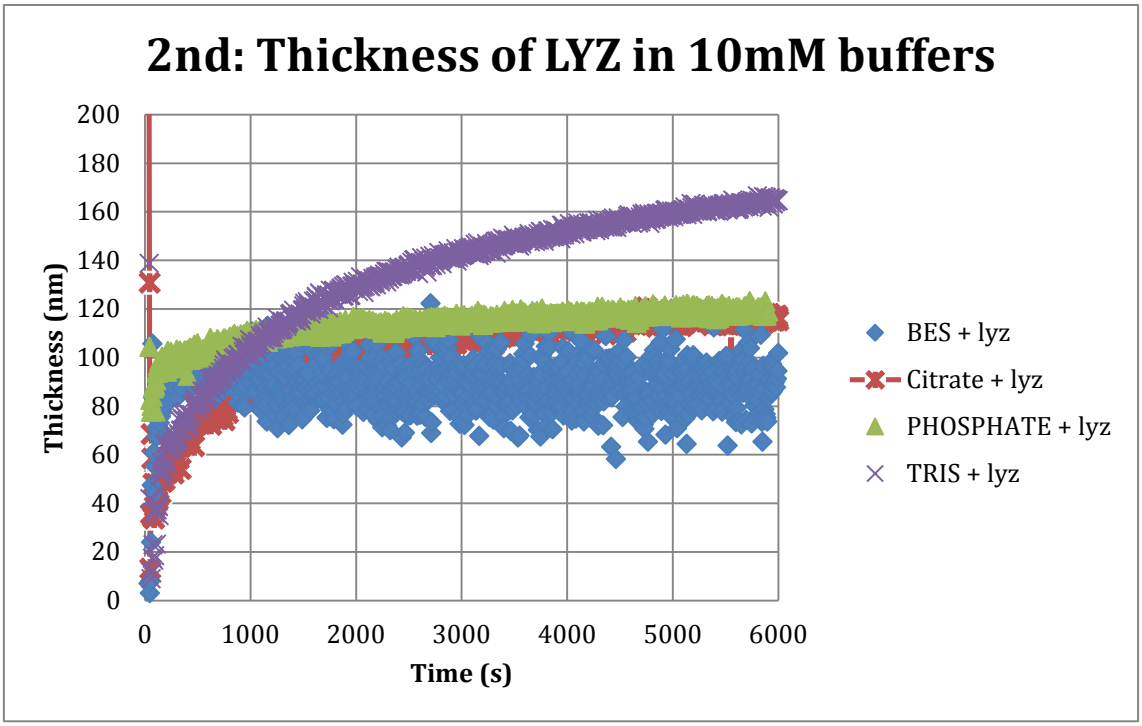
10mM buffers with lysozyme



Graph 42. The thicknesses of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

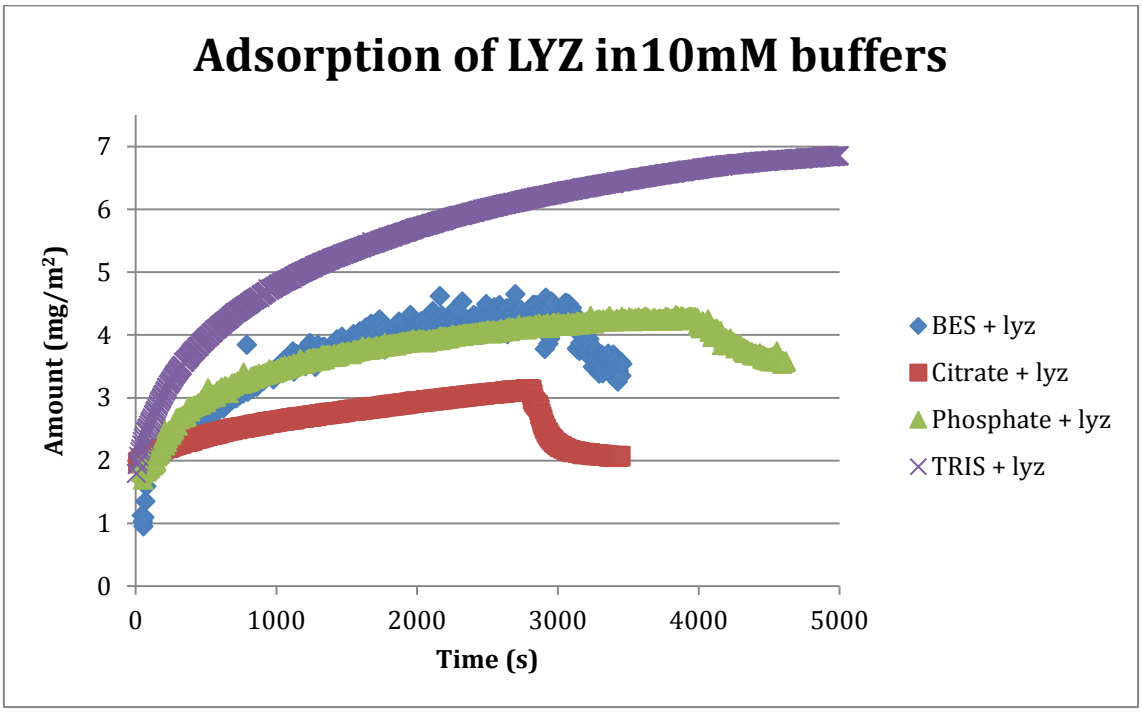
As those were the first measurements ever taken, they have been repeated:

Desorption was not measured for the TRIS buffer. In 10mM concentration, citrate and TRIS seem to have a bigger lysozyme layer.



Graph 43. The thicknesses of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

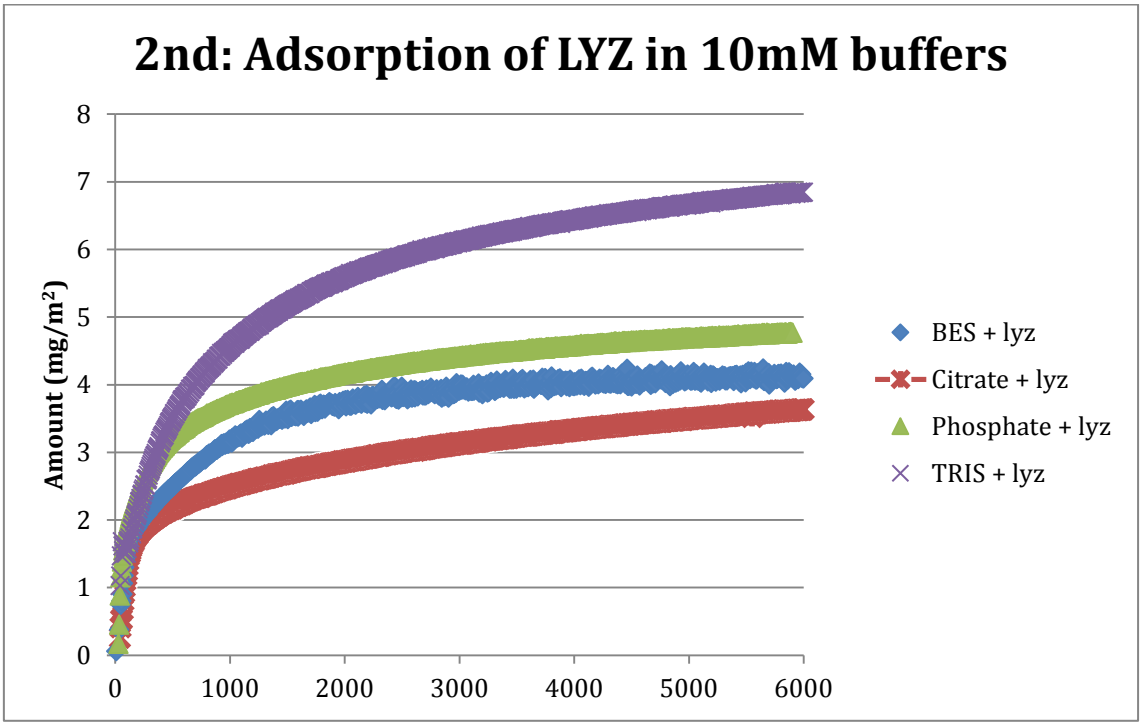
Those are the second measurements of the same solutions as the previous graph. Values in the BES solution are noisy because of the stirring.



Graph 44. The adsorptions of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

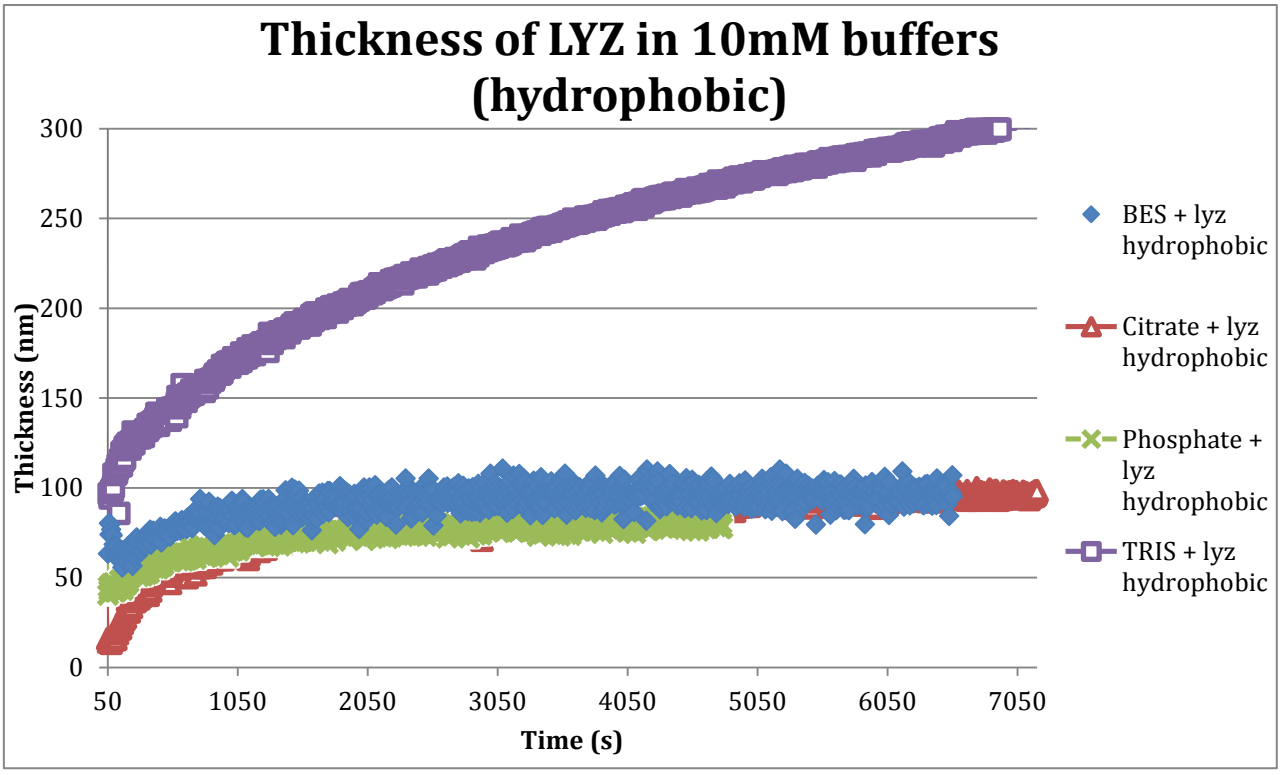
As those were the first measurements ever taken, they have been repeated. Desorption has been monitored.

Lysozyme adsorption decreases: TRIS>BES>Phosphate>Citrate

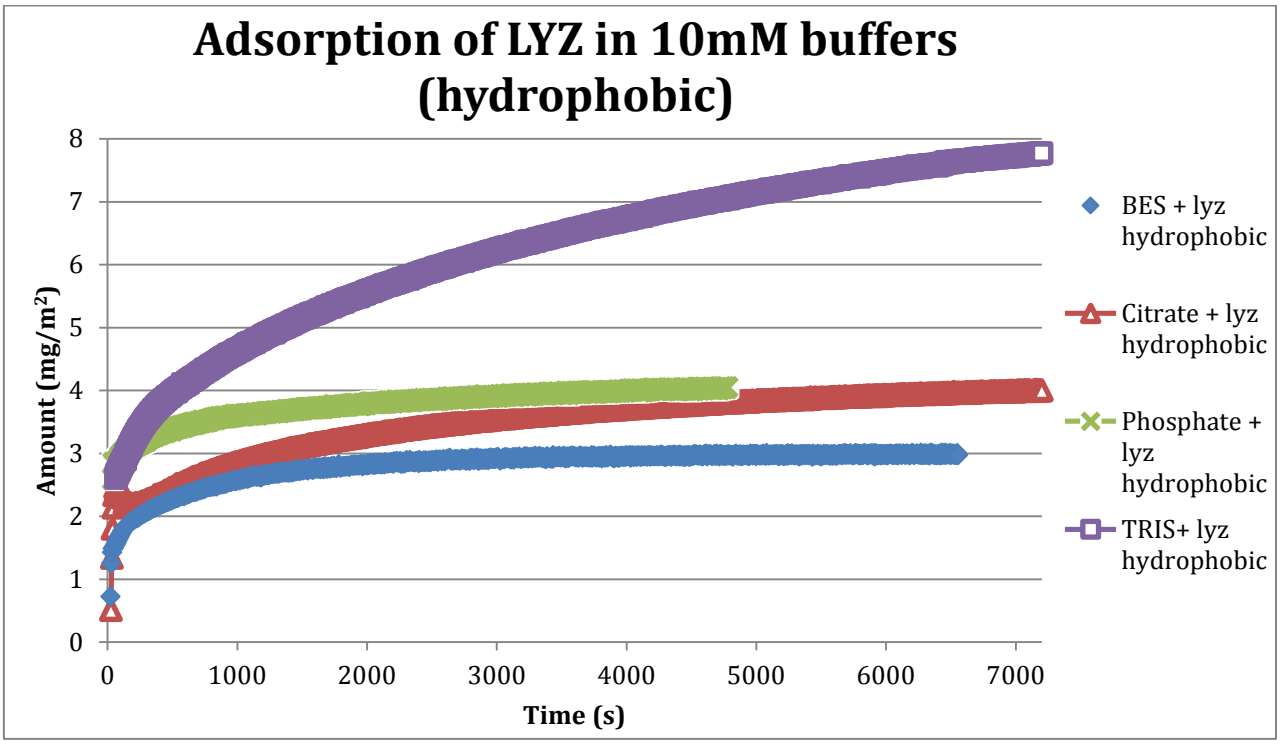


Graph 45. The adsorptions of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

Those are the second measurement of the same solutions as the previous graph without desorption.

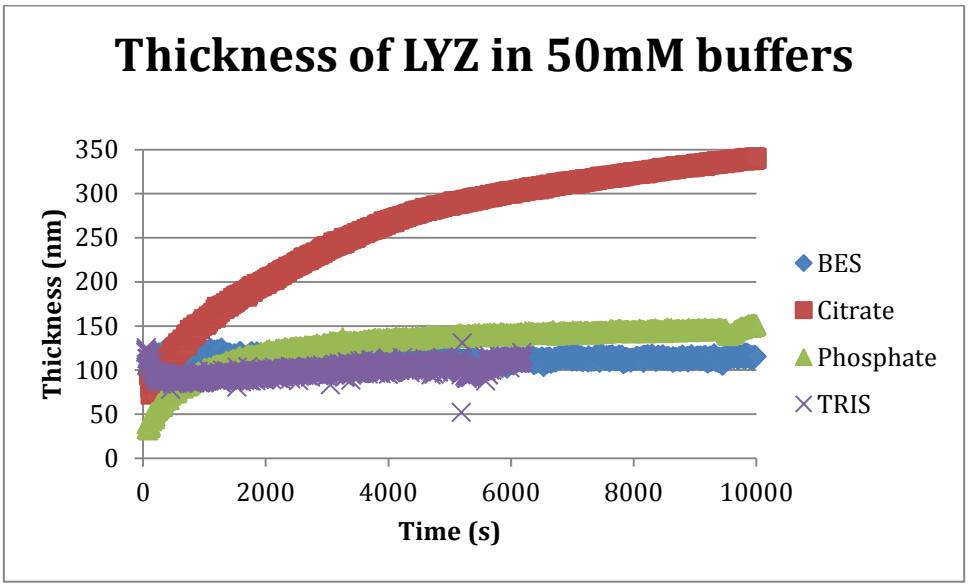


Graph 46.. The thicknesses of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized silica surface are compared.

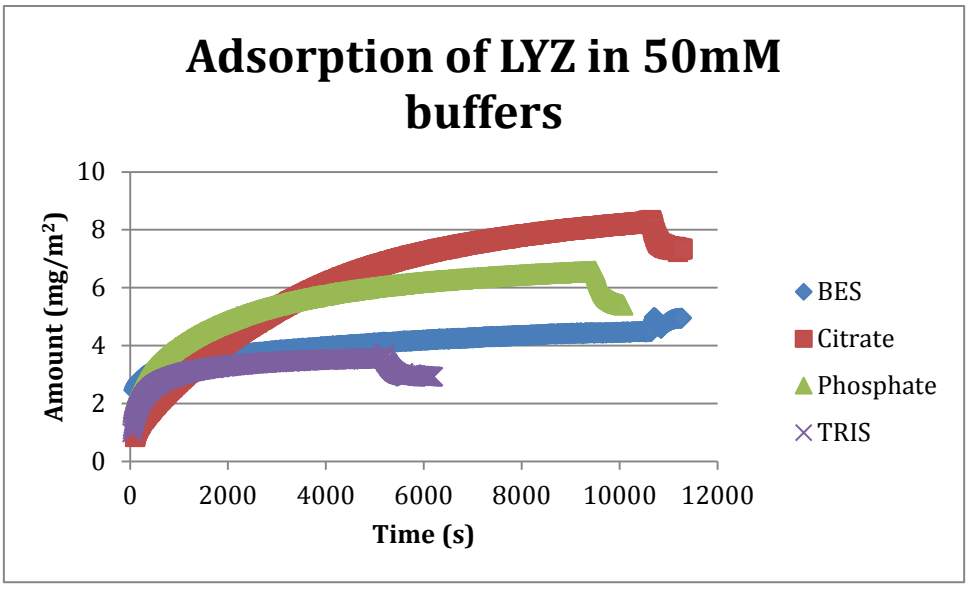


Graph 47. The adsorptions of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized silica surface are compared.

Lysozyme adsorption decreases in: TRIS>Phosphate>Citrate>BES

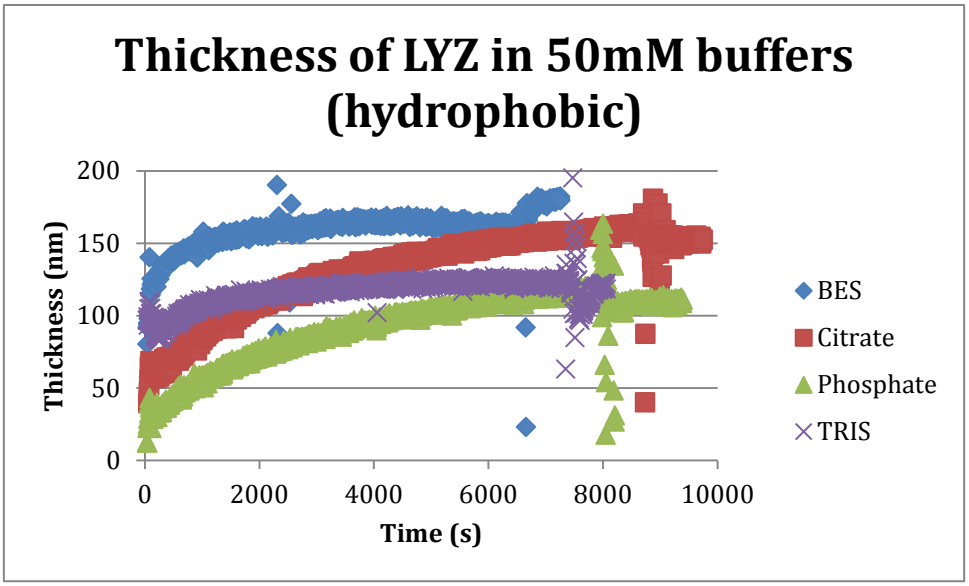


Graph 48. The thicknesses of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

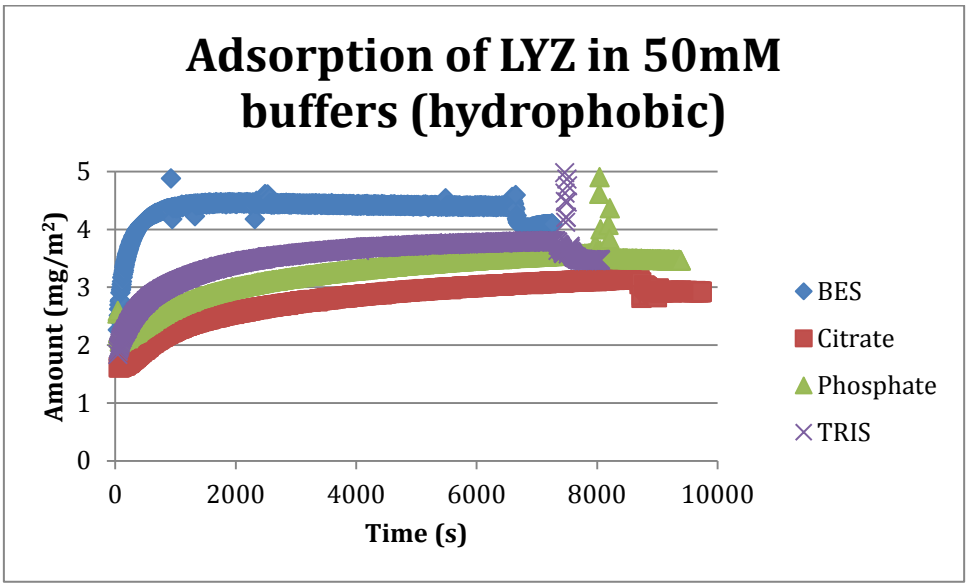


Graph 49. The adsorptions of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared

Lysozyme adsorption decreases in: Citrate>Phosphate>BES>TRIS



Graph 50. The thicknesses of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized surfaces are compared.

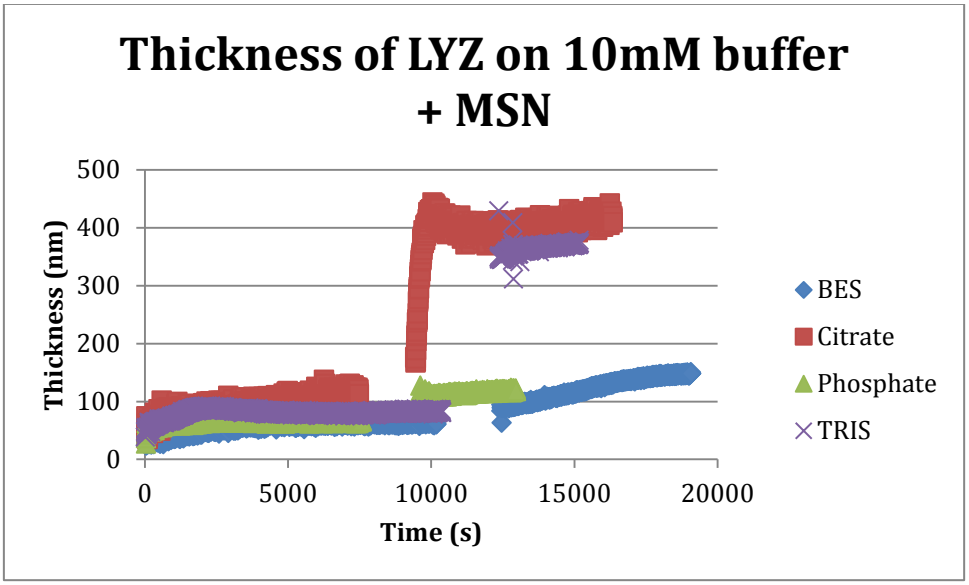


Graph 51. The adsorptions of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized surfaces are compared.

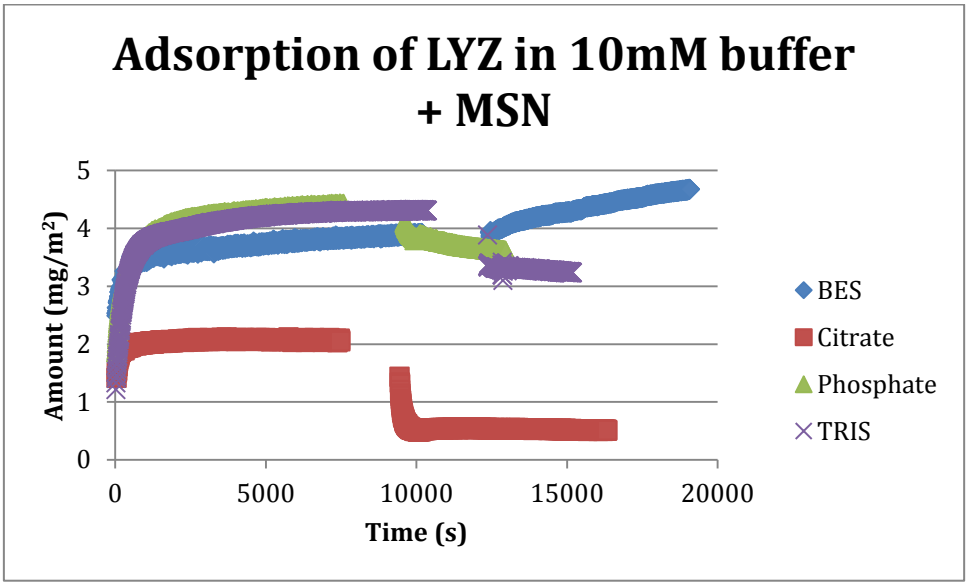
Lysozyme adsorption decreases in: BES>TRIS>Phosphate>Citrate

Here lysozyme adsorption has been revealed from each kind of buffers salts on silica surfaces as well as hydrophobized silica surfaces.

The discontinuation in the graph is due to the rinsing out process.

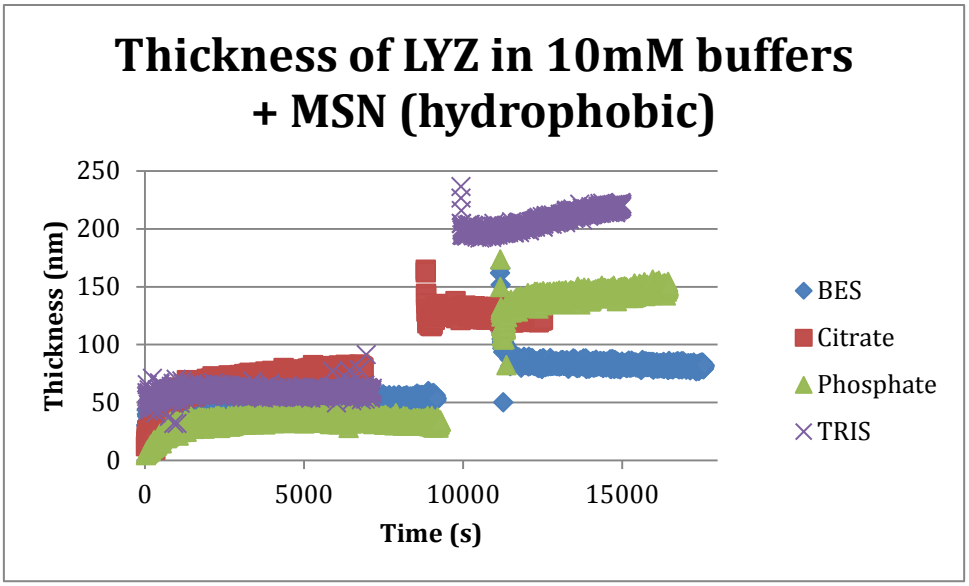


Graph 52. The thicknesses of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

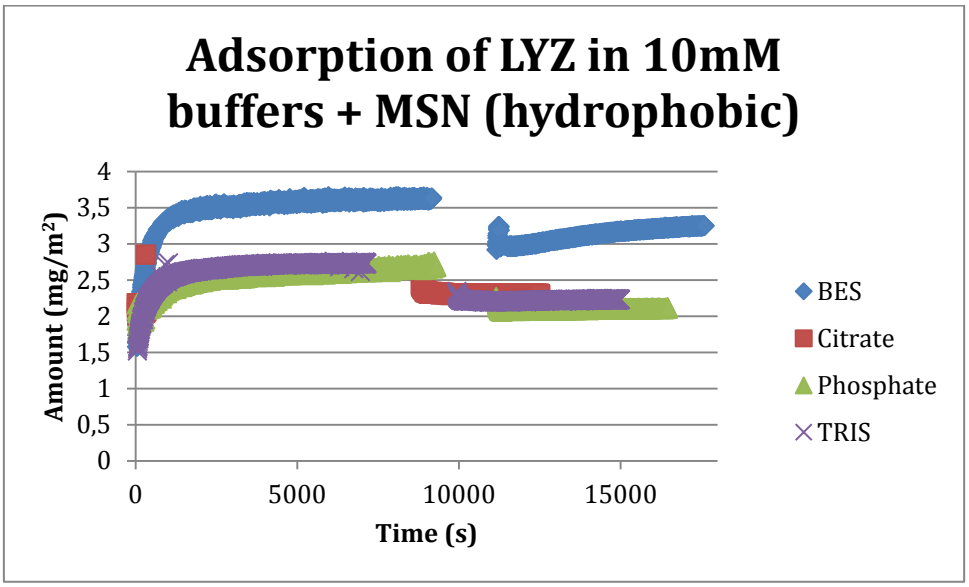


Graph 53. The adsorptions of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared. After the adsorption, 0.010g of MSN-NH_2 were added and left in the cuvette for 30 minutes and then these were rinsed out. The discontinuation in the graph is due to the rinsing out process.

The addition of MSNs have a strong effect on lysozyme desorption from its surface.

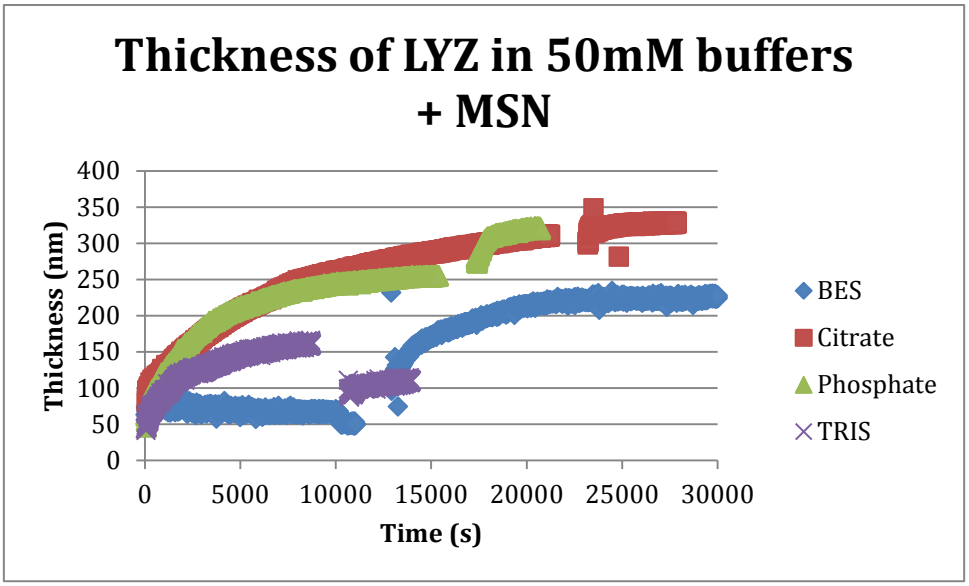


Graph 54. The thicknesses of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized silica surface are compared.

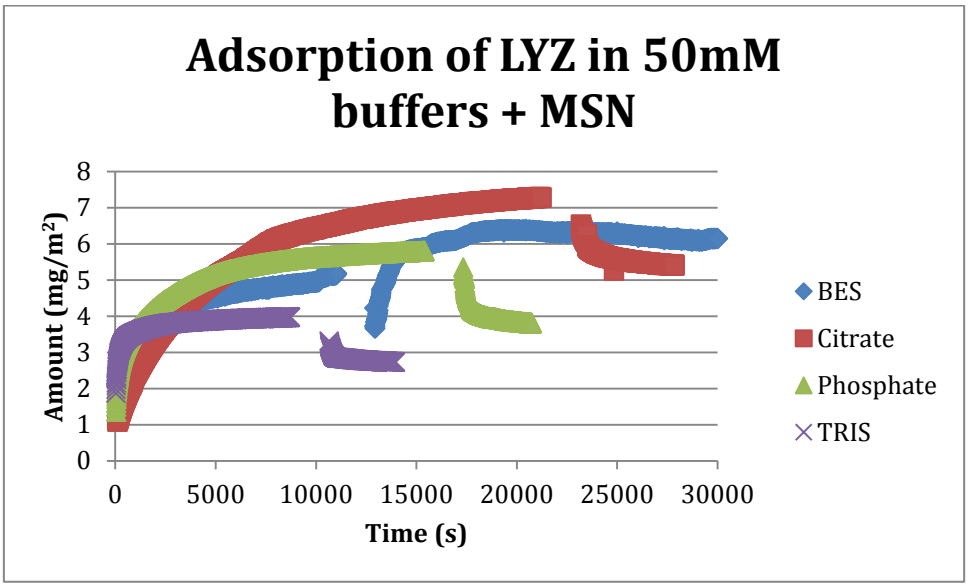


Graph 55. The adsorptions of all 10mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized silica surface are compared.

Adsorption values on hydrophobized silica surfaces are lower with opposite interactive effects.

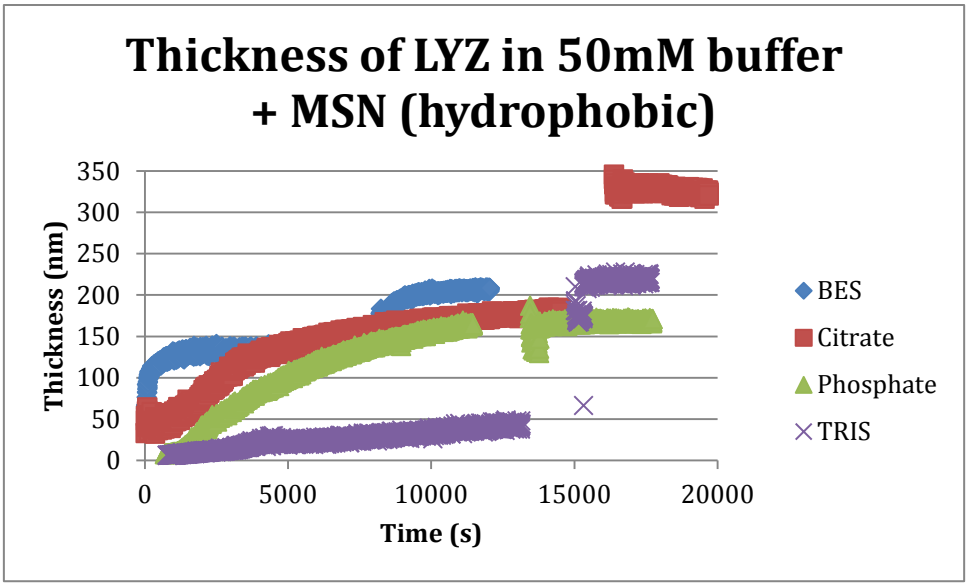


Graph 56. The thicknesses of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

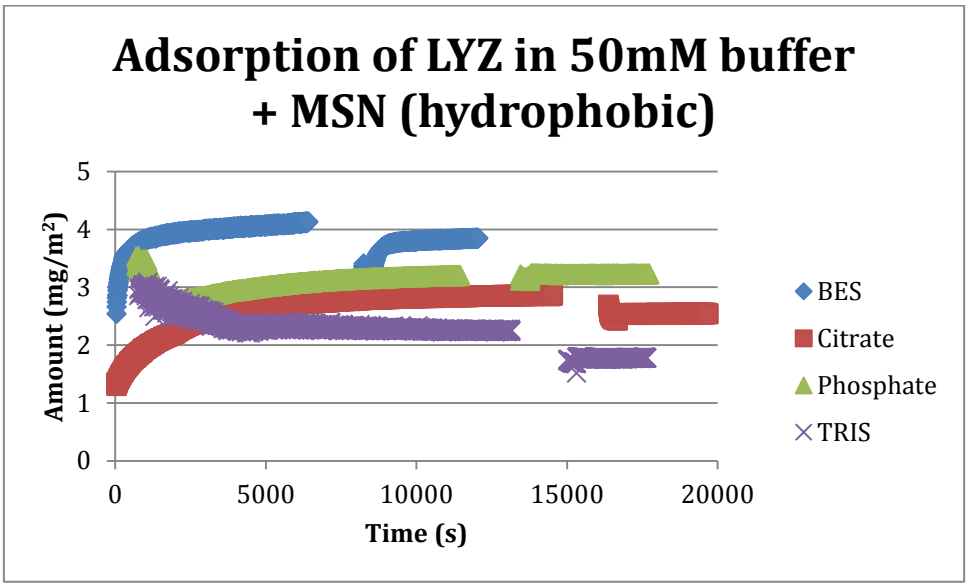


Graph 57. The adsorptions of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme are compared.

Adsorption from 50mM buffers in the presence of MSNs is higher and more affected by the nanoparticles.



Graph 58. The thicknesses of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized silica surface are compared.



Graph 59. The adsorptions of all 50mM buffer solutions with 0.5 mL of 10mg/mL lysozyme on a silanized silica surface are compared.

It is observed that adsorption from 50 mM buffers in the presence of MSNs on hydrophobized silica surfaces is lower than on hydrophilic silica surfaces.

6.0 CONCLUSIONS

Light scattering data was not conclusive due to macro-aggregations of suspended particles or dust related issues. In the appendices, there are evidences showing proper results in size/number data, however, the values taken through DLS take also into consideration size/intensity data which made the averages incorrect.

Ellipsometric values show a high reproducibility between the same set of measurements taken at different times.

Thicknesses layers are usually slimmer when a hydrophobized surface is being used.

The BES solution presents a totally different behavior regarding lysozyme desorption. After exchanging 20 time the cuvette volume for rinsing, instead of desorbing, it keeps adsorbing,

At a lower concentration (10mM), BES and TRIS solutions seem to have a higher lysozyme absorbance due to their molecular properties. On hydrophobized silica surfaces, these results relatively change; TRIS solution presents the highest adsorbance and BES the lowest. The citrate and phosphate solution quite show the same behavior.

In 10mM buffers, MSNs affect the lysozyme desorption of all buffers considerably but in the citrate buffer is most affected. On the hydrophobized surfaces, they all show a similar behavior except that the adsorbance is the highest in BES solution.

At higher concentrations (50mM), citrate and phosphate solutions present the highest lysozyme adsorptions. On hydrophobized silica surfaces, the amount adsorbed is generally lower and all buffers show similar values although BES has the highest adsorbance.

In 50mM buffers, MSNs play even a stronger effect on desorption. However, this strong effect is not shown on hydrophobized surfaces.

Thus, MSN-NH_2 have a large effect on lysozyme desorption in any buffer. This is due to the formation of a protein corona which its surface allows the detachment from the silica surface.

From my point of view, further studies should also be conducted regarding the addition of other salts into each buffer.

At a personal level, I have leant many things at Lund University regarding new physical chemistry techniques, organization and general safety.

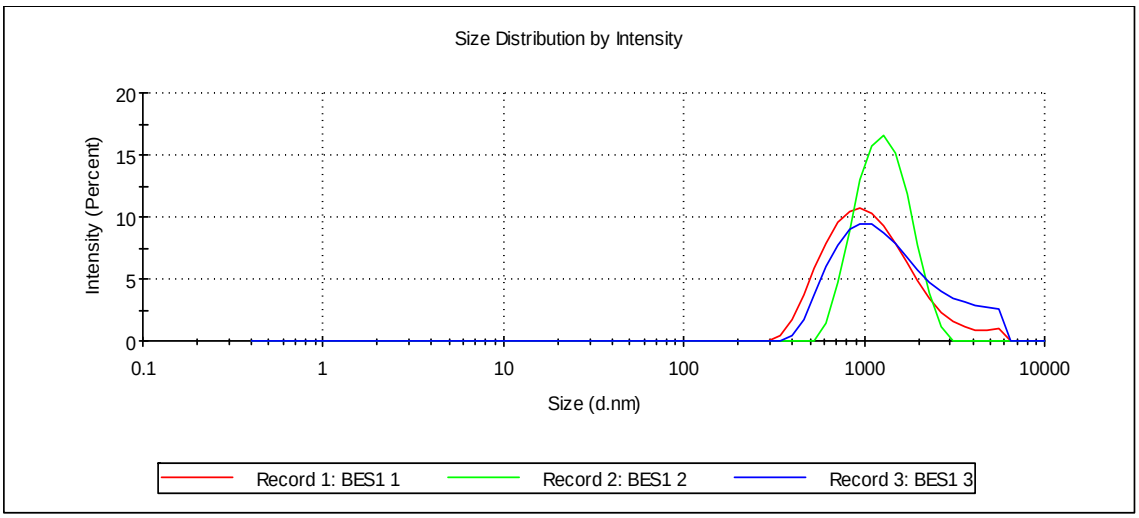
7. REFERENCES

1. V. Nairi et al. Interactions between bovine serum albumin and mesoporous silica nanoparticles functionalized with biopolymers*. Chemical Engineering Journal.* Vol. 340, 15 May 2018, 42-52
2. F. Cugia et al. Are specific buffer effects the new frontier of Hofmeister phenomena? Insights from lysozyme adsorption on ordered mesoporous silica*. RSC Adv.***,** 2016,**6**, 94617-94621
3. T. Arnebrant and T. Nylander. Ellispometry as a tool for in situ studies on adsorption. *Departement of Food Technology, Univeristy of Lund*. Box 124. 22240 Lund, Sweden.
4. Debby P. Chang et al. Adsorption of Lipid Liquid Crystalline Nanoparticles on Cationic, Hydrophilic, and Hydrophobic Surfaces*. ACS Appl. Mater. Interfaces* 2012, 4, 2643−2651
5. Nguyen, V. H., & Lee, B. J. (2017). Protein corona: a new approach for nanomedicine design. *International journal of nanomedicine*, *12*, 3137–3151.
6. General Principles and Strategies for Salting-Out Informed by the Hofmeister Series Alan M. Hyde, Susan L. Zultanski, Jacob H. Waldman, Yong-Li Zhong, Michael Shevlin, and Feng *Peng Organic Process Research & Development* 2017 21 (9), 1355-1370
7. Hofmeister F. (1888). *Arch. Exp. Pathol. Pharmacol.* 24, 247-260
8. Yang Z (2009). "Hofmeister effects: an explanation for the impact of ionic liquids on biocatalysis". *Journal of Biotechnology*. 144 (1): 12–22
9. Zhang Y, Cremer PS (2006). "Interactions between macromolecules and ions: The Hofmeister series". *Current Opinion in Chemical Biology*. 10 (6): 658–63.
10. M. Adreev; A. Chremos; J. de Pablo; J. F. Douglas (2017). "Coarse-Grained Model of the Dynamics of Electrolyte Solutions". *J. Phys. Chem. B*. 121 (34): 8195–8202.
11. M. Adreev; J. de Pablo; A. Chremos; J. F. Douglas (2018). "Influence of Ion Solvation on the Properties of Electrolyte Solutions". *J. Phys. Chem. B*. 122 (14): 4029–4034
12. Chaplin, Martin (August 6, 2014). "Hofmeister Series". *Water Structure and Science*. London South Bank University. Retrieved 2014-09-05
13. F. A. Long and W. F. McDevit. Activity Coefficients of Nonelectrolyte Solutes in Aqueous Salt Solutions. *Chemical Reviews* **1952** *51* (1), 119-169
14. Berne, B.J.; Pecora, R. Dynamic Light Scattering. Courier *Dover Publications* (2000)
15. Shaw, D.J. (1992). Introduction to Colloid And Surface Chemistry, Butterworth Heinemann, UK.

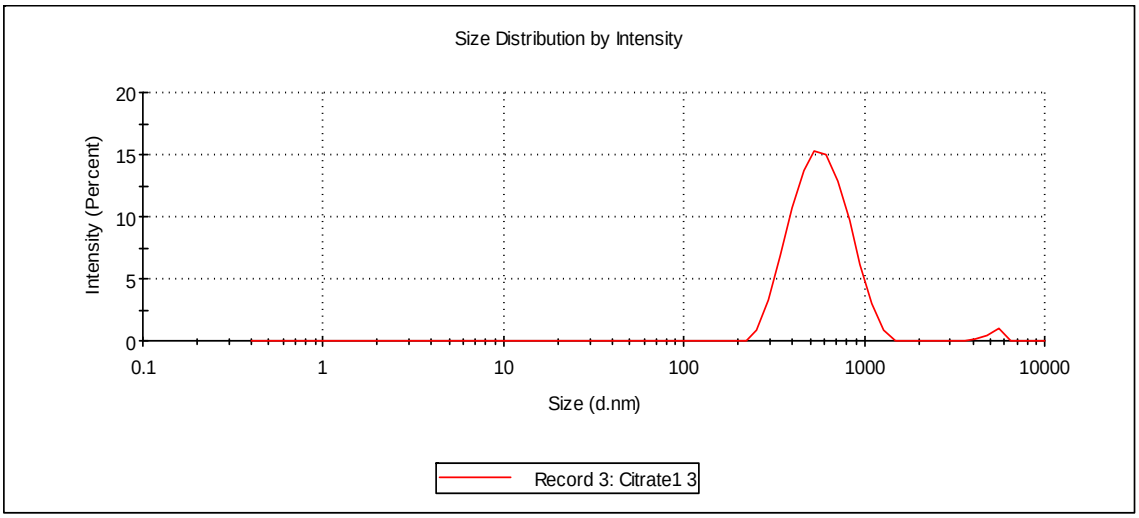
SOURCES:

1. Joy Wolfram. The nano-plasma interface: Implications of the protein corona. *Colloids and Surfaces B: Biointerfaces.* Vol. 124, 2014, 17-24

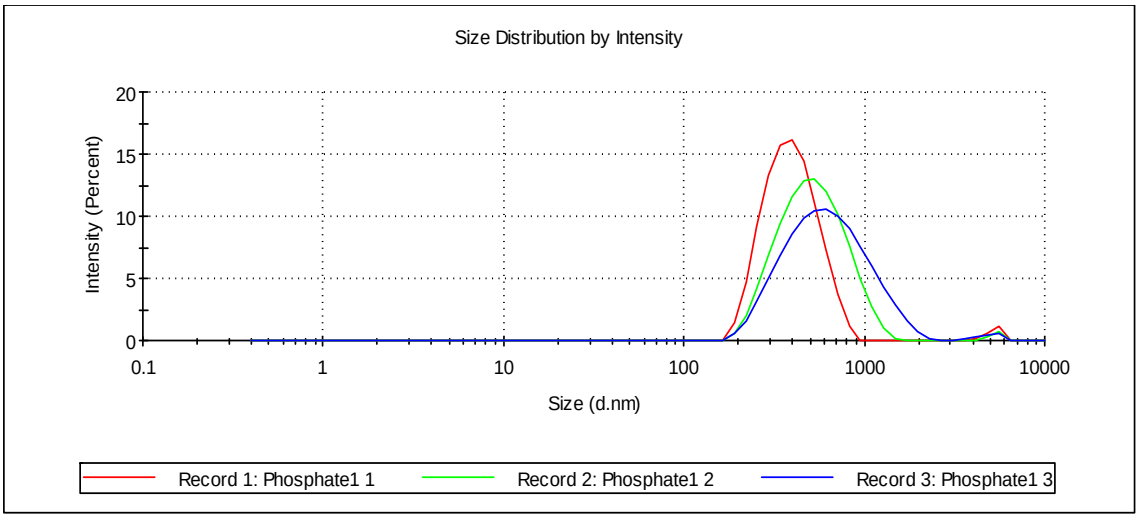
8. APPENDICES



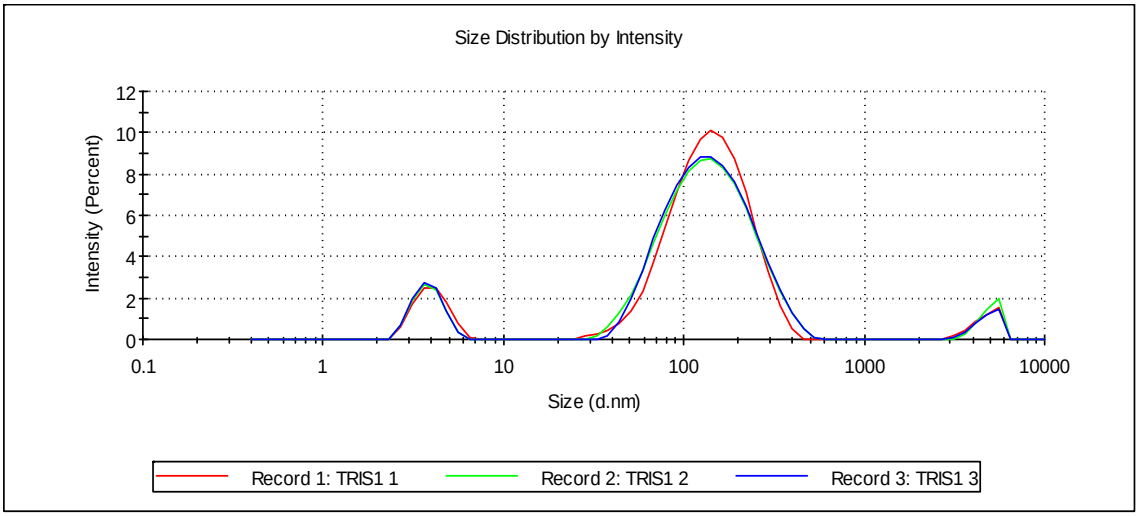
Appendix 1. Dynamic light scattering on a 10mM BES buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer.



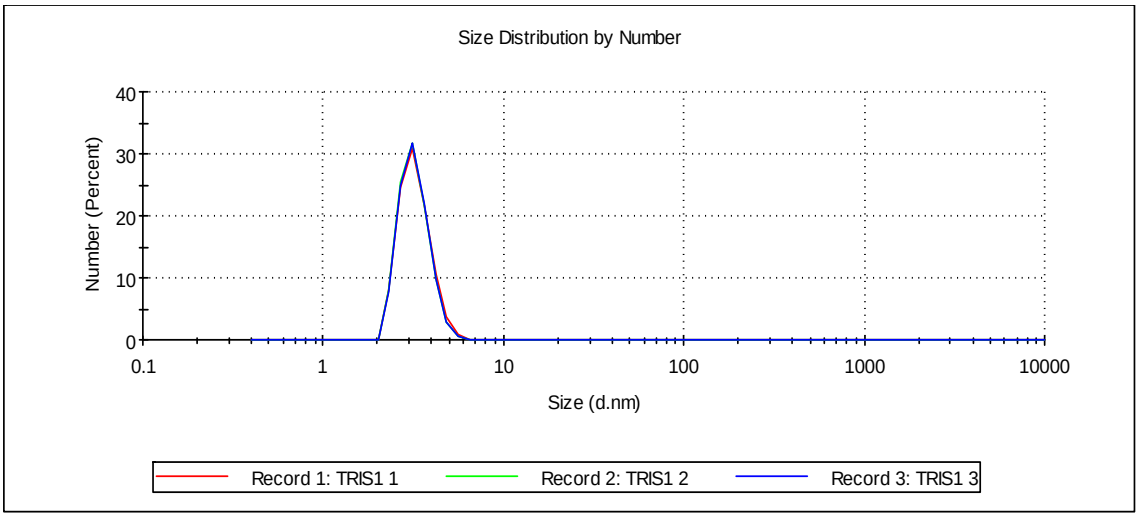
Appendix 2. Dynamic light scattering on a 10mM citrate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer.



Appendix 3. Dynamic light scattering on a 10mM phosphate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer.

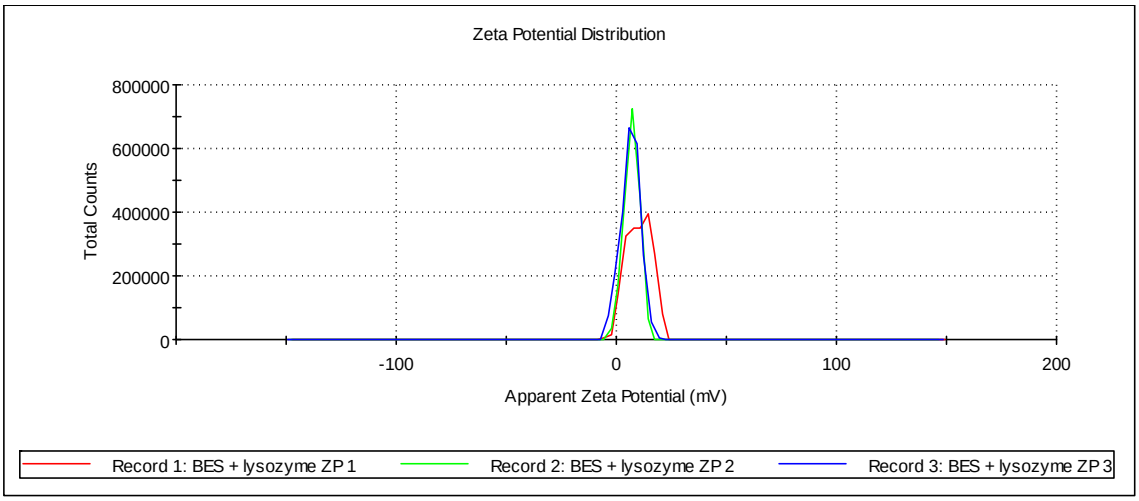


Appendix 4. Dynamic light scattering on a 10mM TRIS buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.

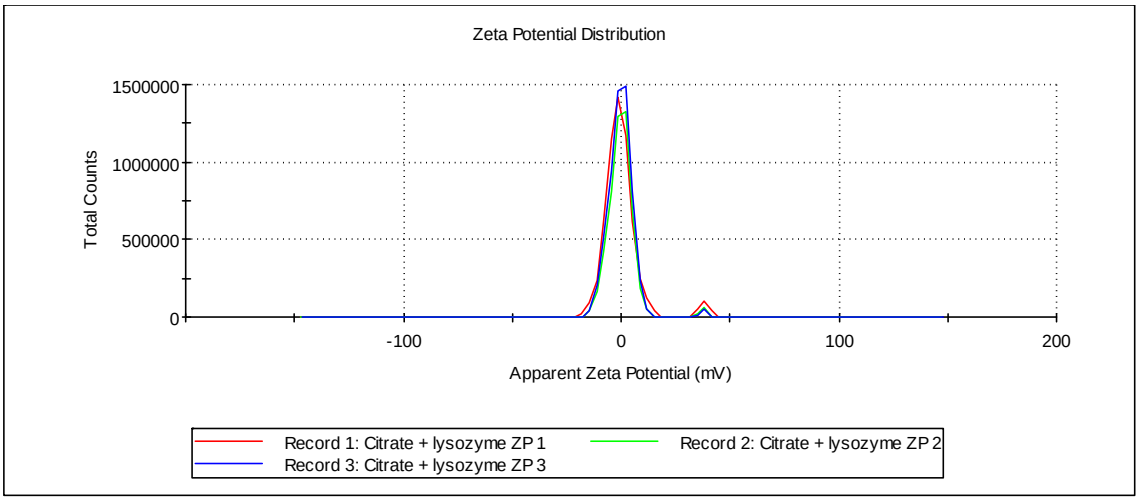


Appendix 5. Dynamic light scattering on a 10mM TRIS buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.

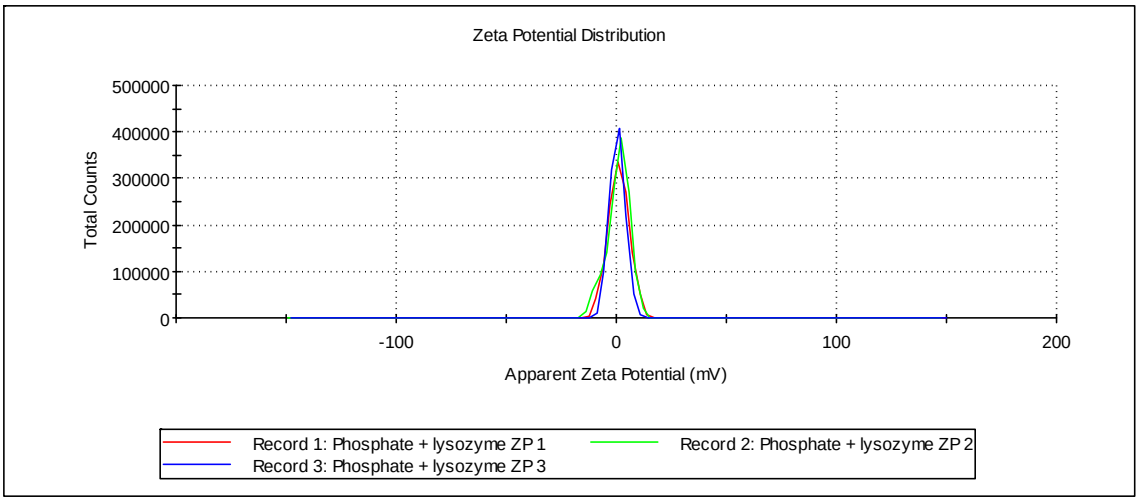
ZETA POTENTIAL ON 10MM BUFFERS:



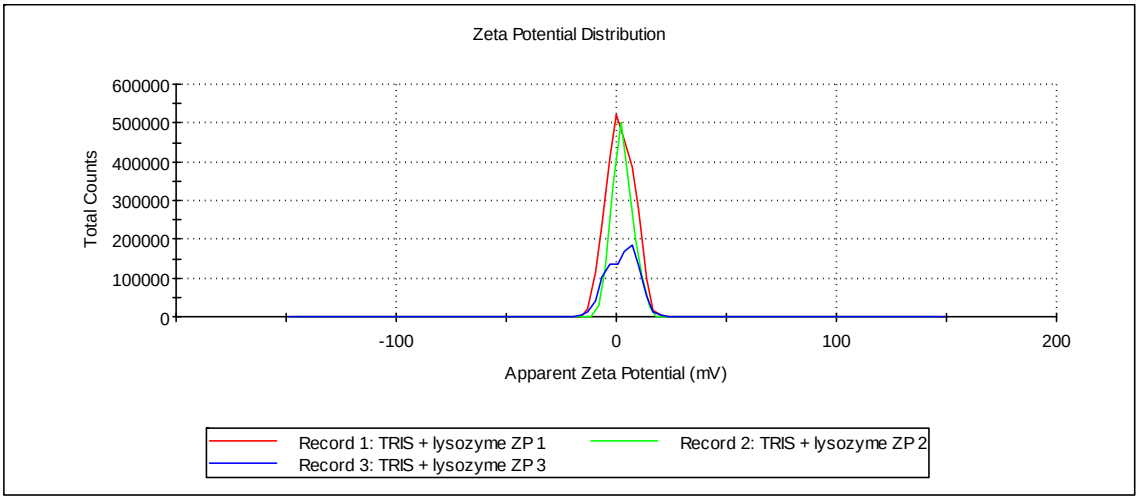
Appendix 6. Zeta Potential Distribution on a 10mM BES buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer.



Appendix 7. Zeta Potential Distribution on a 10mM citrate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer.



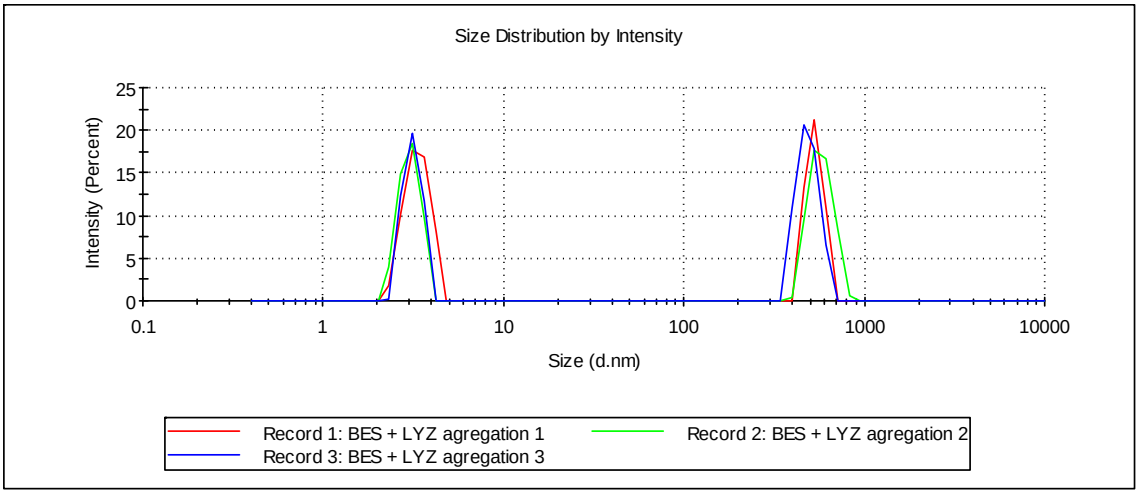
Appendix 8. Zeta Potential Distribution on a 10mM phosphate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer.



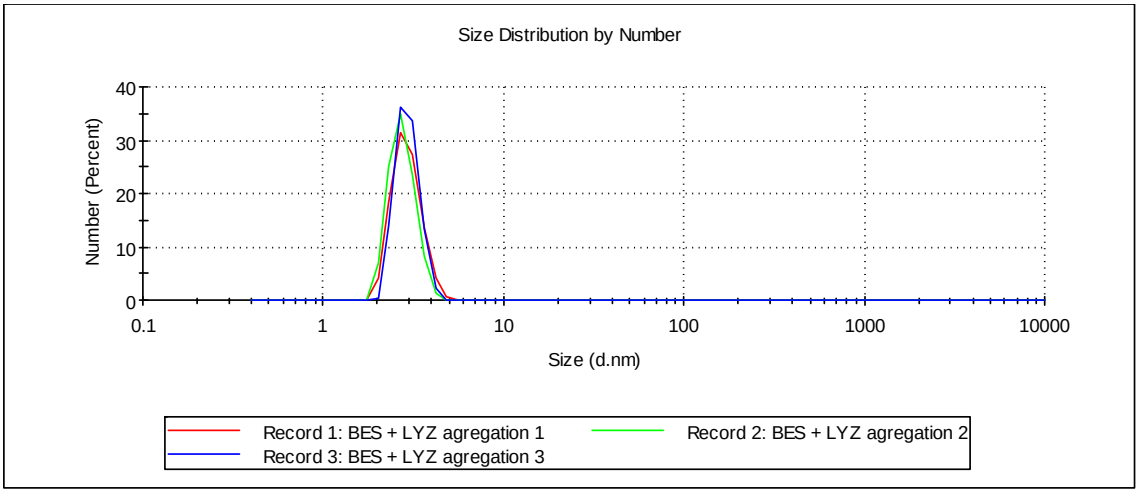
Appendix 9. Zeta Potential Distribution on a 10mM TRIS buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer.

ZETA SIZER ON LYSOZYME AGGREGATION OF 10MM BUFFERS:

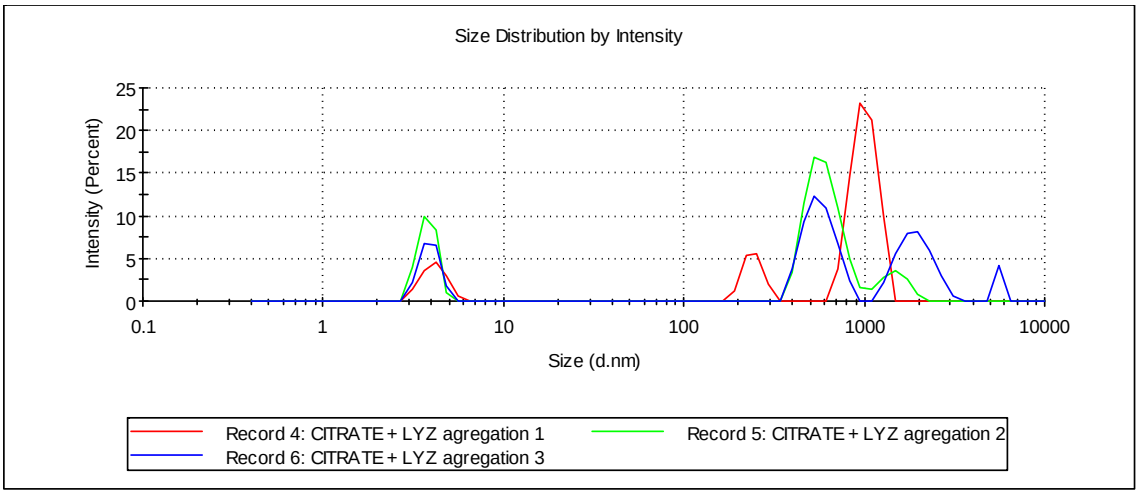
As some previous data differed, implying a higher deviation, the samples were left overnight (24H) so I could reveal whether or not the dispersions formed aggregations.



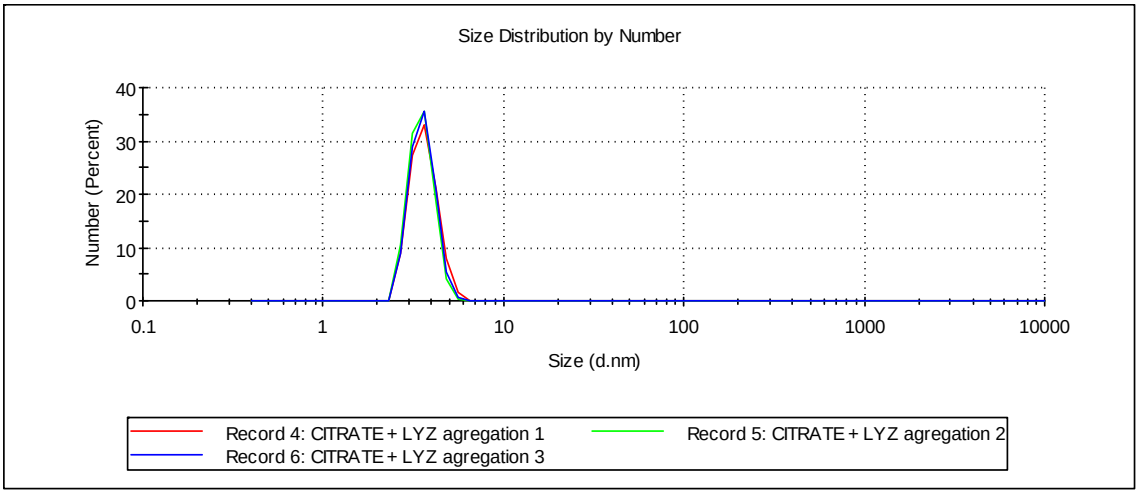
Appendix 10. Dynamic light scattering on a 10mM BES buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.



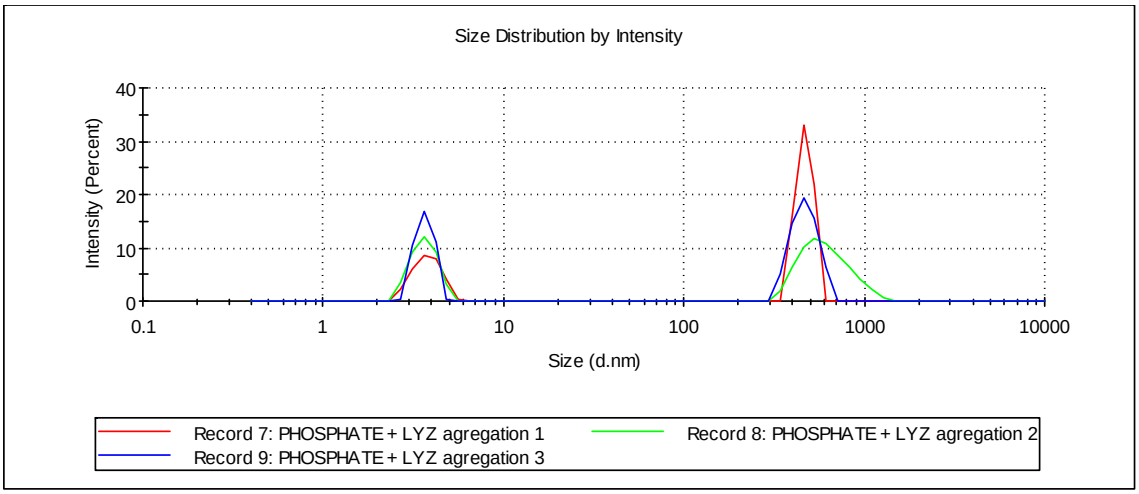
Appendix 11. Dynamic light scattering on a 10mM BES buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.



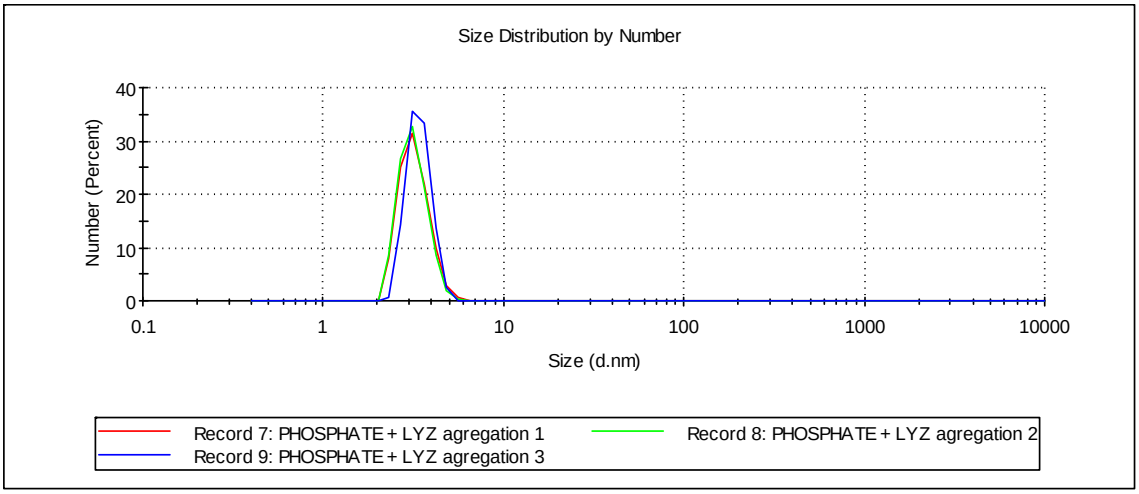
Appendix 12. Dynamic light scattering on a 10mM citrate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.



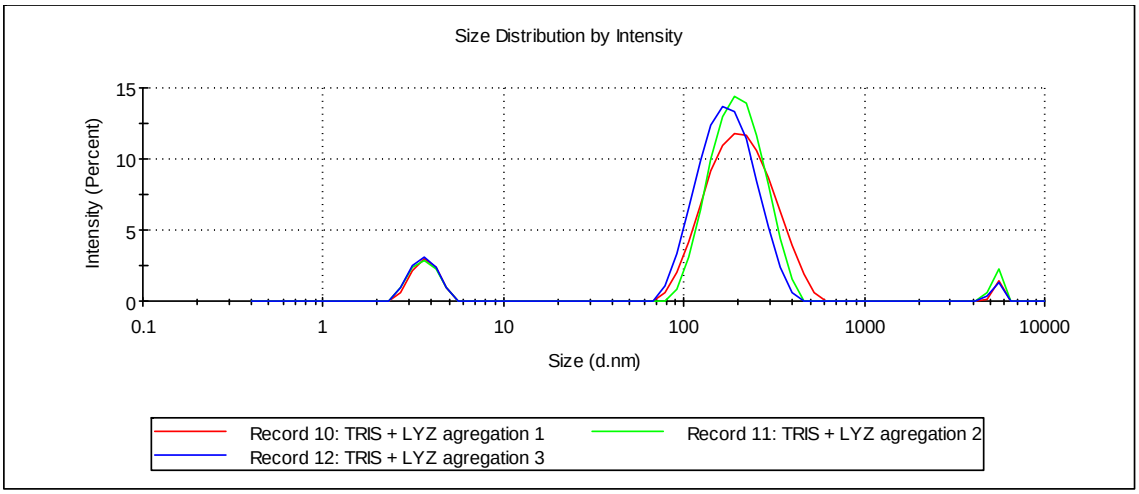
Appendix 13. Dynamic light scattering on a 10mM citrate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.



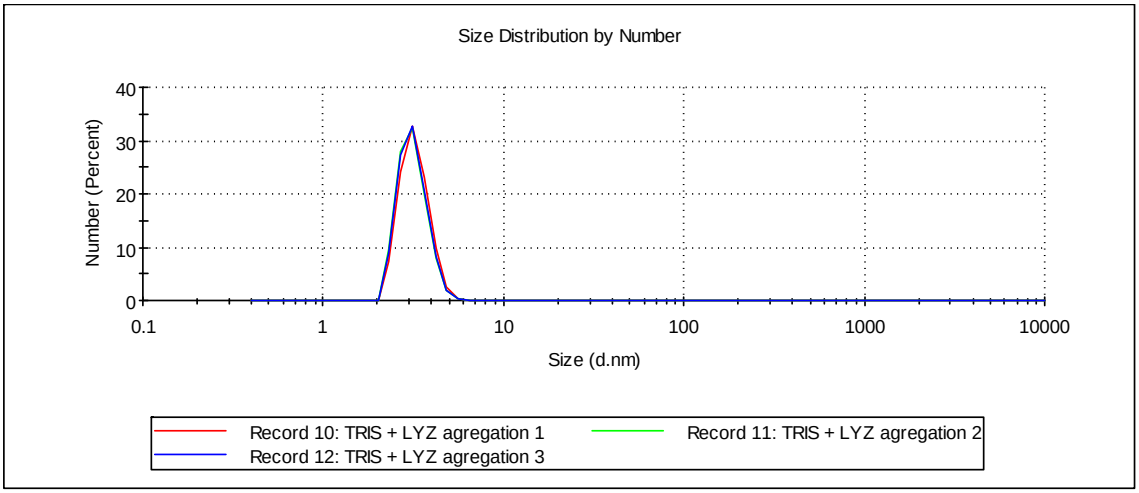
Appendix 14. Dynamic light scattering on a 10mM phosphate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.



Appendix 15. Dynamic light scattering on a 10mM phosphate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.

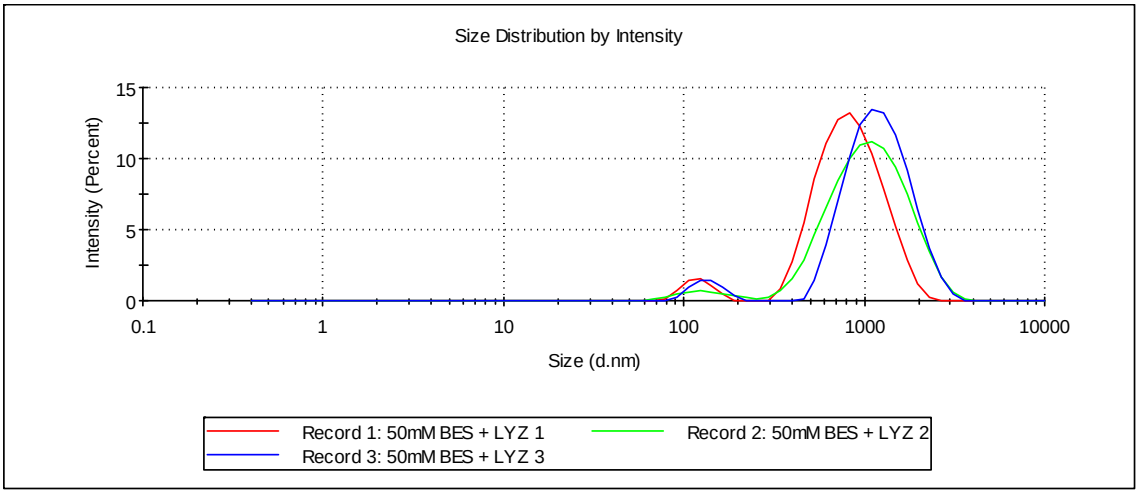


Appendix 16. Dynamic light scattering on a 10mM TRIS buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.

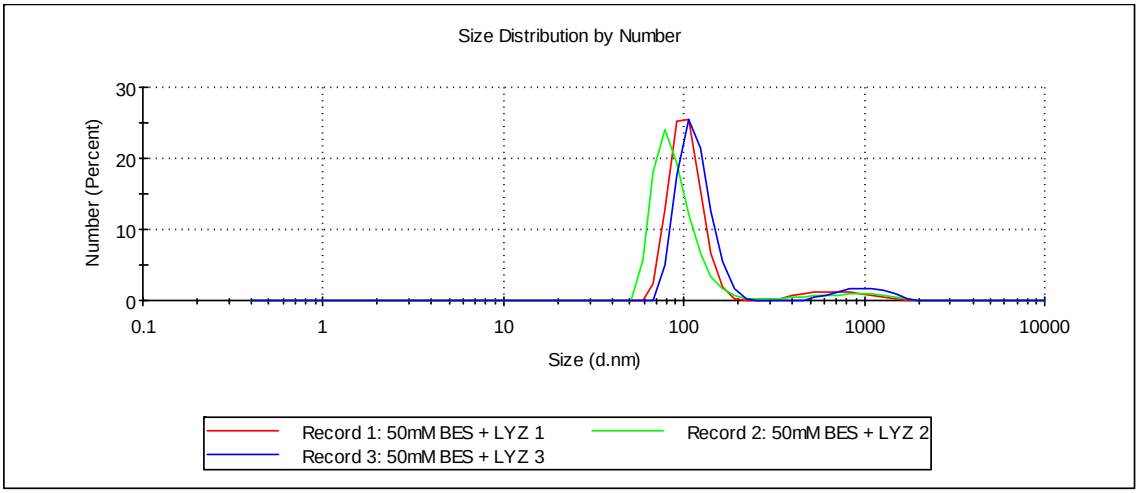


Appendix 17. Dynamic light scattering on a 10mM TRIS buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.

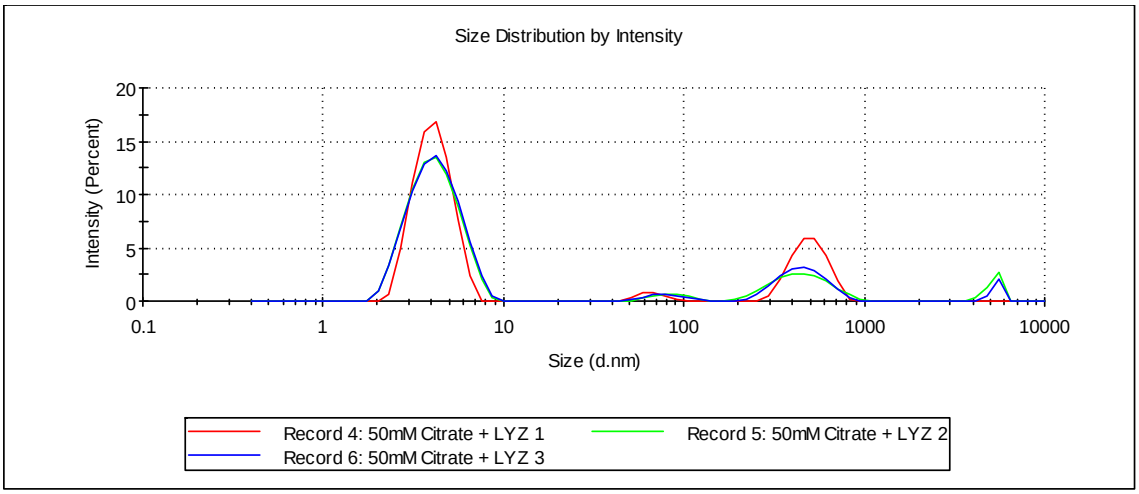
ZETASIZER ON 50MM BUFFERS



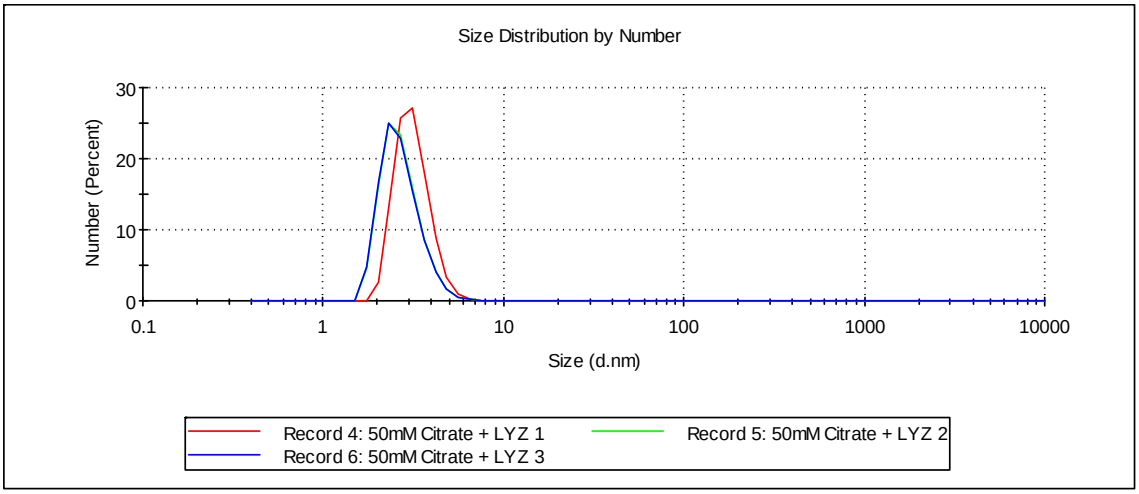
Appendix 18. Dynamic light scattering on a 50mM BES buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.



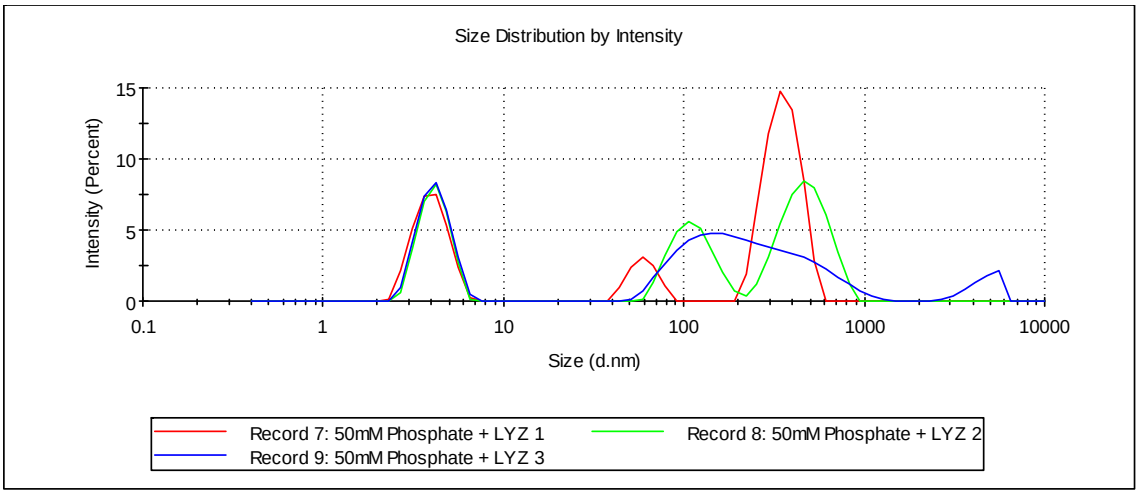
Appendix 19. Dynamic light scattering on a 50mM BES buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.



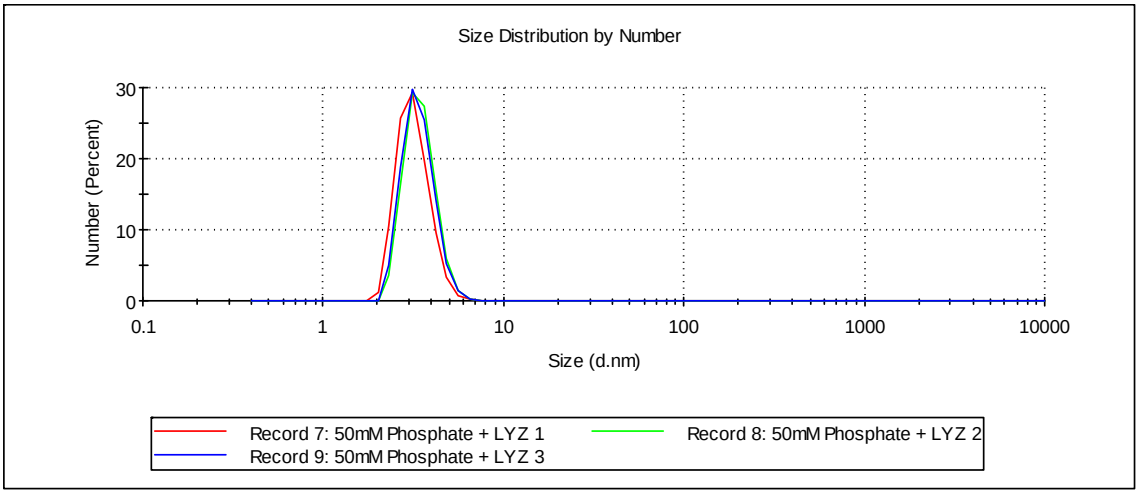
Appendix 20. Dynamic light scattering on a 50mM citrate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.



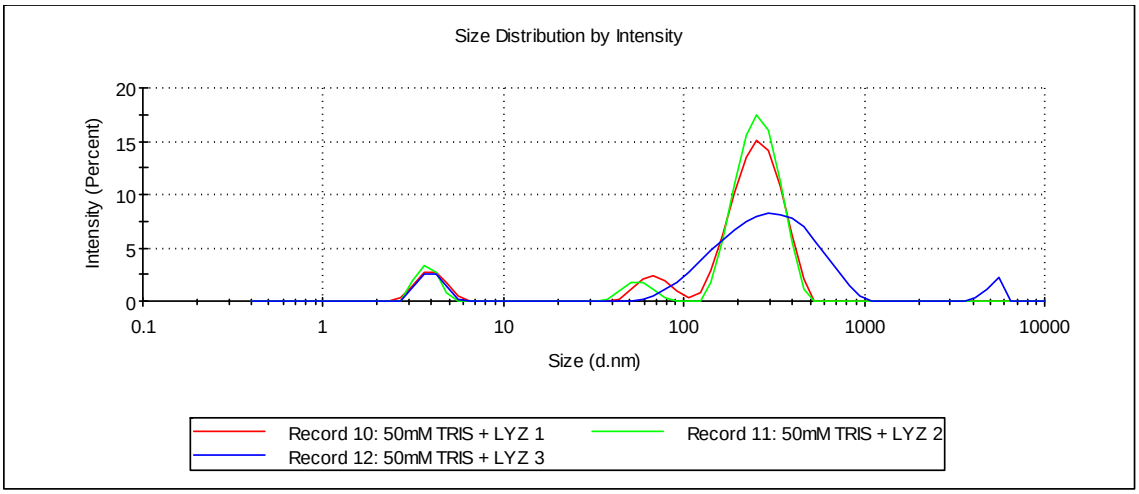
Appendix 21. Dynamic light scattering on a 50mM citrate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.



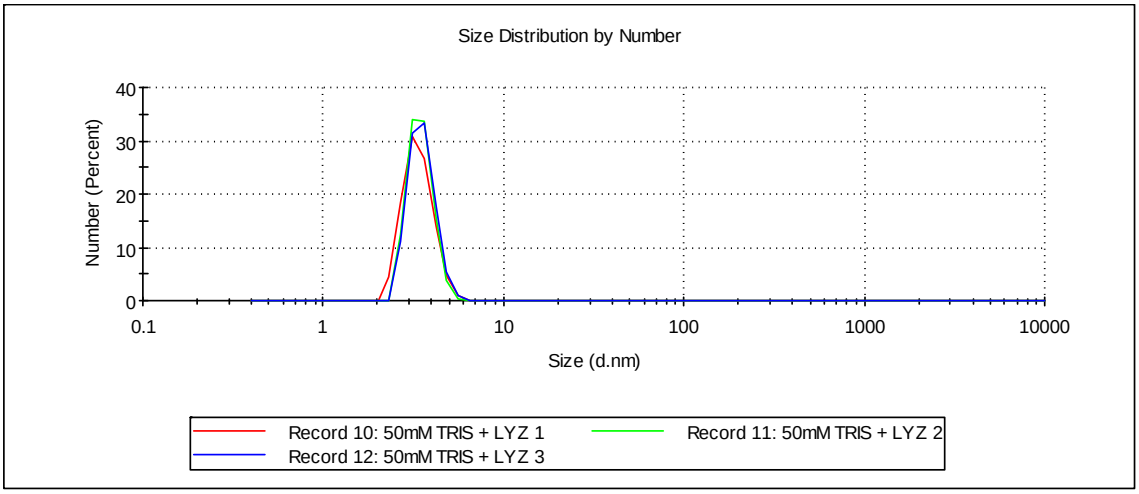
Appendix 22. Dynamic light scattering on a 50mM phosphate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.



Appendix 23. Dynamic light scattering on a 50mM phosphate buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.



Appendix 24. Dynamic light scattering on a 50mM TRIS buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Intensity.



Appendix 25. Dynamic light scattering on a 50mM TRIS buffer with lysozyme. 0,1mL of lysozyme was added in a cuvette with 1mL of buffer. Size Distribution by Number.