DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

UTILIZATION OF NANOSTRUCTURES WITH HIGH BINDING CAPACITY TO REMOVE PESTICIDE POLLUTION FROM WATER

by
Türkan UÇAR

February, 2012
İZMİR

UTILIZATION OF NANOSTRUCTURES WITH HIGH BINDING CAPACITY TO REMOVE PESTICIDE POLLUTION FROM WATER

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MSc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"UTILIZATION OF NANOSTRUCTURES WITH HIGH BINDING CAPACITY TO REMOVE PESTICIDE POLLUTION FROM WATER"** completed by **TÜRKAN UÇAR** 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.

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Türkan UÇAR

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ABSTRACT

Use of pesticides to control crop destroying insect has increased exhibited in last two to three decades. Pesticides and their residues were entered in soil, air and may be leached into ground and surface waters due to their widespread usage. Therefore, removal of pesticides from environmental medium is very important. Hence, poly (HEMA-MAT) nanostructures, novel affinity chromatography matrix for adsorption of deltamethrin synthesized and the optimum conditions for the adsorption of deltamethrin from water samples were also determined in this study. Poly (HEMA-MAT) nanostructures were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and elemental analysis. Maximum adsorption capacity was found to be 315.6 mg/g and the adsorbed amounts per unit mass of nanostructures reached to a plateau value at about 800 mg/L. It was observed that adsorption was more favour in acidic medium. Amounts of deltamethrin in real water samples such as river and ground waters were determined and removed efficiently using poly (HEMA-MAT) nanostructures. Also, we concluded that the poly (HEMA-MAT) nanostructures can be effectively used in deltamethrin adsorption without detectable losses in their initial adsorption capacities.

Keywords: Deltamethrin, pesticides, nanostructures, adsorption, hydrophobic interaction chromatography

YÜKSEK BAĞLAMA KAPASİTELİ NANOYAPILAR KULLANARAK SULARDAN PESTİSİD KİRLİLİĞİNİN UZAKLAŞTIRILMASI

ÖZ

Ekinlerin böcek ile yıkımını kontrol amacıyla pestisit kullanımı son 20-30 yılda artmıştır. Pestisit ve kalıntıları, toprak ve havaya karışmıştır, hatta yeraltı ve yüzey sularına da ulaşmış olabilir. Pestisitlerin yaygın kullanımı yüzünden, çevre ve sağlık problemleri ortaya çıkmıştır. Bu yüzden, çevresel bileşenlerden pestisitlerin uzaklaştırılması çok önemlidir. Bu nedenle, deltamethrinin sulardan uzaklaştırılması amacıyla kullanılmak üzere bu çalışmada yeni afinite kromatografik matriksi [poli (HEMA-MAT) nanoyapısı] sentezlendi ve su örneklerinden deltametrinin adsorpsiyonu için uygun koşullar belirlendi. Sentezlenen poli (HEMA-MAT) nanoyapılar, Fourier dönüşümlü kızılötesi spektroskopisi (FTIR), taramalı elektron mikroskobu (SEM) ve elementel analiz ile karakterize edildi. Maksimum adsorpsiyon kapasitesi 315,6 mg/g olarak bulundu ve nanoyapının birim kütlesi başına adsorplanan deltametrin miktarı yaklaşık 800 mg/L'de plato değerine ulaştı. Adsorpsiyonun asidik ortamda daha istemli olduğu belirlendi. Dere ve yeraltı suyu gibi gerçek su örneklerindeki deltametrin miktarları belirlendi ve poli (HEMA-MAT) nanoyapılar kullanılarak etkin bir şekilde uzaklaştırıldı. Ayrıca, poli (HEMA-MAT) nanoyapıların adsorpsiyon kapasitelerinde önemli bir azalma olmaksızın tekrar tekrar kullanılabilecekleri sonucuna varıldı.

Anahtar Kelimeler: Deltametrin, pestisid, nanoyapılar, adsorpsiyon, hidrofobik etkileşim kromatografisi

CONTENTS

Page

CHAPTER ONE
INTRODUCTION

1.1 Pesticides

A pesticide is any product used to manage, destroy, attract or repel a pest (A pest can be considered to be any unwanted organism, such as an insect, weed, rodent, bacterium, fungus, etc.). The word pesticide is often used incorrectly to refer to products for controlling insects. Pesticide is a generic term, covering all products used for controlling any kind of pest. Under the broad heading of pesticide, there are many sub-categories, including:

- herbicides for controlling one or more species of weeds
- insecticides for insects
- fungicides for fungi and moulds
- nematicides for nematodes (parasitic microscopic worms living in the soil)
- bactericides for bacteria
- algicides for algae (such as in swimming pools)
- rodenticides for mice and rats
- miticides for mites (small insects in the spider family)

Pesticides can be categorized as organic or inorganic in nature. Organic compounds are those that contain carbon. They can either be naturally occurring compounds derived from a living organism or they can be manufactured products. A few pesticides are naturally occurring compounds found in plants (e.g., rotenone). Others are micro-organisms, such as the bacteria, *Bacillus thuringiensis* (Bt). However, the vast majority of pesticides are manufactured organic compounds. Inorganic compounds are those that do not contain carbon. Prior to World War II, most of the products used as pesticides were inorganic in nature. Several of these contained toxic heavy metals such as lead,

arsenic or mercury. Because of the persistent risk to human health they pose, pesticides containing heavy metals have been banned. Only a very few inorganic

compounds continue to be used as pesticides, and they are used only in very limited applications (e.g., ant bait) (http://www.hc-sc.gc.ca/pmra-arla/english/pdf/fact/fs_pmra-e.pdf, n.d.).

1.1.1 Pyrethrins and Pyrethroids

Pyrethrins are insecticides that are derived from the extract of chrysanthemum flowers (pyrethrum) (Klaasen, Amdur, & Doull, 1996). The plant extract, called pyrethrum contains pyrethrin I and pyrethrin II collectively, called pyrethrins. Pyrethrins are widely used for control of various insect pests (http://ace.orst.edu/info/nptn/, n.d.).

Pyrethroids are synthetic (human-made) forms of pyrethrins. There are two types that differ in chemical structure and symptoms of exposure. Type I pyrethroids include allethrin, tetramethrin, resmethrin, d-phenothrin, bioresmethrin, and permethrin. Some examples of type II pyrethroids are cypermethrin, cyfluthrin, deltamethrin, cyphenothrin, fenvalerate, and fluvalinate (Ray, 1991; Klaasen *et al*., 1996).

1.1.2 Deltamethrin

Deltamethrin which was known as decamethrin is in the chemical class of pyrethroids (World Health Organization (WHO), 1990). Unlike other pyrethroids, deltamethrin consists of one pure compound. Deltamethrin have eight possible stereoisomers, but only two isomers, 1R ,3R, S(benzyl) and 1R, 3S, S(benzyl) have insecticidal activity (U.S. Department of Health and Human Services, 2004). The commercial product, deltamethrin, contains only the former (*cis*) isomer; product containing the latter is known as *trans*-deltamethrin. Deltamethrin has an α-cyano group on the 3-phenoxy-benzyl alcohol and is a type II pyrethroid.

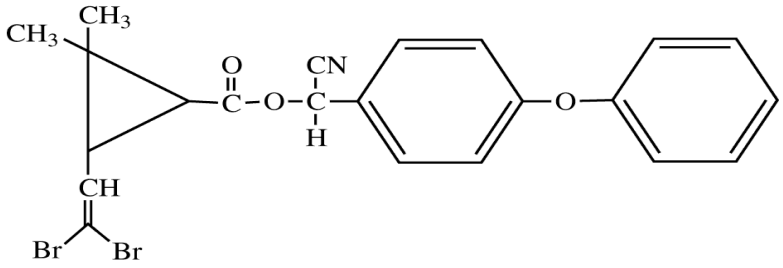


Figure 1.1 Molecular structure of deltamethrin, $C_{22}H_{19}Br_2NO_3$.

Physical and chemical properties of deltamethrin are summarized as follows:

- Technical grade deltamethrin (≥98% pure) consists of odorless crystals that are non-corrosive and colorless or white to light beige (Klaassen, 1996)
- Vapor pressure: $1.5x10^{-8}$ mmHg at 25°C (WHO, 1990)
- Octanol-Water Partition Coefficient (log Kow): 6.1 (WHO, 1990)
- Henry's constant may be determined by estimation or experimentally derived. Reported values include: $1.2x10^{-4}$ atm.m^3/mol at 25°C and $5.0x10^{-5}$ atm·m^3/mol, depending on the technique used. (WHO, 1990; U.S. Environmental Protection Agency(EPA), 1999)
- Molecular weight: 505.2g/mol (WHO, 1990)
- Solubility (water): ranges from 0.002-0.0002 mg/L (WHO, 1990; EPA, 1999)
- Soil Sorption Coefficient (Koc): adsorption ranges from $7.05x\ 0^5$ to $3.14x10^6$; desorption ranges from $1.14x10^6$ to $4.54x10^6$ (EPA, 1999)

Deltamethrin is used commonly to control caterpillars on apples, pears and hops, and for the control of aphids, mealy bugs, scale insects, and whiteflies on glasshouse cucumbers, tomatoes, peppers, potted plants, and ornamentals. It is also registered for use on livestock and for public health use. Depending on the product formulation, deltamethrin pesticides may range in toxicity from EPA toxicity class I to class III (I=most toxic, IV=least toxic), bearing the words DANGER-POISON, WARNING or CAUTION on the label. Deltamethrin products may be general or restricted use pesticides. Most deltamethrin products persist from one to two weeks in environment, with shorter times in direct sunlight (http://ace.orst.edu./cgi-bin/mfs/01/pips/deltamethrin.htm, n.d.).

Deltamethrin produced different signs of poisoning than other pyrethroids. When exposed to deltamethrin, mammals exhibit typical type II motor symptoms, which include a writhing syndrome in rodents, as well as copious salivation. The acute oral LD_{50} in male rats has been reported as low as 128 mg/kg to greater than 5.000 mg/kg depending on the carrier and condition of the study. Some studies have shown deltamethrin to cause skin irritation. Especially characteristic of deltamethrin poisoning is rolling convulsion. The sequence of the signs of poisoning is clearly defined, progressing from chewing, salivation, and pawing to rolling convulsions, tonic seizures, and death (http://ace.orst.edu./cgi-bin/mfs/01/pips/deltamethrin.htm, n.d.). In humans, symptoms of poisoning include ataxia, convulsions leading to muscle fibrillation and paralysis, dermatitis, edema, diarrhea, dyspnea, headache, hepatic microsomal enzyme induction, irritability, peripheral vascular collapse, rhinorrhea, serum alkaline phosphatase elevation, tremors, vomiting and death due to respiratory failure. Deltamethrin is a suspected endocrine disruptor and hydrolyzed by esterase and hydroxylated by cytochrome P450s (Kim, Bartlett, Anand, Bruckner, 2006). Deltamethrin is also toxic to fish, aquatic organisms, amphibians and bees (www.beyondpesticides.org, n.d.). At present, there is concern that deltamethrin and possibly other pyrethoids, like certain organophosfates, may exhibit potantiol to be developmental neorotoxicants in infants and children (Eriksson & Fredriksson, 1991; Shafer, Meyer, & Crofton, 2005).

Although the use of chemicals to combat agricultural pests dates from antiquity, the large-scale utilization of chemicals as major components of pest management systems is a twentieth century development. However, types of chemicals in use have changed substantially in response to environmental concerns that have arisen since their introduction. As late as 1950, many inorganic chemicals were still in use, including calcium arsenate, copper sulfate, lead arsenate, and sulfur (Klassen, Ridgway, & Inscoe, 1982), but, with the exception of sulfur, these materials were almost completely displaced by synthetic organic pesticides in subsequent years. The 1940s and 1950s were productive years in terms of new synthetic organic chemistry. The chemical industry faced a major challenge in its efforts to synthesize and manufacture replacements for materials that were critically needed to protect crops

from insect pests and protect personnel in tropical areas from malaria and other insect-borne diseases. At the end of World War II, newly developed chemical technologies became the basis for the manufacture of a number of new insecticides, particularly the application of the Diels-Alder reaction to synthesize cyclodiene insecticides. Because their acute mammalian toxicity was generally low and their spectrum of activity was very broad, such insecticides could be used to control many agricultural insect pests (Plimmer, 2001).

The primary consideration of pesticide manufacturers has been to develop products that are extremely effective against target pests. This initial goal was achieved fairly rapidly and effective pesticides became available to growers at a low cost. Pesticides eliminated the need for manual weeding and made it possible to raise crops in areas where destructive pests formerly had made it uneconomical. However, when synthetic organic pesticides were applied in increasingly large quantities over large areas, the disadvantages of widespread routine application became apparent (Plimmer, 2001). Due to their widespread usage, environment and health problems were emerged. Pesticides and their residual were entered in soil, air and may be leached into ground and run off-waters.

1.1.3 Toxicity of Pesticides

Unintentional poisonings kill an estimated 355 000 people each year (WHO, 2003). Two-thirds of these deaths occur in developing countries, where such poisoning is strongly associated with excessive exposure to, and inappropriate use of, toxic chemicals. In many such settings, the toxic chemicals may be emitted directly into soil, air and water — from industrial processes, pulp and paper plants, tanning operations, mining and unsustainable forms of agriculture — at levels or rates well in excess of those tolerable to human health (United Nations Development Programme, 1998; World Bank Report, 2002; Yanez *et al*., 2002). There are many pesticides, with thousands of trade names. Two-thirds of their total use is in agriculture (De Silva, Samarawickrema, & Wickremasinghe, 2006). This pesticides that was extremely used in agriculture negatively affects to human health because pesticides

are inherently toxic. Many different pesticides in use with very different modes of action and levels of toxicity. Chemical matters can display toxic effect with two types. The first, acute toxicity is affect that is arisen with define of symptoms or in short time. The second, chronic toxicity is arisen toxicity with repeated take under lethal dose in long term. Measurement of acute toxicity is value of LD_{50}. This value is defined as dose which is form to rate %50 of death in population. If LD_{50} value of a compound is low, its toxicity is high.

Table 1.1 The WHO recommended clasification of pesticides by hazard

		LD_{50} FOR THE RAT (mg/kg BODY WEIGHT)			
		ORAL		DERMAL	
CLASS		SOLIDS	LIQUIDS	SOLIDS	LIQUIDS
Ia	Extremely hazardous	≤5	≤20	≤10	≤40
Ib	Highly hazardous	5–50	20–200	10–100	40–400
II	Moderately hazardous	50–500	200–2000	100–1000	400–4000
III	Slightly hazardous	>500	>2000	>1000	>4000
III+	Unlikely to present hazard in normal use	>2000	>3000	—	—

Contamination of soils, ground water and surface water by pesticides is currently a concern throughout the world because many of these compounds are detrimental to both human health and the environment (Suciu & Capri, 2009). The increasing use of pesticides in agriculture and domestic activities is increasingly contaminating water resources. Environmental regulations in developed countries have become very strict for drinking water treatment over the last few years, especially regarding pesticide compounds (levels decreased to only 0.1 mg/L) (Martin-Gullon & Font, 2001). Reports about their harmful effects on humans and ecosystems have further stressed towards the removal of these pesticides in whole media; particularly in ground and drink water. Maksimum permissible limit of 0.1 μg/L for indivadual pesticides and releted prodacts, 0.5 μg/L for total pesticides in drinking water and 1-3 μg/L for surface waters has been recommended (Akhtar, Hasany, Bhanger, & Iqbal, 2007). Hence, removal of pesticides is very important.

So far, numerous research studies have been reported on pesticides removal from aqueous solutions, aiming at the purification of process streams and/or the recovery of valuable compounds (Yang, Hubble, Lockett, & Rathbone, 1997*;* Kyriakopoulos, Doulia, & Anagnostopoulos, 2005). There are many methods available to treat raw water for the separation of possible harmful organics before offering it to the public use. Conventional treatment technologies including chemical coagulation, sedimentation, filtration, clarification, disinfection, have been widely used but they were not always successful. Pesticides which are insoluble in water, such as DDT, or are easily decomposed, such as carbamate insecticides, generally can be removed effectively by the conventional methods. However, conventional treatment has often been proved ineffective for the separation of the majority of pesticides or their metabolites. Therefore, a number of innovative water treatment methods have been developed to create more efficient systems, particularly for the hydrophilic organic compounds (Frimmel *et al.*, 1999). The applied methods are classified in two categories. The first category, which includes adsorption and membrane technology methods, is based on the removal of toxic organic substances (Ayranci & Honda, 2004). The second category of methods, such as oxidation, ozonation, voltammetry, photocatalysis is based on pesticides decomposition (Chiron *et al.*, 2000). Adsorption is one of the most frequently applied methods because of its efficiency, capacity and applicability on a large scale. This process has been also widely used to remove pesticides from drinking water. The more common adsorbents are: carbon, polymers, agricultural products and soils (Kyriakopoulos & Doulia, 2006).

In literature, there have been a lot of studies about removal of pesticides. But there isn’t any study about removal of pesticide with affinity chromatography and nano polymeric adsorbent in literature. In the study of Ibrahim and Jbara, a phillipsite–faujasite tuff (faujasite) from Jordan has been activated and characterized to evaluate its efficiency in removing paraquat from synthetic wastewater and to specify optimum conditions with maximum efficiency of the faujasite tuff. Removal of paraquat by using faujasite directly gave average removal efficiency equal to 59%. Removal of paraquat from wastewater using charcoal and the non-activated faujasite increased efficiency to about 82%. Thermal activated faujasite at 200 °C and 300 °C

increased the uptake up to about 93% and 99.5%, respectively. Therefore thermal activation of faujasite is necessary to improve its uptake performance (Ibrahim & Jbara, 2009).

Benitez et al., membrane filtration of four phenyl-urea herbicides (linuron, diuron, chlortoluron, and isoproturon) dissolved in ultrapure water was studied in a laboratory cross-flow device in batch concentration mode (with recycling of the retentate stream). Three UF (MWCO of 20 000, 5000 and 2000 Da) and three NF (MWCO of 150–300 Da) membranes were used. The influences of the main operating conditions (transmembrane pressure, tangential velocity, temperature, pH, and MWCO of the membranes) on the steady-state permeate fluxes and the retention factors of the phenyl-ureas were evaluated. The herbicide mass adsorbed onto the membranes was also determined, and the contribution of the fouling resistance to the total resistance to permeate flux was much lower than the inherent resistance of the clean membranes (Benitez *et al*., 2009).

In the other study, sorption potential of rice (*Oryza sativa*) bran and rice husk for the removal of triazophos (TAP), an organophosphate pesticide, has been studied. The specific surface area was found to be 19±0.7m^2 g^{-1} and 11±0.8m^2 g^{-1} for rice bran and rice husk, respectively. Rice bran exhibited higher removal efficiency (98±1.3%) than rice husk (94±1.2%) by employing triazophos solution concentration of 3×10^{-5}M onto 0.2 g of each sorbent for 120 min agitation time at pH 6 and 303 K. The concentration range (3.2–32)x10^{-5}M was screened and sorption capacities of rice bran and rice husk for triazophos were computed by different sorption isotherms. The energy of sorption for rice bran and rice husk was assessed as 14±0.1 and 11±0.2 kJ mol^{-1} and kinetics of the sorption is estimated to be 0.016±0.002 and 0.013±0.002 min^{-1}, respectively. Intraparticle diffusion rate was computed to be 4±0.8 and 4±0.9 nmol g^{-1} $min^{-1/2}$. Thermodynamic constants ΔH, ΔS and ΔG at varying temperatures (283–323 K) were also calculated (Akhtar *et al*., 2009).

Zadaka et al., atrazine removal from water by two polycations pre-adsorbed on montmorillonite was studied. Batch experiments demonstrated that the most suitable

composite poly (4-vinylpyridine-co-styrene)-montmorillonite (PVP-co-S90%-mont.) removed 90–99% of atrazine (0.5–28 ppm) within 20–40 min at 0.367% w/w. Calculations employing Langmuir's equation could simulate and predict the kinetics and final extents of atrazine adsorption. Columns filter experiments (columns 20 _ 1.6 cm) which included 2 g of the PVP-co-S90%-mont. Composite mixed with excess sand removed 93–96% of atrazine (800 ppb) for the first 800 pore volumes, whereas the same amount of granular activated carbon (GAC) removed 83–75%. In the presence of dissolved organic matter (DOM; 3.7 ppm) the efficiency of the GAC filter to remove atrazine decreased significantly (68–52% removal), whereas the corresponding efficiency of the PVP-co-S90%-mont. Filter was only slightly influenced by DOM. At lower atrazine concentration (7 ppb) the PVP-co-S90%-mont. Filter reduced even after 3000 pore volumes the emerging atrazine concentration below 3 ppb (USEPA standard). In the case of the GAC filter the emerging atrazine concentration was between 2.4 and 5.3 mg/L even for the first 100 pore volumes. Thus, the PVP-co-S90%-mont. Composite is a new efficient material for the removal of atrazine from water (Zadaka *et al*., 2009).

Kyriakopoulos et al., the adsorption of herbicides alachlor, amitrole, trifluralin and prometryn on porous polymeric adsorbents has been studied. Two adsorbent resins were investigated, the highly hydrophobic Amberlite XAD-4 (polystyrene divinylbenzene copolymer) and the functionalized more hydrophilic XAD-7 (nonionic aliphatic acrylic polymer). Equilibrium adsorption experiments using buffered aqueous solutions were conductedto estimate the types of isotherms and their parameters. The effect of chemical composition and structure of the herbicides was investigated. The pH range studied was 3–6.5, the temperature range was 288–303 K and the ionic strength was maintained at 0.01 M. In the case of trifluralin and prometryn adsorption on both resins and amitrole adsorption on XAD-4 resin, the heats of adsorption were negative (8.1–33.6 kcal/mol). On the contrary, in alachlor adsorption on both resins and amitrole adsorption on XAD-7 resin, the estimated heats of adsorption were positive (Kyriakopoulos *et al*., 2005).

Sarkar et al., the production of potable water from pesticide contaminated lake and river water was investigated by a coagulation–adsorption–nanofiltration approach. Isoproturon (IPU) was selected as a target pesticide and spiked in distilled water and then in surface water. Various adsorbents such as powdered activated charcoal (PAC), bentonite, and chitosan were tried at different dosages to evaluate their efficiency in IPU removal. The values of adsorption capacity calculated from Langmuir and Freundlich equations were 104.21 mg/g and 69.4 mg/g respectively indicating favorable adsorption of IPU on powdered activated charcoal surface. After optimizing the coagulation and adsorption protocol, nanofiltration (NF) was performed on pretreated water in a test cell in dead end mode. The NF permeate was analyzed for pH, turbidity, TDS, COD, TOC, conductivity, hardness, and colony count. Reverse osmosis (RO) was done after NF if required. The quality of NF/RO permeate was found comparable to the standards of drinking water (Sarkar *et al*., 2007).

As can be seen from the brief of literature above, the methods used for the removal of pesticides may require some extra processes. These methods need very long sample pre-treatment and pre-cleanup procedures in addition to take up time and expensive. Therefore, it may be useful to removal of deltamethrin by affinity chromatography techniques with a new nano-sized polymeric material that is simple and useful.

1.2 Affinity Chromatography

Affinity sorption is already a well-established method for identification, purification and separation of complex biomolecules. This may be achieved by a number of traditional techniques such as gel permeation chromatography, high performance liquid chromatography, chromatofocusing, electrophoresis, centrifugation, etc., in that the process relies on the differences in the physical properties (e.g., size, charge and hydrophobicity) of molecules to be treated. In contrast, affinity sorption techniques exploit the unique property of extremely specific biological recognition (Chaiken, 1983; Gribnan, 1982; Scopes, 1982). This

is due to the complementarity of surface geometry and special arrangement of the ligand and the binding site of the biomolecule. All biological processes depend on specific interactions between molecules. These interactions might occur between a protein and low molecular weight substances (e.g., between substrates or regulatory compounds and enzymes; between bioformative molecules-hormones, transmittors, etc., and receptors, and so on), but biospecific interactions occur even more often between two or several biopolymers, particularly proteins. Affinity chromatography enables the separation of almost any biomolecule on the basis of its biological function or individual chemical structure. Examples can be found from all areas of structural and physiological biochemistry, such as in multimolecular assemblies, effector-receptor interactions, DNA-protein interactions, and antigen-antibody binding.

Affinity chromatography owes its name to the exploitation of these various biological affinities for adsorption to a solid phase (Jonson & Ryden, 1998; Wilcheck *et al.*, 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 1.2.

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 1.2 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

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 *et al*., 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 1.3 summarizes some of these techniques. As can be seen from Table 1.3, some of these subcategories have become accepted useful techniques (Wilcheck, & Miron, 1999).

Table 1.3 Subcategories of affinity chromatography

Affinity Chromatography	
	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
	19. Affinity Capillary Electrophoresis
	20. Centrifuged AC
	21. Affinity Repulsion Chromatography

Principle of affinity chromatography is schematically shown in Figure 1.2. A wide variety of ligands may be covalently attached to an inert support matrix, and subsequently packed into a chromatographic column.

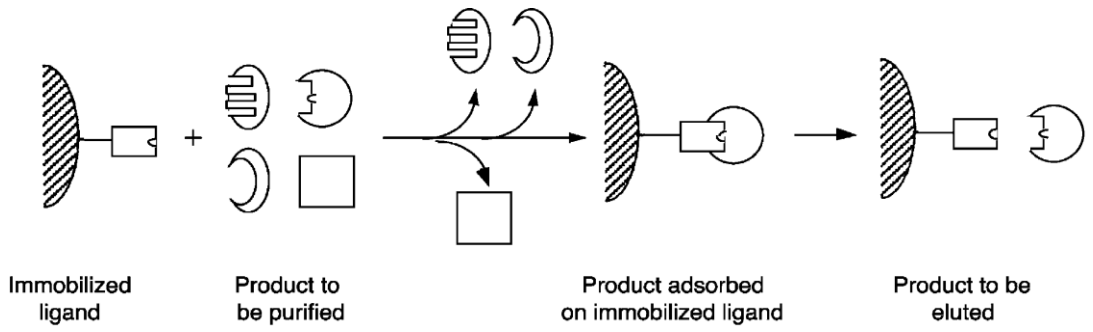


Figure 1.2 Principle of affinity chromatography.

In such a system, only the protein molecules which selectively bind to the immobilized ligand will be retained on the column. Washing the column with a suitable buffer will flush out all unbound molecules. There are several techniques permit to desorb the product to be purified from the immobilized ligand. Because affinity chromatography proper relies on the functional properties, active and inactive forms can often be separated. This is however, not unique to affinity methods. Covalent chromatography can do the same thing when the activity depends on a functional thiol group in the protein. By affinity elution, ion-exchange chromatography is also able to separate according to functional properties. These are, however, exceptions to what is a rule for the affinity methods.

Very often the use of affinity chromatography requires that the investigator synthesizes the adsorbent. The methods for doing this 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 counter ligand. 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) grouping 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 Solid Matrix Support

An essential prerequisite for affinity chromatography is the availability of appropriate chromatographic matrices with the covalently bound specific ligand. Unlike the classic chromatographic procedures, which require only a few basic types of matrices, such as dextrans varying degrees of cross-linking or moleculer sieves or ion exchanger of different charge or ionic strenght, a matrix for affinity chromatography, with regard to the substance to be seperated or purified, is adapted to the given purpose by correspondingly selected ligand. In many cases the matrix can be used for a specific purfication step only; that is it's tailored to the purfication of the corresponding substance.

The working steps of afinity chromatography –adsorption, washing and elution – are mostly simple to perform without major problems. The time-consuming and frequently limiting step is search for an appropriate complex partner and the preparation of bioaffine matrix. At present a variety of bioffine matrices is commercially available as so-called ready-to-use adsorbents. In many cases it is necassary and appropriate to have a homemade matrix for affinity chromatography (Mohr & Pommerening, 1985). The synthesis of a matrix for affinity chromatography requires:

1. A matrix support suitable for chromatography
2. A substance with specific or selective affinity to the substance to be purified.
3. A chemical reaction for covalent linkage of the two partners

Table 1.4 Some specific properties of supports for the affinity chromatography

Hydrophilic	but	insoluble in water
Macroporus	but	mechanically stable
Chemically stable	but	easily chemical modified
Great surface	but	inert, not nonspecific adsorption

1.2.1.1 General Properties of Solid Matrix

Up to now, a variety of matrix supports has been used with more or less success for affinity chromatography. The correct choice of matrix support and the covalent linkage between the matrix and the bioaffine ligand may be essential for the success of the chromatography. The supporting matrix plays not only a passive role as the solid-phase component, it can also have considarable effect on the stability of the complex formed, or it may even the bioaffine ligand itself. Furthermore, the manner and site of linking with the bioaffine ligand may have an essential influence on the effectivity of affinity chromatography.

The matrix should have properties generally required for a chromatograpic matrix and, furthermore, qualities that derive from specific of affinity chromatography. The general properties of matrix should be adequate particles size and shape, sufficient stability and surface. Increasing particle size reduces flow resistance and seperation power. On the other hand, matrices with very low particle size in consequence have too high a flow resistance and soon become clogged. Irregularly shaped particles lead to unequal path lenghts for the substance to be seperated and, consequently, to band broadening. Best suited is spherical shape.

Also important are mechanical and chemical stability and resistance against microorganisms. A large surface is desirable which can be reached best by using a porous and swellable material with a highest possible ratio of the inner to the outer surface (Lederer & Lederer 1957; Determann, 1969; Krauss & Krauss, 1981). A matrix for affinity chromatography should possess several specific properties, some of which appear contradictory but associated with nature of the substances to be purified and with the kind of interactions to be utilized. The aqueous milieu is an essential condition for structure and/or function of biologically active compounds. In some cases water molecules are a part of the native configuration of a biomacromolecule. This is why affinity chromatography is generally performed in the aqueous phase. Thus, the matrix must not only be water insoluble but also hydrophilic, swellable, or, at least, well we table with water (the matrix itself or at

least its surface). The matrix should be either macro porous or consist of a wide-wash network to allow free diffusion of the bio macromolecules (proteins, enzymes, nucleic acids, antigens and the others) to the binding site and not prevent biospecific complex formation by a molecular sieve effect. High porosity or wide-wash network generally decreases the mechanical stability of the matrix material. Furthermore, the matrix should be chemically stable in a wide range (pH 2-12). On the other hand chemical modification must be possible in a simple way.

The supporting material should have the greatest possible surface and at the same time completely inert and have no unspecific interactions with the substance to be purified, such as electrostatic or hydrophobic interactions. But depending on the nature of the matrix, this never can be completely excluded and have been observing increasingly after covalent fixtion of the bioaffine ligand either through the incorporation of dissociable groups as result of the functionalization or hydrophobic spacers. This must not necessarily be a disadvantage. These ‘’nonspecific interactions’’ may amplify, for example, complex formation. As hydrophobic chromatography shows, such interactions may in extreme case lead to a new principle of separation. Up to the present time there is no supporting matrix that has all the requirements in an optimal way. Thus, various supporting materials have been used more or less successfully (Mohr & Pommerening, 1985) (Table 1.5).

Table 1.5 Types and chemical structures of various supporting materials

Type	Chemical structure
Biopolymers (polysaccharides)	Agarose,cross-linked dextran, cellulose
Synthetic polymers	Polyacrylamide;poly(hydroxyethylmetacrylate); polystyrene
Inorganic material	Porous glass , Iron Oxide (magnetogels)
Biopolymers/synthetic copolymers	Agarose polyacrylamide
Inorganic materials/synthetic copolymers	Silica/hydrophilic polymers

1.2.1.1.1 Biopolymers. Hydrophilic biopolymers play a dominating role as supporting materials for affinity chromatography. Starting materials are natural polysacharides, such as agarose, dextran, cellulose, and, to a lesser extent, starch.

The modification necessary for affinity chromatography can be carried out in a relatively simple way via the OH groups. In spite of many common features, there are distinct differences in properties and applications relating to the chemical structure of the biopolymers (Madden & Thom, 1982).

The conformations of the chains and the interactions between them have an influence on the use of polysaccharide as chromatographic support. The geomerty of the chains determined by the relative orientation of adjacent sugar residues around the glycosidic bonds. But in aqueous solutions a disordered random coil form dominates, because of the flexibility of the chains. Under definable conditions, however non-bounded energy-terms (hydrogen bridge, ionic interactions and others) can compensate for confirmation entropy and fix the polysaccaride chains in an ordered configuration. Of great practical importance for the optimization of the polysaccarides as chromatographic support is that it's possible to influence the factors responsible for the equilibrium between an ordered and non-non ordered configuration in aqueous solution. On the basis of their covalent structure, the polysaccarides can be divided into two classes:

1. Simple periodic structures
2. Interrupted periodic structures

In the first class the chains contain only identical sugar residues which are linked through identical positions and configurations.

The second class is characterized by chains containing periodic sequences with ordered conformations, which are interrupted by deviations from regularity. The blance between ordered and soluble regions leads to highly hydratized gels with many fold applications. Agarose is representative of the interrupted periodic type. Other types such as alginates and pectins interrupted ribbon configurations are of great interest in the application of immobilized whole cells but not for chromatographic supports (Mohr & Pommerening, 1985).

Agarose is a linear water-soluble polysaccharide composed of alternating 1,3-linked β -D-galactose and 1,4-linked 3,6-anhydro-α-L-galactose units. That aqueous solutions gel spontaneously when cooled below 50°C has led to the development of agarose gels in bead, pellet, or spherical form.

Dextran is a polysaccharide produced by microorganisms of genus Leuconostos. Its composed of 1,6-linked α-D-glucose units (over %90) and can be brunched out by 1,2 1,3 or 1,4 binding. Dextran cross-linking with epichlorohydrin in alkaline solution is one of the classic supports for gel permeation chromatography. Depending on chain length of dextran and the degree of cross-linking, gel with well-defined molecular screening effects can be produced.

Since the introduction of the chromatographic procedures, cellulose has been one of the standard supporting materials. Cellulose, a vegetable pollysaccharide, is composed of linear 1,4-β-D-glucose units. The polysaccharide chains aggregated to fibers separated by amaorphousgions (Mohr & Pommerening, 1985).

The first examples of affinity chromatography were made with insoluble starch without recognizing the importance of this method, such as the complex formation between α-amylase and insoluble starch or the separation of α- and β- amylase. Today starch plays a secondary role as matrix.

1.2.1.1.2 Inorganic Supports. Like cellulose, inorganic materials are usually applied to many chromatographic methods (adsorption, distribution, gas chromatography, and others). Their advantages include high mechanical and chemical stability (particle and pore size) and resistance against microorganisms. At present, inorganic matrices are predominantly used in the field of immobilized enzymes, but as a result of the studies by Weetall and co-workers it has been possible to use porous glass as a matrix for affinity chromatography (Weetall, 1973; Weetall and Filbert, 1974).

Table 1.6 Some functional monomers for coating

Allylamine N-Allyl-1-bromoacetamide 6-Acrylamidohexanoic acid N-Hydroxysuccinimidyl-6-acrylaminohexanoic acid ester N-Hydroxysuccinimidylacrylic acid ester

1.2.1.1.3 Synthetic Polymers. Besides supports based on biomacromolecules, synthetic polymers have been explored as a potential supporting material for affinity chromatography. This development proceeded in close connection with the investigation of immobilized enzymes. To date, a variety of materials of varying composition has been described but none have attained the universal application of agarose. In most cases they are cross-linked macroporous vinyl polymers in beaded or spherical form with defined size and porosity. The properties can be obtained by proper choice of monomers and conditions of polymerization. The chemical structures of these supports are characterized by the polyethylene backbone, which influences chemical stability, and physical or structural stability, and the modifiable side chains (Mohr & Pommerening, 1985).

The most important synthetic polymers are polyacrylamide gels and hydroxyalkylmethacrylate supports. Polyacrylamide gels are copolymerizates between acrylamide and N,N-methylene-bis-acrylamide as cross-linking agents containing a hydrocarbon framework with carboxamide side chains:

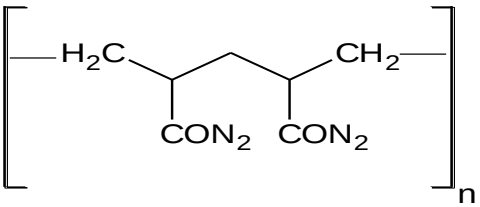


Figure 1.3 N,N-methylene-bis-acrylamide containing a hydrocarbon framework with carboxamide side chains.

Polyacrylamide gels mainly are offered by Bio-Rad (Richmond, California) under the trade name Bio-Gel-P as a carrier for gel permeation, which then can be functionalized for affinity chromatography.

Another well-known matrices of this type are hydroxyalkylmethacrylate supports. In the early 1970s this type was synthesized by Coupek and coworkers (Coupek *et al.*, 1973) and introduced to affinity chromatography. Spheron and Separon are prepared by heterogeneous suspension copolymerization of hydroxyethylmethacrylates and ethylenedimethacrylates in aqueous solution in the presence of inert solvents. This gives a neutral, hydrophilic gel from heavily cross-linked microparticles with micropores, aggregating to macroparticles with macroporous structure. The inner structure, pore size, and distribution, specific surface, and quantity of reactive OH groups can be varied, with a molecular weight exclusion limit from 20000 to 20000000. In contrast to other hydrophilic types of matrix, the macroporous structure is kept in dry state. The gel does not change in volume with changes of pH or in organic solvents and is not attacked by microorganisms as are other synthetic polymers. The gel can be used with good results in organic solvents. This is a great advantage for the modification of the matrix by polymer-analogous reactions. The mechanical and chemical stability is higher than that of matrices based on acrylic acid derivatives. Because of their high rigidity, this matrix shows excellent flow properties.

The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications. Nanosized particles can produce larger specific surface areas and, therefore, may result in high immobilization capacity for biomolecules. Therefore, it may be useful to synthesize nanosized particles with large surface areas and utilize them as suitable carriers for the adsorption of biomolecules.

1.2.1.1.4 Nano-polymers. The word nano is from the Greek word “dwarf”. Its prefix refers for units of 10^{-9}. Nanobiosystem science and engineering is one of the most challenging and fastest growing components of nanotechnology. The

confluence of biology and nanoscience will contribute to unifying concepts of science, engineering, technology, medicine, and agriculture. The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications.

Nanoscience is concerned with the study of unique properties of matter at its nano level exploits them to create novel structures, devices and sysytem for variety of different uses. Particles having size less than 100nm are generally called nanoparticles. This has strikingly different properties due to their small size and thus is found useful in many applications. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties (Murray, Kagan & Bawendi, 2000). Now they have entered a commercial exploration period (Mazzola, 2003; Paull, Wolfe, Hebert & Sinkula, 2003; Salata, 2004). Nanoparticles have been investigated intensively due to their potential applications in many areas, such as biology, medicine and environment, because nanoparticles can produce larger specific surface area due to their nanoscopic size and, therefore, may result in high binding capacity and also they are highly strong and cheaper. Therefore, it may be useful to synthesize nanosized particles with large surface areas and utilize them as suitable carries for biological molecules.

1.2.2 Hydrophobic Interaction Chromatography

Biomolecules are purified using chromatography techniques that separate them according to differences in their specific properties. A wide variety of protein purification techniques are available today, however, different types of chromatography have become dominant due to their high resolving power. In gel filtration chromatography, ion-exchange chromatography, affinity chromatography and hydrophobic interaction chromatography (HIC), the proteins separation is dependent on their biological and physicochemical properties: molecular size, net charge, biospecific characteristics and hydrophobicity, respectively (Kennedy, 1990; Garcia & Pires, 1993). HIC takes advantage of the hydrophobicity of proteins promoting its separation on the basis of hydrophobic interactions between

immobilized hydrophobic ligands and nonpolar regions on the surface of proteins. The adsorption increases with high salt concentration in the mobile phase and the elution is achieved by decreasing the salt concentration of the eluent (Melander & Horvath, 1977; Fausnaugh & Regnier, 1986; Roe, 1989). Therefore, the term 'salt-promoted adsorption' could be used for this type of chromatography (Porath, 1986).

Hydrophobicity is the repulsion between a nonpolar compound and a polar environment such as water. When a non-polar compound is inserted into water, an overall increase in the degree of order of water molecules surrounding the hydrophobic group is observed with a decrease in entropy ($\Delta S<0$). Since the enthalpy change (ΔH) is small compared with the $T\Delta S$ value, there is a positive change in free energy ($\Delta G>0$), according to equation:

$$\Delta G=\Delta H-T\Delta S \quad (1)$$

Therefore, this process does not occur spontaneously because it is thermodynamically unfavorable. On the other hand, if non-polar compounds are put into water they spontaneously associate due to hydrophobic interactions. An increase in entropy ($\Delta S>0$) is observed, resulting from a displacement of the ordered water molecules around the non-associated hydrophobic groups to the more unstructured bulk water. This implies a negative change in free energy ($\Delta G<0$) and a thermodynamically favorable process. In other words, the interaction between two or more hydrophobic molecules in aqueous solutions proceeds spontaneously and is mainly determined by the change in entropy (Lewin, 1974; Jennissen, 1976; Ochoa, 1978; Tanford, 1980; Shansky, Wu, Figueiroa, & Karger, 1990).

The binding mechanism of HIC was investigated by many research groups and several calculations of binding forces and strength have been published (Geng, Guo, & Chang, 1990). The hydrophobic ligands are presumed to interact with hydrophobic side chains of the protein where an electron donor-acceptor complex is formed. The hydrophobic character of a protein is promoted by high salt concentrations. Since the binding is promoted by high salt, a close contact between the ligand and the region of

the protein that is involved in adsorption might be necessary for binding. The salt, however, not only affects the molar concentration of water, but changes the conformational structure of the proteins, the hydrophobic interaction forces and the hydration shell of the protein (salting out effect). Thus, the water molecules are the displacing agents in this process. The proteins are desorbed by decreasing concentrations of salt after the hydrophobic properties of the proteins are reduced during the gradient.

The influence of different salts on hydrophobic interactions follows the Hofmeister (lyotropic) series (Figure 1.4) for the precipitation of proteins from aqueous solutions (Pahlman, Rosengren, & Hjerten, 1977; Roe, 1989):

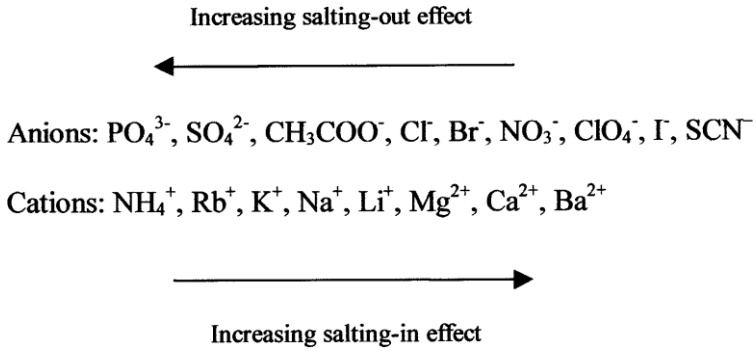


Figure 1.4 Hofmeister (lyotropic) series.

The salts at the beginning of the series promote hydrophobic interactions and protein precipitation (salting-out effect) and are called antichaotropic. They are considered to be water structuring, whereas salts at the end of the series (salting-in or chaotropic ions) randomize the structure of the liquid water and thus tend to decrease the strength of hydrophobic interactions (Porath, 1987).

Chen et al. (1997), Huang et al. (2000), Lin et al.(2000), and Tsai et al. (2002) suggested that the mechanism has five sequential sub-processes: (a) the dehydration or de-ioning (removing the electrical double layer) process of the protein; (b) dehydration or de-ioning process of the gel; (c) Van der Waals forces between proteins and hydrophobic resin; (d) the structure of the protein is arranged upon

adsorption; and (e) the excluded water or ion molecules in a bulk solution is rearranged.

To obtain success in a chromatographic process for a specific application, two major elements have to be considered a stationary phase and a fluid mobile phase. The various kinds of stationary phases could differ on the type of ligand, the ligand chain length, ligand density and on the type of matrix or support. The most widely used ligands for HIC are linear chain alkanes with or without a terminal amino group. Phenyl (and other aromatic groups) is also used as a ligand with good results due to mixed hydrophobic and aromatic (π- π) interactions. At a constant degree of substitution on the matrix the *n*-alkane ligands constitute a homologous series in a hydrophobicity scale (Tanford, 1972):

methyl < ethyl < propyl < butyl < pentyl < hexyl < heptyl < octyl

The hydrophobicity and the strength of interaction increase with the increase in *n*-alkyl chain length but the adsorption selectivity may decrease.

The most widely used ligands are linear chain alkanes (as butyl, octyl) and some aromatic groups (such as phenyl). An increase in the chain length of an alkyl ligand increases the strength of hydrophobic interaction between the protein and the resin; also, the specificity of the resin towards the adsorbed protein is changed (Er-el et al., 1972). However, resolution decreases when chain length is higher (Queiroz et al., 2001). On the other hand, an increase in the substitution degree of the resin leads to an increase in the binding capacity of the stationary phase, due to the higher probability of forming multipoint attachment, and at times, it can be hard to elute the bound protein without denaturation (Hjerten et al., 1974). Using the same type of ligand and the same type of base support, the selectivity of an HIC resin can be manipulated by changing the ligand density (Lin et al., 2000).

For all HIC gels, the density of the ligands has to be optimized, and not always the highest amount of immobilized ligand results in the best chromatographic

performance. A very high density of ligand, even a weak hydrophobic one, will reduce the recovery and the resolution. Therefore, the immobilization technique for the production of HIC gels must be kept highly reproducible. The steric accessibility of the ligand itself is also directly related to a suitable capacity of an HIC gel since the ligand itself must interact in a selective mode with the compound to be isolated. Thus, spacers or linear polymer chains (tentacles) were introduced to enable an appropriate spacing of functional groups. Graft polymerization is a powerful modification method in which a flexible polymer chain can be covalently attached to chromatographic supports and thus provide a favourable binding property. In contrast to spacers, such flexible polymer chains also result in minimized non-specific interactions with proteins.

The most widely used supports are hydrophilic carbohydrates (e.g. cross-linked agarose), silica or synthetic copolymer materials. Using the same type of ligand, the selectivity of the stationary phases can change in function of the different type of supports. The protein retention in HIC depends not only on the stationary phase but also on characteristics of the mobile phase, such as the type and concentration of the salt, pH, temperature and additives (Queiroz, Tomaz, & Cabral, 2001)

The pH of mobile phase can be an important factor that affects the protein retention in HIC (Hofstee, 1973; Strop *et al*., 1983; Sanz *et al*., 1998). Usually, an increase in the pH value (up to 9–10) decreases the hydrophobic interaction between proteins and the hydrophobic ligands, due to the increased hydrophilicity promoted by the change in the charge of the protein (Hjerten, 1973; Porath *et al*., 1973). On the other hand, a decrease in pH results in an apparent increase in hydrophobic interactions.

The first type of hydrophobic stationary phases derived from coupling various non-polar amino acids to an inert support or matrix (Rimerman & Hatfield, 1973). Agarose was the support most widely used, but cellulose, dextran and silica have also been employed.

Porath et al. (1973), Hjerten et al. (1974) were the first to synthesize charge-free hydrophobic adsorbents. Hjerten used a coupling method based on glycidyl ether (with an epoxide, oxirane functional group) that today is widely used for production of the commercially available gels octyl- and phenyl-Sepharose. A free charge gel was also synthesized by Maisano et al. (1985) by activation of agarose with a bisepoxide (1,4-butanediol diglycidyl ether) and then coupled with an alkyl-mercaptan (Maisano et al., 1985). The polysaccharide gels (e.g. agarose) are the supports most widely used in traditional low-pressure HIC, but several rigid microparticulate stationary phases based on organic or inorganic supports, that can be used at high pressure HIC, have been introduced. Thus, silica and polymeric supports were derivatized through their hydroxyl groups with butyl, phenyl and polyether functions.

Hjerten et al. (1986) introduced a rigid highly cross-linked agarose for use in HIC of proteins under HPLC conditions. Also, rigid materials (Separon-HEMA) that are copolymers of 2-hydroxyethyl methacrylate and ethylene dimethacrylate were used in HIC-HPLC (Kleinmann, Plicka, Smıdl, & Svoboda, 1989; Smidl, Kleinmann, Plicka, & Svoboda, 1990). Macroporous agarose beads were converted into non-porous particles by shrinkage and cross-linking in organic solvents using butanediol diglycidyl ether as cross-linker (Hjerten & Liao, 1988). These beads are stable up to pH 14 contrary to silica based materials and can be used for high performance HIC without derivatization.

In HIC, increasing the temperature enhances protein retention and lowering the temperature generally promotes the protein elution (Hjerten, Rosengren, & Pahlman, 1974). In fact, the HIC is an entropy-driven process, as earlier mentioned, where the Gibbs free energy is giving according to the Eq. (1). Since ΔH may be a small positive or negative value, ΔG is controlled by a positive entropy change and thus increases with temperature. The capacity factor k' in HIC increases with increasing temperature according to the equation (El Rassi, 1996):

$$\ln k' = \ln \varphi - \Delta G/RT \quad (2)$$

where R is the gas constant, φ is the phase ratio and T is the absolute temperature.

However, an opposite behavior can occur on protein retention, due to temperature effect on the conformational state of different proteins and on their solubility in aqueous solutions. Even though the role of temperature in HIC is not so simple, this parameter can be used to achieve weaker interaction and promote elution and separation of proteins under mild conditions without denaturation (El Rassi, 1996).

The main factors affecting protein chromatographic behaviour in HIC are salt type and ionic strength of the mobile phase and chemical nature of the backbone, type of hydrophobic ligand, and substitution level of the stationary phase. The effect on protein retention time in HIC of each of these factors could be evaluated by computational experiments, using the different methodologies described.

Wastewater treatment methods include coagulation, nanofiltration, membrane process, oxidation, ozonation and adsorption etc (Akhtar, Iqbal, Bhanger, Zia-Ul-Haq, & Moazzam, 2009). Adsorption is very popular method for removal of pesticide from water. Activated carbon, kaolin, montmorillonite, bentonite clays, and low cost sorbents as rice brain and rice husk have been widely used as adsorbent for adsorption. But in literature, there is no studies used nanoparticules as an affinity matrix for the adsorption of pesticides such as deltamethrin. Therefore, we aimed to synthesize a novel nano-sized affinity chromatography matrix [poly (HEMA-MAT) nanostructures] and to determine the optimum conditions for the adsorption of deltamethrin. We also studied removal deltamethrin from real water samples.

CHAPTER TWO
EXPERIMENTAL METHODS AND MATERIALS

2.1 Materials

HEMA (Sigma Chem., St. Louis, USA) and ethylene glycol dimethacrylate (EGDMA, Aldrich, Munich, Germany) were distilled under vacuum (100 mmHg). Deltamethrin purchased from Sigma Chem. All other chemicals were of the highest purity commercially available and were used without further purification. 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-MAT) Nanostructures

For the synthesis of nano-poly (HEMA-MAT) the following experimental procedure was applied: N-methacryloyl-(l)-tryptophan methyl ester (MAT) was prepared using the method in the study of Akgöl et al. (2007). The stabilizer, polyvinyl alcohol (PVAL) (0.5 g), was dissolved in 50 mL deionized water for the preparation of the continuous phase. Then, the comonomer mixture was added to this dispersion phase, which was mixed in an ultrasonic bath for about half an hour. Before polymerization, initiator was added to the solution and nitrogen gas blown through the medium for about 1–2 min to remove dissolved oxygen. Polymerization was carried out in a constant temperature shaking bath at 70°C, under nitrogen atmosphere for 24 h (Table 2.1)

Table 2.1 Component of polymerization

Dispersion Phase	Organic Phase
50 mL deionized water	0.01 mL MAT
0.0198 g Potassium persulfate (KPS)/45 mL water	0.6 mL HEMA
0.5 g PVAL/45 mL water	0.3 mL EGDMA

After the polymerization, the polymeric nanostructures were cleaned by washing with methanol and water several times to remove the unreacted monomers. For this

purpose, the nanostructures were precipitated at the rate of 18000 g for 2 h in a centrifuge (Zentrifugen, Universal 32 R, Germany), the collected precipitate was resuspended in methanol and water several times. After that poly (HEMA–MAT) nanostructures were further washed with deionized water. Poly (HEMA) nanostructures were produced by same formulation without MAT comonomer. In this study poly (HEMA–MAT) nanostructures having the size range of 100 nm were used as a solid matrix for the adsorption of deltamethrin. Figure 2.1 shows that the hypothetic structure.

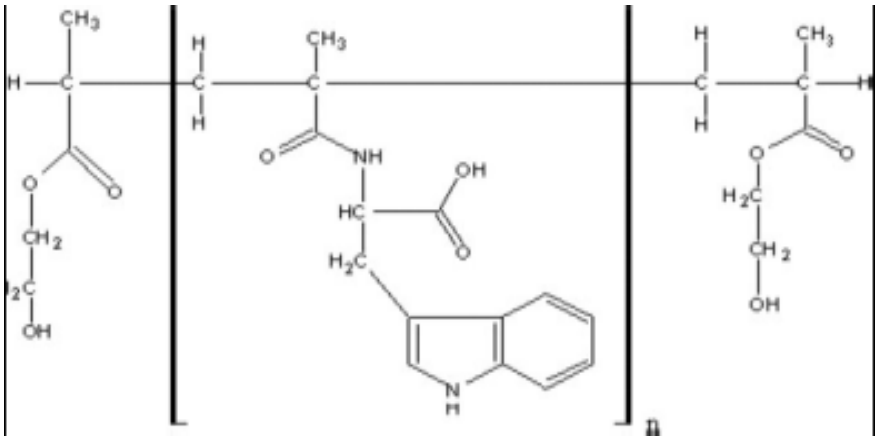


Figure 2.1 Hypothetic structure of poly (HEMA–MAT) nanostructures.

2.2.1 Characterization of Poly (HEMA-MAT) Nanostructures

Fourier transform infrared spectroscopy (FTIR) spectra of the nanostructures were obtained by using FTIR spectrophotometer (Perkin Elmer). The dry nanostructures (about 0.01 g) were thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a tablet form, and the spectrum was then recorded.

The particle size, the size distribution, and the surface charge were determined by Zeta Sizer (Malvern Instruments, Model 3000 HSA, England). The surface morphology of the poly (HEMA–MAT) nanostructures was examined by using a scanning electron microscope (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 SEM (Phillips, XL-30S FEG, Germany). The surface of the sample was then

scanned at the desired magnification to study the morphology of the nanostructures. The degree of MAT incorporation in the synthesized poly (HEMA–MAT) nanostructures was determined by elemental analyzer (Leco, CHN932, USA).

2.3 Deltamethrin Adsorption Studies From Aqueous Solution

Deltamethrin adsorption studies were performed in a batch system with 60 min period at 25^{o}C while stirring continuously in the adsorption medium. Some variables such as time, pH, initial deltamethrin concentration, temperature and ionic strength were studied to optimize adsorption conditions. To determine effect of equilibration time of adsorption, adsorption was carried out at different times such as 15, 20, 30, 40, 50, 60, 90 and 120 min. To observe the effects of pH, pH of the solution was changed between pH 2.0 and 8.0 using different buffer systems (0.1 M citrate buffer was used for pH 2.0-3.0, 0.1 M acetate buffer was used for pH 4.0-5.0, 0.1 M phosphate buffer was used for pH 6.0 – 8.0). Initial deltamethrin concentration was changed between 2-1000 mg/L. To determine the effects of temperature, temperature values were changed between 5^{o}C and 45^{o}C. NaCl concentrations were changed between 0.01-1M to study the effect of ionic strength. The amount of adsorbed deltamethrin per unit mass of the nanostructure was calculated by using the following expression.

$$Q = \frac{C_0 - C_e}{M} V \qquad (3)$$

Where;

Q: adsorbed deltamethrin amount (mg/g)

C_0: initial deltamethrin concentration (mg/L)

C_e: deltamethrin concentration at equilibrium (mg/L)

V: volume of deltamethrin solution (L)

M: amount of nanostructure used (g)

The adsorption experiments were conducted for 60 min which was the equilibrium period for the adsorption of deltamethrin at 25^{o}C. Initial and final deltamethrin concentrations were determined by high performance liquid chromatography (HPLC). This method also applied on determination of deltamethrin in real water samples. All water samples were filtered with filter paper and 0.45 μm Whatman paper. The filtered water samples were spiked at different concentrations using 1 mg/L of deltamethrin standard solution and spiked samples applied on HPLC for determination of deltamethrin.

2.3.1 High Performance Liquid Chromatography (HPLC) Analyses

Many different analyses conditions were tested and the one giving the best chromatography performance in terms of peak shape and retention time was selected. Deltamethrin analyses were carried out according to the method involved in the study of Trajkovska and Petrovska-Jovanovie with some modifications (Trajkovska & Petrovska-Jovanovie, 2002). HPLC analysis of deltamethrin was performed using a Shimatzu SCL-10AVP HPLC system (Shimadzu Corporation, Japan): isocratic pump (LC-10ADVP), degasser (DGU-14A), manual injector (4200), UV-VIS detector (SPD-10AVP), 150x3 mm i.d. C18 column (Thermo ODS Hypersil) and system controller with a PC control program. Mobile phases were in double deionized water (A) and in acetonitrile (B). The conditions were as follow: A 15%, B 85% during the analyses. Other chromatographic conditions were as follows: flow rate, 0.8 mL/min; injection volume, 20 μL and run time, 10 min. Spectral data was collected at 210 nm. Stock solution of deltamethrin standard was prepared in acetonitrile.

2.3.2 Studies in Real Water Samples

Deltamethrin adsorption studies from water samples such as tap, river and ground water, were performed in a batch system with 60 min period at 25^{o}C while stirring continuously in the optimized adsorption medium. Tap water was obtained from tap in our laboratory, while river and ground water were collected from Gümüldür,

İzmir. River water was collected from a river especially through tangerine gardens. All water samples were filtered with filter paper and 0.45 μm Whatman paper before use. The filtered water samples were spiked with deltamethrin standard and then 1.2 mL spiked water samples added to the 0.3mL of poly (HEMA-MAT) solution. The mixtures were shaked for 60 min at 25°C. After shaking, samples were centrifuged for 60 min at 12000 x g, and then supernatant was taken and applied to the HPLC for the measurement of deltamethrin. The adsorption capacities were calculated by using the equation (3).

CHAPTER THREE
RESULTS AND DISCUSSION

3.1 Characterization of Poly (HEMA-MAT) Nanostructure

In this study, we suggested a novel method of introducing a hydrophobic group into the polymeric nanostructures via polymerization of HEMA and MAT. The distinctive feature of this method is the elimination of the activation and ligand coupling steps during the preparation of the affinity matrices. Some other advantages over other methods include the use of a known amount of ligand in the polymer preparation mixture and the good reproducibility of the affinity matrix. Figure 3.1 shows the FTIR spectra of poly (HEMA) and poly (HEMA–MAT) nanostructures. As seen in Figure 3.1, stretching vibration of hydroxyl groups of both poly (HEMA) and poly (HEMA–MAT) nanostructures was observed at 3531 cm^{-1}. The bond in this wave number of poly (HEMA–MAT) nanostructures is sharper than the bound of the poly(HEMA). It may be possible because the bound also contains N-H stretching vibrations in the poly (HEMA–MAT) nanostructures. The bound around 750 cm^{-1} indicates the aromatic characteristic of the MAT structure. These data confirmed that the poly (HEMA–MAT) nanostructures were formed with MAT functional groups.

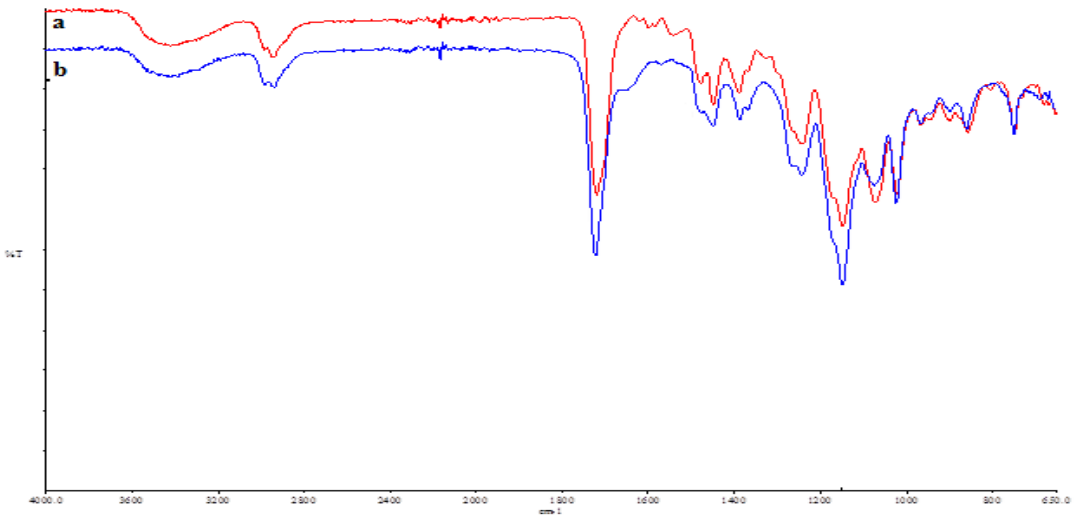


Figure 3.1 FTIR spectrum of; (a) poly (HEMA), (b) poly (HEMA–MAT) nanostructures.

As seen in Figure 3.2, particle size of the poly (HEMA–MAT) nanostructures were measured by Zeta Sizer and determined about 100 nm with 1.189

polydispersity. The particle size was an average of minimum 30 measurements, and the size distribution was recorded automatically by the software of these repeated measurements. To evaluate the degree of MAT incorporation into the polymeric structure, elemental analysis of the synthesized poly (HEMA–MAT) nanostructures was performed. The incorporation of the MAT was found to be 1.95 mmol/g polymer using nitrogen stoichiometry. Nanostructures can produce larger specific surface area and therefore their loading capacity may be very high. The specific surface area was calculated as 1856 m^2/g for poly (HEMA) and 1914 m^2/g for poly (HEMA–MAT) nanostructures.

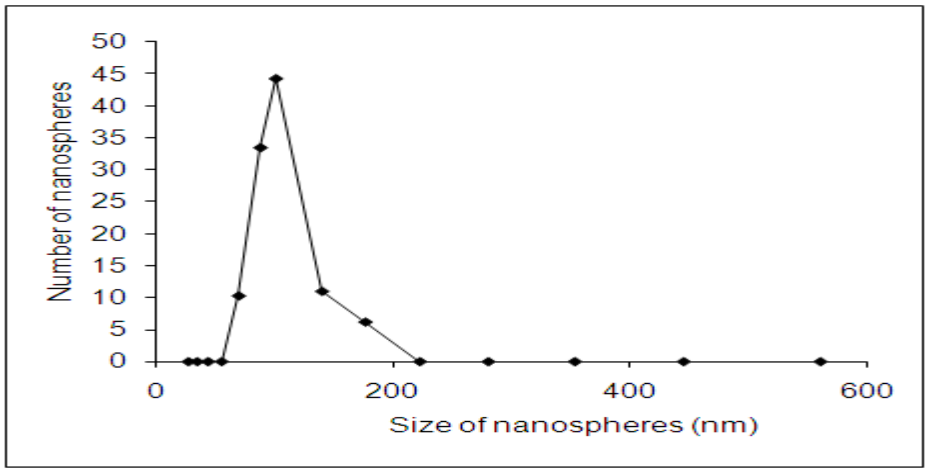


Figure 3.2 Size analyses of poly (HEMA–MAT) nanostructures.

As seen in Figure 3.3, the SEM micrographs clearly show the spherical character of poly (HEMA–MAT) nanostructures. In addition, the total monomer conversion was determined as 98.6% (w/w) for poly (HEMA–MAT) nanostructures.

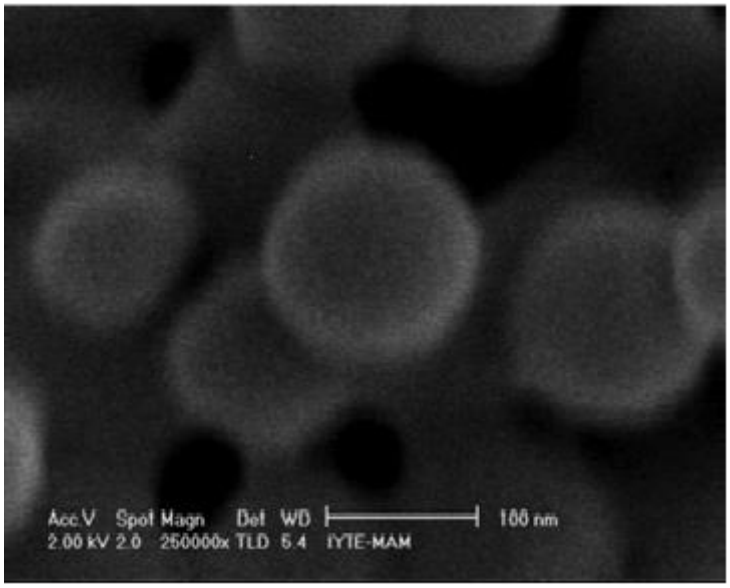


Figure 3.3 Microscopic observations; SEM images of poly (HEMA– MAT) nanostructures.

3.2 HPLC Analysis

Many different analyses conditions were tested and the one giving the best chromatography performance in terms of peak shape and retention time was selected. Deltamethrin analyses were carried out according the method involved in the study of Trajkovska and Petrovska-Jovanovie with some modifications (Trajkovska & Petrovska-Jovanovie, 2002). Mobile phases were determined as double deionized water (A) and acetonitrile (B). The conditions were as follow: A 15%, B 85% during the analyses. Other chromatographic conditions were as follows: flow rate, 0.8 mL/min, in room temperature and in 210 nm wavelength. Optimal conditions for separation of the deltamethrin were established by evaluating commercial HPLC columns and evaluations of mobile phase. The HPLC profiles of standard and water samples (ground and river water) were shown in Figure 3.4 and Figure 3.5, respectively. The retention time of the deltamethrin standard was 2.78 min at flow-rate of 0.8 mL/min. But, retention time of deltamethrin in water samples occurred to chance because of matrix effect. Run times per sample were 10 min.

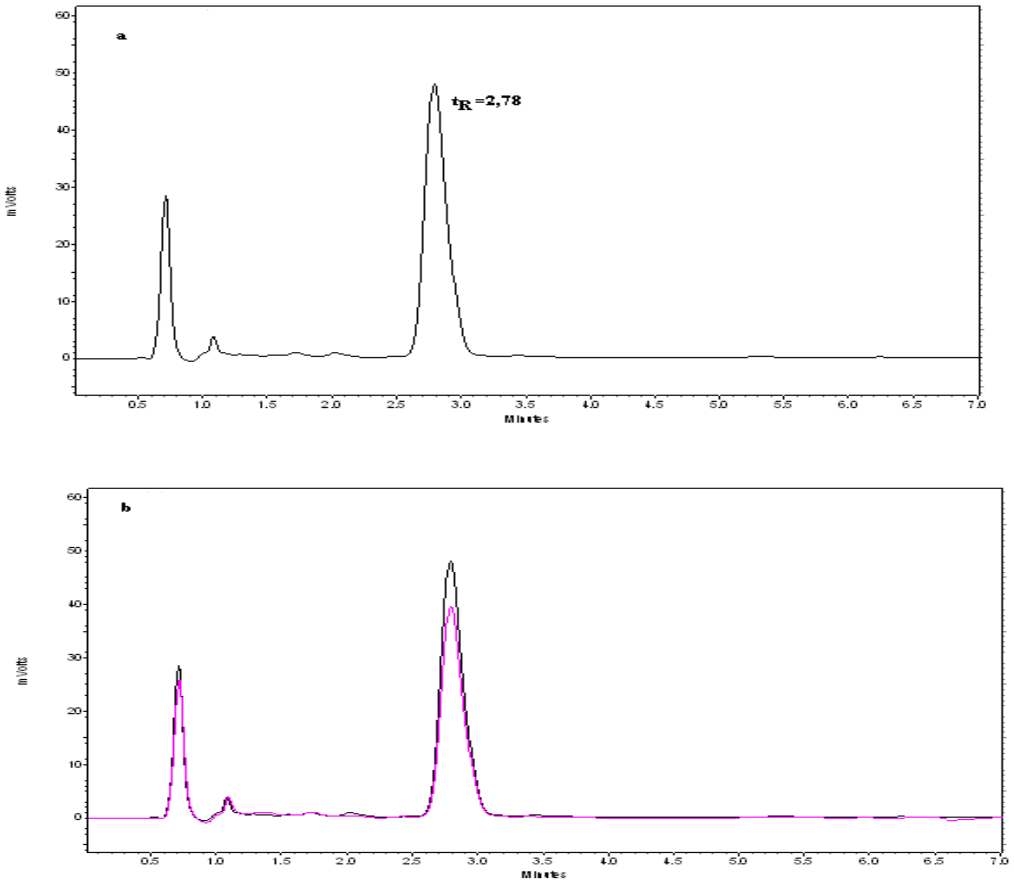
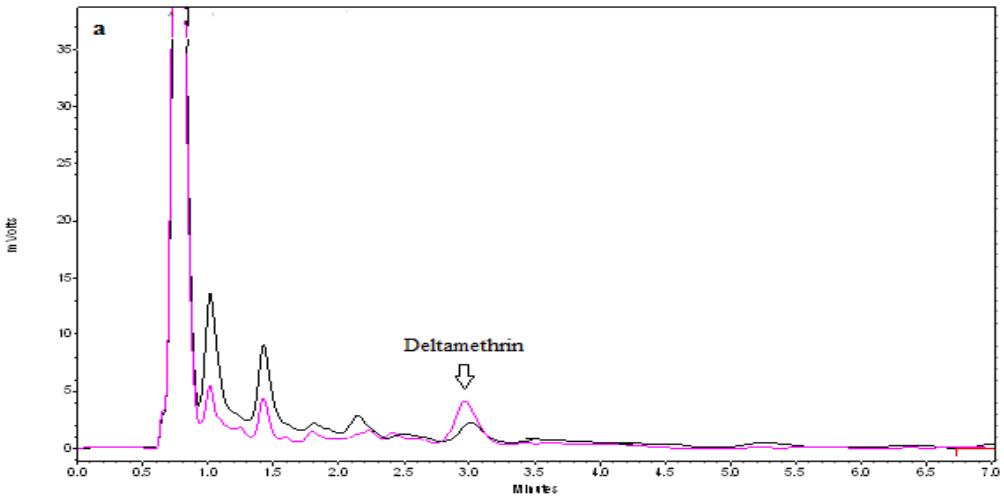


Figure 3.4 a) Chromatogram of 2 mg/L standart solution b) Chromatogram of 2 mg/L standart solution before and after adsorption.



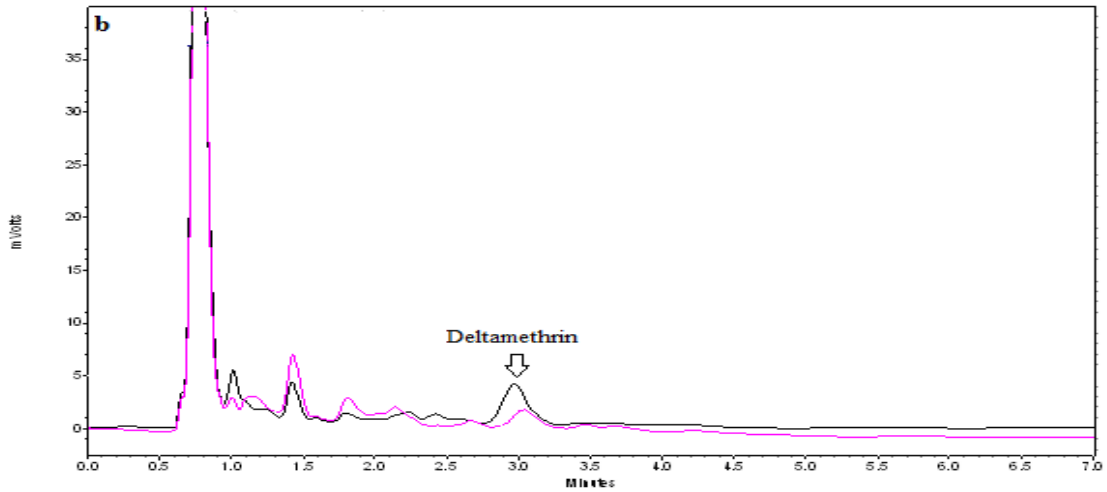


Figure 3.5 Chromatogram of water samples a) Chromatogram of 0.5 mg/L spiked and not spiked ground water sample b)Chromatogram of 0.5 mg/L spiked and not spiked river water sample.

The sensitivity of the method for deltamethrin was determined by construction of a calibration curve in the low concentration region (10–50 µg/L). The limit of detection was calculated as three times the ratio between the standard division (SD) and the slope of the low concentration curve (LOD = 3.SD /slope) and the limit of quantification as ten times the same ratio (LOQ = 10.SD /slope) (Trajkovska & Petrovska-Jovanovie, 2002). Under these chromatographic conditions deltamethrin shows the best sensitivity. LOD and LOQ were calculated as 1.57 µg/L and 28.30 µg/L, respectively.

The within day repeatability of deltamethrin standard peak area was performed by 6 successive injections of 1mg/L of deltamethrin standard. The between days repeatability of deltamethrin standard peak area was performed by 2 successive injections of 1 mg/L of deltamethrin standard for 5 days. % RSD for within day and between days was calculated 2.8 and 3.2, respectively.

3.3 Optimization of Deltamethrin Adsorption

3.3.1 Effect of Contact Time

To determine the effect of contact time on deltamethrin adsorption onto the poly (HEMA-MAT) nanostructure, adsorption studies were performed from 0 to 120 min. As shown in Figure 3.6, deltamethrin adsorption increased with time and reached a plateau of saturation at 60 min. Therefore, all adsorption studies were performed at 60 min.

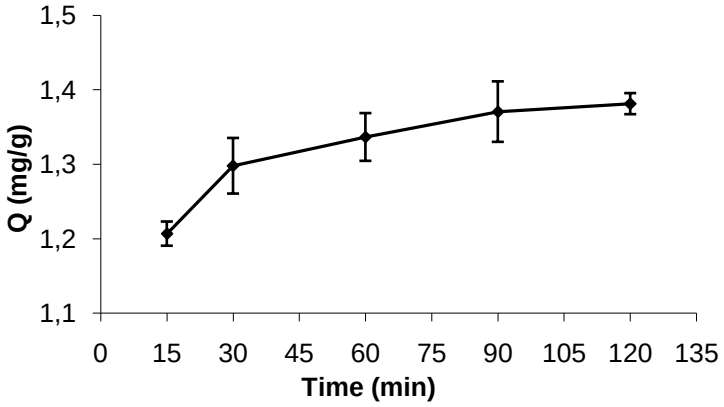


Figure 3.6 Effect of contact time on the deltamethrin adsorption (C:2 mg/L, T:25°C).

3.3.2 Effect of Initial Deltamethrin Concentration

The concentration of deltamethrin in the adsorption medium was varied between 2 and 1000 mg/L. The results of the adsorption studies for poly (HEMA-MAT) nanostructures are presented in Figure 3.7. We observed that the amount of adsorbed deltamethrin increased with the initial deltamethrin concentration. Maximum adsorption capacity of the prepared nanostructures was found to be 315.6 ± 12.0 mg/g and adsorbed amounts per unit mass of poly (HEMA-MAT) nanostructure reached a plateau at about 800 mg/L. It should also be noted that negligible amount of deltamethrin (35.3 ± 2.5 mg/g) adsorbed non-specifically on the plain of the poly (HEMA) nanostructures. Incorporation of MAT significantly increased deltamethrin

adsorption capacity of the nanostructures (about 9-fold). This significant increase may be due to a strong specific interaction between deltamethrin and MAT.

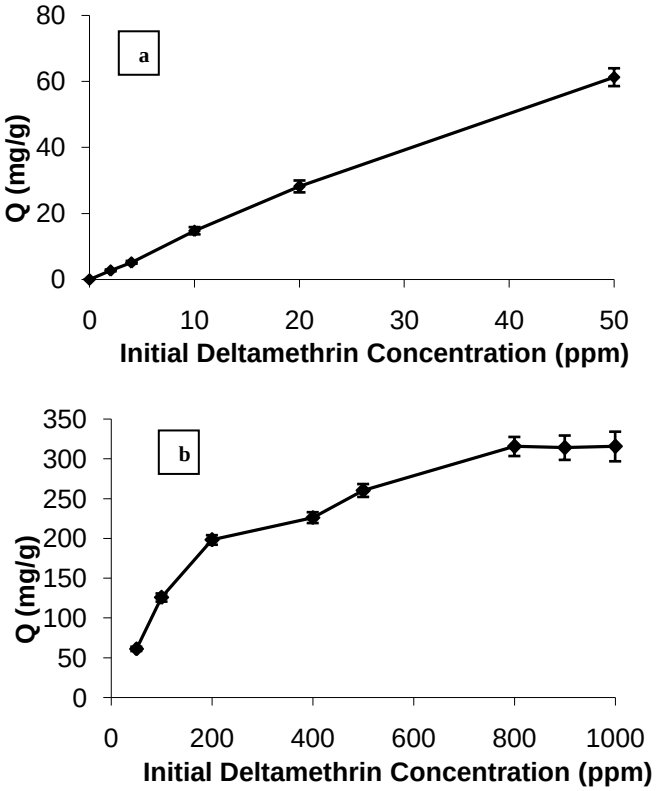


Figure 3.7 Effect of initial deltamethrin concentration a) low deltamethrin concentration effect b) high deltamethrin concentration effect (T: 25°C, 60 min).

3.3.3. Effect of pH

The effect of pH on the adsorption of deltamethrin onto poly (HEMA–MAT) nanostructures was studied in the pH range of 2.0–8.0 at 25°C. It is evident from Figure 3.8 that as pH values increases from 2 to 8, maximum adsorption of deltamethrin onto the poly (HEMA-MAT) nanostructures decreases from 7.69 ± 0.11 to 6.26 ± 0.16 mg/g. This means that adsorption of this pesticide is enhanced in the acidic medium. In acidic medium protonation occurs to the amino groups and the pesticides become positively charged. For this reason more electrostatic attraction may occur between the pesticides and the adsorbent (Al-Qodaha, Shawaqfeh, & Lafi, 2007).

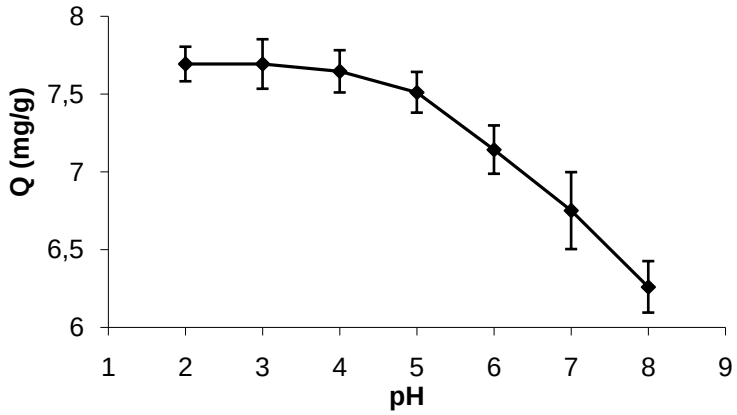


Figure 3.8 Effect of pH (C:2 mg/L, T:25 °C, t: 60 min)

3.3.4 Effect of Temperature

The adsorption studies were performed over a range of temperatures from 5 to 45°C. As seen in Figure 3.9, the adsorption capacity of the poly (HEMA-MAT) nanostructures was increased with increasing the temperature (about 27.4%). Increasing the temperature is known to increase the rate of diffusion of the adsorbate molecules across the external boundary layer and in the internal pores of the adsorbent particle, owing to the decrease in the viscosity of the solution. Furthermore, increasing the temperature will increase solubility of pyrethroids (pesticides) in water (Al-Qodaha, Shawaqfeh, & Lafi, 2007). In addition, this significant increase observed between 5-45°C may be explained according to the theories developed for the interaction of hydrophobic solutes in water. These theories proposed that the binding of molecules to HIC adsorbents is entropy driven, which implies that the interaction increases with an increase in temperature (Builder, 1993).

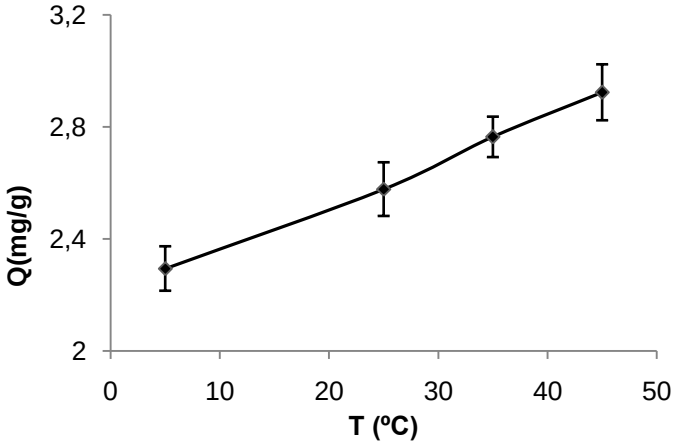


Figure 3.9 Effect of temperature (C:2 mg/L, t: 60 min).

3.3.5 Effect of Ionic Strength

To determine the effect of ionic strength on deltamethrin adsorption, adsorption studies were carried out in the range of 0.0-1.0 M NaCl. As shown in Figure 3.10, the adsorption capacity of poly (HEMA-MAT) nanostructures increased about 2.8 % as the NaCl concentration was increased from 0 to 0.1 M, whereas the adsorption capacity of poly (HEMA-MAT) nanostructures was not significantly changed in the concentration range of 0.1-1.0 M NaCl. The addition of various structure-forming salts to the equilibration buffer or sample solution promotes ligand-sorbate interaction in HIC. As the concentration of such salts is increased, the amount bound molecules also increases (Builder, 1993). The salt-depended increase in the adsorption capacity of the poly (HEMA-MAT) nanostructures may be due to the increased hydrophobic interaction between deltamethrin and hydrophobic indole ring of tryptophan in these nanostructures.

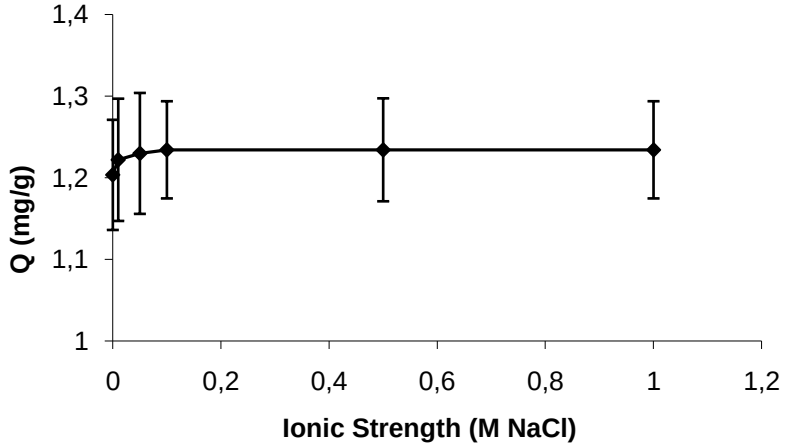


Figure 3.10 Effect of ionic strength (C:2 mg/L, T:25 °C, t: 60 min).

3.3.6 Desorption Studies and Repeated Use

Different desorption agents such as THF, THF containing 0.00001% CTAB, 0.0001% TritonX-114 and 0.00001% SDS, 0.1 M ammonium sulfate and ethyl acetate were used for the desorption of deltamethrin from the poly (HEMA-MAT) nanostructures. Desorption of deltamethrin from the prepared nanostructures was carried out in a batch system with these desorption agents for 1 h at 25°C and repeated two times. Up to 100% of the adsorbed deltamethrin was desorbed from the poly (HEMA-MAT) nanostructures in THF containing 0.00001% CTAB (Figure 3.11).

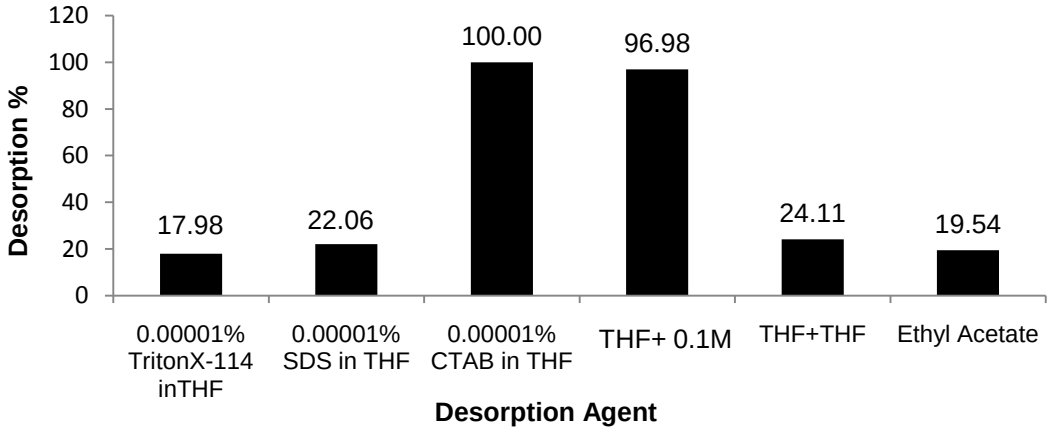


Figure 3.11 Desorption of deltamethrin with different desorption agents.

In order to show the reusability of the poly (HEMA-MAT) nanostructures, the adsorption-desorption cycle was repeated five times using the same nanostructures. There was no remarkable reduction in the adsorption capacity of the nanostructures (Figure 3.12). The result showed that poly (HEMA-MAT) nanostructures can be repeatedly used in deltamethrin adsorption without detectable losses in their initial adsorption capacities.

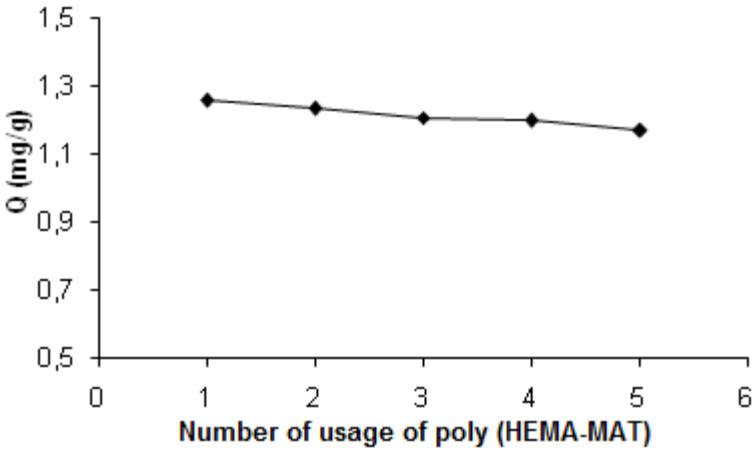


Figure 3.12 Repeated use of poly (HEMA-MAT) nanostructures (C:2 mg/L, T:25 °C, t: 60 min).

3.4 Removal of Deltamethrin from Real Water Samples

Deltamethrin in real water samples was determined using HPLC as described above. Deltamethrin was not detected in tap water, while amount of deltamethrin were determined in river and ground water as 20.06 and 13.82 μg/L, respectively.

All water samples (tap, river and ground) were spiked with 0.01, 0.1 and 0.5 mg/L deltamethrin standard for determination of deltamethrin amount in real water samples. Deltamethrin adsorption studies from water samples such as river and ground water were performed in a batch system for 60 min at 25°C while stirring continuously. Deltamethrin determined in real water samples were adsorbed onto the poly (HEMA-MAT) nanostructures at the rate of 100%. Deltamethrin in real water samples was desorbed with THF containing 0.00001% CTAB from poly (HEMA-MAT) nanostructures in proportion as 91.2% for river water sample and 76.48% for

ground water sample. With these result, it could be concluded that the poly (HEMA-MAT) nanostructures can also be effectively used for the removal of deltamethrin from real water samples.

CHAPTER FOUR
CONCLUSION

A larger surface area for the attachment of hydrophobic molecules may be provided by the hydrophobic smaller particles. Therefore, it may be useful to synthesize hydrophobic nano-sized particles with large surface area and utilize them as suitable carriers for the adsorption of hydrophobic molecules. Adsorption of hydrophobic molecules, especially hydrophobic pesticides, through the hydrophobic interaction is based on the association of hydrophobic groups on the surface of hydrophobic molecules with hydrophobic groups on the surface of matrix. In this study, hydrophobic tryptophan containing nanostructures were prepared by the polymerization of HEMA and MAT. The adsorption of deltamethrin, a hydrophobic pesticide, on hydrophobic nanostructure was investigated using various reaction conditions. Very high amounts of adsorption were determined due to the relatively larger surface area of the nanostructure used in this study. An important advantage in proving process economics is the reusability of the adsorbent. It was also determined that desorption ratios were very high (up to 100%) and the nanostructures prepared in this study were suitable for repeated use of deltamethrin adsorption without noticeable loss of capacity.

In addition, deltamethrin in different water samples was successfully removed with the poly (HEMA-MAT) nanostructures (about 100%). Recovery values of river and ground water samples were determined as 91.25 and 76.48%, respectively. Finally, it can be concluded that the hydrophobic tryptophan containing nanostructures revealed good adsorption properties as a nano-support and will be useful in the removal of deltamethrin from different water sources.

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