SURFACE IMPRINTED SILICA PARTICLES FOR CONCANAVALIN A PURIFICATION

CONCANAVALİN A SAFLAŞTIRILMASI İÇİN YÜZEY BASKILANMIŞ SİLİKA PARTİKÜLLER

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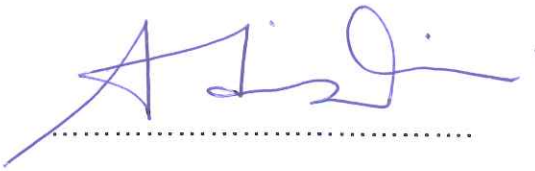
Head ..... 

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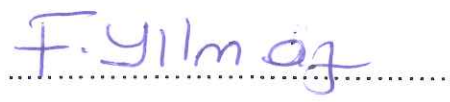
Member ..... 

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☐ **Serbest Seçenek/Yazarın Seçimi**

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Galiya Razym

ÖZET

CONCANAVALİN A SAFLAŞTIRILMASI İÇİN YÜZEY BASKILANMIŞ SİLİKA PARTİKÜLLER

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Concanavalin A (Con A) lektin olarak bilinen bitki protein grubu üyesidir. Çoğu lektin proteinleri hücre bölünmesi ve hücre yüzeyi araştırma çalışmalarında faydalıdır. Con A genelde afinite kromatografi araştırmalarında karbohidrat içeren yapıların saflaştırılması ve karakterizasyonu için kullanılmaktadır. Bu çalışmada çekirdek kabuk yüzey baskılama yöntemini kullanarak Con A saflaştırılması için spesifik adsorbent tasarlanmıştır. Con A molekülüne karşı yüzey moleküler baskılanmış polimer olarak silika mikroküreler kullanılmıştır. Silika yüzeyi asit ile işlenmiş ve sonrasında 3-metakriloiloksipropil trimetoksisilan (MPTMS) ile modifiye edilmiştir. Con A moleküllerini silika partiküllerin yüzeyine baskılamak için N-metakriloil-L-histidin metil ester fonksiyonel monomer olarak kullanılmıştır. Çekirdek-kabuk silika partikülleri Zeta Size, SEM cihazı ve FTIR spektroskopi ile incelenmiştir. pH, Con A derişimi ve sıcaklık gibi parametrelerin Con A adsorpsiyonuna etkileri test edilmiş ve hesaplanmıştır. Con A adsorpsyonunun ilerlemesini incelemek için zaman taraması yapılmıştır. 1.0 M NaCl çözeltisi desorpsiyon ajanı olarak kullanılmıştır. Moleküler baskılanmış polimerlerin etkisini

görmek için aynı yöntem ile moleküler baskılanmamış polimerler hazırlanıp test edilmiştir. Ayrıca Con A baskılanmış partiküllerin bağlanma boşluklarının seçiciliğini araştırmak için lizozim ve hemoglobin molekülleri kullanılmıştır. Baskılanmış silika partiküllerin seçiciliği Con A içeren Jack Bean (fasulye) özütünü kullanarak incelenmiştir. Con A'nın saflığı sodyum dodesil sülfat poliakrilamit jel elektroforezi (SDS-PAGE) ile incelenmiştir. Son olarak Con A baskılanmış silika partiküllerin tekrar kullanılabilirliği 10 gün boyunca ardarda kullanarak seçiciliğin değişmemesi ile ıspatlanmıştır.

Anahtar Kelimeler: Concanavalin A, lektinler, moleküler baskılanmış polimerler, silika partiküller.

ABSTRACT

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Concanavalin A (Con A) is a representative of plant proteins group known as lectins. Many of lectin proteins have useful characteristic for studies of cell division and cell surfaces. It generally employed in affinity chromatography for characterization and purification of cell structures and carbohydrate containing molecules. In this research, a new adsorbent for specific separation of Con A were prepared by applying core-shell surface imprinting method. Silica microspheres were employed as a core material to prepare specific surface imprinted polymer contrary to Con A. Silica surface were stimulated by acidic treatment and modified with 3-methacryloyloxypropyl trimethoxysilane (MPTMS). N-methacryloyl-L-histidine methyl ester was used as a functional monomer to imprint Con A molecules on the surface of silica particles. The core-shell silica particles were characterized using Zeta Size analysis, SEM equipment and FTIR spectroscopy. The effect of parameters such as pH, concentration of Con A and temperature on the adsorption of Con A were tested and calculated. Progress of adsorption of Con A was checked by measuring it in different period of time. 1.0 M NaCl was used as removal agent. To compare the effectivness of MIPs, Non-Imprinted Polymers

(NIP) were also prepared and tested the same way as MIP. Additionally we used lysozyme and hemoglobin molecules instead of Con A to check the specificity of cavities on imprinted silica particles. The selectivity of the imprinted silica particles was also investigated by isolating Con A from Jack Bean. The purity of the Con A was tested by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Finally Con A imprinted silica particles were tested for reusability by exploiting it in 10 subsequent cycles.

Keywords: Concanavalin A, lectins, molecularly imprinted polymers, silica particles.

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SYMBOLS AND ABBREVIATIONS

Symbols

Q	Adsorption capacity

Abbreviations

MIT	Molecular Imprinting Technology
MIP	Molecularly Imprinted Polymers
NIP	Non Imprinted Polymer
Con A	Concanavalin A
UV-Vis	Ultraviolet-Visible Spectroscopy
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
MAH	N-methacryloyl-L-histidine methyl ester
MPTMS	3-methacryloyloxypropyl trimethoxysilane
HEMA	2- hydroxyethyl methacrylate
MBAAm	N,N′-methylenebisacrylamide
TEMED	N,N,N’,N’-tetramethylenediamine
APS	ammonium persulphate
TEOS	tetraethylorthosilicate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
pI	isoelectric point
kDa	kilo-Dalton

1. INTRODUCTION

Concanavalin A (Con A) is a lectin obtained from the Jack Bean seeds. Con A is extensively exploited in science and industry. Con A usually utilized as reagent in chemistry, glycoprotein, and in biological researches, for instance affinity chromatography based on lectin matrixes. One of the most common enzyme urease is derivative of Jack Bean. Concanavalin is able to connect with lipoproteins, immunoglobulins, cell surface matrixes and carcino-embryonary antigen also Con A can interact with carbohydrates containing mannose [1].

Con A has demonstrated itself as a useful molecule in applications which requires glycoenzyme immobilization as a solid-phase, particularly those that have proved difficult to immobilize via traditional covalent binding [2].

Con A's binding ability renders it as an efficient agent for lectin based histochemical staining. This is perfect tool in researching the sugar composition of cell surfaces. Con A is also used in affinity chromatography as a solid phase agent to purify proteins. Recent research proposes that the ability of Con A to bind to specific glycoproteins could be used to help in hepatoma treatment. The glycoprotein internalize hepatoma cells, activating cell death by autophagy. Also, Con A as a T-cell mitogen, can activate an immune response in the liver [3]. Finally, Con A is the most studied and most used plant lectin and is employed as a prototypical legume lectin in order to investigate the molecular base for protein-carbohydrate identification for other lectins [3,4]. Protein-carbohydrate recognition is a crucial system for intercellular communication. Even though Concanavalin A is a legume lectin, it can be used in animal cells; it has a mitogenic role in stimulating T-cells and agglutinates erythrocytes.

Scientists all over the world are intrigued by the structure and function of Con A because of its role in the blood and in studying different immune responses to tumor cells.

There is a high demand for Con A in a global market. Hence purification of Con A by easy, cheap and effective methods is among the most priority issues in science. In this study we used molecularly imprinted silica particles to purify as much of Con A as possible.

Molecular Imprinting Technology (MIT) used to construct synthetic receptors with a prearranged affinity for certain target molecules [5]. Additionally the spatial shape (3D) of the target molecule and the functional monomer are remembered by the cross linker units for binding to the molecule of interest while purification [6].

The main excellence of molecularly imprinted polymers (MIPs) is that they have high selectivity for the imprinted molecules. Additionally, they are also low-cost to be synthesized and also polymers have a long storage life. They can keep their recognition ability for couple of years at room temperature [7].

There are several types of Molecular Imprinting Techniques such as bulk, epitope and surface imprinting. In our case we used surface imprinting method because it has several superiority. For example the synthesis procedure is quite simple to observe and posseses imprinted binding sites which can be reached quickly, easily by target molecules during binding and rebinding steps [8].

In this research, Con A imprinted poly(2-hydroxyethyl methacrylate-co-N-methacryloyl-L-histidine methyl ester) silica microspheres were prepared and characterized by Zeta Size anasysis, FTIR spectroscopy and Scanning Electron Microscope (Carl Zeiss EVO 50 EP, Germany). Adsorption conditions optimization was held by different values of pH. The highest possible adsorption quantity was detected at pH 6.0, adsorbed Con A amount is 21 mg/g. The effect of Con A concentration showed that the adsorbed Con A amont is rised together with the rise of Con A concentration in a solution. The highest point was reached at 1.5 mg/ml concentration adsorbing 300 mg/g of Con A. Effect of temperature was also observed and according to its results we understand that the adsorption ability of the system was reduced as temperature was rised [9]. Due to chemical structure of functional monomer used in experiments, there were hydrogen bonding interactions between Con A-imprinted core–shell silica particles and target molecules. High temperature broke down that chemical interactions, hence MIP and Con A could not match each other. At 40^0C adsorbing of Con A reached the lowest point (15 mg/g). To see the progress of adsorption of Con A we measured the soultion in different period of time. The result showed us that adsorption goes on gradually and reaches the highest point approximately in 2 hours (20 mg/ml). To examine the target selectivity of the imprinted cavities lysozyme and

hemoglobin molecules were used instead of Con A. Selectivity of Con A- imprinted silica particles showed 26 times more efficiency comparing to lysozime and 6 time more selectivity comparing to hemoglobin. The selectivity of the imprinted silica particles was also researched by extracting Con A from Jack Bean. The adsorption amount for ½ concentrated Jack Bean solution was 90 mg/g. The imprinted silica particles was used in 5 subsequent cycles without considerable loss in adsorption capacity (93.5%).

Learning of possible application of Con A in medicine and scientific purposes is still among topical themes. By our research we proved the possibility of purification of Con A by Molecularly Imprinted Silica Particles. The outcomes of adsorption tests of the core-shell imprinted silica particles demonstrated fast association-dissociation kitetics, high binding and good recognition ability for Con A. Also dirung practical experiments our Con A imprinted silica microspheres showed high selectivity and reusability. Furthermore, this effective method can be applied for separation and adsorption of different proteins by imprinting them instead of Con A.

2. GENERAL INFORMATION

2.1. Lectins

Lectins, which noncovalently cooperate with carbohydrates, belongs to a class of proteins. They are found in most organisms like bacteria, viruses, animals, and plants [10]. Various types of lectins owe various sequences , structures and also familiarity with certain carbohydrates. Among lectins wide vital activities there are some main of them like: host pathogen interactions, cell to cell interactions. Vegetative tissues, leaves and roots contain lectins. Lectins as glycoproteins can specifically interact with mono or oligosaccharides without changing its covalent nature [11].

Lectins are mostly purified from lectin containing leguminous vegatables. The overall weight of grown germs consist of lectin up to 4%. Mostly, lectins from legume are main model for researching carbohydrates and other types of lectıns. The types of lectins are showen in the Table 2.1.

Table 2.1. Four groups of mature lectins according to their structure

Types of lectin	Description of the type
Merolectins	Merolectins are monovalent and has domain which can bind to only one carbohydrate. Hence, agglutinattion of cells and precipitation of glycoconjugates are not possible. For example: hevein, obtained from latex of rubber tree, is a chitin binding protein.
Hololectins	Hololectins consist of two domains with similar carbohydrate binding ligands, which can interact with structurally similar sometimes the same carbohydrates. Hololectins are multivalent or divalent. Hence, they agglutination of cells or precipitattion of glycoconjugates are possible.
Chimerolectins	Chimerolectins have domains which can bind to many types of carbohydrates. Chimerolectins are able to function like hololectins at the same time like merolectins relying on amount of domains with carbohydrate binding ability.
Superlectins	Superlectins owe at least two domains with carbohydrate binding abilities. These domains can structurally identify dissimilar carboohydrates. Superlectins represent the specific class of chimerolectins with two types of domains with carbohydrate binding abilities which function and structure are tandemly arranged.

2.1.1. Structure of lectin

Three dimentional skeleton of lectin consist of forepart of the monomer that is made of a curved 7 twisted β- plates and reverse side that is built up of a flat 6 twisted β-plates (Figure 2.1). Either the forepart and the reverse side are interconjugated with loops and turns to form a flatt cupola like construction. 4 loops are placed on the top of the cupola creating the site of binding for the monosaccharides [12].

Lectins of legume have not an α-helix, that is why lectins refer to a β-proteins group. Tetrameric lectins are tetravalent and have four carbohydrate binding sites whereas dimeric lectins are divalent. 2 monomers are bound to create canonical dimer (Figure 2.2). 222 (D2) symmetry forms in the tetrameric molecule if two dimers are connected by its reverse walls where 2 monosaccharides binding sites take place at two sides of the tetramer [13]. As a result, carbohydrate binding sites appear at four corners of the tetrahedron (Figure 2.3).

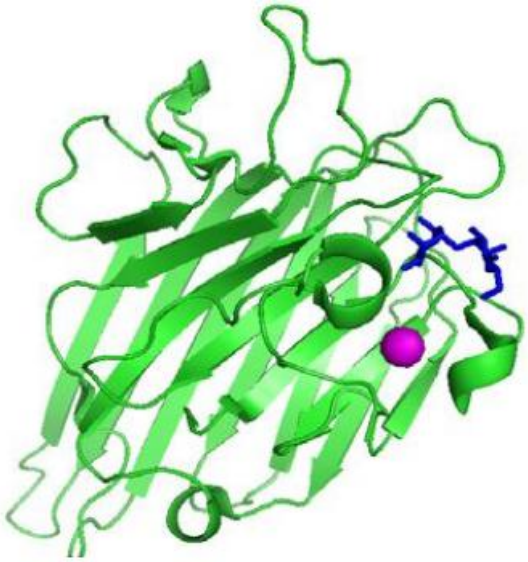

Figure 2.1. Con A's crystal structure. Pink circle is the Manganese atom and blue strand stands for the dimer [11].

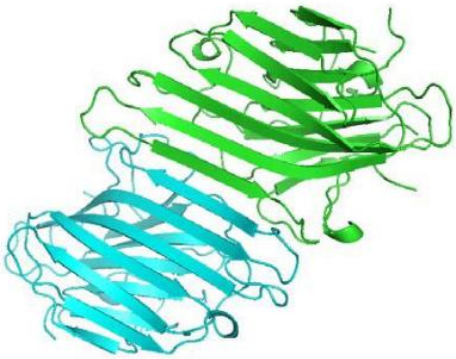

Figure 2.2. A dimer of Con A. 2 monomers are connected non covalently. Together they create anti parallel β-list via intermolecular strands [11].

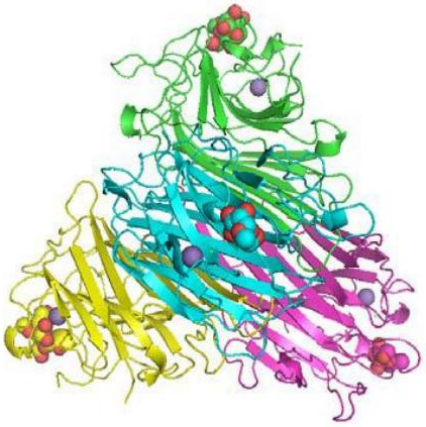

Figure 2.3. Quadruple structure of Con A [11].

2.1.2. Concanavalin A

Concanavalin A (Con A) is a class of plant proteins which is called lectins. Con A, one of the most researched lectin, is well known to cooperate very strongly with the cell surface glycoconjugates containing a- D –mannose and a- D –glucose [14].

It is a single-cell protein. Con A contains metals like Mn^{+2} ions and Ca^{+2} ions.So it is a metalloprotein. Under the impact of pH Con A can be as a tetramer or a dimer. Con A able to occur as dimer with two meatlls in two various forms: in dimeric form and in tetrameric form [15].

2.1.3. Properties of Concanavalin A

Con A was crystallized by Sumner and Howell in 1936. Con A is a homotetramer protein and every subunit is 26.5 kDa. Con A is able to agglutinate erythrocytes via interaction with immunoglobulin glycopeptides. Con A refers to a lymphocyte mitogen and moreover able to interact with bacterias. There are metal ions in Con A structure which assists in interaction with other molecules and help to balance its structure. However buffers like metal cholators, EDTA break down its affinity to carbohydrate binding. Con A is a plant mitogen. Con A can activate T-cell subsets of mouse which, in its turn, activates to 4 types of T-cell populations like precursor to suppressor T-cell, that is one unit of human suppressor T-cells which has an affinity to Con A [16].

2.1.4. Chemical build of Concanavalin A

The structure of Con A has been researched as an I222 crystal of the saccharide-free protein was discovered and described. Since the structure of Con A had been clarified it seemed that structure of the saccharide-binding site of the solution of the would follow shortly. This expectation was not satisfied; chiefly because the I222 crystal dissolves on addition of saccharide. Hardman and Ainsworth in 1976 found where the saccharide locate by placing the iodine atoms in the complexes of concanavalin A with two iodo-derivatives of glucose in a C222, crystal at 6 A resolution. Con A has four binding sites, each one is found in the four subunits. Every subunit owe 1 saccharide connecting site and 2 transition metal connecting sites. Single molecule of Con A interacts with single Mg^{+2} ion and single Ca^{+2} ion. Either furanosides and pyranosides connect to the identical Con A sites.

2.1.5. Practical usage of Concanavalin A

Con A is used in various areas of science and also have different practical implementations. Con A is exploited to efficiently immobilize enormous amount of glycoenzymes. Non covalent binded Con A and glycoenzyme can be reversed by decreasing its pH or adding sugars. Con A is valuable for researching cell surface,

cell division, immune regulations via different immune cells, glycoproteins characterization, isolating and purification of glycosylated macromolecules by lectin based affinity chromatography.

Con A also causes cell autophapy. Moreover Con A is also known as T cell mitogen because of its ability to activate hepatitis in mice via activating NKT cells and switching on $CD4^+$ T cells. Con A's anti cancer effects is caused by the simulation of $CD8^+$ T cells which sets up cancer immune recognazing and suppressing cancer [17].

Con A has been employed as a recognizing agent to differentiate the degree of glycosylation associated with metastasis and malignant tumors from benign. Hence, Con A has a great prospective application in the future as a cancer medicine [18].

2.2. Affinity chromatography

Affinity chromatography is widely used approach for the identification, and of macromolecules based on highly specific molecular recognition. In this approach, special ligand or binder is immobilized on a suitable insoluble support matrix, which is usually a polymeric material in bead or membrane form. The molecule to be isolated analyte or target is selectively adsorbed by the complementary ligand immobilized on the matrix by simply passing the solution containing the target through the chromatographic column under needed conditions. The target molecules are then eluted or desorbed by using proper elutants, using specific solvents or competitive free ligands, so that the interaction between the ligand and target is broken and the target molecules are obtained in a purified form [19].

There are three types of affinity chromatography depending on the use of a general ligand (lectins), specific ligands (enzyme inhibitors, hormones) and antibodies. Immobilized lectin is a general form of affinity chromatography. Lectin-glycoprotein interactions are reversible and can be inhibited by simple sugars. Numerous lectins have been identified with the most widely used lectins to be Con A and wheat germ agglutinin.

Several parameters about using lectin-affinity chromatography are important and are as following: first interaction between protein of interest and specific lectin needs to be empirically tested. Sometimes due to different glycosylation enzymes present in different tissues, the same glycoprotein expressed in different tissues of the same animal may not have the same lectin-binding specificity. Second, a specific lectin-affinity chromatography purifies a group of proteins with a specific type of glycosylation, thus this method is unable to achieve the extent of purification as ligand or antibody chromatography. Third, lectins are sensitive to certain types of detergents. Although nonionic detergents, for example Triton X-100, have negligible effects on the binding of concanavalin A or wheat germ agglutinin with their ligands, some ionic detergents, for example SDS, may inactivate lectins [19].

2.2.1. Ligand-affinity chromatography

The key to a successful ligand-affinity chromatography is that the affinity between the ligand and the receptor needs to be sufficiently high to allow binding and washing during the purification step. The ligand is usually immobilized onto the affinity support through cross-linking. The affinity between the ligand (or inhibitor) and receptor may be altered due to the presence of detergent. As such, choosing a detergent that does not significantly lower the receptor affinity for its ligand will be critical if ligand-affinity column is to be used for its purification.

2.2.2. Antibody-affinity chromatography

If the antibody is available, using immobilized antibody against the specific membrane protein is the most powerful method of purification. Antibodies are relatively stable in nonionic detergents and thus are compatible with the presence of detergents in solubilized membrane preparations. The challenge may be the elution of the protein from the immunoaffinity column.

2.2.3. Ligands in affinity chromatography

A wide variety of functional molecules, including enzymes, coenzymes, cofactors, antibodies, amino acids, oligopeptides, proteins, nucleic acids, and oligonucleotides may be used as ligands in the design of novel sorbents. These ligands are extremely specific in most cases. However, they are expensive, due to high cost of production and/or extensive purification steps. In the process of the preparation of specific sorbents, it is difficult to immobilize certain ligands on the supporting matrix with retention of their original biological activity. Precautions are also required in their use (at sorption and elution steps) and storage [20].

2.2.4. Pseudospecific ligands

Pseudospecific ligands are used to purify many biomolecules, hence offering more structural flexibility as compared with biospecific ligands. Pseudospecific ligands have low binding constants and consequently, belong to the family of weak affinity ligands. Despite that, they can exhibit selectivity resulting from the cumulative effects of multiple weak binding events, such as electrostatic, hydrophobic and hydrogen bonding and van der Waals interactions, with fast kinetics. Recently, it has been found that amino acids as pesudospecific ligands may hold certain advantages for industrial bioaffinity separations, as they are not likely to cause an immune response in the case of leakage into the product. These ligands are also much more stable than protein ligands because they do not require a specific tertiary structure for maintaining biological activity. They offer additional advantages over biological ligands in terms of economy, ease of immobilization and high adsorption capacity [20].

2.2.5. Dye Ligands

Dye ligands are able to replace biological ligands, and they have lots of applications in affinity chromatography. Dye ligands are commercially available, cheap and can easily be immobilized by covalent bonding on polymeric matrices. Both cibacron blue F3GA and alkali blue 6B textile dyes which are also called

reactive dyes contain a chromophore linked to a reactive group. Aromatic structures and acidic groups of dye ligands interact with side chains of amino acids on protein molecules. These interactions constitute of electrostatic, hydrophobic and hydrogen bondings [21].

A textile dyes, known as reactive dyes, have been utilized for protein purification in dye-ligand affinity systems, because they can bind a variety of proteins in a selective and reversible way. Many of the reactive dyes exploited in dye-affinity systems contain a chromophore either azo dyes, anthraquinone, or phathalocyanine, linked to a reactive group often a mono- or dichlorotriazine ring. They also have sulfonic acid groups to provide the desired solubility of the molecule in aqueous media.

Nowadays, triazinyl-based reactive dyes are mostly used in protein purification. Cyanuric chloride (1,3,5-trichloro-sym-triazine) is the basic substance used in the synthesis of these dyes. The presence of electronegative atoms makes the three carbon atoms highly positive, and therefore very susceptible to nucleophilic attacks. Chromophore molecules are easily attached to this molecule to form the dichlorotri- azinyl dyes. By further reactions of these molecules with other nucleophilic substituents such as aniline or sulfanilates, monochlorotriazinyl dyes are synthesized.

2.3. The matrix

Choosing of the appropriate supporting matrix is the first important consideration in affinity systems. The matrix must show very low nonspecific adsorption, which may be because of charged or hydrophobic groups on its surface, which compromise the specificity of the affinity sorbent. This is crucial because the efficiency of affinity sorption depends on specific interaction between the immobilized ligand and the target molecules [19].

The matrix must have functional surface groups hydroxyl, carboxyl, amide, etc. for further derivatization and immobilization of ligands. Also the matrix should be highly porous to allow high amount of ligand immobilization, and therefore, high enough adsorption capacity for the target, which is defined as the amount of

molecules specifically bound per unit weight or volume of the sorbent. However, it should be mentioned that a high level of matrix substitution is not always mean higher adsorbent capacity. The pores should be large, because in most cases, the ligand and target molecules are large size proteins. This loose structure allows the target molecules easily bind in and out during the separation steps, which means fast sorption–elution. In Table 2.2. are shown the most frequently used support materials.

Table 2.2. Some commercially available affinity support materials [19]

Support material	Supplier	Trade name
Conventional affinity chromatography		
Agarose	Pharmacia LKB, Sweden	Sepharose
	Bio-Rad, USA	Bio-gel
	Bio-Rad	Affi-gel blue
Cellulose	Amicon, USA	Matrex Cellufine
Dex tran	Pharmacia LKB	Sephadex
Agarose/Polyacrylamide	IBF, France	Ultrogel
Polyacrylamide/dextran	Pharmacia LKB	Sephacryl
Polyacrylamide	Röhm Pharma, Germany	Eupergit C
	IBF	Trisacryl
	Bio-Rad	Affi-gel
PHEMA	Tessek, Czechoslovakia	Separon H 1000
Methacrylate	Merck, Germany	TSK-Gel Toyopearl
	Separon	Alltech, USA
Controlled pore glass	Pierce, USA	CPG
High performance liquid affinity chromatography		
Polymer-clad silica	J.T. Baker, USA	Prepscale
Silica	Dupont, USA	Zorbax
	Shandon, UK	Hypersil WP300
	Merck	Lichrospher
	Beckman, USA	Ultrasphere
	Waters, USA	Spheron
Methacrylate	Alltech	Eupergit
Synthetic polymer	Dyno Particles, Norway	Dynospheres
Vinyl polymer	Merck	Toyopearl
Polystyrene	PerSeptive Biosystems, USA	Poros-50
Membrane affinity chromatography		
Silica-PVA	FMC	Acti-Disk
Glass	Schott Glass	Bioran-M

2.3.1. Ligand immobilization

The most important clues for a successful ligand immobilization are given below. Firstly, immobilization must be attempted through the least critical region not from the active site of the ligand molecule, to ensure minimal interference on the specific interaction between the immobilized ligand and the target molecules. Note that chemicals and experimental conditions applied may cause deleteriotion of the

ligand molecules during activation or coupling steps, hence should neatly be selected.

There are two alternative procedures schematically represented in Figure 2.4. The matrix is first activated with an activation agent, then the spacer arm is attached covalently to the matrix through the active points. The ligand is then reacted with the other end of the spacer molecules. Alternatively, the ligand-spacer arm conjugate is first synthesized and then attached to the carrier in one single step.

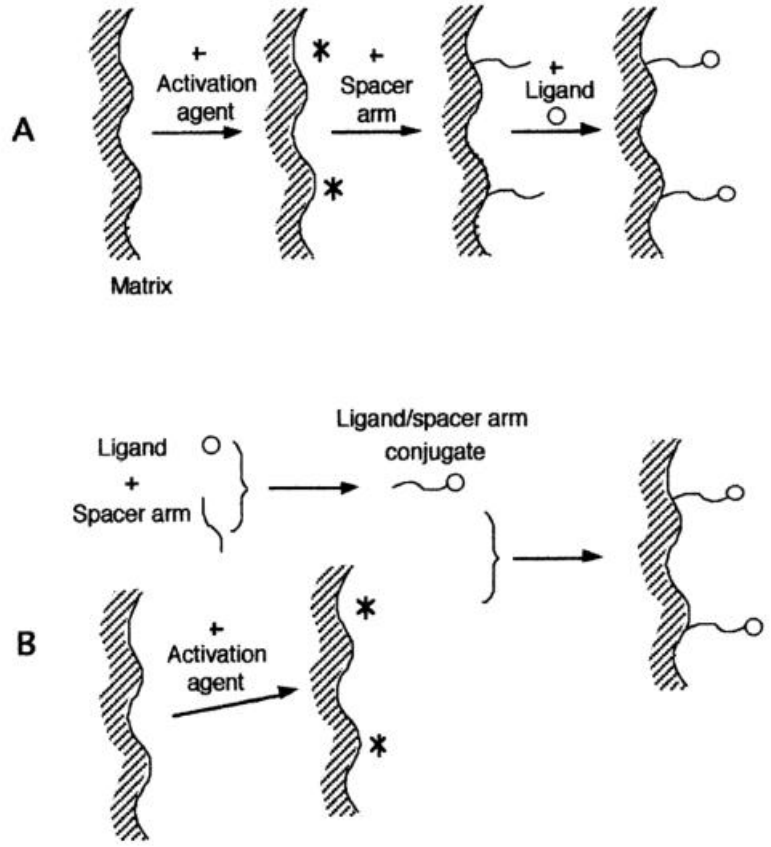

Figure 2.4. Strategies for coupling of ligands to the support matrix; (A) coupling through spacer arm; (B) coupling through spacer arm-ligand conjugates [19].

2.4. Purification of lectins

Although the presence of lectins in numerous organisms had been widely recognized, until the 1970s, very few lectins had been purified and almost all by conventional techniques involving a series of precipitations with salts and solvents. One of the major breakthroughs in lectins purification came with the advent of

affinity chromatography for the capture of lectins using cross-linked dextran gels by Goldstein and his coworkers (Agrawal and Goldstein, 1965). Since then, most purification strategies include an affinity chromatography step in columns containing polysaccharides to take advantage of the specificity of lectins for sugars and the ready availability of sugar-based gel matrix for chromatography, specially the glucose-based Sephadex (cross-linked dextran) and the galactose-based Sepharose [22].

In general, the purification of lectins from crude extracts has been made through several chromatographic steps, such as affinity chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, and gel filtration chromatography. There are many types of ligands used in chromatography methods and some of them are shown in Table 2.3.

Table 2.3. Affinity ligands used in purification of lectins [22]

Ligands	Lectin	Reference
Fetuin	*Macrophomina phaseolina* *Phaseolus acutifolius* *Katsuwonus pelamis* *Castanea crenata*	Bhowal *et al.*, 2005; Jung *et al.*, 2003; Nomura *et al.*, 1998; Reynoso-Camacho *et al.*, 2003
Avidin	*Amansia multifida*	Costa *et al.*, 1999
Guar-gum	*Ptilota serrata* *Pterocladiella capillacea* *Vidalia obtusiloba* *Moringa oleifera*	Melo *et al.*, 2004; Oliveira *et al.*, 2002a, 2002b; Sampaio *et al.*, 1999; Santos *et al.*, 2009
Mucin	*Ulva lactuca* *Nemopilema nomurai*	Imamichi and Yokoyama, 2010; Sampaio *et al.*, 1998a, 1998b
IgG anti-CvL	*Cinachyrella apion*	Medeiros *et al.*, 2010
Ovalbumin	*Serpula vermicularis*	Molchanova *et al.*, 2007
Stroma	*Axinella corrugata*	Dresch *et al.*, 2008
GlcNAc	*Macoma birmanica*	Adhya *et al.*, 2009
Mannose	*Canavalia cathartica* *Vatairea macrocarpa*	Cavada *et al.*, 1998; Suseelan *et al.*, 2007
Chitin	Araucaria angustifolia	Santi-Gadelha *et al.*, 2006
Lactose	*Halichondria okadai* *Perinereis nuntia* ver. *vallata* *Zea mays* *Erythrina speciosa* *Erythrina indica*	Kawsar *et al.*, 2008; Kawsar *et al.*, 2009; Konozy *et al.*, 2003; Konozy *et al.*, 2003; Martínez-Cruz *et al.*, 2001

2.5. Molecular Imprinting Technology

Molecular imprinting technology (MIT) makes it possible to prepare substances with sites that have an ability to memorize target molecule according to its size, shape and chemical functionality. Molecular recognition is connected with

biochemical processes such as the ligand–receptor interactions, enzyme catalysis and immuno response. To get a selective recognition with high resolution of a desired target proteins, target molecule is integrated while the imprinting polymers synthesis as a template. After the polymerization is done, the protein is removed, as a result, leaving behind a three dimensional chemical and physical signature of itself. There are different types of protein-imprinted matrices such as hydrogels, sol-gels, cryogels and monoliths that prepared via bulk imprinting [23].

2.5.1. Historical Background of MIT

MIT's very early researches backdates to the beginning of 1930s and the first unit used for it was silica particles. In the 1940s the research mostly set up traditional researches upon the mechanism of antibody antigen interactions. Imprinting approach was used to evidence the theory which states that initially antibodies developed their selectivity upon presence of the antigen [24]. But the theory of the formation of antibody was afterwards showed to be incorrect. Also studies of MIT have found the limitations of silica matrixes hence a recession in MIT studies was natural. In 1972 Wulff at al. presented preparing of MIT by covalent bonding. In the beginning of 1980s a prosperous synthesis of the molecularly imprinted polymer by non covalent synthesis approach was introduced by Mosbach et al.. New methods were a big progress in a way of synthesizing molecular imprints in organic materials (Figure 2.5.).

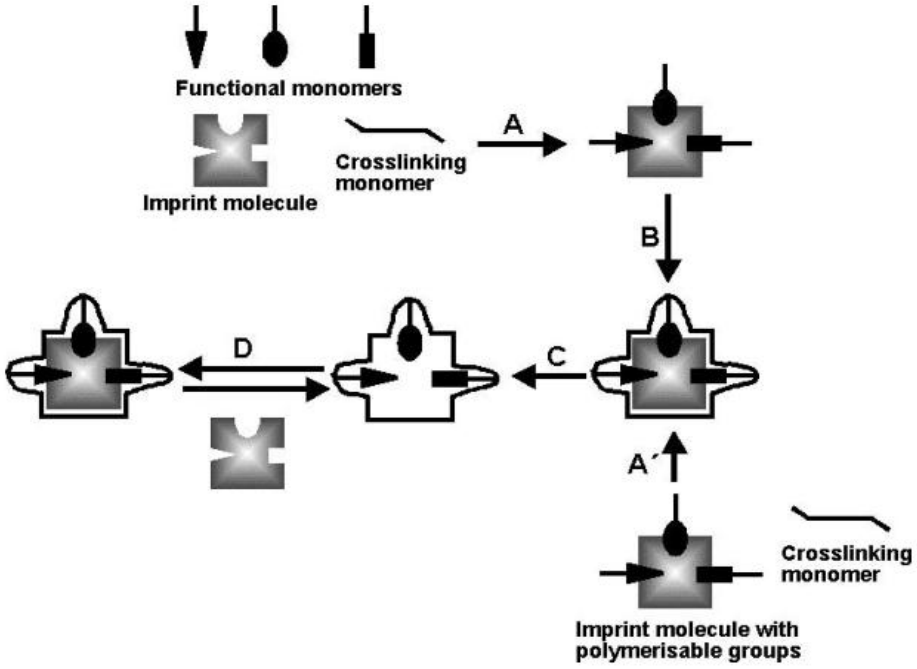

Figure 2.5. Preparation of MIP in schematics. The non-covalent method (A): An imprint molecule, functional monomers, radical initiator and cross-linking monomers are putted in a certain solvent to design cavities. (B) During polymerisation reaction the functional monomers are surrounded and locked in its native state, and (C) after imprinted molecule is removed (D) then the MIP have an ability to bind to the imprinted molecule. The covalent approach: (A') Imprint molecules with replacements of polymerisable groups are mixed with crosslinking monomers and radical initiator in appropriate solvent then polymerisation is activated. (C) After polymerisation is completed the covalent bonds between the imprint molecule and the MIP can be broken down and (D) the MIP can react covalently with the imprinted molecule [24].

2.6. Molecularly imprinted polymers

Synthesis of new mimic enzymes with high catalytic activity and shape-selectivity has great importance in various fields such as catalysis, surface organometallic chemistry, material chemistry and surface chemistry. To prepare polymers possessing molecular recognition ability for particular molecules, molecular

imprinting methods which create template-shaped cavities using suitable template molecules in polymer matrices have been developed in the past decade [25]

Molecular imprinting is an approach for creating special binding cavities in artificial polymers by using a molecular pattern. For imprinting crosslinked polymers molecule of interest is exploited as templates. After fixing of template molecules into the polymer network during polymerization and removal of them via extraction, there exist highly specific recognition sites based on both shape and conformational memory [26, 27]. The selectivity of the imprinted polymers relies on different factors such as the shape and the size of the cavity and rebinding interactions. Covalent and non-covalent interactions, metal ion coordination and electrostatic interactions might be applied to synthesize the functional monomers throughout template molecules. The molecular imprinted polymers (MIPs) have successfully used for biomaterials as biosensors, bioseparations, diagnostic assays and catalysis. MIPs have also been exploited in efforts to design polymeric materials for synthetic enzyme-mimic catalysis applications.

2.6.1. Prepolymerization studies of MIPs

One of the most important points when preparing MIPs is a good interaction between template molecule and monomer. Depending on that prepolymerization interactions there are two strategies used for MIP synthesis:

- Self-assembling method, which utilizes such biological recognition interactions like non-covalent forces, hydrogen bonds, hydrophobic forces, ion and Van der Waals interactions,
- preorganized method. This method uses reversible covalent bonds in which binding sites are quite homogeneous and non specific sites are decreased. But, removing of templates from the cavities necessaries to break covalent bounds down.

2.6.2. Templates

A large range of template molecules like amino acids, drugs, proteins, carbohydrates, hormones, co-enzymes and pesticides have been successfully exploited. Functional monomers are chosen taking into account the nature of the template molecules. To achieve a good-defined interaction between functional monomers and template molecule, target should have functional groups which can bind to monomers and it should be stable after the polymerization process. To provide high selectivity and stability of template is important to create high accuracy for recognition cavities and reduce the change of conformational state of the template after binding and rebinding [28].

2.6.3. Functional monomers used in MIPs

The choice of monomer is an issue of major importance to create highly specific cavities. The most frequently used and typical functional monomers are shown in Figure 2.6. The best monomer for molecular imprinting is chosen due to the chemical structure and strength of the interaction forces between monomer and template molecules. Functional monomers are used to activate binding capasities of the imprinted binding cavities to make silica particles selective for certain molecules. There are many types of functional monomers, that are chemically and structurally different. Nowadays all of them are commercially selled and other types can be also designed according to requests of the experiments.

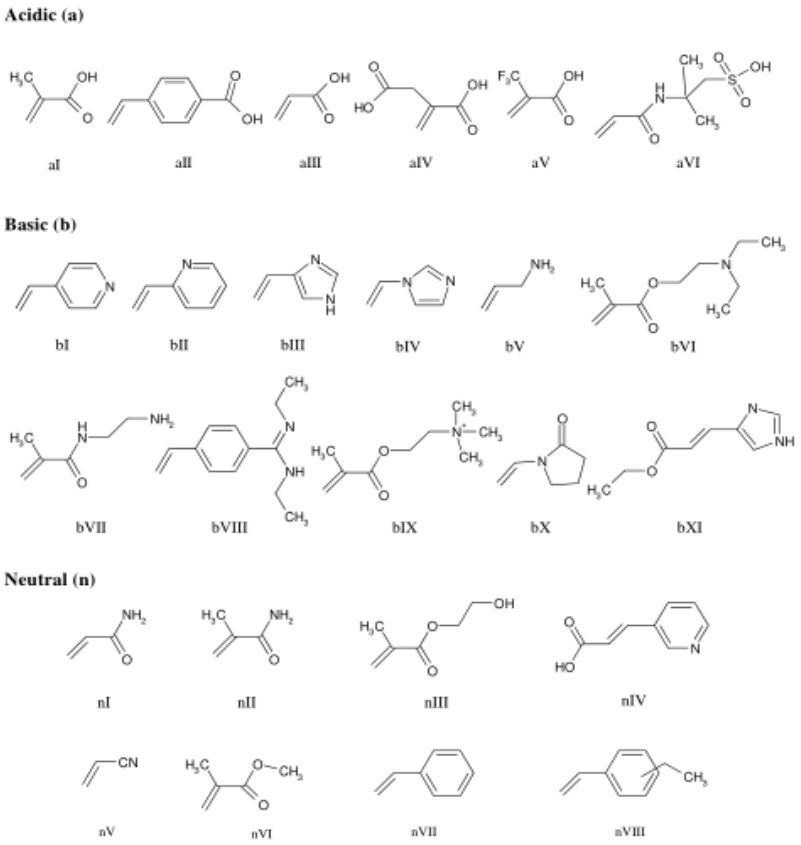

Figure 2.6. Monomers that are exploited for non covalent imprinting. “Acidic: aI: methacrylic acid (MAA); aII: p-vinylbenzoic acid; aIII: acrylic acid (AA); aIV: itaconic acid; aV: 2-(trifluoromethyl)-acrylic acid (TFMAA); aVI: acrylamido-(2-methyl)-propane sulfonic acid (AMPSA). Basic; bI: 4-vinylpyridine (4-VP); bII: 2-vinylpyridine (2-VP); bIII: 4-(5)-vinylimidazole; bIV: 1-vinylimidazole; bV: allylamine; bVI: N,N ? -diethyl aminoethyl methacrylamide (DEAEM), bVII: N-(2-aminethyl)-methacrylamide; bVIII: N,N diethyl-4-styrylamidine; bIX: N,N,N,-trimethyl aminoethylmethacrylate; bX: N-vinylpyrrolidone (NVP); bXI: urocanic ethyl ester. Neutral; nI: acrylamide; nII: methacrylamide; nIII: 2-hydroxyethyl methacrylate (2-HEMA); nIV: trans-3-(3-pyridyl)-acrylic acid; nV: acrylonitrile (AN); nVI: methyl methacrylate (MMA); nVII: styrene; nVIII: ethylstyrene” [29].

2.6.4. Cross-linkers

In molecularly imprinted polymers synthesis cross-linkers serve an significant role. The cross-linker helps to balance the imprinted binding sites, control the morphology of the polymer and gives mechanical stability to the polymer material

for keeping molecular memorizing ability [30]. The most common cross-linkers are given in Figure 2.7.

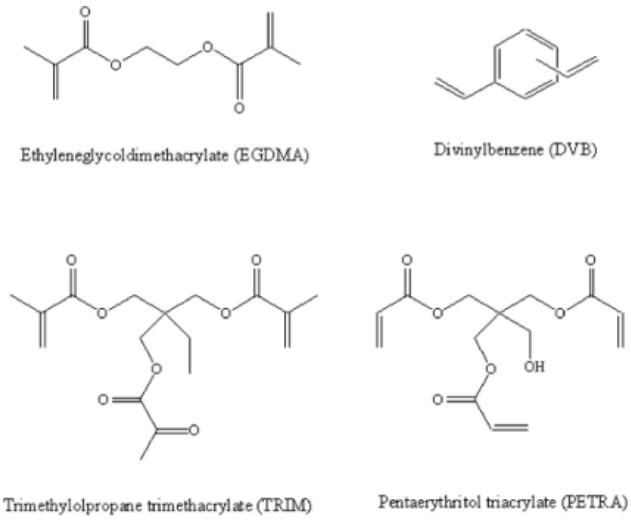

Figure 2.7. Structures of the most frequently used cross-linkers [29].

2.6.5. Solvents

Nature and amount of the solvent influence the polymer morphology greatly in the molecular imprinting process. Most of molecular imprinting approaches using non-covalent method are conducted using low polarity and aprotic organic solutions such as chloroform, toluene, dichlorometane and acetonitrile. Solvent solubilizes the elements of polymerization solution, serves to take all of the elements (template, monomer, cross-linker and initiator) into one phase. Furthermore solvent are able to make pores in macroporous polymers. Solvent are supposed to generate big pores to create a well environment for the process of adsorbance. That is why sometimes solvents are called “porogen”.

2.6.6. Initiators

Initiator agents are utilized to give a start to free radical polymerizations in the existence of template molecules. The chemical structures of the most common polymerization initiators are shown in Figure 2.8.

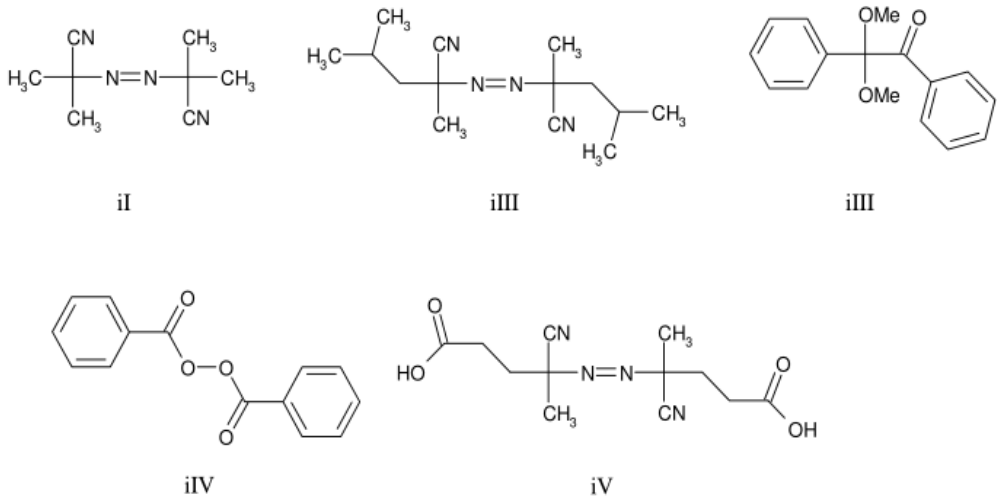

Figure 2.8. Chemical compositions of most common “initiators: iI: azobisisobutyronitrile (AIBN); iII: azobisdimethylvaleronitrile (ABDV); iIII: dimethylacetal of benzil; iIV: benzoylperoxide (BPO); iV: 4,4 -azo(4-cyanovaleric acid)” [31].

2.7. Methods of MIPs synthesis

By using various methods depending on its target applications, MIPs could be prepared in a diversity of physical and chemical forms. Classic MIPs synthesized as monoliths using bulk polymerization of monomer mixtures consisting of template molecules as well as porogen solvent. Obtained monolith made of polymer then passes the process of grounding and sieving to generate tiny particles with reachable molecularly imprinted cavities. In fact, the approach described above was the only approach for preparing of MIP. Obviously this method is rough approach and inappropriate for the commercially production of MIPs.

2.7.1. Molecular Recognition of Proteins

The development of new techniques and methods for the separation and purification of proteins has been essential for many of the recent advancements in biotechnology and biomedicine [32].

The design of stable molecular recognition units for proteins is a difficult task, due to the complexity of the proteins. Affinity separations of proteins could be achieved using enzymes, antibodies or receptors which are able to recognize protein epitopes. Since natural bio macromolecular receptors are not stable and require high costs, the recognition and selection of proteins with high selectivity and affinity is a very big challenge. Hence production of artificial recognition elements, which simulates the natural counterparts is a great demand which requires low cost, selective, reusable, effective alternatives.

Separation of proteins using non biological affinity approaches is based on the existence of special functional groups. So protein surface imidazoles, thiol groups, hydrophobic side-chain amino acids or diols in glycoproteins could be targeted using appropriate ligands immobilized on solid supports. The difficulties involved with using the technique for the template-imprinting of proteins are their large molecular sizes, the fragility and complexity of the molecules [33].

Proteins have high molecular weight and size which results poor mass transport abilities when binding through MIP monolithic columns, which consist of quite dense polymeric units [69]. Usually proteins have a lot of functional groups over its huge surface area. Complex structure of protein surface should be considered when choosing needed functional monomers for protein imprinting in order to avoid inappropriate weak interactions that cause nonspecific binding. Polymerization conditions of imprinting lead to denaturation of proteins or may alter the conformational states of proteins [34]. In addition, proteins are water soluble units, which is not matching with the traditional MIP processes because most of molecular imprinting procedures realizes in organic solution.

2.7.2. Surface Imprinting

Surface imprinting is a widely used approach to improve the performance of MIPs by solving the problems of mass transfer limitations and removal of template molecules generally related with traditional molecular imprinting technique. This method is especially significant for imprinting of macromolecules like proteins since mass transfer limitations for these large molecules is an important factor. The creation of thin films onto solid supports is one of the common approaches of surface imprinting method. This strategy enhances rebinding kinetics significantly because of the easy transportation of molecules through the binding sites on the or near the surface of the material. Other important advantage of this method is simple removal of target molecules leaving large amount of recognition sites onto the solid support. These features are especially significant for macromolecules like proteins. The purification of proteins using this type of materials which have thin films created onto the solid support is feasible regarding to important features explained above [35]. Surface imprinting gives free access for bio macromolecules like proteins to binding cavities by utilizing specific surface binding areas of proteins [36]. One of the spread and common approaches to synthesize surface imprinted polymers consist of two steps: first, the template molecule is captured and immobilized into the solid support and secondly, the support is disintegrated after polymerization with cross-linkers and monomers. Surface imprinting approach was exploited to synthesize various types of materials like silica beads [37,38] and nanowires [39], (Figure 2.9).

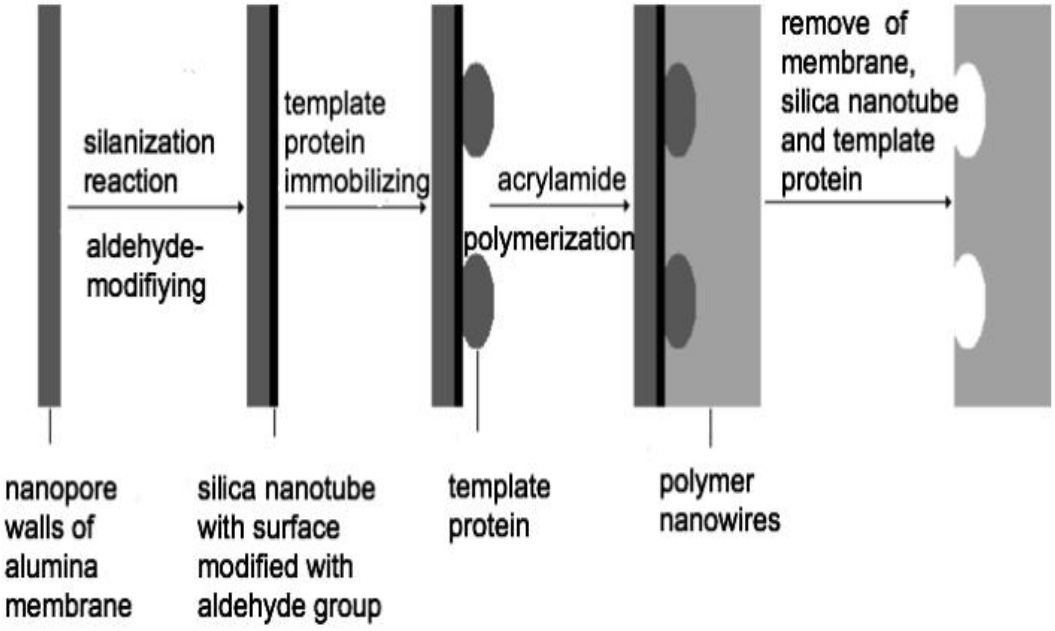

Figure 2.9. Schematic representation of nanowires synthesized by surface imprinting approach [40].

2.7.3. Core-shell imprinting

Core–shell imprinting is the novel techniques exploited to design homogeneously apportioned binding cavities that are very selective and accessible to target molecules (Figure 2.10). The main advantage of this approach is that particles imprinted by core–shell method with binding cavities on surface layer are utilized to quick selection and purification of target proteins. In contrast with classic imprinting methods, core–shell surface imprinting silica particles possess plenty of excellences such as: easily perceptible synthesis procedure, binding cavities that can quickly and easily get to the imprinting sites during adsorption [41].

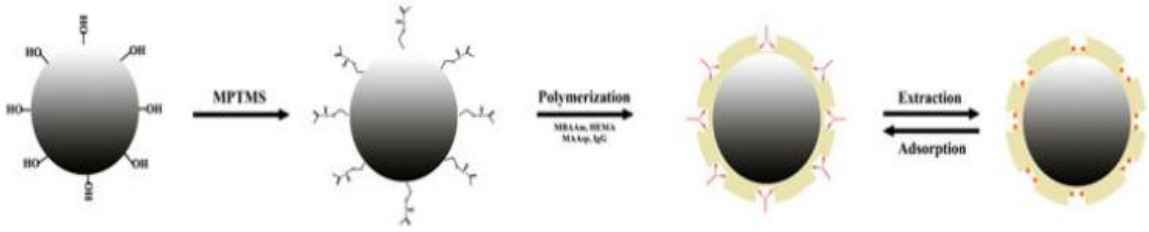

Figure 2.10. Steps of of Con A polymerization process by core–shell imprinted silica particles [38].

Such type of core-shell microspheres could be synthesized using treated amorphous silica particles which have surfaces with polymerizable units on it. Silica microspheres could be suspended in solvent which contains templates and monomers, after polymerization leading to designing on the surface of microspheres MIP coating. Nevertheless, controlling of polymerization process is pretty difficult. Contolling is important for prevention of undesired development of polymers or coagulation. However, using certain templates and monomers in the synthesis seems to be possible without considerable losses. But even though, this strategy emonstrating good outcomes, it does not seem to be suitable for different monomers and templates.

Specific recognition in the imprinted silica particles depends on the type of interaction created between the monomer and the template before polymerisation, which can be non-covalent, covalent or a combination of two. Covalent interactions are very strong and much more selective. But after the capturing target molecules it is almost impossible to remove them from the imprinted polymer. Non-covalent imprinting is more adaptable and easy way due to fast kinetics in the binding-rebinding of the molecules. Also non-covalent interactions allow to imprint wide range of template proteins. There are many examples of proteins that were successfully imprinted using hydrogen bonds, hydrophobic and electrostatic interactions.

Morphology of the polymer is an important point in the synthesis of the MIP. In most studies bulk imprinting were used due to its simple synthesis. But bulk

polymerization has numerous disadvantages such as irregularity of the produced particles, absence of control of the process and waste of polymers. So new methos was developed to control the morphology of the polymers offering new possible applications of MIP in other fields. The new method is particle imprinted polymers. The main advantages of the particle imprinting are avoiding of grinding and huge surface areas where more ligands can be placed.

Core-shell methodology offers an effective way to control and predict the final number and size and hence surface area of imprinted silica particles, as well as the possibility to apply specific properties such as magnetism or fluorescence into the cores. The binding sites must be limited to the thin particle shells where they will have good exchange kinetics and accessibility for ligand binding [42].

2.7.4. Protein recognition via silica-based materials

The first effort of protein imprinting reported in literature used for this purpose silica materials. These results clearly showed that silane imprints can distinguish varieties of spatial composition of proteins. After that, protein imprinting on the surfaces of polysiloxanes and silicates were studied by lot of research institutions next years. In particular, Venton and Gudipati, proved that organo-functional side chains demonstrated an ability to affinity bind with units located on the surface of proteins. The polymer consisting of tetraethylorthosilicate and 3-aminopropyl-triethoxysilane in the existence of certain protein was able to memorize it and then bind specifically to the protein, previously used for imprinting.

2.7.5. Protein recognition via Affinity Nanofiber Membranes

Affinity nanofiber chromatography is a promising method for protein purification having both high efficiency of nanofibers and selectivity of chromatographic materials [43]. Purification of protein is performed usually by packed bed chromatography. But there are some drawbacks of this technique such as long operation time, high-pressure loss and hard scaling up because mass transfer of proteins is performed with diffusion through 18 beads micro channels, which is used as a packing material in the packed bed. Moreover, channeling caused by

non-homogenous distribution of beads through the column may appear. That, in its turn, causes the decrement of column efficiency.

To avoid these technical limitations of the packed beds porous membranes were used as powerful alternatives. The most important excellence of the membrane chromatography is that proteins convection through the binding sites. Pressure loss of membranes is considerably lower comparing with packed beds. The binding usually does not depend on of feed flow rate and so it has an ability to work with high flow rates. One more advantage of this method is the ability to scale up. The interaction between the protein and the material is carried out via the interconnected pores of the membranes (Figure 2.11). Since molecules are transferred through diffusion via dead-ended pores of beads the convection is dominant mass transfer process. The diffusional resistance is overcomed when membranes are used and hence only mass transfer resistance play role as the film diffusion on the membrane surface. Mass transfer restrictions are considerably lowered since film diffusion is much quicker method than pore diffusion. Even though the membranes are much thinner than beads, pressure losses are decreased by using interconnected pores of the membranes and hence efficiency and working flow rates will be enhanced.

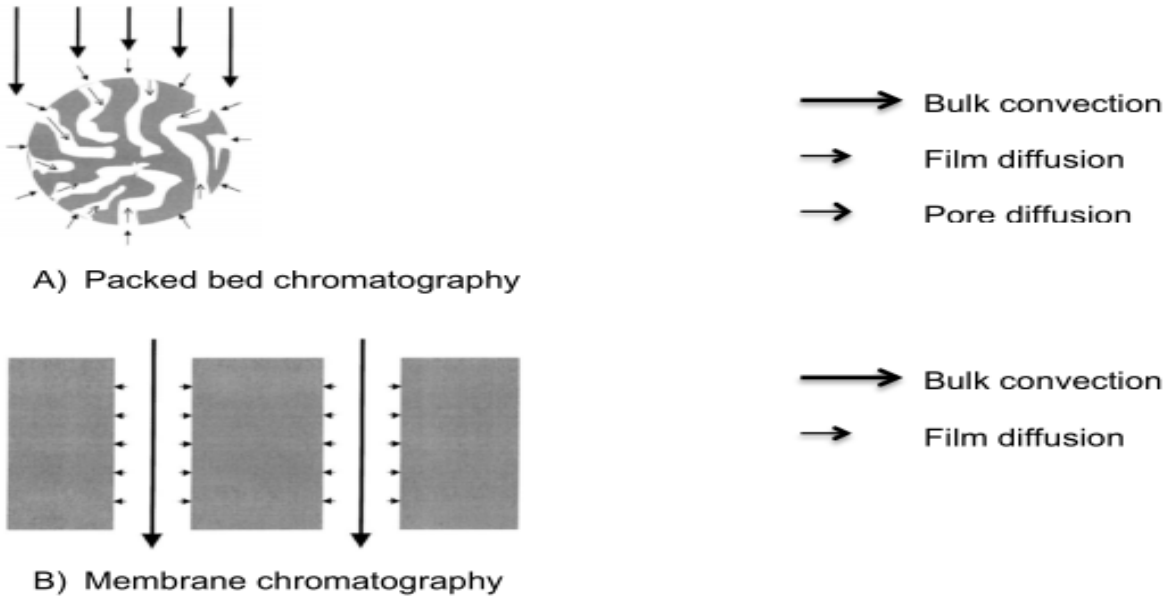

Figure 2.11. Mass transfer mechanisms performed via bead (A) and membrane (B) [44].

2.7.6. Porous silica as a core support

Beaded form of rigid, porous and inorganic supports can be exploited as a scaffold for the synthesis of MIPs. Beaded silica with a narrow size distribution and small bead size (5–10 mm) is broadly commercially available because of its frequent application as a solid phase in affinity chromatography and HPLC. Moreover, MIPs synthesis by such silica beads is quite easy and quick: enough to imbibe silica matrix with mixture which contains initiators, templates as well as appropriate solvent. It is important to control that solvent's point should be under the point of "incipient wetness" of support. After discovering the easiness of the method, the early applications have done in 1985s. Nevertheless this good start have not been followed by next studies, perhaps because of some disadvantages that were soon determined during further experiments with the complex supports. Their relatively low effectiveness and poor recognition were main reasons. Fortunately in the beginning of the new century a few novel researches revitalized the studies in this area (Figure 2.12).

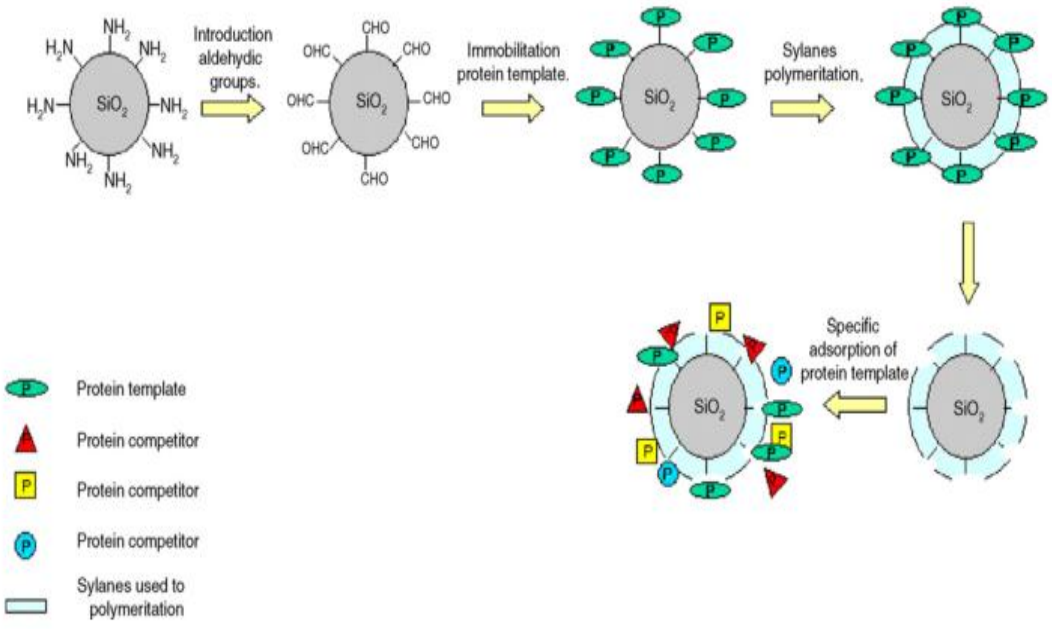

Figure 2.12. Schematic representation of the protein imprinting on silica beads using immobilized template [45].

2.8. Application of MIPs

MIPs have the unique characteristics. This is the main reason why they are an irreplaceable object in various researches such as purification and separation sciences, biosensors, drug delivery etc. Chromatography studies on the base of MIPs is the main usage area of MIPs. Because it is extremely favorable and best material for the synthesis of unique, special solid phases with target affinity. There is a high need in optically uncontaminated biomaterials where capability of MIPs for chiral separations is other major application of it. Particularly MIP is useful to separate drugs racemic resolution [46, 47]. Moreover MIPs is a novel affinity sorbent and is used for solid phase extraction approaches for cleaning of samples and selecting of target molecules from different solutions.

MIPs have been proved to be used instead of natural antibodies due to synthesized binding imitations on its surface. Hence MIPs could serve as affinity units in immunoassay studies. For now MIPs and many kinds of transducers were interacted together. Some approaches seems to reach a successful assembling of transduction platform with polymers [48]. Successful studies were performed on application of MIPs as catalyzators of certain chemical reactions. Special catalytic abilities of MIPs are applicable for enzyme-like catalysis [49, 50]. MIPs have a great challenge to be used in medical therapy and therapeutics. The ability of MIPs to interact with some molecules under certain circumstances gives MIT a great possibility for designing drug delivery systems.

2.8.1. Separation Approaches via MIPs

Chromatography based on molecularly imprinted matrixes is the classic usage area of MIPs. Particularly, liquid chromatography is generally prepared via bulk imprinting whichis prepared by mechanically grinding, sieving and finally putting into column as a solid phase [51]. Nevertheless, the mechanical treatment causes irregular particles with rather broad size distribution. As a result, bulks have irreproducible quality. That is why MIP monolithic columns recently was synthesized inside of the chromatographic columns [52]. Such MIP monolithic columns possess less inappropriate sites comparing to bulk MIPs coumns. A lot of

attemps to lower heterogeneous size distribution were performed via designing monodispersed and spherical beads as HPLC stationary phases.

2.8.2. Biosensors by MIPs

MIP technology could be utilized as artificial antibody units due to their chemical inertness and insolubility [53]. The integration sensors with MIPs can be possible via in situ polymerization employing a thermal and photochemical initiators [54] as well as via surface grafting using UV or chemical initiation [55,56]. Benefit of the novel technique is that it makes possible to control modification of inert electrode surfaces with thin layers containing special polymers.

The first MIP sensors was designed by monolith synthesized imprinted polymers. The aquired MIPs then have been deposited in close vicinity to the electrode by association of particles indise of a supporting agarose gel or inside the carbon paste of screen-printed electrode. It tuned out that signals from sensors completely depend on closeness of transduction element and the MIP on effectiveness of the electrical communication between them.

3. EXPERIMENTAL PROCEDURES

3.1. Materials

During the experiments, Lectin from Canavalia Ensiformis (Con A), 3-methacryloyloxypropyl trimethoxysilane (MPTMS), $NaHSO_3$ and $(NH_4)_2S_2O_8$, ethanol, N,N′-methylenebisacrylamide (MBAAm), 2- hydroxyethylmethacrylate (HEMA), tetraethylorthosilicate (TEOS), sodium bisulfite ($NaHSO_3$), ammonium persulfate, hemoglobin, lysozyme (L-6876, 14.3 kDa), potassium dihydrogen phosphate (KH_2PO_4), potassium hydrogen phosphate (K_2HPO_4) and L-histidine and methacryloyl chloride were obtained from Sigma. Acrylamide, bisacrylamide, N,N,N',N'-tetramethylenediamine (TEMED), ammonium persulphate (APS), sodium dodecyl sulphate (SDS) and tris were also purchased from Sigma Aldrich, USA.

3.2. Solutions

1.0 M Sodium chloride solution was used as a removal agent (NaCl). 500 mL of solution was prepared by adding 29.22 g of NaCl into distilled water. Different phosphate buffers were prepared according to Table 3.1.

Table 3.1. Preparation of buffer solutions

pH	1M (NaOH)	1M Acetic Acid	Volume
4	3.7 mL	25 ml	250 mL
5	15.87 mL	25 ml	250 mL
	K_2HPO_4	KH_2PO_4	
6	0.308 g	1.150 g	250 mL
7	0.6018 g	0.294 g	250 mL
7.4	0.6697 g	0.134 g	250 mL
8	0.7131 g	0.0381 g	250 mL

Solutions for SDS-page: 30% acrylamide/bis-acrylamide stock, 10% SDS solution, 10% APS solution, 1.5 M tris solution, pH 8.8.

3.3. Equipments

- Fourier Transform Infrared Spectroscopy
- Scanning Electron Microscopy
- Zeta Size Analysis
- Lyophilization unit
- UV–visible spectrophotometer
- All water exploited in the experiments was purified using a Barnstead (Dubuque, IA)
- Centrifuge, (Eppendorf Minispin)

3.4. Synthesis and chemical modification of silica particles

The silica particles were synthesized by Stöber method, according to the literature [57]. Briefly, 1.57 mL of concentrated ammonia solution was added into aqueous ethanol solution (EtOH 37 mL, H_2O 5.0 mL) and the resulting solution was stirred vigorously for 30 min. 3.0 mL of TEOS was added into the solution and was stirred for additional 6h. 2.0 mL of MPTMS was added into 50 mL of EtOH solution containing 0.5 g of silica microspheres and the resulting mixture was stirred for 24 h. The obtained MPTMS-modified silica microspheres (vinylated silica) were washed with EtOH three cycles of centrifugation and re-suspension in water.

3.4.1. Synthesis of Con A-imprinted core–shell silica paticles

13 mg of N-methacryloyl-L-histidine methyl ester, 26.0 µL of HEMA, 116.0 mg of MBAAm and 27.0 mL water were mixed and well shaked. 13.0 mg of Con A was added to the first solution. After that suspension of 0.6 g of silica treated by MPTMS was added, dispersed in 5mL of deionized water. The obtained solution was then moved to polymerization reactor. $NaHSO_3$ (57.0 mg) and $(NH_4)_2S_2O_8$

(63.0 mg) were added as initiators. Process of polymerization was performed at 42°C on magnetic stirrer at 550 rpm for 24 hours. Imprinted silica particles were washed with deionized water and EtOH. Sice the reaction is done, Con A-imprinted core–shell particles were extensively treated with 1.0 M NaCl solvent to wash away the template molecule Con A, until no Con A is seen in the supernatant with UV–vis spectrophotometer at 280 nm. After that, silica particles were rewashed with deionized water to wash away NaCl remains. Con A molecules were removed from particles with efficiency around 92%, according to the measurements by UV-vis spectrophotometer. To compare the efficiency of MIP, the non-imprinted particles were synthesized by similar method but there were no Con A in the polymerization process. Then the imprinted particles were placed in lyophilization equipment. Process was performed at 0.0011 mbar, at −55°C for 24 hours.

3.4.2. Characterization of Con A-imprinted Core–Shell Silica Particles

External characterization of Con A-imprinted core–shell particles were tested via SEM. Firstly, the Con A-imprinted core–shell particles were dried at room temperature. Small sample from the core–shell particles was analyzed by SEM. The FTIR spectra was conducted for dried silica, Con A-imprinted and non-imprinted silica particles using FTIR spectroscopy. Zeta Sizer equipment helped to calculate the silica size and its distribution. Concentration of the protein in the solution was calculated via UV–visible spectrophotometer equipment.

3.4.3. Adsorption studies

Adsorption study was conducted with 5 mg of Con A-imprinted and non-imprinted silica particles treated with 1.6 mL of Con A solutions which owe various pH values and then set up accurately at 250 rpm for 2 hours. After that, silica particles were centrifuged at 7500 rpm for 15 min. Concentration of Con A in obtained supernatant was tested by UV–vis spectrophotometer at 280 nm. The effects of pH, Con A concentration and temperature on adsorption of Con A on silica particles were studied. Furthermore, the adsorption kinetics was tested by altering

adsorption time for 2 hours at the initial concentration of Con A at 0.1 mg/mL. Selectivity of the Con A-imprinted particles was determined with lysozyme and hemoglobin molecules instead of Con A taking 1.0 mg/mL of each molecules under the same experimental conditions. Non-imprinted particles were studied in order to check Con A-imprinted particles specificity under similar conditions.

3.4.4. Preparation of Jack Bean Extract

The seeds of Jack Bean were treated by warm water overnight to make them soft. Then 10 g of the softened and crushed seeds were homogenized at 4°C with 100 mL of 0.1 M, pH 7 phosphate buffer overnight. Then, the obtained extraction solution was centrifuged at 700 rpm for 20 min. Supernatant which contains the molecules of Con A proteins was obtained and then used for SDS-PAGE electrophoresis and adsorption studies.

3.4.5. Estimation of Protein Concentration by Bradford method

50 mg of Coomassie Brilliant Blue was dissolved in 50 mL of methanol and 100 mL of 85% phosphoric acid (H_3PO_4) was added. Acidic solution mixture was gradually added into 500 mL of water and waited until the dye dissolved completely. Before use, the solution was filtred via a Whatman #1 paper to remove the precipitates. 2 mL of Bradford solution wad added to 200 µL of extracted Jack Bean supernatant and adsorbance measured by UV-vis spectrophotometer at 595 nm.

3.4.6. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The purity of the Con A was carried out via a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Studies were conducted as described below: to 20 µL amount of every solution added 20 µL of reducing sample buffer mixed. Then samples were loaded into 5–10% trisglycine gel. The

method was conducted via SE 600 Ruby electrophoresis system. After running, SDS-PAGE gel was stained with Coomasie brilliant blue dye.

4. RESULTS AND DISCUSSION

4.1. Preparation and characterization of Con A-imprinted core–shell silica particles

4.1.1. Fourier-Transform Infrared Spectroscopy (FTIR)

MIPs are difficult to characterize due to their insoluble nature. Nevertheless, there are some analytical techniques that can help to learn chemical and morphological characterization of MIPs such as NMR and Fourier Transform Infrared Spectroscopy (FTIR) that hepls to take chemical and quantitative datas upon polymer structure. This method is very useful when various chemical matrixes in the analytes shows good resolved diagnostic signals. FTIR is employed to test non covalent bondings like hydrogen bonds. Usually FT-IR studies are used to characterize MIPs via the examining of functional groups interacted in the polymer and via the comparison between functional monomer and polymer spectra to see the disappearance or decreasing of certain signals like carbon double bond stretching from vinylated functional monomers.

The band at the 1040-1150 cm^{-1} range is assigned to the stretching vibration band of Si-O, which corresponds to the band at 1055 cm^{-1} at Figure 4.1. Also the band at 803 cm^{-1} corresponds to the Si-O-Si vibration and band at 474 cm^{-1} corresponds to the Si-O-Si bending vibrations. After addition of imprinted and non-imprinted polymeric layer, characteristic C=O band appears at 1700 cm^{-1} approves introduction of polymeric layers. Asymmetric methyl bending bands can be also seen at around 1500 cm^{-1}.

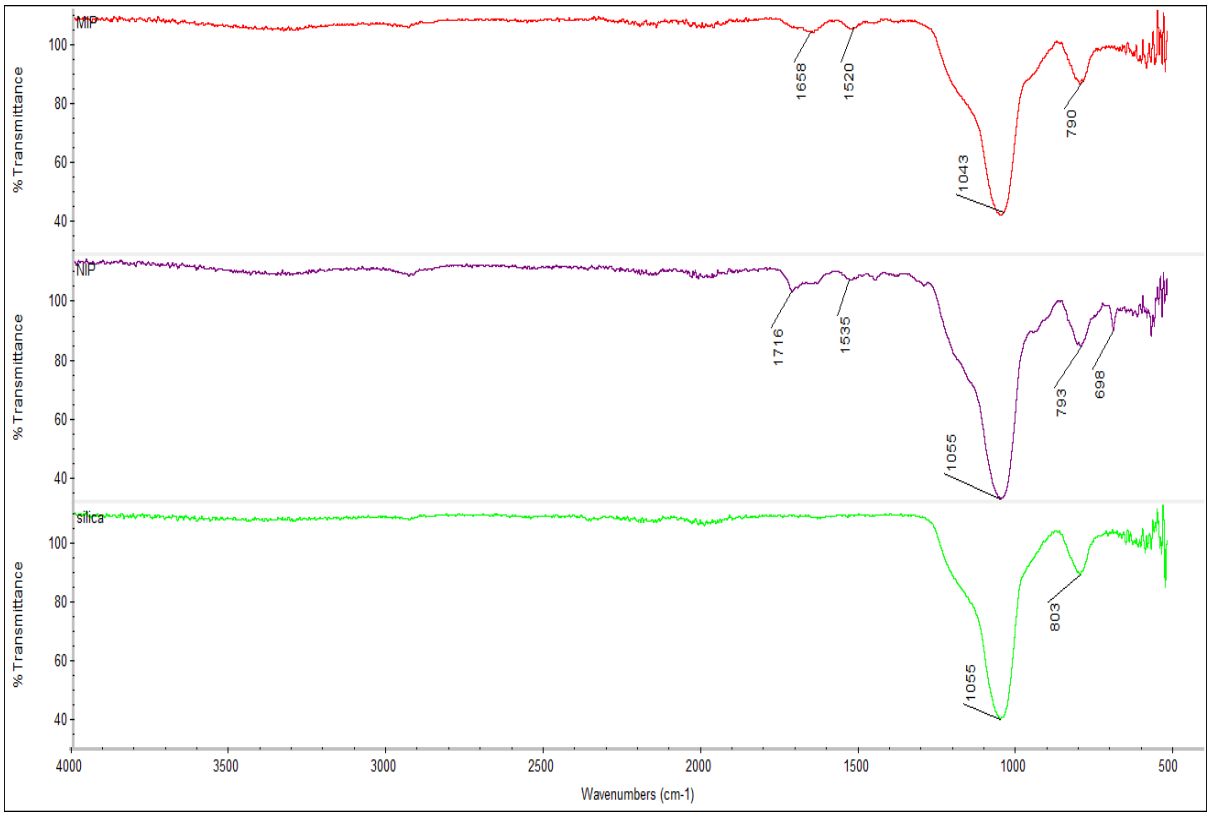

Figure 4.1. FTIR spectra of Con A-imprinted silica particles (MIP), non-imprinted silica particles (NIP) and untreated silica particles.

4.1.2. Morphological characteristics of Con A-imprinted silica particles

Properties of MIPs such as morphology and physical state could be perfectly studied by microscopy approaches like scanning electron microscopy (SEM) to take good images of polymer macropores. Surface morphology and distribution of particle size of silica particles were determined via SEM. According to SEM images we can see that the Con A-imprinted particles have smooth surface and similar size. The adsorbents having high surface area are known to have a high binding capacity with rapid binding kinetics. In this context, the use of silica particles to increase the surface area of particles in the separation of biomolecules is preferred due to the absence of diffusion resistance [58]. (Figure 4.2.).

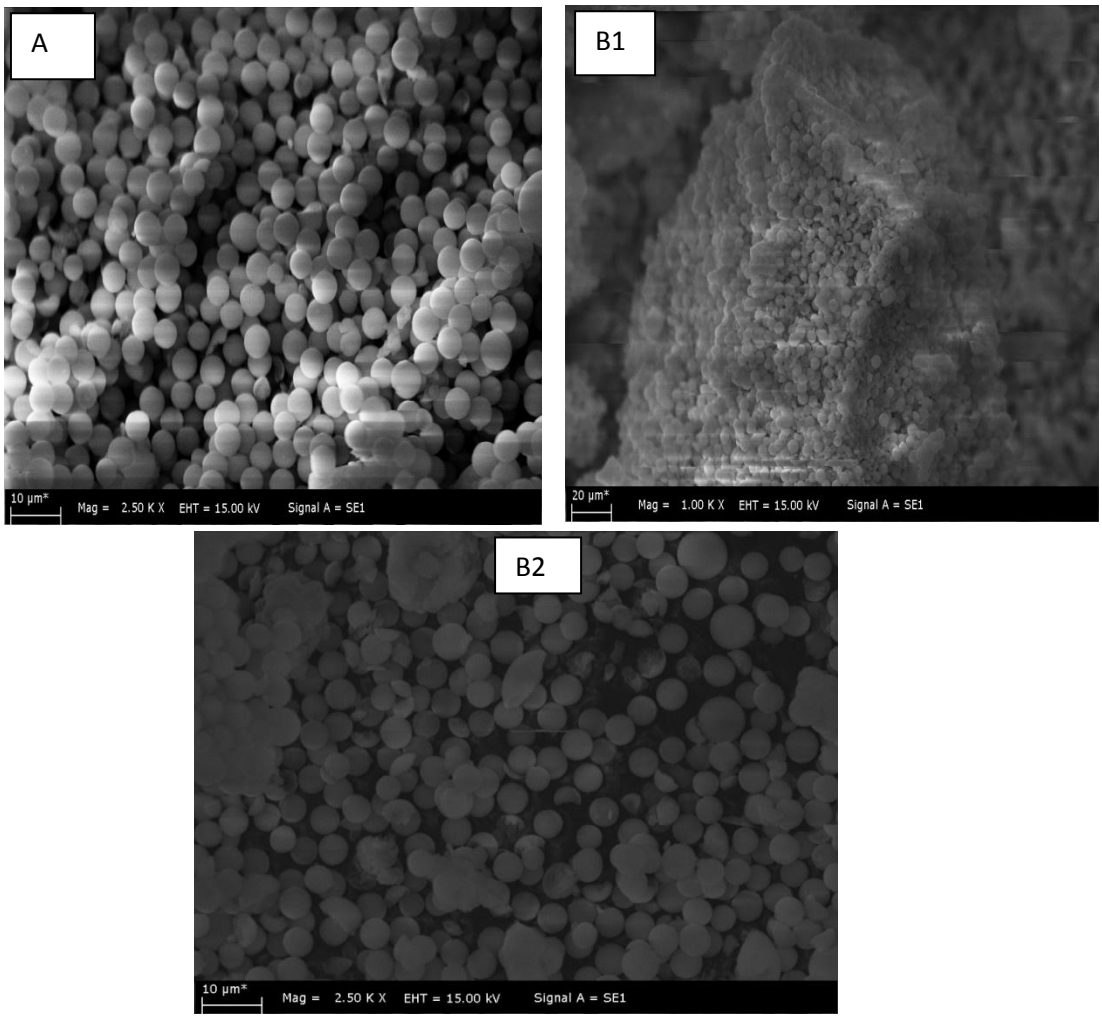

Figure 4.2. SEM images demonstrating the surface morphology and size distribution of silica microspheres. Untreated silica particles (A) and Con A-imprinted silica particles (B1, B2).

4.1.3. Zeta sizer measurements results

According to information obtained from Zeta sizer measurements particle size and its dispersion of the Con A-imprinted particles and silica particles were about 5.2 and 4.3 µm respectively. There is 1 µm difference between non imprinted and imprinted polymers which is come from the polymerization process. When silica particles are imprinted they obtain extra polymer coat on its surface. As a result,

imprinted silica particles bigger than non imprinted ones. Also polydispersity index was 0.309 and 0.092 respectively (Figure 4.3. and Figure 4.4).

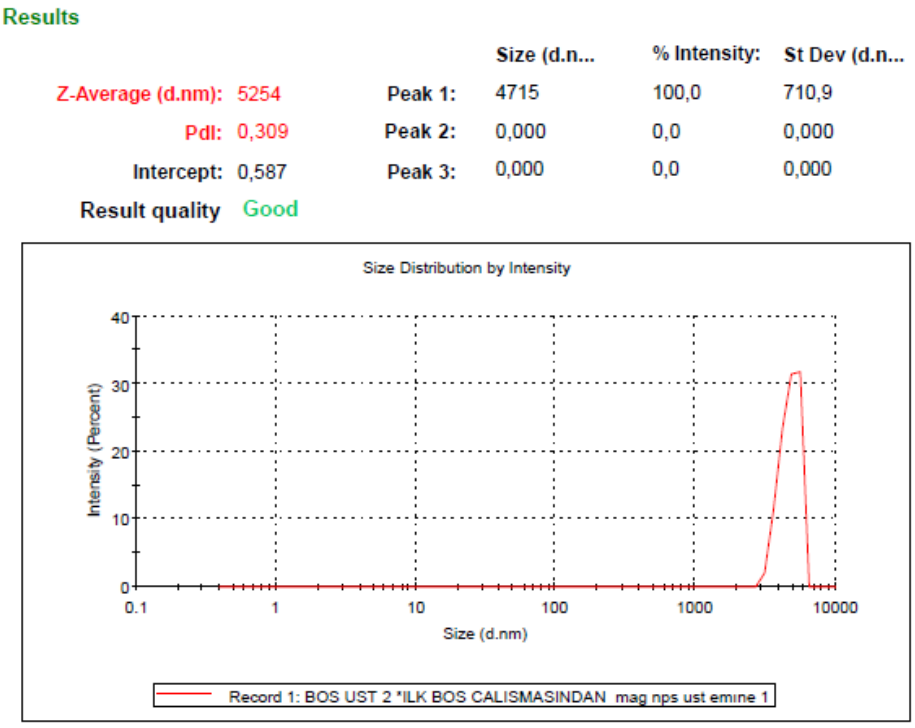

Figure 4.3. Size distribution of Con A-imprinted silica particles

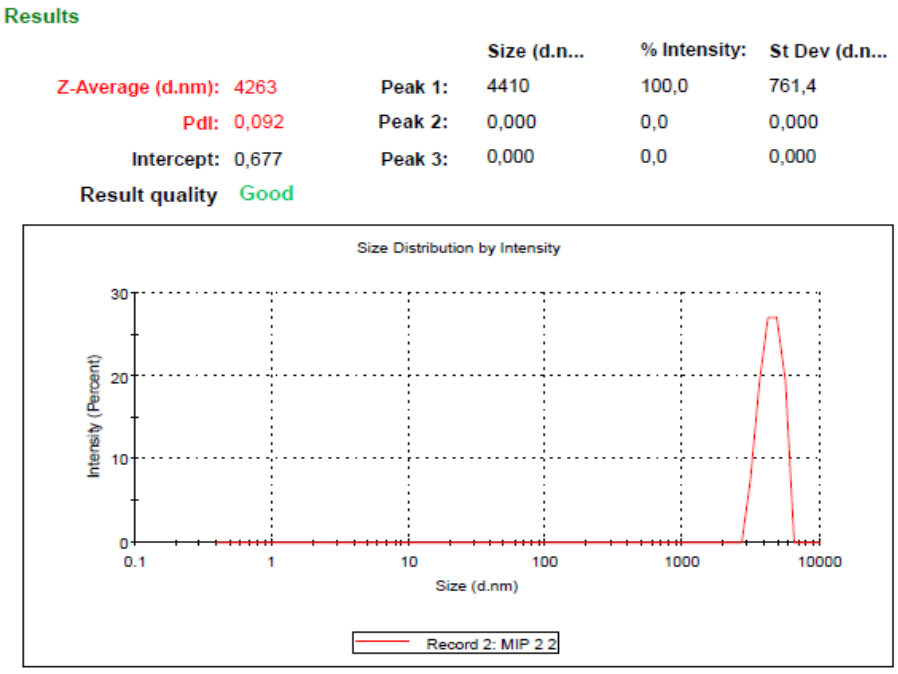

Figure 4.4. Size distribution of silica particles

4.2. Adsorption conditions of Con-A-imprinted silica particles

4.2.1. Effect of pH

Protein adsorption onto specific adsorbents is pH dependent. Con A adsorption studies were performed in phosphate buffer at different pH values. Proteins are affected by conditions of their environment and pH is one of the most important influence on protein folding. During the molecular imprinting process template molecule is imprinted forming microcavity corresponding to the structure how the template is in that polymerization medium. It is mentioned in many molecular imprinting studies that pH of solution used for adsorption tests is generally same with the pH of polymerization medium of molecular imprinting procedure utilized to obtain MIP [59, 60, 61]. In our case, imprinting of Con A was prepared at pH 6 phosphate buffer. In the figure below it is seen that maximum adsorption capacity was obtained at pH 6 phosphate buffer via the conformational memory fitting to recognition microcavity through the shape of the template molecule. It must be noted that the isoelectric point of Con A is between 4.5-5.5. When pH value is less than 5.6 Con A exists as a dimer. Between 5.6-7 pH values Con A is tetrameric and when pH is more than 7 it exists in aggregate form. The significant decreased in adsorption capacity below and above pH 6.0 confirmed that the charge density and geometry of Con A molecules was well-fitted to interact with functional groups of silica surface, i.e. imidazole ring, therefore the maximum adsorption was reached at pH 6.0 [62]. As Figure 4.5 shows, the maximum adsorption quantity was detected at pH 6.0 as 20.2 mg/g for Con A-imprinted particles.

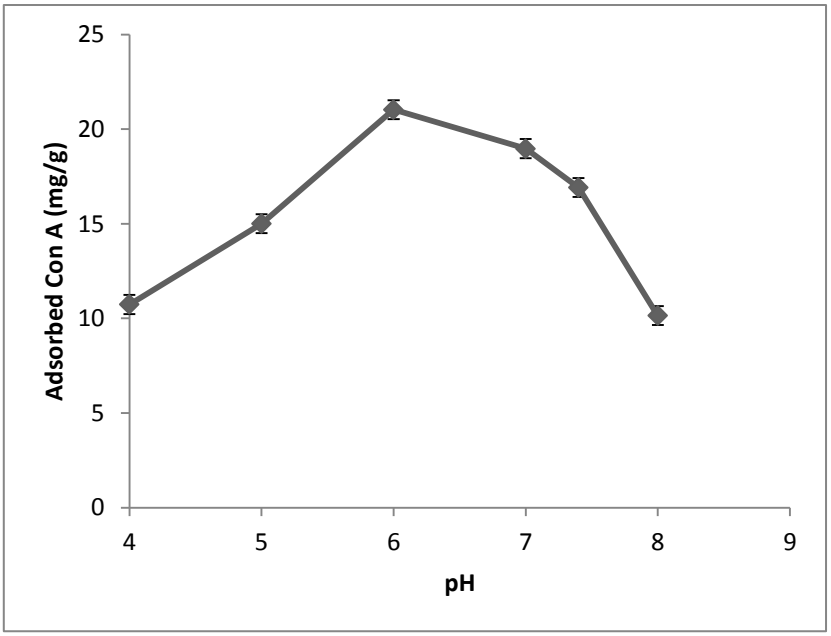

Figure 4.5. The influence of pH on the adsorption of Con A. Adsorption time: 2h; Con A concentration: 0.1 mg/mL; T: 25°C.

4.2.2. Effect of temperature

The conformational state of proteins strongly depends on temperature. The effect of temperature on adsorption process was also studied and adsorption experiments of Con A conducted at 4, 25, 35 and 40°C (Figure 4.6). The maximum adsorption capacity was seen at 4°C. The ralationship between temperature and Con A adsorption offers important information for understanding the common interactions of adsorption nature. The underlying interaction mechanism between histidine methyl ester and Con A is thought to involve hydrophobic, electrostatic and non-specific interactions of water mediated hydrogen bonds [63].

According to the results of temperature tests it is found that the adsorption ability of the MIP particles was reduced while temperature is rising. Due to functional monomer, interactions between Con A-imprinted particles and Con A molecules were electrostatic. High temperature broke down that non covalent interactions, hence MIP and Con A could not bind. At 40°C temperature adsorption of Con A reached the lowest point (15 mg/g).

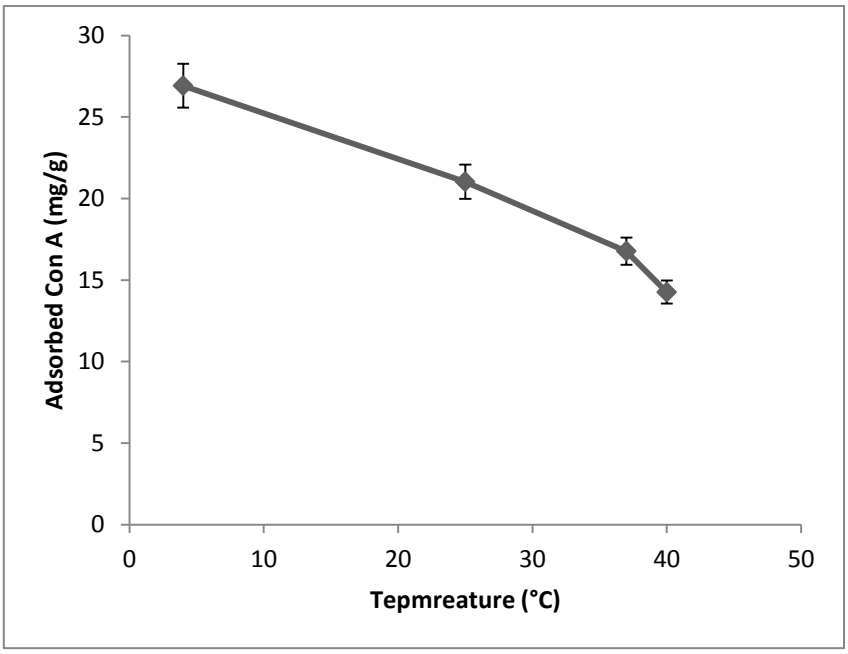

Figure 4.6. Effect of temperature on the adsorption of Con A. Adsorption time: 2h; pH 6.0; Con A concentration: 0.1 mg/mL.

4.2.3. Effect of Con A concentration

The Figure 4.7. demonstrates that the bound Con A value is increased at the same time with increasing of Con A concentration and reached the plateau value after 1.0 mg/mL Con A concentration. It means that after 1.0 mg\mL concentration there are no available binding sites left. Hence the linear graph is seen after that concentration. A driving force for adsorption, which is the concentration difference (ΔC), rises the same rate as concentration is rising. A rising is detected in adsorbance ability at the same time with the increase in motive force [64]. These outcomes suggest that the adsorption capacity of silica particles is also correlated with the amount of MAH ligand on each silica surface [65].

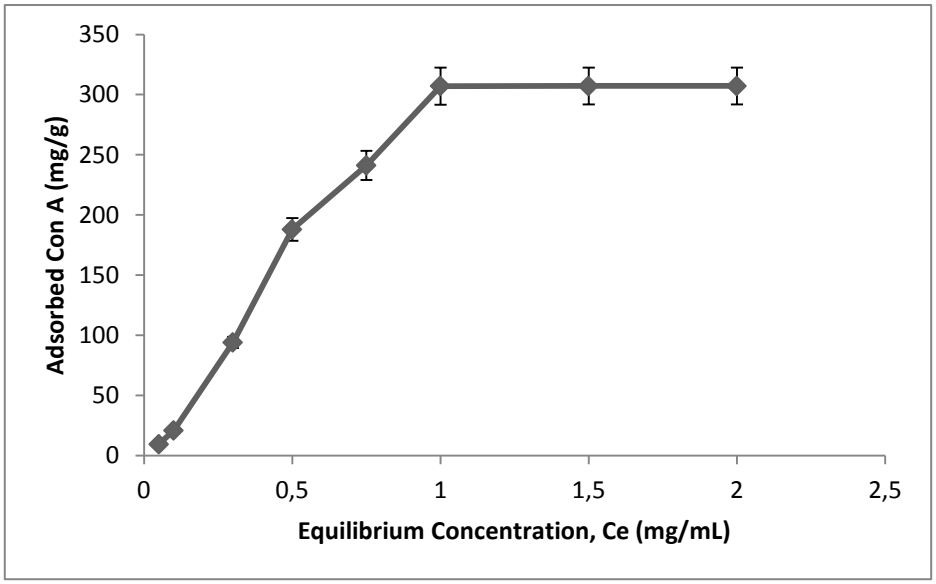

Figure 4.7. Effect of equilibrium concentration to the adsorption of Con A. Adsorbance time: 2h; pH 6.0; T 25°C.

4.3. Adsorption Isotherms

The kinetics and the adsorption equilibrium are the two major approaches for calculating of adsorption mechanism. An adsorption isotherm characterizes the interactions of molecules in the solvent with the adsorbents. In other words, adsorption isotherm is a ratio of concentration of the molecules in a solvent and the number of Con A bound to the MIP surface when the two phases are at equilibrium. The isotherm model by Langmuir supposes that the molecules are bound at a certain amount of synthesized sites, each one is able to bind just one molecule. Synthesized sites are also supposed to be energetically similar and distant from each other to avoid interactions between molecules adsorbed on neighboring sites.

The Freundlich isotherm is the other common isotherm, which is usually exploited to describe adsorption behavior. Freundlich isotherm calculation method is another type of the Langmuir method for adsorption on the heterogeneous surface. Total summing of adsorption on all existing binding sites is the amount of adsorbed protein. The Freundlich isotherm calculates reversible adsorption.

Equilibrium modelling information was carried out utilizing Langmuir and Freundlich isotherms. The isotherm method by Langmuir is broadly used to characterize the adsorption of solutes from aqueous solutions. The Freundlich isotherm is an empirical ratio between the concentrations of a solute of an adsorbent to the concentration of a solute in the liquid with which it is in contact. The Langmuir (4.1) and Freundlich (4.2) isotherms are described by equations below:

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \left[\frac{1}{q_{max}b}\right]\left(\frac{1}{C_e}\right) \qquad (4.1)$$

$$ln\, q_e = \frac{1}{n}(ln\, C_e) + ln\, K_F \qquad (4.2)$$

K_F is the Freudlich constant, *b* is the Langmuir isotherm constant (mL/mg), and *n* is the Freudlich exponent, q_e is experimental adsorbance ability (mg/g), q_{max} is theoretical adsorption capacity (mg/g) and C_e is equilibrium analyte concentration (mg/mL). The Figure 4.8. shows that, Langmuir isotherms for Con A-imprinted core–shell silica particles (R^2 = 0.969) were extremely suited to process. Freundlich isotherms (Figure 4.9) had correlation coefficient R^2 = 0.8974. According to Langmuir model, theoretical maximum adsorption abilities (q_{max}) of MIP was 307.2 and experimental results (305.2 mg/g) supports the theoretical data. The results also showed good ratio between the adsorption process in the experiment and Langmuir model. In accordance with the correlation coefficients of isotherms Langmuir isotherm model is more suitable.

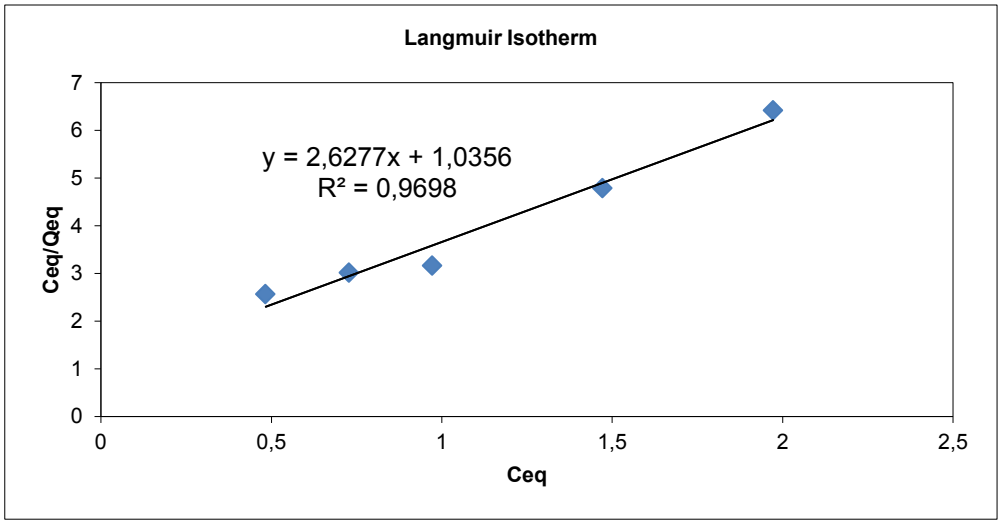

Figure 4.8. Langmuir adsorption correlation and constant coefficients for Con A-imprinted particles.

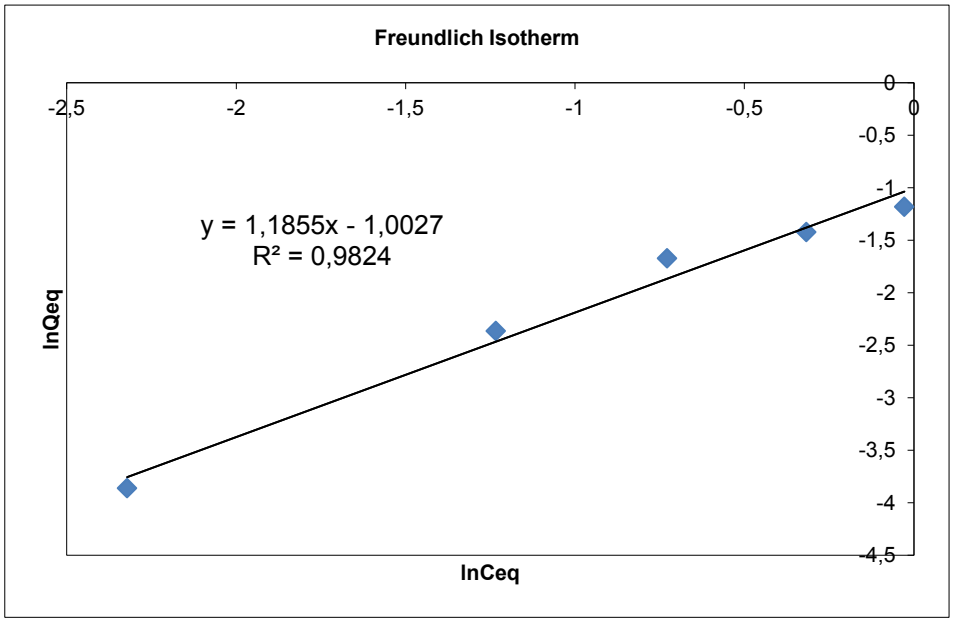

Figure 4.9. Freudlich adsorption correlation and constants coefficients for Con A-imprinted particles.

4.4. Binding Kinetics

The main advantage of MIPs is that they have fast binding kinetics and high recognition ability. The recognition of target molecules by MIP matrixes templating various molecules and prepared with various methods varies from 5 min to 10 h.

Figure 4.10. shows that the first recognition of Con A molecules by Con A-imprinted silica particles appears in 5 minute. That fast response was enabled because of highly specific recognition of Con A molecules through easily accessible binding sites [66]. However, the maximum adsorption of Con A from the solution happens in 120 minutes. Hence, adsorption of Con A conductes slowly but surely during two hours.

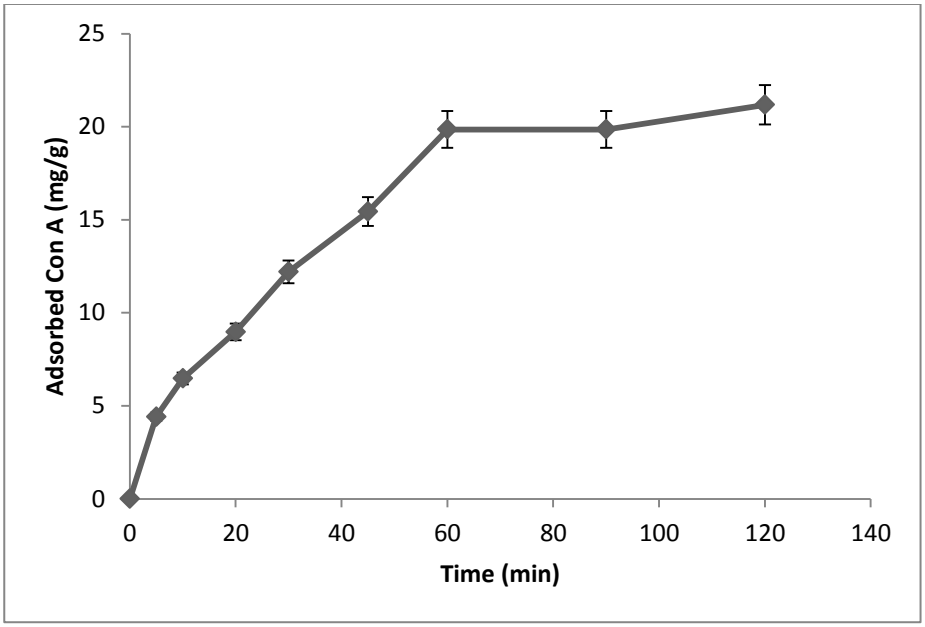

Figure 4.10. Binding kinetics of Con A-imprinted MIP silica particles. Experimental conditions; Con A : 0.1 mg/mL , T: 25°C, pH: 6.0.

As the Figure 4.10. shows, the adsorption is going gradually and transport of target molecules in the solvent phase are conducted by film diffusion, bulk diffusion, and intraparticle diffusion. In MIPs that means the adsorption of target molecules on the surface of adsorbent material. It is important to determine how target molecules interconnected to MIP matrixes. There are two types of interactions between molecules: physical and chemical. In order to learn the basal mechanism of Con A binding on MIP particles, two kinetic models used to check experimental data are as follows:

- Pseudo-first kinetic equation:

$$\frac{dq}{dt} = k_1(q_{eq} - q) \qquad (4.3)$$

where k_1 is the rate constant of first-order adsorption, q_{eq} and q are the amounts of adsorption capacity of silica particles at equilibrium and time t respectively;

- Pseudo-second kinetic equation:

$$\frac{dq}{dt} = k_2(q_{eq} - q)^2 \qquad (4.4)$$

where k_2 stands for the rate constant of second-order adsorption.

Pseuso-first order kinetic and pseudo-second order kinetic models were calculated and shown in Figure 4.11 and 4.12 respectively.

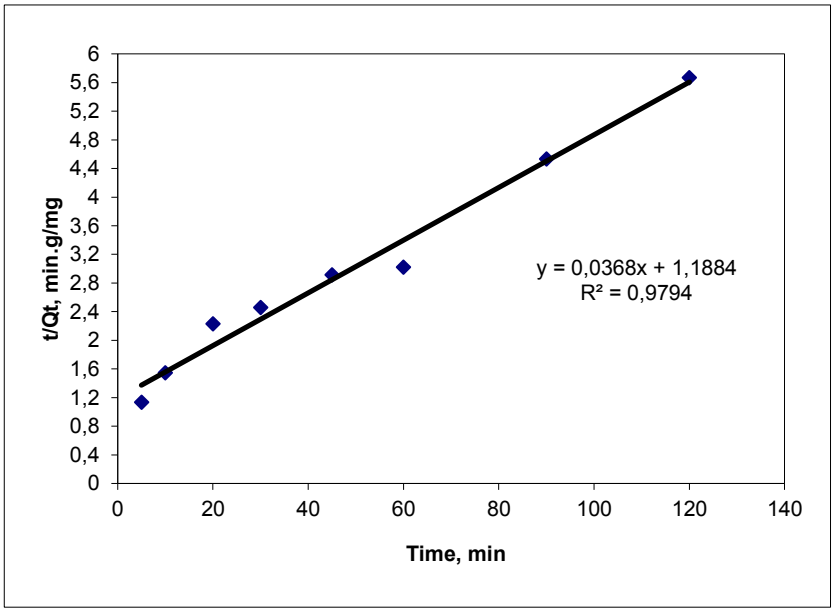

Figure 4.11. Pseudo-first kinetic equation results for the Con A-imprinted silica particles

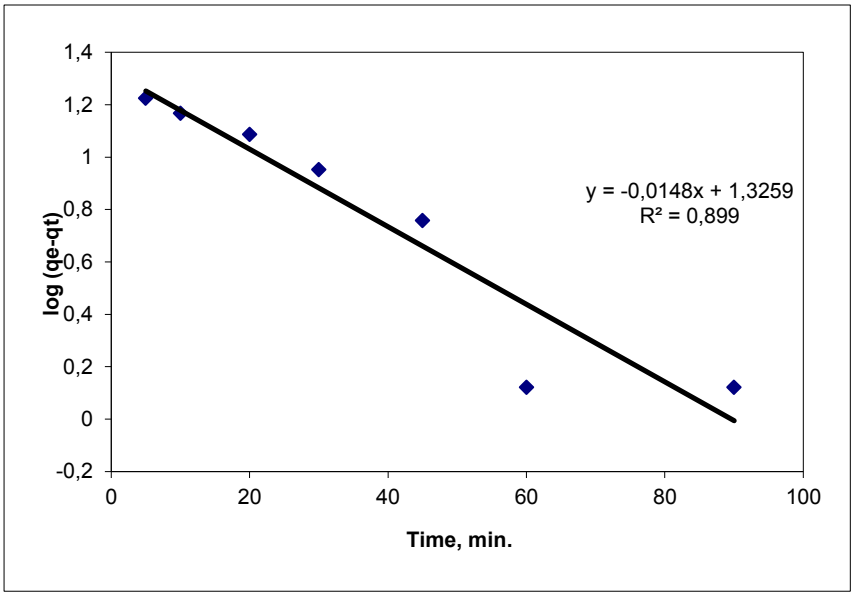

Figure 4.12. Pseudo-second kinetic equation results for the Con A-imprinted silica particles.

The correlation coefficient for the linear plot of $-\log(q_{eq}-q_t)$ vs. t of the pseudo-first kinetics equation is fewer comparing to the correlation coefficient of the pseudo-second kinetics equation. The obtained data demonstrates that this binding method is not characterized good via pseudo-first kinetic isotherm equation. According to these results, Con A-imprinted adsorbent system assumed that the pseudo-second kinetics adsorbance process is the most primary and the general rate of the Con A adsorbance is controlled by physical diffusion, but chemical processes have also demonstrated high values.

4.5. Selectivity Studies

Molecular recognition selectivity is the most important parameter in characterization of MIP microspheres because molecular recognition properties are essential for MIP applications in various areas [67].

The selectivity of MIP silica particles based on the level of interaction of protein surface and imprinted binding sites. Functional groups are spatially located and destined complementary to target protein surface chemistry. The selectivity of binding sites of Con A-imprinted particles were investigated by competitive adsorptions of Hemoglobin and Lysozyme. Selective recognition of MIP silica

particles were studied using template protein and competing proteins which vary in isoelectric point and molecular weight: Con A is 104 kDa, lysozyme is 14.4 kDa and hemoglobin is 64 kDa.

Figure 4.13. obviously demonstrates that MIP particles have highest affinity to Con A since other competing proteins did not fit to Con A-imprinted microcavities. All the competing non-template proteins did not interact with the imprinted microcavities because recognition mechanism is a dominating factor in selectivity.

To better understand the chemical and physical processes happening in adsorption time and determine characteristics of adsorbent outlines mathematical models are usually used to calculate the datas. The partition coefficients (k_d) for molecules showing equilibrium distribution of molecules among solid phase and aqueous phase are shown below:

$$k_d = \frac{\left[\frac{C_i - C_f}{C_f}\right] V}{m} \qquad (4.5)$$

where k_d stands for the partition coefficient for the molecules (mL/g); C_i and C_f are initial and final concentrations of molecules (mg/mL), respectively. V represents the volume of the solvent (mL) and m is the weight of the adsorbent (g). The affinity coefficients (k) representing the affinity of target molecules towards competing molecules were obtained via calculating relations of partition coefficients as k = k_d (target) /k_d (competitor), while comparative affinity coefficients (k′) meaning the relative affinity obtained using imprinting procedures were determined by the ratios of affinity coefficients of Con A-imprinted and non-imprinted adsorbents as k′ = k imprinted /k non imprinted.

As seen in Table 4.1. the relative selectivity coefficients (k′) demonstarting imprinting excellency were calculated as 26.6 and 6.12 torward Lysozyme and Hemoglobin, respectively. Data demonstrated that surface imprinted particles owe great selectivity against molecules of Con A, with proportion: 26.6-fold to Lys and 6.12-fold to Hb. Table 4.2 demonstrates the molecular weight and isoelectric points of Con A, lysozyme and hemoglobin.

Table 4.1. Partition coefficient (k_d), affinity coefficient (k), and relative affinity coefficient (k′) values of Lysozyme and Hemoglobin towards Con A.

	NIP		**MIP**		
	K_d(mL/g)	**K**	**K_d (mL/g)**	**k**	**k'**
Con A	59.40	-	1081.86	-	-
Lyz	6.63	8.95	4.53	238.62	26.64
Hb	2.64	22.49	7.85	137.72	6.12

Table 4.2. Molecular weight and isoelectric point of Con A, lysozime and hemoglobin.

	Molecular weight	İsoelectric point (pI)
Con A	104 kDa	4.5-5.5
Lysozime	14.4 kDa	11.2
Hemoglobin	64.5 kDa	6.8

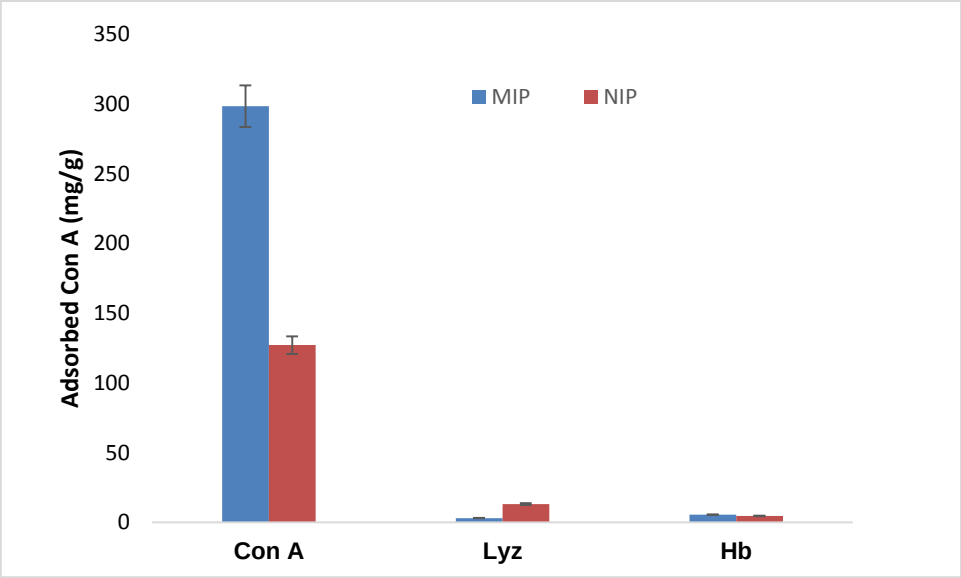

Figure 4.13. The adsorption capacity of Con A and competing proteins on Con A-imprinted silica particles. Experimental conditions: Con A 1 mg/mL; Lyz 1mg/mL; Hb 1 mg/mL; T 25°C, pH 6.0.

4.6. Adsorption studies of Con A from Jack Bean

Jack Bean seed crude extract was treated with 0.1 M phosphate buffer (pH 7). The obtained supernatant containing Con A was used as a solution for Con A-imprinted silica particles and non-imprinted silica particles. As seen in Figure 4.14. the adsorption capacity of MIP was quite impressive. From ½ and ¼ diluted Jack Bean supernatant Con A-imprinted particles adsorbed 90 mg/g and 40 mg/g Con A respectively, whereas NIP adsorbed approximately 10 mg/g and 4 mg/g respectively.

4.7. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Figure 4.15. shows the SDS-PAGE analysis of Jack bean before and after interaction with imprinted particles. Lane 1 corresponds to the Jack Bean seed crude extract treated with phosphate buffer. Lane 2 corresponds to the extract after treated with imprinted particles. Lane 3 corresponds to the elution solution from adsorbent and Lane 4 is wide range biomarker. As seen in the figure elution

contains only one band. The molecular weight corresponds with the molecular weight of Con A, 104 kDa. This approves the one step purification of Con A with the surface imprinted silica particles. Hence, it has been confirmed that the protein of interest that was isolated and obtained in purified form by the above methods was Concanavalin A.

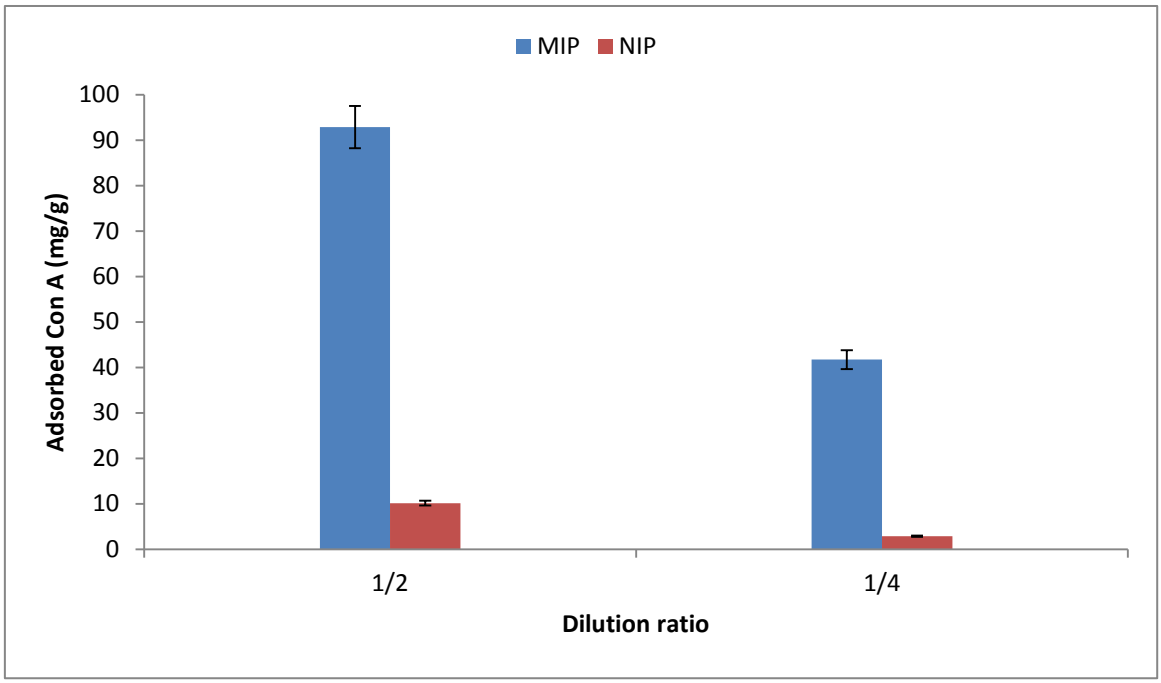

Figure 4.14. The adsorption analysis of Jack Bean extract by Con A-imprinted and non-imprinted silica particles. Experiment conditions: Jack Bean extract solution 2 mL; T 25°C; time 2 h.

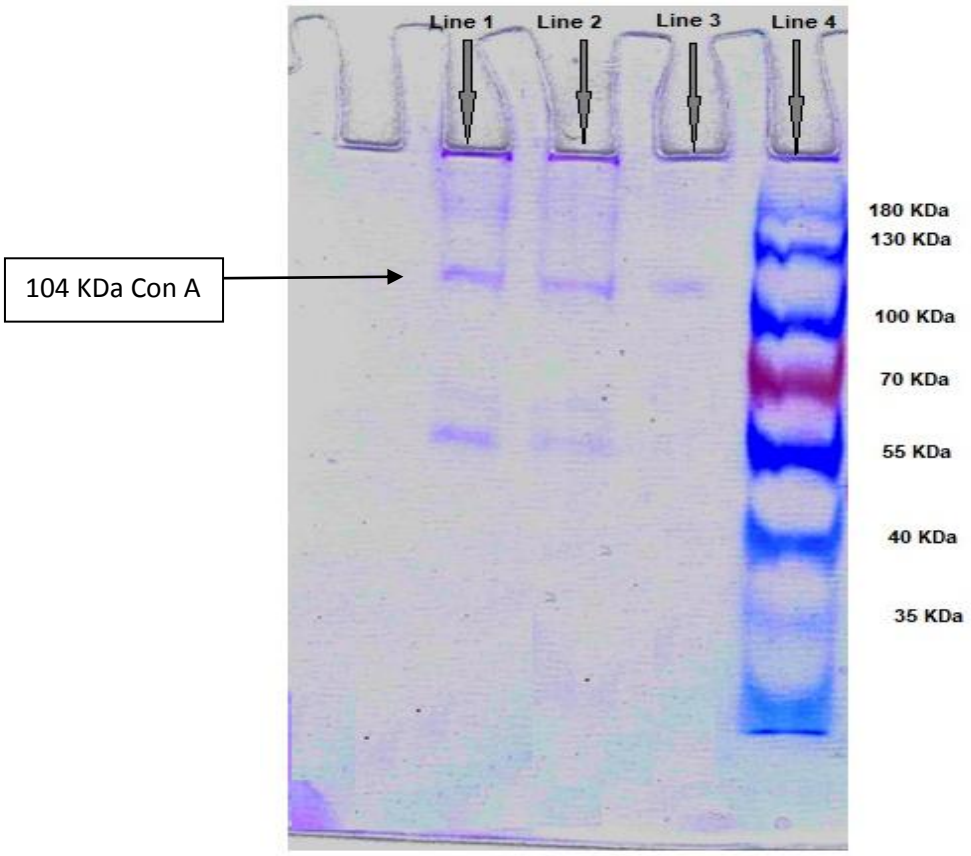

Figure 4.15. SDS-PAGE analysis of Jack bean, Lane 1: Before adsorption Jack Bean seed crude extract treated with phosphate buffer diluted (1/10), Lane 2: After adsorption of Jack Bean seed crude extract treated with phosphate buffer diluted (1/10), Lane 3: Bound eluted fractions (1/10) and Lane 4: Molecular mass markers.

The gel was scanned with an ImageQuant 300 (Amersham, USA) instrument and analyzed by using ImageQuant Software (Molecular Dynemics) in order to calculate the purity of Concanavalin A based on the percentage of Concanavalin A to total protein staining. Based on the results the purity of Concanavalin A is found as 89 %.

4.8. Reusability studies

The reusability of MIPs is one of the important parameters for its practical use. To test the reusability of Con A-imprinted silica particles, ten adsorption-desorption-regeneration cycles were conducted with 0.1 mg/mL of Con A solution. According

to the data shown in Figure 4.16. MIPs demonstrates the same adsoprtion capacity when injected the same Con A concentration at different times after Con A desorption. The reusability of the MIPs was approximately 93.5%. The results prove that Con A-imprinted silica particles can be used for several cycles with excellent reversibility and with no considerable decrease in their adsorption abilities.

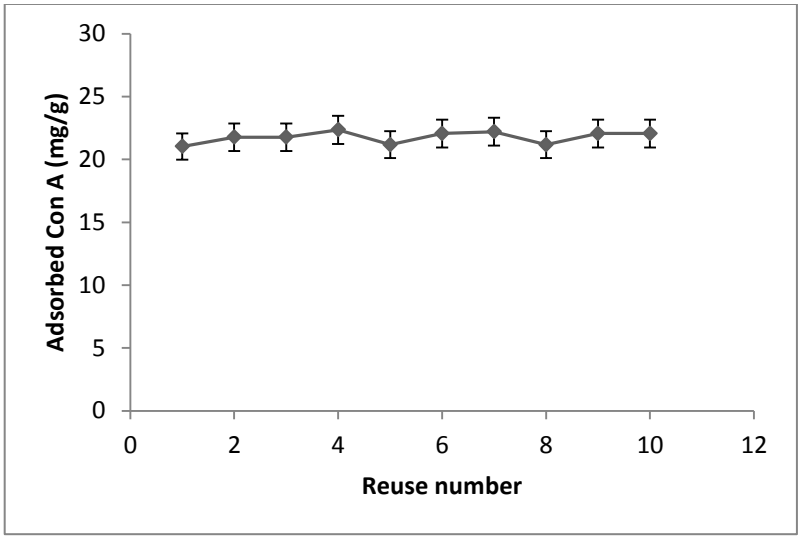

Figure 4.16. Adsorption-desorption cycle showing the reusability of Con A-imprinted silica particles. Experiment conditions: concentration of Con A-0.1 mg/mL; adsorption time 2h; T 25°C.

5. CONCLUSION

- Silica microspheres were successfully synthesized by Stöber method and showed high porosity.

- N-methacryloyl-L-histidine methyl ester was used as an effective functional monomer for selective binding of Con A.

- Con A-imprinted silica particles and non-imprinted silica particles were prepared on the surface of silica particles activated by 3 methacryloyloxypropyl trimethoxysilane (MPTMS). The imprinting was successfully contucted.

- Surface imprinting provides almost 100% adsorption capacity comparing to other molecular imprinting approaches due to its soft and uniform microspheres where binding sites are exposed to target molecules.

- Morphological and physical properties of Con A-imprinted silica particles were investigated by FTIR spectroscopy and obtained data showed that silica particles do interconnected with MPTMS agent and imprinting was realised. SEM images also demonstrated the physical states of silica particles before and after imprinting.

- Zeta sizer measurements helped to obtain information regarding the particle size and its locating of the Con A-imprinted particles and non-imprinted particles and they were 4410 and 1047 nm respectively. Also polydispersity index was 0.092 and 0.705 respectively.

- After treatment of MIPs by different pH ranges, highest possible binding capacity was detected at pH 6.0 as 20.2 mg Con A/g particle. Maximum binding ability was reduced considerably in acidic and alkaline pH values.

- Temperature experiments showed that the higher temperature the lower adsorption. It is because of the chemical reactions involved in interactions

between MIPs and Con A. The worst adsorption rate was detected at 40C and it was 15 mg/g.

- The Con A adsorption in MIP silica particles increases as the Con A concentration in buffer solution is increased, and the system was saturated with excess Con A binding when Con A concentrations above 1.5 mg/mL. There was not available binding sites for Con A molecules until that concentration rate. The maximum adsorption capacity was 307.2 mg/g.

- The Langmuir and Freudlich equilibrium isotherms were exploited to calculate binding characteristics of Con A-imprinted silica particles. The Langmuir isotherm was more appropriate with higher regression coefficient.

- To determine the suitable kinetic process, pseudo-first kinetics and pseudo-kinetics were used to understand the adsorption procedure of Con A molecules through resultant Con A-imprinted silica particles. The obtained calculations suggested that the pseudo-second kinetics adsorption method is dominant and that the total rate of the Con A adsorbance seemed to be driven by chemical mechanisms.

- The selectivity capabilities of Con A-imprinted silica particles were studied by different non-template proteins. MIP silica particles show highest affinity to Con A molecules due to the well-defined cavities designed complementary to Con A molecules by shape and functional groups.

- Crude Con A was extracted from Jack Bean seeds and examined for its purity and selectivity.

- SDS-PAGE electrophoresis analysis showed lanes which corresponds to Con A molecular weight 104 kDa.

- The purity of Jack Bean extracted Con A was determined and it was 89%.

- Con A imprinted MIPs showed high selectivity to crude Con A from Jack Bean.

- 10 adsorption-desorption cycles were conducted to estimate the reusability of MIPs. The results showed that Con A-imprinted silica particles can be used for many times without any considerable loss of its adsorption capacity. Also it was proved that the approach of preparation of MIP was able to work successfully.

- The synthesized Con A-imprinted silica particles demonstrate unique recognition characteristics. Since silica particles is cost-effective matrix with distinct properties, the surface molecularly imprinted silica particles synthesized in this thesis introduce a great alternative for protein purification.

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Oral and Poster Presentations

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GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
THESIS/DISSERTATION ORIGINALITY REPORT

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