Extracorporeal Affinity Therapy

Ekstrakorporal Afinite Tedavisi

Review Article / Derleme

Adil Denizli
Hacettepe University, Department of Chemistry, Biochemistry Division, Beytepe, Ankara, Turkey

ABSTRACT

Extracorporeal therapies are directed at the removal of potential toxic substances including bilirubin, pathogenic antibodies, various circulating immune-complexes, cholesterol, toxic metal ions, etc. from human plasma. The conventional extracorporeal therapies including plasma exchange, hemodialysis, hemofiltration and hemoperfusion are non-selective techniques. In addition the requirement for plasma substitutes such as albumin is very high. Moreover, the dangers of hepatitis or immune reactions accompany these therapies while using plasma for plasma products. Today, one of the most promising procedures for extracorporeal therapy is specific affinity adsorption. Affinity carriers may be used in hemoperfusion system, where blood is directly perfused through the column filled with these carriers. This type of application is effective, simple and inexpensive. In this article, some interesting applications on extracorporeal affinity therapies for removal of toxic substances from human plasma are briefly reviewed.

Key Words
Affinity therapy, affinity carriers, hemoperfusion, extracorporeal treatment, bilirubin removal.

ÖZET

Ekstrakorporal tedaviler bilirubin, patojenik antibadiler, dolaşımdaki çeşitli immün kompleksler, kolesterol, toksik metal iyonları gibi olası toksik maddelerin insan plazmasından uzaklaştırılmasına yöneliktir. Plazma değişimi, hemodiyaliz, hemofiltrasyon ve hemoperfüzyonu içeren geleneksel ekstrakorporal tedaviler seçici olmayan yöntemlerdir. Ayrıca, albümin gibi plazma desteklerine olan ihtiyaç oldukça fazladır. Dahası, plazma ve plazma destekleri kullanılırken hepatit veya immün reaksiyonların ortaya çıkma tehlikesi bulunmaktadır. Günümüzde ekstrakorporal tedavinin en umut verici uygulaması spesifik afinite adsorpsiyonudur. Afinite taşıyıcılar, kanın bu taşıyıcılarla doldurulmuş kolondan doğrudan geçirildiği hemoperfüzyon sisteminde kullanılabilir. Bu tür uygulama etkili, basit ve ucuzdur. Bu derlemede, ekstrakorporal tedavinin insan plazmasından toksik maddelerin uzaklaştırılması için kullanıldığı bazı ilginç uygulamalar kısaca verilmiştir.

Anahtar Kelimeler
Afinite tedavisi, afinite taşıyıcılar, hemoperfüzyon, ekstrakorporal tedavi, bilirubin uzaklaştırma.

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Correspondence to: Adil Denizli, Hacettepe University, Department of Chemistry, Biochemistry Division, Beytepe, Ankara, Turkey
Tel: +90312 297 7983 Fax: +90312 299 2163 E-Mail: denizli@hacettepe.edu.tr

INTRODUCTION

Conventional hemoperfusion is an extracorporeal treatment method in which the blood from the patient is circulated through adsorption column in order to remove exogeneous and endogeneous toxic substances from the blood (Figure 1) [1]. The blood removed from patient must be anticoagulated and generally separated into cells and plasma. The treated plasma is then combined with the previously separated cells and returned to the patient. Hemoperfusion is frequently utilized for acute blood purification as in the case of drug overdose, in which case the carrier is charcoal covered with a biocompatible coating layer [2]. The charcoal adsorbs medium to high molecular weight blood components due to the non-selectivity of conventional hemoperfusion. It would be most desirable to selectively remove any undesired substances from human blood. For this purpose, affinity adsorption was suggested as an alternative to conventional hemoperfusion for removing undesired substances from the plasma of patient. Plasma exchange is the most commonly used treatments for extracorporeal therapy. Table 1 summarizes the advantages of extracorporeal therapy with affinity carriers compared with plasma exchange. Table 2 gives several diseases potentially suitable for treatment by extracorporeal affinity therapy.

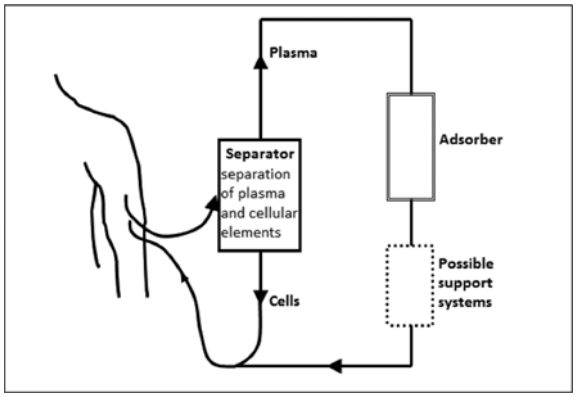

Figure 1. Schematic representation of an extracorporeal affinity therapy.

Table 1. Benefits of extracorporeal affinity therapy in comparison to plasma exchange [3].

- The patients own plasma is returned, there is no need for substitution materials.
- The risk for infectious diseases (hepatitis, ALS) is averted.
- Costs are lower.
- There are only slight deprivations of normal plasma components, including clotting factors.
- Simultaneous drug therapy is not affected, its combination with the affinity therapy is possible.

Affinity therapy in hyperbilirubinemia

Bilirubin is a negatively charged pigment formed in the normal metabolism of heme proteins in senescent red blood cells. Figure 2 shows the chemical structure of bilirubin. There are two kinds of serum bilirubin. One is mono- or di-glucuronide conjugated at the carboxylic acid groups, and the other is the non-conjugated type, free bilirubin [4]. The non-conjugated bilirubin is not water soluble and predominantly bound to albumin. It is transported to the liver as a complex with albumin and excreted into the bile [5]. Indirect bilirubin corresponds to non-conjugated bilirubin, and direct bilirubin corresponds to conjugated bilirubin. The free bilirubin is highly toxic to many cell types, intracellular organelles and physiological processes [6]. It is believed that the toxicity of bilirubin results from its hydrophobicity, which may lead to its aggregate in phospholipid membranes and subsequently damage the integrity of the membrane and inhibit the chemical performance of membrane bound enzymes. High concentration of free bilirubin can evoke hepatic or biliary tract dysfunction and permanent brain damage or death in more severe case [5]. Neurological dysfunctions as kernicterus or bilirubin encephalophathy may develop if the

Table 2. Diseases potentially suitable for treatment by extracorporeal affinity therapy.

- Hyperbilirubinemia
- Familial hypercholesterolemia
- Autoimmune disases such as systemic lupus erythematosus, rheumatoid arthritis
- Hemophilias, complicated by antibodies to clotting factors VIII or IX
- Thrombocytopenia
- Certain neurological diseases (Myasthenia gravis, multiple sclerosis)

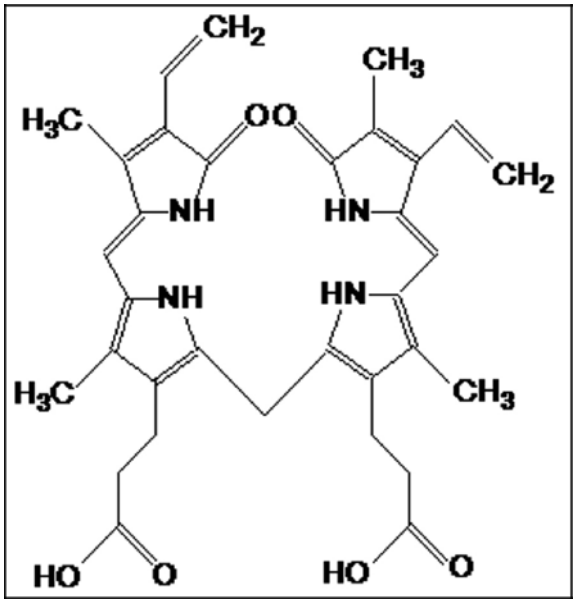

Figure 2. Structural formula of bilirubin ($C_{33}H_{36}N_4O_6$, molar mass 584.66 g/mol).

bilirubin concentration in the plasma rises above 15 mg/dL. Disorders in the metabolism of bilirubin may cause a yellow discoloration of the skin and other tissues.

There have been many bilirubin removal methods reported in literature. Table 3 shows various kinds of methods that have been applied clinically for the treatment of hyperbilirubinemia. Phototherapy is one of the most commonly used treatments for mild cases. However, the effectiveness of phototherapy is limited by the fact that the light could only penetrate a few millimeters of skin and not reach a large proportion of the total bilirubin pool. It is also found that phototherapy may induce DNA damage [7]. Treatment with plasma exchange, however, requires large volumes of fresh frozen plasma, which is expensive and difficult to obtain. In exchange transfusion, infant's blood is replaced with bilirubin-free adult blood. This procedure have been under concerns with the fact its relatedness to hypoglycemia, hypocalcemia, acidosis, and more importantly the transmission of infectious diseases like hepatitis or acquired immune deficiency syndrome [8]. Hemodialysis relays upon the diffusive transport, therefore, is a rather slow process. This method is not specific also. It should be noted that these systems are still complex and expensive. Hemoperfusion, i.e., circulation of blood through extracorporeal column containing affinity carriers for bilirubin removal, has become the most promising technique [9-18].

Table 3. Clinically applied bilirubin removal methods [4].

Phototherapy
Plasma exchange (plasmapheresis)
Exchange transfusion
Hemodialysis
Hemodialysis using highly permeable membrane
Hemofiltration
Hemoperfusion using activated charcoal
Hemoperfusion using anion exchange resin

Sideman et al. suggested the application of hemoperfusion to the removal of the bilirubin from jaundiced newborn babies by using albumin deposited macroreticular carrier [9]. The first clinical trial of hemoperfusion with anion exchange synthetic fibers for bilirubin removal was reported by Idezuki et al in 1981 [10]. Brown prepared oligo-peptide conjugated polyacrylamide particles as affinity carrier for bilirubin removal [11]. Polyacrylamide resin with oligopeptide sequences bound to the functional sites form effective carrier for bilirubin. It was shown that the main driving force for the binding of bilirubin at the active sites is an electrostatic interaction between the carboxylate group of bilirubin and the positively charge of the oligopeptide pendants on the carrier. Chandy et al. used polylysine attached chitosan particles for selective bilirubin removal [12]. Several layers of polylysine have been coated covalently onto chitosan particles, using nitrogen glow discharge plasma. It seemed that the surface modified chitosan particles could possibly provide an improved supportive therapy for hepatic failure especially for patients with hepatic coma and hyperbilirubinemia. Yamazaki et al. developed poly(styrene-divinyl benzene) based carrier and succesfully applied in the treatment of more than 200 patients with hyperbilirubinemia [13]. Morimoto et al. used plasma exchange and plasma adsorption with styrene-divinyl benzene carrier and removed bilirubin from hepatectomized patients [14]. In the clinical evaluation of patients receiving plasma adsorption with these columns containing styrene-divinyl benzene particles, there was a remarkable improvement of total bilirubin depletion in one case. This plasma adsorption system provided a possibility for an improved supportive therapy for hepatic failure, especially for patients with hepatic coma and

hyperbilirubinemia. Avramescu et al. conjugated bovine serum albumin with ethylene vinyl alcohol (EVAL) adsorptive membranes and they reported high bilirubin binding capacity [15]. They also employed the microporous EVAL membranes for the preparation of mixed matrix adsorber membranes by incorporation of ion exchange particles. The mixed adsorber systems combine the advantages of membrane technology (easy scale up, low pressure drop and high throughputs) with those of column chromatography (high selectivity and high binding capacity). Yu et al. prepared amine-containing cross-linked chitosan carrier and investigated adsorption behavior of conjugated bilirubin [16]. They reported that electrostatic and hydrophobic interactions are the main driving forces for the binding. Kuroda et al. studied selective adsorption of bilirubin by macroporous poly(glycidyl methacrylate-divinyl-benzene) particles [17]. When the surface of the particles was previously coated with albumin, discriminating binding of the bilirubin in the serum was attained and the adsorption of albumin was reduced. Ahmad et al. demonstrated the suitability of rat serum albumin loaded poly(lactide-glycolide) biodegradable microspheres in removal of bilirubin from systemic circulation of hyperbilirubinemic rats [18]. On evaluating the potential of microspheres in depletion of bilirubin from the systemic circulation, rat serum albumin carrying microspheres were found to be competent in removing bilirubin from the serum.

Conventional extracorporeal therapies used to remove bilirubin perform poorly due to low accessibility, insufficient adsorption capacities, low efficiency and economic limitations. Denizli and his coworkers proposed a packed-bed column containing dye affinity poly(hydroxyethyl methacrylate) particles to remove bilirubin from human plasma. Alkali Blue 6B, Congo Red and Cibacron Blue F3GA were covalently attached onto the PHEMA particles [19]. Optical photographs of PHEMA particles carrying dye molecules are given in Figure 3. Chemical structures of the reactive dyes are given in Figure 4. As seen here, chemical structures of dye molecules are quite different from each other, and contain several active points (amino, azo and triazine groups) for binding with bilirubin. Cibacron Blue F3GA is a widely used dye-ligand in the literature [20]. It is accepted that ether linkages are formed between the reactive triazine ring of the Cibacron Blue F3GA and the hydroxyl groups of the PHEMA particles. In the case of Congo Red and Alkali Blue 6B, covalent bonds are formed as a result of the condensation reactions between the aromatic amine groups of the dye molecules and the hydroxyl groups of the PHEMA particles. Note that the dye-attached particles were extensively washed until to ensure that there is no leakage from any of the dye-attached PHEMA particles and in any media used at adsorption-elution steps. They showed the dye-affinity PHEMA carriers removed bilirubin efficiently from human plasma [21-23]. It was found in their study that increasing temperature may enhance the adsorption and maximum adsorption was achieved at 37°C. They also found that the adsorption of albumin to the particles contributed to the removal of bilirubin from human plasma. However, increasing flow rate of plasma could decrease the bilirubin adsorption capacity of the PHEMA particles [23].

In recent years, microporous membranes were modified and various affinity ligands were attached for use as alternative carriers for protein purification studies [24]. Microporous membranes have the advantages of large surface area, short diffusion path and low pressure drop. As a result of the convective flow through the pores, the mass transfer resistance is significantly reduced and the adsorption kinetics dominates the binding process. This results in a rapid processing, which greatly improves the adsorption step. The choice of the membrane material may be difficult as a compromise must be found regarding the reactivity of the material, stability in polar solvents, pore

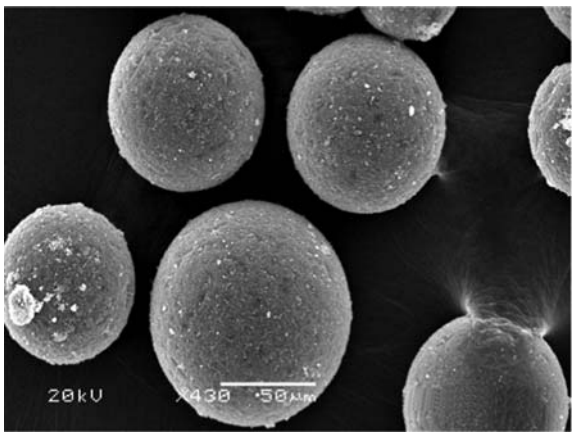

Figure 3. SEM photograph of PHEMA particles.

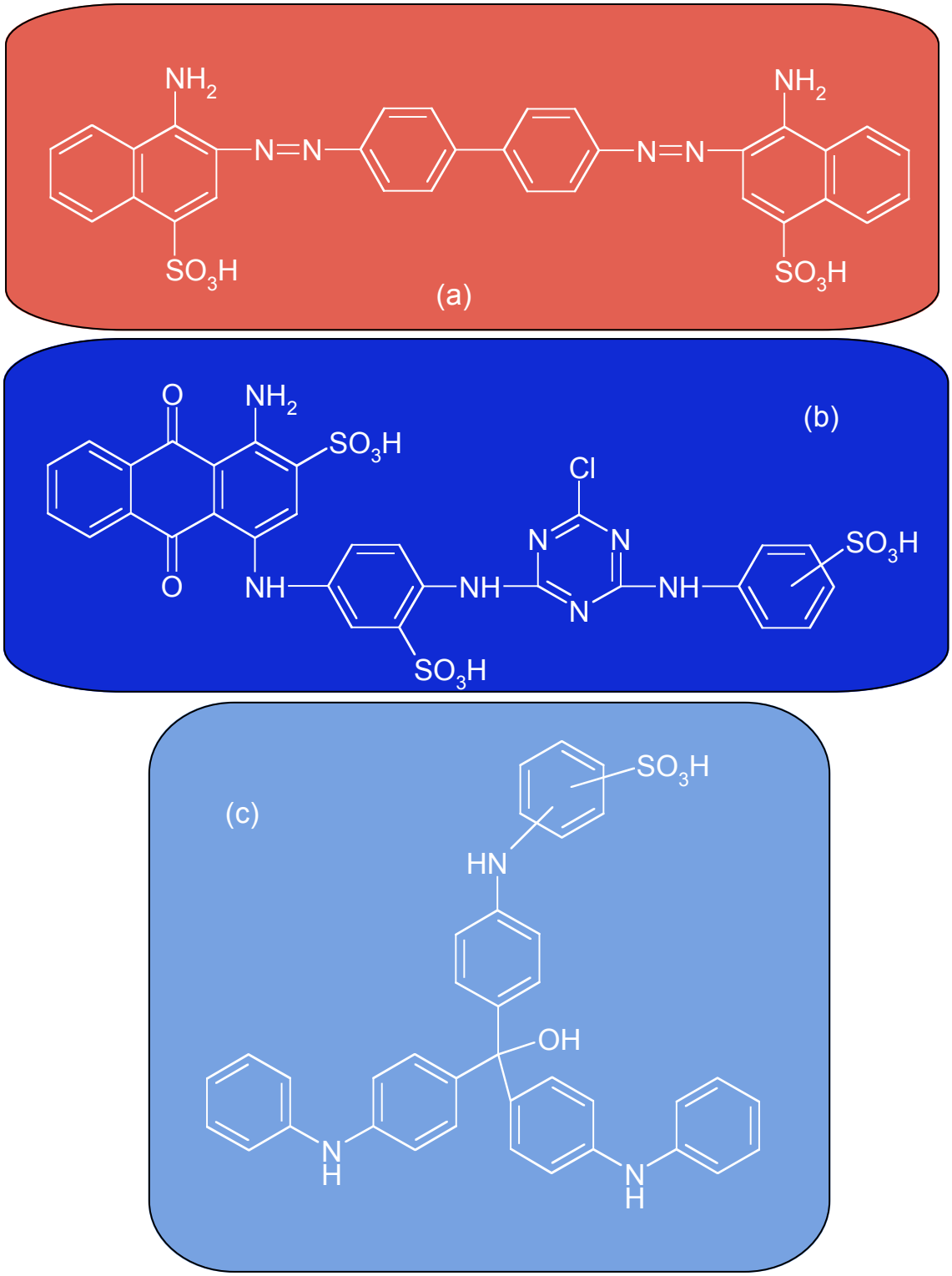

Figure 4. Chemical structures of Congo Red, Cibacron Blue F3GA and Alkali Blue 6B.

size and biocompatibility. A suitable membrane for biological application must fulfill the requirements of high hydrophilicity and low non-specific protein adsorption, fairly large pore size, a narrow pore size distribution, chemical and mechanical resistance as well as having enough reactive functional groups.

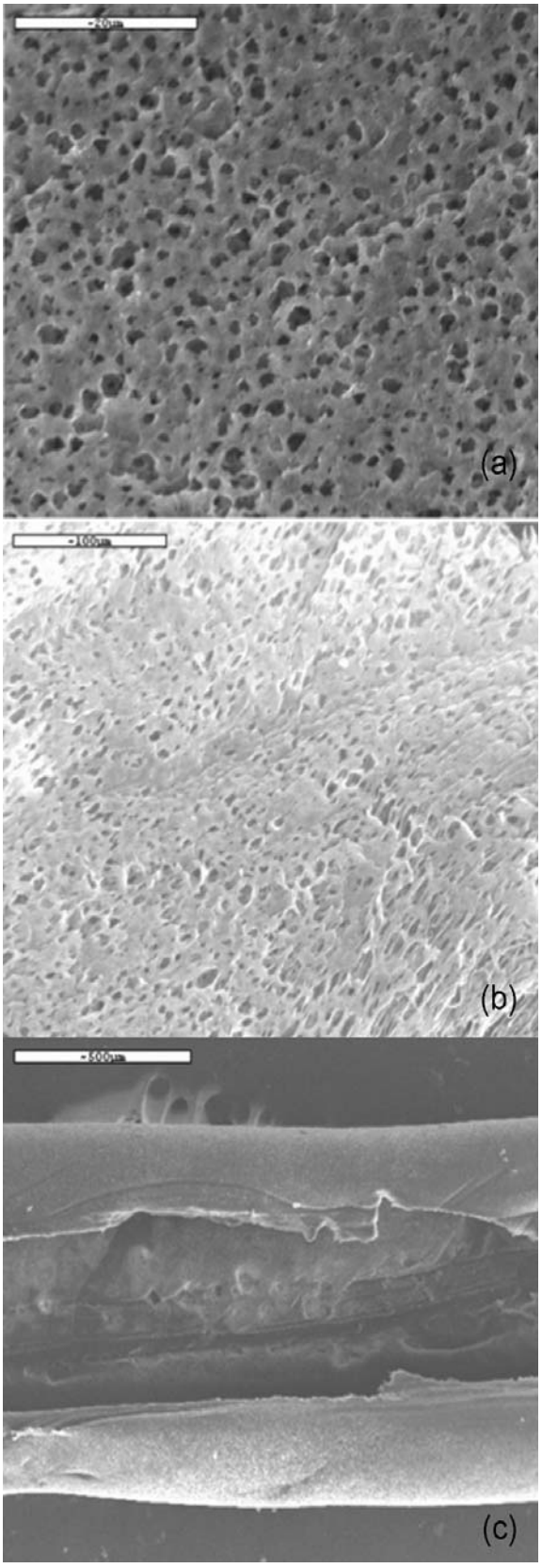

Figure 5. Representative SEM photographs of polyamide hollow-fibre membranes; (a) Inner surface; (b) Outer surface; (c) Cross-section [25].

Şenel et al used polyamide hollow-fiber membrane carrying dye molecules for bilirubin molecules [25]. Polyamide hollow-fiber membrane may meet most of these requirements, since they have a narrow pore size distribution and good mechanical rigidity. The pore radii of the polyamide hollow fiber membranes changed between 200 nm and 450 nm. This indicated that the hollow fiber membranes contained mainly macropores. The scanning electron microscope (SEM) photographs given in Figure 5 show the surface structure and the cross-section of the polyamide hollow-fiber membranes. As seen in these photographs, the smallest pore structure of the hollow fiber was highly asymmetric. Furthermore, the smallest pores occurred at the lumen side of the hollow fiber while the pore size at the shell side was much larger.

Uzun and Denizli used magnetically stabilized fluidized bed (MSFB) column containing human serum albumin (HSA) attached magnetic poly(hydroxyethyl methacrylate) particles for selective bilirubin removal from human plasma for the first time in literature [26]. They used MSFB as a hemoperfusion column for in-vitro bilirubin removal. HSA was used as the affinity ligand for specific binding of bilirubin molecules. HSA is the natural carrier for bilirubin in the blood. It is generally agreed that each HSA molecule may have as many as 12 binding sites for bilirubin, but only two of the sites bind bilirubin molecules strongly. The association constant is 9.5×10^7 M [27]. They reported that the non-specific bilirubin adsorption on the mPHEMA particles was 0.47 mg/g. Higher bilirubin adsorption amounts, up to 88.3 mg/g, were obtained with the HSA-immobilised mPHEMA particles (Figure 6A). Bilirubin adsorption capacity decreased significantly from 75.0 mg/g to 40.0 mg/g polymer with the increase of the flow-velocity from 0.5 ml/min to 4.0 ml/min (Figure 6B). Bilirubin adsorption increased with increasing temperature (Figure 6C). Adsorption behavior of bilirubin could be modelled using the Langmuir isotherm.

Chromatographic columns appear to have a distinct advantage over batch systems because the adsorption rate depends on the concentration of solute in solution being treated. For column system the carriers are continuously in contact with a fresh solution. As a result the concentration of solute in

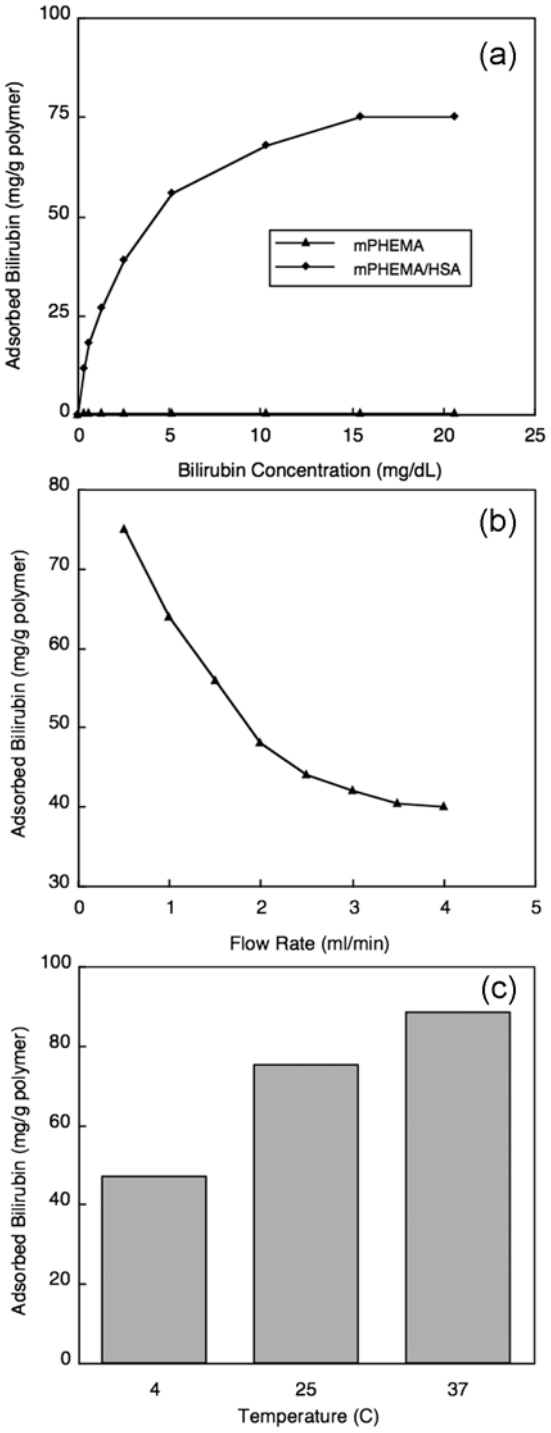

Figure 6. Bilirubin adsorption onto the HSA immobilized mPHEMA particles; (a) effect of initial bilirubin concentration; (b) effect of flow rate; (c) effect of temperature [26].

contact with a given layer of carrier is relatively constant. For batch treatment, the concentration of solute in contact with a specific quantity of carrier steadily decreases as adsorption proceeds,

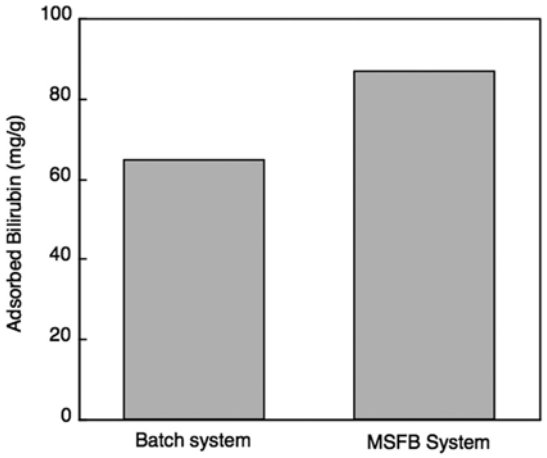

Figure 7. Comparison of bilirubin adsorption capacities in batch and MSFB column systems.

thereby decreasing the effectiveness of the carrier for removing the solute. Figure 7 compares the adsorption capacity of bilirubin in batch and MSFB column operations. The MSFB column capacity is found to be higher than the batch capacity [26,28]. This means, in equilibrium binding experiments, maximum adsorption capacity is 35% lower as compared to the value obtained in MSFB. The higher column capacity may be due to the fact that the continuously large concentration gradient at the interface zones occured as to passes through the column, while the concentration gradient decreases with time in batch experiments.

One of the most important properties of a affinity carriers is the adsorption capacity. In the last decade, research interest focused on the preparation of bilirubin carriers with reactive ligands including different aminoacids, albumin, peptide sequences and dye ligands. A list of the adsorption capacity of affinity carriers reported in literature is given in Table 4.

Molecularly imprinted polymers in bilirubin removal studies

Highly specific carriers underline various affinity-based separation techniques [46]. For example antibodies are routinely used as analytical reagents in clinical and research laboratories [47]. For many practical reasons attempts have been made to replace antibodies with more stable counterparts. One method that is being increasingly adopted for the generation of biomimetic antibodies is molecular imprinting

Table 4. Adsorption capacities for bilirubin of various carriers.

Material	Ligand	Adsorption capacity (mg/g)	[R]
Polyacrylamide particles	L-Lysine, L-Ornithine	0.2-75	[8]
Macroreticular resin	Albumin	2-24	[9]
Chitosan particles	Poly-L-lysine	1.5	[12]
Poly(ethylene vinyl alcohol)	Bovine serum albumin	25.0	[15]
Poly(GMA-DVB) copolymer	Albumin	30	[17]
PHEMA particles	Dye molecules	6.8-32.5	[19-21]
Polyamide hollow fiber	Cibacron Blue F3GA	48.9	[25]
mPHEMA particles/MSFB	Human serum albumin	88.3	[26]
mPHEMA particles/Batch	Human serum albumin	64.7	[28]
Chitosan coupled nylon membrane	Cibacron Blue F3GA	63.4	[29]
Styrene-divinyl benzene	Quaternary ammonium salt	4.0-80	[30]
Poly(tetrafluoroethylene) membrane	Cibaron Blue F3GA	76.2	[31]
Polyamide resin	Aminoacid	5-80	[32]
IONEX Polypropylene fiber	Tertiary amine	7.7	[33]
Polyacrylonitrile membrane	Hepatoycte receptor	2.8	[34]
Poly(butadien-HEMA) gels	Bovine serum albumin	3.1	[35]
Partially aminated polyacrylamide	β-cyclodextrin	42.2	[36]
Cellulose acetate fiber	Cibaron Blue F3GA	4.0	[37]
Polyamide/chitosan membrane	Polylysine	28.6	[38]
Polyamide membrane	Polylysine	32.4	[39]
Poly(GMA-AAm-MBA)	Polyethyleneimine	16.6	[40]
Poly(tetrafluoroethylene) membrane	Human serum albumin	71.2	[41]
Poly(tetrafluoroethylene) fiber	Bovine serum albumin	9.6	[42]
Aluminum oxide-silica membrane	Lysine	17.6	[43]
Poly(pyrrole)-alumina membrane	Lysine	32.4	[44]
Poly(glycidyl methacrylate)	Cibaron Blue F3GA	241.5	[45]

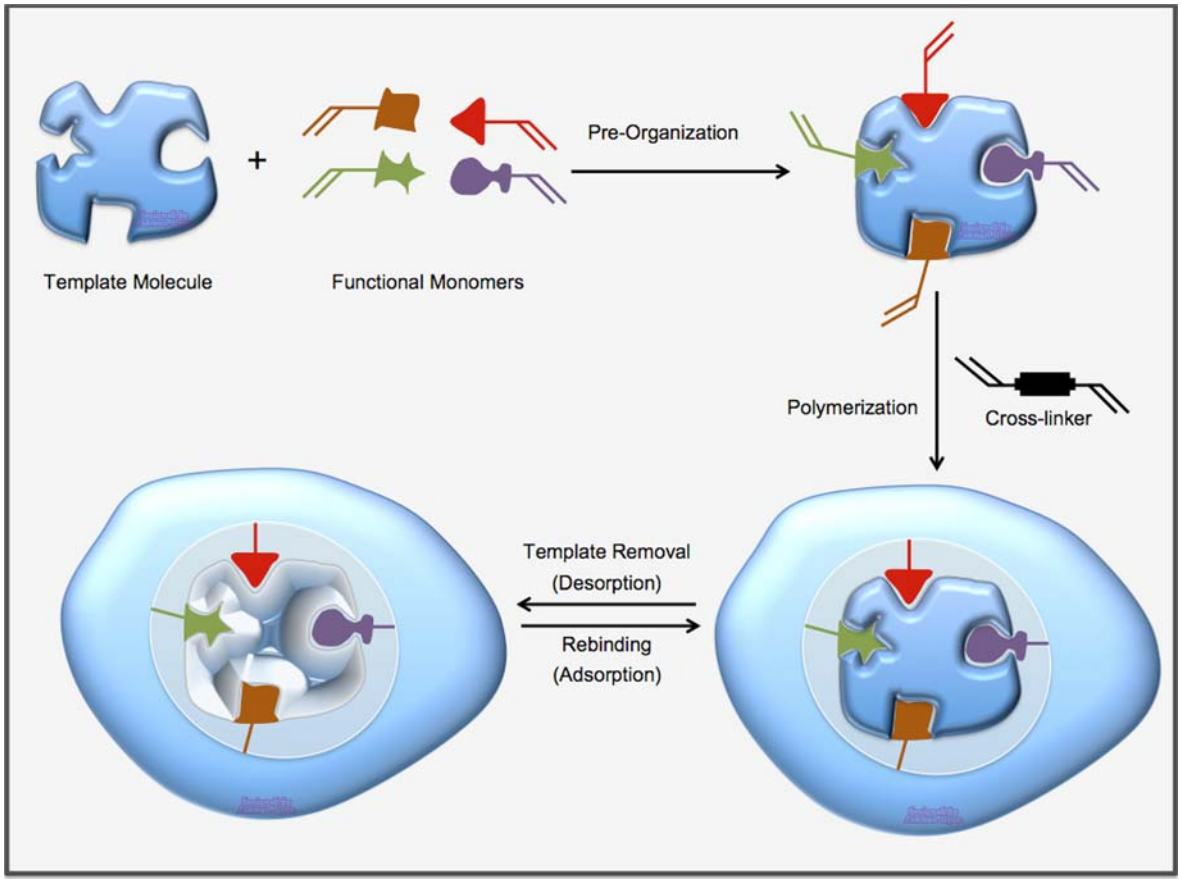

Figure 8. Schematic drawing of molecular imprinting method.

of polymers [48]. Given the advantage of easy preparation and chemical stability, molecularly imprinted polymers (MIPs) possess a high potential for use in a variety of applications such as chromatographic stationary phases, immunoassay-type analyses and sensor development [49-52]. Generally molecular imprinting is a synthetic strategy that is used to assemble a molecular receptor via template-guided synthesis (Figure 8). To prepare MIP, a template molecule is used to guide the assembly of functional monomers. Polymerization reaction is then employed to fix the preassembled binding groups around the template molecule. Following removal of the template molecule, the polymer revealed retains specific binding sites that can selectively rebind the original template molecule. Depending on the interactions between the template molecule and the functional monomers/groups involved at the imprinting and rebinding step, molecular imprinting has two different approaches: non-covalent and covalent [53-55]. In the non-covalent approach, various non-covalent interactions such as hydrogen bond, ionic interactions and hydrophobic effects are utilized. Given the fact that the non-covalent molecular interactions are prevalent in the biological world, exploitation of these binding forces, as it has turned out, has proven to be the most efficient and preferred method for generating robust, biomimetic materials [53].

During the development of biocompatible materials, aminoacids are main substances for the improvement of biocompatibility. For example, the molecular design and synthesis of a new aminoacid based monomer with a tyrosine and its copolymer had been reported [56]. These new bilirubin-imprinted polymeric particles were prepared for selective removal of bilirubin from hyperbilirubinemic human plasma. In the first step, functional monomer N-methacryloyl-(L)-tyrosine methylester (MAT) was synthesized using methacryloyl chloride and (L)-tyrosine methylester as a complexing monomer (Figure 9). Then, bilirubin was complexed with MAT and the bilirubin-imprinted

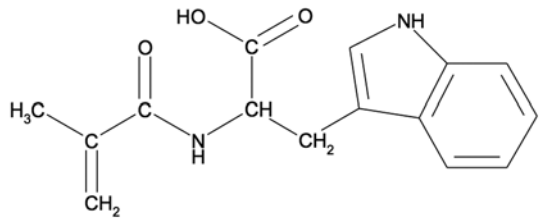

Figure 9. The molecular formula of MAT.

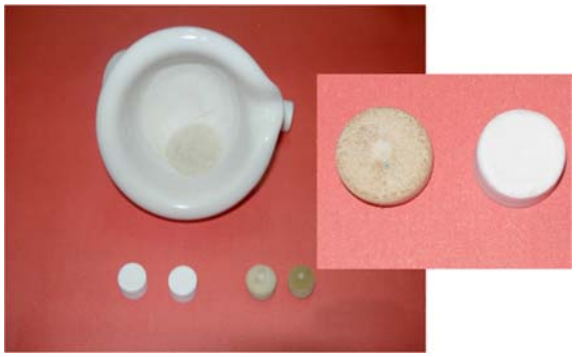

Figure 10. Optic photographs of MIP (right) and NIP (left) particles.

Poly(HEMA-MAT) [MIP] was produced by bulk polymerization. After that, the template molecules (i.e., bilirubin) were removed using Na_2CO_3 and NaOH. Figure 10 represents the optic photographs of NIP (left) and MIP (right) particles. It is clearly seen that the MIP particles have a characteristic yellow-green bilirubin color. The surface roughness and porosity of MIP and NIP particles are examined by the scanning electron photographs (SEM). Figure 11A shows the MIP particles. They are composed of small and interconnected microparticles which form a porous structure. The size of the microparticles was determined roughly to be in a 0.5-2 μm in range, according to the enlarged SEM photograph; it should be noted that this size is about five-fold less than those of conventional porous particles packed in chromatographic devices. It is clearly seen that the MIP particles have similar porous structure to the corresponding the NIP particles (Figure 11B). This similarity is important in quality of competitive studies.

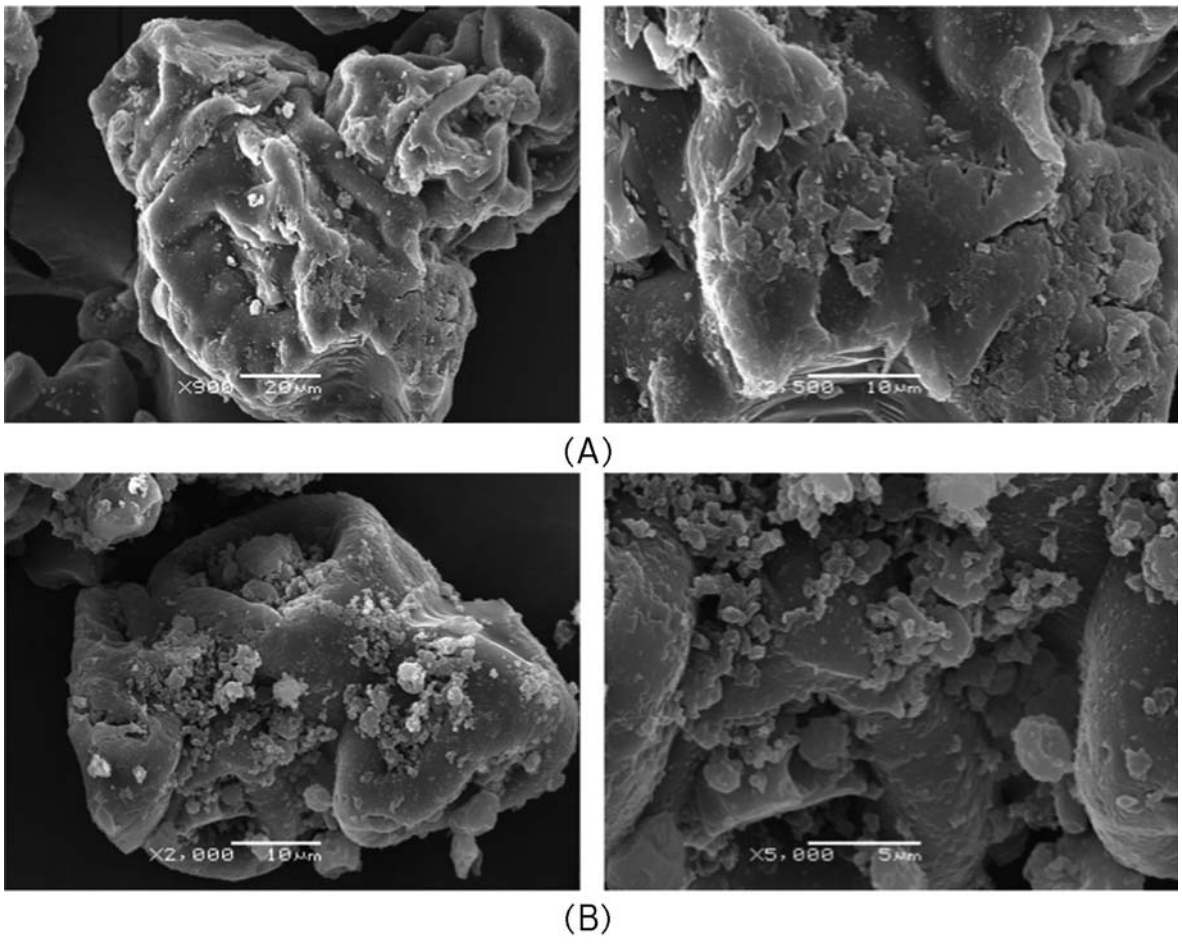

Figure 11. SEM photographs of (A) MIP and (B) NIP particles.

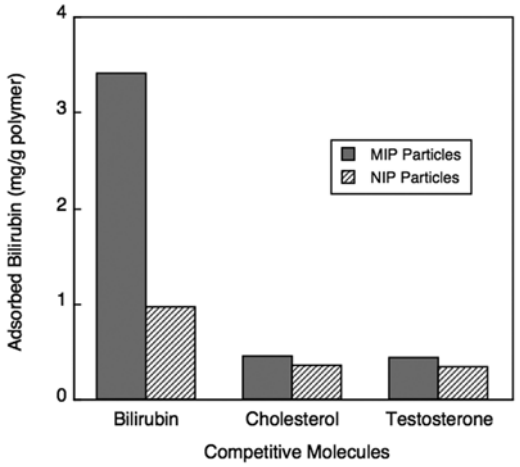

Figure 12. Adsorption behaviour of bilirubin, cholesterol and testosterone both in MIP and NIP particles [56].

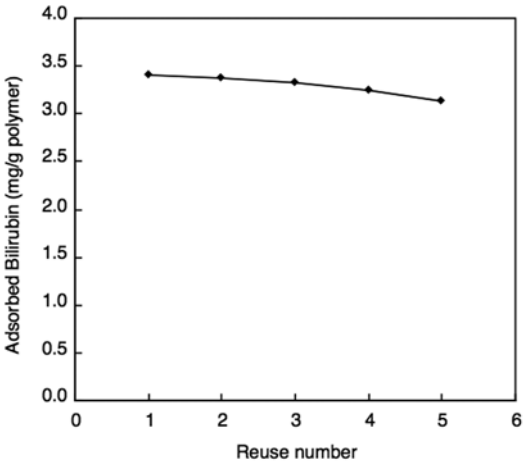

Figure 13. Repeated use of MIP particles [56].

The specific surface area of the MIP particles was found to be 27.8 m^2/g. The pore diameter of the MIP particles changed between 20 and 245 Å and average pore diameter is 25.0 Å. Template molecules (i.e., bilirubin) were removed from the polymer structure in the ratio of 87% of the initial concentration. Bilirubin adsorption increased with the increase in bilirubin concentration up to 0.8 mg/ml. The maximum bilirubin adsorption capacity was 3.4 mg/g of the dry weight of particles. The MIP particles were 6.3 and 3.0 times selective with respect to the cholesterol and testosterone, respectively (Figure 12).

There is considerably more potential for reducing costs by increasing the useful life of affinity carriers, whose adsorption capacity decreases with repeated use [57]. Reusability of the MIP particles was also investigated (Figure 13). The MIP particles showed negligible loss in the bilirubin adsorption capacity after five adsorption-desorption cycles with the same adsorbent. A list of the adsorption capacity of MIP particles reported in literature is given in Table 5.

Some selected extracorporeal affinity applications

Cadmium overload studies

Distinct biological roles for most metal ions are now well recognized. Metal ions play roles in catalysis, hormone action, gene and other regulatory functions, structural stabilization of macromolecules, muscle contraction, nerve conduction and transport. The alkali and alkaline earth metal ions as well as cobalt, copper, iron, manganese, molybdenum, nickel, zinc and others are known to be essential, many of them in the mechanism of action of specific enzymes.

Table 5. Bilirubin adsorption capacities of MIP particles.

Material	Interaction Type	Adsorption capacity (mg/g)	[R]
Poly(HEMA-MAT) particles	Molecular recognition	3.41	[56]
Poly(MAA-EGDMA)	Molecular recognition	1.04	[58]
Poly(MAA-EGDMA)	Molecular recognition	0.24-0.85	[59]
B-Cyclodextrin	Molecular recognition	60.1	[60]
Poly(vinyl pyridine-DVB) film	Molecular Recognition	61.0	[61]
Poly(MAA-EGDMA)	Molecular recognition	0.48	[62]
Poly(MAA-EGDMA)	Molecular recognition	0.83	[63]
Poly(HEMA-MAT) cryogel	Molecular recognition	3.6	[64]
PHEMA-MIP composite cryogel	Molecular recognition	10.3	[65]

However, the adverse effects of metal ions will manifest when a metal ion level exceeds e certain threshold level. The toxicities of heavy metals may be caused by the following mechanisms: blocking the essential functional groups of biomolecules; displacing essential metal ions from biomolecules; modifying the active conformation of biomolecules; disrupting the integrity of biomembranes and modifying some other biologically active agents [66]. Toxic metals are absorbed through the air passages and alimentary canal with food and drinking water. They disturb the economy of endogenous metals and biochemical equilibrium. They have an etiological effect on hypertension, cancer, decrease the ventila of the lungs and other lung diseases. Toxic metal ions are the source of degeneration, reduction of pancreatic efficiency and disfunction of kidneys [67].

The chronic toxicity of cadmium compounds includes kidney damage with proteinuria of low-molecular-weight molecules. An epidemic of Japanese itai-itai disease is believed to be the result of chronic ingestion of Cd^{2+}, with altered renal tubular function, impaired regulation of calcium and phosphorus, manifesting bone demineralization, osteomalacia, and pathological fractures. No specific treatments for acute or chronic cadmium poisoning are available. However, in addition to supportive therapy and hemodialysis, heavy metal poisoning is often treated with a chelating agent. Different chelating agents that are available commercially for the treatment of cadmium poisoning are British anti-lewisite and calcium disodium ethylene diamine tetraacetic acid (EDTA). But there is histopathological evidence for increased toxicity in animals when calcium disodium EDTA is utilized [68]. Recently, one of the most promising technique for blood detoxification is extracoporeal affinity adsorption [69]. So far, only a few affinity adsorbents were reported for metal detoxification [70,71].

Metallothioneins are a group of non-enzymatic (6-7 kD) low molecular mass proteins with 61-68 aminoacid residues including 20 cysteins, bound to certain bivalent ions such as Zn^{2+}, Cd^{2+}, Hg^{2+}, Bi^{2+}, Sn^{2+}, Ni^{2+}, Rb^{2+} or Tc^{2+} with high affinity [72]. The binding is also observed with univalent ions such as Cu+, Ag^{+} and Au^{+}. Metallothionein and its analogues are widely distributed among organisms, from bacteria and fungi to plants and mammals. Metalloproteins play an important role in the metabolism and kinetics of metals including transport of metals, removal of metals, protection from metal toxicity, free radical scavenger, storage of metals, metabolism of essential metal ions, immune response and genotoxicity and carcinogenecity. Cysteinyl hexapeptide (CysHP) is a small metallo-peptide (molecular mass: 627.8) composed of six amino acids, three of them being cysteine residues. The thiol groups in the CysHP structure permit it to form metal clusters including Cd^{2+}, Zn^{2+}, Hg^{2+}, Cu^{+} and some other bivalent and divalent metal ions. This makes them attractive ligands and metalloproteins are among the most selective chelators known for metal ions for biomedical applications [73].

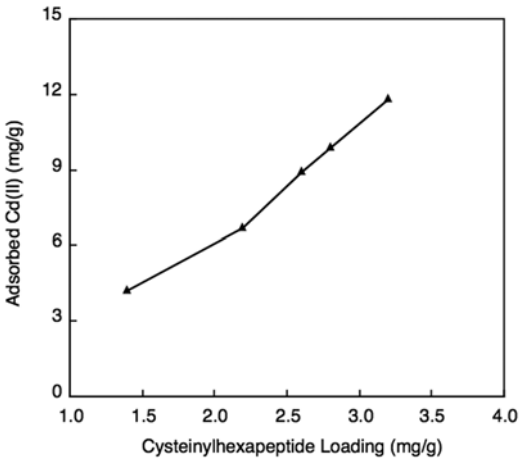

Figure 14. Effects of CysHP loading on Cd^{2+} adsorption [76].

Denizli et al. prepared thionein carrying PHEMA particles for Cd^{2+} removal from human plasma both in batch and packed-bed column system [74-76]. Monochlorotriazinyl dye ligand Cibacron Blue F3GA was attached onto the PHEMA particles. Later, a metallo-peptide (i.e., cysteinylhexapeptide) was attached to the dye-attached particles. Then, they were used for Cd^{2+} removal from human plasma poisoned with Cd^{2+} in a column system. PHEMA particles were selected as the basic polymer matrix which carries functional hydroxyl groups for further modification. PHEMA particles have hydrophilic character, good blood-compatibility properties, minimal non-specific protein interactions, high chemical and mechanical stabilities for column applications and resistance towards microbial and

enzymatic attacks [77-79]. It was reported that obtained results made these thionein carrying PHEMA particles potential candidates for future detoxification studies. It has been found that the cysteinylhexapeptide loading has a great effect in the capacity of particles for adsorbing Cd^{2+} ions from human plasma (Figure 14).

Iron overload studies

Iron is an essential trace element for almost all organisms for a broad spectrum of biological processes which include electron transfer, transport, storage and activation of oxygen, nitrogen fixation and DNA synthesis [80]. The toxic effects of iron overload are well known especially since the human body has no physiological route for the elimination of excess iron [81]. Chronic iron overload may occur in a variety of diseases where the administration of parental iron is necessary (e.g. thalassemia, aplastic anemia). Acute iron intoxication is also a frequent, sometimes life-threating form of poisoning, especially among young children. The toxicity of iron is related to its ability to induce oxidative stress in cells [82]. In an occupational setting, inhalation exposure to iron oxide may cause siderosis. In the nonoccupational population, ingestion of large quantities of iron salts may cause nausea, vomiting and intestinal bleeding. There is accumulating evidence suggesting that an increase an iron storage may be associated with an increasing risk of developing cancer [83]. Studies have demonstrated that there is an increased risk for developing colorectal carcinoma following ingestion of high amounts of iron [84]. There is also an increase in hepatocellular carcinoma in patients with hereditary hemochromatosis, an inherited disorder in which there is hyper-absorption of iron from the intestinal tract and in lung cancer from exposure to asbestos fibers, which contains approximately 30% iron by weight [85].

For transfusional iron overload and acute iron poisoning, the only available supportive treatment is chelation therapy and the only available clinical drug for this treatment is desferrioxamine B (DFO) a linear hydroxamate, a natural siderophore [84]. The use of DFO has already been shown to result in prolonged life expectancy, reduced liver iron and the establishment of negative iron balance. However, the major limitation to the use of DFO is its lack of effectiveness when administered orally, the short half-life time in plasma and its potential toxicity when present in high concentrations [86]. DFO is highly expensive also. For this reason a number of orally active iron chelators are being tested but none of them are still satisfactory [85,87]. To overcome the drawbacks of soluble iron chelators in the treatment of iron overload, attachment of iron chelating ligands have been studied [88]. Comparing to soluble iron chelators, iron chelating resins might have advantages in stability, reusability and minimal damage to biological substances.

Yavuz et al prepared ferritin immobilized PHEMA membranes due to several advantages of membrane systems such as high porosity, large internal surface area, high chemical, biological and mechanical stability [89]. Ferritin is a widely distributed iron-storage protein thought to be very important to provide protection against the catalysis of deleterious oxidation of biomolecules by iron [90]. It is an almost spherical major iron storage plasma protein of molecular mass of 440 kDa. It composed of 24 equivalent subunits arranged in a shell-like manner with a hollow core of diameter 7.5 nm. Its outer shell diameter is 12.5 nm, as determined by transmission electron microscopy [91]. Based on these data, it was concluded that the PHEMA membrane has effective pore structures for attachment of ferritin. Ferritin contains approximately 2500 iron binding (chelation) center. This makes it an attractive chelating ligand for the removal of iron in iron poisoning. Then, they were used ferritin immobilized PHEMA for iron removal from aqueous solutions and human plasma. They showed that these ferritin modified membranes are suitable for repeated use for more than six cycles without noticable loss of adsorption capacity.

Figure 15 gives removal rates of Fe^{3+} ions onto the PHEMA membranes from human plasma. In chronic dialysis an average treatment time is around 3-5 h. High mass transfer rates are necessary to keep treatment time to a minimum and meet therapeutic demands. This equilibrium removal period seems to be satisfactory for extracorporeal therapy for Fe^{3+} removal from human plasma. Yavuz et al reported that Fe^{3+} levels in blood plasma can be

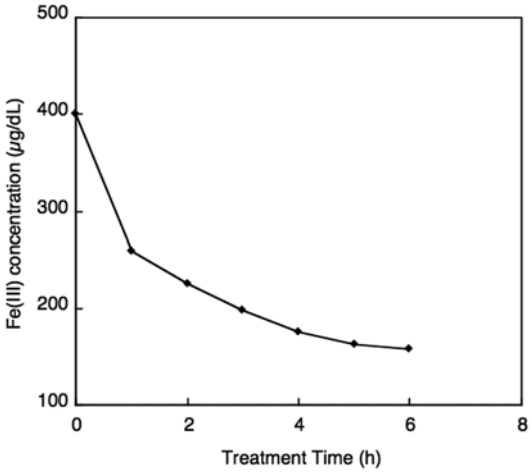

Figure 15. Iron removal rate using PHEMA membranes [89].

reduced significantly with relatively fast adsorption rates by using ferritin modified PHEMA membranes developed in this study (Figure 15).

Aluminum removal studies

Aluminum has recently been considered as a causative agent in dialysis encephalopathy, osteodystrophy, and microytic anemia occuring in patients with chronic renal failure who undergo long-term hemodialysis. Only a small amount of Al^{3+} ions in dialysis solutions may cause these disorders. Encephalopathy has also occured in children consuming aluminum hydroxide as a phosphate binder for renal disorders. Al^{3+} has also been implicated in neurotoxicity associated with amyotrophic lateral sclerosis, a form of parkinsonism and in Alzheimer's disease. Demircelik et al prepared Al^{3+}-imprinted PHEMA beads [MIP] with the assistant functional monomer

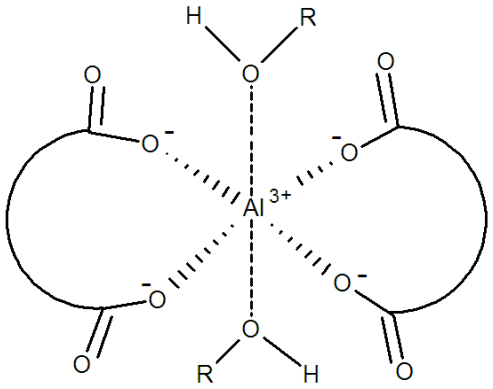

Figure 16. Schematic presentation of a monodentate binding mode of Al^{3+} hexagonally coordinated to 2 equivalents of $MAGA_2^-$ and 2 equivalents of ROH solvent molecules, where R is H or $C_2H_5^-$ or a combination of both.

of N-methacryloyl-L-glutamic acid [MAGA] to be used in selective removal of Al^{3+} out of human plasma overdosed with Al^{3+} ions [92]. MAGA was synthesized as the metal complexing monomer by the reaction of L-glutamic acid and methacryloyl chloride, with the goal preparing a solid-phase which has the high selectivity for Al^{3+} ions (Figure 16). The carboxyl groups of MAGA monomer were complexed with Al^{3+} ions.

The surface morphology and bulk structure of MIP beads are shown by the SEM in Figure 17. The MIP beads have a spherical form with the size range of 40-50 μm and rough surface due to the large pores, which formed during the polymerization process. The photograph in Figure 2B shows the presence of macropores within the bead bulk structure due to the formation of ionic cavities. The roughness of the bead surface should be considered as a factor providing an increase in the surface area.

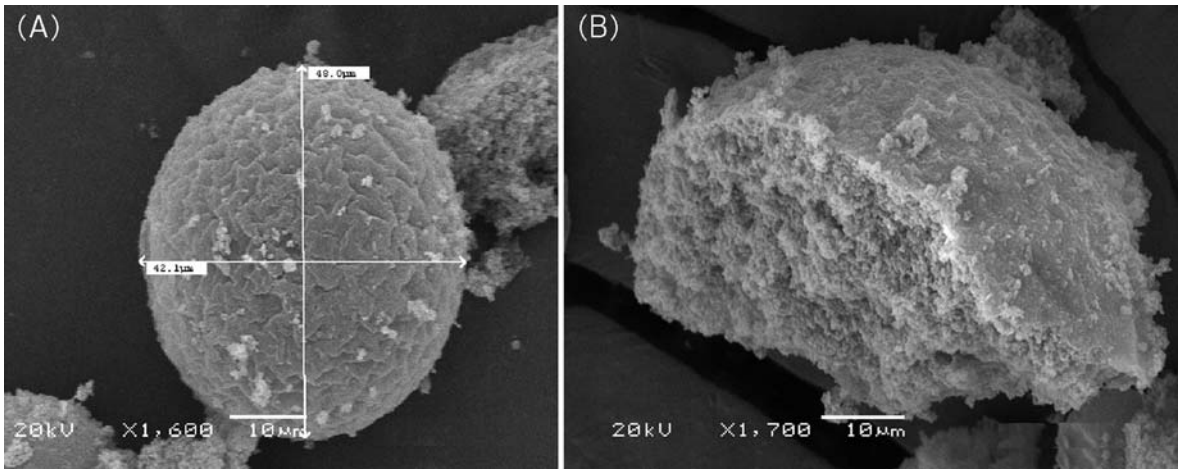

Figure 17. SEM photographs of MIP beads: (A) Surface morphology and (B) bulk structure.

In addition, the large pores reduce convective mass transfer resistance and facilitate mass. The specific surface area of MIP beads was found to be 55.6 m^2/g on the average.

The template Al^{3+} ions could be reversibly detached from the matrix using a 50 mM solution of EDTA. The MIP and non-imprinted [NIP] beads were then tested in a set of adsorption experiments for their capabilities to remove Al^{3+} selectively out of human plasma overdosed with Al^{3+} in the presence of Fe^{3+}, Cu^{2+} and Zn^{2+} ions. The relative selectivity coefficients (k') of the MIP beads for Al^{3+}/Fe^{3+}, Al^{3+}/Cu^{2+} and Al^{3+}/Zn^{2+} were found to be almost 4.5, 9.0 and 32.5 times greater than those of the NIP beads, respectively. In addition to these results, Figure 18 illustrates the adsorbed template and competitive ions both in the MIP (dark grey) and the NIP (light grey) beads. As clearly seen here, the competitive adsorption amount for Al^{3+} ions in the MIP beads is 0.762 mg/g polymer in the presence of competitive ions (Fe^{3+}, Cu^{2+} and Zn^{2+}). They recovered these MIP beads and reused many times, with no significant decrease in their adsorption capacities.

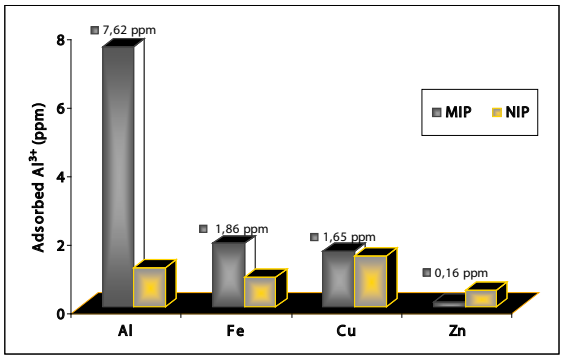

Figure 18. Adsorbed template and competitive ions both in PHEMAGA-Al3+ and PHEMAGA beads. Flow rate: 0.3 mL/min, ion concentration: 20 mg/L; polymer amount 0.2 g; T:20°C.

Other several applications

Immunoadsorption therapy based on the latter principles of affinity is thus an attractive method chiefly used for cleaning biological fluids such as blood and plasma [93]. Jia et al immobilized protein A on the membrane cartridge for extracorporeal IgG and immune complex removal from blood. The protein A membrane cartridge (6 mg protein A/g dry matrix) adsorbed 23.5 mg IgG per g dry matrix from human plasma. Experiments in vitro and in vivo confirmed that protein A membrane cartridge mainly adsorbed IgG and little of other plasma proteins, and that blood cell damage was negligible. Phenylalanine or tryptophan-containing imunoadsorbents based on poly(vinyl alcohol) particles have been used for rheumatism and myasthenia gravis therapy [94,95]. Protein A has been used to remove IgG from serum [96]. Protein A apheresis has also been applied successfully to remove autoantibodies in the treatment of severe forms of various autoimmune diseases and after chemotherapy [97]. In some haemophilia cases, antibodies to factor VIII are present and various neurological, nephrological and haemological conditions have been treated using extracorporeal affinity therapy using immunoadsorption columns, packed with sepharose-protein A [98].

Concluding remarks

Hemoperfusion is a process in which the blood is passed over a carrier in a packed bed column. Hemoperfusion is frequently used for acute blood purification as in the case of drug overdose using charcoal as an adsorbent. The charcoal adsorps medium- to high molecular weight blood components. Charcoal hemoperfusion is non-specific in nature, removing many desirable blood components as well as toxic substances. Consequently, a number of scientists have examined specific extracorporeal removal of toxic substances including bilirubin, metal ions, etc. In this review, we outlined the developments in the extracorporeal affinity therapies. It has been demonstrated that a variety of affinity carriers including both specific and pseudospecific can be used for the removal of toxic substances from human blood.

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