**Izmir Institute of Technology**

**The Graduate School**

# APPLICATION OF ZEOLITES IN BIOTECHNOLOGY: PROTEIN ADSORPTION



**A Thesis in**

**Biotechnology and Bioengineering**

**Şebnem Karasu Özgü**



Submitted in Partial Fulfillment
of the Requirements
for the Degree of

**Master of Science**
**in**
**Biotechnology**

**July 1998**

We approve the thesis of **Şebnem Karasu Özgü**

1/7/98

**Prof. Dr. Semra Ülkü**
Supervisor
Department of Chemical Engineering

1/7/98

**Asst. Prof. Dr. Funda Tıhmınlıoğlu**
Department of Chemical Engineering

1/7/98

**Asst. Prof. Dr. Canan Tarı**
Department of Food Engineering

1/7/98

**Assoc. Prof. Dr. Şebnem Harsa**
Head of Interdisciplinary Biotechnology Program

# ACKNOWLEDGEMENTS

I hereby would like to thank my advisor, Prof. Semra Ülkü, for her guidance throughout the study and appreciate her knowledge and dedication particularly in zeolites; and to my co-advisor, Assoc.Prof. Şebnem Harsa, for her cooperation and expertise with proteins. I also thank my former teacher and my current boss, the President of Izmir Institute of Technology, Prof. Erdal Saygın for his support and patience in giving me the opportunity to carry on with my career during work hours. I respectfully recognize Prof. Halis Püskülcü for his contributions especially in using computerized statistical programs. I would also like to extend my warm feelings and gratitude to Şerife Şahin and Nilgün Özgül who have helped tremendously in the laboratory for this study.

# ABSTRACT

Recovery of proteins from various culture broths is a complex engineering problem, involving multi-step schemes that lead to significant loss of the desired bioproduct by conventional methods. The bioproduct is not only present in very low concentrations, but also it is subject to chemical/enzymatic degradation. An alternative cost-effective method for recovery of proteins in a highly purified form may be through adsorption, which is a separation technique based on specific and reversible binding, with the use of zeolites that have favorable adsorption characteristics over the other adsorbents. Protein adsorption characteristics of a natural zeolite (clinoptilolite from Turkey) and a synthetic (3A) zeolite were determined through various experiments, altering parameters as pH (range 3.5-6), the amount of zeolites used for adsorption (0.01-0.05 g/ml) and the initial protein concentration (0.01-0.1%). Within the concentration and pH ranges studied, the adsorption capacity of the zeolites was around 50 mg protein/g zeolite. For natural zeolite, approximately 98% was adsorbed within the first 2 minutes, while for the synthetic zeolite, 70% was adsorbed within the first 15 minutes. Uptake diagrams were obtained, adsorption isotherms were determined and Langmuir method was used to describe the isotherms. Desorption was also investigated after treating the zeolites with salt and acid, but further work is necessary for obtaining better recovery. It can be concluded that clinoptilolite is capable of adsorbing proteins in a short time. This study may be the preliminary step, followed by further laboratory work and necessary scale-up experiments, towards the use of zeolites in the recovery of proteins in industry as an alternative to conventional methods.

# ÖZ

Proteinlerin değişik kültür ortamlarından saflaştırılması zor bir mühendislik problemi olup, konvansiyonel yöntemler kullanıldığında arzu edilen biyo-ürünün önemli ölçüde kaybına sebep olan çok-basamaklı prosesleri içermektedir. Biyo-ürünün çok az konsantrasyonda bulunmasının yanısıra, aynı zamanda kimyasal/enzimatik bozunma da meydana gelmektedir. Proteinlerin yüksek derecede saflaştırılmasında alternatif bir yöntem, diğer adsorbentlere göre daha iyi adsorpsiyon özellikleri olan zeolitlerin kullanımıyla, spesifik ve tersinir bağlanmaya dayalı bir ayrım tekniği olan adsorpsiyon yöntemidir. Doğal (Türkiye'den clinoptilolite) ve sentetik (3A) zeolitlerin protein adsorpsiyon özellikleri; pH (3.5-6 bölgesinde), adsorpsiyonda kullanılan zeolit miktarı (0.01-0.05 g/ml), ve başlangıç protein konsantrasyonu (%0.01-0.1) parametreleri değiştirilerek incelenmiştir. Çalışılan konsantrasyon ve pH bölgelerinde, zeolitlerin adsorpsiyon kapasitesi yaklaşık 50 mg protein/g zeolit olarak bulunmuştur. Doğal zeolit ilk 2 dakikada %98 protein adsorplarken, sentetik zeolit ilk 15 dakikada %70 adsorplamıştır. Çıkış grafikleri ve adsorpsiyon izotermleri belirlenmiş, izotermleri yorumlamak için Langmuir metodu kullanılmıştır. Desorpsiyonu incelemek için zeolitler tuz ve asitle muamele edilmiştir, ancak bu konuda daha çalışma gerekmektedir. Sonuç olarak; zeolitler, proteinleri kısa zamanda adsorplama yeteneğine sahiptir. Diğer laboratuvar ve ölçek büyütme denemeleri ile desteklendiği takdirde, bu çalışma, zeolitlerin sanayide protein saflaştırmasında kullanımı için konvansiyonel yöntemlere alternatif olabilecek ilk basamaktır.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# INTRODUCTION

Recovery of proteins from the various culture broths is a complex engineering problem, involving multi-step schemes that lead to significant loss of the desired bioproduct by conventional methods. The bioproduct is not only present in very low concentrations, but also it is subject to chemical/enzymatic degradation. An alternative cost-effective method for bioseparation/recovery of proteins in a highly purified form may be through adsorption, which is a separation technique based on specific and reversible binding via biological reactions [1]. Research on protein adsorption include adsorption onto titanium powder, hydroxyapatite and non-biological glasses. All studies indicate increase in adsorption with decreasing pH, although not all follow the same model. It has been observed that natural zeolites have favorable adsorption characteristics over the other adsorbents. This study is the first one in examining protein adsorption onto zeolites. In this study, protein adsorption characteristics of a natural and a synthetic zeolite were determined through various experiments. The adsorption of bovine serum albumin onto zeolite has been studied as a function of pH, amount of zeolites and protein concentration. Isotherm data have been analyzed using the Langmuir model, the adsorption parameters were calculated. and will provide the basis for the efficient use of zeolites in protein purification/recovery.

# CHAPTER 1

# ZEOLITE

## Definition

The word "zeolite" derives from a Greek word *zeein* meaning to boil. Natural zeolites were discovered by a Swedish mineralogist, Redrich Cronstedt, in 1756 as ordinary volcanic minerals. It was not until the second half of the 18$^{th}$ century that these mineral specimens were found to contain high amount of zeolite. Many types were then discovered which include clinoptilolite, mordenite, erionite that have commercial value. This led to the development of synthetic zeolites as commercial cation exchangers in the early 20$^{th}$ century which were primarily used in water softening [2]. Low temperature synthesis and discovery of natural zeolite deposits of sedimentary origin enhanced the use of zeolites. The first application of dehydrated zeolites as molecular sieves in the separation of gas mixtures was demonstrated by Barrer in 1945. Synthetic zeolites were first utilized commercially as molecular sieve adsorbents in 1954 [2].

Zeolites have recently gained importance and become recognized as one of the most abundant zeolite mineral species on earth [3]. Zeolite may be defined as a mineral, characterized by a framework of linked tetrahedral enclosing open cavities in the form of channels and cages that commonly are occupied by water molecules and cations. These channels are large enough to allow the passage of guest species. Zeolites show different behavior when their framework is different [4].

## Formation and Areas Found

Zeolite is formed as a volcanic eruption product. Information on zeolite is collected by studying "epiclastic" volcanic sediments, which are formed through normal sedimentary processes. In order for zeolite to be formed, length of time for which ash remains at the optimum temperature is vital. External water is also needed for the formation ("zeolization"). Acidity of the zeolitizing fluid is very important; zeolitization

requires neutral to alkaline conditions. On the contrary, recent eruptions are highly acidic and zeolite cannot be formed [5].

Zeolite is found in almost every continent on earth. Commercial use is increasing in many countries which are also rich in zeolite deposits, with Bulgaria, Italy, U.S.A., Japan, South Africa and Cuba on the lead. In Turkey, most zeolite is in the form of clinoptilolite and analcime, and was first detected in 1971.

There are more than 150 synthetic and 40 natural zeolite forms [6]. One of the reasons for using natural zeolites is an economic one; since they are potentially much cheaper than synthetic zeolites, they are preferred for "low" technology applications [7].

## Structure

Zeolite has a tetrahedral structure with aluminum and silicate ions in center and oxygen ions on the corners. Its geometry allows the zeolite to separate mixtures of molecules in gas and liquid phases. It is considered microporous with a uniform pore size which makes it convenient for selective separation and purification processes [6]. This is why the terms "zeolite" and "molecular sieve" are frequently used interchangeably although not all zeolites are molecular sieves, e.g., natrolite contract on dehydration, whereas molecular sieves have a relatively rigid framework structure that do not change on dehydration [7].

In the determination of structure-related properties, various methods are used: infrared spectrophotometer (IR), X-ray diffraction (XRD), atomic adsorption spectrophotometer (AS), induced coupled plasma (ICP), and scanning electron microscope (SEM), X-ray fluorescence, neutron and electron diffraction, and high resolution electron microscopy. X-ray powder diffraction data gives the basis for determining structure where X-ray irradiation of zeolite powders produce a pattern from the regular arrays of ions within the structure. By studying the angle of pattern, structure can be determined. Thermal properties of the zeolite can be determined with differential thermal analysis (DTA), thermo-gravimetric analysis (TGA), differential

scanning calorimeter (DSC), and microcalorimeter. Adsorption-related properties are estimated by volumetric and gravimetric methods.

Zeolites show different behavior with different silicate/aluminum ratios. Aluminous zeolites are excellent desiccants, where siliceous zeolites act as organophilic non-polar sorbents [8]. Table 1 shows the comparison of low and high Si/Al ratios:

Table 1. Comparison of Low and High Silicate/Aluminum Ratios

| | **Low Si/Al** | **High Si/Al** |
|---|---|---|
| Stability | | |
| to acids | low | moderate to very good |
| to alkalis | good | moderate to low |
| thermal | good | very good |
| hydrothermal | low to moderate | moderate to good |
| Polarity | high to moderate | moderate to low |
| Catalytic activity | yes | yes |
| Catalytic carriers | yes | yes |

## Clinoptilolite

Clinoptilolite is the most abundant natural zeolite on earth. Occurrences of clinoptilolite are formed in marine and lake basins as a result of burial diagenesis or hydrothermal metamorphism at shallow depths. It comes in colors of white, gray, greenish or rose, depending upon the presence of accessory minerals. Clinoptilolite tuffs contain 70-90% clinoptilolite along with "impurities" as montmorillonite, celadonite, chlorite, low cristobalite, mordenite, as well as high temperature minerals as quartz, plagioclase, biotite and potassium feldspar [9]. Clinoptilolite and heulandite exhibit similar morphology; and heat treatment and elemental analyses are required for positive identification of these minerals [10]. At present, mining of clinoptilolite rocks is carried out in Japan, U.S.A., Bulgaria, Czechoslovakia, Hungary and Cuba abundantly. Deposits in Turkey are in Manisa-Gördes, Balıkesir-Bigadiç, Emet-Yoncaağaç, Kütahya-Saphane, Gediz-Hisarcık, İzmir-Urla, Cappadocia.

Clinoptilolite is alkali rich (Na + K > Ca + Mg), and has a Si/Al ratio between 4.3 - 5.3. Its hydrated form's pore openings range between 4.0 and 7.2 A. Clinoptilolite has three types of channels: Channel A (7.2 x 4.4 A), Channel B (4.0 x 5.5 A), and Channel C (4.1 x 4.0 A). Channels A and B are parallel to each other and are intersected by Channel C. Channel A is occupied by Ca, Mg, and Na; Channel B is occupied by Na and Ca, and Channel C is occupied by K. These cations are coordinated with the framework oxygen atoms and/or water molecules. Change of location affects types of cations, pore and channel dimensions, which has a strong effect on adsorption and ion exchange properties [11]. Typical maximum theoretical cation exchange capacity is around 2.6 meq/g [12]. Na- or K-rich clinoptilolite is usually thermally stable up to 700°C. It is quite stable when exposed to high concentration of acids and retains its crystalinity. Acid resistance can be increased with thermal treatment due to migration of cations to more inaccessible sites. Increase in porosity and adsorption capacity has been observed by some investigators with acid treatment [9]. It is less hard than quartz sand; on Moh's scale the hardness of clinoptilolite is 3.5-4, whereas that of quartz is 6-7; but can successfully replace quartz in the purification of drinking water. It was found that clinoptilolite filters purify water not only against coarse-grained, suspended and colloidal particles of mineral and organic origin, but also harmful chemicals as heavy metals and ammonia.

Its other applications include drying (air, ethanol, hydrocarbons), waste water treatment, fishery (removal of ammonium, ammonia, heavy metal ions), nuclear waste treatment and storage (Cs, Sr), air pollution control ($SO_2$, $CO_2$, $NO_x$), energy recovery, heating-cooling systems, animal feed additive, soil conditioner (efficient use of fertilizer and water), odor control, filler in paper and cement [11,13,14,15]. These are explained in more detail later in the chapter.

## 3A Zeolite

3A Zeolite is used as a synthetic zeolite model in our experiments. The number besides the letter suggests the pore dimensions, and difference between the other (4A, 5A) zeolites is in the cation present. 3A Zeolite contains the potassium ion, which

permits the entry of molecules smaller than 3 A° where only $H_2O$ and $NH_3$ can penetrate through the window. 3A Zeolite is used as a sealed cartridge in refrigeration circuits to dry the refrigerants, in vehicle breaking and air conditioning systems, and in heavy duty transformers, as well as heat and sound insulators. 3A Zeolite is preferred in drying cracked gas, ethylene, propylene and methanol [3].

## APPLICATION OF ZEOLITES

The major factors which determine the commercial use of zeolites include: (1) structural, thermal, adsorption and ion exchange properties; (2) availability; (3) cost.

Applications of zeolites have grown rapidly with increasing awareness of zeolite properties and their extensive potential uses. Some of the areas are as follows:

**a) Use as an ion exchanger**

Although the ion exchange process was discovered in 1850, it was not used in industrial separation process until 1905 in water softening and to remove certain metal ions, especially iron and manganese. From 1905 to 1935, aluminosilicates were the only ion exchangers available, operated in neutral pH regions. After 1935, sulfonated coal ion exchangers and their derivatives were commercially developed. Due to lack of knowledge, zeolites did not attract significant interest as commercial ion exchangers until the early 1960's. However, their superb qualities led the discovery of many zeolite structures and expanded their use. First commercial use involves processing radioactive wastes, followed by its use in wastewater treatment [12].

Zeolites provide unique combinations of selectivity, capacity and stability not available in other ion exchangers. Zeolites, particularly clinoptilolite, have a high exchange capacity for cations which makes them possible to be used in waste water, nuclear waste, and hard water treatment; and since they are especially $NH_4$-selective, removal of $NH_4$ from water which has a toxic effect. Many zeolites exhibit high selectivity for various heavy metals. Especially removal of Cd, Cu, Pb, and Zn from waste waters were studied [2].

Its use as a detergent additive [16] by removing $Ca^{++}$ and $Mg^{++}$ ions through selective exchange does not cause any environmental problems, as well. Since the 1960s, the use of phosphates as builders in laundry detergents has been criticized because of their contaminating effect. Regulations in many countries require reduction or total ban of the use of phosphates in detergents. Use of zeolites as phosphate substitutes has won recognition since 1978 and production of Zeolite A has been increasing ever since. Addition of zeolite boosts the detergency performance and reduces deposits on fabrics [17].

Zeolite is also used in medicine for artificial kidney dialysate regeneration [2]. Hemodialysis treatment in artificial kidney systems involves the transfer of uremic wastes through membranes by dialysis to a dialysate fluid while the small pores of the membranes prevent loss of desirable blood components. Since large volumes of dialysate solutions are needed for a single treatment, a process to remove the waste products from the spent dialysate solution, thus enabling its reuse was developed with the use of zeolites.

Zeolite's porous and rigid structure, as well as its stability across the range of common soil pHs and ion selectivity make it possible to be used extensively in agriculture. Clinoptilolite is used as agent for soil conditioning and remediation [18,19,20,21,22,23]. Even though mixed results have been found in using zeolites for slow-release fertilization, in general, zeolites have the potential to improve fertilizer-use efficiency by slowing nitrification, reducing volatilization losses, reducing leaching losses, and/or slowly releasing $NH_4^+$, $K^+$, and other nutrient cations. Their use as soil conditioners are to improve the physical properties of the soil and the remediate acidic or contaminated soils.

The term "zeoponics" has recently received attention as an important process in agronomy and horticulture which involves cultivation of plants in a synthetic soil that includes zeolites as an important component. Most zeoponic applications took place in Bulgaria, Cuba and the U.S. where large variety of crops were involved commercially. The applied systems take advantage of the chemical stability, hardness, rigid and porous

structure and nutrient-buffering capacities of the zeolite [23]. Slow release fertilization, zeoponics and soil conditioning techniques have great commercial potential because of demands for better fertilizer efficiency and environmental protection.

Clinoptilolite is used in animal feeding [24,25]. Extensive studies in Japan and the U.S. show increased weight gain, increased feeding efficiency, reduced incidence of intestinal and other diseases, e.g., ammonia toxicity, reduced death rates, increased egg shell quality, and lower odor of animal excrement [12,26]. It has been suggested as a dietary supplement to improve absorption of immunoglobulins, total protein, iron, and copper in newborn calves [27]. In ruminant animals, clinoptilolite alters rumen fermentation, thereby modifying fatty acid production by rumen bacteria and changing milk and body fat content. Zeolites may also be used in the sanitation of manure and animal bedding as an effective deodorizer and dehydration material [28]. Zeolites also protect pigs, chickens and turkeys from mycotoxins present in contaminated grains and reduce aflatoxin concentration in milk from cows fed aflatoxin-contaminated feeds. Although the exact mechanism is not yet known, ion exchange properties are likely of great importance. Widespread application awaits further quantification of animal responses [29].

Zeolite (clinoptilolite) is widely used in aquaculture; when extensive water reuse is practiced, the ammonia released directly by the fish and bacterial growth in the feed will reach toxic levels if not removed. Zeolites are used to remove ammonia which escapes the biological filter or as a backup system independently to remove $NH_4$ [12].

It should be noted, however, that the successful use of zeolites as ion-exchangers depends on the mineralogical, physical and chemical characteristics of the particular tuff and physicochemical parameters as equilibrium coefficient and kinetic coefficient must be known prior to use [30].

**b) Use as an adsorbent**

Natural zeolites are good adsorbents for $H_20$, $NH_3$, $H_2S$, NO, $NO_2$, $SO_2$, $CO_2$, but their use as sorbents in industry is limited. Synthetic zeolites (especially A and X)

are used for gas drying. Although alumina and silica are still used industrially because of their desirable thermal and mechanical properties, natural zeolites can replace them in some applications as natural gas drying, air drying and $NH_3$ removal during coal-gasification [13,31,32,33]. Some of the areas used can be summarized as: dehydration [32], removal of carbon dioxide and nitrogen, air pollution control (removal of Hg, $NO_x$, $SO_x$), bulk separation, energy conservation, solar energy and heat pump through solid-gas adsorption cooling system [13], various purification processes, vacuum freeze drying of food [34], aspirin adsorption to reduce side effects [35], aflatoxin adsorption [36].

**c) Catalyst**

Selective catalytic reduction is the most effective technique for removing $NO_x$ from flue gasses. Under low NO concentration and high $O_2$ access, zeolite is found to catalyze NO reduction and $NH_3$ oxidation [37]. In the hydration of acetylene, the Cd-exchanged form of natural clinoptilolite is found as one of the best catalysts [26]. Some other areas of zeolites used as catalysts are: Hydrocarbon transformation, hydrogenation and dehydrogenation, metanization, carbohydrate separation [38], catalytic and hydrocracking, hydroisomerization, dewaxing, methanol to gasoline conversion, benzene alkylation [3], dehydration, organic catalysis, inorganic reactions as $H_2S$ oxidation, CO oxidation and breaking of $H_2O$ to $H_2$ and $O_2$ [2].

**d) Other**

The most ancient use of zeolites is their use as building materials. Zeolites were found in the ruins of Etruscan, Grecian and Roman walls. This use has continued until the present time especially in Italy. Zeolitic tuffs have mechanical properties comparable with those of other natural building materials. The main characteristic is their ability to act as a thermal store due to their ability to adsorb and desorb water molecules reversibly [13]. Nevertheless, buildings are cool during the day and warm at night since the zeolite removes heat from the environment by desorbing water molecules during the warmest hours of the day, and returns this heat during the coolest hours at night by re-adsorbing water. Thus, thermal regulation is combined with the environmental humidity [39].

# CHAPTER 2

# ADSORPTION

## Definition

Adsorption is the adhesion of molecules on the surface of solids. As described by *Sing, et.al., a*dsorption is the enrichment of one or more components in an interfacial layer [40].

Substance in the fluid phase which is capable of being adsorbed is called "*adsorptive*", fluid already adsorbed is "*adsorbate*" and the solid used for adsorbing is the "*adsorbent*".

There are two kinds of adsorption: physical and chemical. Table 2 shows the comparison of the two:

Table 2. Characteristics of Physical and Chemical Adsorption

| Physical Adsorption | Chemical Adsorption |
|---|---|
| Low adsorption temperature | High adsorption temperature |
| Non-specific | Specific |
| Single and multi-layer adsorption | Only single layer adsorption |
| Definite in relatively lower temperature | Wide range of temperature |
| Fast and reversible | Slow and Irreversible |
| No electron transfer even though adsorbate polarization occurs | Chemical bonds are formed during electron transfer |

"*Physisorption*" occurs whenever an adsorptive is in contact with the adsorbent. In addition to the dispersion-repulsion forces, specific molecular interactions (e.g., polarization, field-dipole, field gradient-quadruple) usually occur as a result of particular geometric and electronic properties of the adsorbent and adsorptive.

*"Desorption"* is the converse process of adsorption, where the amount adsorbed decreases. *"Adsorption hysteresis"* arises when the reverse isotherm does not follow the

same profile as the forward process and adsorption and desorption curves do not coincide.

In "*monolayer*" adsorption, all the adsorbed molecules are in contact with the surface layer of the adsorbent; whereas in "*multilayer*" adsorption, more than one layer is involved.

## Adsorbents

It is important to define the characteristics of the adsorbent before deciding if it is appropriate to be used for a specific adsorption process. Adsorption is directly related to the features of the adsorbent as: shape, porosity, surface area, polarity, surface energy and number of adsorption sites [41]. Among these, the effect of porosity on adsorption has been the most extensively studied physical property.

Adsorbents of high surface area are generally porous. In adsorption, pore size of an adsorbent and total pore volume is important, as well as the shape of the pore. IUPAC has adopted classification of pores according to the width:

a. macropores (> 50 nm)
b. mesopores (2-50 nm)
c. micropores (< 2 nm)

However, these are arbitrary since the pore filling mechanisms are affected by the pore size and shape, as well as the properties of the adsorptive [42]. Commercial adsorbents are generally made from microporous solids, which have a well-defined pore size distribution [43].

There are three classes of adsorbents: Inorganic adsorbents, synthetic adsorbents, and composite adsorbents (combination of the first two):

1. Inorganic adsorbents: They are not very specific, have a low capacity and are difficult to generate (activated carbon, silica, calcium phosphate).

2. Synthetic adsorbents: They usually consist of cross-linked polymer chains.
3. Composite adsorbents: They combine the good mechanical properties of inorganic adsorbents with favorable adsorption properties of polymeric adsorbents [44].

## Adsorption Isotherms

When an adsorbent is in contact with a fluid, adsorption takes place, and after a sufficiently long time, the adsorbent and the fluid reach equilibrium. This can be seen through adsorption isotherms. Nature of the adsorption process can be told by interpreting the adsorption isotherm, which shows the relation between the amount adsorbed and the equilibrium pressure of gas at constant temperature in gas systems or the equilibrium concentration of the adsorbent in liquid systems [45].

Adsorption isotherms are classified by IUPAC into six groups according to the nature of adsorption. If the tendency to adsorb is high, isotherms with a steep initial slope ("high affinity isotherms"-Type I) are obtained. The straight line shows that the adsorbate has filled all the empty spaces of the adsorbent and adsorption occurs as a single layer. According to the Type I isotherm, initial part of the isotherm is associated with micropore filling, and low slope of the plateau is due to multilayer adsorption on the small external area. Type I isotherm is seen with microporous adsorbents, where micropore volume rather than internal surface area is the governing factor [40]. The maximum amount adsorbed is related to the surface available for adsorption [44].

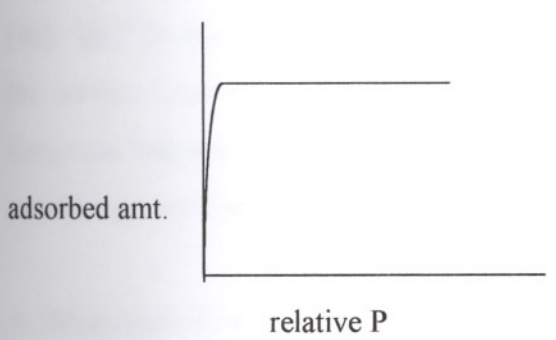



Figure 1. Adsorption isotherm - Type I

In the description of adsorption isotherms, various models are used. Among them, Langmuir model is the one used in the description of Type I isotherms:

$$\frac{q}{qs} = \frac{bc}{1+bc} \quad \text{..... (Eq.1)}$$

q = concentration of adsorbed protein at equilibrium

$q_s$ = adsorbent saturation capacity

c = free protein concentration at equilibrium

b = constant

When using the Langmuir model, several assumptions are made as: there is no change in structure upon adsorption; adsorption is reversible; there is no interaction between adsorbed molecules; adsorption occurs in a monolayer; and all adsorption sites are identical [12], which are not valid for all the cases.

## Determination of Surface Area

In determining the surface area, it becomes important to distinguish between external and internal surfaces, especially since adsorbents of high surface area are porous. It is noted that external surfaces include all the prominence and surface of those cracks which are wider than deep; and that internal surfaces comprise the walls of all cracks, pores and cavities which are deeper than wide and accessible to the adsorptive [40]. BET (Brunauer-Emmett-Teller) method is generally used for the determination of the surface area of porous materials. This method generalizes the treatment of Langmuir, and incorporates the concept of multimolecular layer adsorption. Following assumptions are made when using this method:

- Molecules on the outer layer of the adsorbate are in equilibrium with fluid
- Molecules on each layer of the adsorbate stay constant
- Adsorption heat of all layers except the first layer is equal to the molar condensation heat.

$$\frac{p}{n(p_o - p)} = \frac{1}{nQ} + \frac{(c-1)}{n_m Q} * \frac{p}{p_o} \quad \text{..... (Eq.2)}$$

n = amount adsorbed

$p/p_o$ = relative pressure

$n_m$ = monolayer capacity

Q = constant (related exponentially to the heat of adsorption for the first layer).

It should be noted that when using the BET method in micropores, different terminology arises. *"Micropore capacity"* is the amount of an adsorptive required to fill all the micropores of an adsorbent. Converting this to the *"micropore volume"*, it is assumed that the pores are filled by liquid adsorptive. However, this assumption excludes the fact that pore size and shape affect the degree of molecular packing in small pores [42]. The whole volume present in micropores may be regarded as adsorption space and the process which then occurs is the "*micropore filling*", which differs from surface coverage that takes place on the walls of open macropores or mesopores.

## Adsorption Properties of Zeolites

One of the main structure-related properties by which a zeolite is classified is its adsorptive property. The amount sorbed gives idea about the porosity of the zeolite; the higher the porosity, the greater the amount sorbed [46], since the surface area and pore volume increase with porosity.

Adsorption properties of a zeolite is affected by its structure, location and size of its cations and presence of molecules formerly adsorbed. It is possible to change the zeolite structure for more efficient use as an adsorbent or catalyst. Some of the methods used are: decationization and cation exchange, dealumination (washing with acids).

Adsorption characteristics are usually presented as isotherms (plot of amount of adsorbed as a function of fluid concentration at constant temperature), isobars (plot of

amount adsorbed as a function of temperature at constant pressure) or isosters (plot of fluid concentration as a function of temperature when the amount adsorbed is constant).

When compared with other adsorbents like activated carbon, silica gel, activated alumina and clay, zeolites have uniform pore sizes, which are also uniform throughout the particle. Although in all other adsorbents adsorption depends on the fluid phase concentration and is low at low concentrations, zeolites are capable of working well under low concentrations. These properties make zeolites convenient for selective separation and purification processes [32].

# CHAPTER 3

# PROTEINS

## Structure and Purification of Proteins

An understanding of the structures and interactions of proteins should form the basis for obtaining selective adsorption.

Proteins are co-polymers of aminocarboxylic acid groups which are joined together by amide linkages formed by the condensation reaction of the amino group of one monomer with the carboxylic acid group of another. Molecular weight of a protein is larger than 10,000, but may be as high as several million. Proteins can be either water insoluble such as fiber-forming proteins (e.g., silk, wood and collagen), or water soluble as blood protein and casein. The fundamental structure consists of a hydrophobic interior and a hydrophilic exterior [47]. The molecular structure of protein is chemically represented as $NH_2$-CHR-COOH where R can be derived from any of 23 different aminocarboxylic acids.

A set of process operations are used within the food and chemical industries dealing with protein purification. This process is classified into four phases:

1. Removal of insolubles from the starting biomass;
2. Product isolation;
3. Product purification;
4. Polishing and packaging.

First phase requires primarily mechanical operations, e.g. cell disruption, extraction, centrifugation, and filtration. Second phase yields a low-resolution separation of product molecule from the other materials that may be present. Third phase employs processes of high-resolution and selectivity to refine the product from molecular species that may be similar in physical or chemical characteristics. Final phase will often employ purification techniques of lower resolution to remove single specific components that passed through the previous refinement stages. This phase employs processes as

formulation, freeze-drying, sterile filtration, etc. to prepare the product for packaging and storage.

Figure 2 shows steps of protein purification [48]:

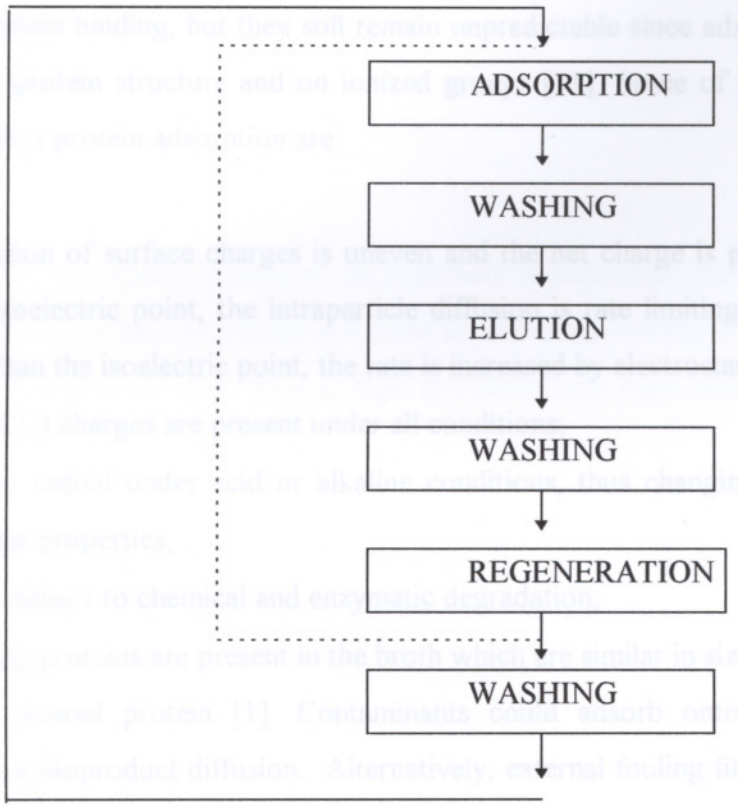



Figure 2. Protein purification

## Protein Adsorption

Various purification techniques have to be used to isolate proteins from their crude source to the desired purity. Researchers studying adhesion and behavior of platelets, endothelial cells, fibroblasts and other cells on substrates are dealing with protein adsorption, as well as food technologists. Research on protein adsorption will also enable scientists to examine the interactions between blood and synthetic materials [49].

Industrial applications of adsorption techniques for protein purification have attracted considerable attention because of high selectivity/cost ratio [50]. Adsorption is

a widely used method in separation processes; however, protein adsorption is not common. Protein adsorption involves the adsorption of protein molecules from a liquid phase onto a solid phase; but yet is a complex process since the surface of proteins is continuously changing because of its metabolic and other growth activities. A number of studies on adsorption have been made to understand the interaction mechanisms involved in protein binding, but they still remain unpredictable since adsorption depends mainly on the protein structure and on ionized groups [51]. Some of the complicating factors that affect protein adsorption are:

- The distribution of surface charges is uneven and the net charge is pH-dependent; at pH near the isoelectric point, the intraparticle diffusion is rate limiting, whereas at pH values lower than the isoelectric point, the rate is increased by electrostatic effects [52];
- Both (+) and (-) charges are present under all conditions;
- Proteins may unfold under acid or alkaline conditions, thus changing their apparent physicochemical properties;
- Proteins are subject to chemical and enzymatic degradation;
- Contaminating proteins are present in the broth which are similar in size, charge, etc. to those of the desired protein [1]. Contaminants could adsorb onto the pores and eventually block bioproduct diffusion. Alternatively, external fouling film on the surface may form and reduce diffusion by introducing mass transfer resistance.
- Protein denaturation is a common problem in chromatographic separations due to significant alteration of protein's structure and properties by changes in pH, temperature, ionic strength, etc. Likewise, addition of solvents or contact with different surfaces can also alter protein formation. Protein recovery is greatly reduced by denaturation, and adsorption mechanisms may be misinterpreted [47].

Nevertheless, strong amphipathic nature of proteins, resulting from their mixture of polar and non-polar groups, causes them to be adsorbed. This property enables proteins to be used as food additives to stabilize food foams and emulsions with prolonged shelf life, as well as separation of proteins using foam fractionation in downstream bioprocessing [53].

Following are the steps of the protein adsorption-desorption process:

(i) transport of protein molecules to the adsorbent;

(ii) attachment;

(iii) rearrangement of the conformation of the adsorbed molecule in response to the change in micro environment;

(iv) detachment;

(v) diffusion away from the surface [54].

In protein adsorption, choice of the adsorbent is important, since it should have an affinity only for the desired protein and none for the other components present in the broth. Immobilized antibodies, having high specificity and high binding affinity, are widely used in protein purification [55].

Zeolites are used as an alternative adsorbent for protein adsorption in this study. Zeolites are made from small particles of the microporous solid formed into macroporous particles of a size convenient for process use. In general, when protein molecules are being adsorbed, they face several mass transfer resistances as: film diffusion resistance (transfer from bulk liquid to the outer surface of the particle), pore diffusion resistance (movement by diffusion into the pores) and surface reaction resistance (interaction at the binding site) [55]. Whether external or internal resistances will be effective will depend on the conditions. Zeolite diffusivity is highly affected with dehydration, where cation locations are also altered [43]. Moreover, mass transfer is affected by the pore size and tortuosity [52]. All these limiting resistances should be taken into account in the application of adsorption process [56].

In highly specific separations, only the desired product adsorbs while others run through; any remaining contaminants are removed with a wash procedure and desired product is eluted in a pure form with an eluent that reduces the affinity of the adsorbate for the adsorbent. Acidic buffers or strong solutions of urea are often used to elute proteins.

Most large-scale adsorption processes are carried out with packed bed. Advantages of using a packed bed can be outlined as:

1. easy scale-up;
2. stages of separation can be automated;
3. high degree of purification is achieved in a single step process [55].

# CHAPTER 4

# MATERIALS AND METHODS

Natural zeolite (clinoptilolite), which was identified in previous studies [13,32] from Manisa region, Turkey, and synthetic (3A) zeolite were crushed, ground, soaked and thoroughly washed with water at 90°C to remove undesired solvable impurities, and preconditioned by thermal activation in a furnace at 250°C for 8 hours. Microscopic examination shows that impurities formerly present were partially removed with this process. They were stored in a vacuum desiccator before use in adsorption experiments. Zeolite samples were reduced in size with the use of balling mill to have uniform size and to increase the surface area available for adsorption.

Protein used in this study was bovine serum albumin (BSA) (See Appendix A).

Batch kinetic experiments were carried out to investigate the effects of the pH, the zeolite amount, and protein concentration. When protein adsorption experiments are carried out in the laboratory, adsorbent is added to a series of flasks containing adsorbate at various concentrations. The flasks are agitated in a water bath at constant temperature until adsorption reaches equilibrium. Concentrations of protein in the liquid phase (c ) is measured, adsorbate concentrations and equilibrium capacities of the adsorbent (q) are calculated by simple mass balances.

For each sample, four different buffer solutions were prepared to determine the effect of pH on adsorption (pH range 3.5-6). Zeolite was conditioned in these buffers for 24 h. to have pH stability Because of the possible denaturation of proteins, protein solution was conditioned in a potassium phosphate buffer [51]. 1 ml 0.1% BSA was added to the known amounts of zeolite samples in flasks in a water bath at 25°C and agitated at 300 rpm. Samples from the liquid phase were taken against time to determine adsorption kinetics and centrifuged for 10 min at 3000 rpm. Protein analyses were carried out using Shimadzu UV 1601 uv-visible spectrophotometer, Lowry method was used to determine the amount of protein with the use of the protein assay kit by Sigma

Diagnostic and standard calibration curve was obtained (see Appendix B). BSA concentrations were determined by uv absorbance at 750 nm and the optical densities were converted to concentrations by reference to calibration data. Results were determined as shown in the sample calculations (see Appendix B).

Knowing the initial protein concentration, the amount of BSA adsorbed by the zeolite was calculated by simple mass balance. In order to put zeolites in favorable conditions for competition, acidity where the optimum adsorption obtained (pH: 3.5) was used to determine the effect of zeolite amount in adsorption by using 3 different amounts (0.01, 0.03 and 0.05 g/ml).

Third parameter that could affect adsorption was the protein concentration. The equilibrium liquid-phase concentrations were obtained by allowing 4 days for the adsorbent-liquid mixtures to come to equilibrium. Three different protein concentrations (0.01, 0.05, and 0.1 g/ml) were used and samples were taken against time for both zeolites.

Desorption was also investigated after treating the zeolites with salt and increasing the pH. pH of the fluid where the zeolite samples were kept was increased to 8.5 for 3 days, however no desorption was observed. When samples were treated with 3M NaCl, desorption did take place.

Adsorption isotherms are obtained for both zeolite samples and Langmuir model was used in the description of adsorption equilibria.

# CHAPTER 5

# RESULTS AND DISCUSSION

Local clinoptilolite was characterized experimentally in a volumetric adsorption system (Micromeritic ASAP 2010M), by using nitrogen gas with high purity as the adsorptive after degassing 1 hour at 90°C, 4 hours at 200°C and 24 hours at 350°C. Following data was obtained:

| | | |
|---|---|---|
| BET surface area: | 29.05 $m^2/g$ | (correlation coefficient = 0.998) |
| Langmuir surface area: | 29.32 $m^2/g$ | (correlation coefficient = 0.998) |
| Medium pore diameter: | 5.8 A | (actual pore size is smaller). |

Since pore diameter of the clinoptilolite is less than 4 A and molecular diameter of nitrogen is around 3.6 A, surface characterization of clinoptilolite using nitrogen is not a suitable method; because the diffusion mechanism for nitrogen entrance to the pores at the liquid nitrogen temperature is very small and sometimes even impossible, and attainment of equilibrium is quite difficult. Nevertheless, this method is conventionally used for comparison of surfaces [57].

In our experiments, we found out that pH does not have a significant effect on the adsorption of local clinoptilolite, whereas in 3A, it is highly critical. Acidic pH is more favorable in protein adsorption on 3A zeolite. As also stated by *Fargues et.al.*, BSA adsorption increases when the pH of the solution decreases [51], whereas *Yamamoto et.al.* shows decrease in adsorption with decreasing pH [58]. It has been noticed that maximum protein adsorption takes place near its isoelectric point which supports our findings. *Yoshida et.al.* states that effect of pH on the isotherm is strong around pH = pI. Around the isoelectric point, interaction between protein and surface becomes weaker and diffusion proceeds more rapidly. Since the pI of BSA is 4.9, the BSA molecules would be negatively charged at pH>5 and may be adsorbed by electrostatic attraction; and as the negative charges of the BSA increase with increasing pH of the solution, the electrostatic attractions becomes stronger [58]. With decreasing

pH, the electrostatic interaction between the protein and the adsorbate surface increases, thus increasing the rate of adsorption [52]. Results are given in figures 3-6 for natural zeolite and figures 8-11 for 3A Zeolite; comparisons are given in figures 7 and 12 for natural and 3A Zeolite, respectively.

As can be seen in Figure 13, the higher the amount of zeolite, the lower the amount adsorbed. Although the curves were expected to overlap, this behavior might indicate that external mass transfer is also important and that shaking is not enough to get a uniform liquid phase concentration around the zeolite particles. When sufficient shaking is achieved, better diffusion and adsorption is obtained. There is a fluctuation observed with zeolite amount 0.01 g/ml. Similar results were obtained by other researchers [59-61], and this was explained due to a result of rearrangements in the structure of already adsorbed molecules. Conformational changes in the molecules lead to some unfolding, which results in an increase in the number of protein sites contacting the surface. Some protein molecules may become detached in favor of the spreading of other adsorbed molecules.

Natural and synthetic zeolites act differently against changes in protein concentration. Synthetic zeolite is found to be more stable against changes. Experimental results and comparisons are given in figures 14-19.

Adsorption isotherms are obtained and Langmuir model is used in the description of adsorption isotherms. In order to test Langmuir isotherm against experimental data, Equation 1 can be rewritten in the form:

$$\frac{1}{q} = \frac{1}{q_s} + \frac{1}{bq_s} * \frac{1}{c} \quad \text{.....(Eq.3)}$$

The plot of 1/q against 1/c should yield a straight line of slope $1/ bq_s$ , intercept $1/ q_s$. Langmuir model gives approximate representation of the system behavior at low concentrations, but breaks down in the saturation region when the effects of molecular interaction become important. SPSS statistical program is used on the computer to obtain the results within the concentration range 0.04-3.58 mg/ml for clinoptilolite, and

0.0001-0.574 mg/ml for 3A Zeolite. As can be observed from the results that are shown in Table 3 and in figures 21-24, predicted values are analogous to experimental results.

Desorption was also investigated when samples were treated with salt and their pH was increased with the addition of an alkali buffer. Increased pH did not have any affect on desorption for neither of the zeolites. When treated with 3M NaCl, 20-25% desorption took place during the first 24 hours, reaching only 35% in 7 days. Further laboratory work is required to obtain better recovery of proteins from zeolites through desorption.

Table 3. SPSS data results for adsorption isotherms

| | **Natural Zeolite** | **3A Zeolite** |
|---|---|---|
| **$1/q_s$ (interception)** | 0.023187 | 0.045523 |
| **$q_s$ (maximum adsorption)** | 43.128 | 21.97 |
| **$1/bq_s$ (slope)** | 0.001273 | 0.0000455724 |
| **b (constant)** | 18.21 | 998.7 |
| **$r^2$** | 0.90711 | 0.9949 |

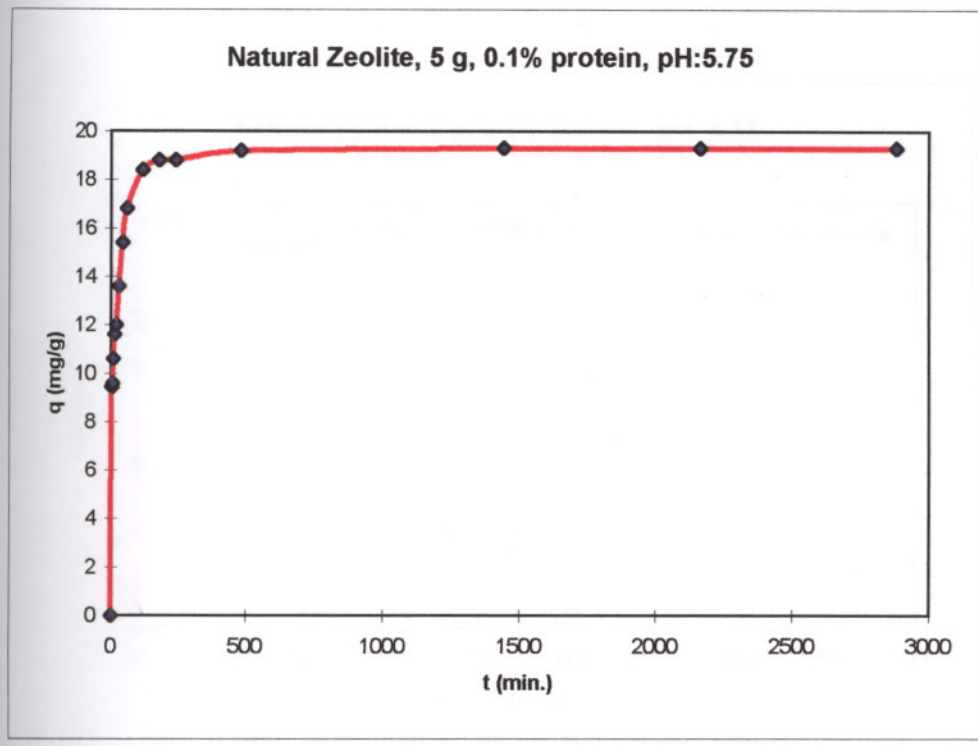



Figure 3. Uptake (q vs.t) Diagram for Natural Zeolite-BSA Pair

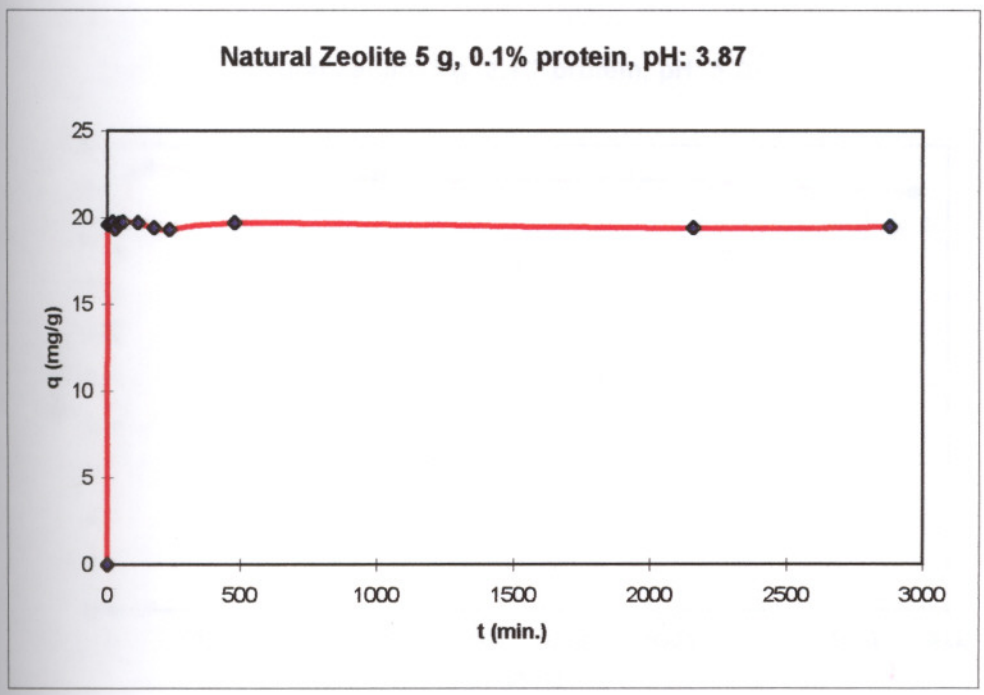



Figure 4. Uptake (q vs.t) Diagram for Natural Zeolite-BSA Pair

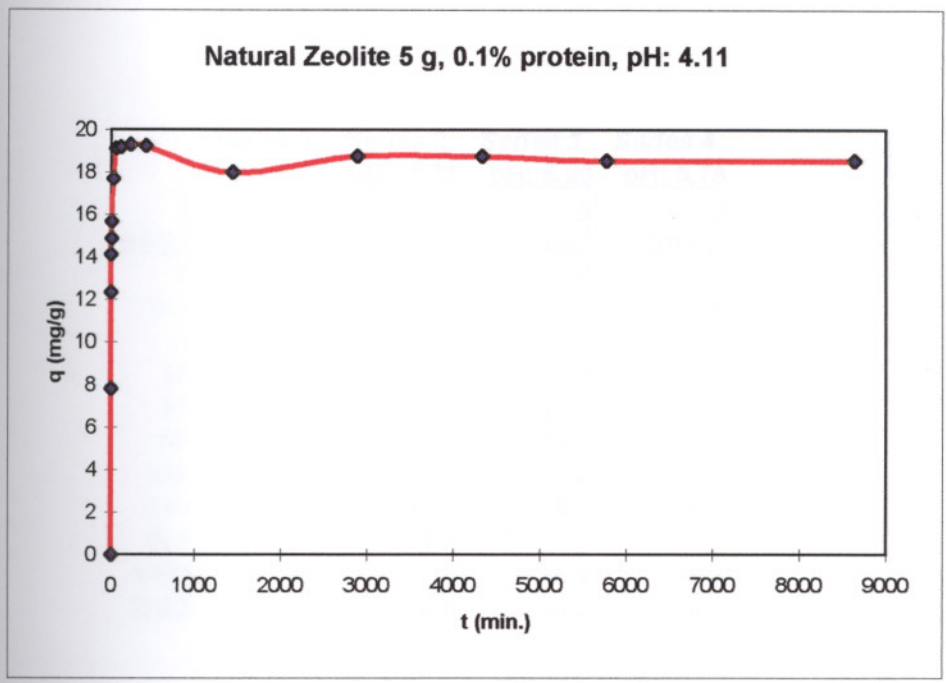



Figure 5. Uptake (q vs.t) Diagram for Natural Zeolite-BSA Pair

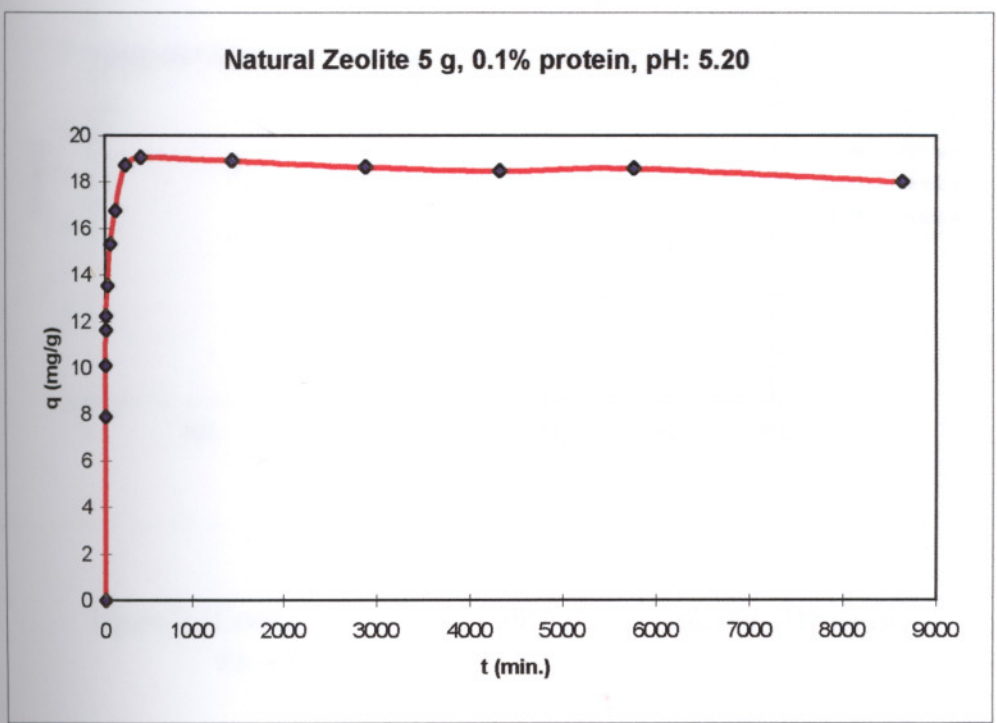



Figure 6. Uptake (q vs.t) Diagram for Natural Zeolite-BSA Pair

| | *Series 1* | *Series 2* | *Series 3* | *Series 4* |
|---|---|---|---|---|
| | **pH: 3.87** | **pH: 4.11** | **pH: 5.20** | **pH: 5.75** |
| **t** | **q** | **q** | **q** | **q** |
| (min.) | (mg/g) | (mg/g) | (mg/g) | (mg/g) |
| 0 | 0 | 0 | 0 | 0 |
| 2.5 | 19.6 | 14.4 | 10.1 | 9.4 |
| 5 | 19.6 | 14.9 | 11.6 | 9.4 |
| 10 | 19.6 | 15.7 | 12.2 | 10.6 |
| 30 | 19.3 | 17.7 | 13.5 | 13.6 |
| 60 | 19.7 | 19.1 | 15.3 | 16.8 |
| 120 | 19.7 | 19.2 | 16.8 | 18.4 |
| 240 | 19.4 | 19.3 | 18.7 | 18.4 |
| 420 | 19.7 | 19.2 | 19 | 19.2 |
| 1440 | 19.7 | 18 | 18.9 | 19.3 |
| 2880 | 19.5 | 18.8 | 18.6 | 18.9 |

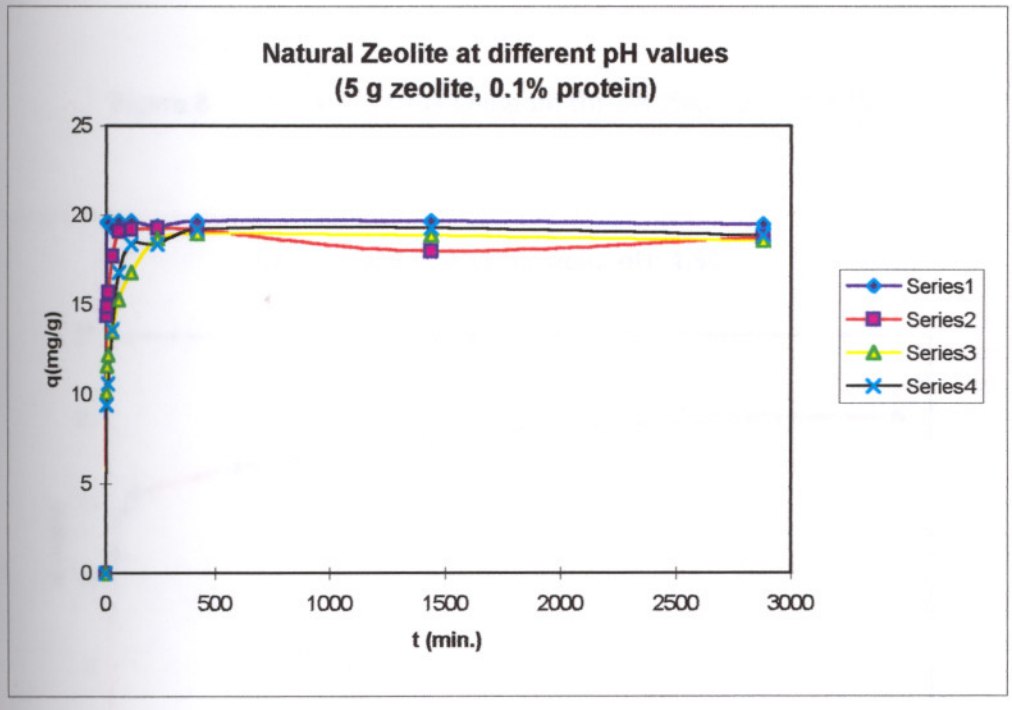



Figure 7. Comparison of q vs. t diagrams of different pH values for natural zeolite

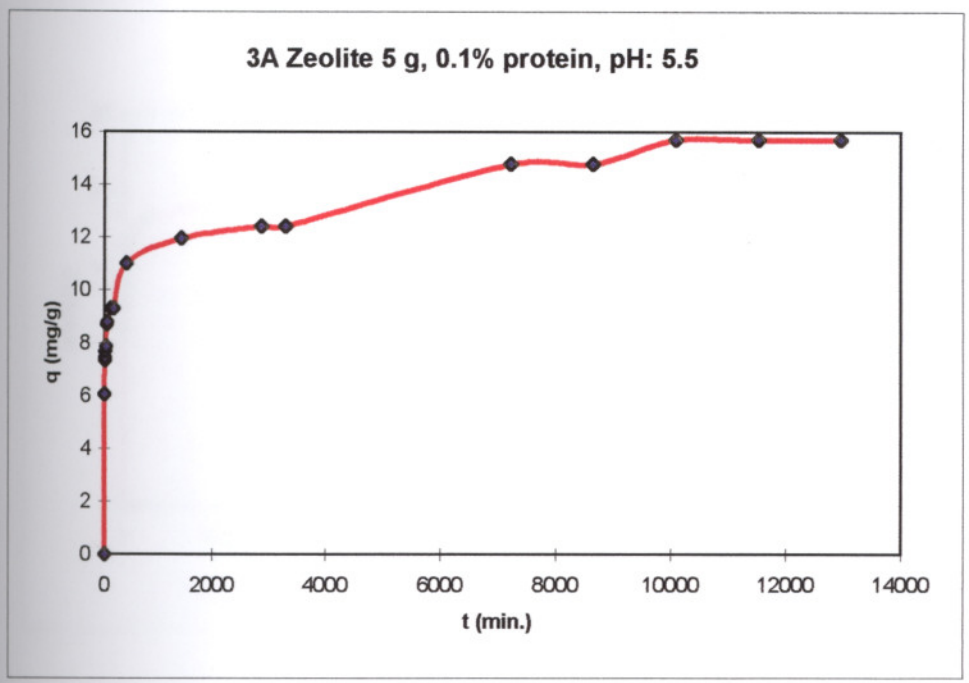



Figure 8. Uptake (q vs.t) Diagram for 3A Zeolite - BSA Pair

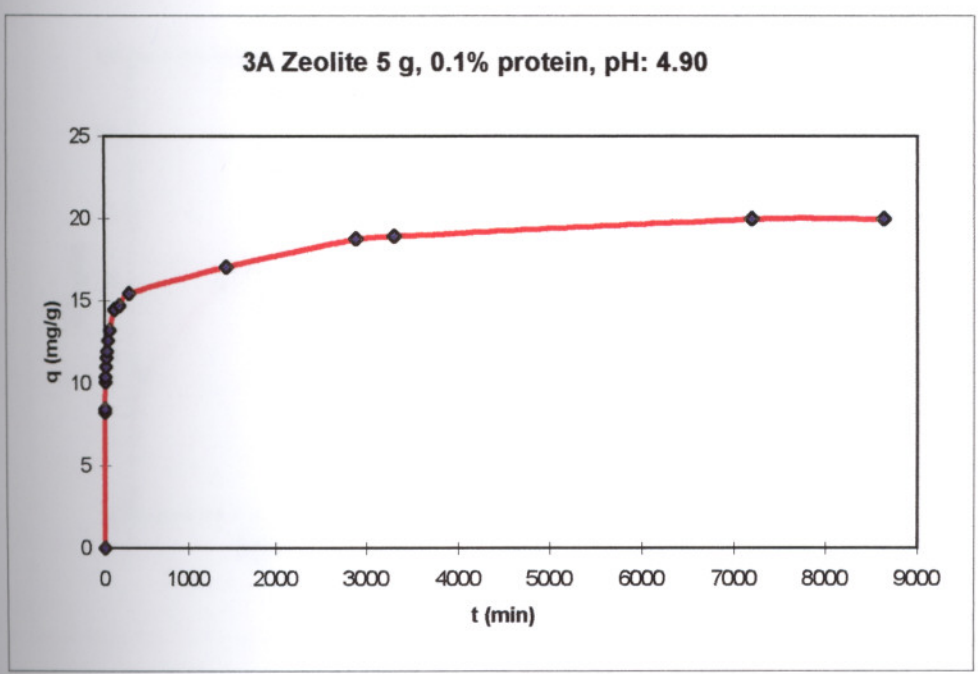



Figure 9. Uptake (q vs.t) Diagram for 3A Zeolite - BSA Pair

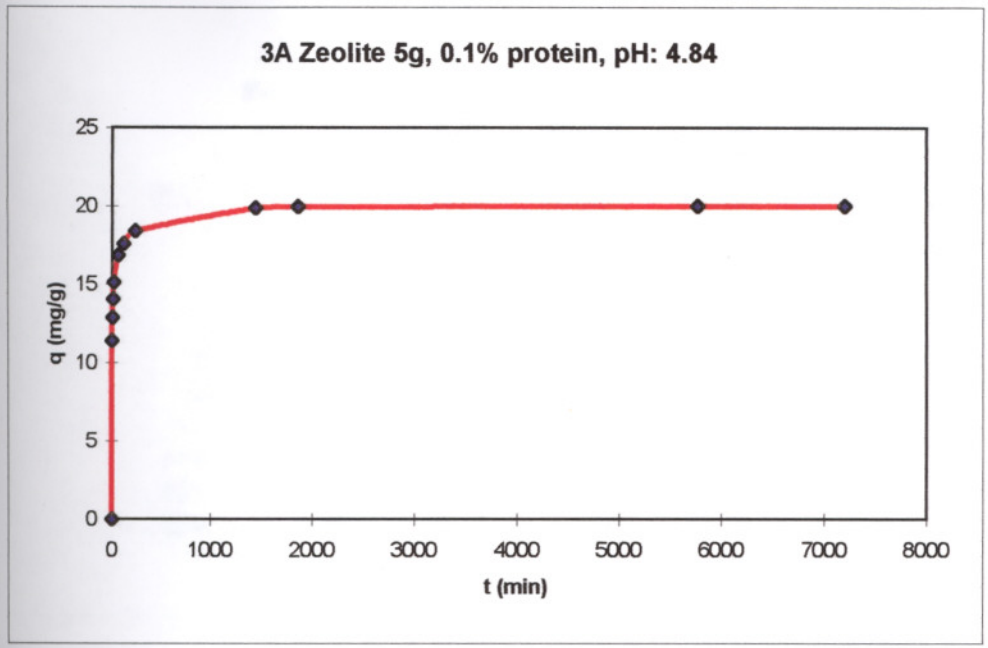



Figure 10. Uptake (q vs.t) Diagram for 3A Zeolite - BSA Pair

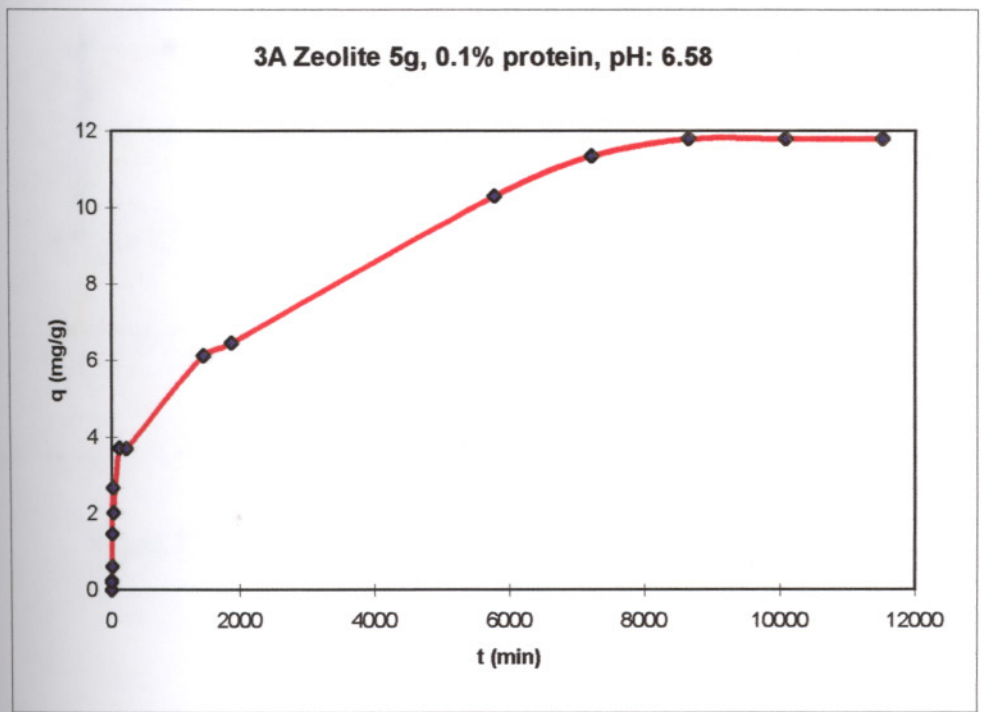



Figure 11. Uptake (q vs.t) Diagram for 3A Zeolite - BSA Pair

| t (min.) | Series 1 pH:4.84 q (mg/g) | Series 2 pH: 4.90 q (mg/g) | Series 3 pH:5.5 q (mg/g) | Series 4 pH:6.58 q (mg/g) |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 1 | 11.4 | 8.24 | 6.04 | 0.23 |
| 5 | 12.86 | 10.06 | 6.06 | 0.6 |
| 10 | 14.05 | 10.3 | 7.46 | 1.47 |
| 20 | 15.14 | 11.94 | 7.86 | 2.01 |
| 60 | 16.83 | 13.24 | 8.8 | 1.12 |
| 120 | 17.59 | 14.46 | 9.3 | 3.72 |
| 1440 | 19.87 | 17.06 | 11.95 | 6.12 |
| 2880 | 20 | 18.76 | 12.43 | 6.46 |
| 8640 | 20 | 19.95 | 14.78 | 11.79 |

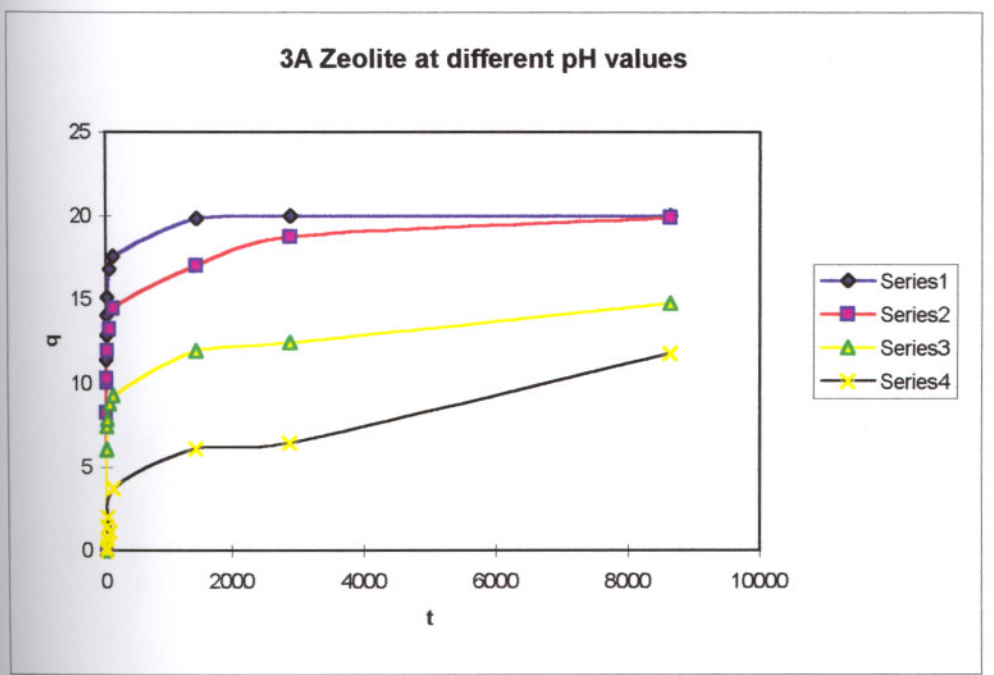



Figure 12. Comparison of q vs. t diagrams of different pH values for 3A Zeolite

| | *Series 1* | *Series 2* | *Series 3* |
|---|---|---|---|
| | **1 g** | **3 g** | **5 g** |
| **t** | **q** | **q** | **q** |
| (min.) | (mg/g) | (mg/g) | (mg/g) |
| 0 | 0 | 0 | 0 |
| 2.5 | 20.06 | 17.28 | 19.6 |
| 5 | 21.67 | 17.55 | 19.58 |
| 10 | 21.74 | 18.76 | 19.56 |
| 30 | 25.27 | 21.05 | 19.33 |
| 60 | 25.27 | 21.43 | 19.74 |
| 180 | 10.06 | 22.36 | 19.4 |
| 360 | 21.89 | 23.68 | 19.72 |
| 1440 | 39.76 | 26.59 | 19.5 |
| 2880 | 35.98 | 26.84 | 19.5 |
| 4320 | 22.93 | 27.05 | 19.7 |
| 7200 | 17.75 | 27.22 | 19.7 |

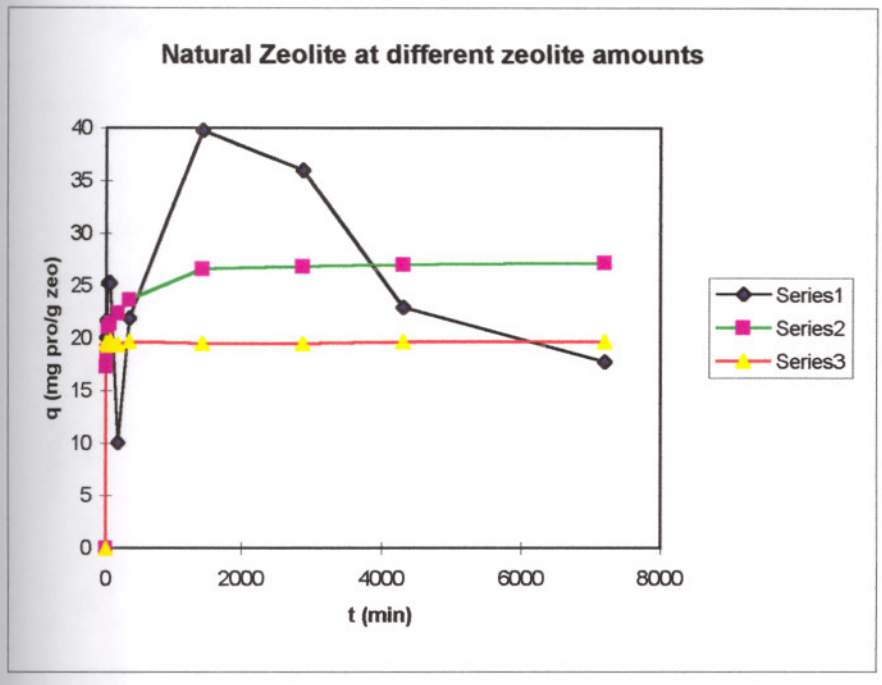



Figure 13. Comparison of q vs. t diagrams of different zeolite concentrations

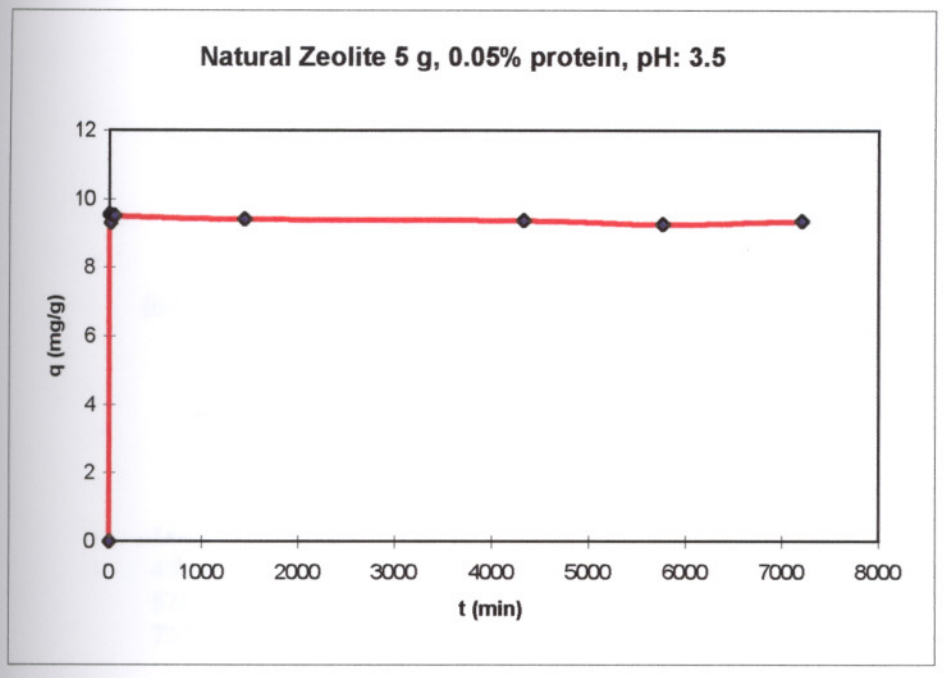



Figure 14. Uptake (q vs.t) Diagram for Natural Zeolite - BSA Pair

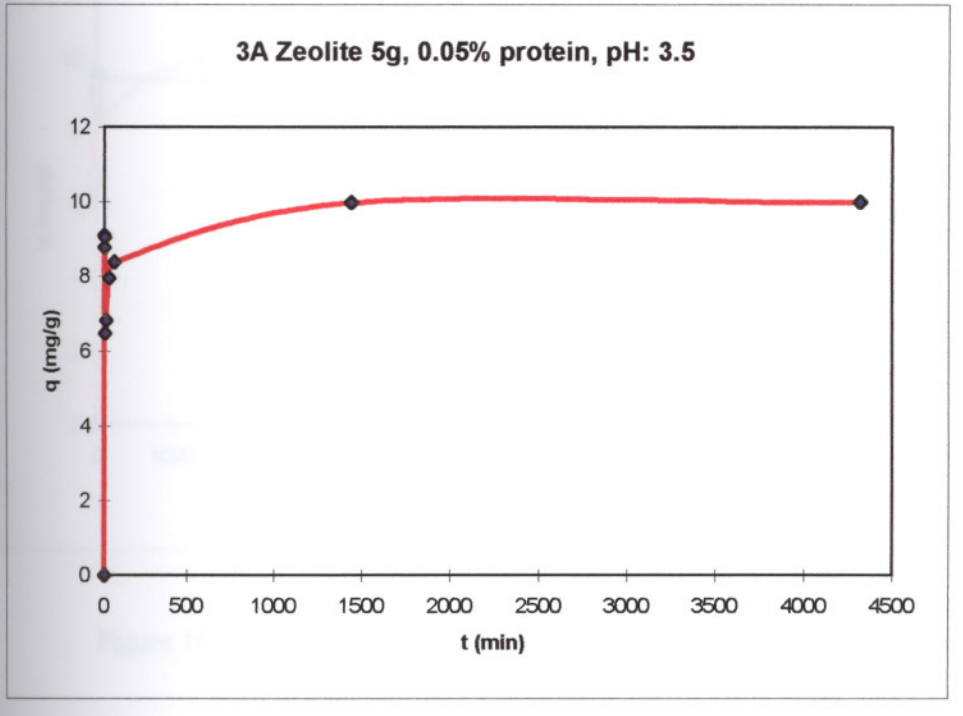



Figure 15. Uptake (q vs.t) Diagram for 3A Zeolite - BSA Pair

| | Series 1 Natural | Series 2 3A |
|---|---|---|
| t (min.) | q (mg/g) | q (mg/g) |
| 0 | 0 | 0 |
| 0.5 | 9.57 | 8.78 |
| 1 | 9.57 | 9.11 |
| 5 | 9.53 | 6.47 |
| 10 | 9.51 | 6.84 |
| 60 | 9.5 | 8.36 |
| 1440 | 9.41 | 9.98 |
| 4320 | 9.38 | 10 |
| 5760 | 9.27 | 10 |
| 7200 | 9.36 | 10 |

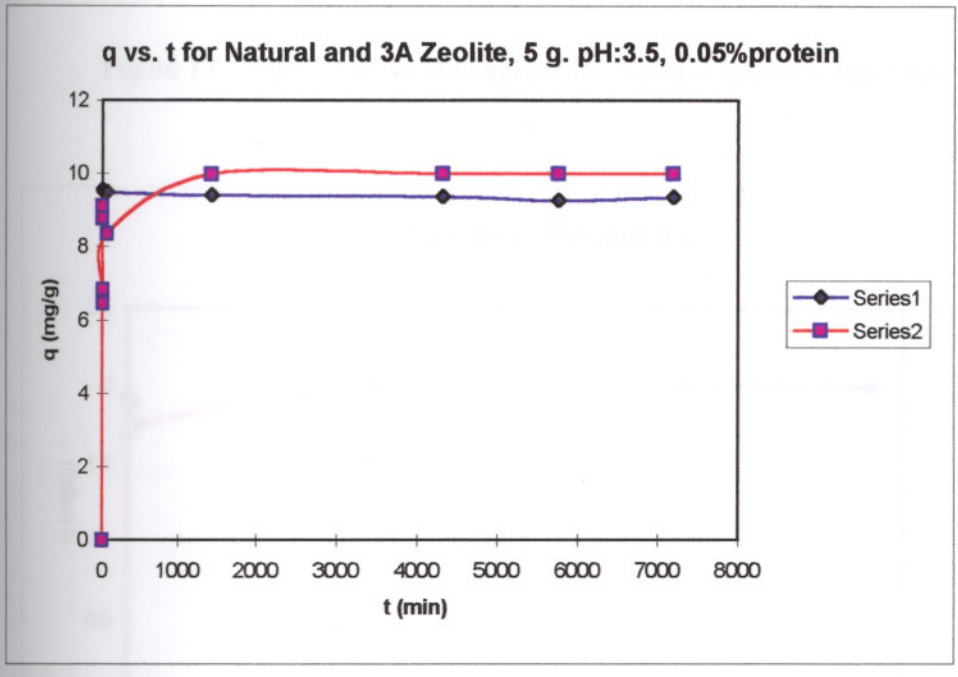



Figure 16. Comparison of q vs. t diagrams for Natural and 3A Zeolite at 0.05% protein concentration

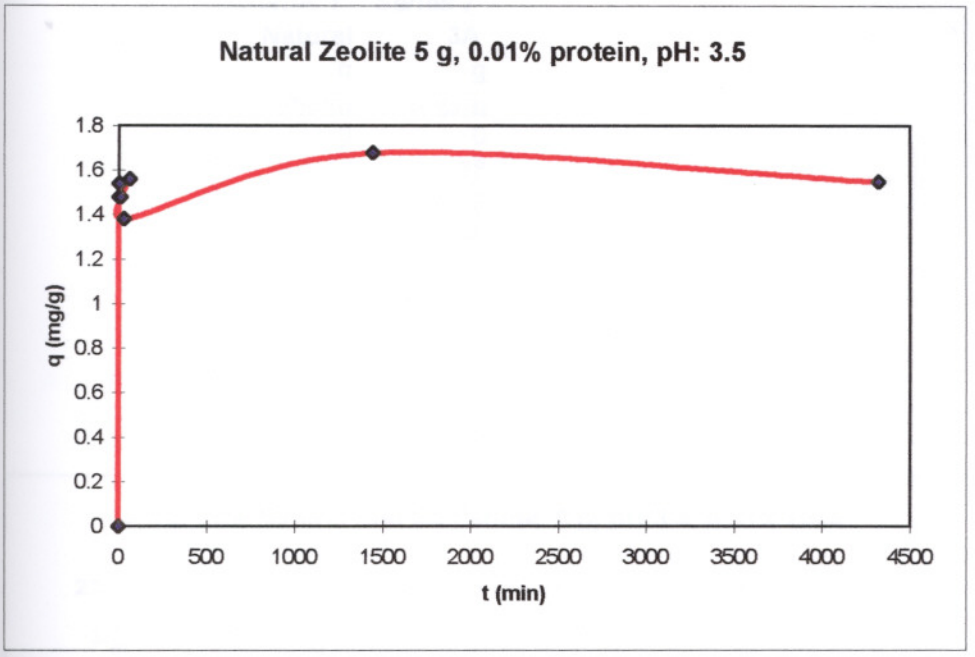



Figure 17. Uptake (q vs.t) Diagram for Natural Zeolite - BSA Pair

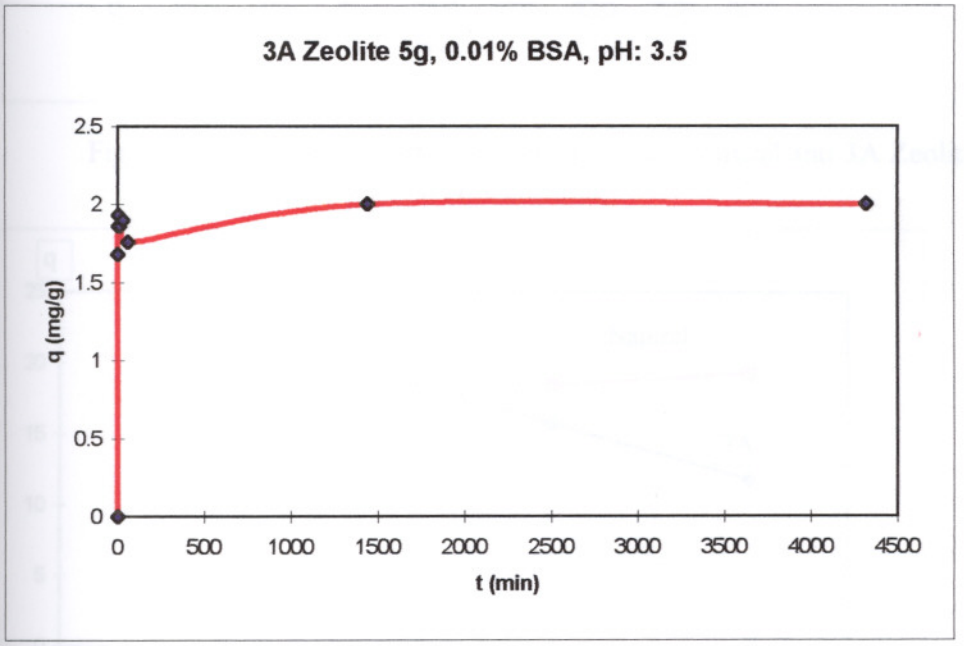



Figure 18. Uptake (q vs.t) Diagram for 3A Zeolite - BSA Pair

| | *Series 1* | *Series 2* |
|---|---|---|
| | **Natural** | **3A** |
| t | q | q |
| (min.) | (mg/g) | (mg/g) |
| 0 | 0 | 0 |
| 0.5 | 1.48 | 1.68 |
| 1 | 1.54 | 1.68 |
| 5 | 1.54 | 1.86 |
| 10 | 1.48 | 1.86 |
| 60 | 1.56 | 1.76 |
| 1440 | 1.68 | 2 |
| 4320 | 1.55 | 2 |
| 5760 | 1.55 | 2 |
| 7200 | 1.55 | 2 |

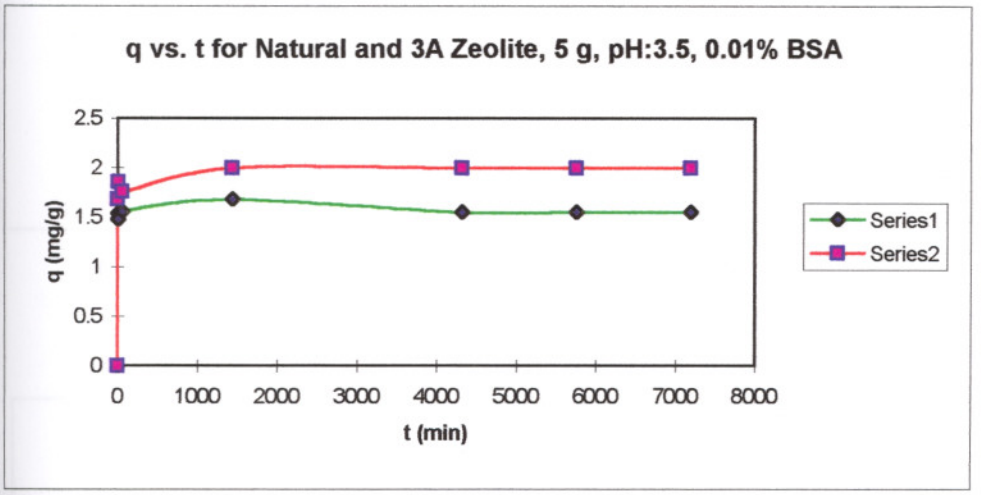



Figure 19. Comparison of q vs. t diagrams for Natural and 3A Zeolite at 0.01% protein concentration

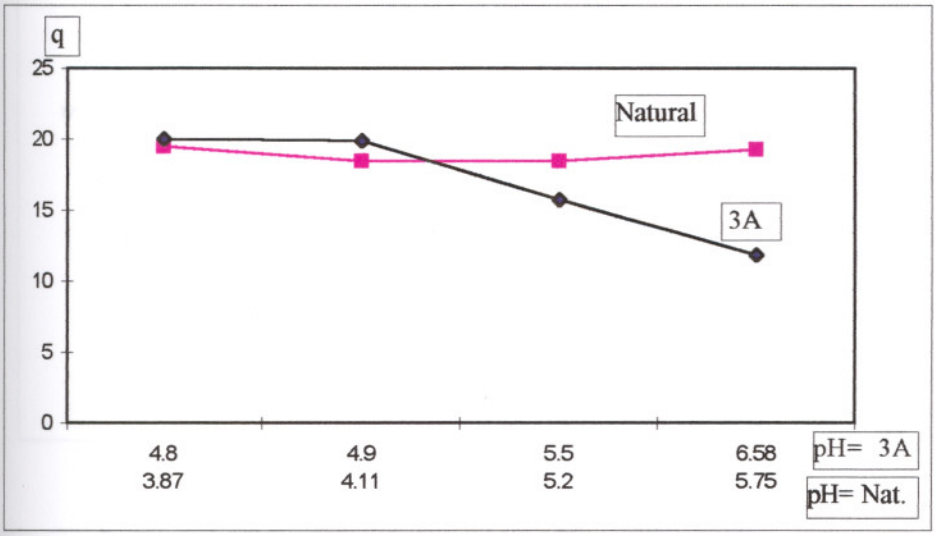



Figure 20. Effect of pH on BSA Adsorption

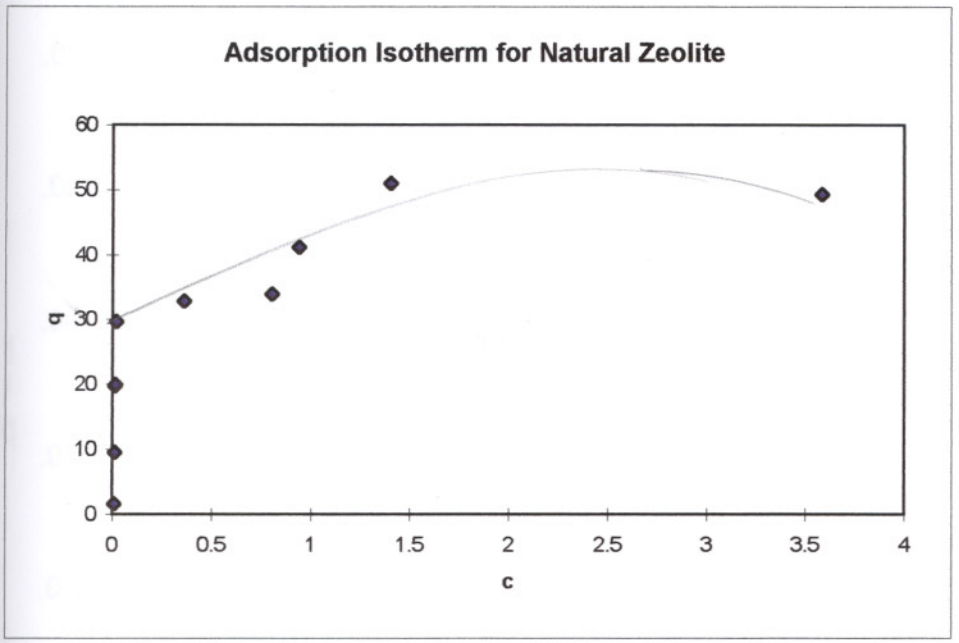



Figure 21. Adsorption isotherm for natural zeolite

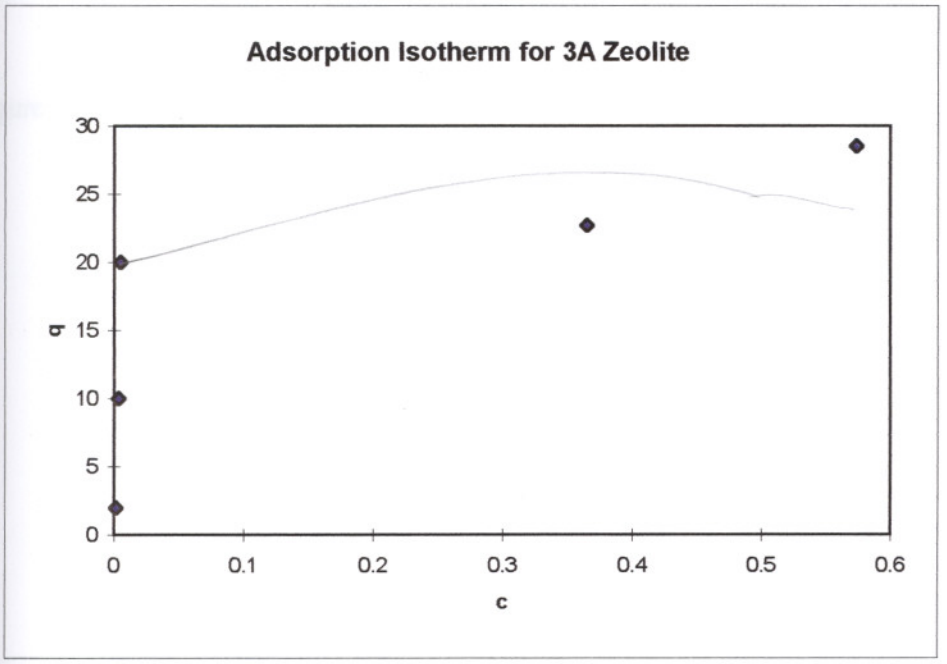



Figure 22. Adsorption isotherm for 3A zeolite

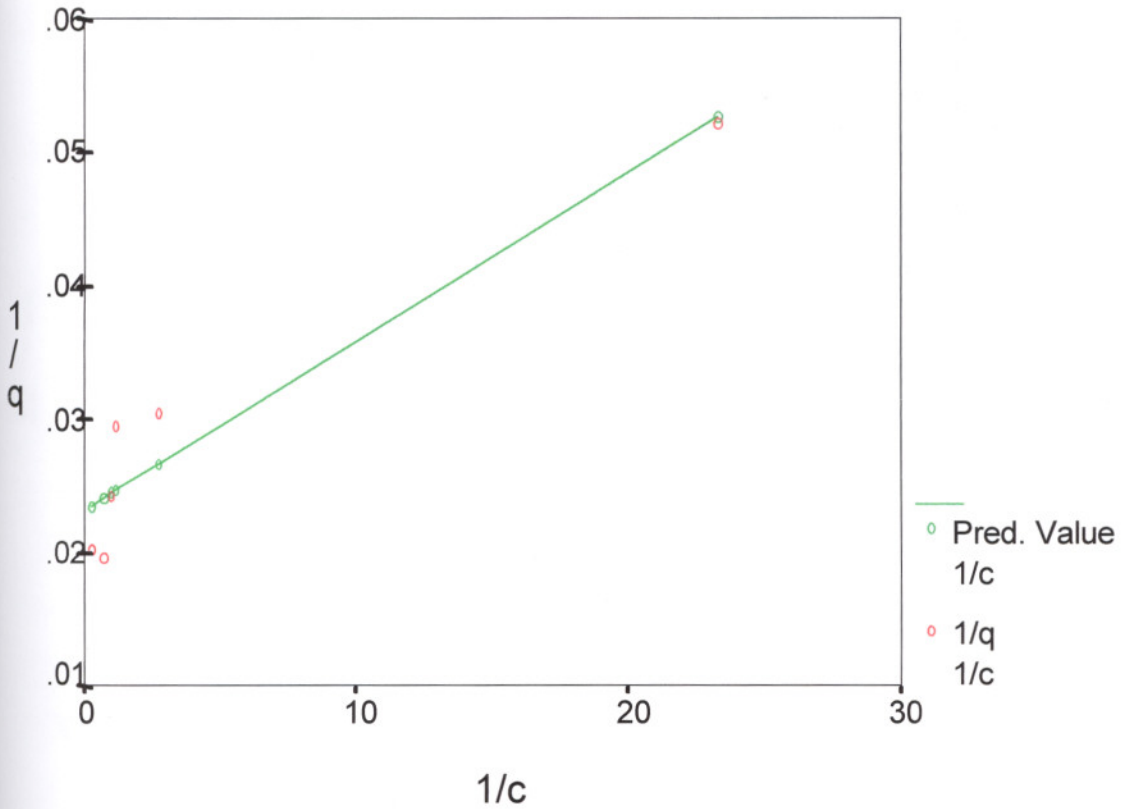



Figure 23. Application of Langmuir Model to describe adsorption isotherms for clinoptilolite with the use of SPSS Statistical Program.

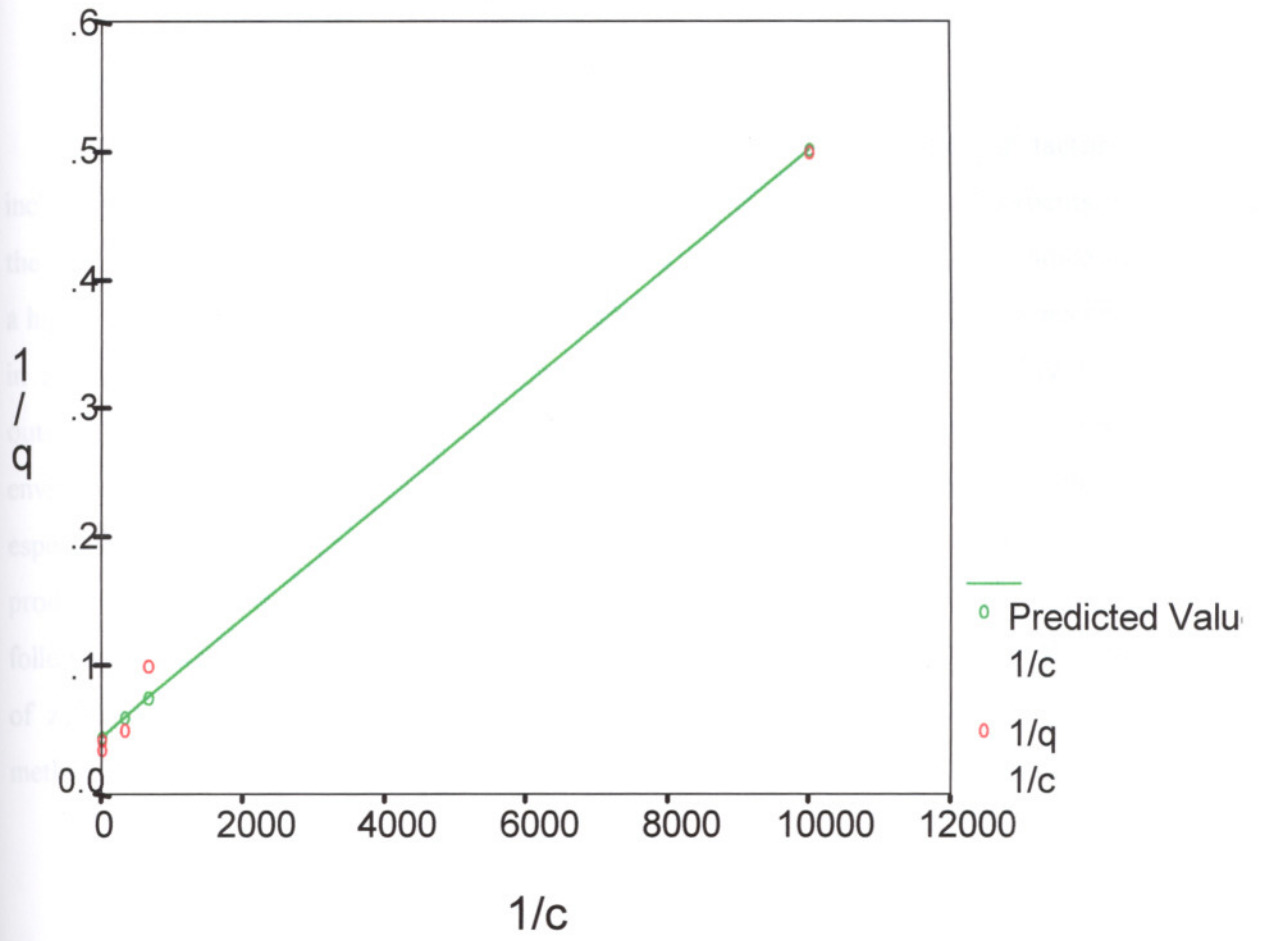



Figure 24. Application of Langmuir Model to describe adsorption isotherms for 3A Zeolite with the use of SPSS Statistical Program.

# CHAPTER 6

# CONCLUSION

The optimal choice of an adsorbent is influenced by a number of factors, including the difficulty of the purification process, the sensitivity of fragile adsorbents to the conditions, and its affinity to the desired product (protein). In general, the choice of a highly specific adsorbent will result in a reduction of the total number of steps needed in a purification procedure, but the expense of the adsorbent and its fragility may outweigh such considerations. However, zeolites, which are very stable against environmental changes, exist extensively. Our study shows the affinity of zeolites, especially clinoptilolite, to adsorb proteins in a short time, thus decreasing the risk of product deterioration during purification. This study may be the preliminary step, followed by further laboratory work and necessary scale-up experiments, towards the use of zeolites in the recovery of proteins in industry as an alternative to conventional methods.

# REFERENCES


1. Somesh C. Nigam, Henry Y. Wang, "Recovery of bioactive molecules and recombinant proteins from heterogeneous culture media", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 241.
2. D.W. Breck, "Potential uses of natural and synthetic zeolites in industry", in *The Properties and Applications of Zeolites,* edited by R.P. Townsend (The Chemical Society, London, 1979), p.397.
3. A. Dyer, "Zeolites as molecular sieves and drying agents", in *An Introduction to Zeolite Molecular Sieves*, (John Wiley and Sons, Chichester, 19..), p.87.
4. Douglas S. Coombs, "Nomenclature for zeolite minerals", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 21
5. Anthony Hall, "Large eruptions and large zeolite deposits", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 26
6. Alan Myers, "Theories of adsorption in micropores", in *Adsorption Science and Technology*, , edited by A.Rodrigues (Kluwer Academic Publishers, 1989), p.15.
7. D.S. White, R.L. Bussey, "Water sorption properties of modified clinoptilolite", *Separation and Purification Technology,* **11**, (1997), 137.
8. R.M. Barrer, "Porous crystals: a perspective", *Journal of Society Chem.Ind.*, **64**, 3.
9. G.V. Tsitsishvili, et.al., *Natural Zeolites*, (Ellis Horwood Ltd., 1992), p.101.
10. J.L. Boettinger, R.C. Graham, "Zeolite occurrence in soil environments: an updated review" in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.28.
11. Fehime Ozkan, Arzu Demirci, Semra Ulku, in *Natural Zeolites '97: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (to be published)
12. John D. Sherman, "Ion exchange separations with molecular sieve zeolites", in *Zeolites: Science and Technology*, (Martinus Nijhoff Publishers, 1984), p. 583.
13. Semra Ulku, "Natural zeolites in energy storage and heat pumps", *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), 1047.

# REFERENCES


1. Somesh C. Nigam, Henry Y. Wang, "Recovery of bioactive molecules and recombinant proteins from heterogeneous culture media", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 241.
2. D.W. Breck, "Potential uses of natural and synthetic zeolites in industry", in *The Properties and Applications of Zeolites,* edited by R.P. Townsend (The Chemical Society, London, 1979), p.397.
3. A. Dyer, "Zeolites as molecular sieves and drying agents", in *An Introduction to Zeolite Molecular Sieves*, (John Wiley and Sons, Chichester, 19..), p.87.
4. Douglas S. Coombs, "Nomenclature for zeolite minerals", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 21
5. Anthony Hall, "Large eruptions and large zeolite deposits", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 26
6. Alan Myers, "Theories of adsorption in micropores", in *Adsorption Science and Technology*, , edited by A.Rodrigues (Kluwer Academic Publishers, 1989), p.15.
7. D.S. White, R.L. Bussey, "Water sorption properties of modified clinoptilolite", *Separation and Purification Technology,* **11**, (1997), 137.
8. R.M. Barrer, "Porous crystals: a perspective", *Journal of Society Chem.Ind.*, **64**, 3.
9. G.V. Tsitsishvili, et.al., *Natural Zeolites*, (Ellis Horwood Ltd., 1992), p.101.
10. J.L. Boettinger, R.C. Graham, "Zeolite occurrence in soil environments: an updated review" in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.28.
11. Fehime Ozkan, Arzu Demirci, Semra Ulku, in *Natural Zeolites '97: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (to be published)
12. John D. Sherman, "Ion exchange separations with molecular sieve zeolites", in *Zeolites: Science and Technology*, (Martinus Nijhoff Publishers, 1984), p. 583.
13. Semra Ulku, "Natural zeolites in energy storage and heat pumps", *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), 1047.

14. Yucel H. Culfaz, "Characterization of clinoptilolite in Western Anatolia", in *Natural Zeolites '86: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1986), p.99.
15. H. Sirkecioglu, Y. Altav, E. Senatalar, "Adsorption of H2S and SO2 on Bigadic clinoptilolite", *Separation Science and Technology*, **13**, (1995), 2763.
16. Izumi Yamane and Tadahisa Nakazawa, "Development of zeolite for non-phosphated detergents in Japan", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 991.
17. C.P. Kurzendorfer, et.al., "Sodium aluminosilicates in the washing process", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 1009.
18. Juana Galindo, A. Elias and M.R. Gonzalez, "The effect of zeolite on ruminal bacteria population and its activity in heifers fed sunflower: sorghum silage", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 1055.
19. J.L. Boettinger, et.al, "Developing sustainable agricultural systems based on nitrogen cycling with clinoptilolite", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 74.
20. John E. Gruener, et.al, "Nutrient uptake of wheat grown in diluted clinoptilolite-natural/synthetic apatite substrates", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 162.
21. Peter J. Leggo, "Experiments with wheat: an investigation of plant growth in an organo-zeolitic substrate", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 206.
22. Ming and E.R. Allen, "Advances in the use of natural zeolites for plant growth", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 29.
23. Allen and D.W. Ming, "Recent progress in the use of natural zeolites in agronomy and horticulture", in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.480.
24. Ioan Bedelean, et.al, "Use of zeolitic volcanic tuffs from Romania in agriculture: crop growing and animal raising", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 60.

25. Domenico Bergero, et.al, "Acid resistance of natural zeolites utilized in animal feeding", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 65.
26. G.Onyestyal, D. Kallo, "Cd-clinoptilolite as a catalyst for the hydration of acetylene to acetaldehyde", in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.435.
27. Vrzgula, et.al, "Clinical and laboratory studies of zeolite in cattle and sheep: Research Report", in *VSV Kosice*, (1985), 253.
28. Minato, "Occurrance and application of natural zeolites in Japan", in *Occurrence, Properties and Utilization of Natural Zeolites*, ed.by D. Kallo and H.Sherry, (Akademiai Kiado, Budapest, 1988), p.395.
29. Wilson Pond, "Zeolites in animal nutrition and health: a review", in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.449.
30. Nikashina, et.al. "Evaluation of colinoptilolite-rich tuffs as ion-exchangers", in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.289.
31. R.Roque-Malherbe, et.al., "Physicochemical properties of natural zeolites used for adsorption of water", in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.299.
32. Funda Tihmillioglu and Semra Ulku, "Use of clinopilolite in ethanol dehydration", *Separation Science and Technology*, **31**, (1996), 2855.
33. Fehime C.Ozkan, Semra Ulku, Arzu Demirci, "$H_2O$ and $CO_2$ adsorption on natural and modified clinoptilolite from Bigadic-Turkey", *5th International Conference on the Occurrence, Properties, and Utilization of Natural Zeolites*, (1997), 241.
34. A. Takasaka and Y. Matsuda, "Vacuum freeze drying of food using natural zeolite", *Material Science*, (1992), 1041.
35. Anabel Lam Barandela, et.al, "Theoretical study of aspirin adsorption in natural zeolite", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 54.
36. Magdalena Tomasevic-Canovic, et.al, "Comparative study of aflatoxin B1 adsorption on different modified clinoptilolite and montmorillonite", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 290.

37. Maria Turco, et.al. "Selective catalytic reduction of NO with $NH_3$ on copper-exchanged natural mordenite", in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.429.
38. John D. Sherman and Chien C. Chao, "Carbohydrate separations using zeolite molecular sieves", *AIChE Symposium Series*, **74**, 1978, 1025.
39. Rosario Aiello, "Zeolitic tuffs as building materials in Italy: A review", in *Natural Zeolites '93: Occurrence, Properties, Use*, ed.by D.W.Ming, F.A. Mumpton, (Intl.Comm.on Natural Zeolites, 1995), p.590.
40. K.S.Sing, et.al, "Reporting physisorption data for gas/solid systems" , in *Pure and Applied Chemistry*, (Singwork, London, 1985), p.605.
41. T.Sato and R.Ruch, "Adsorption of polymers from solution", in *Stabilization of Colloidal Dispersions by Polymer Adsorption*, (M.Dekker Inc., New York, 1980), p.3.
42. K.S.W.Sing, "Characterization of adsorbents", in *Adsorption Science and Technology*, edited by A.Rodrigues (Kluwer Academic Publishers, 1988), p.3.
43. D.M.Ruthven, "Adsorption kinetics", in *Adsorption Science and Technology*, edited by A.Rodrigues (Kluwer Academic Publishers, 1989), p.87.
44. R.A.Williams, *Colloid and Surface Engineering: Applications in the Process Industries* (Butterworth-Heinemann, 1992), p.180.
45. Sebnem Harsa, "Pektinaz enzimlerinin iyon-değiştiricilerde adsorpsiyonu", Gıda Mühendisliği Kongresi, (1992), 202.
46. E.L. Wu, G.R. Randolt and A.W. Chester, "Hydrocarbon adsorption characterization of some high silica zeolites", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 547.
47. Roger D.Whitley, Xiaomang Zhang, N.H.Linda Wang, "Protein denaturation in non-linear isocratic and gradient eultion", *AICHE Journal*, **40**, (1994), 1067.
48. I.S.Gosling, D.Cook and M.Fry, "The role of adsorption isotherms in the design of chromatographic separations for downstream processing", *Chemical Engineering*, **67**, (1989), 23.3.
49. Krishnan Chittur, "Protein adsorption to Biomaterials", http://eb-b5/eb/uah.edu, 1995.

50. S.Harsa, D.L.Pyle and C.A.Zaror, "Effect of pH on polygalacturonase adsorption in CM-Sephadex gels", in *New Developments in Zeolite Science and Technology*, (Kodansha-Elsevier Pub., 1986), p. 345.
51. Clarie Fargues, Michel Bailly and Georges Grevillot, "Adsorption of BSA and Hemoglobin on hydroxyapatite support: equilibria and multicomponent dynamic adsorption", *Adsorption,* **4**, (1998), 5.
52. S.Harsa, C.A.Zaror and D.L.Pyle, "Adsorption of Kluyveromyces marxianus pectinase on CM-Sephadex gels", in *Enzyme Microbiology Technology*, (Butterworth-Heinemann, 1992), p.180.
53. Daechul Cho, E.Franses, N.H.Wang, "Dynamics of protein adsorption at air-water interfaces", *Biotechnology Progress,* **8**, 167.
54. G.T.Keller and R.T.Yang, "Technological maturity of sorption processes and sorbents", in *New Directions in Sorption Technology*, edited by G.E. Keller (Butterworths, Boston, 1992), p.44.
55. H.A.Chase, "Optimization of adsorption techniques for the purification of biomolecules", *Adsorption: Science and Technology,* (1989), 539.
56. Felix Franks, "Purification of proteins", in *Protein Biotechnology*, (Humana Press, New Jersey, 1993), p.533.
57. Fehime Ozkan, "Adsorbent yatakların dinamik davranışının incelenmesi ve doğal kaynakların adsorbent olarak değerlendirilmesi", PhD thesis, Ege University, Izmir, 1992.
58. Hiroyuki Yoshida, Hideki Nashara and Takeshi Kataoka, "Adsorption of BSA on DEAE-Dextran: Equilibria", *Separation Science and Technology,* **17**, (1994), 2233.
59. Diana T. Hughes Wassell, Rachel C. Hall, Graham Embery, "Adsorption of bovine serum albumin onto hydroxyapatite", *Biomaterials,* **16**, (1995), 697.
60. Diana T. Hughes Wassell, Graham Embery, "Adsorption of bovine serum albumin onto titanium powder", *Biomaterials,* **17**, (1996), 859.
61. A.Krajewski, R.Malavolti, A.Piancastelli, "Albumin adhesion on some biological and non-biological glasses and connection with their z-potentials", *Biomaterials,* **17**, (1996), 53.

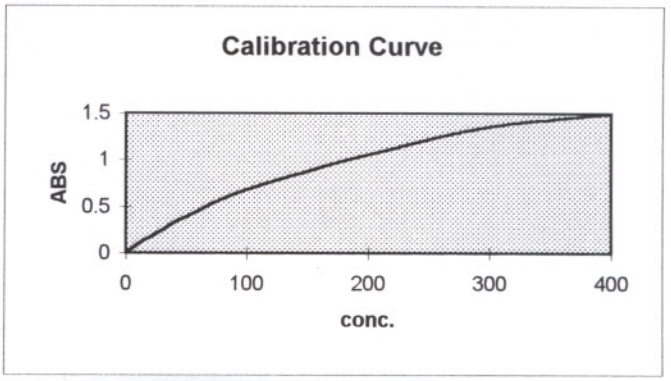
# APPENDIX-A

## Properties of BSA

BSA is widely used as a model protein to study protein adsorption since it is well-characterized. BSA has a molecular mass of 66000 daltons and an isoelectric point (pI) of 4.9. It has the shape of an ellipsoid with axes of 14 and 4 nm. Proteins are polyelectrolytes, so the charge varies with pH. Net charge of the molecule is 0 at the pI. The net charge at pH 5, 6 and 7 is -2, -12 and -18, respectively. Several isomers of BSA exist at various pH's. Main structural transitions occur at pH = 4.3 and pH = 8.0.

# APPENDIX-B

| St. # | conc. | ABS |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 10 | 0.093 |
| 3 | 20 | 0.168 |
| 4 | 30 | 0.248 |
| 5 | 40 | 0.327 |
| 6 | 50 | 0.387 |
| 7 | 100 | 0.682 |
| 8 | 200 | 1.058 |
| 9 | 300 | 1.359 |
| 10 | 400 | 1.495 |




## LOWRY METHOD

Lowry method is used in the determination of protein concentrations. After samples were taken and diluted with water, 1 ml. reagent solution was added. After waiting for 20 minutes, 0.5 ml phenol was added with intense stirring. After 30 minutes of waiting period, samples were ready for uv-spectrophotometer to determine the amount of protein.

***Formulation used to calculate q and c values***

$$W * q = V (ci - c)$$

q = amt. of adsorbed protein per g. of zeolite
ci = initial protein concentration
c = protein concentration of liquid (non-adsorbed protein)
W = total amount of zeolite
V = volume of liquid adsorption experiment is carried out

***Sample calculation:***

Absorbance at 750 nm = 0.145
Protein concentration of the diluted sample = 20.065 μg/ml
Since sample is diluted by 1/4=20.065 x 4=80.26μg/ml=0.080 mg/ml( c)
Adsorbed amount = 1* - 0.080 = 0.920 mg/ml
Adsorbed amt. per g.zeolite=0.920 / 5** x100*** =18.39 mg pro/g.zeo (q)

* *initial protein conc. used for this particular sample (mg/ml)*
** *total zeolite amount used (g)*
*** *total volume used (ml)*

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 3.87**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 2.5' | 0.037 | 5.010 | 0.020 | 0.980 | 19.599 |
| 5' | 0.039 | 5.280 | 0.021 | 0.979 | 19.578 |
| 7.5' | 0.040 | 5.420 | 0.022 | 0.978 | 19.566 |
| 10' | 0.041 | 5.550 | 0.022 | 0.978 | 19.556 |
| 15' | 0.038 | 5.140 | 0.021 | 0.979 | 19.589 |
| 20' | 0.023 | 3.110 | 0.012 | 0.988 | 19.751 |
| 30' | 0.062 | 8.430 | 0.034 | 0.966 | 19.326 |
| 45' | 0.031 | 4.190 | 0.017 | 0.983 | 19.665 |
| 1h | 0.024 | 3.240 | 0.013 | 0.987 | 19.741 |
| 2h | 0.028 | 3.780 | 0.015 | 0.985 | 19.698 |
| 3h | 0.055 | 7.470 | 0.030 | 0.970 | 19.402 |
| 4h | 0.058 | 7.880 | 0.032 | 0.968 | 19.370 |
| 8h | 0.026 | 3.450 | 0.014 | 0.986 | 19.724 |
| 24h | 0.018 | 2.470 | 0.010 | 0.990 | 19.802 |
| 36h | 0.058 | 7.878 | 0.032 | 0.968 | 19.370 |
| 48h | 0.044 | 6.010 | 0.024 | 0.976 | 19.519 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 5.75**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 2.5' | 0.817 | 131.870 | 0.527 | 0.473 | 9.450 |
| 5' | 0.818 | 132.070 | 0.528 | 0.472 | 9.434 |
| 7.5' | 0.806 | 129.660 | 0.519 | 0.481 | 9.627 |
| 10' | 0.740 | 116.770 | 0.467 | 0.533 | 10.658 |
| 15' | 0.685 | 106.460 | 0.426 | 0.574 | 11.483 |
| 20' | 0.651 | 100.270 | 0.401 | 0.599 | 11.978 |
| 30' | 0.542 | 81.250 | 0.325 | 0.675 | 13.500 |
| 45' | 0.391 | 56.650 | 0.227 | 0.773 | 15.468 |
| 1h | 0.284 | 40.250 | 0.161 | 0.839 | 16.780 |
| 2h | 0.150 | 20.720 | 0.083 | 0.917 | 18.342 |
| 3h | 0.114 | 15.640 | 0.063 | 0.937 | 18.749 |
| 4h | 0.151 | 20.860 | 0.083 | 0.917 | 18.331 |
| 8h | 0.076 | 10.390 | 0.042 | 0.958 | 19.169 |
| 24h | 0.066 | 9.030 | 0.036 | 0.964 | 19.278 |
| 36h | 0.098 | 13.447 | 0.054 | 0.946 | 18.924 |
| 48h | 0.100 | 13.634 | 0.055 | 0.945 | 18.909 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 5.20**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 1' | 0.915 | 152.450 | 0.610 | 0.390 | 7.804 |
| 2.5' | 0.776 | 123.650 | 0.495 | 0.505 | 10.108 |
| 5' | 0.675 | 104.700 | 0.419 | 0.581 | 11.624 |
| 10' | 0.633 | 97.035 | 0.388 | 0.612 | 12.237 |
| 30' | 0.538 | 80.709 | 0.323 | 0.677 | 13.543 |
| 1h | 0.403 | 58.512 | 0.234 | 0.766 | 15.319 |
| 2h | 0.286 | 40.477 | 0.162 | 0.838 | 16.762 |
| 4h | 0.115 | 15.850 | 0.063 | 0.937 | 18.732 |
| 7h | 0.086 | 11.730 | 0.047 | 0.953 | 19.062 |
| 24h | 0.099 | 13.549 | 0.054 | 0.946 | 18.916 |
| 48h | 0.124 | 17.050 | 0.068 | 0.932 | 18.636 |
| 3 days | 0.138 | 18.990 | 0.076 | 0.924 | 18.481 |
| 4 days | 0.129 | 17.703 | 0.071 | 0.929 | 18.584 |
| 6 days | 0.180 | 25.027 | 0.100 | 0.900 | 17.998 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 4.11**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 0.915 | 152.450 | 0.610 | 0.390 | 7.804 |
| 1' | 0.625 | 95.690 | 0.383 | 0.617 | 12.345 |
| 2.5' | 0.495 | 73.409 | 0.294 | 0.706 | 14.127 |
| 5' | 0.437 | 63.912 | 0.256 | 0.744 | 14.887 |
| 10' | 0.376 | 54.337 | 0.217 | 0.783 | 15.653 |
| 30' | 0.207 | 28.833 | 0.115 | 0.885 | 17.693 |
| 1h | 0.080 | 10.872 | 0.043 | 0.957 | 19.130 |
| 2h | 0.075 | 10.163 | 0.041 | 0.959 | 19.187 |
| 4h | 0.065 | 8.868 | 0.035 | 0.965 | 19.291 |
| 7h | 0.069 | 9.389 | 0.038 | 0.962 | 19.249 |
| 24h | 0.179 | 24.922 | 0.100 | 0.900 | 18.006 |
| 48h | 0.114 | 15.663 | 0.063 | 0.937 | 18.747 |
| 3 days | 0.114 | 15.663 | 0.063 | 0.937 | 18.747 |
| 4 days | 0.132 | 18.236 | 0.073 | 0.927 | 18.541 |
| 6 days | 0.132 | 18.236 | 0.073 | 0.927 | 18.541 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**3A ZEOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 4.90**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 1' | 0.890 | 147.010 | 0.588 | 0.412 | 8.239 |
| 2.5' | 0.880 | 144.950 | 0.580 | 0.420 | 8.404 |
| 5' | 0.778 | 124.150 | 0.497 | 0.503 | 10.068 |
| 7.5' | 0.757 | 120.020 | 0.480 | 0.520 | 10.398 |
| 10' | 0.764 | 121.370 | 0.485 | 0.515 | 10.290 |
| 15' | 0.719 | 112.740 | 0.451 | 0.549 | 10.981 |
| 20' | 0.682 | 105.960 | 0.424 | 0.576 | 11.523 |
| 30' | 0.654 | 100.780 | 0.403 | 0.597 | 11.938 |
| 45' | 0.608 | 92.566 | 0.370 | 0.630 | 12.595 |
| 1h | 0.561 | 84.527 | 0.338 | 0.662 | 13.238 |
| 2h | 0.470 | 67.342 | 0.269 | 0.731 | 14.613 |
| 3h | 0.450 | 66.271 | 0.265 | 0.735 | 14.698 |
| 5h | 0.393 | 56.974 | 0.228 | 0.772 | 15.442 |
| 24h | 0.260 | 36.710 | 0.147 | 0.853 | 17.063 |
| 48h | 0.113 | 15.543 | 0.062 | 0.938 | 18.757 |
| 55h | 0.098 | 13.380 | 0.054 | 0.946 | 18.930 |
| 5 days | 0.005 | 0.678 | 0.003 | 0.997 | 19.946 |
| 6 days | 0.005 | 0.678 | 0.003 | 0.997 | 19.946 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**3A ZEOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 5.50**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 1' | 1.012 | 174.590 | 0.698 | 0.302 | 6.033 |
| 5' | 1.011 | 174.350 | 0.697 | 0.303 | 6.052 |
| 7.5' | 0.941 | 158.150 | 0.633 | 0.367 | 7.348 |
| 10' | 0.935 | 156.850 | 0.627 | 0.373 | 7.452 |
| 15' | 0.922 | 154.060 | 0.616 | 0.384 | 7.675 |
| 20' | 0.949 | 160.050 | 0.640 | 0.360 | 7.196 |
| 30' | 0.912 | 151.730 | 0.607 | 0.393 | 7.862 |
| 45' | 0.861 | 141.120 | 0.564 | 0.436 | 8.710 |
| 1h | 0.868 | 142.400 | 0.570 | 0.430 | 8.608 |
| 2h | 0.826 | 133.650 | 0.535 | 0.465 | 9.308 |
| 3h | 0.837 | 135.840 | 0.543 | 0.457 | 9.133 |
| 7h | 0.717 | 112.420 | 0.450 | 0.550 | 11.006 |
| 24h | 0.653 | 100.620 | 0.402 | 0.598 | 11.950 |
| 48h | 0.620 | 94.674 | 0.379 | 0.621 | 12.426 |
| 55h | 0.620 | 94.674 | 0.379 | 0.621 | 12.426 |
| 5 days | 0.445 | 65.188 | 0.261 | 0.739 | 14.785 |
| 6 days | 0.445 | 65.188 | 0.261 | 0.739 | 14.785 |
| 7 days | 0.373 | 53.786 | 0.215 | 0.785 | 15.697 |
| 8 days | 0.373 | 53.786 | 0.215 | 0.785 | 15.697 |
| 9 days | 0.373 | 53.786 | 0.215 | 0.785 | 15.697 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**3A ZEOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 4.84**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 1' | 0.689 | 107.260 | 0.429 | 0.571 | 11.419 |
| 5' | 0.589 | 89.283 | 0.357 | 0.643 | 12.857 |
| 10' | 0.501 | 74.337 | 0.297 | 0.703 | 14.053 |
| 20' | 0.417 | 60.697 | 0.243 | 0.757 | 15.144 |
| 1h | 0.280 | 39.581 | 0.158 | 0.842 | 16.834 |
| 2h | 0.215 | 30.128 | 0.121 | 0.879 | 17.590 |
| 4h | 0.145 | 20.065 | 0.080 | 0.920 | 18.395 |
| 24h | 0.012 | 1.645 | 0.007 | 0.993 | 19.868 |
| 31h | 0.003 | 0.378 | 0.002 | 0.998 | 19.970 |
| 4 days | 0.000 | 0.000 | 0.000 | 1.000 | 20.000 |
| 5 days | 0.000 | 0.000 | 0.000 | 1.000 | 20.000 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**3A ZEOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.1%**
**pH = 6.58**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 1' | 1.264 | 247.140 | 0.989 | 0.011 | 0.229 |
| 2.5' | 1.265 | 247.540 | 0.990 | 0.010 | 0.197 |
| 5' | 1.258 | 242.450 | 0.970 | 0.030 | 0.604 |
| 10' | 1.218 | 231.680 | 0.927 | 0.073 | 1.466 |
| 20' | 1.170 | 216.680 | 0.867 | 0.133 | 2.666 |
| 30' | 1.197 | 224.850 | 0.899 | 0.101 | 2.012 |
| 45' | 1.259 | 245.490 | 0.982 | 0.018 | 0.361 |
| 1h | 1.231 | 235.890 | 0.944 | 0.056 | 1.129 |
| 2h | 1.124 | 203.450 | 0.814 | 0.186 | 3.724 |
| 4h | 1.138 | 207.440 | 0.830 | 0.170 | 3.405 |
| 24h | 1.007 | 173.560 | 0.694 | 0.306 | 6.115 |
| 31h | 0.989 | 169.270 | 0.677 | 0.323 | 6.458 |
| 4 days | 0.763 | 121.160 | 0.485 | 0.515 | 10.307 |
| 5 days | 0.694 | 108.190 | 0.433 | 0.567 | 11.345 |
| 6 days | 0.664 | 102.690 | 0.411 | 0.589 | 11.785 |
| 7 days | 0.663 | 102.440 | 0.410 | 0.590 | 11.805 |
| 8 days | 0.627 | 95.970 | 0.384 | 0.616 | 12.322 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 1 g.**
**Initial protein = 0.1%**
**pH = 3.5**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 1.100 | 197.060 | 0.788 | 0.212 | 21.176 |
| 1' | 1.110 | 199.830 | 0.799 | 0.201 | 20.068 |
| 2.5' | 1.110 | 199.830 | 0.799 | 0.201 | 20.068 |
| 5' | 1.096 | 195.820 | 0.783 | 0.217 | 21.672 |
| 10' | 1.095 | 195.630 | 0.783 | 0.217 | 21.748 |
| 30' | 1.061 | 186.830 | 0.747 | 0.253 | 25.268 |
| 1h | 1.061 | 186.830 | 0.747 | 0.253 | 25.268 |
| 3h | 1.197 | 224.850 | 0.899 | 0.101 | 10.060 |
| 6h | 1.094 | 195.270 | 0.781 | 0.219 | 21.892 |
| 24h | 0.911 | 150.590 | 0.602 | 0.398 | 39.764 |
| 2 days | 0.949 | 160.050 | 0.640 | 0.360 | 35.980 |
| 3 days | 1.084 | 192.660 | 0.771 | 0.229 | 22.936 |
| 5 days | 0.131 | 205.620 | 0.822 | 0.178 | 17.752 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 3 g.**
**Initial protein = 0.1%**
**pH = 3.5**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 0.845 | 137.650 | 0.551 | 0.449 | 14.980 |
| 1' | 0.825 | 133.500 | 0.534 | 0.466 | 15.533 |
| 2.5' | 0.750 | 120.400 | 0.482 | 0.518 | 17.280 |
| 5' | 0.749 | 118.350 | 0.473 | 0.527 | 17.553 |
| 10' | 0.700 | 109.270 | 0.437 | 0.563 | 18.764 |
| 30' | 0.607 | 92.390 | 0.370 | 0.630 | 21.015 |
| 1h | 0.589 | 89.280 | 0.357 | 0.643 | 21.429 |
| 3h | 0.548 | 82.280 | 0.329 | 0.671 | 22.363 |
| 6h | 0.489 | 72.360 | 0.289 | 0.711 | 23.685 |
| 24h | 0.352 | 50.530 | 0.202 | 0.798 | 26.596 |
| 2 days | 0.340 | 48.710 | 0.195 | 0.805 | 26.839 |
| 3 days | 0.328 | 46.937 | 0.188 | 0.812 | 27.075 |
| 5 days | 0.321 | 45.840 | 0.183 | 0.817 | 27.221 |
| 6 days | 0.176 | 24.379 | 0.098 | 0.902 | 30.083 |
| 7 days | 0.157 | 21.694 | 0.087 | 0.913 | 30.441 |
| 9 days | 0.155 | 21.469 | 0.086 | 0.914 | 30.471 |
| 14 days | 0.098 | 13.362 | 0.053 | 0.947 | 31.552 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.05%**
**pH = 3.5**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 0.078 | 10.669 | 0.021 | 0.479 | 9.573 |
| 1' | 0.078 | 10.669 | 0.021 | 0.479 | 9.573 |
| 2.5' | 0.087 | 11.750 | 0.024 | 0.477 | 9.530 |
| 10' | 0.090 | 12.290 | 0.025 | 0.475 | 9.508 |
| 30' | 0.129 | 17.670 | 0.035 | 0.465 | 9.293 |
| 1h | 0.093 | 12.630 | 0.025 | 0.475 | 9.495 |
| 5h | 0.179 | 24.870 | 0.050 | 0.450 | 9.005 |
| 24h | 0.108 | 14.762 | 0.030 | 0.470 | 9.410 |
| 3 days | 0.113 | 15.458 | 0.031 | 0.469 | 9.382 |
| 4 days | 0.132 | 18.133 | 0.036 | 0.464 | 9.275 |
| 5 days | 0.116 | 15.902 | 0.032 | 0.468 | 9.364 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**CLINOPTILOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.01%**
**pH = 3.5**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 0.096 | 13.175 | 0.026 | 0.074 | 1.473 |
| 1' | 0.083 | 11.320 | 0.023 | 0.077 | 1.547 |
| 2.5' | 0.083 | 11.320 | 0.023 | 0.077 | 1.547 |
| 5' | 0.083 | 11.320 | 0.023 | 0.077 | 1.547 |
| 10' | 0.095 | 13.030 | 0.026 | 0.074 | 1.479 |
| 1h | 0.080 | 10.970 | 0.022 | 0.078 | 1.561 |
| 5h | 0.114 | 15.590 | 0.031 | 0.069 | 1.376 |
| 24h | 0.059 | 7.980 | 0.016 | 0.084 | 1.681 |
| 3 days | 0.082 | 11.180 | 0.022 | 0.078 | 1.553 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**3A ZEOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.05%**
**pH = 3.5**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 0.218 | 30.502 | 0.061 | 0.439 | 8.780 |
| 1' | 0.160 | 22.146 | 0.044 | 0.456 | 9.114 |
| 2.5' | 0.171 | 23.663 | 0.047 | 0.453 | 9.053 |
| 5' | 0.583 | 88.245 | 0.176 | 0.324 | 6.470 |
| 10' | 0.529 | 79.083 | 0.158 | 0.342 | 6.837 |
| 30' | 0.355 | 51.041 | 0.102 | 0.398 | 7.958 |
| 1h | 0.289 | 40.934 | 0.082 | 0.418 | 8.363 |
| 24h | 0.003 | 0.443 | 0.001 | 0.499 | 9.982 |
| 3 days | 0.000 | 0.000 | 0.000 | 0.500 | 10.000 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**3A ZEOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.01%**
**pH = 3.5**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 0.059 | 8.079 | 0.016 | 0.084 | 1.677 |
| 1' | 0.059 | 8.079 | 0.016 | 0.084 | 1.677 |
| 2.5' | 0.013 | 1.744 | 0.003 | 0.097 | 1.930 |
| 5' | 0.027 | 3.596 | 0.007 | 0.093 | 1.856 |
| 10' | 0.024 | 3.248 | 0.006 | 0.094 | 1.870 |
| 30' | 0.018 | 2.421 | 0.005 | 0.095 | 1.903 |
| 1h | 0.044 | 5.989 | 0.012 | 0.088 | 1.760 |
| 24h | 0.000 | 0.000 | 0.000 | 0.100 | 2.000 |
| 3 days | 0.000 | 0.000 | 0.000 | 0.100 | 2.000 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**3A ZEOLITE**
**Total zeolite amount = 5 g.**
**Initial protein = 0.05%**
**pH = 3.5**

| time | ABS | pro. conc of dilute sample (μg/ml) | pro. conc. of liquid (c) (mg/ml) | adsorbed amount (mg/ml) | adsorbed pro/g zeo (q) (mg/g) |
|---|---|---|---|---|---|
| 0.5' | 0.218 | 30.502 | 0.061 | 0.439 | 8.780 |
| 1' | 0.160 | 22.146 | 0.044 | 0.456 | 9.114 |
| 2.5' | 0.171 | 23.663 | 0.047 | 0.453 | 9.053 |
| 5' | 0.583 | 88.245 | 0.176 | 0.324 | 6.470 |
| 10' | 0.529 | 79.083 | 0.158 | 0.342 | 6.837 |
| 30' | 0.355 | 51.041 | 0.102 | 0.398 | 7.958 |
| 1h | 0.289 | 40.934 | 0.082 | 0.418 | 8.363 |
| 24h | 0.003 | 0.444 | 0.001 | 0.499 | 9.982 |
| 3 days | 0.000 | 0.000 | 0.000 | 0.500 | 10.000 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**Adsorption isotherm points**

**Clinoptilolite**

| c (mg/ml) | q (mg/g) | 1/c | 1/q |
|---|---|---|---|
| 0.023 | 1.540 | 43.478 | 0.649 |
| 0.021 | 9.570 | 47.620 | 0.105 |
| 0.043 | 19.130 | 23.260 | 0.520 |
| 0.361 | 32.790 | 2.770 | 0.030 |
| 0.805 | 33.900 | 1.240 | 0.029 |
| 0.941 | 41.180 | 1.063 | 0.024 |
| 1.403 | 51.940 | 0.713 | 0.019 |
| 3.582 | 48.360 | 0.279 | 0.020 |

**3A**

| c (mg/ml) | q (mg/g) | 1/c | 1/q |
|---|---|---|---|
| 0.001 | 2.000 | 10,000 | 0.500 |
| 0.002 | 10.000 | 666.700 | 0.100 |
| 0.003 | 20.000 | 333.300 | 0.050 |
| 0.365 | 22.700 | 2.740 | 0.044 |
| 0.574 | 28.520 | 1.742 | 0.035 |

# APPENDIX-C
# EXPERIMENTAL RESULTS

**Desorption analysis - Clinoptilolite**
**(after increasing pH of the solution)**

| time | ABS | protein (mg/ml) | c (mg/ml) | Desorbed (mg/ml) | q (mg/ml) |
|---|---|---|---|---|---|
| 15' | 0.593 | 89.850 | 0.180 | 2.820 | 56.406 |
| 24h | 0.696 | 108.480 | 0.217 | 2.783 | 55.661 |
| **(after adding 3M NaCl)** | | | | | |
| 15' | 0.410 | 59.706 | 0.299 | 1.761 | 35.229 |
| 24h | 0.457 | 67.195 | 0.336 | 1.724 | 34.481 |
| 4 days | 0.078 | 10.601 | 0.053 | 2.007 | 40.140 |

**Desorption analysis - 3A**
**(after increasing pH of the solution)**

| time | ABS | protein (mg/ml) | c (mg/ml) | Desorbed (mg/ml) | q (mg/ml) |
|---|---|---|---|---|---|
| 15' | 0.565 | 85.170 | 0.170 | 1.330 | 26.593 |
| 24h | 0.736 | 116.040 | 0.232 | 1.268 | 25.358 |
| **(after adding 3M NaCl)** | | | | | |
| 15' | 0.419 | 61.043 | 0.305 | 1.185 | 23.696 |
| 24h | 0.443 | 64.968 | 0.325 | 1.165 | 23.303 |
| 4 days | 0.606 | 92.133 | 0.461 | 1.029 | 20.587 |
| 7 days | 0.590 | 89.384 | 0.447 | 1.043 | 20.862 |