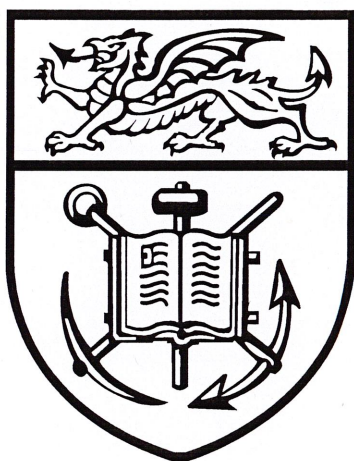


FOULING AND REGENERATION OF SILICA ADSORBENTS FOR PROTEIN SEPARATION

By

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**A Dissertation Submitted to the University of Wales for the Degree of
Master of Science**

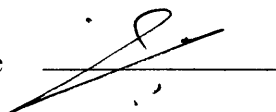
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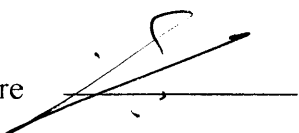
STATEMENT

This Dissertation is a description of the work carried out in the Department of Chemical Engineering, University of Wales, Swansea, between October 1997 until January 1999.

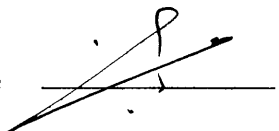
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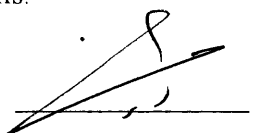
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My ever warmest gratitude and appreciation are forwarded to my beloved parents, sisters and brothers.

SUMMARY

This Thesis describes an experimental study of the fouling of a chromatographic adsorbent used to separate proteins. Experimental procedures have been designed for adsorption, desorption and regeneration of a rigid, silica-based, weak anion exchanger adsorbent, BioPrep WAX 40/300, using Bovine Serum Albumin (BSA) as a model protein.

Adsorption isotherms of BSA have been obtained in the experiments and fitted by a linearised Langmuir isotherm. Also studied was the influence of the various parameters on the adsorption isotherms. The salt concentration in the buffer solution used in the adsorption stage has been decided to be 2.5 g/l of sodium chloride. At this concentration the capacity was better defined and it reached equilibrium fast. The time for attainment of equilibrium was 24 hours. 23 g/l sodium chloride concentration was the minimum concentration to desorb protein from the adsorbent.

Experiments were made on study the procedure for measuring the UV absorbance. The results show that the optimum filling of the UV/VIS spectrophotometer cell was 75%. Supernatant liquid from centrifuging was best transferred to the cell by pouring.

Fouling decreases the capacity parameter q_m of the Langmuir isotherm. Regeneration was studied with 6-10 M urea. Experiments using tyrosine were developed to prove that urea had no effect on the specific absorption of BSA and that urea does not itself absorb at 254 nm wavelength. The regeneration process was not very effective and adds little to the amount of protein already removed by desorption with sodium chloride. The regeneration was only slightly more effective when the urea concentration was increased. There was no evidence that microbiological organisms grew on the fouled protein within a timescale of 28 days.

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Chapter 1 Introduction

1.1 The importance of separating proteins

The need for pure proteins, particularly in the fractionation of human blood proteins and the recovery of high-value proteins from cheese whey and animal blood for use in veterinary medicine, in food stuffs, and as substitutes for human plasma fractions, are the main reasons why we are interested in isolation of pure proteins.

The aims of removing proteinaceous materials from any aqueous solution are either for their subsequent use in different food formulations utilising their nutritional value and functional properties, or because they are considered undesirable contaminants.

The problem arises now of providing a method for isolating the protein from such a solution in maximum purity without affecting its native structure.

1.2 Downstream processing

Downstream processing is the general term used to describe separation processes for recovery of biological products, that act at some stage in their production. It encompasses a wide and diverse range of products and processes.

A typical bioprocess includes a bioreaction stage (fermentation or bioreactor) and subsequent product recovery stages. The particular separation techniques useful for any given bioprocess depend not only on the location of the product (intracellular or extracellular), the size of the molecule, charge and solubility of the product but also on the scale of the process itself and the product value.

The operation sequence through which a bioreactor broth must pass for a highly purified product is typically as follows:

1. Removal of Particulate (insoluble).

Common operations here are filtration, centrifugation, and/or settling /sedimentation /decanting.

2. Primary Isolation.

Solvent extraction, sorption, precipitation and ultrafiltration. During primary isolation, desired product concentration increases considerably, and substances of widely differing polarities are separated from the product.

3. Purification.

These operations often select for impurity removal as well as further product concentration. Approaches include fractional precipitation, many kinds of chromatography and absorption.

4. Final Product Isolation

The last steps must provide the desired product in a form suitable for final formulation and blending, or for direct shipping. Processes here including centrifugation and subsequent drying of a crystallised product, drum or spray drying, freeze drying (lyophilization), or organic solvent removal.

1.3 Chromatography

It is difficult to separate mixtures between components of closely similar physical and chemical properties. Many mixtures, which are difficult to separate by other methods, can be separated by chromatography.

There are two basic kinds of chromatographic procedures: adsorption and partition. Adsorption techniques have a higher capacity than partition techniques and should, therefore be a first choice, especially in the initial stages of a purification scheme where there is more volume to process and more contaminants to remove.

Chromatographic adsorption techniques for proteins include ion exchange, hydrophobic interaction and affinity chromatography.

1.3.1 Gel filtration

In gel filtration, molecules are partitioned between the stationary phase (separation medium) and the mobile phase (solvent system). Large molecules, which cannot penetrate into the pores of the stationary phase, are excluded from the liquid inside the diffuse into a limited proportion of the stationary phase, while small molecules diffuse freely into the stationary phase.

1.3.2 Affinity chromatography

Affinity chromatography is an exquisitely selective technique that takes advantage of biospecific interaction that occurs in nature. Because the desired degree of purity can sometimes be achieved in a single step, affinity chromatography is an excellent purification tool for biotechnology.

Flow rates used in affinity chromatography are high, columns are short and wide and it is an easy to perform technique. There are many types of matrices and many chemistries available for coupling liquid to the gel matrix.

1.3.3 Hydrophobic interaction chromatography (HIC)

HIC is another adsorption technique. This technique takes advantage of the existence of hydrophobic 'pockets' on the surface of many proteins and peptides. Generally, the affinity of proteins for separation media surface increases as the chain length of the ligand increases. The driving force of the interaction between the protein and the functional group is an increase in entropy.

HIC is quite useful in the early stages of purification since it is compatible with high salt that may be used in an initial precipitation step. High ionic strengths, salts which have a high degree of 'salting-out' ability, and decreased pH, promote the binding of hydrophilic as well as hydrophobic proteins. It is often possible to precipitate the bulk of the contaminants and bind the components of interest at a salt concentration just below that which causes them to precipitate.

1.3.4 Ion exchange

In ion exchange chromatography, proteins and peptides are separated due to their charge differences. This technique is well suited for large scale applications because it has a high capacity, (dynamic capacity typically 30g total protein per litre of adsorbent), is selective, has a concentrating effect, and can be used to isolate many proteins in one step. Flow rates are high, and short, wide columns are used. Ion exchange is chemically a milder technique for proteins than affinity or hydrophobic interaction chromatography and is less likely to denature the protein. It is therefore the most often need of the various chromatographic methods for proteins.

It is important to select an ion exchanger that can be used in the pH range in which the molecule of interest is stable. In selecting the ion exchanger the following should be considered. Capacity for the molecules of interest, ability of the matrix and functional group to withstand high flow rates and the ability of the separation media to be cleaned in place. In this work we have used a weak anion exchange form of the Whatman BioPrep series of adsorbents.

1.4 Soft gels versus rigid adsorbents

1.4.1 Adsorbent materials

There are some requirements applying to the particles which are used for chromatographic separation of protein. These requirements are related to the size and function of proteins and the need to bring the protein molecules into contact with as much active (binding) surface on the adsorbents as possible. Thus, the requirements are:

1. The particles will preferably be hydrophilic since proteins are more stable in aqueous environment.
2. The particles will be highly porous because proteins are large molecules and for the concern of adsorption capacity and diffusion kinetics, the size as well as the number of pores are of great importance.

3. The particles will also preferably be rigid and incompressible, where the rigidity of the particles permits high operating pressure, high flow rate, and correspondingly rapid separations.
4. The materials from which the particles are made should be chemically inert so that they are insoluble and will not react except in the specific way for which they have been chosen.

1.4.2 Soft gels

The adsorbent particles most often used hitherto for liquid chromatography (LC) separations of proteins have been soft matrices, e.g., agarose, cellulose and dextran. Soft gels are hydrophilic (good for adsorbing protein) have large surface area, large porosity and withstand large range of pH. However, these soft materials cannot withstand high pressure drop, and consequently, low flow rates or large particles need to be used. This implies longer processing times and thus higher operation cost. Another disadvantage is that soft gels tend to swell and contract in size as the salt concentration and pH is cycled during operations. This produces an increase pressure drop across the column and reduces its life.

1.4.3 Rigid adsorbents

Small rigid particles have recently developed as LC media for large-scale protein separations. Rigid chromatographic adsorbent can be classified into the following four categories:

1. A synthetic organic polymer available in two forms, hydrophilic and hydrophobic.
2. Synthetic polymer/biopolymer composites, e.g., polyacrylamides and agarose, which are popular in affinity applications.
3. Inorganic, such as macroporous silica. They are more rigid than synthetic polymer but tend to dissolve in aqueous solution above pH 8.
4. Inorganic coated with synthetic polymers or biopolymers. The adsorbents have both rigidity and stability over a wide range of pH (2-12).

The advantages of rigid adsorbent are that they allow higher process throughputs and longer column life. Additionally, their structure, shape, particle size and pore size can

be easily characterised. The structure of adsorbents is an important parameter in the theoretical modelling of the kinetics of protein adsorption, since the rate of mass transfer, pore diffusion and film mass transfer are closely related to the structure of the particles. Smaller particles with wide pores will result in faster adsorption.

Amongst the four categories and the four requirements the most successful of materials currently appear to be synthetic organic polymers and especially hydrophilically coated macroporous silica. The BioPrep ion exchange adsorbent that we have used in this work is of this type.

1.5 Fouling of the adsorbent by protein

Small organic molecules normally adsorb/desorb rapidly and easily because they usually have only one chemical functional group by which they attach to the surface.

Proteins, however, are high molecular weight polymers of amino acids possessing a large number of functional groups. When protein adsorbs on the surface the initial attachment may be through one or two groups to the protein surface but in time some of the protein molecules may re-orient themselves and become attached to many points. Such molecules may then be much more strongly attached and may be difficult to desorb from the surface.

The ion exchange process separating the protein is a batch-wise process, involving repeated adsorption/desorption cycles. If the desorption process is incomplete some protein is left behind on the adsorbent and this may build up in time over several cycles to reduce the capacity of the adsorbent. This is described as fouling of the adsorbent by protein.

1.6 Literature survey

Proteins play a major role in the fouling. Protein interactions with solid surfaces have been studied for decades, and several thorough reviews are available. Numerous observations have been made indicating the presence of weakly and tightly bound proteins: for example, rinsing a surface after protein contact does not remove all of the protein, yet some of the remaining protein is removable during a second, longer buffer rinse (Andrade 1985, Norde 1986, Horbett and Brash 1987). Elwing (1988) has described some general concepts about what should control protein behaviour at solid surfaces as listed in Table 1.1. Unlike contact-surface and solution-property influences on fouling, the molecular origins of protein surface activity are not readily observable.

Protein molecules are assumed, in general, to change conformation to a greater extent on hydrophobic surfaces than on hydrophilic surfaces. This is due to the presence of hydrophobic interactions between the solid surface and hydrophobic (pockets) in the protein molecule. This interaction gives the molecule an extended structure, covering a relatively large area of the surface. The repulsive force normally acting between native protein molecules is probably decreased for such conformationally changed molecules (Elwing 1988).

Table 1.1 Some factors that can affect protein behaviour at solid surfaces

Protein characteristics:	Isoelectric points; net charge and charge distribution; size, structure and stability in solution; placement and nature of hydrophobic patches.
Solution characteristics:	pH; temperature; ionic strength; solute types and concentrations; hydrodynamics.
Solid-surface characteristics:	Composition; chemical and energetic heterogeneity; topography; electrochemical potential; hydrophilic/hydrophobic balance; temperature.

Many workers have reported that protein can adsorb onto a surface in more than one layer (Arnebrant 1985). Their results showed that on clean hydrophilic surfaces a thick, highly hydrated layer is obtained, which can be partially removed by aqueous buffer rinsing. They suggested that the protein adopts a bilayer formation on the surface, with the bottom layer unfolded and attached by strong polar bonds to the surface and not removable by rinsing.

Luey (1991) has made some observations from adsorption equilibrium, showing how various conditions affect the adsorbed mass of protein. The effect of pH and ionic strength on protein adsorption depends on which type of interactions predominate (e.g., van der Waals interactions). The relationship between adsorbed mass and changes in pH and ionic strength becomes intimately tied to protein conformational stability. In general, pH and ionic strength conditions that lead to a less stable conformation for the protein in solution will lead to an increased adsorbed mass, assuming that the protein molecules is more stable on the solid surface.

Another observation of importance (McGuire 1991) is that protein adsorption is often an apparently irreversible process. The adsorbed mass remains constant or decreases very little when the solution in contact with the solid surface is depleted of proteins. This irreversibility is more pronounced as protein-surface contact time increases.

1.7 Scope of this thesis

Ion exchange chromatography is widely used for protein separations and rigid adsorbents are increasingly being used for improved performance. The process operates by repetitive batch adsorption. However, there is a problem with this repetitive process that the capacity falls with increasing the number of cycles. Most of the workers involved in studying the performance of rigid adsorbents have faced this problem (Hayek 1994, Li 1997 and Ashmead 1998), which necessitated use of virgin adsorbents. In practical separations, however, regeneration is needed periodically.

According to our literature survey in Section 1.6 little quantitative work has previously been published on the fouling and regeneration processes particularly on rigid adsorbents.

This thesis aims to study the effect of fouling by protein and the way to regenerate the adsorbent. Some of the previous studies relevant to fouling have shown that the capacity of the adsorbent for protein decreases, but the size of this decrease has not been studied quantitatively. In this work we have used the changes in the isotherm capacity parameter as a measure of the progress of fouling and subsequent regeneration.

The choice of regenerating agent needed to be considered. Sodium chloride was adopted as a common agent for desorption but is not strong enough to remove all fouling protein. Amongst stronger agents suitable for regeneration, sodium hydroxide is often used with soft gels, but is unsuitable for rigid silica adsorbents because of its high pH. Whatman, the manufacturer of the rigid BioPrep silica adsorbent used in this study, does not recommend any particular regenerating (chaotropic) agent, but J.T. Baker (1990/91) recommend 6-10 M urea for similar silica adsorbents. We therefore decided to study the effect of urea as a regenerating agent. Procedures for adsorption, desorption and regeneration have been developed.

This thesis also considers many factors involved in the isotherm measurements, such as the way to fit the Langmuir isotherm, decide a standard value of sodium chloride concentration in the buffer solutions used for adsorption, the minimum amount of solution in the spectrophotometer cell, the effect of time on attainment of equilibrium and the minimum sodium chloride concentration to desorb protein.

Chapter 2 Experimental Methods

2.1 Materials

2.1.1 Adsorbent

Whatman Partisil BioPrep (40/300 WAX) adsorbents have been used in this study. The particles are irregular in shape and therefore have a high surface area per unit weight. Partisil BioPrep WAX is a weak anion exchanger with a Dimethyl Amino Propyl group (Figure 2.1).

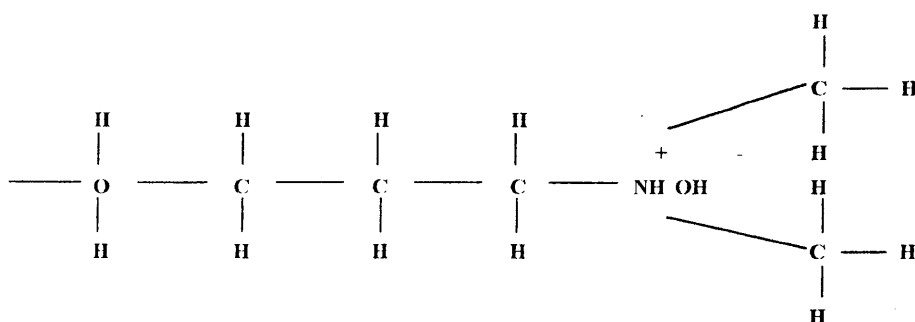


FIG. 2.1 Functional group bonded to the porous silica matrix

Whatman provide three sizes of particle in the Partisil BioPrep range (BioPrep 10, 20 and 40) and they have following particle size distributions (Whatman HPLC Product Guide):

Table 2.1 Particle size distributions of BioPrep Media (Whatman HPLC product guide).

Medium	Mean Particle Size, μ m	Particle Size Distribution, μ m
BioPrep 10	10.5	5 to 15
BioPrep 20	16.5	13 to 24
BioPrep 40	50	35 to 70

- Partisil BioPrep 10 is designed for high efficiency separations.
- Partisil BioPrep 20 is designed for high efficiency with lower operating pressures.
- Partisil BioPrep 40 is designed for lower efficiency separations with low back pressures.

2. Experimental Methods

Partisil BioPrep media are available in three pore sizes; 75, 150 and 300 Å and the corresponding surface area and pore volumes are shown in Table 2.2.

Table 2.2 Pore Size of Partisil BioPrep Media

Mean Pore Size, Å	Surface Area, m ² /g	Pore Volume, cm ³ /g
75	485	0.76
150	300	1.1
300	275	1.8

The pore size should be selected in accordance with the solute to be separated. 75 Å for small molecules with a molecular weight between 2,000 to 50,000 daltons, e.g. peptides and 300 Å for large molecules, e.g. proteins, with a molecular weight of 50,000 to 500,000. Although pore sizes have been designed to accommodate a wide range of molecular weights, Whatman recommends that a mean pore volume of 300 Å be used for macromolecules (MW 50,000 to 500,000); this was used in the current study. Whatman showed that the 300 Å media has a surface area of 275 m²/g and a pore volume 1.8 cm³/g.

2.1.2 Protein

The Bovine Serum Albumin used in all experiments was Fraction V powder of purity 98% (Sigma A7906) prepared with charcoal treatment and extensive dialysis to reduce low molecular weight substances. The pH of a 1% (w/v) aqueous solution was approximately 7. It is assumed in all experimental work that BSA has a molecular weight of 66500 (Cantor and Schimmel 1980) and an isoelectric point of 4.9 (Lehninger, Nelson and Cox 1993). Experiments were conducted at pH 7.5, thus giving the BSA molecules an overall net negative charge, so that they will undergo ion exchange with an anion exchanger.

2. Experimental Methods

2.1.3 Buffers

The purpose of using buffers in protein adsorption is to provide a suitable environment for the optimum adsorption to take place. The properties of the functional groups (proteins and ion exchange) may be affected by both pH and salt concentration of buffer solutions used. The buffer components themselves are charged species which may interact with the protein and ion exchanger resulting in reduction of the binding capacity of the adsorbent, so that buffer materials should be chosen carefully to eliminate those interaction. This is particularly important for hydrophobic interactions where the adsorption capacity is strongly affected by the type and concentration of buffer solutions. The buffers were prepared by dissolving the salts (either 2.5 g/l or 5.0 g/l NaCl and 0.02 M Tris (hydroxymethyl)methylamine) in de-ionized water, which was produced in the Department by membrane filtration and reverse osmosis to remove solid particles and unwanted ionic species. The pH of the buffers was adjusted by titrating with HCl or NaOH solutions to 7.5.

2.2 Equipment

2.2.1 UV/VIS Spectrophotometer

The Unicam 8625 Series UV/VIS Spectrophotometers have been designed to measure the absorbance and concentration with a specification:

1. Wavelength range between 195 to 1100 nm.
2. Wavelength setting speeds 100 nm/s and bandwidth 8 nm.
3. Wavelength accuracy: +/- 1 nm.
4. Wavelength repeatability: +/- 0.5 nm.
5. Environmental operating ranges: temperature (5 to 40 °C) and humidity (20 to 80%).

2.3 Experimental procedure

2.3.1 Warm-up of UV/VIS Spectrophotometer

1. Use Edit button on the upper panel to set wave length to 254 nm.
2. Wait until the flash on the right hand side of the screen stops then press upper arrow until the screen shows Lo ABS, then press zero and wait for 20 minutes.

2.3.2 Make buffer solution

1. Add 0.02 M of Tris (hydroxymethyl) methylamine (2.422 g/l) to the beaker.
2. Add 2.5 g/l or 5.0 g/l sodium chloride to the beaker (In some of the experiments we used 2.5 g/l NaCl and the other we used 5.0 g/l NaCl).
3. Add 600 ml of reverse osmosis water to the beaker.
4. Use pH detector probe to measure the pH. Replace the probe in potassium chloride solution.
5. Titrate to pH 7.5 with hydrochloric acid or sodium hydroxide.
6. Add this solution to 1 litre flask and add more of reverse osmosis water until it reaches 1 litre level, and mix.

2.3.3 Calibration curve experiment

1. Prepare five different concentrations of protein made up in buffer solution; e.g. 1 g/l of protein in 1 litre buffer solution for the first isotherm point.
2. Zero the UV/VIS Spectrophotometer with buffer solution in the cell. Empty and wash the cell with reverse osmosis water.
3. Put these different concentrations of protein solution in the cell and take the UV reading from the spectrophotometer.

2. Experimental Methods

2.3.4 Batch equilibrium studies for BSA

1. Make up two solutions, (A) containing Bovine Serum Albumin (10 g/l) in a 0.02 M Tris buffer solution containing either 2.5 g/l or 5.0 g/l sodium chloride at pH 7.5, (B) containing Tris and sodium chloride only.
2. Place a pre-determined (Table 2.3) volume of solution (A) into each of a series of test tubes containing 0.2 g BioPrep 40/300 WAX adsorbent.
3. Dilute BSA solution with solution (B) to give desired initial protein concentration (C_i) of between 0.5 and 10 g/l with a total solution volume of 10 ml.
4. Place the test tubes on a shaker for 24 hours at a constant temperature of 25 °C for the adsorption to proceed.
5. Take 1.5 ml sample from each tube, put it in sampling (centrifuge) tube and centrifuge for 20 minutes at 7000 rpm to remove any adsorbent particles.

Table 2.3 Volumes of solution A and B into a series of tubes

Tube no.	Initial conc. C_i , g/l	Volume of buffer, ml	Volume of protein solution, ml
1	0.5	9.5	0.5
2	1	9	1
3	1.5	8.5	1.5
4	2	8	2
5	2.5	7.5	2.5
6	3	7	3
7	3.5	6.5	3.5
8	4	6	4
9	4.5	5.5	4.5
10	5	5	5
11	5.5	4.5	5.5
12	6	4	6
13	6.5	3.5	6.5
14	7	3	7
15	7.5	2.5	7.5
16	8	2	8
17	8.5	1.5	8.5
18	9	1	9
19	9.5	0.5	9.5
20	10	0	10

2. Experimental Methods

6. Transfer supernatant liquid from sampling tube to UV cell. Measure the absorbance and final protein concentration (C_o) of each sample at 254 nm wavelength using UV/VIS spectrophotometer.
7. Determine using calibration chart (Appendix A Figure A.1) the amount of protein adsorbed per unit mass of adsorbent.

$$q = \frac{V}{M_s} * (C_i - C_o) \quad (2.1)$$

Where q is the solid concentration, g/g solid. V is the volume of protein in each tube, ml. M_s is the mass of the adsorbent, g and C_i , C_o are the initial and final protein concentrations, g/l respectively. A plot of q versus C_o provides the isotherm for the system.

2.3.5 UV/VIS Spectrophotometer cell

Experiments were carried out to study the procedure for increasing the UV absorbance. Three aspects of the procedure were studied:

- A. To see if any fine adsorbent particles remained in the supernatant liquid transferred from the centrifuged sampling tube to the UV cell and if this was affected by whether the transfer was made by pouring or pipetting.
- B. To see whether the UV absorbance depended on whether sample was pipetted from the upper or middle part of the solution in the centrifuged sampling tube.
- C. To see whether the absorbance depended on the depth to which the UV cell was filled.

The initial protein concentration was 5.0 g/l. Transfer the solution from the sampling tube to UV cell using three ways:

1. Pour direct from the sampling tube to UV cell.
 - 1.1. Pour the solution direct from the tube to cell of spectrophotometer. The sampling tubes are designed with a conical bottom so that a small amount of solution,

2. Experimental Methods

containing centrifuged solid, remains in it. The cell is full 100% .The UV absorbance reading was then 0.031.

1.2. Remove 25% of the solution from the cell, so the remaining amount is about 75% of the solution in the cell. The reading was the same, 0.031.

1.3. Remove another 25% of the solution from the cell, so the remaining is about 50% of the solution in the cell. The reading was the same, 0.031.

1.4. Remove another 25% of the solution from the cell, so the remaining is about 25% of the solution in the cell. The reading increased to 0.362.

It appears that, when the transfer is made by pouring, a minimum 50% filling of the cell is sufficient.

2. Take the upper part from the solution using pipette.

2.1. Add 25% of the solution to the cell. The reading was 0.352

2.2. Add another 25% of the solution, which means that 50% of the cell full with the solution. The reading will be 0.023.

2.3. Add another 25% of the solution, which means that 75% of the cell is full with the solution. The reading was 0.031.

2.4. Remove the rest of the solution and put it in the cell .The reading was 0.267.

It appears that, when the transfer is made by pipetting the upper part of the solution from the sampling tube, the volume to be transferred should be 75% of the cell capacity. More than this causes a higher absorbance reading than the correct 0.031 value, indicating possible transfer of the fine adsorbent particles from the bottom of the sampling tube.

3. Take the middle part from the solution using pipette

3.1. Add 50% of the solution to the cell. The reading was 0.020.

3.2. Add another 25% of the solution, which means that 75% of the is full with the solution. The reading was 0.031.

3.3. Fill the cell with the solution without removes bottom part from the sampling tube. The reading was 0.035.

The conclusion for method 3 is the same as for method 2.

2.3.6 Effect of time on attainment of equilibrium

Same procedures in Section 2.3.4 except in part 4 where the time will be variable and the initial protein concentration is constant in all series of tubes at 4.5 g/l.

2.3.7 Determine minimum concentration of sodium chloride to desorb protein.

To determine the minimum concentration of sodium chloride required to desorb protein, a series of nine tubes was set up, as described in Section 2.3.4. Conditions were chosen to give the same (high) equilibrium value of about 4.5 g/l protein concentration, i.e. the initial protein concentration in solution was 4.5 g/l in all tubes. The initial sodium chloride concentration was 2.5 g/l in all cases. Experiments were performed in two stages.

Stage 1

First, the equivalence of all the tubes was tested by allowing adsorption to progress towards equilibrium for two hours and determining the near-equilibrium values of C_e and q obtained.

Stage 2

Second, sufficient sodium chloride was added to each tube to bring the total sodium chloride concentration to pre-determined value. This was done as follows, as described for a pre-determined value of 7.5 g/l, i.e. a 5 g/l increase on the initial (adsorption) value of 2.5 g/l.

1. Add 5 ml of sodium chloride solution of concentration x g/l to the test tubes to increase the concentration by 5 g/l, which means that in the second tube we will have 7.5 g/l of the sodium chloride with 15 ml of solution. The value of x varied from tube to tube (Table 2.4).

E.g. calculation in second tube. Let x be concentration of NaCl solution to be added to the tube by concentration of mass.

2. Experimental Methods

(New mass of NaCl in tube)=(Old mass of NaCl in tube) + (Added mass of NaCl in tube)

$$(7.5 \text{ g/l} * 15 \text{ ml})=(2.5 \text{ g/l} * 10 \text{ ml}) + (x \text{ g/l} * 5 \text{ ml})$$

Therefore $x = 17.5 \text{ g/l}$

This NaCl concentration was made up in a quantity of 150 ml in a graduate beaker (though a better technique would be to use a pipette or weigh the water and use a smaller quantity of water).

Table 2.4 Added mass of NaCl in tubes

Tube no.	Old conc., g/l	Add conc., g/l	NaCl g /150 ml
1	2.5	0	0
2	7.5	17.5	2.625
3	12.5	32.5	4.875
4	17.5	47.5	7.125
5	22.5	62.5	9.375
6	27.5	77.5	11.625
7	32.5	92.5	13.875
8	37.5	107.5	16.125
9	42.5	122.5	18.375

2. Place the tubes on a shaker for 24 hours at a constant temperature of 25 °C for the desorption to proceed.
3. Transfer 1.5 ml samples from each tube to sampling tube and centrifuge for 20 minutes at 7000 rpm to remove any adsorbent particles.
4. Measure the absorbance and hence calibration chart (Appendix A Figure A.1) final protein concentration (C_o) of each sample at 254 nm wavelength using the UV/VIS spectrophotometer.
5. Determine the amount of protein adsorbed per unit mass of adsorbent.

$$q_o = q_i + (C_{oi}V_{oi} - C_oV_o)/M_s \quad (2.2)$$

2. Experimental Methods

Where q_i is the solid concentration, C_{oi} the liquid concentration and V_{oi} the volume of protein in each tube at the end of Stage 1. M_s is the mass of the adsorbent. C_o is the liquid concentration and V_o is the volume of protein in each tube at the end of Stage 2.

2.3.8 Adsorption/Desorption cycles

1. The adsorption procedures are the same as we used in Section 2.3.4 except we add 1.5 ml of reverse osmosis water to the BioPrep before any BSA solution was added. This was done to match later adsorption/desorption cycle in which a minimum level of 1.5 ml of solution was left in the tube after washing as a safety measure to avoid removing the adsorbent while we wash it. The resulting dilution effect was taken into account in the calculation. Mark the level of water (1.5 ml) you reach in the tube. The contents of the tube at the end of the adsorption stage are 11.5 ml. Ten tubes were set up in this way to cover the isotherm.
2. Add 5 ml of 23 g/l sodium chloride to the test tubes and place it on a shaker for 24 hours at a constant temperature of 25 °C for the desorption to proceed. (The added sodium chloride solution should have been of a higher concentration to allow for the dilution from 11.5 to 16.5 ml total concentration.)
3. Take 1.5 ml as a sample and measure the absorbance of each sample at 254 nm wavelength using UV/VIS spectrophotometer.
4. Remove the solution from the tube until you reach the marked level.
5. Wash the adsorbent three times with reverse osmosis water to make sure you clean the adsorbent very well. Repeat the adsorption/desorption cycle several times.

2.3.9 Tyrosine check experiment

1. Make up solution of 0.5 g/l tyrosine in water.
2. Determine the absorption of 1 ml of this solution diluted three parts with water to 4 ml total, using UV/VIS spectrophotometer.
3. To 1 ml of tyrosine solution add 3 ml of 8 M urea solution to give 4 ml of 6 M urea.
4. Determine UV absorption of this solution using UV/VIS spectrophotometer.

2. Experimental Methods

5. If UV absorption is similar in 2 and 4, then urea has no effect on specific absorption of tyrosine, and properly therefore has no effect on specific absorption of BSA.

2.3.10 Regenerate adsorbent with urea

1. After procedure described in Section 2.3.8., tube contains adsorbent and 1.5 ml wash water.
2. Make up urea solution of desired concentration and determine its absorbance (blank).
3. Add 10 ml of urea solution of the desired concentration.
4. Place it on a shaker for 24 hours at a constant temperature of 25 °C for the regeneration to proceed.
5. Take samples from the tubes to determine UV absorption of this urea using UV/VIS spectrophotometer. Calculate protein concentration from difference between this value and the blank value in step 2.
6. Wash the adsorbent three times and then repeat the adsorption procedure.

2.4 Methods of fitting Langmuir isotherms

The isotherm is a plot of the concentration of protein adsorbed against the residual liquid concentration at equilibrium, at constant temperature. Since the work involves measuring isotherms to follow the protein adsorption, desorption and regeneration processes associated with fouling, we have to fit our curve to a Langmuir isotherm curve.

The experimental data presented in the next chapter show that the Langmuir isotherm equation could be used satisfactorily to fit the curves. The Langmuir isotherm is characterized by two important parameters, q_m the maximum capacity of the adsorbent corresponding to the complete filling of the pores, and k_D the dissociation constant.

2.4.1 Nonlinear least squares method

The Langmuir isotherm equation is

$$q = \frac{q_m C_o}{C_o + k_D} \quad (2.3)$$

where q is the point concentration in solid phase, (g/g solid), q_m is maximum monolayer adsorption capacity (g/g solid), k_D is dissociation constant (g/l) and C_o is protein concentration in bulk liquid, g/l.

In principle, the optimization method for the best fit is similar to a least squares fit for a straight line where the sum of the squares of the error between the fitted line and data points is minimized. For a straight line the value of the constants can be obtained analytically but an iterative method is required for a nonlinear form such as the Langmuir equation.

The iterative method requires starting values of q_m and k_D so that Langmuir curves can be calculated to compare the data points and the initial estimate of the fitted curve. From these values, a new pair of q_m and k_D values are calculated, to reduce the magnitude of the sum of the squares of the errors. This procedure is repeated until the differences in the calculated value of q_m between successive iterations are less than a specified limit.

This fitting procedure was carried out with a software program called EASYPLOT.

2. Experimental Methods

2.4.2 Linear fitting method

Equation (2.3) can be converted to a linear form in terms of q_m and k_D as,

$$\frac{k_D}{q_m} + \frac{C_o}{q_m} = \frac{C_o}{q} \quad (2.4)$$

A plot of C_o / q versus C_o is a straight line of slope $1/q_m$ and intercept of k_D / q_m from which k_D and q_m are determined.

The fitting was carried out by linear regression using MICROSOFT EXCEL.

Chapter 3 Results and Discussion

3.1 Fitting Langmuir isotherm in two different ways

The Langmuir isotherm fitting could be in two ways, nonlinear fitting and linear fitting. Both of these fits used the Langmuir isotherm equation (equations 2.3 and 2.4). The resulting values for the maximum capacity of the adsorbent corresponding to the complete filling of the pores q_m , and the dissociation constant k_D are shown in Table 3.1. The values of q_m from the two kinds of fit are almost the same for 2.5 g/l sodium chloride concentration and for 5.0 g/l. However, the two kinds of fit give different values of k_D . This parameter affects the initial slope of the isotherm and the curvature of the isotherm.

Table 3.1 Comparison between linear and nonlinear fits

2.5 g/l NaCl	q_m , g/g	k_D , g/l
Linear fitting	0.204	0.107
Nonlinear fitting	0.202	0.079
5.0 g/l NaCl	q_m , g/g	k_D , g/l
Linear fitting	0.210	0.938
Nonlinear fitting	0.206	0.858

The Langmuir isotherm, fitted both ways, was a poor fit for 2.5 g/l NaCl because the low salt concentration gives a very strongly curved, almost rectangular isotherm. At 5.0 g/l the fit was good due to the less curved isotherm at the higher salt concentration. We can notice from Figures 1 and 3 that 2.5 g/l sodium chloride for the nonlinear fit had a good fit in the initial points but a bad fit at higher concentrations, as where the linear fit showed the opposite behavior with a good fit at higher concentrations. Referring to Figure A.2, the linearised curves were much more sensitive to the high concentration points, which is why it fitted well at high concentrations.

3. Results and Discussion

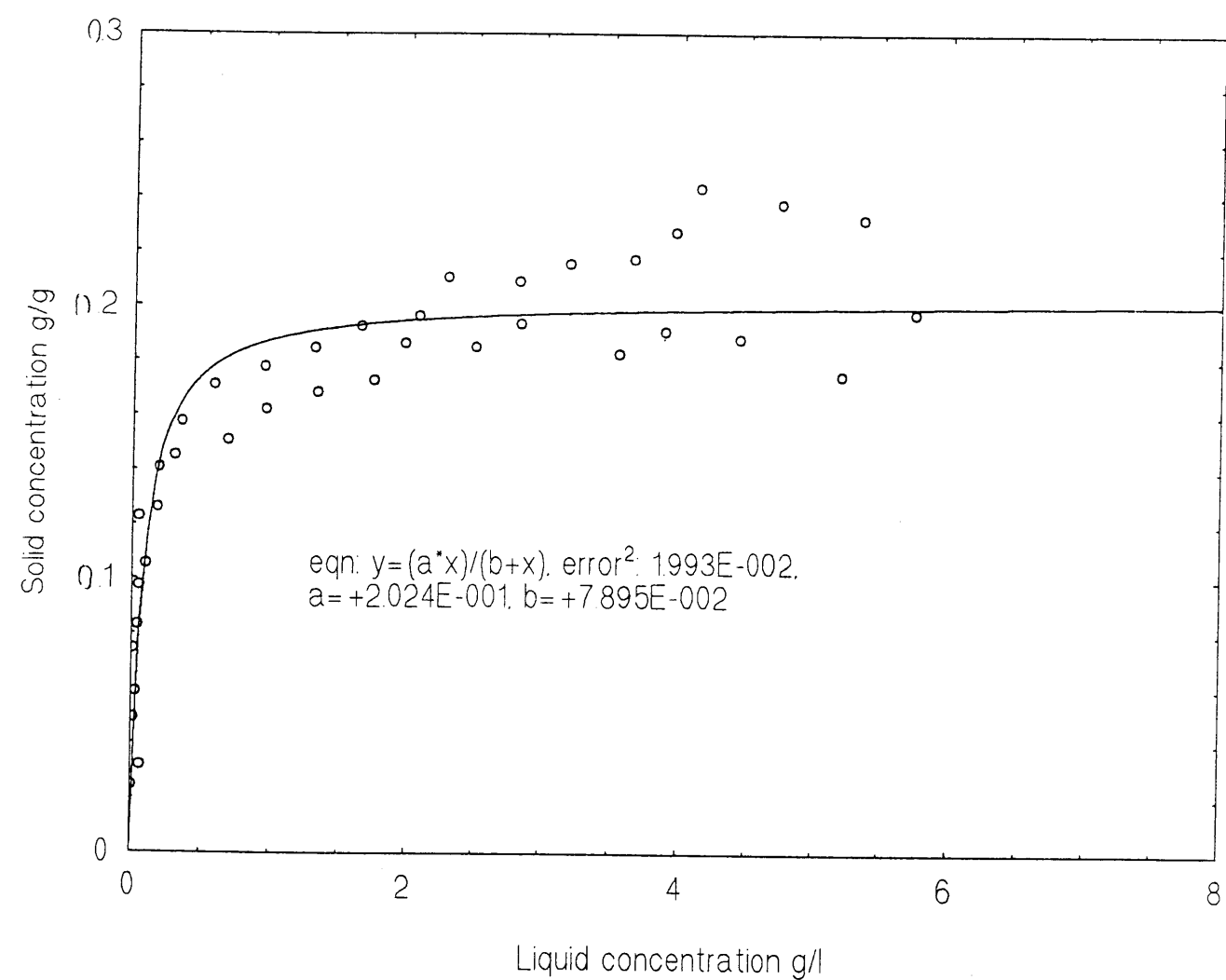


FIG. 1 Nonlinear fit for Langmuir isotherm, 2.5 g/l NaCl

3. Results and Discussion

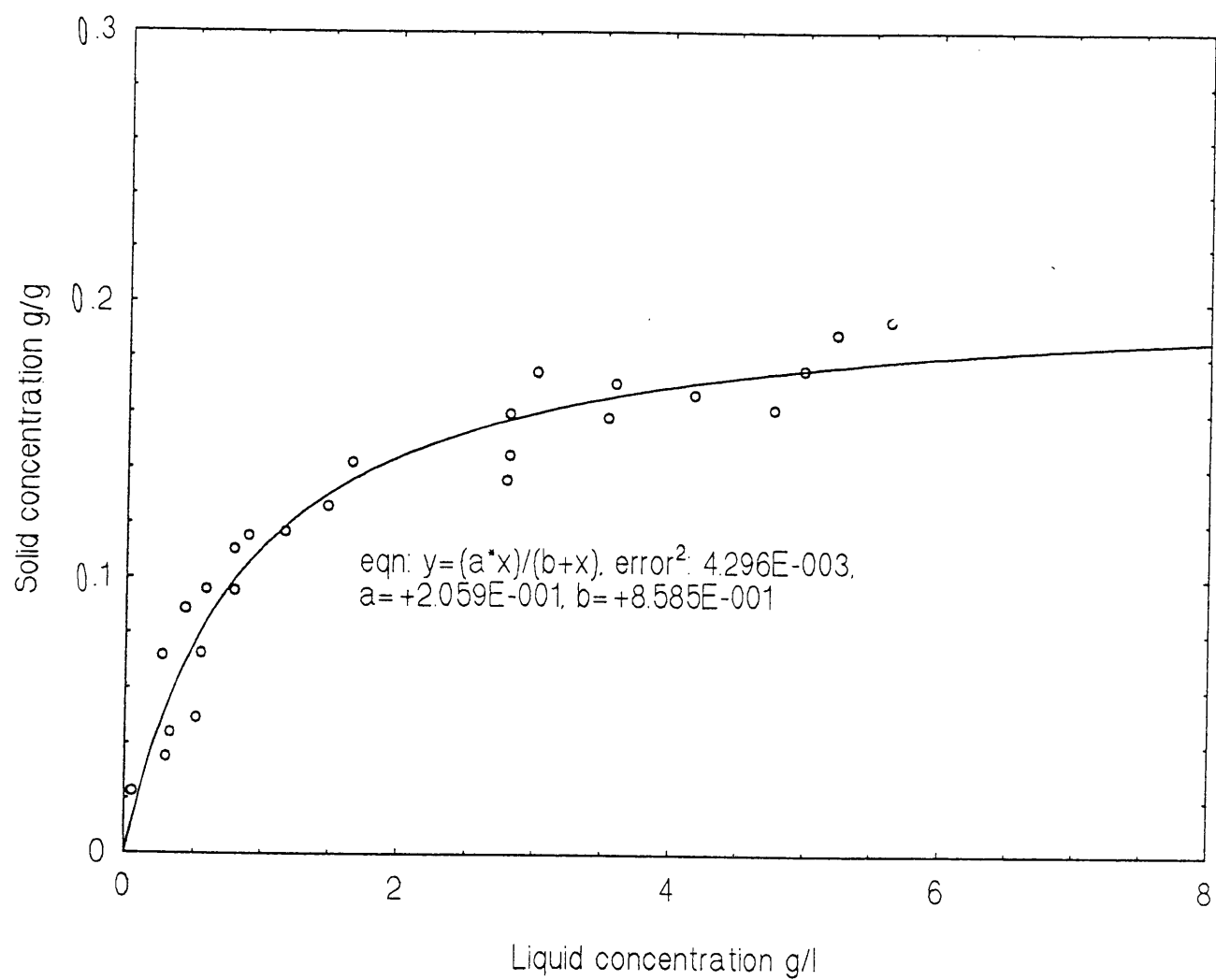


FIG. 2 Nonlinear fit for Langmuir isotherm, 5.0 g/l NaCl

3. Results and discussion

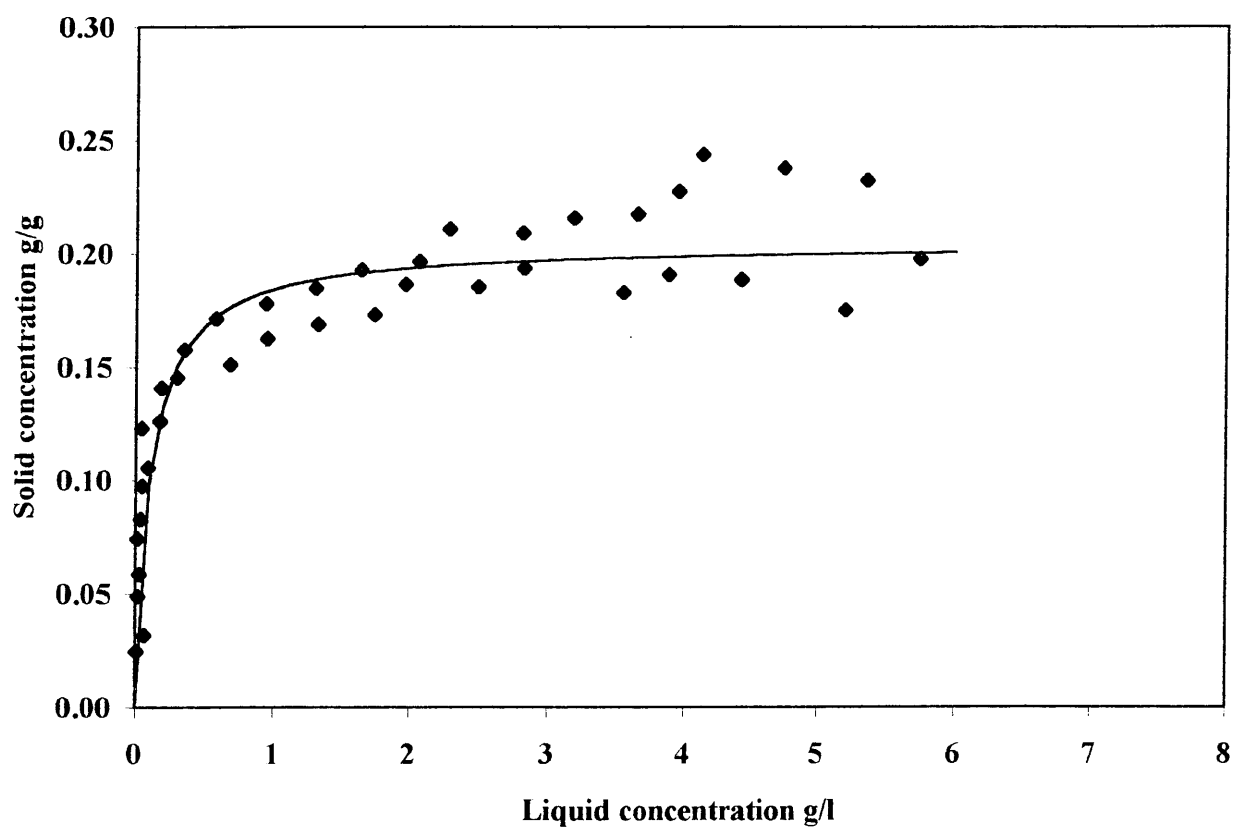


FIG. 3 Linear fit for Langmuir isotherm, 2.5 g/l NaCl

3. Results and discussion

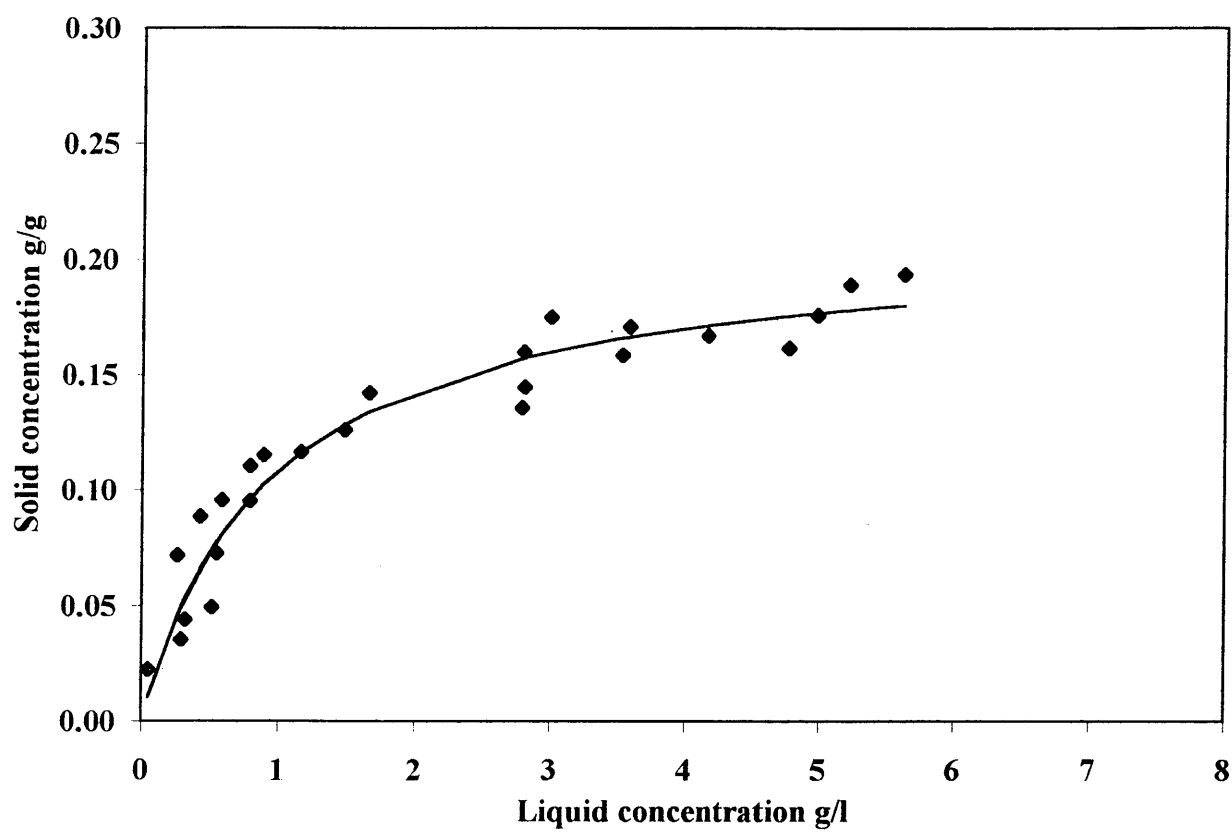


FIG. 4 Linear fit for Langmuir isotherm, 5.0 g/l NaCl

3. Results and Discussion

We used linear fit for this study for two reasons. First, the better overall fit for 2.5 g/l sodium chloride concentration was the linearised one. Second, the linear fit would be expected to give better q_m because it concentrated on high concentration points. The capacity values q_m are particularly important in this study of fouling.

3.2 Deciding a standard value of sodium chloride concentration in the buffer solution

The proper way to decide a standard value of sodium chloride concentration in the buffer solution to be used for the fouling study was to draw the Langmuir isotherm curves with different sodium chloride concentrations and analyze the two figures.

According to Figure 3 and 4 the capacity was similar for the two concentration. However at 2.5 g/l sodium chloride concentration q_m was better defined, i.e. we found that for the liquid concentrations between 2 g/l and 6 g/l the Langmuir isotherm curve was flatter. The main reason we chose 2.5 g/l NaCl as the standard value was that it reached equilibrium faster.

3.3 UV/VIS Spectrophotometer cell

Experiments were carried out, as described in Section 2.3.5, to study the procedure for measuring the UV absorbance. The results described in that section shows that:

- A. Some fine adsorbent particles may still remain in the sampling tube after centrifuging. They may affect the absorbance reading if transferred to the UV cell. Transfer by pouring appeared to give less risk of affecting the reading than did transfer by pipetting. Pouring was also more efficient since the UV cell gave the same reading with solution volumes down to 50% of the cell capacity.
- B. If pipetting was used, the optimum filling of the cell was 75%, regardless of whether the solution was withdrawn from the upper or middle part of the sampling tube.

3. Results and Discussion

It was therefore decided to make the transfer by pouring, and to fill the UV cell to 75% of its capacity, in all subsequent work.

3.4 Effect of time on attainment of equilibrium

According to the procedure showed in last chapter the time we used to shake the tubes was 24 hours. Now we would like to show why we decided this time. We chose an initial protein concentration of 4.5 g/l, giving a high value of solid concentration $q = 0.178$ g/g (Figure 3). Then we had chosen different times for the isotherm measurement. (Figure 5/ Table A.4). The plot in Figure 5 shows that a period of 24 hours was needed to ensure that equilibrium was attained. Therefore we decided to take 24 hours as the standard time.

3.5 Minimum sodium chloride concentration to desorb protein

Desorption using sodium chloride was used to removed protein from the adsorbent after each adsorption stage, i.e. to complete the normal adsorption/desorption cycle. Therefore we needed to determine the minimum sodium chloride concentration needed for desorption. The procedure in previous chapter had divided in to two stages. Stage 1 is shown as the upper line in Figure 6 and Table A.5. This shows that all the tubes reached a similar degree of equilibrium after 2 hours. The lower line in Figure 6 and Table A.6 shows the protein concentrations remaining on the solid after desorption. From the lower line it seems that 23 g/l NaCl was the minimum concentration to desorb protein from the adsorbent.

3.6 Measuring the fouling process

Fouling would occur due to a series of adsorption/desorption processes but we do not have evidence how this fouling affects the isotherm measurements. We believe that fouling will decrease the capacity q_m of the Langmuir isotherm but the size of this change is the question.

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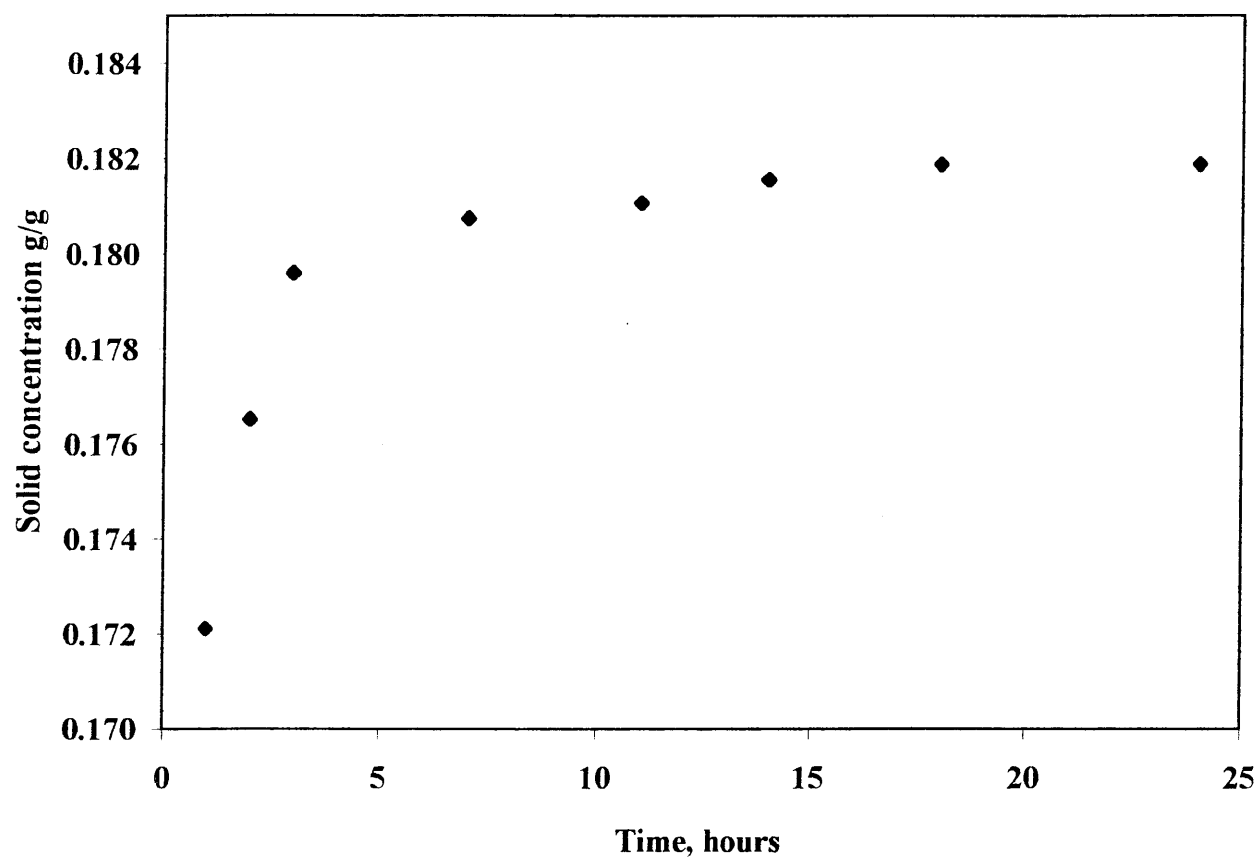


FIG.5 Effect of time on attainment of equilibrium

3. Results and discussion

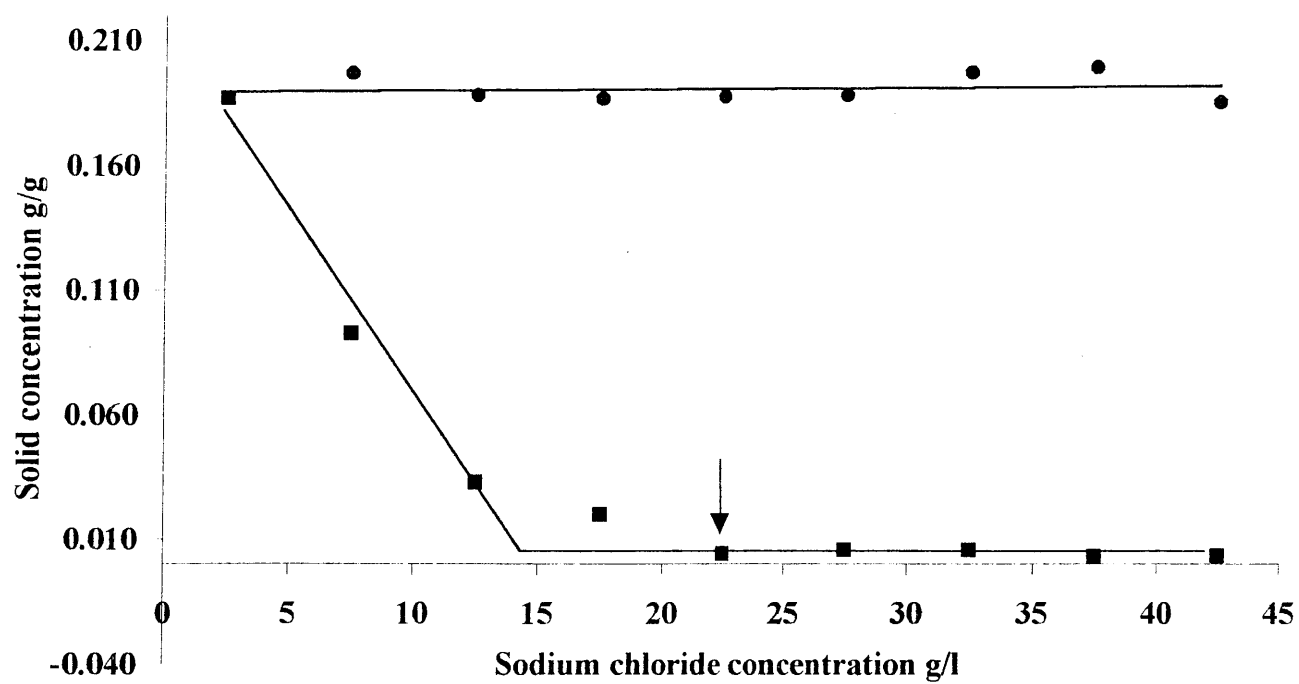


FIG. 6 Effect of sodium chloride addition on the solid concentration after 24 hours

3. Results and Discussion

Figure 7 shows that for the first cycle with virgin adsorbent, q_m was 0.273 g/g. Through four cycles of adsorption/desorption it declined to 0.112 g/g. However as we increase the number of adsorption/desorption cycles the gaps between successive Langmuir isotherms declined.

This evidence suggest that the desorption processes was not sufficient to remove all the protein which remained in the adsorbent. Therefore we need to obtain a way to remove this fouled protein which remained in the adsorbent.

3.7 Tyrosine experiment check

According to the literature survey we found the proper way to regenerate silica rigid adsorbent was urea (6-10 M). Our strategy to determine the effectiveness of the regeneration used two ways. First by UV/VIS spectrophotometer on solutions, if possible and second by determine the Langmuir isotherm after regeneration.

To make the spectrophotometer measurement successfully it was necessary to make two checks. First that urea had no effect on the specific absorption of BSA. Second that urea did not itself absorb UV radiation at 254 nm. Absorption of 254 nm by proteins is due to aromatic rings in the constituent amino acids, tyrosine, phenylalanine and tryptophan. Tyrosine can therefore be used as a model for BSA as a UV absorber. The UV absorption of tyrosine was measured before and after adding urea, as described in Section 2.3.9. The results are shown in Table 3.2 below.

The conclusions from this experiment were, first, that the urea had no effect on the specific absorption of tyrosine and therefore had no effect on the specific absorption of BSA. Second, that urea does not itself absorb 254 nm UV. Then we could use the UV reading before adding urea and after adding urea to BSA solutions to determine the amount of fouling protein removed from BioPrep.

Table 3.2 Tyrosine experiment results

UV reading of tyrosine solution before adding urea	UV reading of tyrosine solution after adding urea
0.148	0.148

3.8 Regenerate adsorbent with urea

3.8.1 First series of experiments

After four cycles of adsorption/desorption processes and the tyrosine check experiment, we were in a position to regenerate with urea (6–10 M). After first regeneration with 6 M urea, the performance of the process was not good enough to remove the protein fouling, as Table A.11 shows that little protein was removed. The measurement of the Langmuir isotherm of the adsorbent after this regeneration shown in Figure 8 gives a capacity q_m 0.069 g/g which is actually lower than the fourth cycle value of 0.112 g/g. At this stage we thought that this surprising result might be due to a change in the calibration curve because the calibration curve had been determined few months earlier. Therefore we checked the calibration curve but there was no significant change in it. It was decided to increase the urea concentration from 6 M to 7 M because, due to the dilution of 6 M urea in the 1.5 ml of water in the tube, the concentration decreased. The supernatant liquid was therefore removed, the adsorbent was washed, and a second regeneration was performed with 7 M urea.

After the second regeneration with 7 M urea there was still not enough change in the amount of the protein removed (Table A.12). The Langmuir isotherm also does not show much change where the capacity was $q_m=0.101$ g/g (Figure 9). Therefore we thought this increase for the concentration was still not enough to remove the protein fouled. Then we decided to increase the urea concentration to 9.6 M (11 M before dilution). However, this increase in the urea concentration has little effect on the efficiency of regeneration. For example, if you take tube no. 10 (Table A.15) the amount of the protein removed was extremely low at 0.007 g. There was a slight improvement in the Langmuir isotherm to a

3. Results and discussion

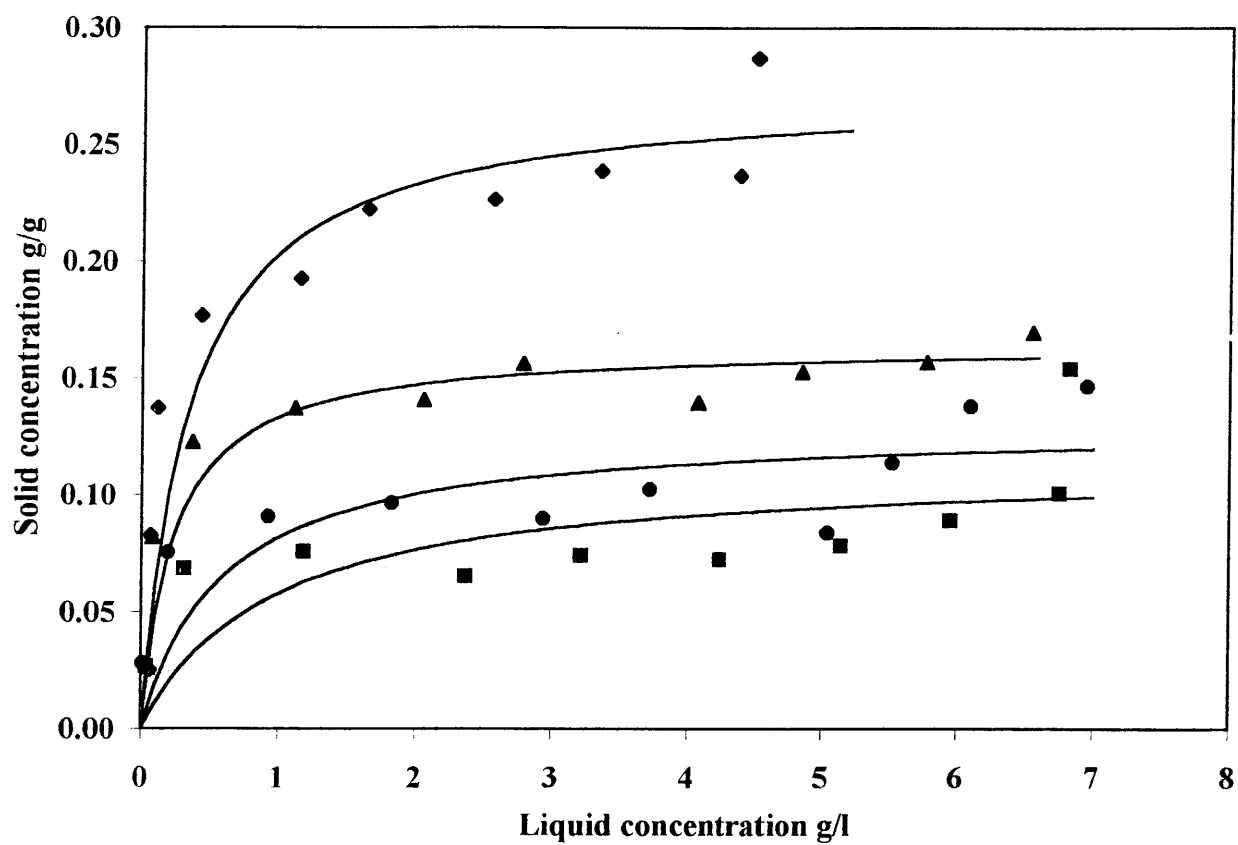


FIG. 7 Linear fit for Langmuir isotherm, 2.5 g/l NaCl, four cycles of adsorption/desorption

3. Results and discussion

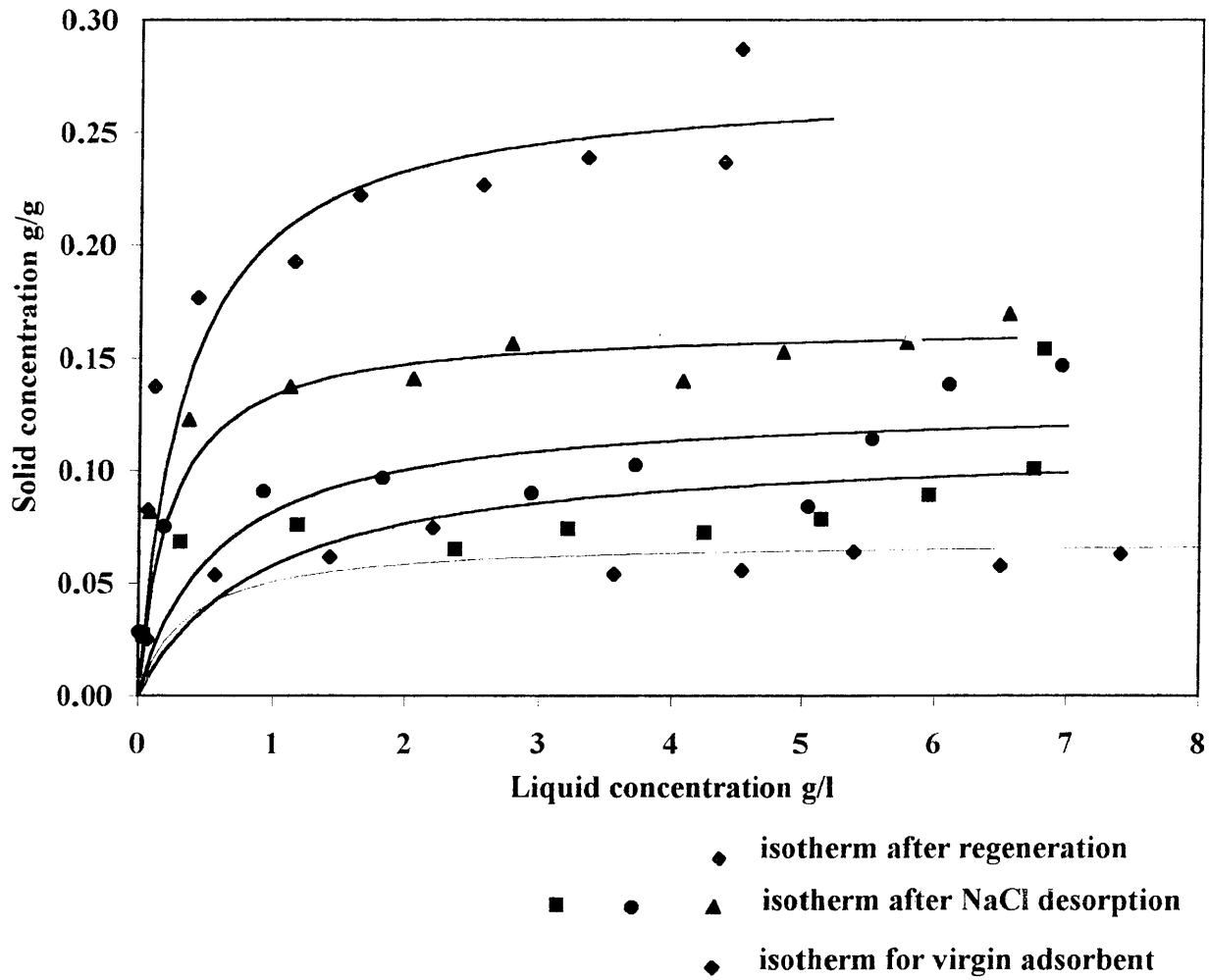


FIG. 8 Linear fit for Langmuir isotherm, 2.5 g/l NaCl, after first regeneration with 6M urea

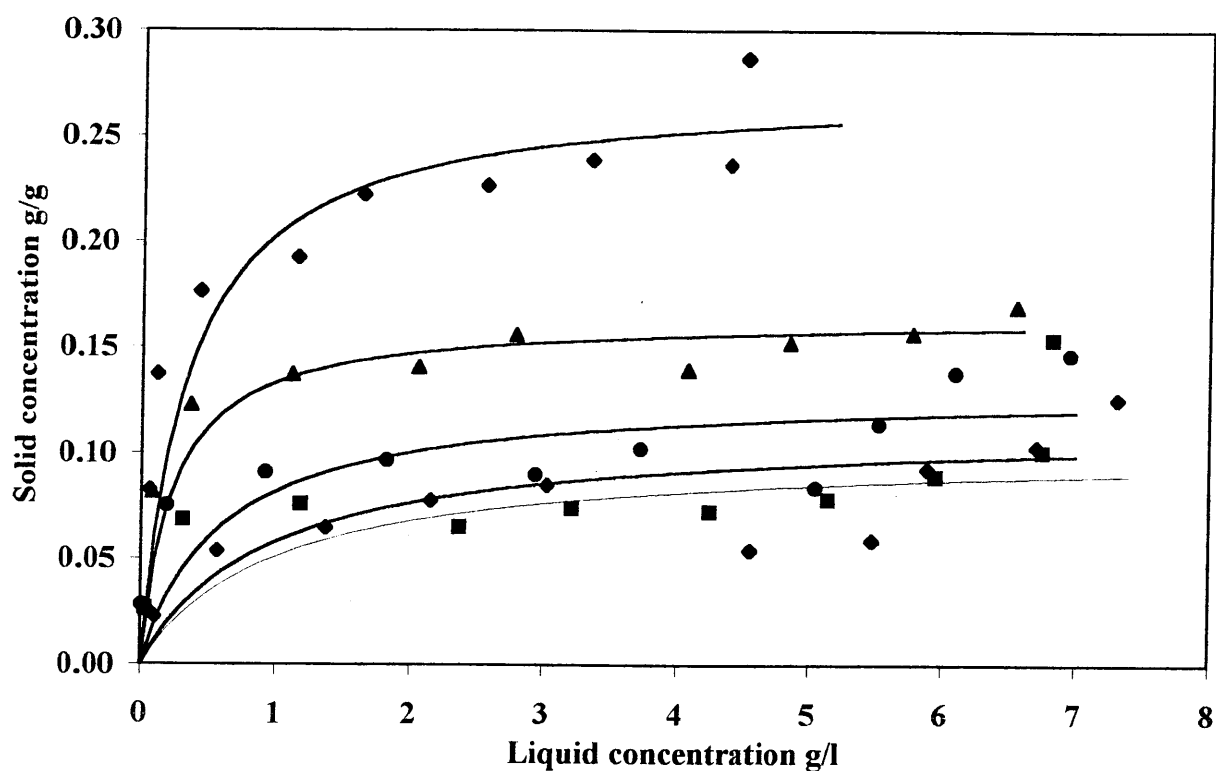


FIG. 9 Linear fit for Langmuir isotherm, 2.5 g/l NaCl, after second regeneration with 7M urea

(The isotherm after first regeneration with 6 M urea is not shown)

3. Results and Discussion

capacity $q_m=0.141$ g/g (Figure 10), which almost reached the fourth cycle of adsorption/desorption.

Since this first series of experiments took about 28 days, it was possible that the regeneration results indicated that there was microbiological fouling present, or even just fouling by protein which had become firmly fixed with time.

3.8.2 Second series of experiments

A second series of experiments was conducted to try to see if microbiological fouling was present and to check the amount of protein gained or lost by the adsorbent using mass balances. What was special about this series of experiments was that it was done in a short period of time (six days). This was to make sure that there is no chance for the microbiological organisms to grow during this short time.

Figure 11 shows that the first adsorption cycle started with virgin adsorbent with $q_m=0.277$ g/g. After desorption the second cycle of adsorption was measured where the capacity declined to 0.184 g/g. At the end of the second cycle, we started the regeneration process with 9.6 M urea (11 M before dilution). However the result on the isotherm measurement was not as we were expected since $q_m=0.114$ g/g, i.e. less than the second cycle. We conclude that the growing of microbiological fouling was not the reason for the bad performance of the regeneration process.

We thought that maybe the wash process after the regeneration with urea before the adsorption was incomplete which means that there was still some urea in the adsorbent. So we decided to desorb with NaCl, wash four times and adsorb again to make sure that the urea had been thoroughly removed. However the isotherm measurement after this procedure still gave a further decline in capacity to $q_m=0.076$ g/g (Figure 12).

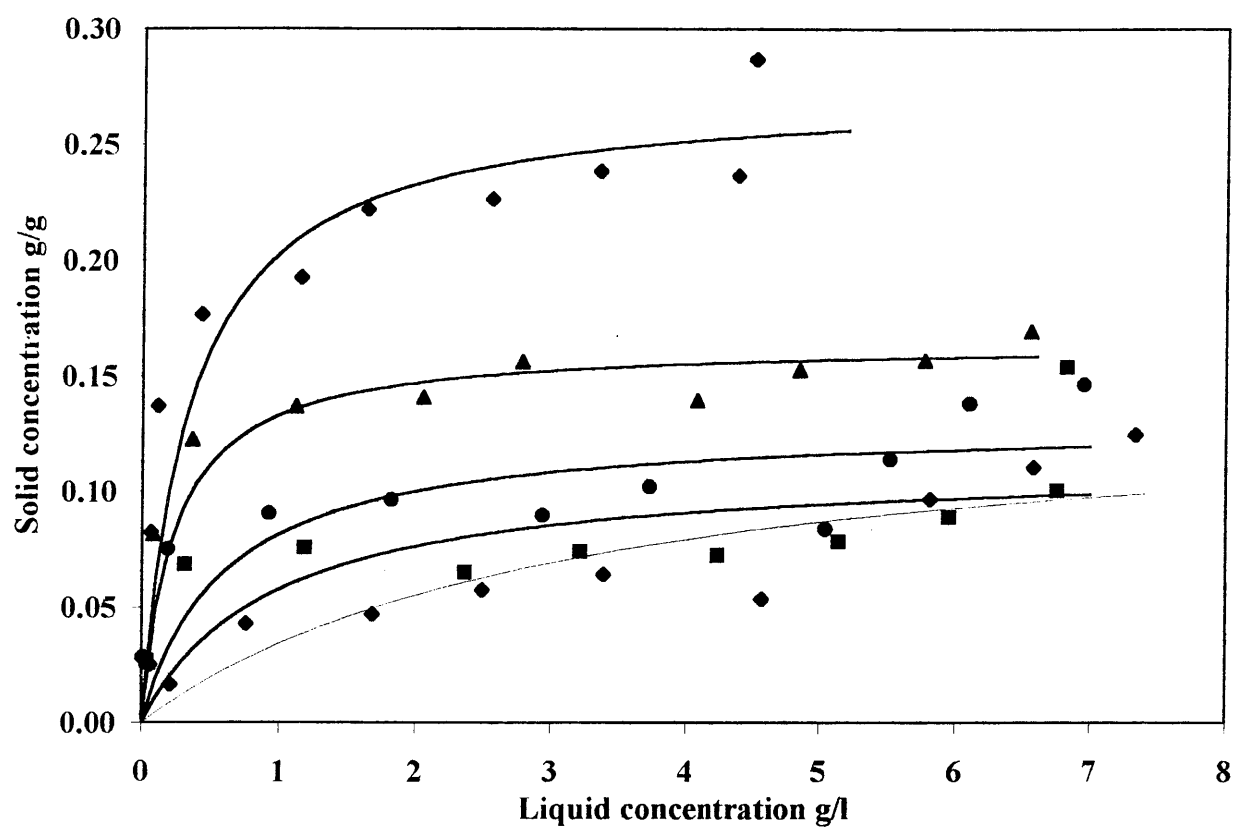


FIG. 10 Linear fit for Langmuir isotherm, 2.5 g/l NaCl, after third regeneration with 11M urea

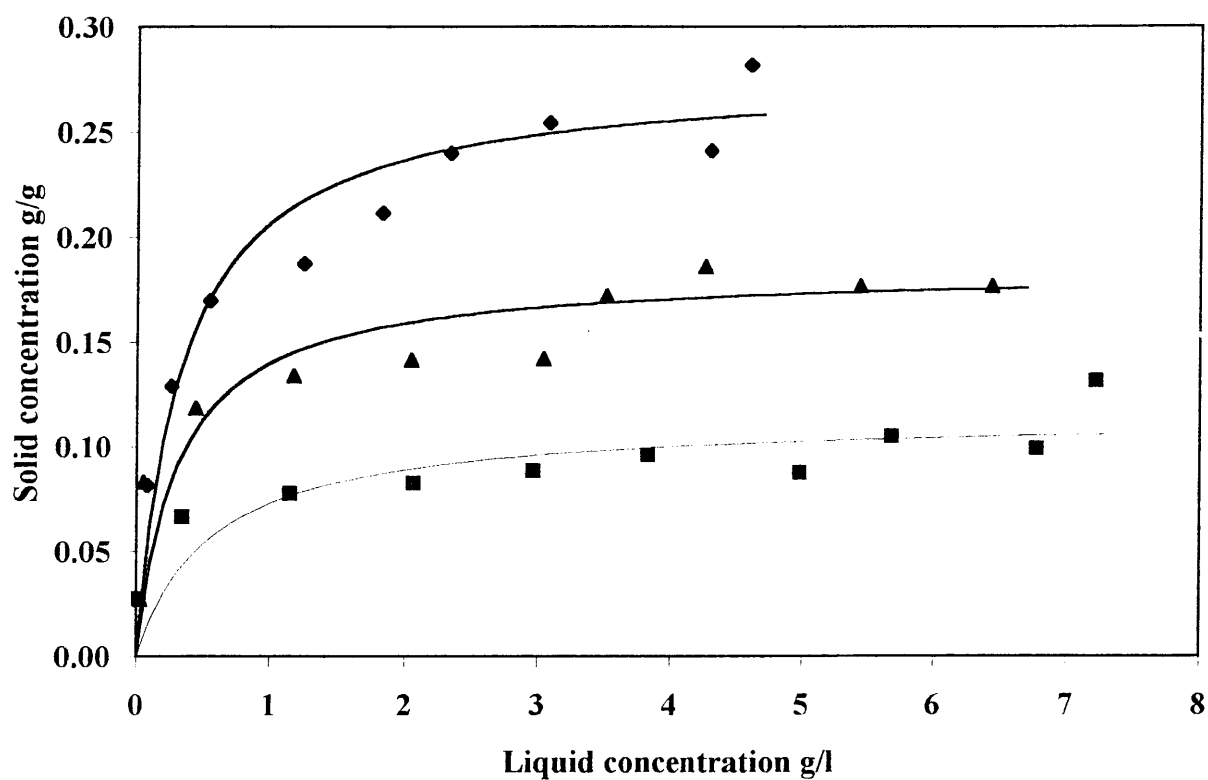


FIG. 11 Linear fit for Langmuir isotherm, 2.5 g/l NaCl, two cycles of adsorption/desorption and regeneration (second series)

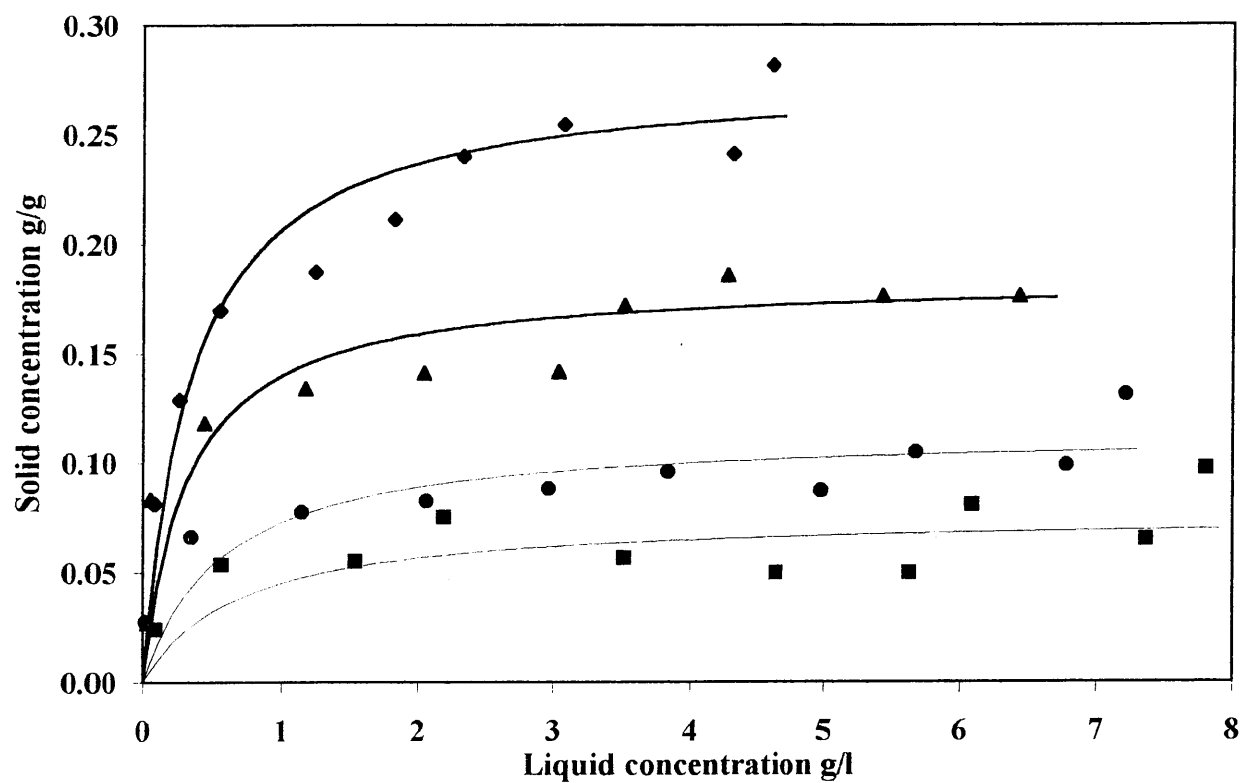


FIG. 12 Linear fit for Langmuir isotherm, 2.5 g/l NaCl, after regeneration with 11M urea and desorption (second series)

3. Results and Discussion

The only explanation for all these results is that each desorption step, and even a desorption plus regeneration step, does not completely reverse the previous adsorption step. There is a net accumulation of protein over a complete adsorption/desorption cycle, which is not overcome by a subsequent regeneration step.

This explanation was confirmed by drawing up complete mass balances for the isotherm measurements through adsorption, desorption and regeneration steps (Tables A.21-A.28). These mass balances are illustrated for tubes 2,5,8 and 10 as examples at different concentrations in (Figures 13,14,15 and 16).

The evidence in the mass balance charts leads to seven concentrated conclusions:

1. Desorption by sodium chloride always desorbs only about two thirds of the protein adsorbed immediately beforehand.
2. Therefore the capacity for the protein was reduced with each adsorption/desorption cycle. I.e. the base level for each adsorption stage was higher with each successive cycle.
3. However, total protein on the adsorbent at the end of each adsorption stage was approximately constant except for tube 5. Therefore fouling could be explained solely in term of protein irreversibly adsorbed, and there was no need to include microorganisms as a fouling agent to explain the fouling phenomena observed.
4. The pattern of successive isotherms before and after regeneration was the same in first series as in second series. Therefore it was likely that there was no significant microbiological fouling occurring within the time scale of either series of experiments (28 days in first series to the second adsorption step after first regeneration; 6 days in second series).

3. Results and Discussion

5. Mass balance charts showed that, although urea does achieve some regeneration, it was only a little more effective than sodium chloride, when the sodium chloride concentration is relatively high.
6. In the first series, three regenerations were performed, first with 5.2 M urea (6 M diluted from 10 ml to total 11.5 ml), second with 6.1 M urea (7 M diluted in same ratio) and third with 9.6 M urea (11 M diluted in same ratio). The first isotherm obtained after the third regeneration step appears to show a similar q_m to that after the first regeneration, though there was substantial scatter in the data. It thus appears that 9.6 M urea was a little more effective for regeneration than 6.1 M urea.
7. Inspection of the mass balance charts showed that after several adsorption/desorption cycles and despite regeneration, the level of irreversible fouling had become quite high and represents the major part of the original adsorption capacity. The same conclusion was seen in the isotherm figures, where q_m for the final isotherm was much less than for the first isotherm.

3. Results and discussion

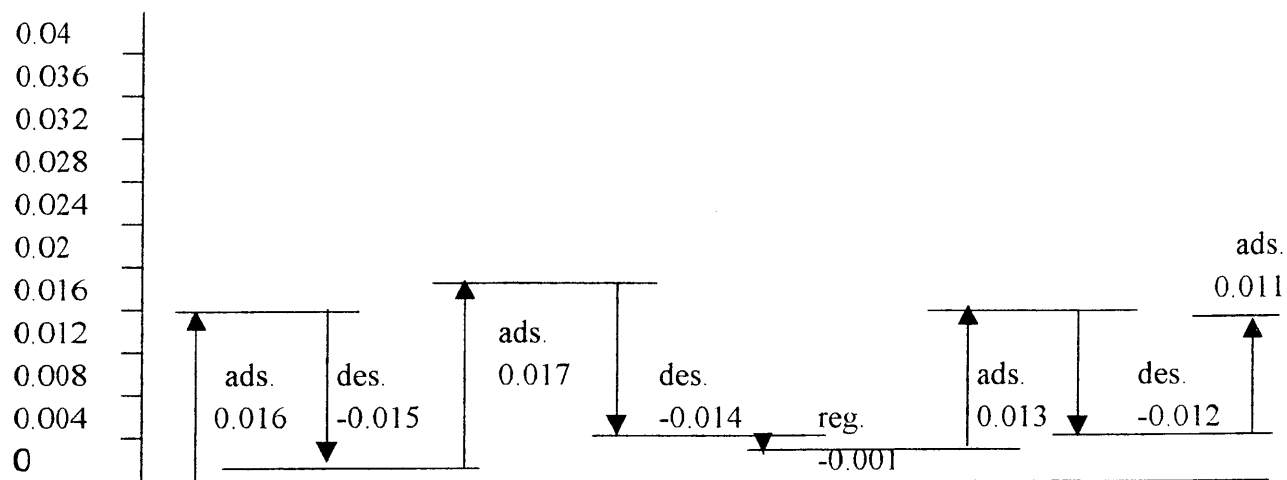


FIG. 13 Mass balance for the isotherm through adsorption, desorption and regeneration with urea for tube number 2

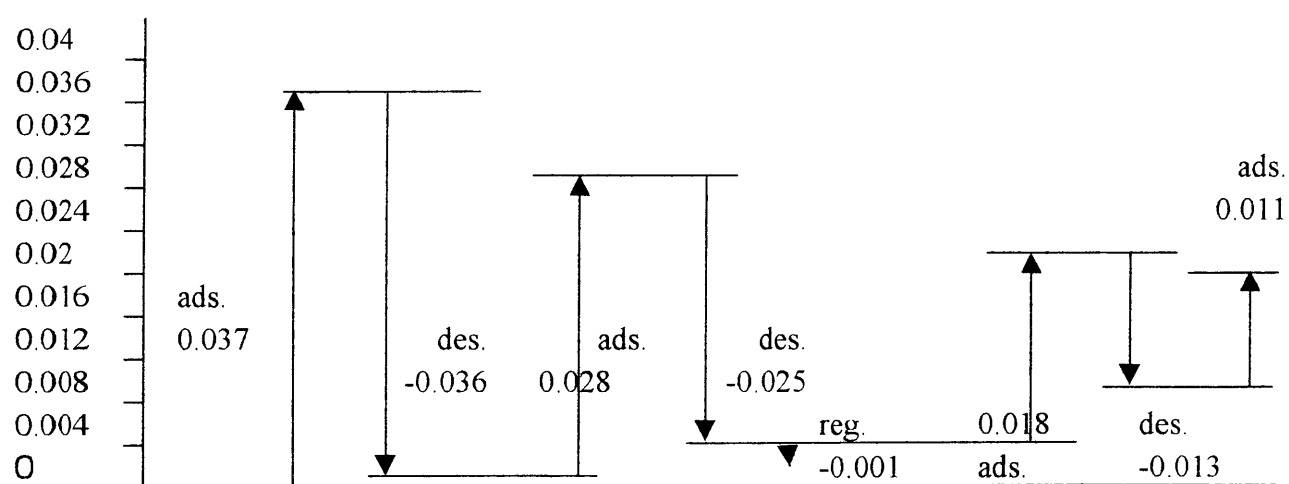


FIG. 14 Mass balance for the isotherm through adsorption, desorption and regeneration with urea for tube number 5

3. Results and discussion

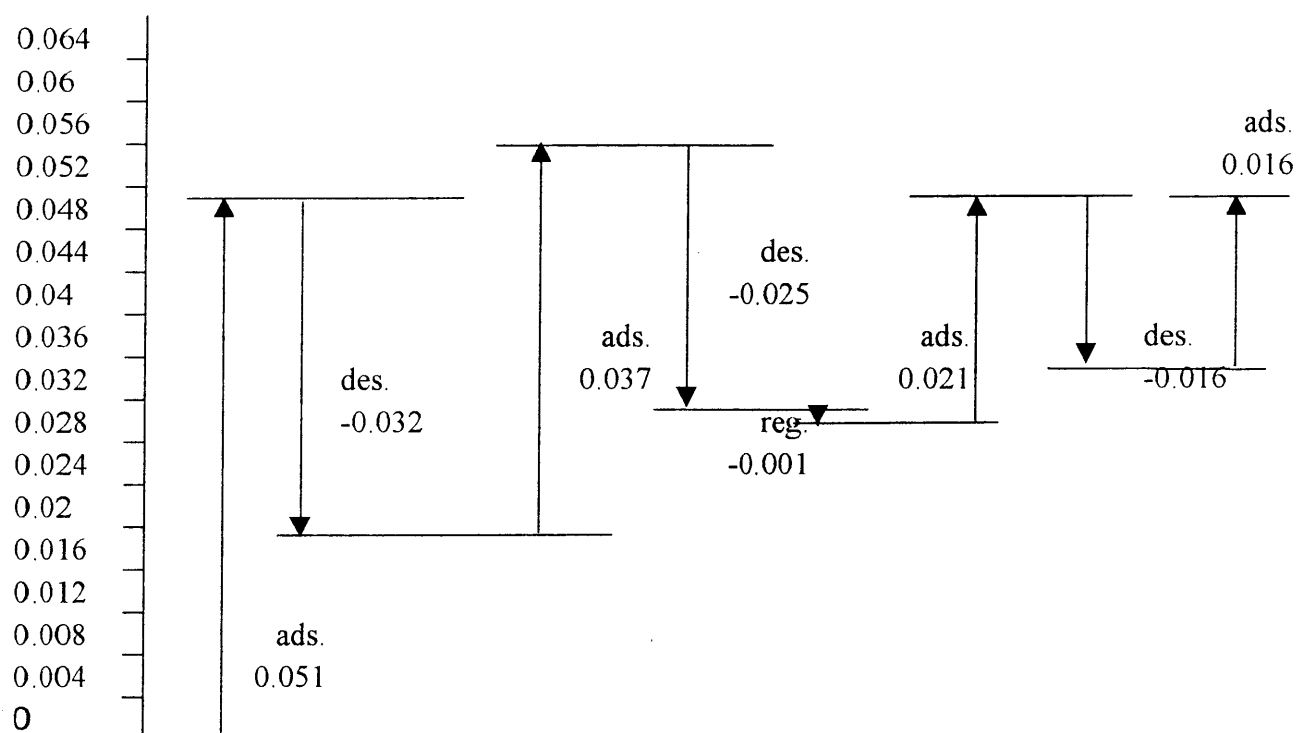


FIG. 15 Mass balance for the isotherm through adsorption, desorption and regeneration with urea for tube number 8

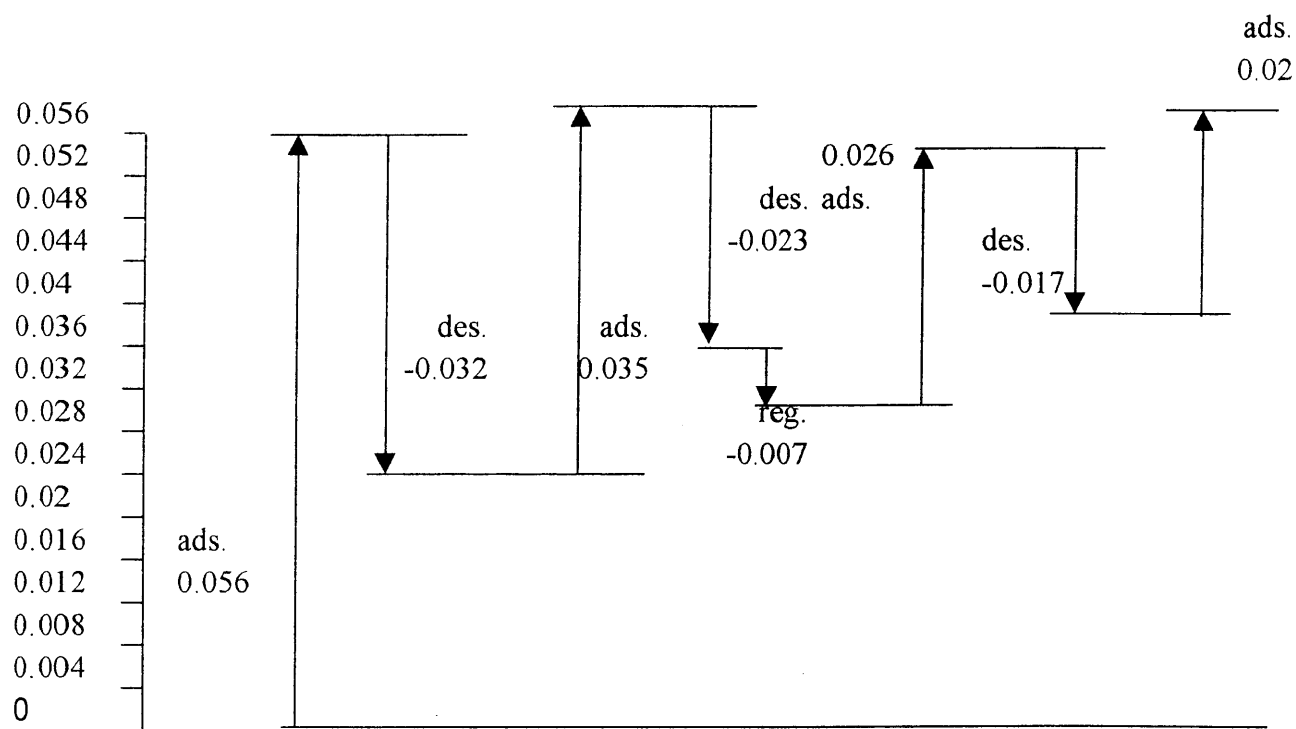


FIG. 16 Mass balance for the isotherm through adsorption, desorption and regeneration with urea for tube number 10

Chapter 4 Conclusions and Recommendations

4.1 Conclusions

1. The minimum amount of solution in UV/VIS spectrophotometer cell should cover 75% of the cell. To avoid carrying over adsorbent fines pour the solution direct from 1.5 ml sampling tube to the UV cell.
2. When the scatter of isotherm data-points is large, a large number of points are needed to determine the shape and parameters of the Langmuir isotherm.
3. At 2.5 g/l sodium chloride concentration in the buffer solution, q_m was better defined than at 5.0 g/l and equilibrium was reached faster.
4. A suitable time for the isotherm measurements on the BSA+ BioPrep 40/300 system to reach equilibrium was 24 hours.
5. 23 g/l sodium chloride concentration was the minimum concentration to desorb protein from the adsorbent.
6. Fouling decreases the capacity q_m for the Langmuir isotherm.
7. Tyrosine experiment proved that urea had no effect on the specific absorption of BSA and that urea does not itself absorb at 254 nm wavelength.
8. There was no evidence that microbiological organisms grew on the fouled protein within a timescale of 28 days.

4. Conclusions and Recommendations

9. The regeneration process using urea is not very effective and adds little to the amount of protein already removed by desorption with sodium chloride.
10. Increasing the urea concentration makes the regeneration only slightly more effective.

4.2 Recommendations

1. Determining the factors (pH, solution temperature, ionic strength, solute types and concentrations) that affect relative adsorption affinity among proteins in contact with surfaces in a quantitative way would be a sound approach to improve our understanding of how mixtures of surface-active molecules will act at an interface, and an important goal for future research.
2. The beaker used to make up 150 ml quantities of NaCl concentrations for the desorption process gave 5-10% error. A better technique would be to use a pipette or weigh the water and to use a smaller quantity of water.
3. Before the desorption process with NaCl solution, 1.5 ml of water was added to the adsorption. This diluted the NaCl solution added subsequently. To compensate for this the added sodium chloride solution should be higher than the desired value of 23 g/l.

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Appendix 1

Tables from A.1 to A.28

Table A.1 Calibration curve

Protein concentration g/l	Absorbance @ 254 nm
0	0
1	0.345
2	0.539
3	0.972
4	1.176
5	1.566

Table A.2 Isotherm measurements for 2.5 g/l NaCl concentration

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.003	0.010	0.025	0.398	0.017
0.7	0.02	0.065	0.032	2.050	0.077
1	0.006	0.020	0.049	0.398	0.032
1.2	0.009	0.029	0.059	0.500	0.044
1.5	0.004	0.013	0.074	0.175	0.022
1.7	0.012	0.039	0.083	0.470	0.055
2	0.015	0.049	0.098	0.500	0.064
2.2	0.028	0.091	0.105	0.864	0.094
2.5	0.013	0.042	0.123	0.344	0.058
2.7	0.054	0.176	0.126	1.392	0.127
3	0.057	0.185	0.141	1.318	0.129
3.2	0.091	0.296	0.145	2.040	0.150
3.5	0.107	0.348	0.158	2.209	0.156
3.7	0.209	0.680	0.151	4.504	0.176
4	0.177	0.576	0.171	3.364	0.172
4.2	0.293	0.953	0.162	5.874	0.183
4.5	0.29	0.944	0.178	5.307	0.183
4.7	0.408	1.328	0.169	7.874	0.189
5	0.401	1.305	0.185	7.063	0.189
5.2	0.535	1.741	0.173	10.066	0.192
5.5	0.505	1.643	0.193	8.522	0.192
5.7	0.605	1.969	0.187	10.553	0.194
6	0.636	2.070	0.197	10.532	0.194
6.2	0.767	2.496	0.185	13.477	0.196
6.5	0.702	2.284	0.211	10.838	0.195
6.7	0.869	2.828	0.194	14.606	0.197
7	0.866	2.818	0.209	13.478	0.197
7.2	1.09	3.547	0.183	19.420	0.198
7.5	0.979	3.186	0.216	14.769	0.197
7.7	1.193	3.882	0.191	20.337	0.199
8	1.122	3.651	0.217	16.791	0.198
8.2	1.362	4.432	0.188	23.526	0.199
8.5	1.215	3.954	0.227	17.394	0.199
8.7	1.598	5.200	0.175	29.716	0.200
9	1.269	4.130	0.244	16.957	0.199
9.5	1.458	4.745	0.238	19.954	0.200
9.7	1.766	5.747	0.198	29.075	0.200
10	1.646	5.356	0.232	23.069	0.200

Table A.3 Isotherm measurements for 5.0 g/l NaCl concentration

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.015	0.049	0.023	2.164	0.010
1	0.09	0.293	0.035	8.283	0.050
1.2	0.099	0.322	0.044	7.340	0.054
1.5	0.158	0.514	0.049	10.431	0.074
1.7	0.081	0.264	0.072	3.670	0.046
2	0.169	0.550	0.073	7.585	0.078
2.2	0.132	0.430	0.089	4.852	0.066
2.5	0.18	0.586	0.096	6.120	0.081
2.7	0.244	0.794	0.095	8.332	0.096
3	0.243	0.791	0.110	7.159	0.096
3.2	0.275	0.895	0.115	7.764	0.102
3.5	0.358	1.165	0.117	9.978	0.116
4	0.454	1.477	0.126	11.713	0.128
4.5	0.509	1.656	0.142	11.650	0.134
5.5	0.856	2.786	0.136	20.524	0.157
5.7	0.862	2.805	0.145	19.379	0.157
6	0.861	2.802	0.160	17.521	0.157
6.5	0.923	3.004	0.175	17.181	0.160
6.7	1.085	3.531	0.158	22.281	0.166
7	1.102	3.586	0.171	21.008	0.166
7.5	1.28	4.165	0.167	24.982	0.171
8	1.465	4.767	0.162	29.495	0.175
8.5	1.532	4.985	0.176	28.369	0.177
9	1.605	5.223	0.189	27.656	0.178
9.5	1.73	5.630	0.194	29.092	0.180

Table A.4 Effect of time on attainment of equilibrium

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Time, hour
4.5	0.325	1.058	0.172	1
4.5	0.298	0.970	0.177	2
4.5	0.279	0.908	0.180	3
4.5	0.272	0.885	0.181	7
4.5	0.27	0.879	0.181	11
4.5	0.267	0.869	0.182	14
4.5	0.265	0.862	0.182	18
4.5	0.265	0.862	0.182	24

Table A.5 Effect of sodium chloride addition on the solid concentration(upper line)

NaCl conc.g/l	initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g
2.5	4.5	0.237	0.771	0.186
7.5	4.5	0.175	0.569	0.197
12.5	4.5	0.228	0.742	0.188
17.5	4.5	0.236	0.768	0.187
22.5	4.5	0.231	0.752	0.187
27.5	4.5	0.227	0.739	0.188
32.5	4.5	0.172	0.560	0.197
37.5	4.5	0.159	0.517	0.199
42.5	4.5	0.247	0.804	0.185

Table A.6 Effect of sodium chloride addition on the solid concentration(lower line)

NaCl conc.g/l	initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g
2.5	4.5	0.237	0.771	0.186
7.5	4.5	0.543	1.767	0.092
12.5	4.5	0.788	2.564	0.033
17.5	4.5	0.841	2.737	0.020
22.5	4.5	0.905	2.945	0.004
27.5	4.5	0.899	2.925	0.006
32.5	4.5	0.899	2.925	0.006
37.5	4.5	0.91	2.961	0.003
42.5	4.5	0.909	2.958	0.003

Table A.7 Isotherm measurements for 2.5 g/l NaCl concentration(first cycle)

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.019	0.062	0.025	2.454	0.042
1.5	0.02	0.065	0.083	0.789	0.044
2.5	0.035	0.114	0.137	0.830	0.068
3.5	0.132	0.430	0.177	2.433	0.152
4.5	0.354	1.152	0.193	5.984	0.210
5.5	0.503	1.637	0.222	7.369	0.226
6.5	0.787	2.561	0.226	11.307	0.241
7.5	1.029	3.349	0.239	14.028	0.248
8.5	1.347	4.383	0.237	18.518	0.253
9.5	1.386	4.510	0.287	15.720	0.254

Table A.8 Isotherm measurements for 2.5 g/l NaCl concentration(second cycle)

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.014	0.046	0.026	1.743	0.027
1.5	0.025	0.081	0.082	0.997	0.042
2.5	0.113	0.368	0.123	2.999	0.101
3.5	0.344	1.119	0.137	8.178	0.136
4.5	0.631	2.053	0.141	14.596	0.147
5.5	0.856	2.786	0.156	17.847	0.152
6.5	1.252	4.074	0.139	29.209	0.155
7.5	1.49	4.849	0.152	31.805	0.157
8.5	1.775	5.776	0.157	36.879	0.158
9.5	2.014	6.554	0.169	38.688	0.159

Table A.9 Isotherm measurements for 2.5 g/l NaCl concentration(third cycle)

nitial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.003	0.010	0.028	0.346	0.002
1.5	0.059	0.192	0.075	2.553	0.032
2.5	0.284	0.924	0.091	10.200	0.079
3.5	0.56	1.822	0.096	18.891	0.098
4.5	0.903	2.938	0.090	32.728	0.108
5.5	1.144	3.723	0.102	36.429	0.112
6.5	1.55	5.044	0.084	60.245	0.116
7.5	1.697	5.522	0.114	48.561	0.117
8.5	1.875	6.102	0.138	44.242	0.118
9.5	2.137	6.954	0.146	47.505	0.120

Table A.10 Isotherm measurements for 2.5 g/l NaCl concentration(forth cycle)

nitial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.011	0.036	0.027	1.341	0.004
1.5	0.096	0.312	0.068	4.575	0.028
2.5	0.364	1.185	0.076	15.660	0.063
3.5	0.728	2.369	0.065	36.429	0.080
4.5	0.988	3.215	0.074	43.517	0.087
5.5	1.304	4.243	0.072	58.729	0.092
6.5	1.58	5.142	0.078	65.824	0.095
7.5	1.83	5.955	0.089	67.038	0.097
8.5	2.075	6.752	0.100	67.195	0.099
9.5	2.097	6.824	0.154	44.348	0.099

Table A.11 Regeneration with 6M urea results.

tube no.	UV absorbence of urea before regeneration	UV absorbence of urea after regeneration	conc. of protein in urea, g/l	mass of protein in urea, g
1	0.096	0.269	0.564	0.006
2	0.096	0.351	0.831	0.010
3	0.096	0.436	1.108	0.013
4	0.096	0.598	1.635	0.019
5	0.096	0.607	1.664	0.019
6	0.096	0.444	1.134	0.013
7	0.096	0.420	1.055	0.012
8	0.096	0.749	2.126	0.024
9	0.096	0.748	2.123	0.024
10	0.096	0.815	2.341	0.027

Table A.12 Second regeneration with 7M urea results.

tube no.	UV absorbence of urea before regeneration	UV absorbence of urea after regeneration	conc. of protein in urea, g/l	mass of protein in urea, g
1	0.0965	0.184	0.285	0.003
2	0.0965	0.452	1.157	0.013
3	0.0965	0.256	0.519	0.006
4	0.0965	0.200	0.337	0.004
5	0.0965	0.251	0.503	0.006
6	0.0965	0.440	1.118	0.013
7	0.0965	0.395	0.971	0.011
8	0.0965	0.382	0.929	0.011
9	0.0965	0.639	1.765	0.020
10	0.0965	0.585	1.590	0.018

Table A.13 Isotherm measurements for 2.5 g/l NaCl concentration
(After regeneration with 6M urea)

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.02	0.065	0.025	2.603	0.011
1.5	0.174	0.566	0.054	10.546	0.042
2.5	0.439	1.429	0.062	23.188	0.055
3.5	0.677	2.203	0.075	29.542	0.059
4.5	1.095	3.563	0.054	66.158	0.063
5.5	1.393	4.533	0.056	81.528	0.064
6.5	1.657	5.392	0.064	84.645	0.065
7.5	1.997	6.499	0.058	112.853	0.065
8.5	2.276	7.406	0.063	117.788	0.066
9.5	2.472	8.044	0.084	96.102	0.066

Table A.14 Isotherm measurements for 2.5 g/l NaCl concentration
(After second regeneration with 7m urea)

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.032	0.104	0.023	4.575	0.010
1.5	0.175	0.569	0.054	10.643	0.037
2.5	0.422	1.373	0.065	21.196	0.059
3.5	0.661	2.151	0.078	27.730	0.070
4.5	0.93	3.026	0.085	35.716	0.076
5.5	1.4	4.556	0.054	83.915	0.083
6.5	1.683	5.477	0.059	93.082	0.086
7.5	1.81	5.890	0.093	63.625	0.087
8.5	2.062	6.710	0.103	65.196	0.088
9.5	2.248	7.315	0.126	58.234	0.089

Table A.15 Third regeneration with 11M urea results.

tube no.	UV absorbence of urea before regeneration	UV absorbence of urea after regeneration	conc. of protein in urea, g/l	mass of protein in urea, g
1	0.1130	0.180	0.2179	0.003
2	0.1130	0.204	0.2960	0.003
3	0.1130	0.211	0.3188	0.004
4	0.1130	0.264	0.4912	0.006
5	0.1130	0.288	0.5693	0.007
6	0.1130	0.271	0.5140	0.006
7	0.1130	0.260	0.4782	0.005
8	0.1130	0.292	0.5824	0.007
9	0.1130	0.326	0.6930	0.008
10	0.1130	0.303	0.6181	0.007

Table A.16 Isotherm measurements for 2.5 g/l NaCl concentration
(After third regeneration with 11M urea)

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.065	0.212	0.017	12.752	0.009
1.5	0.232	0.755	0.043	17.623	0.028
2.5	0.517	1.682	0.047	35.786	0.050
3.5	0.768	2.499	0.058	43.429	0.063
4.5	1.04	3.384	0.064	52.755	0.074
5.5	1.404	4.569	0.054	85.331	0.084
6.5	1.549	5.041	0.084	60.072	0.087
7.5	1.788	5.818	0.097	60.175	0.092
8.5	2.022	6.580	0.110	59.597	0.096
9.5	2.253	7.332	0.125	58.802	0.099

Table A.17 First cycle of adsorption (second series)

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.006	0.020	0.028	0.707	0.015
1.5	0.025	0.081	0.082	0.997	0.053
2.5	0.08	0.260	0.129	2.022	0.120
3.5	0.169	0.550	0.170	3.242	0.171
4.5	0.382	1.243	0.187	6.638	0.218
5.5	0.561	1.826	0.211	8.641	0.234
6.5	0.716	2.330	0.240	9.717	0.242
7.5	0.945	3.075	0.254	12.087	0.249
8.5	1.324	4.308	0.241	17.877	0.257
9.5	1.414	4.601	0.282	16.336	0.258

Table A.18 Second cycle of adsorption(second series)

initial conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.009	0.029	0.027	1.082	0.016
1.5	0.016	0.052	0.083	0.625	0.026
2.5	0.136	0.443	0.118	3.741	0.108
3.5	0.360	1.171	0.134	8.750	0.145
4.5	0.629	2.047	0.141	14.511	0.159
5.5	0.933	3.036	0.142	21.430	0.167
6.5	1.080	3.514	0.172	20.473	0.169
7.5	1.312	4.269	0.186	22.984	0.171
8.5	1.670	5.434	0.176	30.830	0.174
9.5	1.977	6.433	0.176	36.486	0.175

Table A.19 Adsorption after regeneration with 11M urea(second series)

nitil conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.006	0.020	0.028	0.707	0.004
1.5	0.106	0.345	0.066	5.194	0.043
2.5	0.353	1.149	0.078	14.784	0.077
3.5	0.634	2.063	0.083	24.971	0.090
4.5	0.911	2.965	0.088	33.577	0.096
5.5	1.178	3.833	0.096	40.002	0.099
6.5	1.53	4.979	0.087	56.923	0.102
7.5	1.745	5.678	0.105	54.217	0.104
8.5	2.083	6.778	0.099	68.474	0.105
9.5	2.218	7.218	0.131	55.000	0.106

Table A.20 Adsorption after regeneration with 11M urea and desorp it again

nitil conc.,g/l	absorbance	liquid conc. Co, g/l	solid conc. q, g/g	Co/q	q(calc.)
0.5	0.026	0.085	0.024	3.542	0.008
1.5	0.174	0.566	0.054	10.546	0.035
2.5	0.474	1.542	0.055	28.015	0.053
3.5	0.673	2.190	0.075	29.076	0.058
4.5	1.08	3.514	0.057	62.020	0.064
5.5	1.424	4.634	0.050	93.050	0.066
6.5	1.731	5.633	0.050	112.983	0.068
7.5	1.872	6.092	0.081	75.232	0.068
8.5	2.264	7.367	0.065	113.127	0.070
9.5	2.398	7.803	0.098	79.993	0.070

Table A.21 Isotherm of first cycle(second series)

tube no.	initial conc., g/l	absorbance	liquid conc., g/l	final mass of protein in solution, g	mass of protein adsorbed, g
1	0.5	0.006	0.020	0.000	0.006
2	1.5	0.025	0.081	0.001	0.016
3	2.5	0.08	0.260	0.003	0.026
4	3.5	0.169	0.550	0.006	0.034
5	4.5	0.382	1.243	0.014	0.037
6	5.5	0.561	1.826	0.021	0.042
7	6.5	0.716	2.330	0.027	0.048
8	7.5	0.945	3.075	0.035	0.051
9	8.5	1.324	4.308	0.050	0.048
10	9.5	1.414	4.601	0.053	0.056
tube no.	solid conc., g/g				
1	0.028				
2	0.082				
3	0.129				
4	0.170				
5	0.187				
6	0.211				
7	0.240				
8	0.254				
9	0.241				
10	0.282				

Table A.22 Desorb isotherm with 23g/l Sodium Chloride after first cycle(second series)

tube no.	initial conc., g/l	absorbance	liquid conc., g/l	mass of protein after desorption into solution, g	mass of protein described into solution, g
1	0.5	0.242	0.788	0.013	0.013
2	1.5	0.301	0.979	0.016	0.015
3	2.5	0.611	1.988	0.033	0.030
4	3.5	0.725	2.359	0.039	0.033
5	4.5	0.941	3.062	0.051	0.036
6	5.5	1.008	3.280	0.054	0.033
7	6.5	1.106	3.599	0.059	0.033
8	7.5	1.257	4.090	0.067	0.032
9	8.5	1.533	4.989	0.082	0.033
10	9.5	1.59	5.174	0.085	0.032
tube no.	decrease in protein conc. on adsorbence				
1	0.065				
2	0.081				
3	0.164				
4	0.195				
5	0.253				
6	0.271				
7	0.297				
8	0.337				
9	0.412				
10	0.427				

Table A.23 Isotherm of second cycle(second series)

tube no.	initial conc., g/l	absorbance	liquid conc., g/l	final mass of protein in solution, g	mass of protein adsorbed, g
1	0.5	0.009	0.029	0.000	0.005
2	1.5	0.016	0.052	0.001	0.017
3	2.5	0.136	0.443	0.005	0.024
4	3.5	0.36	1.171	0.013	0.027
5	4.5	0.629	2.047	0.024	0.028
6	5.5	0.933	3.036	0.035	0.028
7	6.5	1.08	3.514	0.040	0.034
8	7.5	1.312	4.269	0.049	0.037
9	8.5	1.67	5.434	0.062	0.035
10	9.5	1.977	6.433	0.074	0.035
tube no.	solid conc., g/g				
1	0.027				
2	0.083				
3	0.118				
4	0.134				
5	0.141				
6	0.142				
7	0.172				
8	0.186				
9	0.176				
10	0.176				

Table A.24 Desorb isotherm with 23g/l Sodium Chloride after second cycle(second series)

tube no.	initial conc., g/l	absorbance	liquid conc., g/l	mass of protein after desorption into solution, g	mass of protein described into solution, g
1	0.5	0.124	0.404	0.007	0.006
2	1.5	0.278	0.905	0.015	0.014
3	2.5	0.491	1.598	0.026	0.021
4	3.5	0.702	2.284	0.038	0.024
5	4.5	0.903	2.938	0.048	0.025
6	5.5	1.087	3.537	0.058	0.023
7	6.5	1.224	3.983	0.066	0.025
8	7.5	1.373	4.468	0.074	0.025
9	8.5	1.578	5.135	0.085	0.022
10	9.5	1.8	5.857	0.097	0.023
tube no.	decrease in protein conc. on adsorbence				
1	0.033				
2	0.075				
3	0.132				
4	0.188				
5	0.242				
6	0.292				
7	0.329				
8	0.369				
9	0.424				
10	0.483				

Table A.25 regenerate with 11M urea(second series)

tube no.	UV Absorbence of Urea before regeneration	UV Absorbence of Urea after regeneration	concentration of protein in urea, g/l
1	0.1052	0.116	0.0351
2	0.1052	0.121	0.0514
3	0.1052	0.122	0.0546
4	0.1052	0.110	0.0156
5	0.1052	0.119	0.0449
6	0.1052	0.125	0.0644
7	0.1052	0.123	0.0579
8	0.1052	0.121	0.0514
9	0.1052	0.123	0.0579
10	0.1052	0.295	0.6176

tube no.	mass of protein, g	decrease in protein conc. on adsorbence
1	0.000	0.002
2	0.001	0.003
3	0.001	0.003
4	0.000	0.001
5	0.001	0.003
6	0.001	0.004
7	0.001	0.003
8	0.001	0.003
9	0.001	0.003
10	0.007	0.036

Table A.26 Isotherm after regeneration with 11M urea(second series)

tube no.	initial conc., g/l	absorbance	liquid conc., g/l	final mass of protein in solution, g	mass of protein adsorbed, g
1	0.5	0.006	0.020	0.000	0.006
2	1.5	0.106	0.345	0.004	0.013
3	2.5	0.353	1.149	0.013	0.016
4	3.5	0.634	2.063	0.024	0.017
5	4.5	0.911	2.965	0.034	0.018
6	5.5	1.178	3.833	0.044	0.019
7	6.5	1.53	4.979	0.057	0.017
8	7.5	1.745	5.678	0.065	0.021
9	8.5	2.083	6.778	0.078	0.020
10	9.5	2.218	7.218	0.083	0.026

tube no.	solid conc., g/g
1	0.028
2	0.066
3	0.078
4	0.083
5	0.088
6	0.096
7	0.087
8	0.105
9	0.099
10	0.131

Table A.27 Desorb isotherm with 23g/l Sodium Chloride for the isotherm after regeneration(second series)

tube no.	initial conc., g/l	absorbance	liquid conc., g/l	mass of protein after desorption into solution, g	mass of protein desorbed into solution, g
1	0.5	0.1	0.325	0.005	0.005
2	1.5	0.295	0.960	0.016	0.012
3	2.5	0.49	1.595	0.026	0.013
4	3.5	0.719	2.340	0.039	0.015
5	4.5	0.878	2.857	0.047	0.013
6	5.5	1.155	3.759	0.062	0.018
7	6.5	1.339	4.357	0.072	0.015
8	7.5	1.512	4.920	0.081	0.016
9	8.5	1.79	5.825	0.096	0.018
10	9.5	1.855	6.036	0.100	0.017

tube no.	decrease in protein conc. on adsorbence
1	0.027
2	0.079
3	0.132
4	0.193
5	0.236
6	0.310
7	0.359
8	0.406
9	0.481
10	0.498

Table A.28 Isotherm after regeneration with 11M urea and desorption with 23 g/l NaCl(second series)

tube no.	initial conc., g/l	absorbance	liquid conc., g/l	final mass of protein in solution, g	mass of protein adsorbed, g
1	0.5	0.026	0.085	0.001	0.005
2	1.5	0.174	0.566	0.007	0.011
3	2.5	0.474	1.542	0.018	0.011
4	3.5	0.673	2.190	0.025	0.015
5	4.5	1.08	3.514	0.040	0.011
6	5.5	1.424	4.634	0.053	0.010
7	6.5	1.731	5.633	0.065	0.010
8	7.5	1.872	6.092	0.070	0.016
9	8.5	2.264	7.367	0.085	0.013
10	9.5	2.398	7.803	0.090	0.020

tube no.	solid conc., g/g
1	0.024
2	0.054
3	0.055
4	0.075
5	0.057
6	0.050
7	0.050
8	0.081
9	0.065
10	0.098

Appendix 2

Sample calculations

Sample calculation of solid concentration q , (g/g) for Table A.2

$$q = \frac{V}{M_s} * (C_i - C_o)$$

Data for run number 1:

V = Volume of protein in each tube = 0.01 liter

M_s = Mass of the adsorbent = 0.2 g

C_i = Initial protein concentrations = 0.5 g/l

C_o = Final liquid concentrations = 0.01 g/l

$$q = [0.01 \text{ (liter)} / 0.2 \text{ (g)}] * [0.5 \text{ (g/l)} - 0.01 \text{ (g/l)}]$$

$$q = 0.025 \text{ g/g}$$

Sample calculation of the point concentration in the solid phase q (calc.), g/g for Table A.2

$$q \text{ (calc.)} = \frac{q_m C_o}{C_o + k_D}$$

Data for run number 1:

q_m = Maximum monolayer adsorption capacity = 0.204 g/g

k_D = Dissociation constant = 0.1068 g/l

C_o = Final liquid concentrations = 0.01 g/l

$$q \text{ (calc.)} = [0.204 \text{ (g/g)} * 0.01 \text{ (g/l)}] / [0.01 \text{ (g/l)} + 0.1068 \text{ (g/l)}]$$

$$q \text{ (calc.)} = 0.017 \text{ g/g}$$

Sample calculation of solid concentration q , (g/g) for Table A.6

$$q_o = q_i + (C_{oi}V_{oi} - C_oV_o)/M_s$$

Data for run number 2:

q_i = Solid concentration at the end of Stage 1 = 0.197 g/g

C_{oi} = Liquid concentration at the end of Stage 1 = 0.569 g/l

V_{oi} = Volume of protein in each tube at the end of Stage 1 = 0.01 liter

M_s = Mass of the adsorbent = 0.2 g

C_o = Liquid concentration at the end of Stage 2 = 1.767 g/l

V_o = Volume of protein in each tube at the end of Stage 2 = 0.015 liter

$$q = 0.197 \text{ (g/g)} + [0.569 \text{ (g/l)} * 0.01 \text{ (liter)} - 1.767 \text{ (g/l)} * 0.015 \text{ (liter)}] / 0.2 \text{ (g)}$$

$$q = 0.929 \text{ g/g}$$

Sample calculation of mass of protein in urea, g for Table A.11

*Mass of protein in urea = [UV absorbance of urea before regeneration - UV absorbance of urea after regeneration] * [Volume of urea in the tube/Calibration curve constant]*

Data for run number 1:

UV absorbance of urea before regeneration = 0.269

UV absorbance of urea after regeneration = 0.096

Volume of urea in the tube = 0.0115 liter

Calibration curve constant = 0.3073

$$\text{Mass of protein in urea} = [0.269 - 0.096] * [0.115 \text{ (liter)} / 0.3073]$$

$$\text{Mass of protein in urea} = 0.006 \text{ g}$$

Appendix 3

Figures from A.1 to A.14

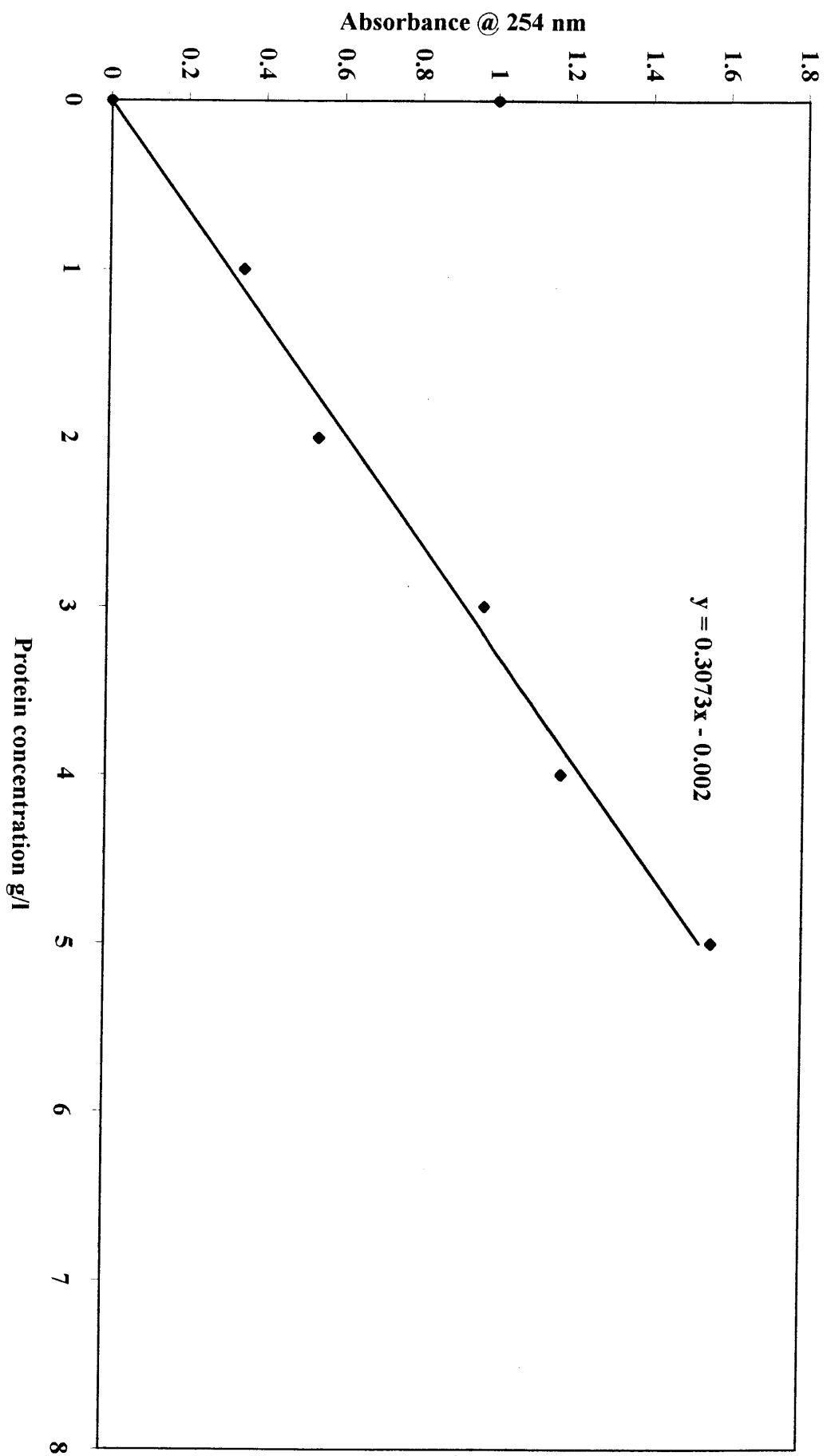


FIG. A.1 Calibration curve

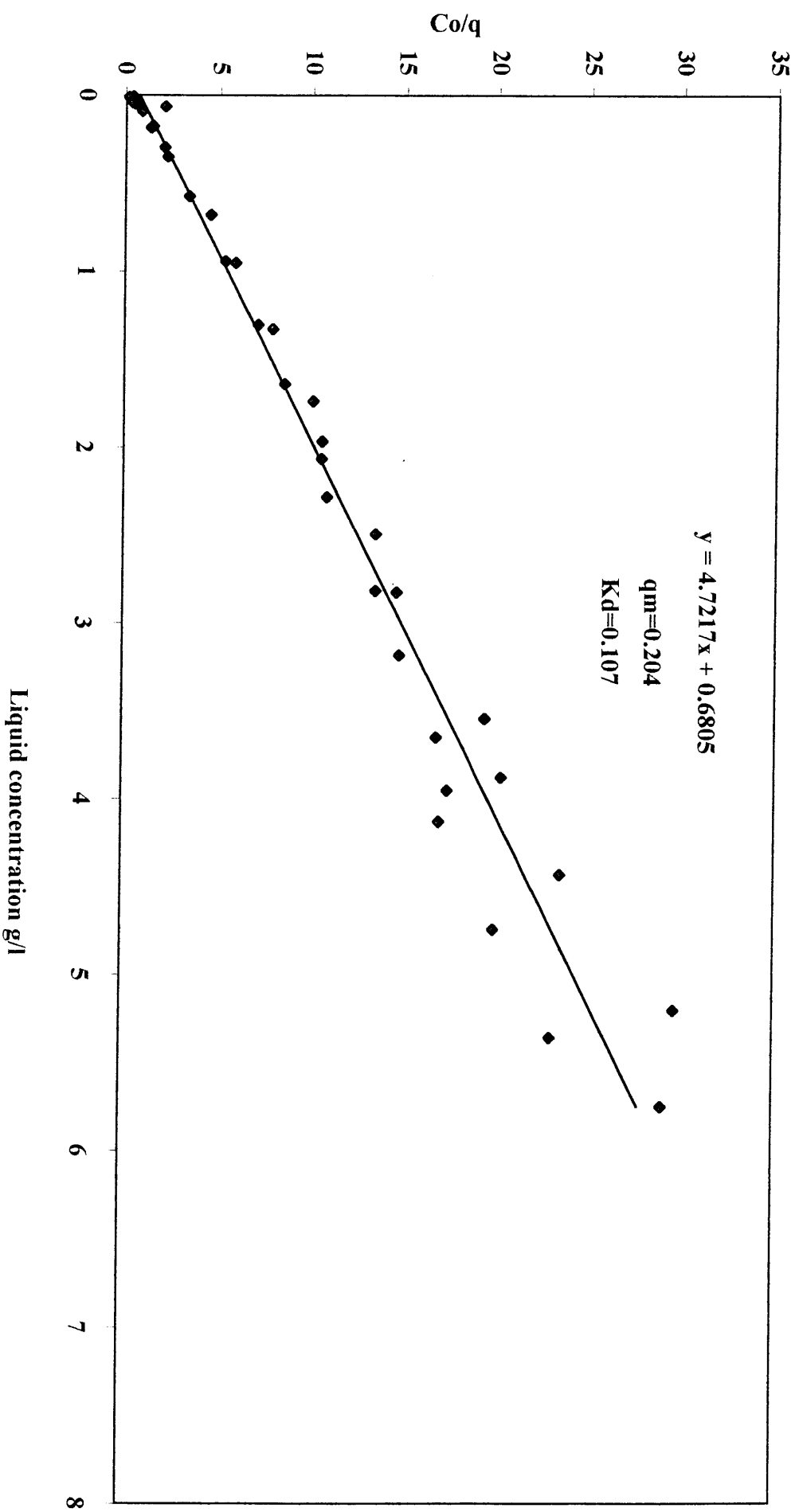


FIG. A.2 Linearised Langmuir isotherm, 2.5 g/l NaCl

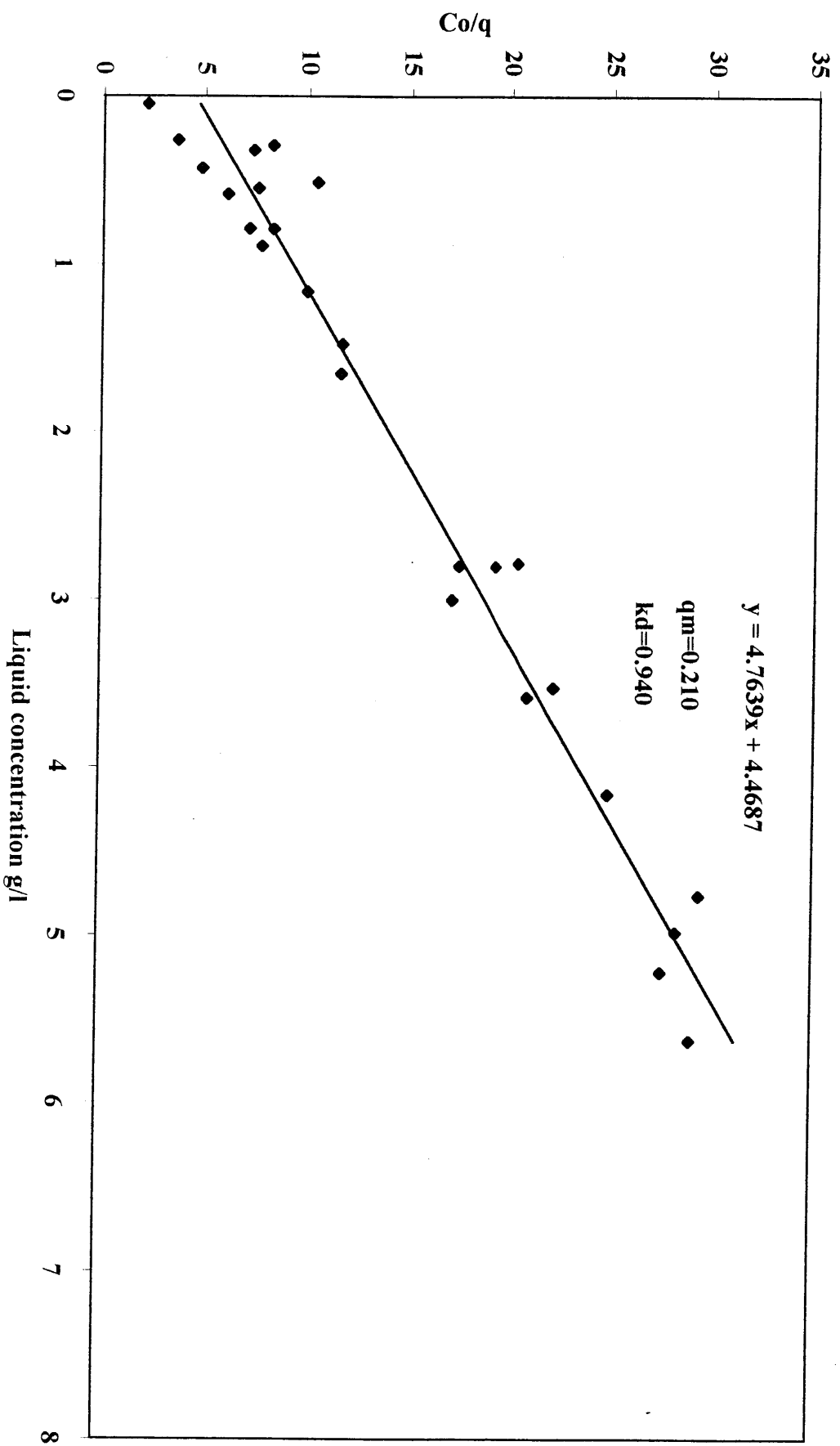


FIG. A.3 Linearised Langmuir isotherm, 5.0 g/l NaCl

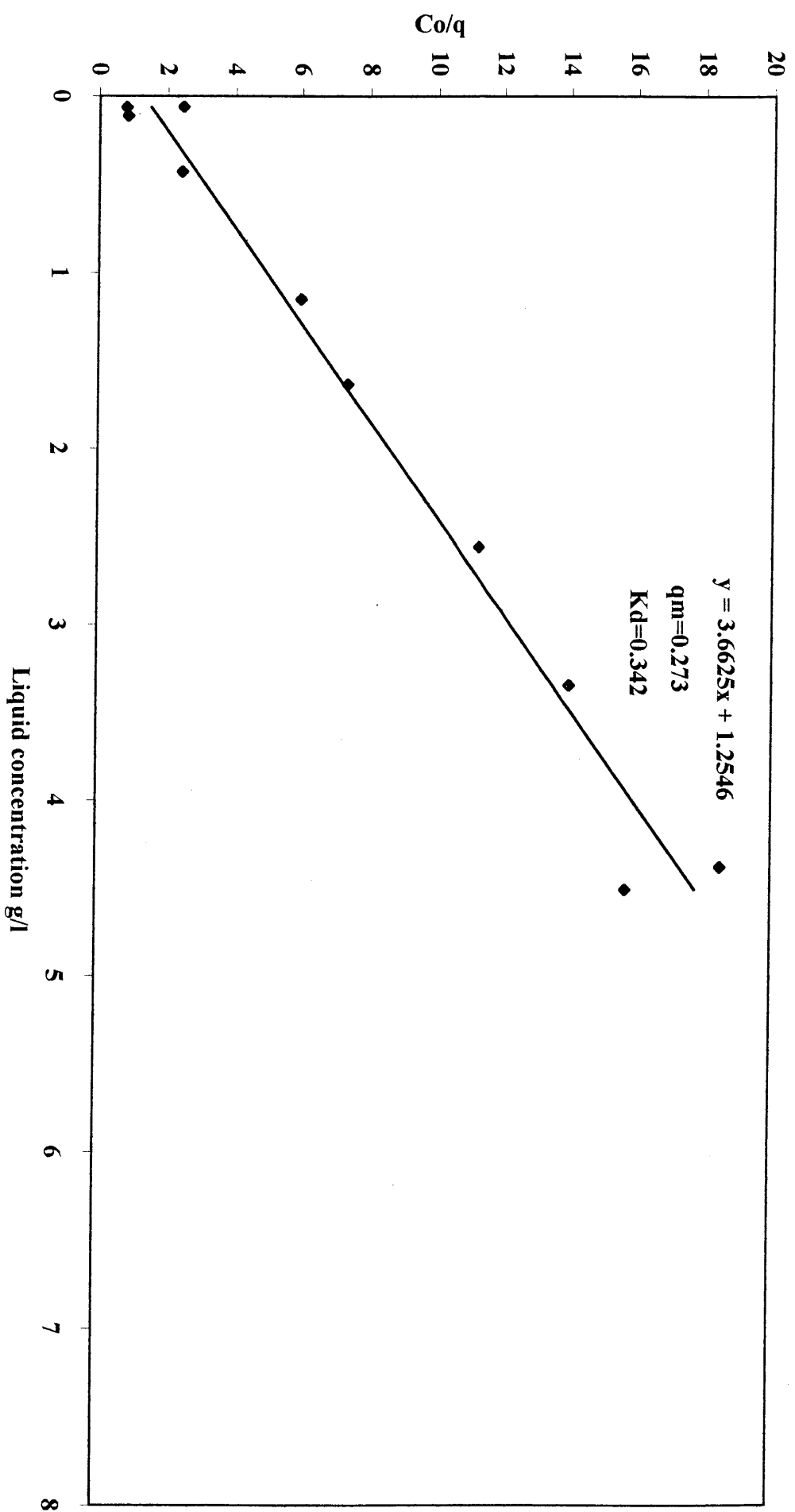


FIG. A.4 Linearised Langmuir isotherm, 2.5 g/l NaCl, first cycle

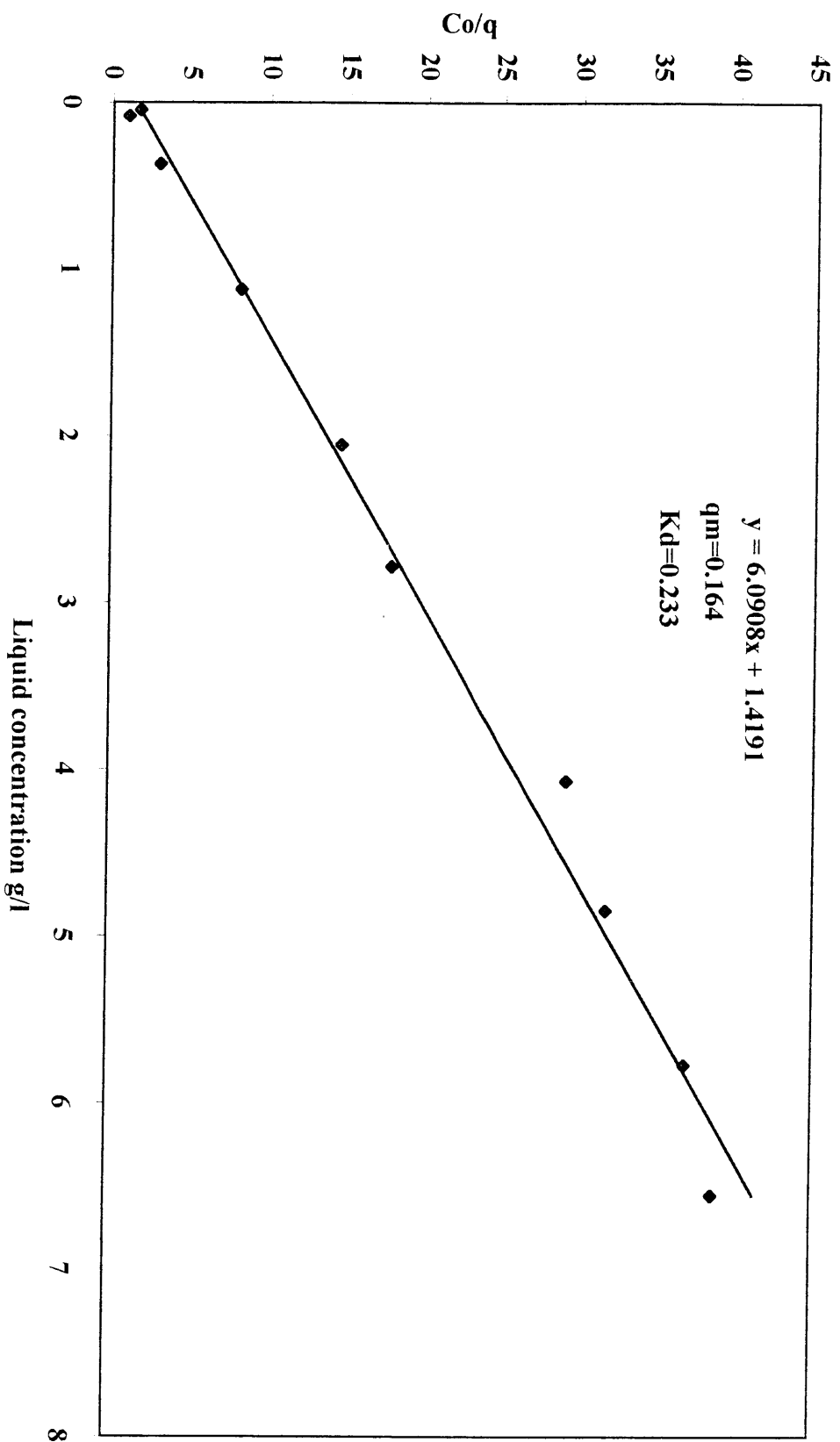


FIG. A.5 Linearised Langmuir isotherm, 2.5 g/l NaCl, second cycle

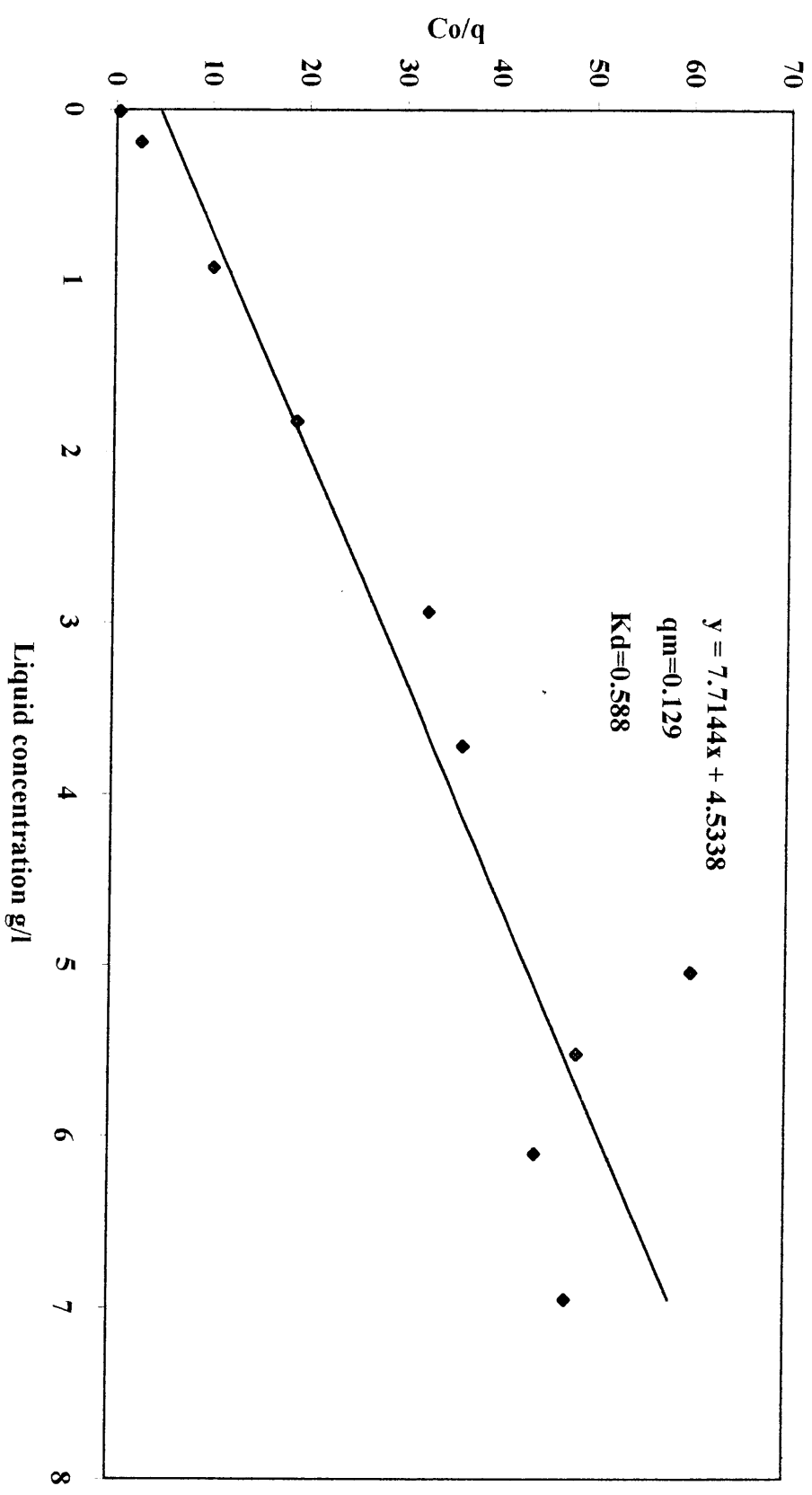


FIG. A.6 Linearised Langmuir isotherm, 2.5 g/l NaCl , third cycle

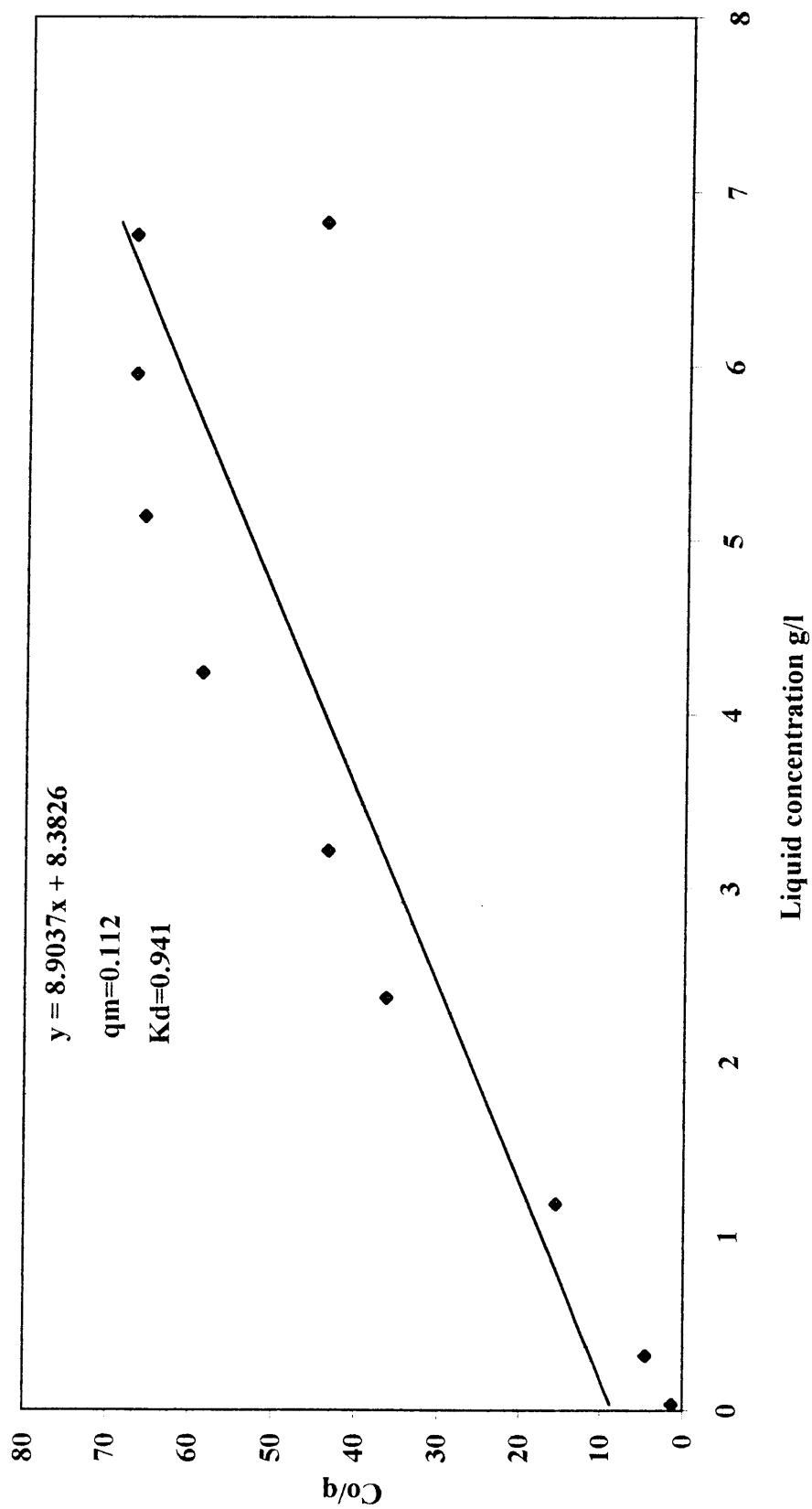


FIG. A.7 Linearised Langmuir isotherm, 2.5 g/l NaCl , fourth cycle

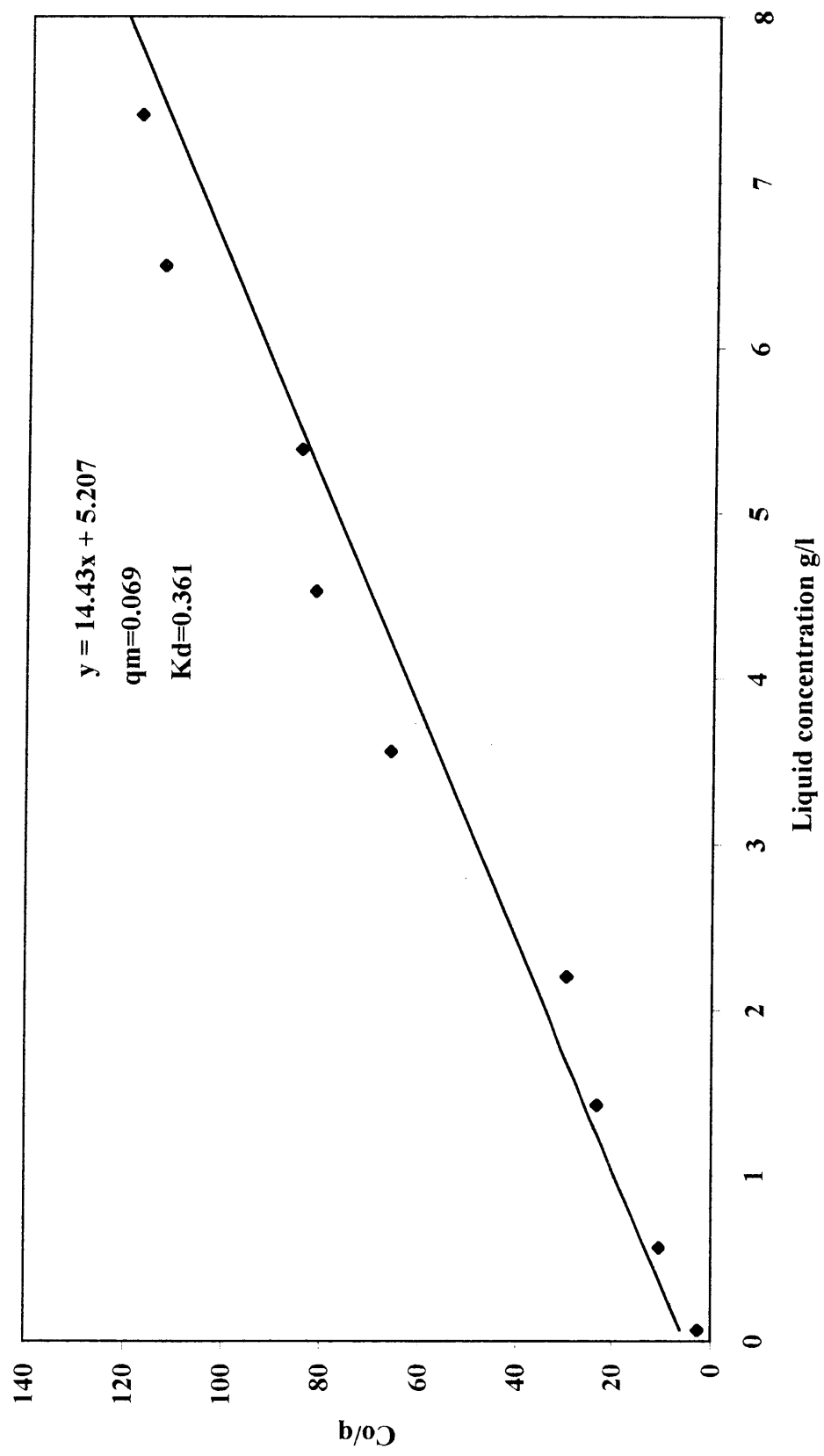


FIG. A.8 Linearised Langmuir isotherm, 2.5 g/l NaCl , after first regeneration with 6M urea

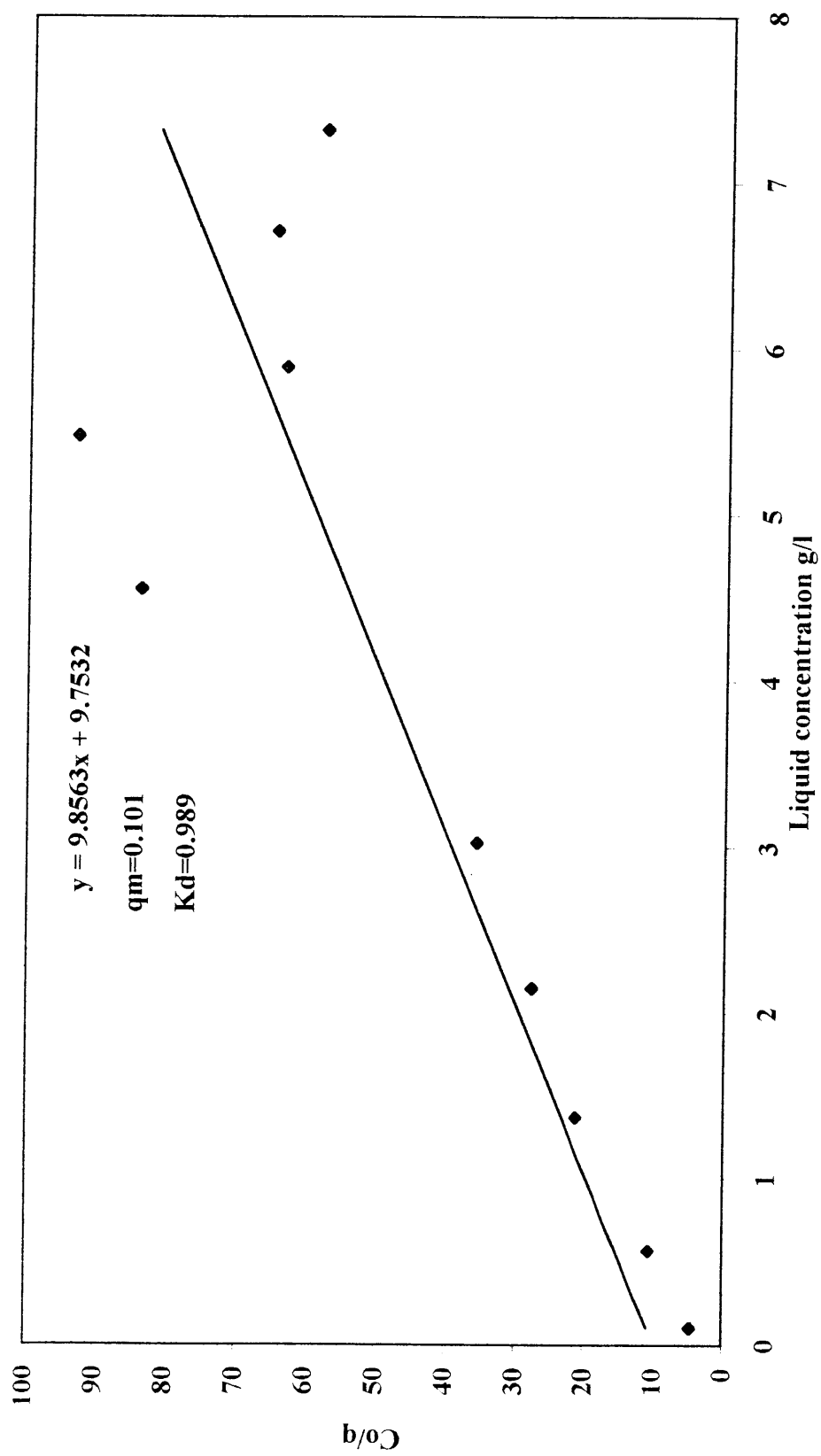


FIG. A.9 Linearised Langmuir isotherm, 2.5 g/l NaCl , after second regeneration with 7M urea

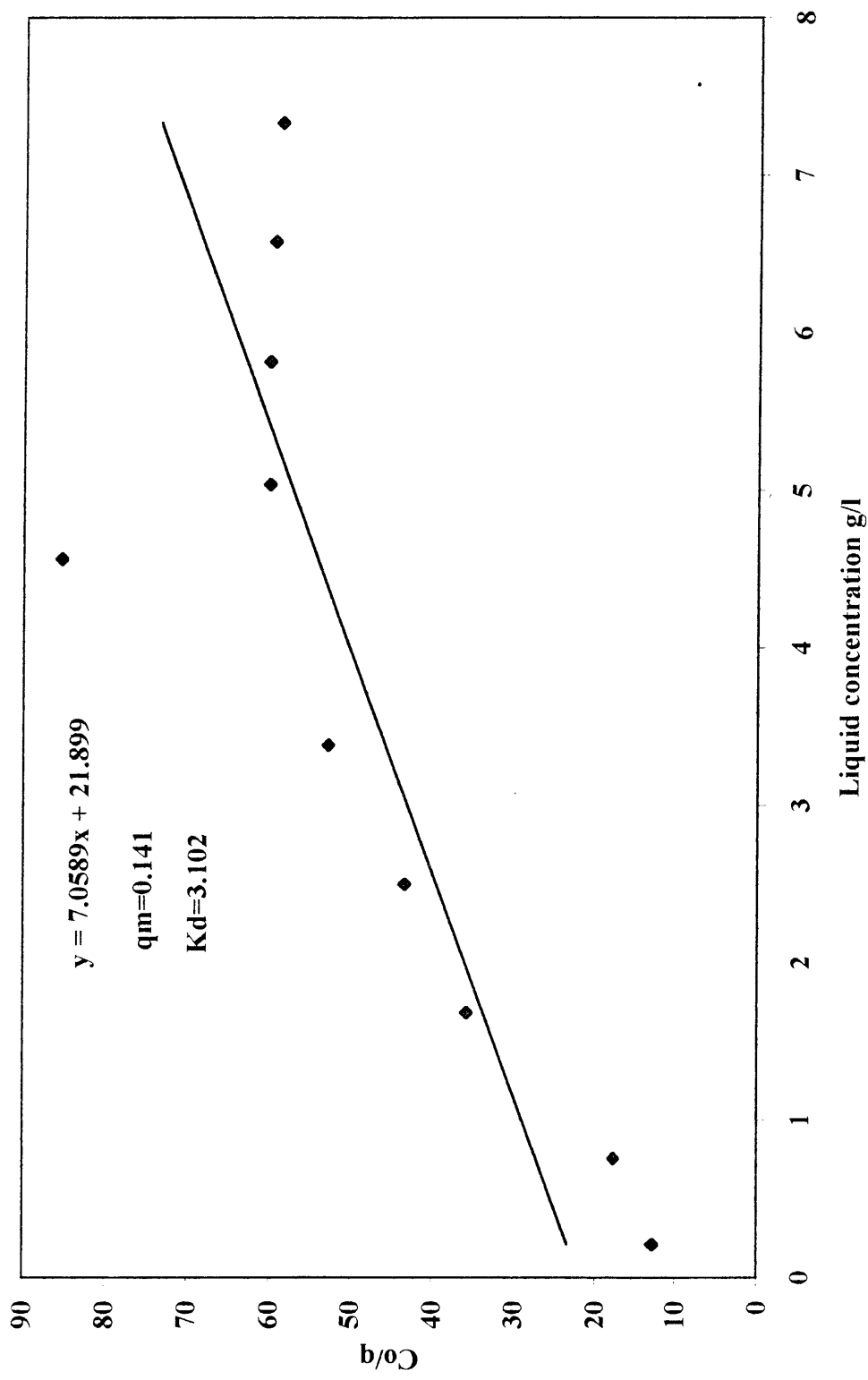


FIG. A.10 Linearised Langmuir isotherm, 2.5 g/l NaCl , after third regeneration with 11M urea

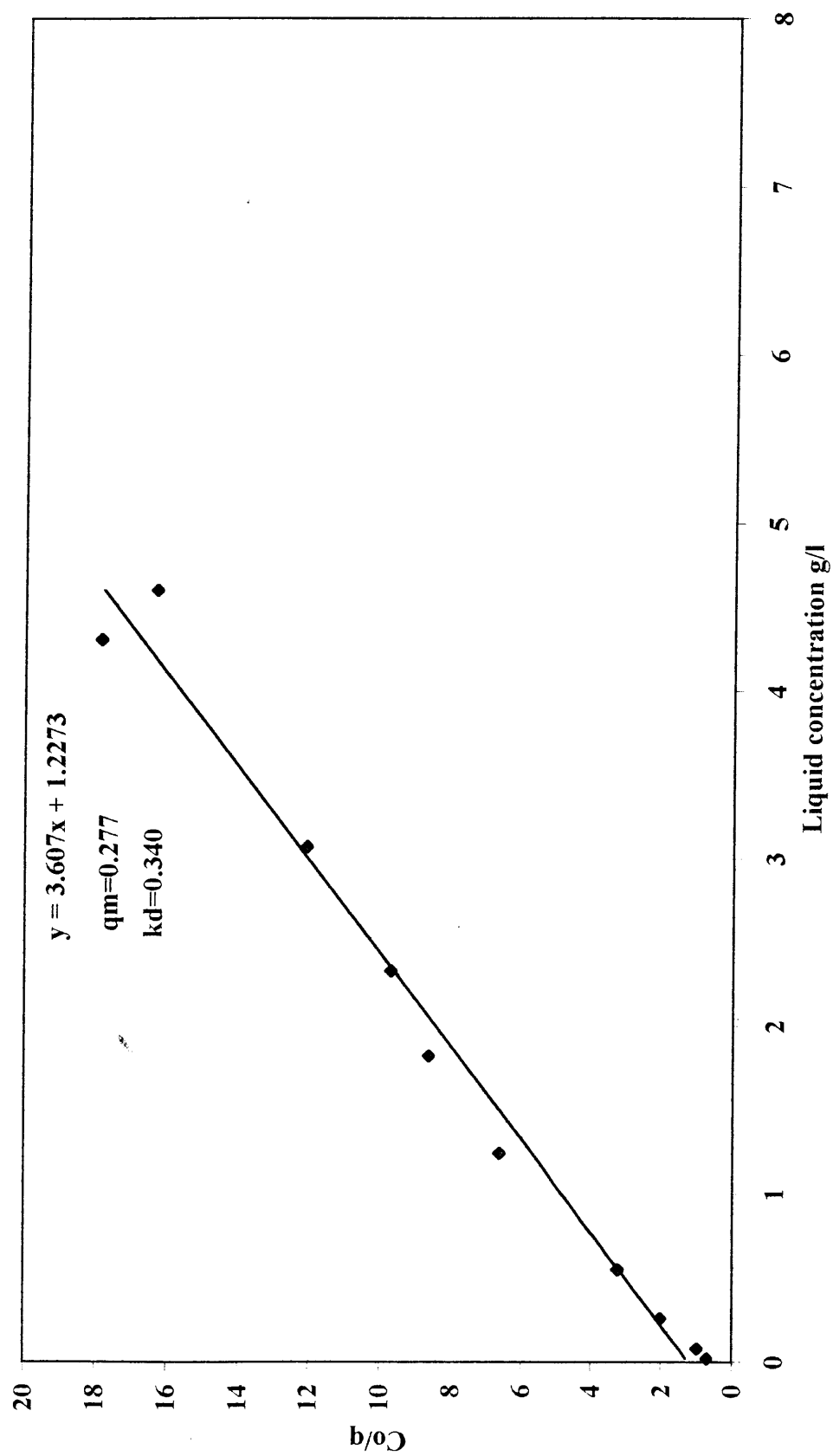


FIG. A.11 Linearised Langmuir isotherm, 2.5 g/l NaCl, first cycle (second series)

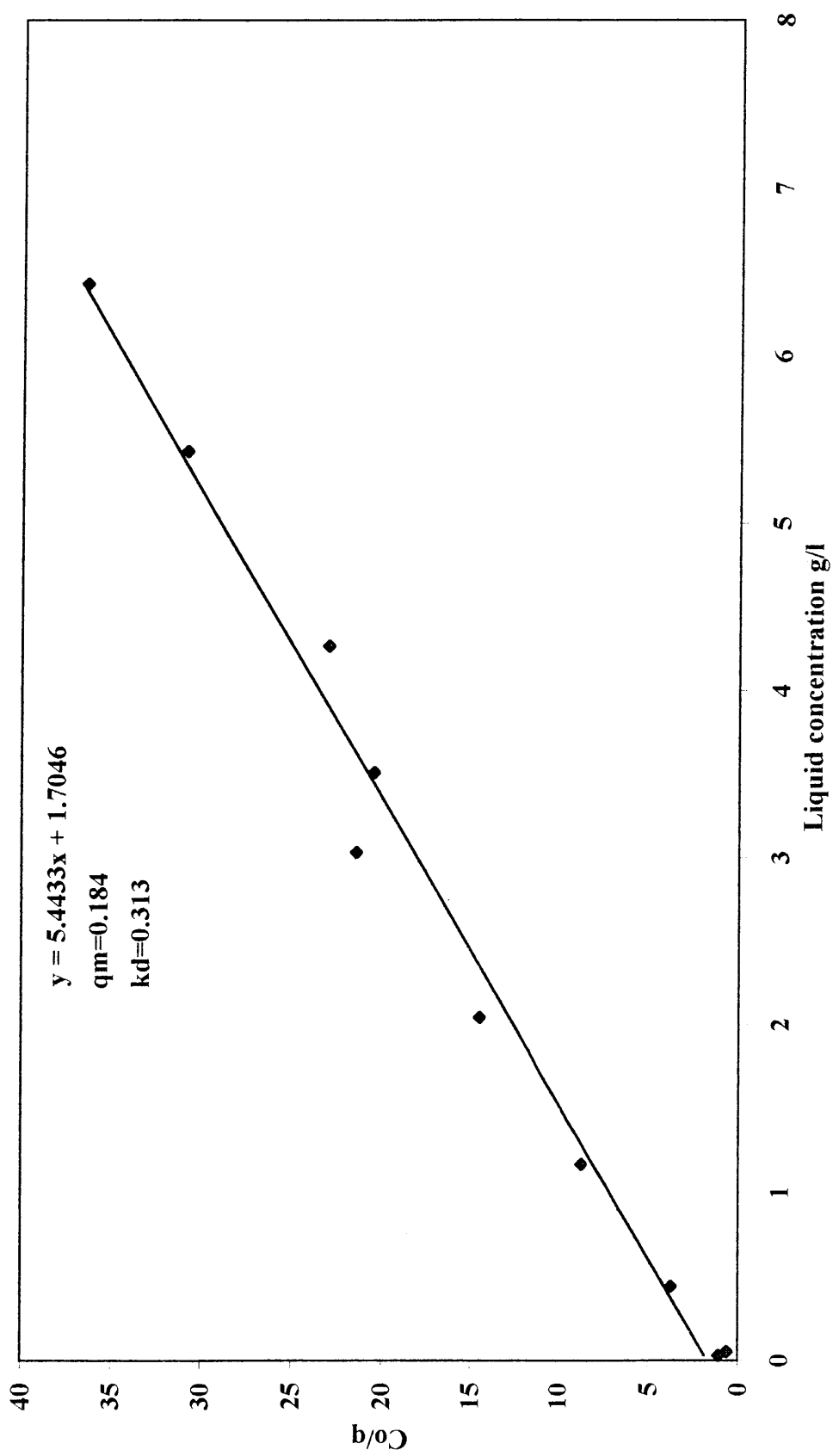


FIG. A.12 Linearised Langmuir isotherm, 2.5 g/l NaCl, second cycle (second series)

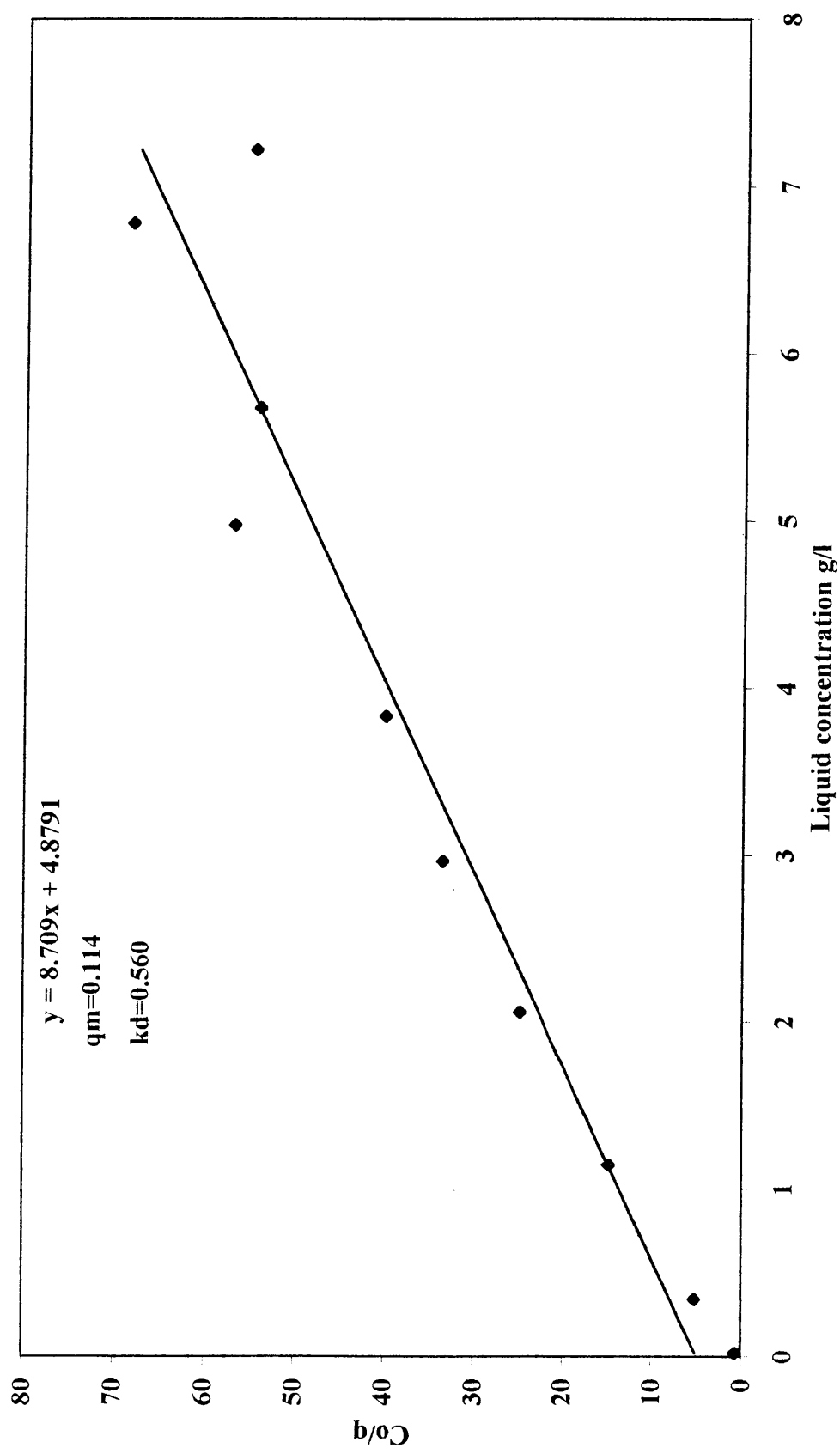


FIG. A.13 Linearised Langmuir isotherm, 2.5 g/l NaCl, after regeneration with 11M urea (second series)

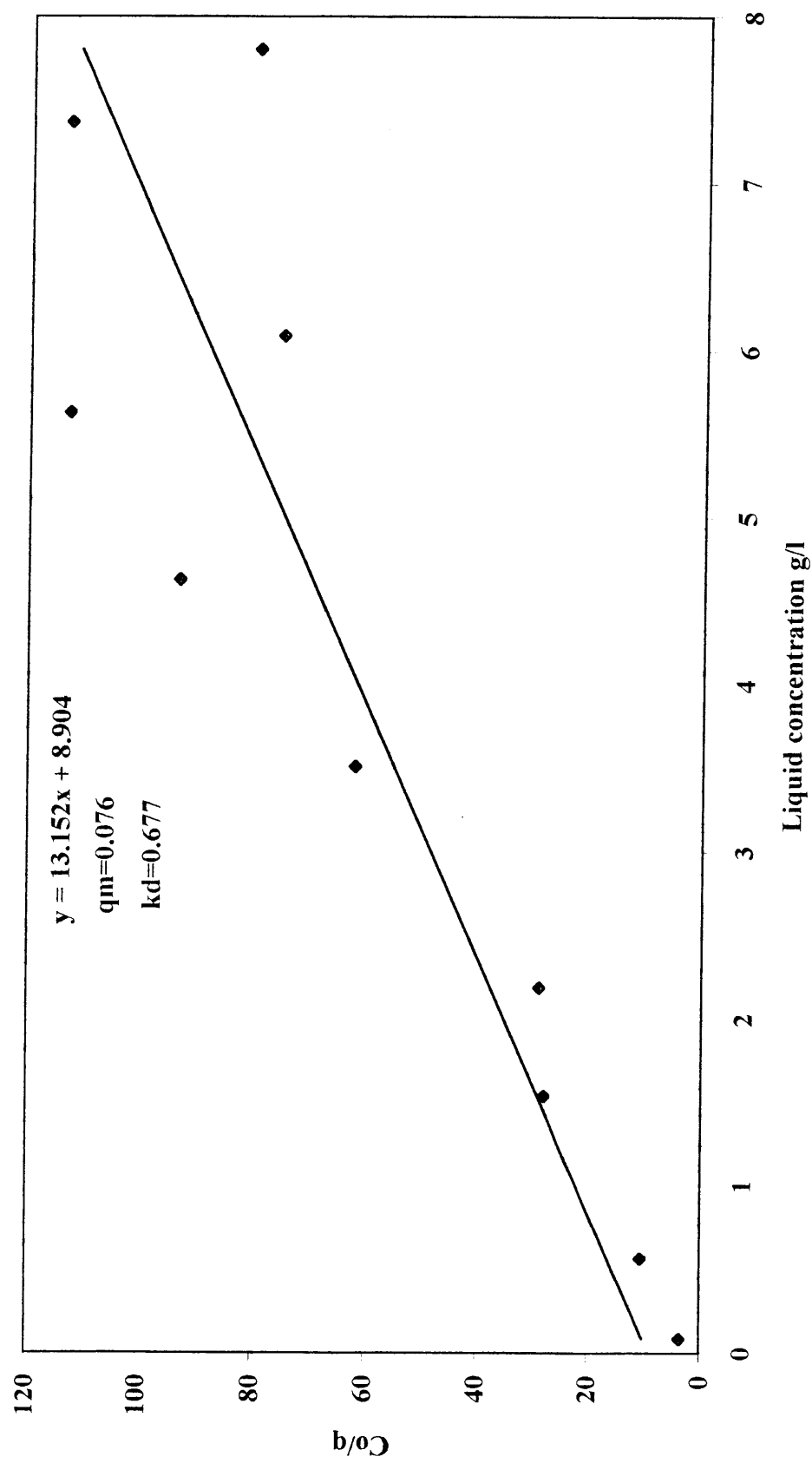


FIG. A.14 Linearised Langmuir isotherm, 2.5 g/l NaCl, after regeneration with 11M urea and desorption (second series)