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High Throughput Screening of Selectivity for Protein Purification

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Dedication

To my kind parents and the whole family

Table of contents

Abbreviations

AI(s)	Adsorption isotherm(s)
c(mg/ml)	concentration (mg/ml)
CV	Column volume
Cyt C	Cytochrome C
FT	Flowthrough
Lys	Lysozyme
FDA	Food and Drug Administration
HPLC	High-performance liquid chromatography
HTPD	high-throughput process development
HTS	High-throughput screening
IEX	Ion Exchange Chromatography
Kd	Binding strength (dissociation constant)
mAb	Monoclonal antibodies
mAU	1/1000 absorbance unit
pI	Isoelectric point
q	Binding capacity
RT	Retention time
RNase A	Ribonuclease A
UV	Ultra-violet

Introduction

Protein purification is a crucial part of many processes in biological and pharmaceutical sciences and the part that usually takes most money and time. Therefore, the development of high throughput screening methods is essential in process development as it will not only save time and money but also sample used, by miniaturization [1, 2].
Generally, chromatographic techniques are used in a very wide range in the biotechnology and related fields, both in academia and industry. Thus, the demand for HTS methods is high [1].

The development of chromatographic purification methods has been essential to all bioprocess and biotech processes. PreDictor™ plates are 96-wells plates prefilled with resins in different volumes (for example 6 and 20µl) allowing high throughput screening of different chromatographic conditions in parallel. Additionally, sample volume can be reduced significantly when using these plates. This high throughput screening (HTS) system allows the screening of up to 96 different varieties of batch conditions including for example different values for salt, pH condition or protein concentrations.

The problem with traditional methods for optimization is the need for doing optimization for the different conditions separately, hence high throughput screening (HTS) methods are essential for process development. Protein chromatography can be performed using different types of bioprocess media like ion exchange (IEX) chromatography. IEX chromatography is widely used for protein purification processes because of its high capacity, robustness and the good understanding of the purification process [1, 3].
Capto™ SP ImpRes (GE Healthcare) is an example of a strong cationic exchanger that can be used for purification of different biomolecules [4].

The miniaturization of the bioprocess development has been employed in several studies over the last years and has been proven to work satisfactorily and therefore has become a very valuable method for process development [1, 4-7].
For example, in a recent study, mAb purification was optimized using a HTS method which enabled the researchers to test a variety of different conditions rapidly and in parallel [1-3].

The PreDictor™ plates HTS system allows the screening of up to 96 different chromatographic conditions in a miniaturized scale, thereafter the results can be verified with large columns using for example ÄKTA chromatography systems (GE Healthcare).Most purification processes nowadays are performed using ÄKTA chromatography systems (GE Healthcare) [2].

The most significant advantage of the HTS is a significant shortening of the time needed to perform the screening, since in HTS it is possible to screen for several parameters in parallel which facilitates the identification of the best parameters required for the purification of new biotechnological products. On the other hand, the traditional chromatographic screening methods using columns take long time, from

days to even weeks, to optimize the chromatography/purification method. Thus, HTS techniques provide the need to speed up both time and to cut development costs [5, 7].

Moreover, the HTS method is amenable to be automated using robots. A robot system manufactured by Tecan Group Ltd. has already been used together with the PreDictor™ plates. The robot could be used in order to save time, reduce human errors, and handing of many samples automatically.

It is not possible to make a continuous gradient of elution buffer for the purification process, thus only batch methods are available. Additionally, the methods used for analyzing the samples being screened are very critical and stumbling blocks for this HTS method. The analysis methods being used the most nowadays are UV absorption and HPLC [8]. There have been many attempts to overcome the analysis problem in order to facilitate the work with HTS methods, a recent study [3] for example proposed a new statistical method to overcome the analysis method.

Adsorption isotherm

Adsorption isotherms studies provide information concerning binding strength (*Kd*) of the desired products to the potential bioprocess media or resin. Additionally, the AIs studies also provide precious information about the potential binding capacity (*qmax*) for certain conditions studied. The PreDictor™ plates can be used for obtaining adsorption isotherms and there are different strategies that can be applied:

1. Keeping the concentration of the tested sample unchanged and changing the phase ratio.
2. Keeping the phase ratio unchanged and changing the concentration of the tested sample.
3. Combination of the two previous approaches (see Figure 1).

The phase ratio is the ratio between the liquid volume (sample) and the media (resin) volume. The phase ratio (β) can be calculated using the following equation:

$$\beta = \frac{V_{liq}}{V_{medium}}$$

Where (β) is the phase ratio,
V_{liq} is the liquid (sample) volume and
V_{medium} is the medium (resin) volume

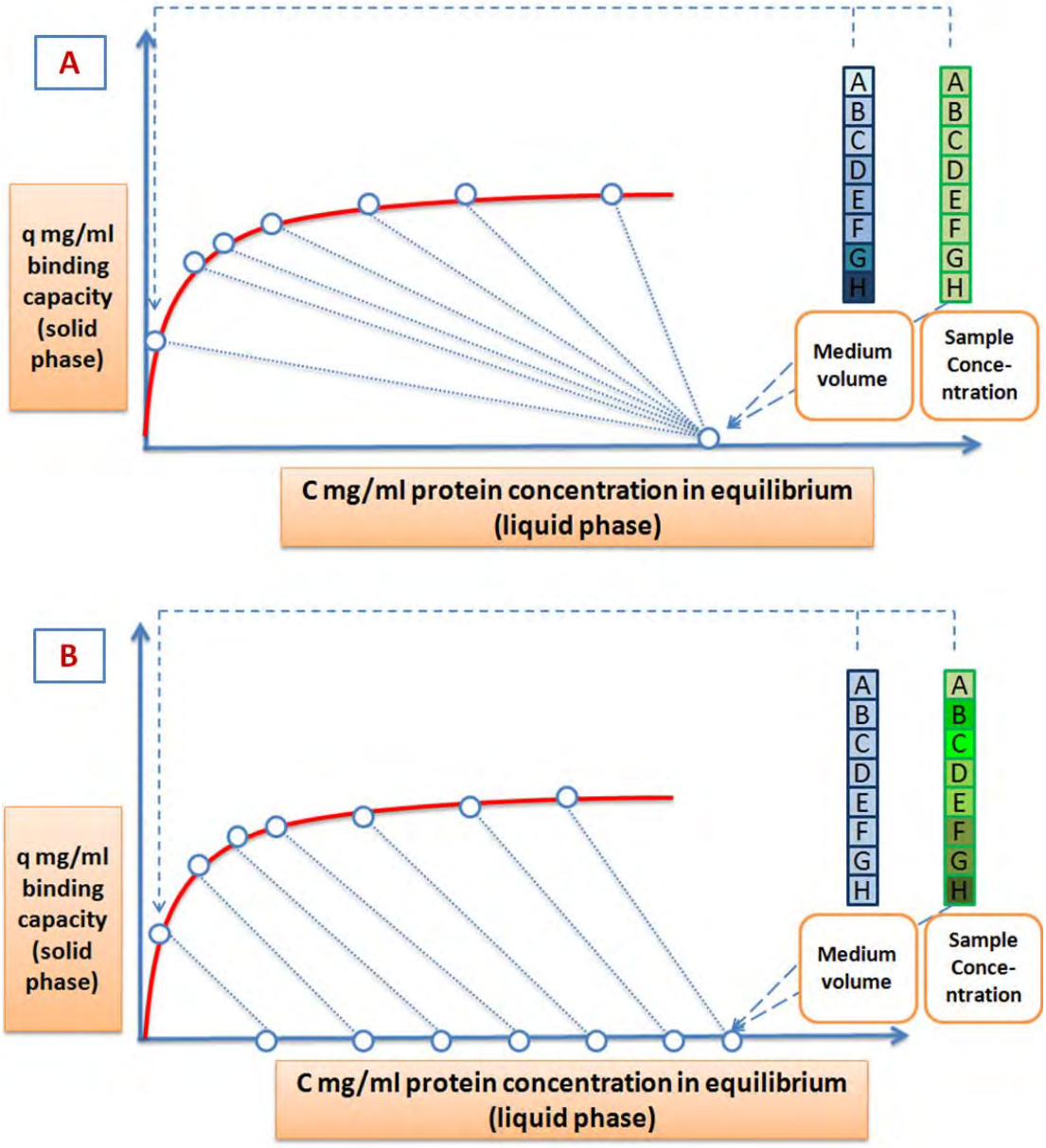

Figure 1 The two approaches of adsorption isotherms determination using PreDictor™ plates in a batch system. A) Keeping the concentration unchanged of the tested sample and changing the phase ratio. B) Keeping the phase ratio unchanged and changing the concentration of the tested sample.

PreDictor™ Plates

Time is a critical factor for any company; the time-to clinic and the time-to-market are important for the biopharmaceutical industry working in a competitive environment. In addition, the regulations established by agencies like FDA increase the need for controlled process development.

In order to shorten the time and effort required to develop a biopharmaceutical product high-throughput tools are increasingly being employed in the process

development. The data obtained from the PreDictor™ plates are correlated to a high extent to the data obtained from column chromatography making them an excellent choice to start the protein purification optimization process.

The plates allow the screening of many different chromatographic conditions parameters. Chromatographic conditions can be screened using PreDictor™ plates (different salt concentrations, different pH, additives…etc.). Although it is a batch system of parameters (e.g. concentration) could be imitated using a small difference in the elution conditions.
The adsorption isotherms conditions could be tested using special PreDictor™ plates or by using single media PreDictor™ plates and varying the protein concentrations instead. Design of Experiment (DoE) is usually done in order to get as much information as possible from the experiment. The Assist software (GE Healthcare) can be used in the guidance of plate experiment, handling of experimental data and data analysis. Elution studies can be also performed using PreDictor™ plates in order to optimize the yield of the targeted product. The recommended volumes of the plates are 20µl to 50µl for elution studies. A variety of parameters can be obtained from the elution studies e.g. pH or salt concentrations. The maximum load of the sample in the PreDictor™ plates is 300µl however; multiple sample loads could be done in order to get the desired load depending on the study being done.

Each well of the 96 wells represents a batch condition or a run on a column in specific condition. The main difference between them is that the separation in a batch occurs once, while the separation in a column experiment is continuous. Therefore the results could be validated using columns [9].

Aim of the project

High throughput screening (HTS) of chromatographic parameters is being used more and more within the biopharmaceutical industry. For selectivity studies there are two main routes: adsorption isotherms and elution study approaches.

The aim of this project was to investigate and study a strategy for screening selectivity for chromatographic conditions using adsorption isotherms and elution study approaches. Both methods have been used for three proteins (cytochrome c, lysozyme and RNase A). The results from the adsorption isotherms and elution studies are scaled up to small columns on the ÄKTA avant system (GE Healthcare) in order to verify the predicted results obtained from both studies. The investigation is done to verify whether either of these or both approaches led to the same result or not.

Materials & Methods

Instruments

Instruments used in the experiments are listed below in table 1.

Table 1 Instruments.

Instrument	Description
Electronic Multichannel pipettes	Eppendorf Research Pro 12 Channel (20-300 µl) & Eppendorf Research Pro 8 Channel (50-1200 µl)
UV plate-reading spectrophotometer	SpectraMax Plus384 Absorbance MicroplateReader (Molecular Devices) using a software package called SoftMax Pro
ÄKTA Avant	Chromatography system supplied with the UNICORN 6 software package

PreDictor™ plates

PreDictor™ plates used in the experiments are listed below in Table 2.

Table 2 PreDictor™ plates used in the experiments:

PreDictor™ plates
PreDictorCapto™ SP ImpRes, 20 µl
PreDictorCapto™ SP ImpRes, 6 µl

PreDictor™ plates were used according to the instructions provided by the supplier (GE Healthcare)[6]with some modifications. The modifications are the equilibration steps for the plates that were performed 3 times with shaking at 1100 rpm followed by incubation of the sample protein for 120 minutes.

Column packaging

Column packaging of the Capto™ SP ImpRes(GE Healthcare) was performed according to the instructions provided by the supplier and the columns were tested using the ÄKTA Avant 25 chromatography system. See Table 3 for the resin used for column packaging.

Table 3 resin used for the column packaging

Resin	Supplier
Capto SP ImpRes	GE Healtchare

Proteins

Three standard proteins were used, see Table 4.

Table 4 standard proteins

Protein	Supplier
Cytochrome c equine heart	Sigma-Aldrich
Lysozyme	Sigma-Aldrich
Ribonuclease A from bovine pancreas	Sigma-Aldrich

Chemicals

Chemicals used for the buffer preparations are given in Table 5.

Table 5 chemicals

chemical	Molecular weight(g/mol)	Supplier
NaH2PO4	137.99	Merck
Na2HPO4	177.99	Merck
NaCl	58.44	Merck

Buffers and solutions

Buffer preparation software

Buffer wand (GE Healthcare) and buffer Mate (GE Healthcare) have been used for the preparation of the buffers used for the experiments (Tables 6-8).

Table 6 Buffers for the adsorption isotherms experiments

Buffer	Phosphate conc(mM)	NaCl (mM)	pH
Phosphate0	50	0	6
Phosphate50	50	50	6
Phosphate100	50	100	6
Phosphate150	50	150	6
Phosphate1000	50	1000	6

Table7 Buffers for the elution experiments

Buffer	Phosphate conc(mM)	NaCl range (mM)	pH
Phosphate	150	0 - 280	4,9
Phosphate	130	0 – 280	5,7
Phosphate	90	0 – 280	6,5
Phosphate	60	0 - 280	7,3

Table 8 Buffers for the column experiments

Buffer	Phosphate conc(mM)	NaCl (mM)	pH	Description
Phosphate0	50	0	6	Binding buffer
Phosphate50	50	300	6	Elution buffer

Protocol for the adsorption isotherms studies using PreDictor™ plates:

1. Equilibration: The plates were incubated 5 min with 300 µl of equilibration buffer with 1100 rpm shaking; this step was repeated 3 times. Equilibration was

performed using phosphate buffer different concentrations and salt content (see table 6).

2. Loading: The medium was incubated 120 min with 300 µl of sample solution with 1100 rpm shaking. Loading was performed with protein samples which were mixed with several phosphate buffers different concentrations and salt content (see table 6). The protein concentrations were varied for protein loading pattern (see figure 2).

3. Collection of the flow through (vacuum was used for cytochrome c and lysozyme, whereas, centrifuge was used for RNase A to avoid foam formation).

Loading protein samples into the PreDictor™ plates performed using digital multichannel pipette according to the layout (see figure 2).

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6
B	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6
C	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6
D	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6
E	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6
F	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6
G	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6
H	0,1	0,25	0,6	0,9	1,4	1,8	2,4	3,1	3,7	4,4	5,2	6

Figure 2 Layout of protein concentration (mg/ml) loaded into PreDictor™ plates (96-well plate).

Protocol for the elution studies using PreDictor™ plates:

1. Equilibration: The plates were incubated 5 min with 300 µl of equilibration buffer with 1100 rpm shaking; this step was repeated 3 times. Equilibration was performed with phosphate buffer 50mM pH 6 without salt.

2. Loading: The medium was incubated 120 min with 300 µl of sample solution with 1100 rpm shaking. Loading protein samples was performed with phosphate buffer 50mM pH 6, without salt. The protein concentration in the sample loading was 1 mg/ml except for RNase A where this concentration was not sufficient to be read by UV microplate reader and an experiment with 3 mg/ml was performed in order to overcome this problem.

3. Wash: The medium was incubated 5 min with 300 µl of equilibration buffer with 1100 rpm shaking, this step repeated 3 times.

4. Elution: The medium was incubated 5 min with 300 µl of elution buffers with 1100 rpm shaking; this step was repeated 3 times (Figure 3).

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0	0
C	50	50	50	50	50	50	50	50	50	50	50	50
D	50	50	50	50	50	50	50	50	50	50	50	50
E	100	100	100	100	100	100	100	100	100	100	100	100
F	100	100	100	100	100	100	100	100	100	100	100	100
G	150	150	150	150	150	150	150	150	150	150	150	150
H	150	150	150	150	150	150	150	150	150	150	150	150

Figure 3 Layout of 50mM Phosphate buffer loaded into PreDictor™ plates with different salt concentrations (numbers indicating NaCl concentrations (mM)).

The following 48-wells buffer plate was prepared using BufferWand (internal GE Healthcare software) and Tecan robot (see figure 4 & 5).

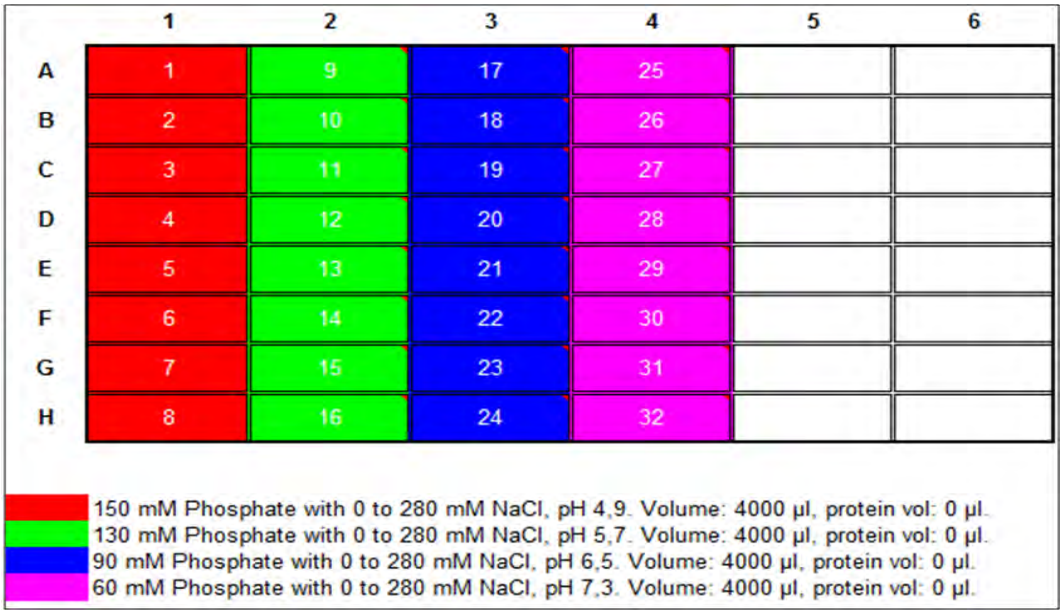

Figure 4 Buffer plate layouts prepared for the elution step, 32 wells loaded in triplicate in PreDictor™ plate (96 wells), see figure legend for details.

The following 96-wells buffer plate was prepared using BufferWand (internal GE Healthcare) and robot showing the elution pattern performed using PreDictor™ plate (Figure 5).

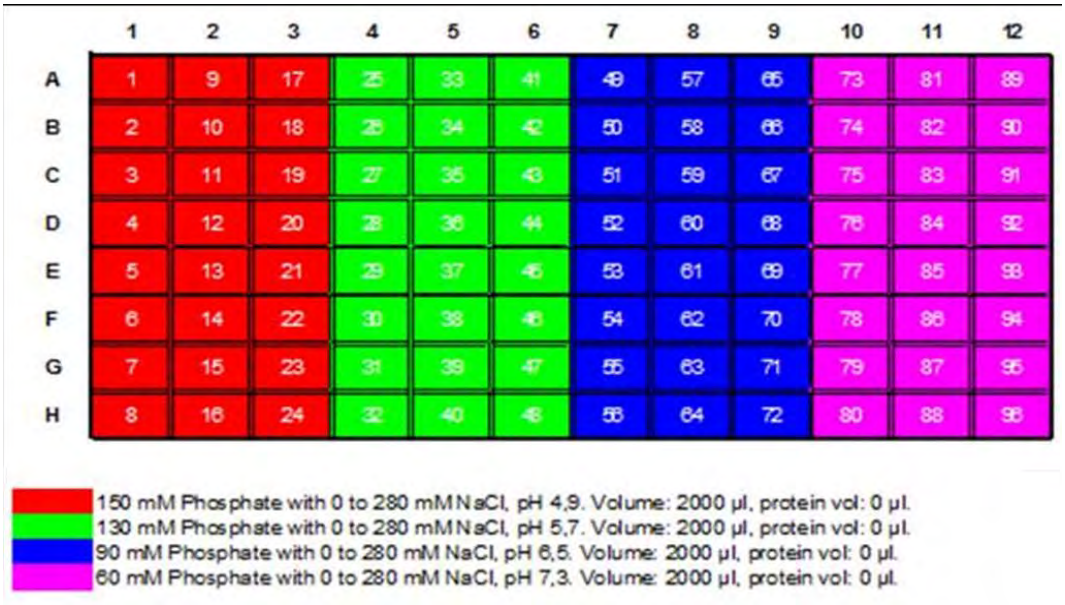

Figure 5 Buffer plate layouts prepared for the elution step in using PreDictor™ plate (96 wells), see figure legend for details.

Analysis using UV spectrophotometer

The absorbance of different wavelengths was used for the analysis; 528nm was used for Cytochrome c, 280nm for RNase A and 280 nm for Lysozyme (300nm for Lysozyme only when at high concentrations). The UV spectrophotometer readings performed using SpectraMax Plus384 Absorbance Microplate Reader (Molecular Devices) and the analysis of spectrophotometric readings using SoftMax® Proenabled software.

Evaluation for the adsorption isotherms studies:

Readings from the flow-through obtained from the PreDictor™ plates (GE Healthcare) were used for the calculations of the adsorption isotherms. The calculations were performed after importing the data to Excel file. The evaluation of the results was performed using an Excel spreadsheet where the plate readings were pasted, organized, analyzed and subtracted from the blanks. The adsorption isotherms were calculated using the following equation:

$$q = \beta\,(C_0 - C)$$

Where q is the binding capacity, $C_0$the initial protein sample concentration, C the final concentration and β is the volume phase ratio (liquid/resin).

Evaluation for the elution studies

The evaluation of the results from the elution was performed after importing the data from the UV spectrophotometer to the Assist software (GE Healthcare). A mass balance and the percentage yield were calculated using the Assist software (GE Healthcare), which helped with the evaluation of the experiment. The contour plots of the elution percentage yield obtained gave a good indication of the protein elution order when scaling up.

Evaluation of the column experiments using ÄKTA Avant and Unicorn

ÄKTA Avant is a preparative chromatography system designed for fast and secure development of scalable methods and processes. The ÄKTA Avant 25 (GE Healthcare) chromatography system has been used for scaling up and to inspect the results gained from both adsorption isotherms and elution studies. The ÄKTA Avant 25 system is operated and controlled with UNICORN software version 6. Evaluation of the results was performed using UNICORN 6 software (GE Healthcare) that was supplied with the ÄKTA Avant system.

Protocol for the column experiments using ÄKTA Avant

A Capto Sp ImpRes 1ml column was packed in the laboratory and tested using the ÄKTA Avant system. The experiment was done using the results and information gained from the previous studies i.e. adsorption isotherms and elution. The protein samples were prepared in the binding buffer separately with a concentration of 10 mg/ml and then mixed together in equal volumes. The sample loading protein was injected using a needle and a connector with a 100µl volume in the run. The elution was then performed using elution buffer using isocratic and gradient elution protocols in some points that correlate with the data points obtained from the previous studies.

Results and discussion

Cytochrome C AI on PreDictor™ plates Capto SP ImpRes

Cytochrome c adsorption isotherms were obtained from Capto SP ImpRes both 6 µl & 20 µl. It was possible to get the adsorption isotherms from both 6 µl & 20 µl volume resin (Figures 6 & 7); however, the q_{max} from the PreDictor™ plates with a 20µl volume resin was clearer than that obtained from the PreDictor™ plates with a 6µl volume resin (Figure 8).

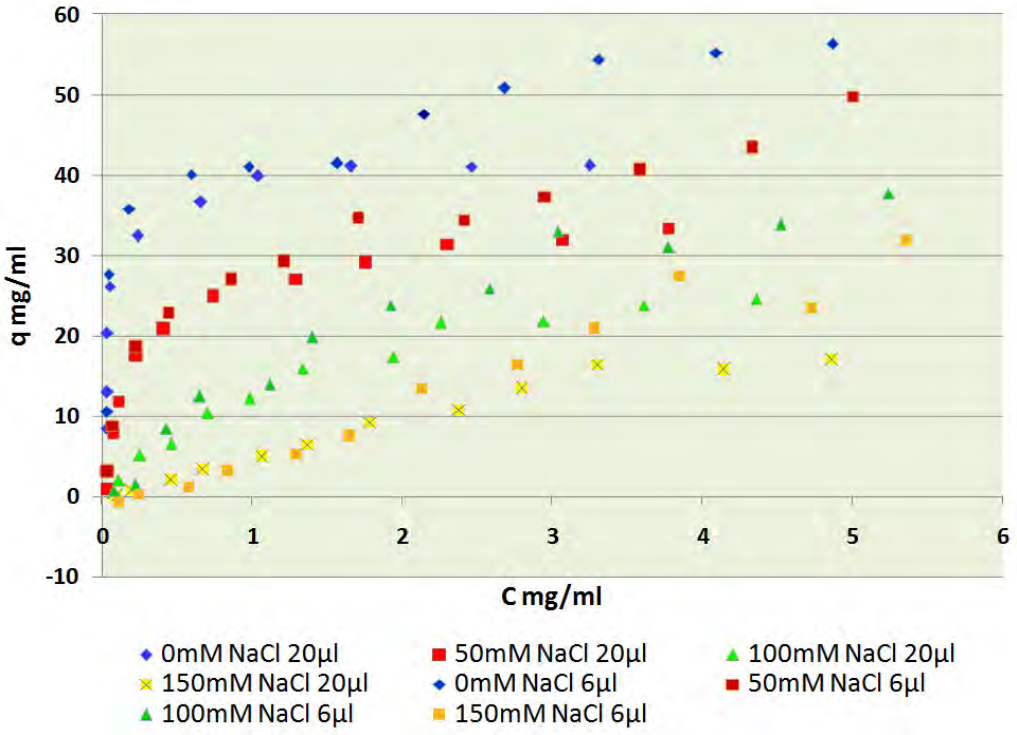

Figure 6 Adsorption isotherms forcytochrome c obtained from PreDictor plates Capto Sp Impress both 6µl & 20µl.

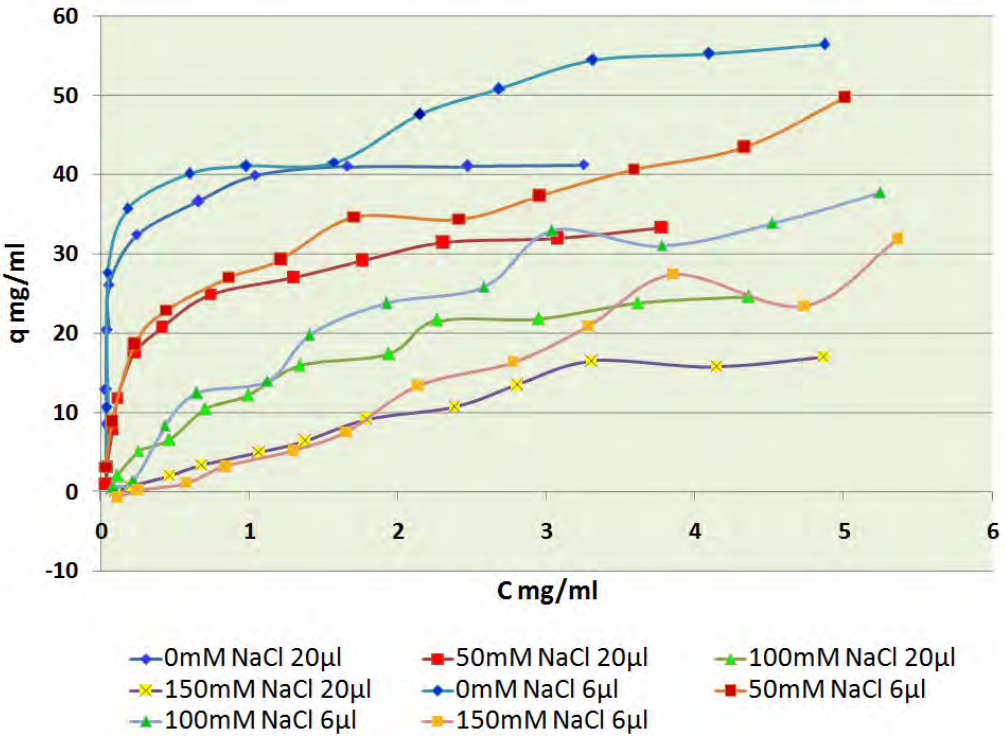

Figure 7 Adsorption isotherms forcytochrome c obtained from PreDictor™ plates Capto Sp Impress both 6µl & 20µl (with lines).

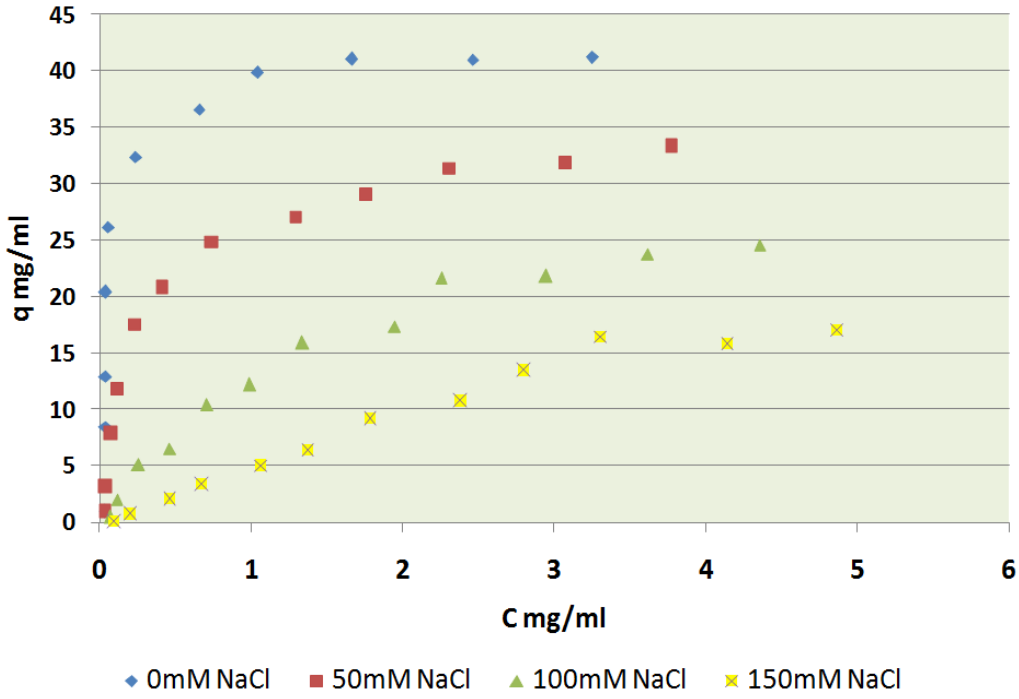

Figure 8 Adsorption isotherms forcytochrome cobtained from PreDictor™ plates Capto Sp Impress 20µl.

Adsorption isotherm for Lysozyme using PreDictor™ plates Capto SP ImpRes

Lysozyme is protein that absorbs quite much at different wavelengths, thus it was important to find a suitable wavelength to measure the protein at high concentrations. From the data obtained from the spectraand the calibration curves below we decided to measure at 300nm when we have high concetrations of lysozyme. Three different wavelegths were compared (295, 254 and 300 nm), and the best R-square (coefficient of determination) was acquired at 300 nm where a regression was obtained for the R^2almost one (Figures 9,10 and 11). The protein was dissolved in different concentrations of salt (NaCl) (0mM, 50mM, 100mM and 150mM) in a 30mM phosphate buffer.

The results for the lysozyme are similar to the results of the cytochrome c where it was possible to get the adsorption isotherms from both 6 µl & 20 µl volume resin, however, the q max from the PreDictor™ plates with a 20µl volume resin was more clear than that obtained from the PreDictor™ plates with a 6µl volume resin (see figures 12 to 15).

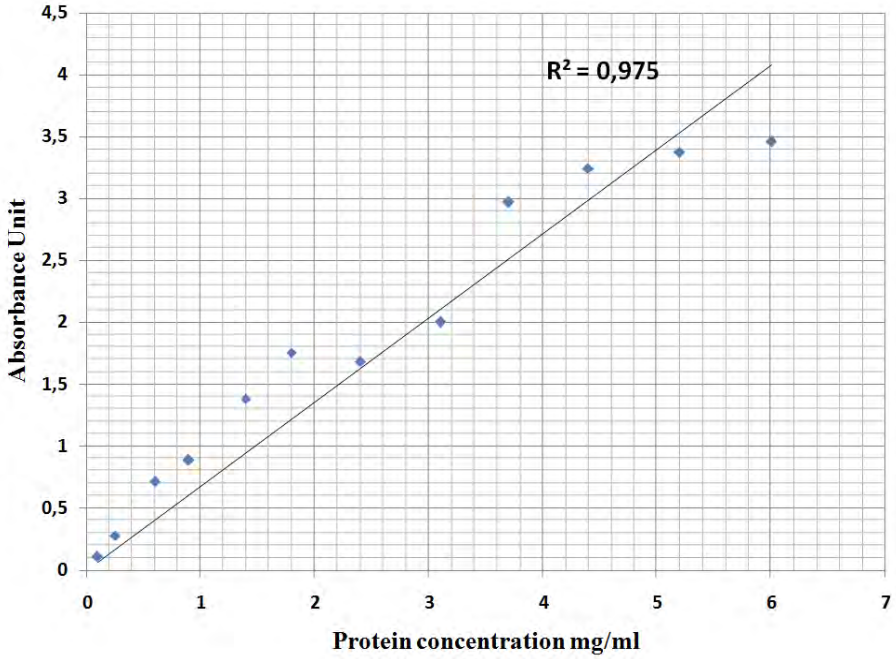

Figure 9 Calibration curves of the lysozyme obtained by measuring absorbance at UV 295 nm in 100mM NaCl .

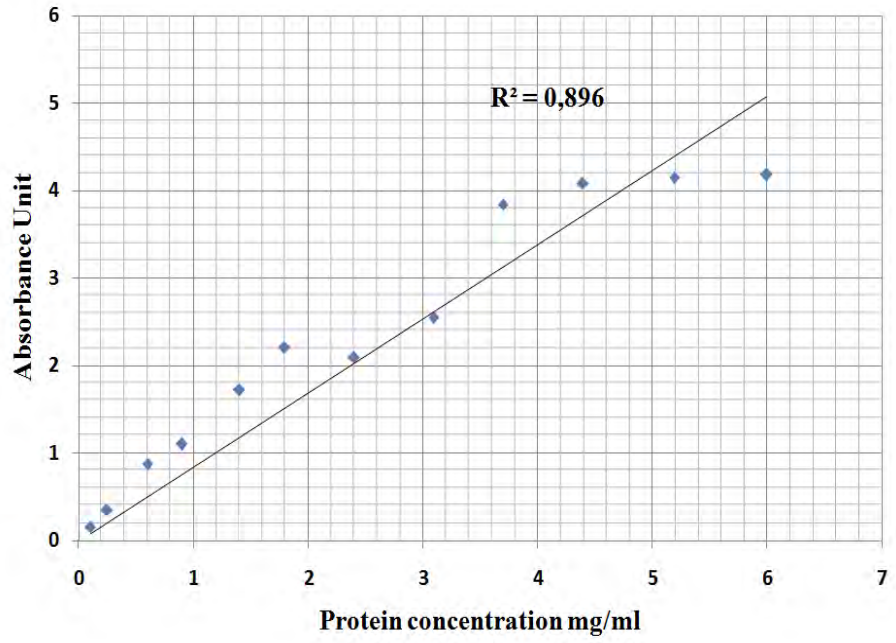

Figure 10 Calibration curves for lysozyme dissloved in 100mM NaCl obtained atUV 254 nm.

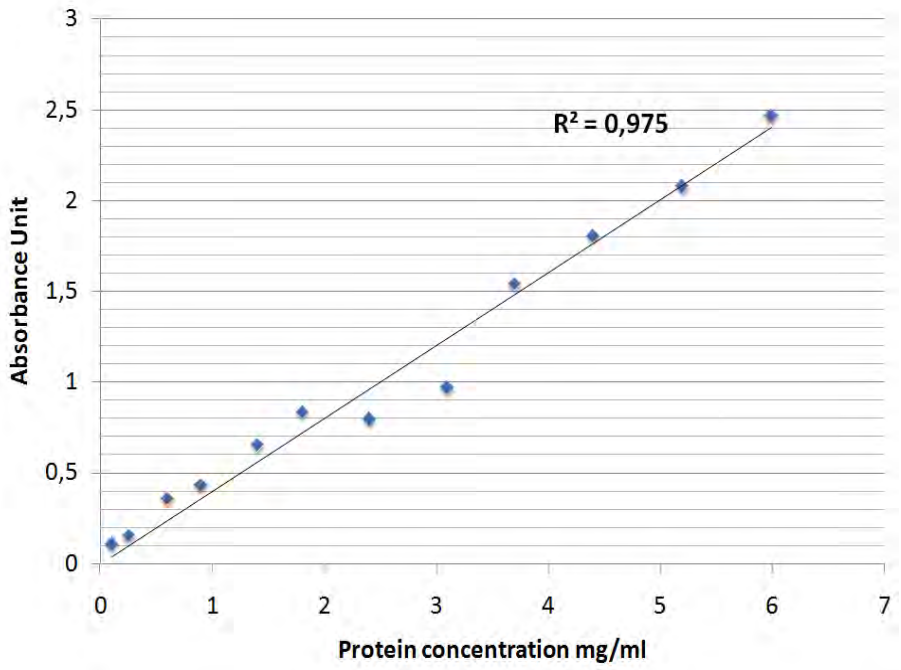

Figure 11 Calibration curves for lysozyme dissloved in 100mM NaCl obtained by UV 300 nm.

A 300nm wavelength was chosen for the measurement of the high concentration of the lysozyme while, 280nm wavelength wasused formeasuring lysozyme concentration when we have lower concentrations of the protein (less than 3mg/ml).

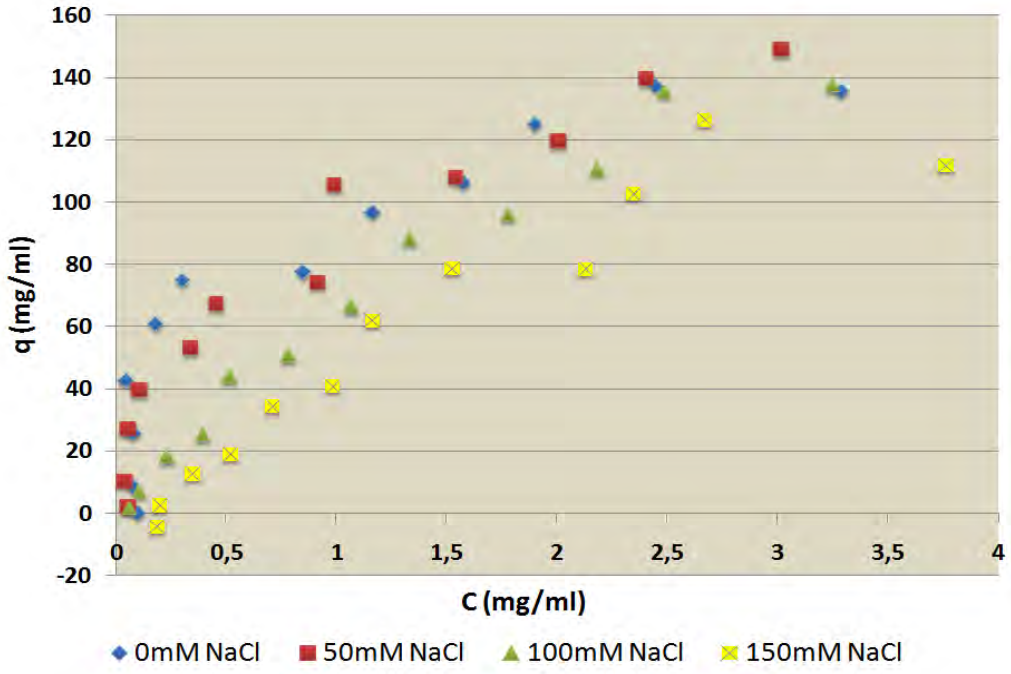

Figure 12 Adsorption isotherms of the lysozyme obtained from PreDictor™ plates Capto Sp Impress 6µl.

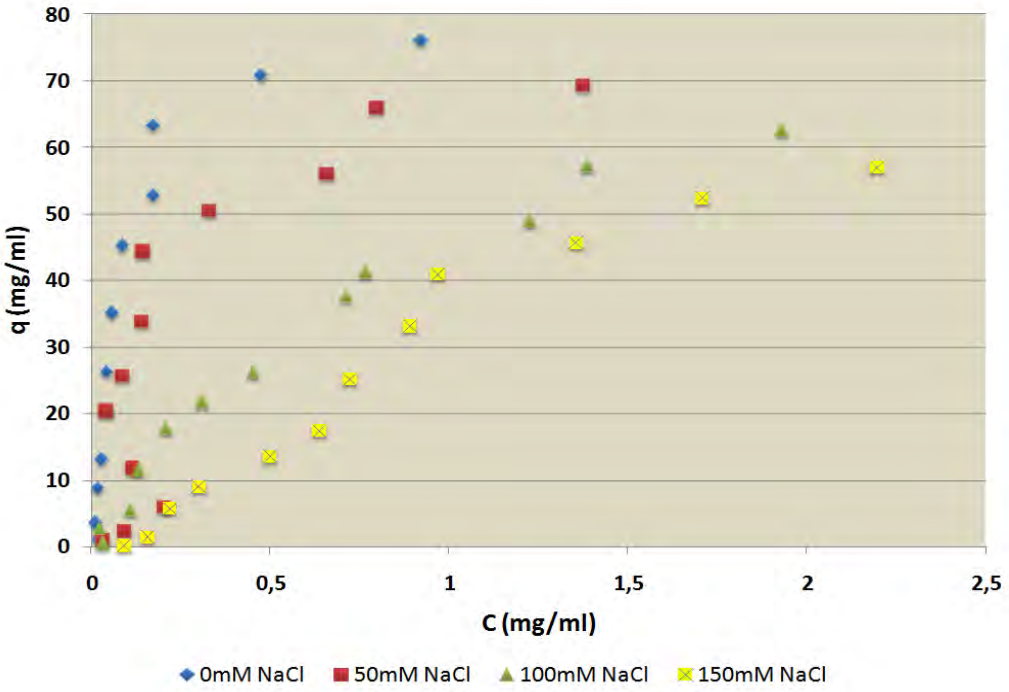

Figure 13 Adsorption isotherms of the lysozyme obtained from PreDictor™ plates Capto Sp Impress 20µl.

Adsorption isotherms of a mixture of Cytochrome C and Lysozyme

A mixture of cytochrome c and lysozyme applied to the PreDictor™ plates Capto SP Impress in order to get the adsorption isotherms taking advantage of the cytochrome c which has a unique wavelength absorption at 528 nm where lysozyme does not absorp (see figures 14 & 15). The adsorption isotherms for both proteins were compared to the AIs obtained from seperated experiments and they were very similar.

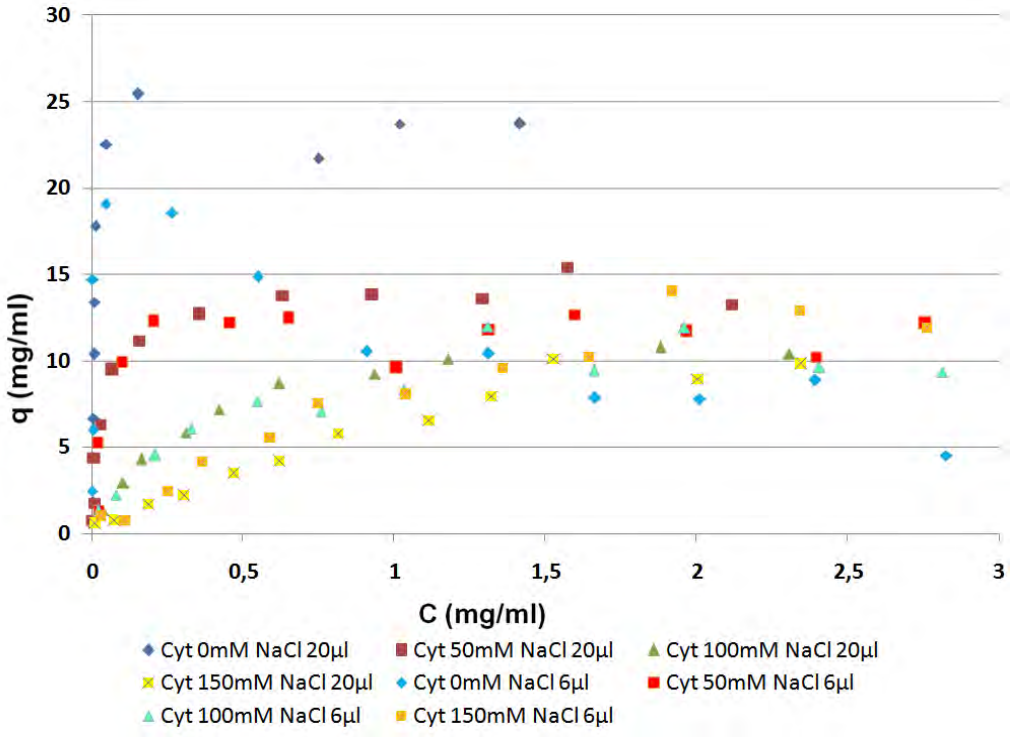

Figure 14 Adsorption isotherms of the cytochrome c obtained from PreDictor™ plates Capto Sp Impress both 6µl & 20µl.

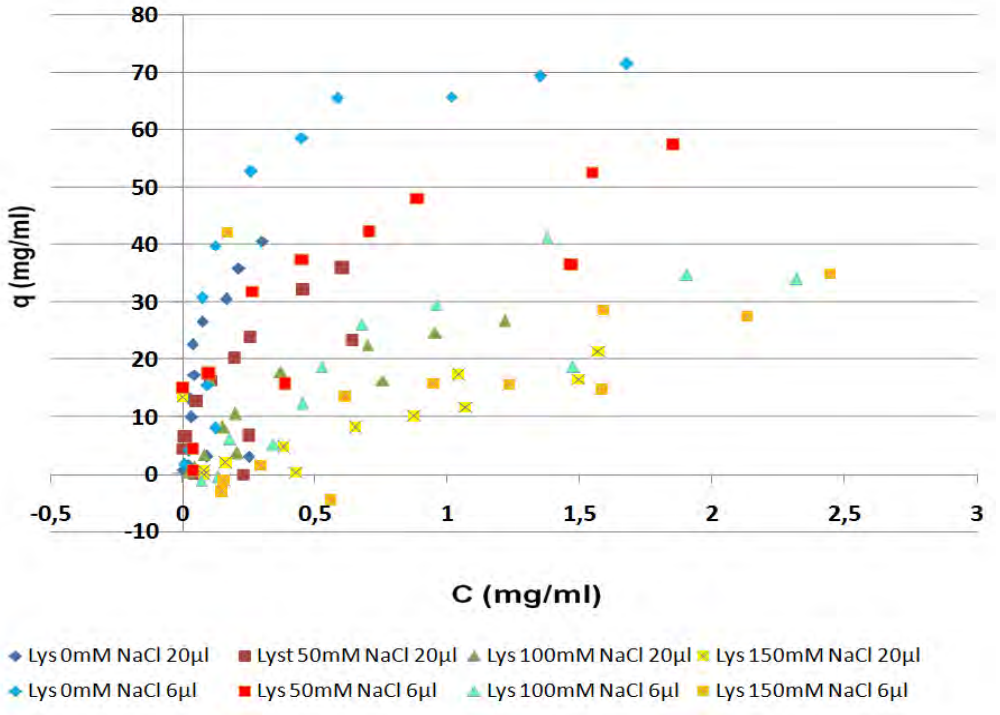

Figure 15 Adsorption isotherms of the lysozyme obtained from PreDictor™ plates Capto Sp Impress both 6µl & 20µl.

RNase A experiments

The calibration curve for RNase A has a good linearity and R^2 value approaching 1(Figure 16). RNase A adsorption isotherms were obtained from Capto Sp ImpRes at both 6 µl & 20 µl, the same as for the two previous proteins. It was possible to get the adsorption isotherms from both 6 µl & 20 µl volume resins, however, the q_{max} from the PreDictor™ plates with a 20µl volume resin was more clear than that obtained from the PreDictor™ plates with a 6µl volume resin (Figures 17, 18 and 19).

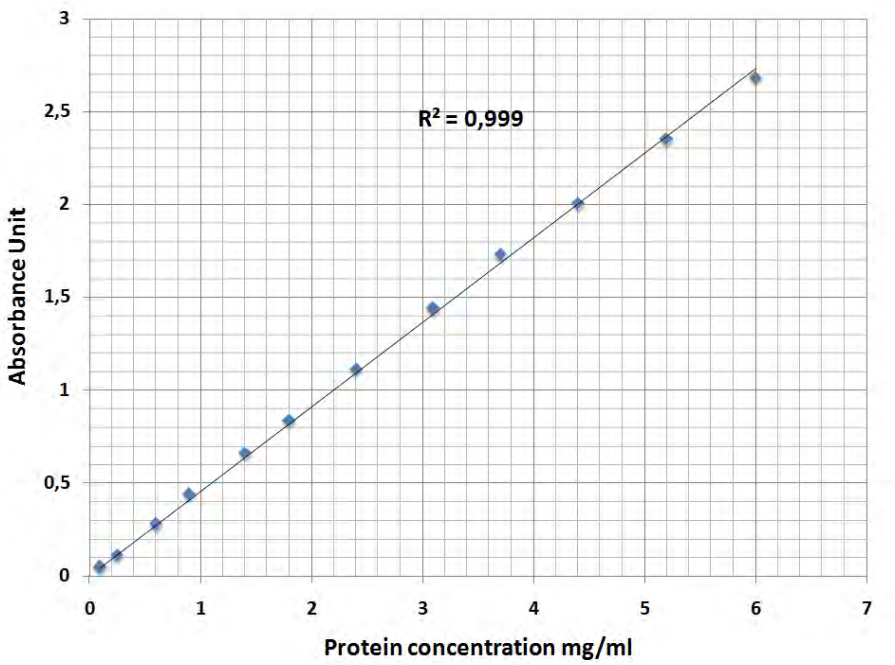

Figure 16 Calibration curves of the RNase A dissloved in 100mM NaClobtained by UV 280 nm.

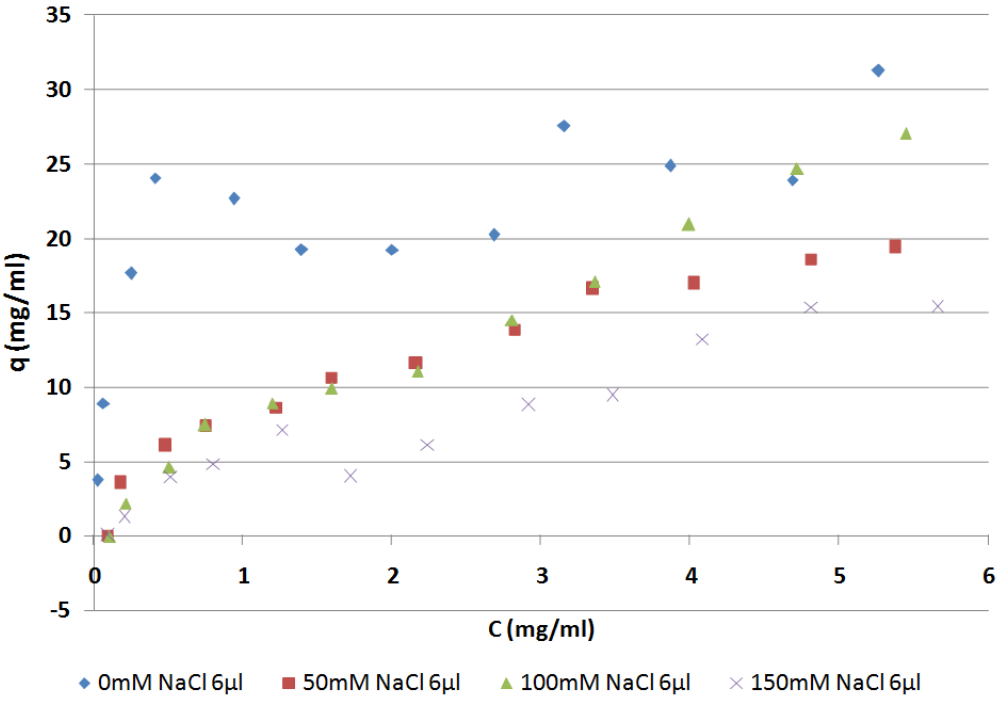

Figure 17 Adsorption isotherms of the lysozyme obtained from PreDictor™ plates Capto Sp Impress both 6µl & 20µl.

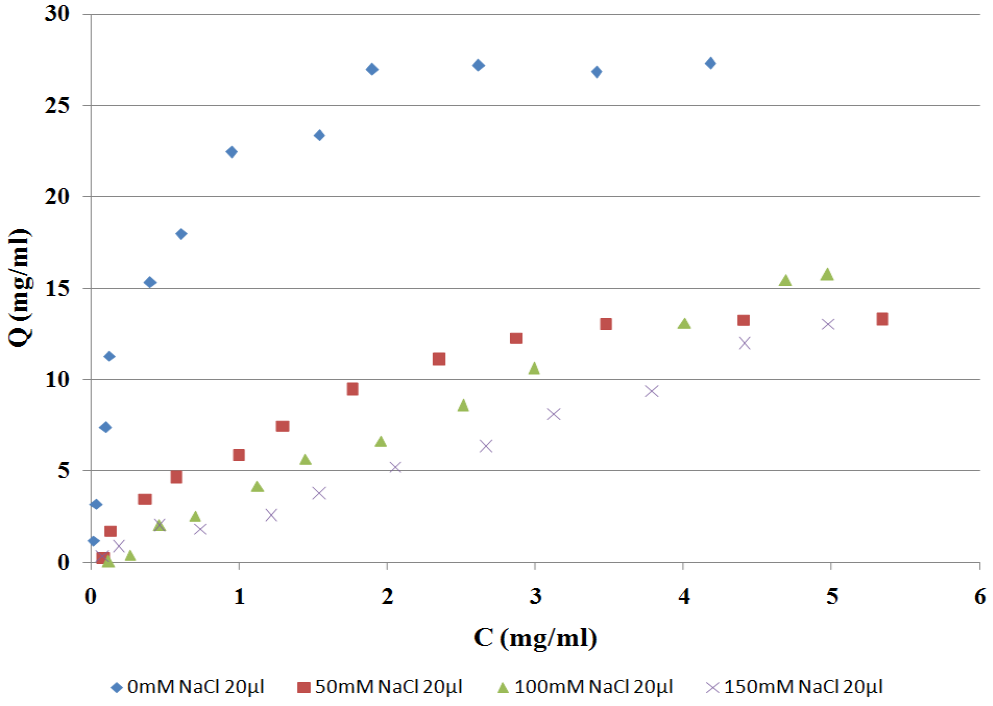

Figure 18 Adsorption isotherms of the lysozyme obtained from PreDictor™ plates Capto Sp Impress both 6µl & 20µl.

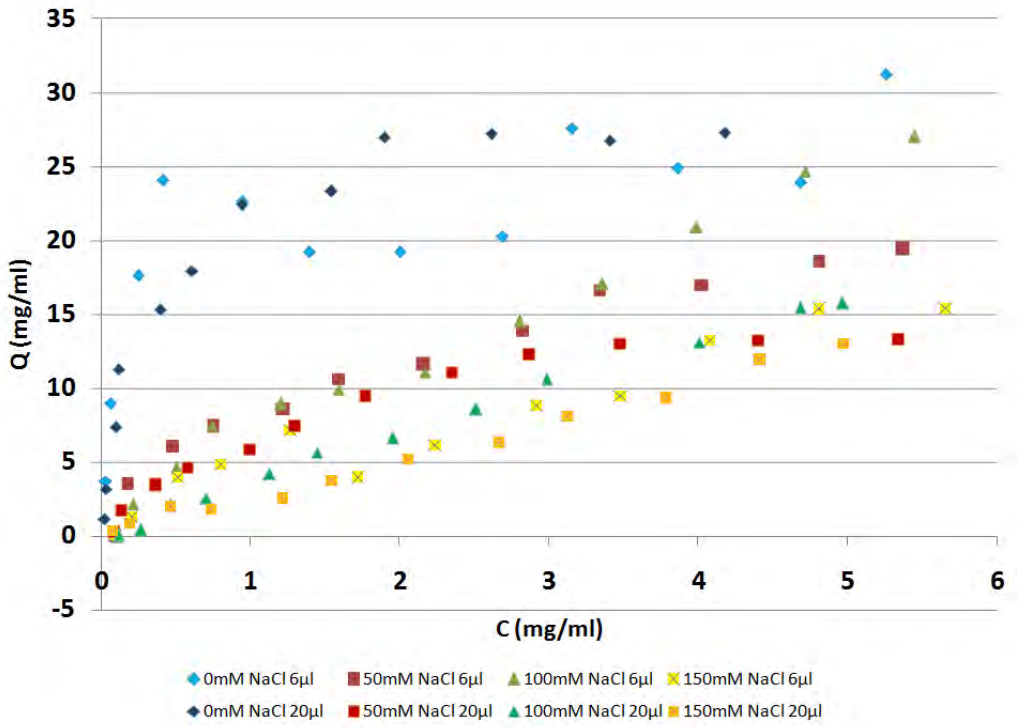

Figure 19 Adsorption isotherms of the lysozyme obtained from PreDictor™ plates Capto Sp Impress both 6µl & 20µl.

Elution studies

Elution studies were performed for all proteins individually and for a mixture of Cytochrome c and Lysozyme. The most important results were extracted from the contour plot representing the yield percentage from the elution in specific points compared to the adsorption isotherms at the same points that gave the same correlation and result. The general result from the elution studies was that some specific point like 100mM NaCl the RNase A will be eluted first, followed by Cytochrome c and Lysozyme when scaling up to the column experiment.

Cytochrome elution study

The Cytochrome c elution experiment is visualized in Figure 20. The results analyzed by the Assist software (GE Healthcare).

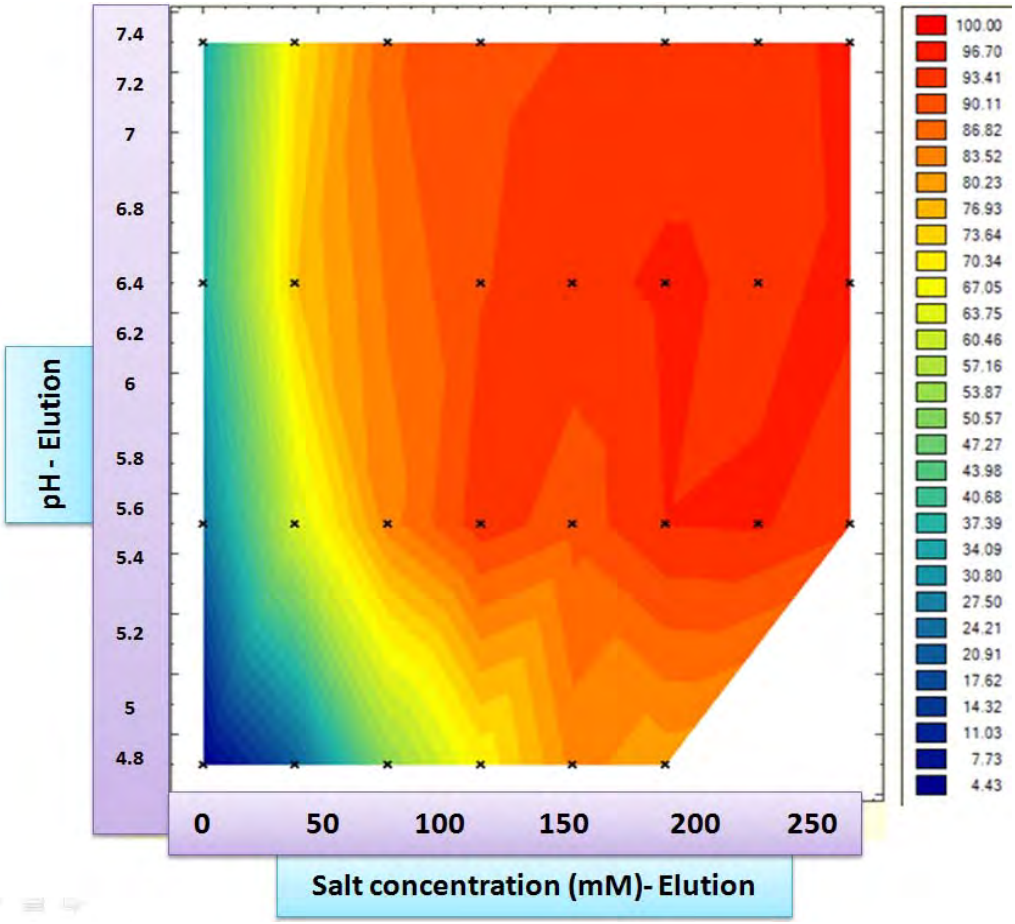

Figure 20 Contour plot of the cytochrome c eluted using 1 mg/ml of the protein(legend right showing the percentage of eluted protein).

Lysozyme Elution study

The lysozyme experiment showed that the protein can be eluted totally from the PreDictor™ plates in the pH range from 4.8 to 6.4 if the NaCl concentration is more than 150 mM (see figure 21).

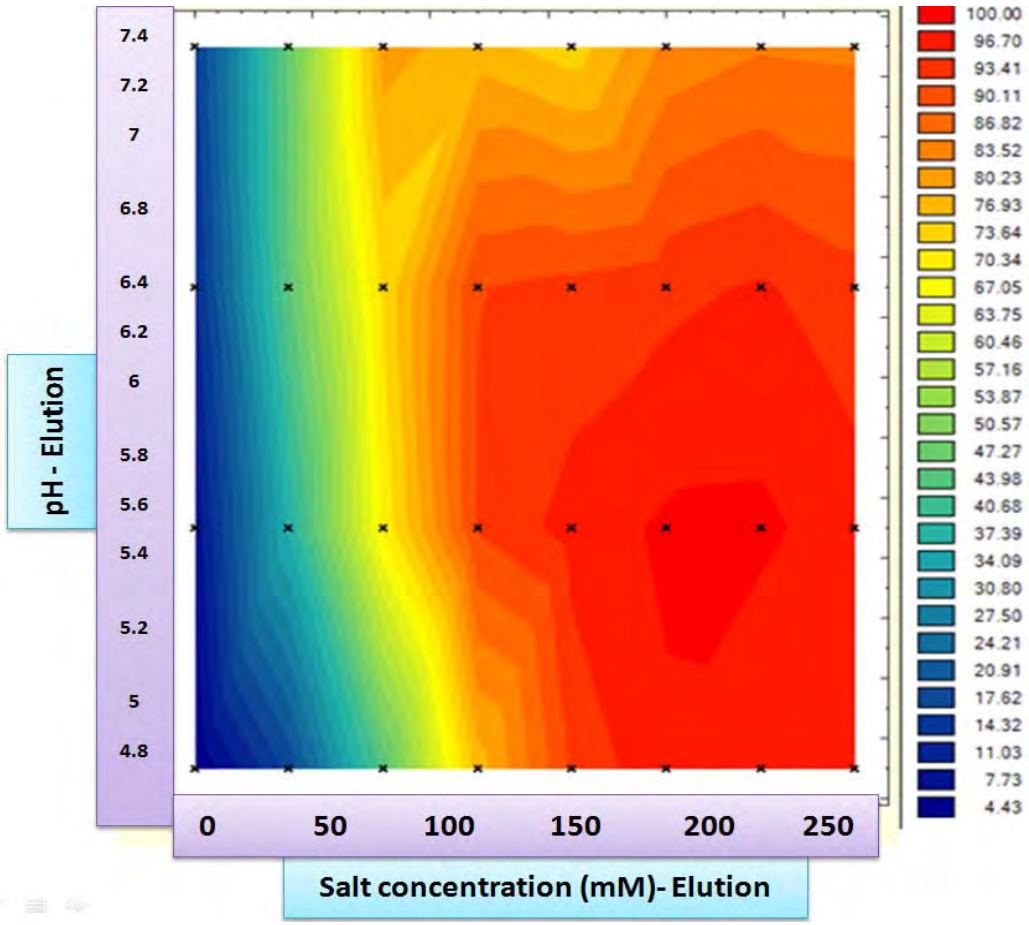

Figure 21 Contour plot of the lysozyme eluted using 1 mg/ml of the protein (legend right showing the percentage of eluted protein).

Cytochrome and Lysozyme mixture

The Cytochrome and Lysozyme mixture experiment showed the following plot. Both plots compared to the previous experiments where individual proteins have been used and they match quite well.

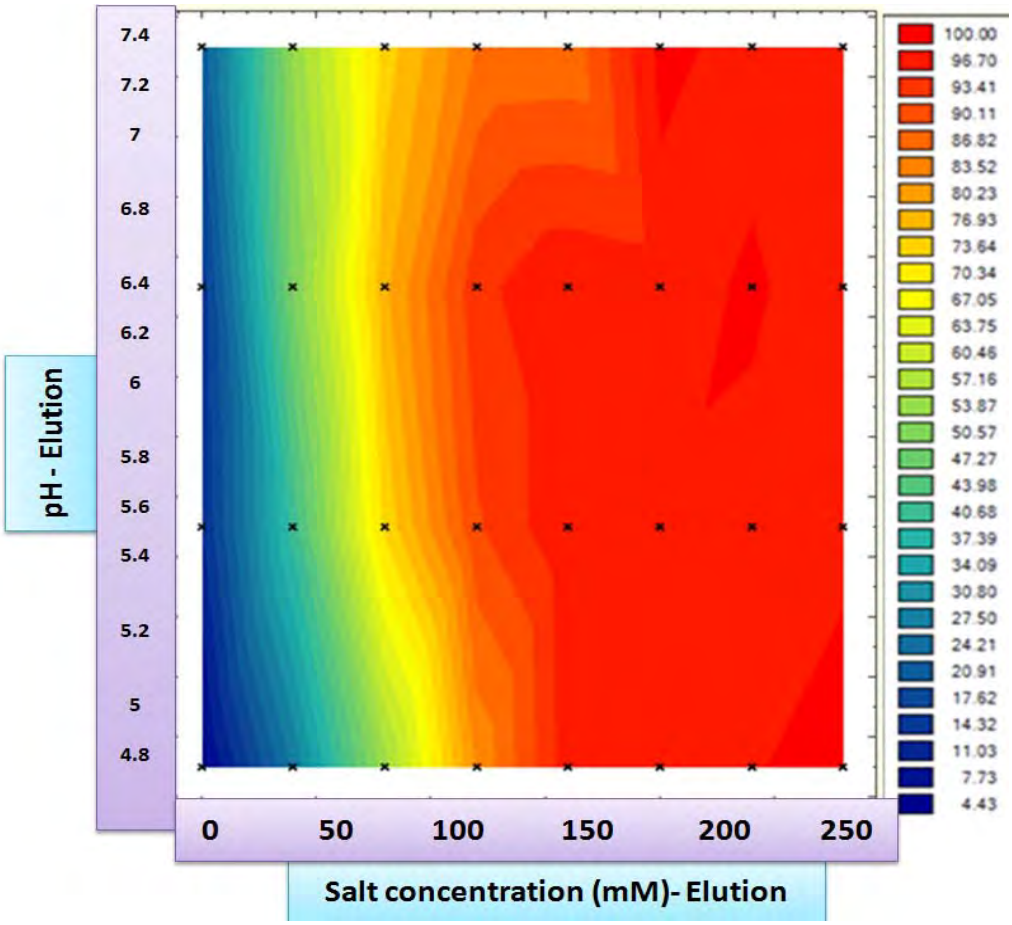

Figure 22 Contour plot of the lysozyme eluted (legend right showing the percentage of eluted protein).

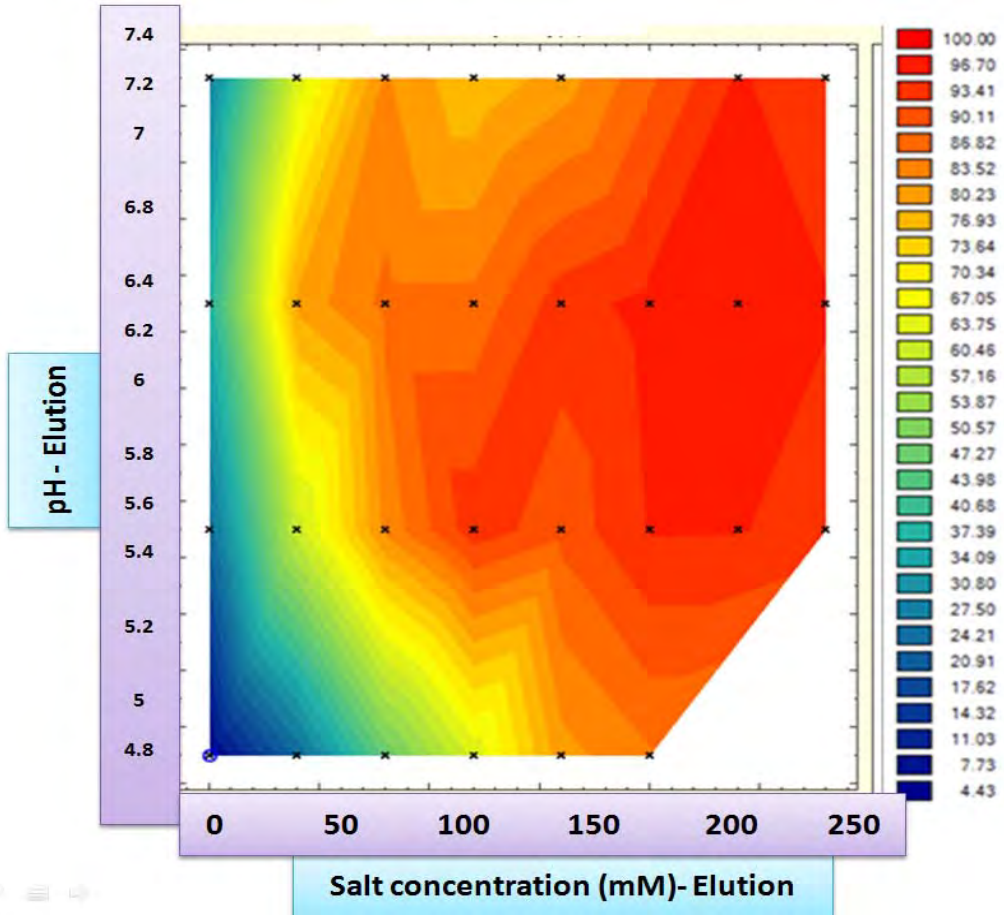

Figure 23 Contour plot of the cytochrome C elution study (legend right showing the percentage of eluted protein).

Elution study of the RNase A

The elution study of the Rnase A performed first using 1mg/ml of the RNase A (Figure 24), since the protein absorbs a lowquantity oflightatlowconcentrations- many points looked blurred and the readings were very vague and unclear (see figure 24). This result required us to repeat the experiment ata higher conecntration of 3mg/ml (Figure 25).

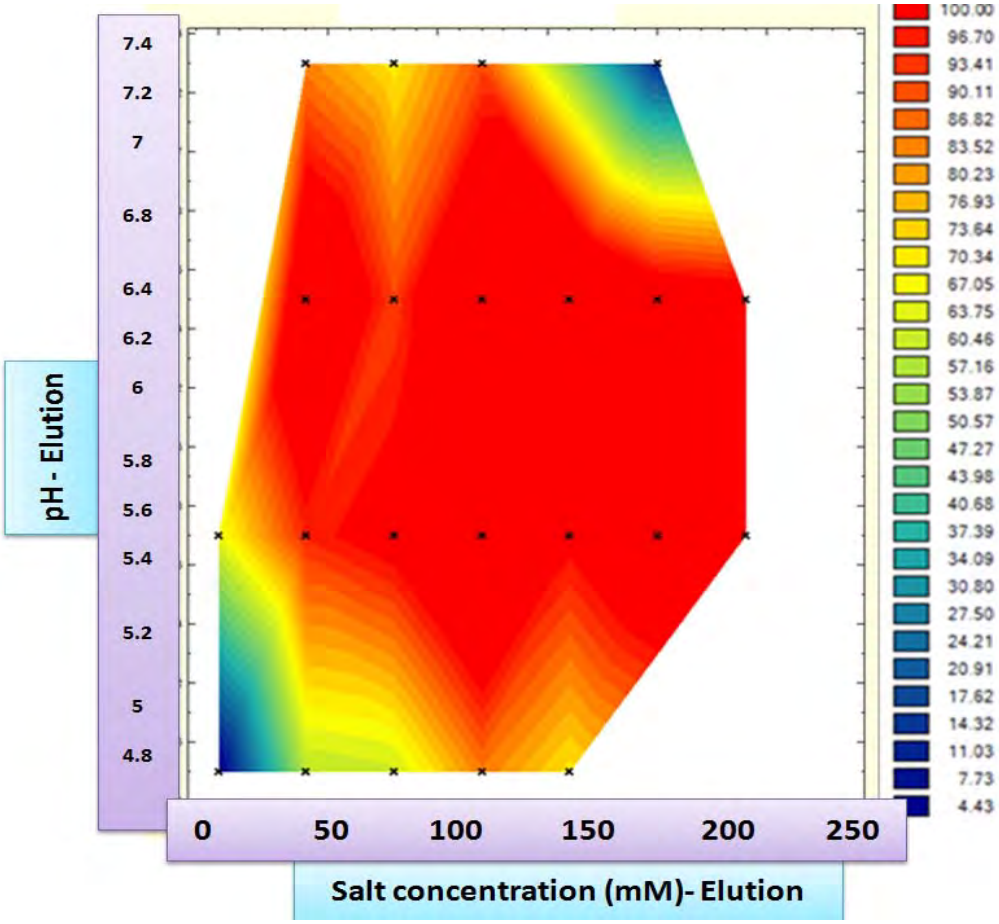

Figure 24 Contour plot of the RNase A eluted using 1 mg/ml of the protein (legend right showing the percentage of eluted protein).

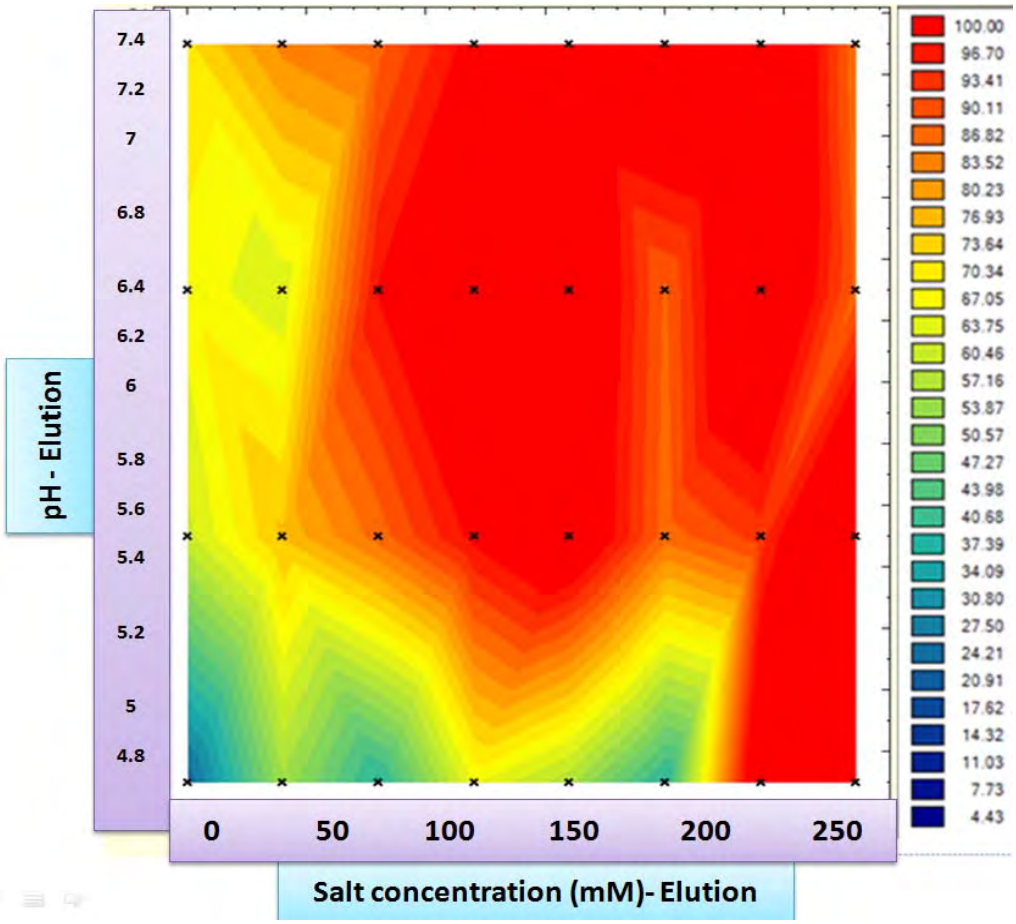

Figure 25 Contour plot of the RNase A elutedusing 3 mg/ml of the protein(legend right showing the percentage of eluted protein).

The results from the elution studies of all proteins indicated that at specific point, for example, pH 6 and 100mM NaCl concentration, the RNase A eluted first followed by Cytochrome c and lysozyme. This prediction correlates with the results obtained from the AIs experiments and could be validated using column experiment.

The previous results were collected into one diagram with a focus on one specific point at pH 6 and 100mM of NaCl where the proteins (RNase A, cytochrome c and lysozyme) have different percentage of protein yield eluted; 100%, 86% and 82%,respectively. The different yield percentage of eluted protein indicating that the proteins will be eluted sequentially when using column purification, the protein with a higher yield percentage will be eluted first from a column (see figure 26).

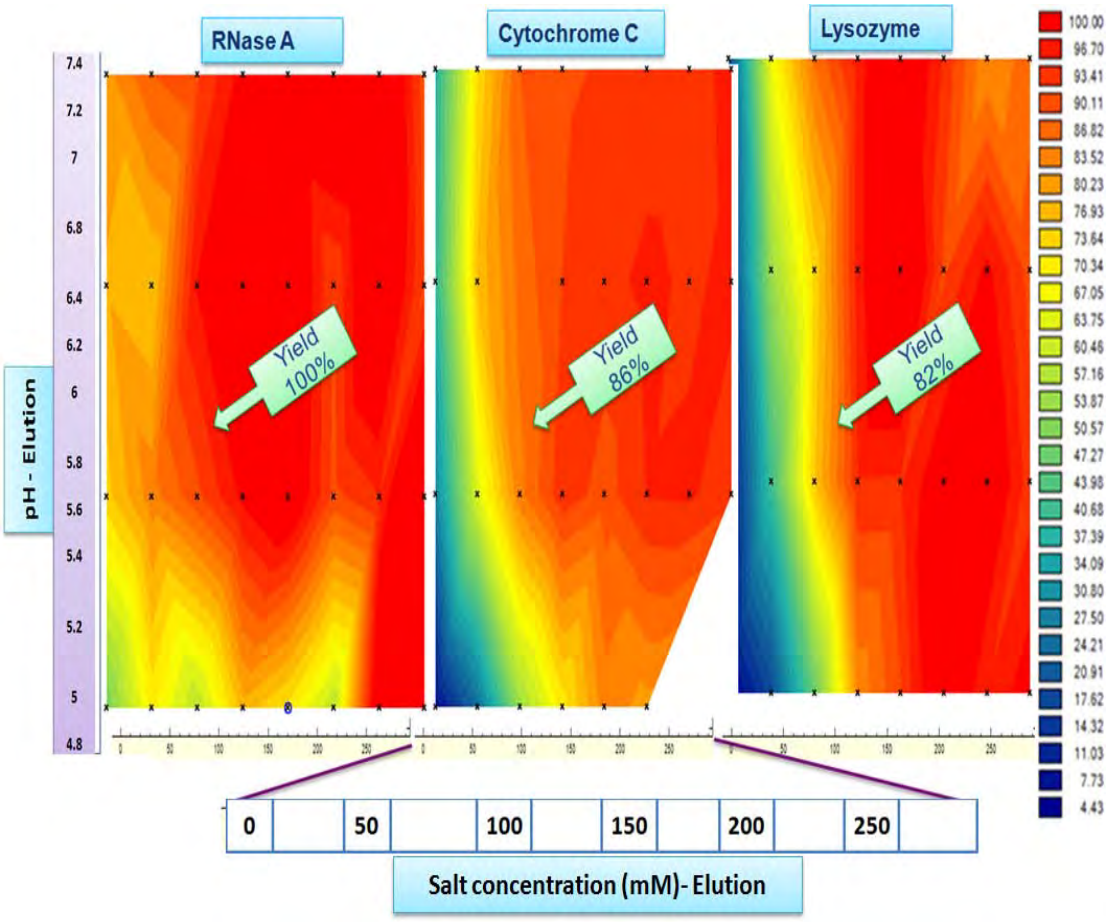

Figure 26 Combinations of previous results for all three proteins showing the yield of

Column experiments

Column experiments performed using ÄKTA avant 25 system (GE Healthcare) in order to verify the results obtained from adsorption isotherms and elution studies.

The elution performed using step, gradient and isocratic elution strategies. The isocratic elution performed using points that correlate with the adsorption isotherms and elution studies in order to verify these results. First a gradient (figure 27) followed by taking a specific point (isocratic elution) 100mM and pH 6 to confirm the previous results from adsorption isotherms and elution studies (see figure 28). The results correlated greatly with the predictions obtained from of the previously mentioned experiments (adsorption isotherms and elution studies).

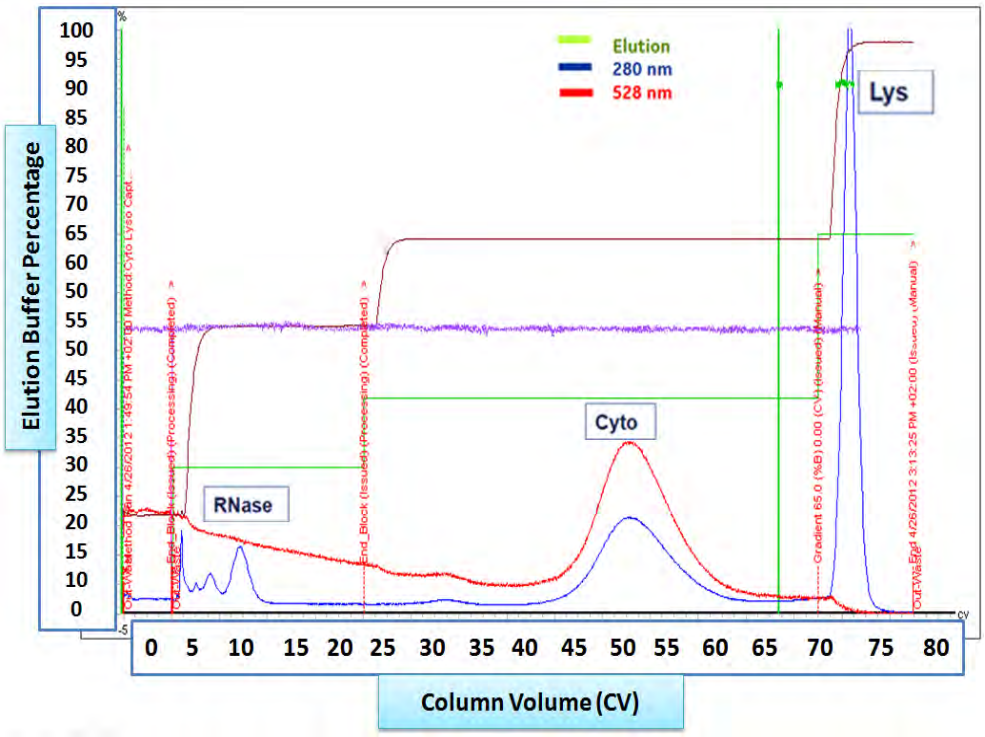

Figure 27 The gradient elution pattern (from 0 to 100% elution buffer) of the mixture of the the three proteins (cytochromec, lysozyme and RNase) .

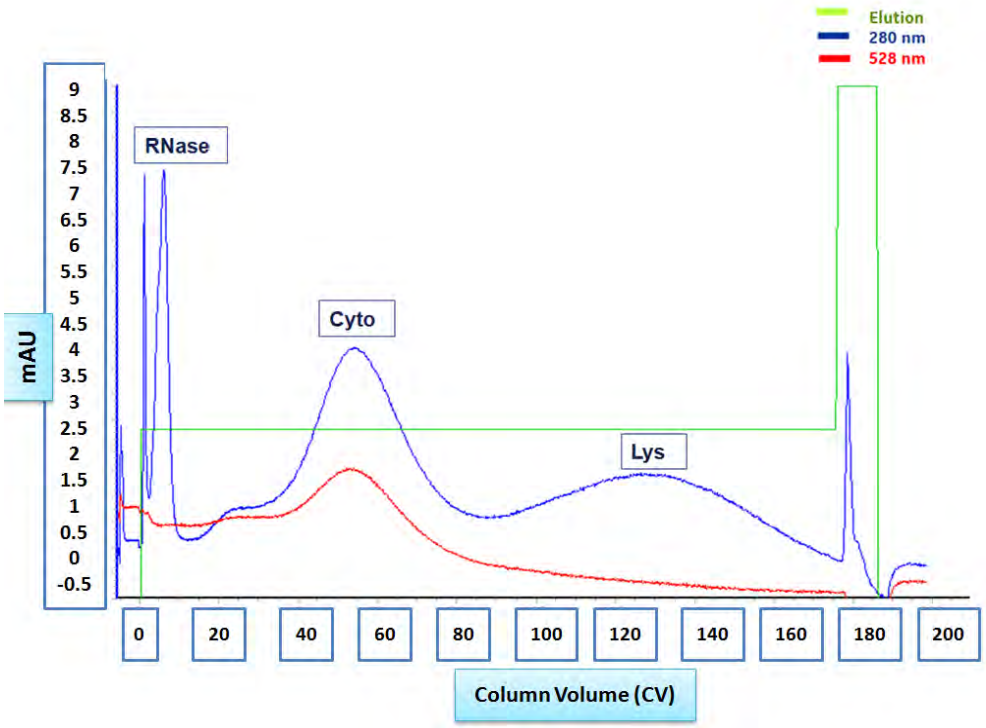

Figure 28 The isocratic elution pattern (from 0 to 100% elution buffer) of the mixture of the the three proteins (cytochromec, lysozyme and RNase) .

Conclusion

It was possible to get the adsorption isotherms of all three proteins (cytochrome c, lysozyme and RNase A) at different concentrations and varied salt concentration (ionic strength) using a single media PreDictor™ plates. All three proteins have different adsorption isotherms; this showed the possibility to separate them in different chromatographic conditions when scaling up is performed.

The elution studies for all three proteins were also successful, in addition to that they are very well-correlated with the results from the adsorption isotherms studies in the way that RNase A eluted first followed by cytochrome c then lysozyme at the end.

The protein purifications were scaled up to lab-scale small columns in ÄKTA avant 25 system (GE Healthcare) to verify the results obtained from both previous approaches. The mixture of three proteins eluted by an isocratic elution using specific conditions obtained from both previous approaches generated three peaks where RNase A, cytochrome c and lysozyme eluted respectively. The results correlated very well with both adsorption isotherms and elution study results.

Both approaches are currently used in biopharmaceutical industries for high throughput screening (HTS) of chromatographic conditions, however, there is no such a comparison between the two methods, especially for the PreDictor™ plates.

As a final point, both selectivity approaches (adsorption isotherms and elution approaches) are acceptable as methods for determination of chromatographic selectivity leading to the same result, taking into consideration that they provide quite different information.

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