DOKUZ EYLUL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

REMOVAL OF CADMIUM WHICH IS A HUMAN CARCINOGEN USING A NOVEL CRYOGELIC PELLETS

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İZMİR

REMOVAL OF CADMIUM WHICH IS A HUMAN CARCINOGEN USING A NOVEL CRYOGELIC PELLETS

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MSc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"REMOVAL OF CADMIUM WHICH IS A HUMAN CARCINOGEN USING A NOVEL CRYOGELIC PELLETS "** completed by **BURAK KELEŞ** under supervision of **ASSOC. PROF. DR. M. NALAN TUZMEN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science in Chemistry.

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ABSTRACT

Removal of wastes from aqueous media has an increasing importance as a result of rapid development of industry causing pollution of water sources. The usage of cadmium in industry; batteries, tin soldering, veneering and also in production of cars, plastic and etc. causes the presence of cadmium in water sources. Cd^{2+} is a toxic element that may cause pathologies like renal tubular disfunction, kidney damage, pulmoner emphysema, osteoporosis and also may compete with trace elements like Zn^{2+}. Beause of this toxic properties cadmium must be removed from wastes of these industries. Conventional methods using removal of heavy metals from industrial wastes are expensive and inefficient. As a new developed method, adsorption, is economic and simple method for removal of heavy metals. In this study, cryogelic matrix poly(HEMA) was synthesized from 2-hydroxyethylmetacrylate monomer at -12^{0}C for 24 hours by free radical polimerization to remove toxic Cd^{2+} ions. Congo Red (CR) and Cibacron Blue F3GA (CB) was immobilized on cryogel membranes as ligands. Characterization of the polymeric structures was carried out with FTIR, SEM and elemental analysis. Effects of some system parameters such as pH, initial concentration, temperature and contact time were investigated for the optimization of adsorption conditions on CR and CB immobilized cryogelic pellets. The maximum adsorption capacities are determined as 28,534 mg/g for poly(HEMA)-CR and as 25,492 mg/g for poly(HEMA)-CB. We also determined that poly(HEMA)-CR and poly(HEMA)-CB cryogelic pellets can be used for the removal of Cd^{2+} ions from human plasma.

Keywords: Cadmium removal, Adsorption, Cryogel, Dye Ligand Affinity, Metal detoxification

YENİ NESİL KRİYOJELİK PELLETLER İLE KARSİNOJEN ETKİLİ KADMİYUMUN UZAKLAŞTIRILMASI

ÖZ

Sudan kirlilik verici maddelerin uzaklaştırılması, hızlı sanayileşme sonucunda su kaynaklarının endüstriyel atıklarla her geçen gün kirlenmesi nedeniyle daha da önemli hale gelmektedir. Kadmiyumun, pil, lehim, metal kaplamacılık, otomobil sanayi, askeri malzeme, vida, cıvata ve plastik yapımında çok kullanılması, bu metalin sulu ortamda bulunmasına neden olmaktadır. Cd^{2+} , renal tübüler disfonksiyon ve ağır böbrek hasarından başlayarak, pulmoner anfizem ve osteoporoz gibi değişik patolojilere yol açabilen, organizmada Zn^{2+} gibi esansiyel iz elementler ile rekabete girebilen toksik bir elementtir. Söz konusu toksik özelliklerinden dolayı kadmiyumun uzaklaştırılması gerekmektedir. Bu konuda geliştirilen yöntemler arasında adsorpsiyon yöntemi; ekonomik ve kolay uygulanabilen bir yöntem olarak oldukça önem kazanmıştır. Sunulan çalışmada, Cd^{2+} iyonlarının uzaklaştırılması amacıyla, 2-hidroksietilmetakrilat monomeri kullanılarak serbest radikal polimerizasyonu ile -12^{0}C'de 24s süreyle poli(HEMA) kriyojelik matriksin sentezi gerçekleştirilmiştir. Sentezlenen poli(HEMA) kriyojel membrana, ligand Congo Red (CR) ve Cibacron Blue F3GA (CB) immobilize edilmiştir. Polimerik yapıların karakterizasyonu, elementel analiz, SEM ve FTIR yöntemleri kullanılarak gerçekleştirilmiştir. CR ve CB immobilize pelletlere Cd^{2+} iyonlarının adsorpsiyonu için en uygun koşulların belirlenmesi amacıyla pH, başlangıç derişimi, sıcaklık ve sürenin etkisi araştırılmıştır ve maksimum adsorpsiyon kapasitesi poli(HEMA)-CR için 28,534 mg/g, poli(HEMA)-CB için 25,492 mg/g olarak kaydedilmiştir. Ayrıca, poli(HEMA)-CR ve poli(HEMA)-CB kriyojelik pelletlerin insan plazmasından Cd^{2+} iyonlarının uzaklaştırılmasında kullanılabileceği belirlenmiştir.

Anahtar sözcükler: Cadmium uzakşaltırma, Adsorpsiyon, Kriyojel, Boya-ligand afinite, Metal detoksifikasyonu

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CHAPTER ONE

INTRODUCTION

1.1 Cadmium

Cadmium (atomic number 48; relative atomic mass 112.40 g/mol) is a metal that belongs, together with zinc and mercury, to group IIB in the Periodic Table. It is a relatively rare element and is not found in the pure state in nature. Cadmium is mainly associated with the sulfide ores of zinc, lead, and copper, although purification first took place in 1817 from zinc carbonate. Commercial production only became significant at the beginning of this century. Cadmium is often considered as a metal of the 20th century; indeed, over 65% of the cumulative world production has taken place in the last two decades (Wilson, 1988). Cadmium has a relatively high vapour pressure. Its vapour is oxidized rapidly in air to produce cadmium oxide. When reactive gases or vapour, such as carbon dioxide, water vapour, sulfur dioxide, sulfur trioxide or hydrogen chloride are present, cadmium vapour reacts to produce cadmium carbonate, hydroxide, sulfite, sulfate or chloride, respectively. These compounds may be formed in stacks and emitted to the environment. Some cadmium compounds, such as cadmium sulfide, carbonate, and oxide, are practically insoluble in water. There is, however, a lack of data on the solubility of these compounds in biological fluids, e.g., in the gastrointestinal tract and lung. These water-insoluble compounds can be changed to water-soluble salts in nature under the influence of oxygen and acids; cadmium sulfate, nitrate, and halides are water-soluble. Most of the cadmium found in mammals, birds, and fish is probably bound to protein molecules.

The speciation of cadmium in soil, plants, animal tissues, and foodstuffs may be of importance for the evaluation of the health hazards associated with areas of cadmium contamination or high cadmium intake. Very few data on the occurrence and speciation of cadmium compounds in nature are available. Cadmium is commonly regarded as a pollutant of worldwide concern. The metal has been reviewed by the International Register of Potentially Toxic Chemicals of the United

Nations Environment Programme. As a result, it has been included on the list of chemical substances and processes considered to be potentially dangerous at the global level (Wilson, 1988).

1.1.1 Using Area of Cadmium

Cadmium has a limited number of applications but within this range the metal is used in a large variety of consumer and industrial materials. The principal applications of cadmium fall into five categories: protective plating on steel; stabilizers for poly-vinyl chloride (PVC); pigments in plastics and glasses; electrode material in nickel-cadmium batteries; and as a component of various alloys. Detailed consumption statistics are only available for a limited number of countries but from these it is apparent that the pattern of use can vary considerably from country to country (Wilson, 1988). Examination of the reported trends in cadmium consumption over the last 25 years reveals considerable changes in the relative importance of the major applications. The use of cadmium for electroplating represents the most striking decrease in 1960 this sector accounted for over half the cadmium consumed worldwide, but in 1985 its share was less than 25% (Wilson, 1988). In contrast, the use of cadmium in batteries has shown considerable growth in recent years from only 8% of the total market in 1970 to 37% by 1985. The use of cadmium in batteries is particularly important in Japan and represented over 75% of the total consumption in l985 (Wilson, 1988). Of the remaining applications of cadmium, pigments and stabilizers are the most important, accounting for 22% and 12%, respectively, of the world total in 1985. The share of the market by cadmium pigments remained relatively stable between 1970 and l985 but the use of the metal in stabilizers during this period showed a considerable decline, largely as a result of economic factors. The use of cadmium as a constituent of alloys is relatively small and has also declined in importance in recent years, accounting for about 4% of total cadmium use in l985 (Wilson, 1988).

1.1.2 Sources of Cadmium

Cadmium is a relatively rare element and current analytical procedures indicate much lower concentrations of the metal in environmental media than did previous measurements. At present, it is not possible to determine whether human activities have caused a historic increase in cadmium levels in the polar ice caps. Cadmium is widely distributed in the earth's crust at an average concentration of about 0.1 mg/kg. However, higher levels may accumulate in sedimentary rocks, and marine phosphates often contain about 15 mg cadmium/kg. Weathering also results in the riverine transport of large quantities of cadmium to the world's oceans and this represents a major flux of the global cadmium cycle; an annual gross input of 15 000 tonnes has recently been estimated (Friberg et al., 1992). Volcanic activity is a major natural source of cadmium release to the atmosphere. Emissions of cadmium take place both during episodic eruptions and continuous low-level activity. Difficulties exist in quantifying the global flux from this source but an estimate of 100-500 tonnes has been made. Deep sea volcanism is also a source of environmental cadmium release, but the role of this process in the global cadmium cycle remains to be quantified. Ice and snow deposits from the polar regions represent a unique historical record of pollutants in atmospheric precipitation. However, the problems of contamination are great and no reliable data are at present available from historic samples; this prevents an insight into temporal changes in the cycling of cadmium. Nevertheless, current ice samples have been analysed; those from the Arctic contain on average 5 pg/g, while corresponding values from the Antarctic (0.3 pg/g) are much lower (Wolff & Peel, 1985). Commercial cadmium production started at the beginning of this century. The pattern of cadmium consumption has changed in recent years with significant decreases in electroplating and increases in batteries and specialized electronic uses. Most of the major uses of cadmium employ cadmium in the form of compounds that are present at low concentration; these features constrain the recycling of cadmium (Friberg et al., 1992). Restrictions on certain uses of cadmium imposed by a few countries may have widespread impact on these applications. Cadmium is released to the air, land, and water by human activities. In general, the two major sources of contamination are the production and consumption

of cadmium and other non-ferrous metals and the disposal of wastes containing cadmium. Areas in the vicinity of non-ferrous mines and smelters often show pronounced cadmium contamination. Increases in soil cadmium content result in an increase in the uptake of cadmium by plants; the pathway of human exposure from agricultural crops is thus susceptible to increases in soil cadmium. The uptake by plants from soil is greater at low soil pH. Processes that acidify soil (e.g., acid rain) may therefore increase the average cadmium concentrations in foodstuffs (Friberg et al., 1992). The application of phosphate fertilizers and atmospheric deposition are significant sources of cadmium input to arable soils in some parts of the world; sewage sludge can also be an important source at the local level. These sources may, in the future, cause enhanced soil and hence crop cadmium levels, which in turn may lead to increases in dietary cadmium exposure. In certain areas, there is evidence of increasing cadmium content in food. Edible free-living food organisms such as shellfish, crustaceans, and fungi are natural accumulators of cadmium. There are increased levels of cadmium in the liver and kidney of horses and some feral terrestrial animals, as in the case of humans. Regular consumption of these items can result in increased exposure. Certain marine vertebrates contain markedly elevated renal cadmium concentrations, which, although considered to be of natural origin, have been linked to signs of kidney damage in the organisms concerned (Friberg et al., 1992).

1.1.3 Environmental Transport, Distribution, and Transformation

1.1.3.1 Atmospheric Deposition

Cadmium is removed from the atmosphere by dry deposition and by precipitation. Total deposition rates have been measured at numerous localities worldwide and values have generally been found to increase in the order: background < rural < urban < industrial (Friberg et al, 1992).

1.1.3.2 Transport from Water to Soil

Rivers contaminated with cadmium can contaminate surrounding land, either through irrigation for agricultural purposes, by the dumping of dredged sediments, or through flooding (Forstner, 1980). For example, agricultural land adjacent to the Neckar River, Germany, received dredged sediments to improve the soil, a practice that produced soil cadmium concentrations in excess of 70 mg/kg (Forstner, 1980).

1.1.3.3 Uptake from Soil by Plants

It has been shown repeatedly that an increase in soil cadmium content results in an increased plant uptake of the metal. This has been demonstrated for soils with naturally elevated cadmium levels (Lund et al., 1981), those contaminated by non-ferrous metal mining, and those that have received cadmium via sewage sludge applications (Davis & Coker, 1980). It is this basic relationship that makes the soil-crop pathway of human exposure susceptible to increased levels of soil cadmium. Indeed, since the major sources of cadmium exposure for the general population are food and tobacco, it is important to assess those soil and plant factors that influence cadmium uptake by crop plants. The most important soil factors influencing plant cadmium accumulation are soil pH and cadmium concentration (Davis & Coker, 1980). Soil cadmium is distributed between a number of pools or fractions, of which only the cadmium in soil solution is thought to be directly available for uptake by plants. Soil pH is the principal factor governing the concentration of cadmium in the soil solution. Cadmium absorption to soil particles is greater in neutral or alkaline soils than in acidic ones and this leads to increased cadmium levels in the soil solution. As a consequence, plant uptake of cadmium decreases as soil pH increases. Other soil factors that influence the distribution of cadmium between the soil and soil solution include cation exchange capacity and the contents of the hydrous oxides of manganese and iron, organic matter, and calcium carbonate. Increases in these parameters result in decreased availability of cadmium to plants owing to a reduction of the level of cadmium in the soil solution.

Much attention has been paid to the plant availability of cadmium in agricultural soils to which sewage sludge has been applied. It has been observed that the repeated application of sludge to soils can alter the availability of cadmium, and although soil cadmium levels may increase, crop levels do not always reflect this increase (Page et al., 1981). The long-term availability of cadmium to plants is uncertain, availability having been reported to remain constant, decrease, or even increase with time. In another study there were no clear changes in the plant availability of cadmium over a period of five years after sewage sludge was applied to the soil (Carlton-Smith, l987).

Concern over the long-term implications of present-day cadmium inputs to European arable soils has led to modelling studies of the future cadmium exposure for the general population. It was estimated by Tjell et al. (1981) that cadmium inputs from phosphate fertilizers and atmospheric deposition will cause an annual increase of 0.6% in Danish soil cadmium levels. The corresponding increases in crop cadmium concentrations would lead to a predicted 70% increase in dietary cadmium intake 100 years hence (Friberg et al., 1992).

1.1.3.4 Transfer to Aquatic and Terrestrial Organisms

In general, cadmium concentrations in terrestrial and aquatic biota from uncontaminated localities are low, corresponding to the geochemical abundance of this metal. However, in certain situations, cadmium displays a propensity for marked bioaccumulation, a feature that has implications for human dietary exposure and may be of toxicological significance for the organisms concerned. It appears that cadmium shows greatest mobility in certain marine ecosystems. Phytoplanktons in areas of oceanic upwelling contain raised cadmium levels (Martin & Broenkow, 1975), and filter-feeding molluscs can accumulate significant concentrations of cadmium even in coastal localities that are only moderately contaminated. Oysters, in particular, are well-known cadmium accumulators, levels of up to 8 mg/kg wet weight having been recorded in New Zealand (Nielsen, 1975). Certain edible crustaceans such as crab and lobster also contain relatively high cadmium

concentrations, the metal being localized in the hepatopancreas or "brown meat" (Buchet et al., l983). Terrestrial mosses and lichens display a high capacity for retention of metals deposited from the atmosphere and these plants have been used to map both local contamination from point sources and regional patterns of cadmium deposition. The fruiting bodies of some macrofungi contain remarkably high cadmium concentrations even in areas uncontaminated with cadmium. This phenomenon has implications for human dietary exposure as some accumulator species are edible. In addition to humans, certain long-lived terrestrial mammals such as the horse and moose may possess considerable cadmium burdens in the kidney and liver (Frank et al., 1981; Jeffery et al., 1989). It has been shown that cadmium accumulates with age in horse kidney. High inhalation exposure to cadmium oxide fume results in acute pneumonitis with pulmonary oedema, which may be lethal. High ingestion exposure of soluble cadmium salts causes acute gastroenteritis. Long-term occupational exposure to cadmium has caused severe chronic effects, predominantly in the lungs and kidneys (Friberg et al., 1992). Chronic renal effects have also been seen among the general population. Following high occupational exposure, lung changes are primarily characterized by chronic obstructive airway disease. Early minor changes in ventilatory function tests may progress, with continued cadmium exposure, to respiratory insufficiency. As has occurred in the past, an increased mortality rate from obstructive lung disease has been seen in workers with high exposure. The accumulation of cadmium in the renal cortex leads to renal tubular dysfunction with impaired reabsorption of, for instance, proteins, glucose, and amino acids. A characteristic sign of tubular dysfunction is an increased excretion of low molecular weight proteins in urine. In some cases, the glomerular filtration rate decreases. Increase in urine cadmium correlates with low molecular weight proteinuria and in the absence of acute exposure to cadmium may serve as an indicator of renal effect. In more severe cases there is a combination of tubular and glomerular effects, with an increase in blood creatinine in some cases. For most workers and people in the general environment, cadmium-induced proteinuria is irreversible.

Among other effects are disturbances in calcium metabolism, hypercalciuria, and formation of renal stones. High exposure to cadmium, most probably in combination with other factors such as nutritional deficiencies, may lead to the development of osteoporosis and/or osteomalacia. There is evidence that long-term occupational exposure to cadmium may contribute to the development of cancer of the lung but observations from exposed workers have been difficult to interpret because of confounding factors. For prostatic cancer, evidence to date is inconclusive but does not support the suggestion from earlier studies of a causal relationship. At present, there is no convincing evidence for cadmium being an etiological agent of essential hypertension. Most data speak against a blood pressure increase due to cadmium and there is no evidence of an increased mortality due to cardiovascular or cerebrovascular disease (Friberg et al., 1992).

1.1.4 Human Exposure

Humans normally absorb cadmium into the body either by ingestion or inhalation Dermal exposure (uptake through the skin) is generally not regarded to be of significance (Lauwerys, 1988). It is widely accepted (WHO, 1988: ATSDR, 1997) that approximately 2% to 6% of the cadmium ingested is actually taken up into the body. Factors influencing cadmium absorption are the form in which cadmium is present in the food, and the iron status of the exposed individual. In contrast, from 30% to 64% of inhaled cadmium is absorbed by the body, with some variation as a function of chemical form, solubility and particle size of the material inhaled. Thus, a greater proportion of inhaled cadmium is retained by the body than when cadmium is taken in by ingestion. For the non-occupationally exposed individual, inhalation exposure to cadmium does not usually contribute significantly to overall body burden. The exception to this generalisation is the cigarette smoker. One model for human cadmium intake (Van Assche, 1998)has estimated that ingestion accounts for 95% of total cadmium intake in a non-smoker. For a smoker, this model estimates that roughly 50% of their cadmium intake arises from cigarettes with the balance due to ingestion and the low levels of cadmium naturally present in ambient air. In the past, occupational exposure was also a significant contributor to total cadmium

intake, but with very stringent occupational standards in place today, occupational cadmium intake is much less of a consideration than it was 20 years ago. Thus, the principal determinants of human cadmium exposure today are smoking habits, diet, and, to a certain extent, occupational exposure.

1.1.4.1 Human Intake of Cadmium

1.1.4.1.1 Ingestion. Much of the cadmium which enters the body by ingestion comes from terrestrial foods. This is to say, from plants grown in soil or meat from animals which have ingested plants grown in soil. Thus, directly or indirectly, it is the cadmium present in the soil and the transfer of this cadmium to food plants together with the cadmium deposited out of the atmosphere on edible plant parts which establishes the vast majority of human cadmium intake. Some have estimated that 98% of the ingested cadmium comes from terrestrial foods, while only 1% comes from aquatic foods such as fish and shellfish, and 1% arises from cadmium in drinking water (Van Assche, 1998).

The cadmium content of terrestrial foods varies significantly as a function of the type of food crop grown, the agricultural practices pursued, and the atmospheric deposition of cadmium onto exposed plant parts. Cadmium levels in the soil, principally derived from natural sources, phosphate fertilisers and sewage sludge will naturally impact upon this cadmium uptake.

1.1.4.1.2 Cadmium Intake from Foods. Many studies have attempted to establish the average daily cadmium intake resulting from foods. In general, these studies show that the average daily diet for non-smokers living in uncontaminated areas is at present at the low end of the range of 10 to 25 μg of cadmium (Elinder, 1985: OECD, 1994: ATSDR, 1997). This general trend is confirmed by decreasing blood cadmium levels in the general population in several countries during this time period (Ducoffre, 1992: MURL, 1989). In a rather recent evaluation, the International Programme on Chemical Safety (IPCS) assessed the average daily intake at the lower end of this range (WHO, 1992).

The World Health Organisation (WHO) has established a provisional tolerable weekly intake (PTWI) for cadmium at 7 µg/kg of body weight. This PTWI weekly value corresponds to a daily tolerable intake level of 70 µg of cadmium for the average 70-kg man and 60 µg of cadmium per day for the average 60-kg woman. Clearly, the daily cadmium intake for the general population from food, which is by far the dominant source of cadmium, is well below the guidelines established by the World Health Organisation. The average daily cadmium intake for the general population in the Western World has shown a distinct downward trend from 1970 through 1992 (Van Assche, & Ciarletta, 1992) a reduction presumed to be due to the marked decreases in direct atmospheric deposition of cadmium onto crops and soils. Other studies have suggested that, over the timeframe of 1980 - 1985, levels of cadmium intake have been relatively constant (OECD, 1994). At an absorption rate of 5% from ingestion, the average person is believed to retain about 0.5 to 1.0 µg of cadmium per day from food.

There is considerable information in the literature regarding the cadmium contents of foods grown in contaminated areas (Elinder, 1985: WHO, 1992: OECD, 1994). Detailed studies have indicated that only a small percentage of these contaminated areas were actually utilised for growing foods which were subsequently consumed with the exception of rice fields in Japan where considerable cadmium did find its way into the average person's diet through rice grown on contaminated rice fields (Elinder, 1985). In specific cases, management measures to reduce the transfer of cadmium from historically contaminated soils into the local food chain have proven successful (Staessen et al., 1991).

1.1.4.1.3 Inhalation. Cadmium inhalation is a far smaller contributor to total cadmium body burden except in the cases of smokers or some highly exposed workers of the past. Today, the inhalation route is well controlled in the occupational setting, and is well-controlled from point sources such as those which directly pertain to the non-ferrous, cadmium or cadmium products industries. Ambient air emissions from fossil fuel power generation plants, the iron and steel industry and other major industries where cadmium may be present as a low concentration impurity, on the

other hand, may be substantial because the volumes of the waste gases generated are substantial.

Cadmium Intake From Cigarette Smoking - Smokers absorb amounts of cadmium comparable to those from food, about 1 to 3 µg of cadmium per day, from the smoking of cigarettes. It has been reported that one cigarette contains about 1 - 2 µg of cadmium and that about 10% of the cadmium content is inhaled when the cigarette is smoked (WHO, 1992). Cigarette construction, the use of filters and variations in the cadmium contents of tobaccos could decrease cadmium exposure by this route, but in general cigarette smoking is a habit which can more than double the average person's daily cadmium intake. Cigarette smokers who are also occupationally exposed may increase their total cadmium intake even further.

Cadmium Intake From Occupational Exposure - Up to the l960's, very elevated cadmium in air exposure levels were measured in some workplaces, sometimes as high as 1 mg/m^3. Since that time, workplace exposures and standards have decreased markedly so that most occupational exposure standards today are in the range from 2 to 50 $\mu g/m^3$. The result has been that occupational exposures today are generally below 5 $\mu g/m^3$, and most cadmium workers are exposed at levels which are considered to be safe (ATSDR, 1997). In rare cases where cadmium air levels are higher, the use of personal protective equipment is obligatory. Extensive preventative hygiene programs and medical follow-up programs have been developed to control the risk related to cadmium exposure at the workplace (ACGIH, 1996: OSHA, 1992: Lauwerys, 1986. Considering present levels of occupational exposure cadmium intake, general dietary intake, and cigarette smoking intake, it still would appear, however, that the average daily cadmium intake is well below the values recommended by the World Health Organisation.

1.1.5 Effects on Humans

Exposure to cadmium produces a wide variety of effects involving many organs and systems. From the point of view of preventive medicine, the detection of early effects on the kidneys is of particular importance in order to prevent more serious renal effects and those on the lungs or bones. Recent studies have been indicated that chronic exposure to cadmium may give rise to cancer (Friberg et al., 1992).

Acute cadmium poisoning and, in some cases, death have been reported among workers shortly after exposure to fumes when cadmium metal or cadmium-containing materials have been heated to high temperatures. Food contamination arose when acid foods and drinks were prepared and stored in contact with cadmium-plated surfaces. Rapid onset with severe nausea, vomiting, and abdominal pain were characteristic symptoms. Effects also occurred following the consumption of drinks with a cadmium concentration of approximately 16 mg/litre from an automatic vending machine in which drinking-water was cooled in a tank constructed with cadmium-containing solder. Lower cadmium concentrations with longer periods of exposure than those described above will cause chronic cadmium poisoning. Fully developed poisoning among industrial workers shows two main effects: renal dysfunction and emphysema. The kidney is most frequently the critical organ, but under certain conditions (short-term peak exposures) it may be the lung. For people in the general environment, exposure is usually by the oral route and the kidney is the critical organ (Friberg et al., 1992).

The available data show that cadmium can affect calcium, phosphorous, and bone metabolism in both industrial workers and people exposed in the general environment. These effects may be secondary to the cadmium effects on the kidneys but there have been few studies of calcium metabolism in people with excess exposure to cadmium. The increased prevalence of renal stones reported from certain industries is probably one manifestation of the cadmium-induced kidney effects. It is not known if factors other than cadmium play a role.

The International Agency for Research on Cancer and the US National Toxicology Program has both concluded that there is adequate evidence that cadmium is a human carcinogen. This designation as a human carcinogen was prompted primarily by repeated findings of an association between occupational cadmium exposure and lung cancer, as well as very strong rodent data, which included the pulmonary system as a target site. Thus, the lung is the most definitively established site of human carcinogenesis from cadmium exposure. In some studies, occupational or environmental cadmium exposure has also been associated with development of cancers of the prostate, kidney, liver, hematopoietic system and stomach. Clearly, further epidemiological and experimental work is necessary to determine the target sites and nature of the carcinogenic risk from cadmium exposure to humans.

Removal of excesses of cadmium ions from wastewaters or plasma is essential due to their extreme toxicity towards aquatic life and humans. Heavy metal ions from wastewaters are commonly removed by chemical precipitation, ion-exchange, and reverse osmosis processes. These methods have several disadvantages such as unpredictable metal ions removal, high reagent requirements, and the generation of toxic sludges which are often difficult to dewater and also require extreme caution in their disposal. These disadvantages can become pronounced at low metal concentrations in contaminated groundwaters, mine tailings effluent and other industrial wastewaters. There is a need for innovative treatment technologies for the removal of heavy metal ions from wastewater. Besides these methods, affinity chromatography is another method which is used for removal of cadmium. This method can be adjusted high selectivity to cadmium ions and also it can be more effective in low concentrations compared to the other methods. In our study, we used affinity chromatography medthod and chose specific matrix for Cd^{2+} ions because of advanatages of affinity chromatography mentioned above.

1.2 Affinity Chromatography

Affinity chromatography separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand coupled to a chromatographic matrix. The technique is ideal for a capture or intermediate step in a purification protocol and can be used whenever a suitable ligand is available for the protein(s) of interest. With high selectivity, hence high resolution, and high capacity for the protein(s) of interest, purification levels in the order of several thousand-fold with high recovery of active material are achievable. Target protein(s) is collected in a purified, concentrated form. Biological interactions between ligand and target molecule can be a result of electrostatic or hydrophobic interactions, van der Waals' forces and/or hydrogen bonding. To elute the target molecule from the affinity medium the interaction can be reversed, either specifically using a competitive ligand, or non-specifically, by changing the pH, ionic strength or polarity. In a single step, affinity purification can offer immense time-saving over less selective multistep procedures. The concentrating effect enables large volumes to be processed. Target molecules can be purified from complex biological mixtures, native forms can be separated from denatured forms of the same substance and small amounts of biological material can be purified from high levels of contaminating substances. Affinity chromatography owes its name to the exploitation of these various biological affinities for adsorption to a solid phase (Jonson, 1998; Wilcheck, 1984). One of the members of the pair in the interaction, the ligand, is immobilized on the solid phase, whereas the other, the counterligand (most often a protein), is adsorbed from the extract that is passing through the column. Examples of such affinity systems are listed in Table 1.1.

Affinity sorption requires that the compound to be isolated is capable of reversibly binding (i.e., sorption-elution) to a sorbent which consists of a complementary substance (i.e., the so-called ligand) immobilized on a suitable insoluble support, i.e., the so-called carrier.

Table 1.1 Examples of Biological Interactions Used in Affinity Chromatography.

Ligand	Counter ligand
Antibody	Antigen, virus, cell
Inhibitor	Enzyme (ligands are often substrate analogs or cofactor analogs)
Lectin	Polysaccharide, glycoprotein, cell surface receptor, membrane protein, cell
Nucleic acid	Nucleic acid binding protein (enzyme or histone)
Hormone, vitamin	Receptor, carrier protein
Sugar	Lectin, enzyme, or other sugar-binding protein

Schematic represantation of bioaffinity is shown in Figure 1.1. Affinity chromatography demonstrated in this figure is based on the simple principle that every biomolecule usually recognize another natural or artificial molecule. A wide variety of ligands may be covalently attached to an inert support matrix, and subsequently packed into a chromatographic column.

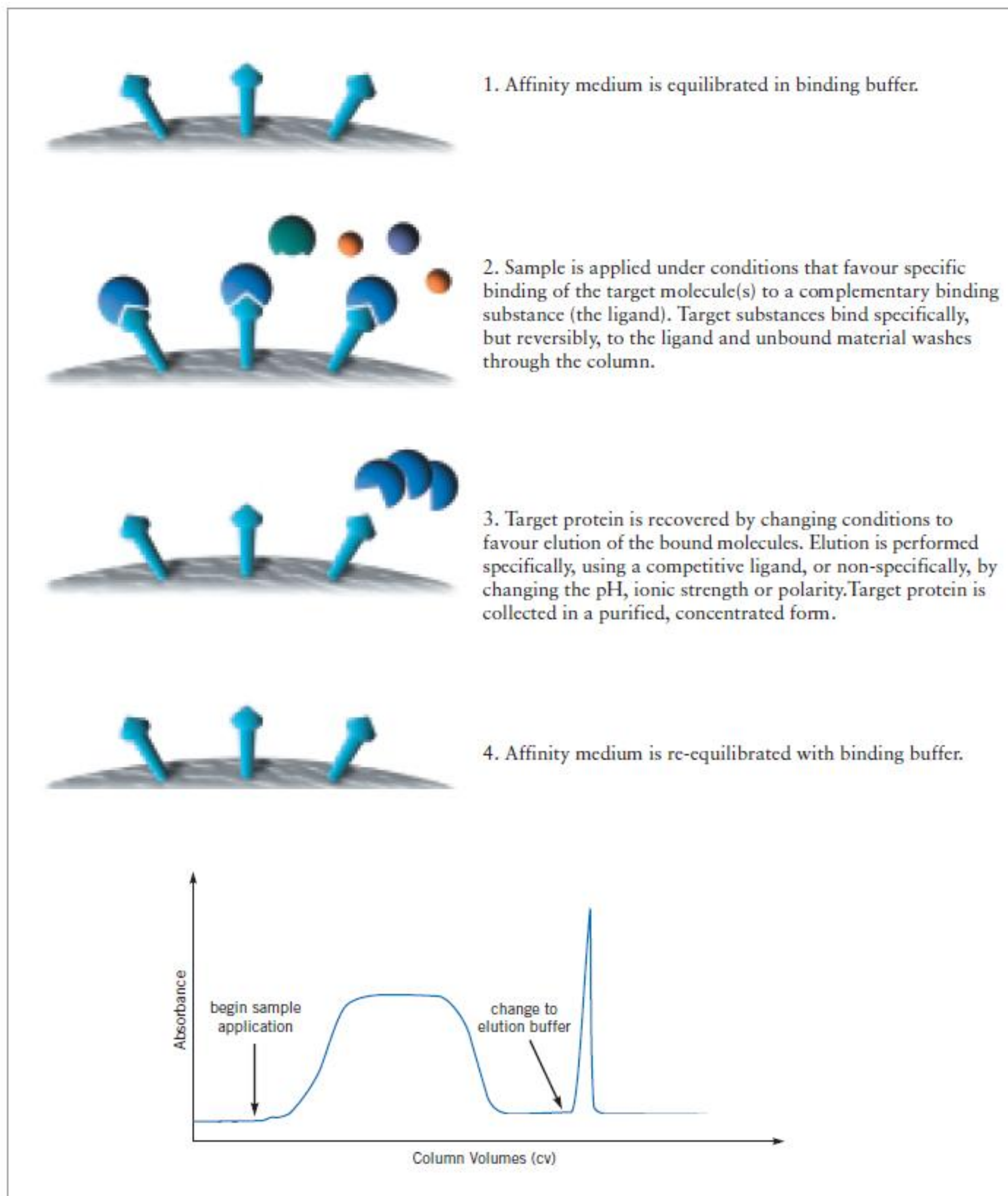

Figure 1.1 Schematic representation of the main steps in affinity chromatoraphy.

The term affinity chromatography has been given quite different connotations by different authors. Sometimes it is very broad, including all kinds of adsorption chromatographies based on nontraditional ligands, in the extreme all chromatographies except ion exchange. Often it is meant to include immobilized metal ion affinity chromatography (IMAC), covalent chromatography, hydrophobic interaction chromatography, and so on. In other cases it refers only to ligands based

on biologically functional pairs, such as enzyme-inhibitor complexes. The term not only to include functional pairs but also the so-called biomimetic ligands, particularly dyes whose binding apparently often occurs to active sites of functional enzymes although the dye molecules themselves of course do not exist in the functional context of the cell. Thus chromatography based on the formation of specific complexes such as enzyme-substrate, enzyme-inhibitor, etc., i.e on biological recognition, is termed bioaffinity or biospecific chromatography and the respective interaction-biospecific adsorption or bioaffinity (Porath, 1973). The original term "affinity chromatography" acquired a broader meaning also including hydrophobic chromatography, covalent chromatography, metal-chelate chromatography, chromatography on synthetic ligands, etc., i.e chromatography procedures based on different, less specific types of interaction. The broad scope of the various applications of affinity has generated the development of subspecialty techniques, many of which are now recognized by their own nomenclature. Table 1.2 summarizes some of these techniques. As can be seen from Table 1.2, some of these subcategories have become accepted useful techniques (Wilcheck, & Miron, 1999).

Table 1.2 Subcategories of affinity chromatography.

Affinity Chromatography	No.	Subcategory
	1.	Hydrophobic Chromatography
	2.	Immunoaffinity Chromatography
	3.	Covalent AC
	4.	Metal-Chelate AC
	5.	Molecular Imprinting Affinity
	6.	Membrane-Based AC
	7.	Affinity Tails Chromatography
	8.	Lectin Affinity
	9.	Dye-Ligand AC
	10.	Reseptor Affinity
	11.	Weak AC
	12.	Perfusion AC
	13.	Thiophilic Chromatography
	14.	High Performance AC
	15.	Affinity Density Pertubation
	16.	Library-Derived Affinity
	17.	Affinity Partitioning
	18.	Affinity Electrophoresis

Very often the use of affinity chromatography requires that the investigator synthesizes the adsorbent. The methods for doing this, which are described later, are well worked out and are also easily adopted for those not skilled in synthetic organic chemistry. To further simplify the task, activated gel matrices ready for the reaction with a ligand are commerically available. The immobilization of a ligand can, in the best cases, be a very simple affair. In addition, immobilizations are just as easy for proteins as for small molecules.

A property that needs special consideration is the association strength between ligand and counterligand. If it is too weak there will be no adsorption, whereas if it is too strong it will be difficult to elute the protein adsorbed. It is always important to find conditions, such as pH, salt concentration, or inclusion of, for example, detergent or other substances, that promote the dissociation of the complex without destroying the active protein at the same time. It is often here that the major difficulties with affinity methods are encountered. Ligands can be extremely selective, but they may also be only group specific. The latter type includes glycoprotein-lectin interactions, several dye-enzyme interactions, and interactions with immobilized cofactors. However, these interactions have also proved to be extremely helpful in solving many separation problems. Good examples are ligands that are group selective against immunoglobulins (e.g., staphylococcal protein A or streptococcal protein G) (Janson & Ryden, 1998).

1.2.1 Dye-Ligand Afinity Chromatography

In affinity chromatography a molecule having specific recognition capability (“ligand” or “binder”) is immobilized on a suitable insoluble support (“matrix” or “carrier”), which is usually a polymeric material in bead or membrane form. The molecule to be isolated (“analyte” or “target”) is selectively captured (“adsorbed”) by the complementary ligand immobilized on the matrix by simply passing the solution containing the target through the chromatographic column under favorable conditions. The target molecules are then eluted (“desorbed”) by using proper eluants

under conditions favoring desorption, by adjusting the pH, ionic strength or temperature, 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. Since its first introduction (Cuatrecasas, Wilchek, & Anfinsen, 1968), thousands of different molecules (enzymes, antibodies, hormones, vitamins, receptors, many variety of other proteins and glycoproteins, RNA, DNA, etc.), even bacteria, viruses, and cells have been separated or purified by affinity chromatography (Deutscher, 1990; Godfrey, 1997; Scouten, 1981; Turkova, 1993). 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. Dye-ligands have been considered as one of the important alternatives to natural counterparts for specific affinity chromatography to circumvent many of their drawbacks, mentioned above (Denizli, & Pişkin, 2001).

Dye-ligands are able to bind most types of proteins, especially enzymes, in some cases in a remarkably specific manner. They are commercially available, inexpensive, and can easily be immobilized, especially on matrices bearing hydroxyl groups (Denizli, & Pişkin, 2001), stable against biological and chemical attack, storage adsorbent without loss of activity, reusable: cleaning and sterilization, high capacity (Boyer, & Hsu, 1992). Although dyes are all synthetic in nature, they are still classified as affinity ligands because they interact with the active sites of many proteins by mimicking the structure of the substrates, cofactors, or binding agents for those proteins (Denizli, & Pişkin, 2001).

1.3 Cryogel

Cryogels are formed in moderately frozen solutions of monomeric or polymeric precursors. Cryogels typically have interconnected macropores (or supermacropores), allowing unhindered diffusion of solutes of practically any size, as well as mass transport of nano- and even microparticles. The unique structure of cryogels, in combination with their osmotic, chemical and mechanical stability, makes them attractive matrices for chromatography of biological nanoparticles (plasmids, viruses, cell organelles) and even whole cells. At present, polymeric gels have applications in many different areas of biotechnology including use as chromatographic materials, carriers for the immobilization of molecules and cells, matrices for electrophoresis and immunodiffusion, and as a gel basis for solid cultural media. A variety of problems associated with using polymer gels, as well as the broad range of biological objects encountered, lead to new, often contradictory, requirements for the gels. These requirements stimulate the development and commercialization of new gel materials for biological applications. One of the new types of polymer gels with considerable potential in biotechnology is 'cryogels' (from the Greek krios (kryos) meaning frost or ice). Cryogels are formed as a result of cryogenic treatment (freezing, storage in the frozen state for a definite time and defrosting) of low- or highmolecular- weight precursors, as well as colloid systems–all capable of gelling. Cryogels were first reported, 40 years ago and their properties, which are rather unusual for polymer gels, soon attracted attention. The biomedical and biotechnological potential of these materials has now been recognized (Lozisky et al., 2003).

Given that the biotechnological applications of cryogels are discussed, they deserve a more detailed consideration, especially regarding the properties that distinguish them from other gel types. The formation of cryogels is schematically presented in Figure 1.2, in which the features of cryotropic gelation processes are also listed. Cryotropic gelation produces polymeric materials with essentially different morphology compared with gels obtained in non-frozen systems. Cryogels could be of any chemical type–covalent, ionic or non-covalent. Obviously, only the

precursors of heat induced (thermotropic) gels cannot be used for the preparation of cryogels. With some exceptions, freeze-dried polymeric materials soaked in solvent (in which the polymer swells without dissolution) can be considered as materials with macro and microstructure similar to that of cryogels. The solvent freezing followed by the sublimation of solvent crystals (ice in case of aqueous systems) forms a system of interconnected pores in the polymeric material. However, no gel formation takes place perse in unfrozen liquid microphase (Figure 1.2, unfrozen liquid microphase, UFLMP). Freeze-dried materials can be produced only as relatively thin objects, for example, films, plates or small beads. The production of freeze-dried cylinders or thick blocks is impractical from a technical point of view. On the contrary, cryogels can be formed in any desirable shape, for example, blocks, cylinders, tubes, granules and disks. Moreover, the production of cryogels is simpler than production of freze-dried materials because solvent removal under reduced pressure is not necessary. A system of large interconnected pores is a main characteristic feature of cryogels; some cryogels possess spongy morphology. The pore system in such sponge-like gels ensures unhindered convectional transport of solutes within the cryogels, contrary to diffusion of solutes in traditional homophase gels. The size of macropores within cryogels varies from tens or even hundreds to only a few micrometers–micrographs in Figure 1.2 illustrate these cases. The interconnected system of large pores makes various cryogels promising materials for the production of new chromatographic matrices tailormade for the separation of biological nano- and microparticles (plasmids, viruses, cell organelles and even intact cells), and also for the implementation as carriers for immobilization of molecules and cells (Lozisky et al., 2003).

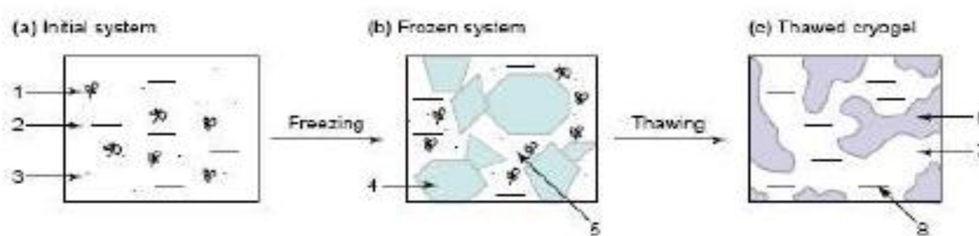

Figure 1.2 1, macromolecules in a solution; 2, solvent; 3, low-molecular solutes; 4, polycrystals of frozen solvent; 5, unfrozen liquid microphase; 6, polymeric framework of a cryogel; 7, macropores; 8, solvent.

The main characteristic features of the cryotropic gelation processes;

1) The reaction mixture containing gel-forming agents is frozen at temperatures a few degrees centigrade below the solvent crystallization point. The frozen system, despite looking as a single solid block, remains essentially heterogeneous and contains so-called unfrozen liquidmicrophase (UFLMP) along with the crystals of the frozen solvent.
2) Gel-forming reagents are concentrated in UFLMP, that is, cryoconcentration takes place. As UFLMP presents only a small portion of total initial volume, the concentration of gel precursors increases dramatically promoting the gel-formation. In fact, owing to cryoconcentration, the gel formation in such frozen systems proceeds sometimes faster than in liquid medium, when using the same initial concentration of precursors.
3) The crystals of frozen solvent perform as a pore-forming agent. When melted, they leave voids, macropores filled with the solvent. The surface tension between solvent and gel phase rounds the shape of the pores, making pore surface smoother. When freezing, the solvent crystals grow till they meet the facets of other crystals, so after thawing a system of interconnected pores arises inside the gel. The dimensions and shape of the pores depend on many factors, the most important are the concentration of precursors and the regimes of cryogenic treatment.
4) The polymer phase of the cryogel has, in turn, micropores formed in between the polymer chains. Thus, cryogels have both heterophase and heteroporous structure (Figure 1.2).

For biomedical and biotechnological applications porous gels are usually modified with a ligand capable of selective interactions with some biological target, e.g., ligands (ionexchange, hydrophobic, affinity) in chromatographic matrices to ensure selective binding of proteins or plasmid DNA; anchoring peptides or proteins in scaffolds for tissue engineering to ensure attachment and proliferation of cells; catalytically active enzymes in biocatalysts etc. (Plieva et al., 2005)

In literature, there has been different studies related cadmium removal. Some of them summarized as follows:

Denizli and his colleagues have attempted to use new chelate-forming microspheres for Cd(II) poisoning. Reactive dye-ligand Cibacron Blue F3GA was covalently attached onto the poly(HEMA–EGDMA) microspheres. Then a metallopeptide (i.e., thionein) was bound to the Cibacron Blue F3GA-incorporated microspheres. They were used for cadmium removal from human plasma contaminated with Cd(II). The maximum adsorption capacity of the beads was determined to be 11.8 $mmol/cm^2$ on the average (Denizli et al., 1998).

In another study, a wood-based activated carbon (AUG WHK) was used to remove Cd^{2+}. It was oxidized electrochemically to enhance its metal binding capacity and subsequently studied for the removal of cadmium ions from aqueous solution. Treated adsorbents were characterized by N_2 adsorption at 77K before and after oxidation, and a quantitative determination of weak-acid surface groups was carried out by direct titration. Equilibrium isotherms were determined at pH 4, 5 and 6 and showed that there was a slight increase in cadmium uptake with increase in pH. The experimental data were fitted by Langmuir and Freundlich isotherms and it was found that the Freundlich isotherm fitted beter in all the cases. Overall, the results indicated a rapid adsorption rate with over 96% fractional uptake of metal occurring in the first 12 minutes(J. R. Rangel-Mendez et al.,2000).

In the study of Luan et al., granular red mud(GRM) was prepared by a novel method and its potential use to remove cadmium ions from aqueous solutions as a low-cost adsorbent was evaluated. The properties of the novel adsorbent were examined and then used for cadmium adsorption experiments. Batch experiments were conducted and equilibrium isotherms at different temperatures (20 ^oC, 30 ^oC, 40 ^oC) have been determined and analyzed with a Freundlich model. Kinetics data at initial pH 6.0 and 3.0 were fitted with Pseudo-second-order model and external mass transfer coefficients, effective particle diffusion coefficients were subsequently calculated for cadmium–GRM system at initial pH 6.0. The column adsorption was

reversal and the regeneration operation was accomplished by pumping 0.1 mol/L hydrochloric acid through the adsorbed column. The maximum adsorption capacity was determined to be 52.1 mg/g at 40 ^{o}C on the average (Zhaokun Luan et al. 2007).

In another study, researchers used ion-imprinted magnetic beads in the selective removal of Cd^{2+} ions out of human plasma overdosed with Cd^{2+} ions. The Cd^{2+} imprinted magnetic poly(HEMA-MAC) (mPHEMAC-Cd^{2+}) beads were produced by suspension polymerization in the presence of magnetite Fe_3O_4 in a nano-powder form. The template Cd^{2+} ions could be reversibly detached from the matrix to form mPHEMAC-Cd^{2+} beads using 0.1M thiourea solution. The specific surface area of the mPHEMAC-Cd^{2+} beadswas found to be 24.7 m^2/g. The MAC and Fe_3O_4 contents of the mPHEMAC-Cd^{2+} beads were found to be 41.8 µmol/g polymer and 8.2% on the average. The Cd^{2+} adsorption capacity of mPHEMAC-Cd^{2+} columns decreased drastically from 48.8 µmol/g to 20.0 µmol/g as the flow rate is increased from 0.50 ml/min to 3.0 ml/min. The maximum adsorption capacity of the mPHEMAC-Cd^{2+} beads was determined to be 48.8 µmol $Cd^{2+/}g$ on the average. The relative selectivity coefficients of themPHEMAC beads for $Cd^{2+/}Pb^{2+}$ and $Cd^{2+/}Zn^{2+}$ were 22.6 and 160.7 times greater than those of the non-imprinted magnetic PHEMAC (mPHEMAC) beads, respectively. The mPHEMAC-Cd^{2+} beads are reusable for many times with no significant decrease in their adsorption capacities (Candan et al., 2009).

Beause of the toxic properties, cadmium must be removed from the industrial wastes. Conventional methods using removal of heavy metals from industrial wastes are expensive and inefficient. As a new developed method, adsorption, is economic and simple method for removal of heavy metals. Therefore we aimed to prepare a new HEMA–based cryogelic dye-ligand affinity pellets for removal of Cd^{2+} ions and to optimize the system parameters affecting adsorption.

CHAPTER TWO

EXPERIMENTAL METHODS AND MATERIALS

2.1 Materials

2-Hydroxyethylmethacrylate (HEMA), N,N,N',N'-tetramethylethylene diamine (TEMED) and amonium persulfate (APS) were supplied by Fluka (Fluka A.G. (Buchs, Switzerland). N,N'-N,N'-methylene-bis(acrylamide) (MBAAm), sodium chloride and cadmium sulfate were obtained from Sigma (St Louis, USA). All other chemicals were of the highest purity commercially available and were used without further purification. All water used in the experiments was purified using a All water used in the adsorption experiments was purified using a Millipore S.A.S 67120 Molsheim-France facility whose quality management system is approved by an accredited registering body to the ISO 9001. Before use the laboratory glassware was rinsed with deionised water and dried in a dust-free environment.

2.2 Preparation of Poly(HEMA) Cryogelic Pellets

For the purpose of cadmium removal, poly(HEMA) cryogelic pellets were synthesized by free radical polimerization started with TEMED and APS. The steps of synthesis can be summerrized as follows: 0.04 g APS was added to the solution included 2.60 mL HEMA and 0.566 g MBAAm, as a starting agent and then let it stir about 10 min at 150 rpm. After stirring process 50 µl TEMED was added to the solution and immediately planted into the glass plates with a syringe. The plates put into the deepfreeze at -12^{0}C for 24h. Synthesized membranes were washed and cut as the shape of pellets and used for the adsorption studies.

2.2.1 Immobilization of Dye Ligands onto the Poly(HEMA) Cryogelic Pellets

Poly(HEMA) cryogelic pellets were washed with 2L water to remove unreacted monomers. CB and CR immobilization were performed using the book of immobilized affinity ligand techinques (Hermanson, Mallia & Smith, 1992). poly(HEMA) pellets were divided into two parts. First part was bottled up into CB solution (100mg Cibacron Blue F3GA dissolved in 30mL deionized water) and second part was bottled up into CR solution (100mg Congo Red dissolved in 30mL deionized water) and same procedure were applied for each part. Dye solutions were shaked at 150rpm for 30min at 60^0C. 1.5g NaCl was added to reaction mixture and shaked for 1h at 60^0C. Then, temperature of reaction was increased to 70^0C and 0.15g Na_2CO_3 added to reaction mixture. Reaction was continued for 4h at 70^0C. Reaction mixture was cooled to room temperature and pellets were washed with water until washing water are colourless. Finally, pellets were washed with ethanol-water (50%-50%) solution. CB and CR immobilized pellets were stored at 4^0C.

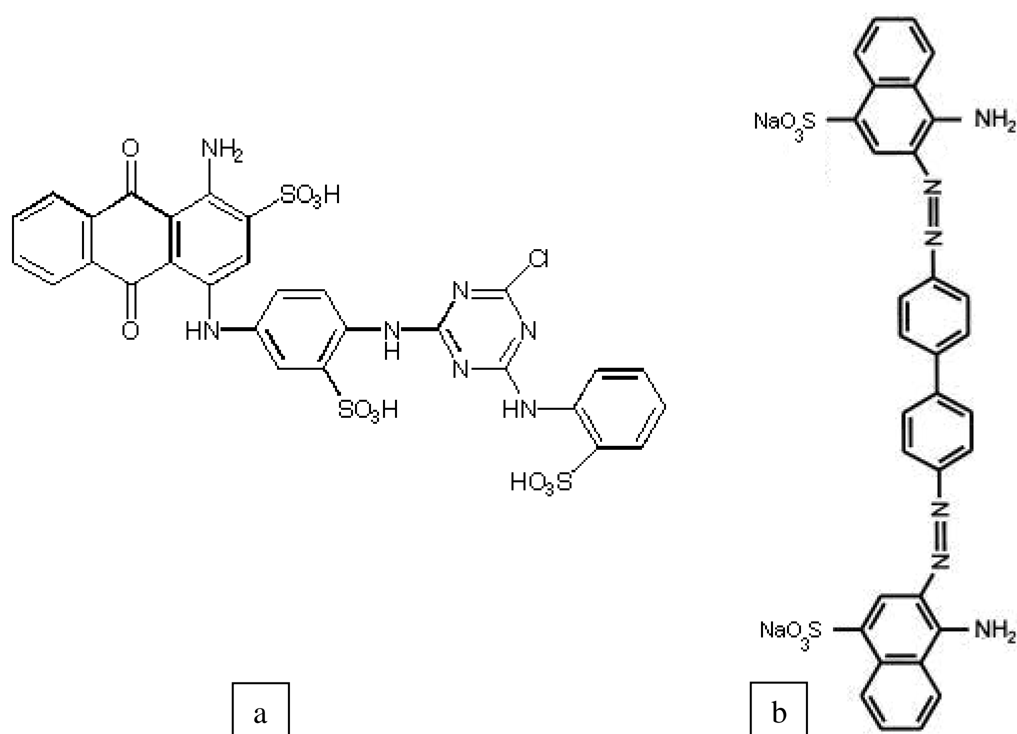

Fig 2.1 Chemical Structure of; (a) Cibacron Blue F3GA (CB) and (b) Congo Red (CR)

2.2.2 Characterization of the Poly(HEMA) Cryogelic Pellets

The swelling degree of the pellets (S) was determined. Pellets were dried to constant mass at vacuum oven at 55^0C and 100 mbar and masses of dried pellets were determined (m_{dry} pellet). The dried pellets were bottled up to 50mL ionized water and masses of swollen pellets were determined regularly for 24h period (m_{wet} pellet).

The swelling degree was calculated as:

$$S = (m_{wet\ pellet} - m_{dried\ pellet}) / m_{dried\ pellet}$$

Percentage of porosity and porosity for macropores were also calculated.

$$\%\ porosity = [(m_{swollen\ pellet} - m_{water\ bound})/m_{swollen\ pellet}] \times 100$$

$$\%\ porosity\ for\ macropores = [(m_{swollen\ pellet} - m_{squeezed\ pellet})/m_{swollen\ pellet}] \times 100$$

The surface morphology of the poly(HEMA) cryogelic pellets were examined using SEM. The samples were initially dried in air at 25°C for 7 days before being analyzed. A fragment of the dried bead was mounted on a SEM sample mount and was sputter coated for 2 min. The sample was then mounted in a scanning electron microscope (SEM, Phillips, XL-30S FEG, Germany). The surface of the sample was then scanned at the desired magnification to study the morphology of the pellets.

FTIR spectra of poly(HEMA) cryogelic pellets and CB and CR-attached poly(HEMA) cryogelic pellets were obtained by using a FTIR spectro-photometer (FTIR 8000 Series, Shimadzu, Japan). The dry pellet (about 0.1 g) was thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a tablet, and the spectrum was then recorded.

2.3 Cadmium Adsorption Studies From Aqueous Solution

Cadmium adsorption studies were performed in a batch system with 1h period at 25°C while stirring continuously in the adsorption medium. Some variables such as time, pH, initial cadmium concentration and temperature were studied to optimize adsorption conditions. To determine effect of time, adsorption was completed at different times such as 15, 30, 45, 60, 75 and 90 min. To observe the effects of pH, pH values were changed between pH 3.0-8.0 by using 0.1M NaOH and HCL solutions. Initial cadmium concentration, was changed between 10-500ppm. Temperature values were changed between 15°C and 45°C to determine the effects of temperature. The amount of adsorbed cadmium per unit mass of the pellet was calculated by using the following expression.

$$q = \frac{C_0 - C_e}{M} V \qquad (1)$$

Where;

q: adsorbed cadmium amount (mg/g)

C_0: initial cadmium concentration (mg/L)

C_e: cadmium concentration at equilibrium (mg/L)

V: volume of cadmium solution (L)

M: amount of pellets used (g)

The adsorption experiments were conducted for 60 min which was the equalibrium period for the adsorption of cadmium at room temperature. Initial and final cadmium concentrations were determined by atomic adsorption Spectrophotometer (AAS).

2.4 Adsorption of Cd^{2+} Ions from Human Plasma

Adsorption of Cd^{2+} ions from human plasma on poly(HEMA)-CR and poly(HEMA)-CB cryogelic pellets was carried out in a batch system. Human blood is collected from thoroughly controlled voluntary blood donors. No preservatives are added to the samples. Human blood was collected into EDTA-containing vacutainers and red blood cells were separated from plasma by centrifugation at 4000 rpm for 30 min at room temperature, then filtered (3 μm Sartorius filter) and frozen at -20°C. Before use, the plasma was thawed for 1 h at 37°C. Then, adsorption stdudies were performed with the overloaded plasma samples by using the same procedure mentioned above.

CHAPTER THREE
RESULTS AND DISCUSSION

3.1. Characterization of Poly(HEMA)-CB and Poly(HEMA)-CR Cryogelic Pellets

Poly(HEMA) cryogel pellets was synthesized by free radical polymerization started with APS and TEMED as initiator/activator pair. The functional hydroxyl groups on the surface of the pores in pellets allowed their modification with the ligands, CR and CB. The FTIR spectra of the poly (HEMA) and poly (HEMA)-dye derived cryogelic pellets are shown in Figure 3.2. The FTIR bands observed around 1160 cm^{-1} was assigned to symmetric stretching of S-O, as also pointed out on the chemical structure of the Cibacron Blue F3GA and Congo Red. The band observed at 3500 cm^{-1} was assigned to the -OH functional group. After CB and CR attachment, the intensity of the -OH band increases due to NH stretching. The split of the band at 3400-3600 cm^{-1} indicates also SO_3H and NH_2 groups. These bands show the attachment of CB and CR within the poly(HEMA) cryogel. The visual observations (the colour of the cryogel) ensured attachment of dye molecules(Fig 3.1)**.**

Figure 3.1 Cibacron Blue and Congo Red attached poly(HEMA) cryogelic pellets.

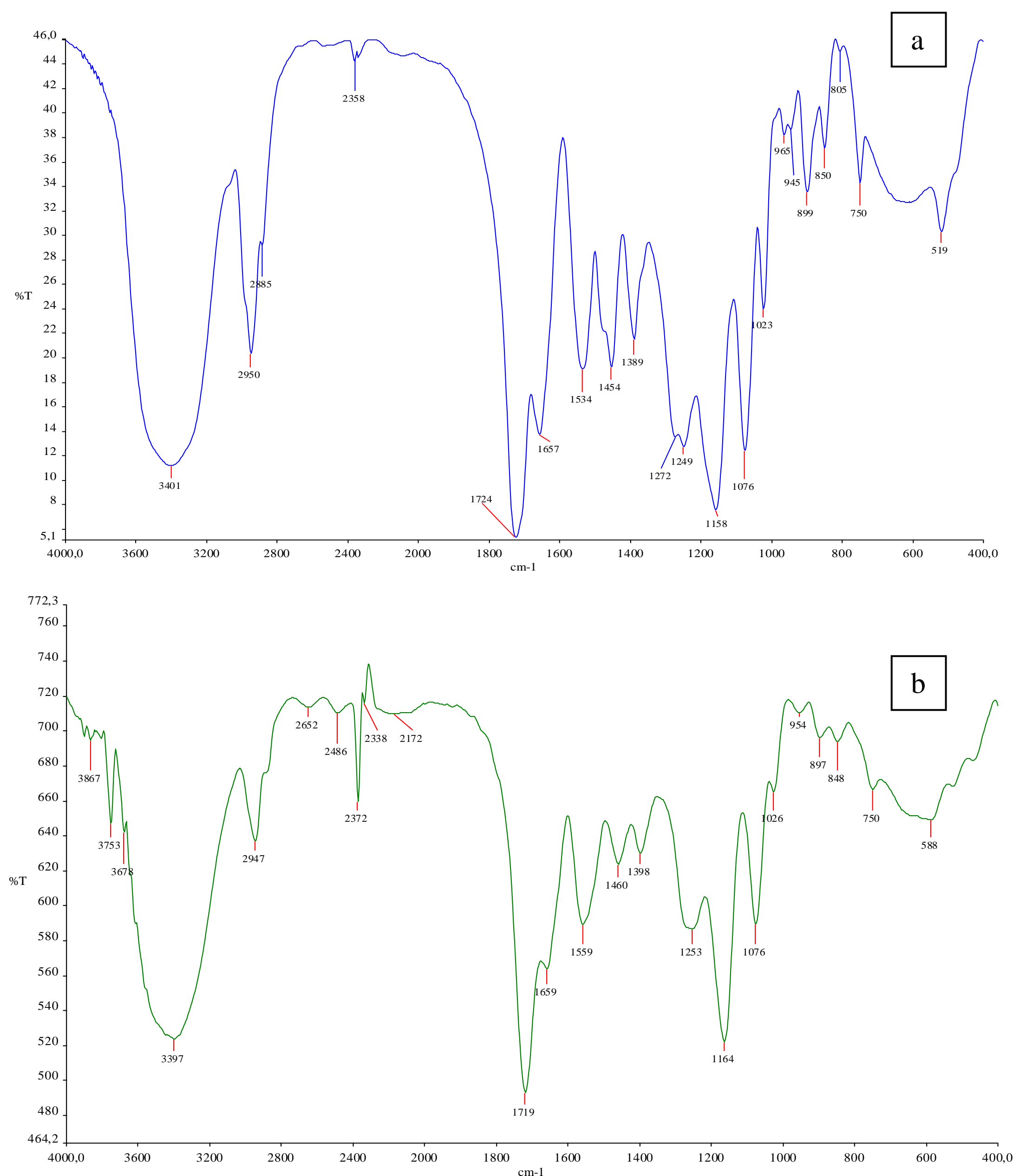

Figure 3.2 FTIR spectrum of; (a) poly(HEMA), (b) poly(HEMA)-dye derived cryogelic pellet

The scanning electron micrograph of the internal structure of the cryogel is shown in Figure 3.3. Poly(HEMA)-dye derived cryogelic pellets produced in such a way have thin polymer walls, large continuous inter-connected that provide channels for the flow through. Pore size of the matrix is large enough allowing the solutions to pass easily. Poly(HEMA) cryogel is opaque and sponge like. This cryogel can be easily compressed by hand to remove water accumulated inside the pores. When the compressed piece of cryogel was submerged in water, it soaked in water and within 1-2 s restored its original size and shape.

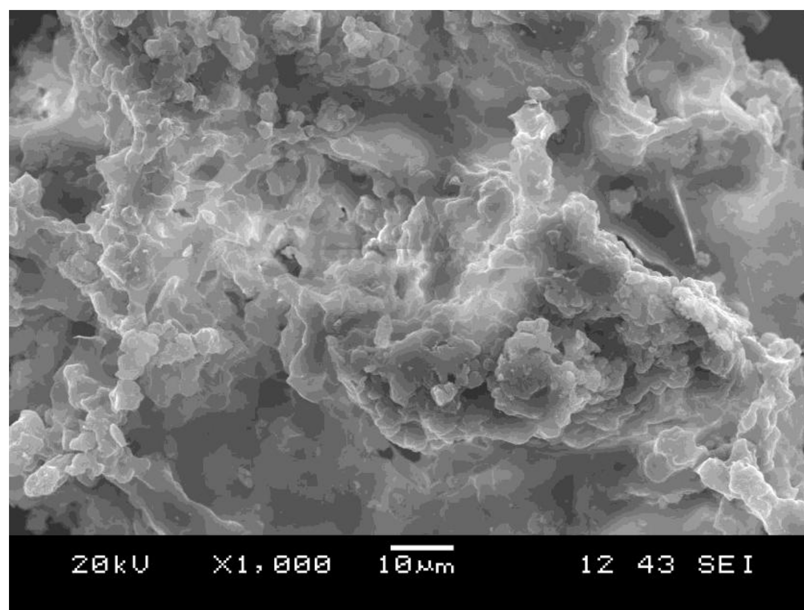

Figure 3.3 Microscopic observations; SEM micrographs of Poly(HEMA)-dye derived cryogelic pellets.

3.2 Optimisation of Cadmium Adsorption

3.2.1 Effect of pH

It is known that solubility, charge species and complex formation of chelating agents are pH dependent (Konishi et al., 1993; Kantipuly et al., 1990). In the absence of complexing chemical substances, the precipitation of the heavy metal ion is affected by the concentration. The solubility of cadmium is governed by hydroxide or carbonate concentration. As discussed in detail by Reed and Matsumoto, precipitation of cadmium ions becomes significant at pH 8.5. For example, the theoretical and the experimental precipitation curves showed that precipitation begins above this pH, which also depends on the concentration of cadmium in the medium. In this study, in order to establish the effect of pH's on the adsorption of Cd^{2+}ions onto the dye-derived cryogelic pellets, we repeated the batch adsorption studies at different pH in the range of 3.0-8.0. Figure 3.4 shows the effect of pH on the specific adsorption (i.e., adsorption by chelating with the dye molecules attached on the poly(HEMA) cryogelic pellets) of Cd^{2+}ions.

As seen in Figure 3.4, adsorption of Cd^{2+} ions increased with increasing pH and maximum adsorption was observed at pH 7.0. The increasing pH of the solution favors complex formation between the dye molecules and Cd^{2+} ions. Cd^{2+} adsorption around pH 2.0-5.0 was very low, may be due to protonation of the functional groups on the structure of dyes. The reason of higher adsorption at higher pH values may be that Cd^{2+} ions interact with CR and CB not only through the nitrogen and oxygen atoms by chelating, but also through $-S0_3H$ groups by cation-exchange, which are unprotonated at high pH. It was also observed with this study that adsorption capacity of the poly(HEMA)-CB cryogelic pellets is higher than the capacity of the the poly(HEMA)-CR cryogelic pellets.

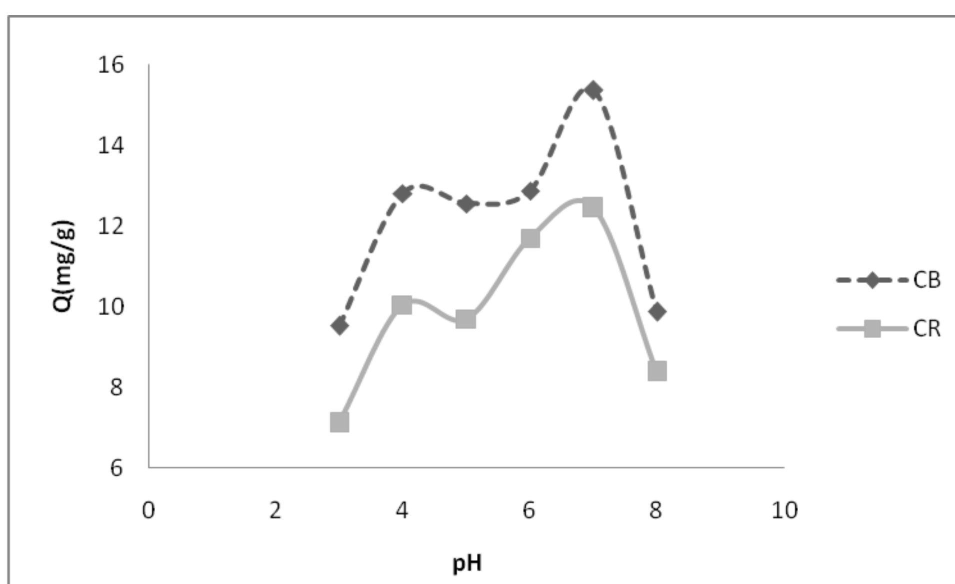

Figure 3.4 Effect of pH on adsorption of cadmium (C: 100 ppm, T: 25 ^{0}C)

3.2.2 Effect of Contact Time

Effect of contact time on cadmium adsorption onto the dye derived poly(HEMA)-cryogelic pellets is shown in figure 3.5. Adsorption capacity increases till 60 minutes for poly (HEMA)-CB cryogelic pellets and after that equilibrium was observed. For poly(HEMA)-CR pellets equilibrium time was observed as 45 minutes.

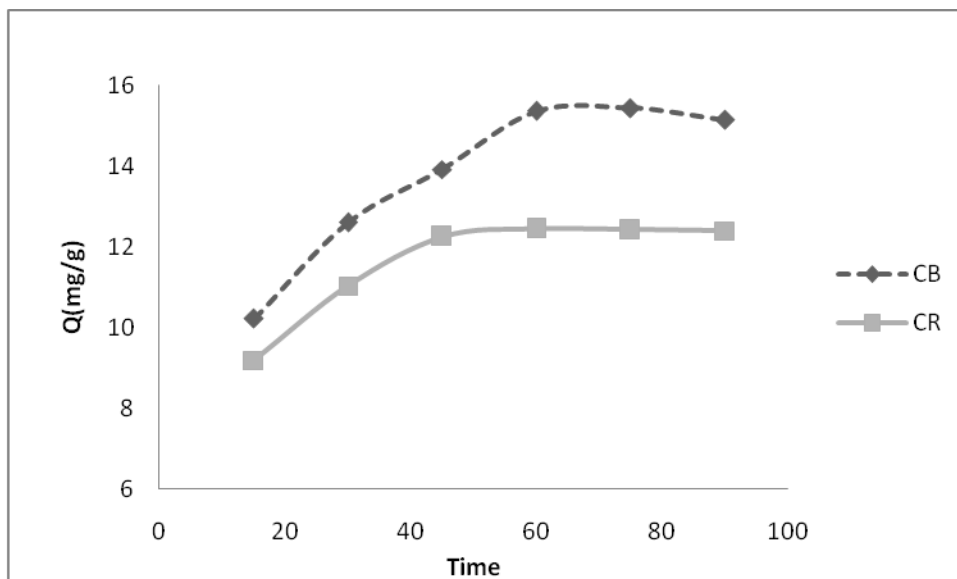

Figure 3.5 Effect of contact time on the cadmium adsorption (C: 100ppm, T: 25^0C, pH:7)

3.2.3 Effect of Initial Cadmium Concentration

Figure 3.6 shows the effect of initial cadmium concentrations on Cd^{2+} adsorption on dye-derived cryogelic pellets. The maximum adsorption capacities are determined as 28,53 mg/g and as 25,49 mg/g for poly(HEMA)-CR and poly(HEMA)-CB, respectively, and the adsorbed amounts per unit mass of cryogel reached to a plateau value at about 200ppm Cd^{2+} concentration. Negligible amounts of Cd^{2+} adsorbed non-specifically on the poly(HEMA) cryogelic pellets (0.18mg/g for Cd^{2+}). It should be also noted that immobilization of dye molecules on the poly(HEMA) cryogelic pellets increases adsorption of Cd^{2+} ions.

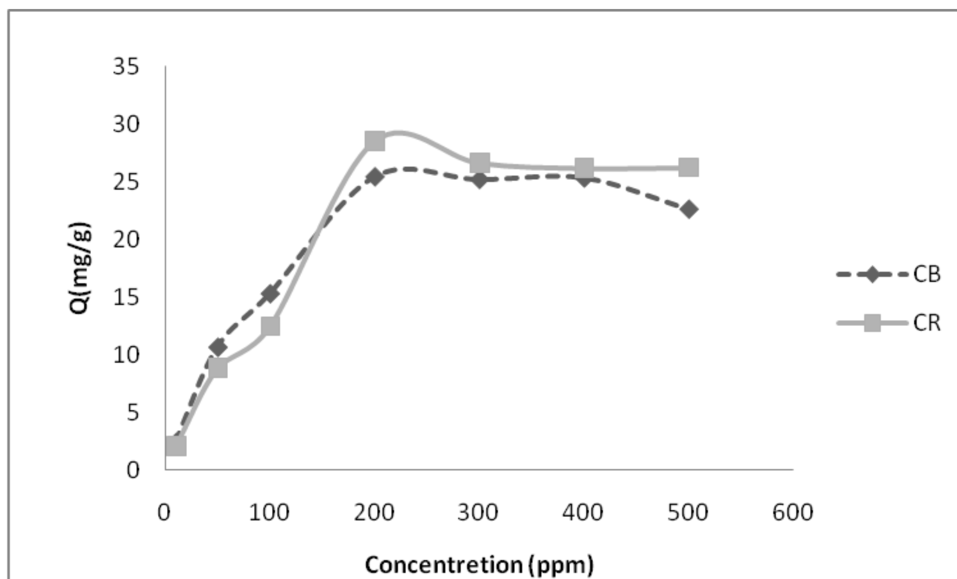

Figure 3.6 effect of initial cadmium concentration (pH: 6 T: 25^0C)

3.2.4 Effect of Temperature

Effect of temperature can be seen in figure 3.7. As seen in figure 3.7, the amount of Cd^{2+} adsorbed onto poly(HEMA)-CR and poly(HEMA)-CB cryogelic pellets decreased about 13.01% and 15.75%, respectively, as temperature changes from 25^0C to 45 ^{0}C. Increasing temperature causes reduction of adsorption capacity because of the exotermic nature of adsorption and may be the physical changes on the matrix surface.

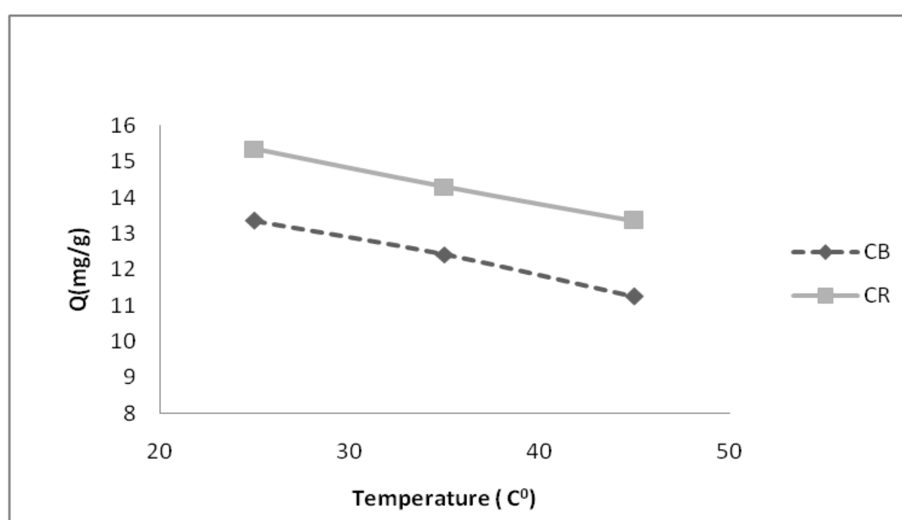

Figure 3.7 Effect of temperature (pH:7 , C:100 ppm, t: 60 dk)

3.2.5 Desorption Studies

Three different desorption agents such as, NaCl, 2-mercaptoethanol and thiourea were used for desorption studies. The desorptions of Cd^{2+} ions are expressed in % of totally adsorbed Cd^{2+} ions. Cadmium adsorbed poly(HEMA)-dye derived cryogelic pellets were placed within the desorption medium containig 0.5M-1.0M NaCl, 0.5M-1.0M mercaptoethanol and 0.5M-1.0M thiourea at room temperature for 6h. Note that there was no CB and CR release in this case which shows that dye-molecules are bonded strongly to poly(HEMA)-dye derived cryogelic pellets. The maximum desorption was observed at 1.0M NaCl solution and desorption values were over than 90% for poly (HEMA)- CB and 95% for poly (HEMA)-CR. With the desorption data given above we concluded that 1.0 M NaCl is a suitable desorption agent, and allows repeated use of the affinity cryogel used in this study.

3.3 Biological Sample Studies

Plasma samples dilueted in the range of 1:0, 1:1, 1:2, and 1:4 and samples were overloaded with cadmium solution then adsorption was performed for 1h under the optimum conditions. The percentages of adsorbed Cd^{2+} ions from human plasma were observed as 5.66%, 15.01%, 43.40% and 47.40% for poly(HEMA)-CB and as 6.61%, 12.27%, 35.85% and 43.40% for the poly(HEMA)-CR, respectively. As seen from these results, adsorption increases with increasing dilution due to decrease of shielding effect of blood components. Considering these results, poly(HEMA)-CR and poly(HEMA)-CB cryogelic pellets can be used for the removal of Cd^{2+} ions from human plasma.

CHAPTER FOUR
CONCLUSION

Polymeric gels have applications in many different areas of biotechnology including use as chromatographic materials, carriers for the immobilization of molecules and cells, matrices for electrophoresis and immunodiffusion, and as a gel basis for solid cultural media. A variety of problems associated with using polymer gels, as well as the broad range of biological objects encountered, lead to new, often contradictory, requirements for the gels. These requirements stimulate the development and commercialization of new gel materials for biotechnological applications. The most attractive feature of polymeric cryogels from the bioseparation view point is the combination of macropores formed by the crystals of frozen solvent and micropores in between polymer macromolecules forming the walls of macropores. The porosity of cryogels makes them appropriate candidates as the basis for such supermacroporous chromatographic materials (Lozinsky et al., 2003). Hence, the introduction of novel polymeric materials with new, sometimes unusual, properties, is of great interest in various areas of biotechnology.

In this study, a supermacroporous cryogelic pellets were synthesized by free radical polymerization of HEMA and two different dyes (CB and CR) were immobilized onto the poly(HEMA) cryogel. These dye derived-poly(HEMA) cryogelic pellets were applied to the removal of cadmium ions from aqueous solutions and human plasma. The maximum adsorption capacities were determined at pH 7.0 as 28,53 mg/g and as 25,49 mg/g for poly(HEMA)-CR and poly(HEMA)-CB, respectively. High desorption ratios observed with this study allow repeated use of the dye derived-poly (HEMA) cryogelic pellets. We also determined that poly(HEMA)-CR and poly(HEMA)-CB cryogelic pellets can be used for the removal of Cd^{2+} ions from human plasma. Our results suggest that dye derived poly(HEMA) cryogelic pellets can be good heavy metal adsorbers for environmental and therpeutic applications.

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