Republic of Iraq
AL-Nahrain University
College of Science

Characterization of new cross linked polymer as potential stationary phase for HPLC separation

A Thesis

Submitted to the College of Science of AL-Nahrain University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

By

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(B.Sc. 2002)

Rabee'a Al-Thany 1426 **May 2005**

بسم الله الرحمن الرحيم

فتعالى اللهُ المِلك الحقُ ولاتَعجل

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(سورة طه ١١٤)

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الإهداء

الى مثلي الاعلى ...وقدوتي في الحياة ...الى نبع المحبة الصافي الى من علمني معنى العطاء و التضحية

والدي

الى من صبرت وسهرت من اجل راحتي ... الى فيض الحنان الدافق

والدتي

الى من شرفني الله بوجودهم ... الى سندي و مشجعي في الشدائد

الى مهد طفولتي و مرح شبابي ... و أحباب قلبي ...

أخوتي

إلى كل من أحبني ... و أراد مودتي ...

أهدي هذا الجهد المتواضع

نكتل

ACKNOWLEDGMENTS

I wish to express my deepest gratitude and great appreciation to my supervisors **Dr. Shahbaz A. Maki** and **Dr. Emad T. Baker** for their patient, supervision and encouragement during the course of my study.

I would like to express my thanks and appreciation to the department of chemistry and the College of Science at Al-Nahrain University for offering me this opportunity to accomplish this thesis.

Finally, sincere thanks and deep respect goes to all my teachers, friends, and my family for their help and support.

Naktal

Summary

A cross linked polymer was synthesized by condensation reaction between polyacrylic acid with succinic acid dihydrazide in the presence of phosphorus oxychloride. The products were characterized by FTIR. The resulted polymer has high rigidity and easily grinded, with high stability. This polymer was used as stationary phase for HPLC. This stationary phase has been packed into stainless steel column. The chromatographic performance of the newly packed column was characterized. The number of theoretical plates (N), high equivalent to theoretical plate (H), capacity factor(K)and selectivity factor(α) were measured by analyzing different analytes on the new packed column using different mobile phase compositions and flow rates. Some of polyaromatic compounds such as phenanthrene ,acenaphthylene, anthracene and naphthalene were separated with this column either using mixture of (40%:60%), hexane and isopropanol or 100% isopropanol, as mobile phases. with flow rate 1ml/min with UV detection at 254 nm.

Furthermore some amino acids (tyrosine , Tryptophane, Phenylalanine, and Histidine) were also separated using this column with a mixture of phosphate buffer at pH 6 in 10% acetonitrile or15% methanol at pH6.5 as mobile phase with flow rate of 1ml/min with UV detection at 254 nm and RI detector.

Calibration curves for all the analyzed compounds were linear from their detection limits to at least 25 ppm with correlation coefficient range from 0.9995 to 0.9999. The detection limits were ranged from 0.05 to 0.1 ppm at signal to noise ratio of three or more for most analyzed compounds.

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List of abbreviation

LC	*Liquid chromatography*
HPLC	High performance liquid chromatography
BPC	Bonded-phase column
PITC	Phenylthiocyanate
PTC	Phenylthiocarbamyl
OPA	Orthophthalaldehyde
EC	Electrochemical
ODS	Octyldecylsilane.
PS -DVB	Polystyrene divinylbenzene
CD	Cyclodextrin
HDI	Hexamethylene diisocyanate
FTIR	Fourier transform infra red
i.d.	Internal diameter
RI	Refractive Index
DMF	dimethylformamide
DMSO	dimethylsulphoxide

Introduction: -

1.1-Chromatography:

Chromatography is a method used primarily for separation of the components of a sample. The components are distributed between two phases; one is a stationary while the other moves (the mobile phase). The stationary phase may be a solid, or a liquid supported on a solid, or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. The mobile phase is either a gas (gas chromatography) or a liquid (liquid chromatography).In gas chromatography, there are two kinds of interactions to consider; those between the solute molecules themselves and between solute molecules and the stationary phase [(1)].In liquid chromatography (LC); however, there are three kinds of interactions. Solute-mobile phase, and solute-stationary phase, in addition to solute-solute interaction [(1)].

1.2. Liquid chromatography (LC):

Liquid chromatography is a method for separation of sample components as they pass down a column packed with a stationary phase, due to the distribution of sample components between two phases the mobile phase and the stationary phase. There are two types of (LC), classical and high performance liquid chromatography (HPLC). Classical (LC) uses a large column approximately 20-50 cm packed with large particles 50-250 μm in diameter. Sample volumes in the milliliters range are often common. The mobile phase is generally a gravity-fed at slow flow rates. Since the deep pores of packing limits mass transfer, the analysis times may be in order of hours. Fraction collection of separated sample components for later spectroscopic and other identification method is a usual practice with this technique.

The second type is (HPLC), which is a technique that separates components of mixture more efficiently that classical (LC) does. HPLC requires special sample-injection system, columns, and pumps to provide uniform flow rates. The stationary phase must be sufficiently rigid so that its dimensions do not change with pressure. A further consequence of the reduction in column and particle size was that the volume of the detector cell which has to be small to accommodate the eluted sample components. The importance of HPLC is evidenced by the fast growth in published scientific papers [(2)], which site the technique as the chosen method of analysis of wide varieties of compounds in comparison with (GC).

1.3. ***Types of liquid chromatography:***

HPLC can be carried out in any of the classical modes such as normal-phase and reversed-phase as well as ion exchange (either cationic or anionic) chromatography [(2)]. The types of liquid chromatography also named after the general type of interaction that occurs between the stationary phase and solutes in the eluent [(6)]. The exact mechanism of chromatographic separation that operates on a given application is determined principally by the nature of the packing [(2).]

1.3.1. Normal phase (Adsorption) chromatography:

The separation in normal phase chromatography is carried out using conventionally polar stationary phase, and a non-polar organic mobile phase [(7)]. In this liquid solid adsorption chromatography the lattice of common porous adsorbent stationary phase (e.g. alumina or silica) is terminated at its surface with polar hydroxyl groups. These groups; provide the mean for surface interactions with solute molecules. The sample is applied to the column;

molecules with polar functional groups are attracted to the active sites on the column packing. Polar molecules of the eluent will subsequently displace the sample components in this mode.

The solvent (a non-polar solvent), commonly hexane, containing a small amount of polar additives, such as 2-propanol is used as eluent. The mechanism of separation involves no partioning of the sample solute in the stationary phase; instead the polar groups of each organic solute interact through primarily hydrogen bonding forces with polar sites of the stationary phase [(8)].

As the chromatography is developed the sample components is passed down the column to be re-adsorbed on the fresh sites of the stationary phase packing. The ease of displacement of solute molecules will depends on their relative polarities, the more polar molecules will be adsorbed more strongly and hence elute more slowly from the column [(2)]

One of the strength of adsorption chromatography is its ability to separate isomers, particularly aromatic functionalized compound with polar groups in the retention order ortho> meta> para as reported by Majors [(9)], who was able to separate nitro aniline isomers in that order.

1.3.2.Reversed phase chromatography:

In reversed phase mode the interactions and separation is based on a non-polar stationary phase and a relatively polar eluent. The retention of an analyte depends on the degree to which it is partitioned into the stationary phase and it's largely determined by the hydrophobic interactions of the analyte with the mobile phase. This can be considering as the repulsion of the analyte molecule from the aqueous mobile phase; its represent interferes with the stable hydrogen bonding between the polar groups [(7)].

The packing materials have been developed in which the stationary phase is chemically bonded to an insoluble matrix (solid support). The main advantage of this bonded-phase column (BPC) packing is its quite stability and cannot be easily removed or lost during use. The availability of a wide variety of functional groups, in BPC allowed both normal and reversed phase chromatography to carry out in relatively simple and straight forward manner. Reversed phase BPC involved a relatively non-polar stationary phase used in conjugation with polar mobile phase to separate a wide variety of less polar solutes [(8)].

BPC packing is prepared by many synthetic methods. Solid support used is either silica or synthetic polymer such as polystyrene-divenylbenzene. Functional groups such as hydrocarbons, amino, ethers, and ion exchange groups such as sulfonic acid and quaternary ammonium salts were attached chemically to the support[(9)]. Tanaka [(68)] prepared alkylated stationary phases for reversed phase liquid chromatography based on polymer particles with aliphatic backbones, having hydroxyl groups, the chromatographic properties were examined in terms of steric selectivity and preference for aromatic and saturated compounds. Polymer supports stationary phase were less hydrophobic than silica-based phases, but they showed preferential retention of aromatic compounds. The preference shown by the polymer-based stationary phases toward rigid, compact molecules over flexible and/or bulky molecules can be explained by the contribution of the polymer network structure, to the retention process. The polymer-based stationary phases showed greater variation of selectivity due to changes in the composition of the mobile phase [(9)].

1.3.3.Ion-Exchange chromatography (IEC):

(IEC) is an electrostatic method for the separation of ionic species based on their differential migration on ion exchange resin column [10]. A process in which a charged solute molecule in the mobile phase solution is electrostically attracted (the ion exchange equilibria) to the oppositely charged stationary phase particles [13].

The most commonly used resin in ion exchange chromatography is copolymer styrene-divinyl-benzene. Functional groups are placed on the benzene rings to provide the ion exchange sites. Depending on the type of ions that have available for exchange in the resin, the process may be called cation or anion exchange resin. The terms strong or weak ion exchanger refer to acid / base strength of the functional groups.

Sulfuric acid, $R\text{-}SO_3^-H^+$, and carboxylic acid, $R\text{-}COO^-H^+$, functional groups are considered as strong and weak cation exchangers, respectively, while the quaternary ammonium groups, $R\text{-}N^+(CH_3)_3Cl^-$, and the tertiary group, $R\text{-}N^+(CH_2CH_3)_2HCl$, are considered as strong and weak anion exchangers, respectively.

Ion exchange is a process in which charged analyte in the sample solution competes with an eluent ion, of like charge for sites having the opposite charge on stationary phase resin.

In the stationary phase, the ions are immobilized and it travels through column with the mobile phase. The separation of analyte ions depends upon the differential affinities of a functional group for different analyte ions. The relative affinities of analyte for the stationary phase are known as the selectivity. Selectivity is determined by many parameters, including type of functional group, stationary phase, concentration and characteristics of eluent

ions, its concentration, and the additives to the mobile phase such as buffer or ion pairing agents, as well as the temperature.

The first two parameters determined by the design of the ion exchange column and usually optimized for inorganic anions or cations and organic acid. The other parameters can be adjusted during analysis.

The number of functional group sites on the stationary phase is known as the ion-exchange capacity of the column and it is usually expressed as the number of equivalents per column or equivalents per gram of resin. A higher capacity results in longer retention of the analyte ion. It is independent on selectivity. Capacity can be increased or decreased without altering selectivity [(14)]. It is determined by taking a weighed amount of cation exchanger in the H^+ form, replacing the H^+ with an alkali metal cation, and titrating the librated H^+ with base. Anion exchangers in the OH^-or Cl^-, which is titrated with standard acid or Ag^+ in similar way.

The general uses of ion exchanger resins are separation of ions and ionizable species, determining the equivalent distribution of mixtures or oligomeric ions, determination of several ions concentration in mixtures, of samples of low concentration. Kang [(16)] developed a chromatographic system with methylene blue as the counter ion for the separation of organic and inorganic anion. Alizarin Red-S has been recently used to modify several anion exchanger resins and found a great application for the selective analysis of several metal ions [(16)].

KI-Won and workers [(17)] have used Amberlite IRA-400 coated with Alizarin Red-S as a functional group for the pre-concentration and separation of

Fe (II) from solution containing Co, Ni, and Pb using classical column and pH 4.5 buffer solution and 0.1 HNO_3 as eluent [18].

Saxons[19] has described the synthesis a chelating polymer matrix by immobilizing Alizarin Red-S on Amberlite XAD-2 and its application to the separate ions of Pb (II), Cd (II), Zn (II) and Ni (II)

1.4.HPLC DETECTION SYSTEM: -

The detectors in HPLC are employed for continuously monitor the column eluent. The detector signal is generally amplified and processed to a potentiometric recorder to obtain a permanent signal record with time in the form of a chromatogram[20]..

Wide varity of HPLC detectors have been developed with high sensitivity and universal detection requirements. The HPLC detectors can be generally classified as either responsive to change in the property of the mobile phase, when a solute (sample component) is present or to a property of the actual solute itself [12]. These include UV-Visible spectrophotometer, refractive index, conductivity, fluorescence, electrochemical detectors and others [21].

UV detector spectrophotometer is the most widely used in HPLC. Its popularity is due to a wide range of applicability, excellent stability, and low cost. It is relatively insensitive to temperature change and flow variation. Detection limit at the nanogram level for a certain type of compounds can easily be achieved. This device has a high sensitivity for many solutes but samples must absorb in the UV-Visible region (190-900nm) to be detected. Both fixed and variable wavelength detectors are commercially available with HPLC equipments [4]. These detectors equipped with a low-volume flow cell (8 µl or less), usually 1 cm in path length. The UV-Visible detector has detection limit

of a bout $1x10^{-9}$ g cm^{-3} for highly absorbing compounds with large extension coefficient.

Janssen and coworkers [23] describe a method for amino acid determination that are used phenylisothiocyanate (PITC) to form phenylthiocarbamyl (PTC) derivatives of amino acids which can be separated by reversed phase HPLC and detected by UV at 254 nm.

The second type of detector is the refractive index (RI). It works by measuring the change in refractive index of the eluent as the solute passes through the sample cell. The RI detector is non destructive and can be used on a continuous basis. It can sense all materials, that's why it is considered as universal detector. RI cannot be used in gradient elution mode since the base line is continuously varied as the solvent mixture changes which causes refractive index variation. The detection limit of RI ranges between 10^{-8}-10^{-6} g cm^{-3} [22].

The third type of detection system is the conductivity detector. Electrical conductivity is a universal property of all ionic solutions. After a chromatographic system equilibrates with the eluent ions, the magnitude of the response is proportional to the difference in conductance of the analyte and the eluent ions as well as the concentration of the analyte. In order to detect a small analyte signal it is necessary to employ an eluent, which gives a relatively low conductance [24]. Response of conductivity detectors is temperature dependent; consequently, temperature must be controlled carefully. This detector has been extremely valuable for analyzing both inorganic and organic ionic substance in aqueous mobile phase.

Fluorescence detector is another important detector used in HPLC. In this detector the solute is exited by UV radiation of a given wavelength (the excitation wavelength) and the fluorescence energy which is emitted at a longer wavelength (the emission wavelength) is detected [(25)].

Fluorescence detector has been successfully employed with compounds which are naturally fluorescent, or which have been chemically reacted to form fluorescent derivatives [(26)]. Fluorescent detection system has given a useful selectivity in trace analyses when either the sample size is small or the solute concentration is extremely low [(27)].

The number of fluorescing species can be enlarging by treatment of samples with reagents that form fluorescent derivatives. 5-dimethylaminonaphthalene-1-sulphonylchloride (dansylchloride), which reacts with primary and secondary amines, amino acids, and phenols to give fluorescent compounds, has been widely used for the detection of amino acids in protein hydrolyzates. Aminoquinoline (AMQ) which reacts with amino acids to form fluorescent derivatives has been used by Cohen and DeAntonis [(28)] to study the separation of amino acid derivatives (AMQ-amino acids) using ODS column with fluorescence detector at excitation wavelength 250 nm and emission wavelength 395 nm).

Most of the HPLC derivatization chemistry is based on well-known reactions reported in organic or inorganic chemistry literature[(29)]. The fluorescent derivatization of primary amines such as amino acids using orthophthalaldehyde (OPA) and mercaptoethanol either in the pre-column or post-column modes has been well studied by Lindroth and Mopper [(29)] to

separate OPA-amino acids derivatives at the nano gram level on 25 cm C 18 column (5μm) with fluorescence detector (excitation wavelength 330 nm and emission wavelength 418 nm).

Electrochemical detectors provide useful selectivity for electro active compounds such as ketons, aldehydes, marcaptans, peroxides, phenols, and aromatic amines. Many electro-reducible and electroxidizable compounds can be detected in column effluents at very low concentrations by selective electrochemical (EC) measurements. With this approach the current between polarizable and reference electrodes is measured as a function of applied voltage [(10)]. The polarizable electrode may be constructed of a material such as platinum, gold, glassy carbon, or graphite electrode. Any reference electrode can be used. Usually the voltage applied to the polarizable electrode is set, and the current monitored with time. The current-generated at a fixed, pre-set potential is amplified to the output response[(30)] Developments in cell technology have led to electro chemical detection with mercury - drop electrode [(10)].

1.5.Column Packing Methods: -

The optimum or "best" procedure for packing columns is determined by the nature and size of the packing particles. The goal is to pack a uniform bed with no cracks or channels and without sizing or sorting the particles within the column. Usually rigid solids and hard gels are packed as densely as possible but without fractioning the particles during the packing procedures [(10)]. The "tap-fill" procedure was recommended for the dry packing of rigid particles with diameter dp >20 μm. The technique for dry-filling high efficiency LC columns

is not very different from that used to prepare high efficiency GC columns [10]. The high-pressure "wet- fill" or slurry-packing techniques were, alternatively, used for packing particles with dp <20 μm. In this technique a suitable liquids were used to wet the particles and eliminate particle aggregation during packing [31].

1.5.1. Down-Flow Method: -

The down-flow method has been most widely used, and it permits the preparation of satisfactory columns of all types of micro particles packing. Rigid particles are required for the best results with this method. An apparatus such as shown in figure (1-1) is used. The slurry mixture is rapidly forced downward into the column blank with a constant pressure pump [10] as describe in the figure (1-1). Slurry flow rate is dependent on the pressure used and as the packed bed is formed, the flow rate decreases. Forcing the slurry mixture into the column blank at highest possible velocity generally produce the best column performance. This operation is carried out by pumping the slurry into the column blank at the highest pressure permitted by compression fittings connecting the column to the slurry-packing apparatus. The pump used for packing could be either reciprocating or penumatic pump [32]. The very high initial velocity as the slurry enters the column blank may fracture weak particles, producing fine particles that tend to plug the column outlet and caused packing structure irregularities [10].

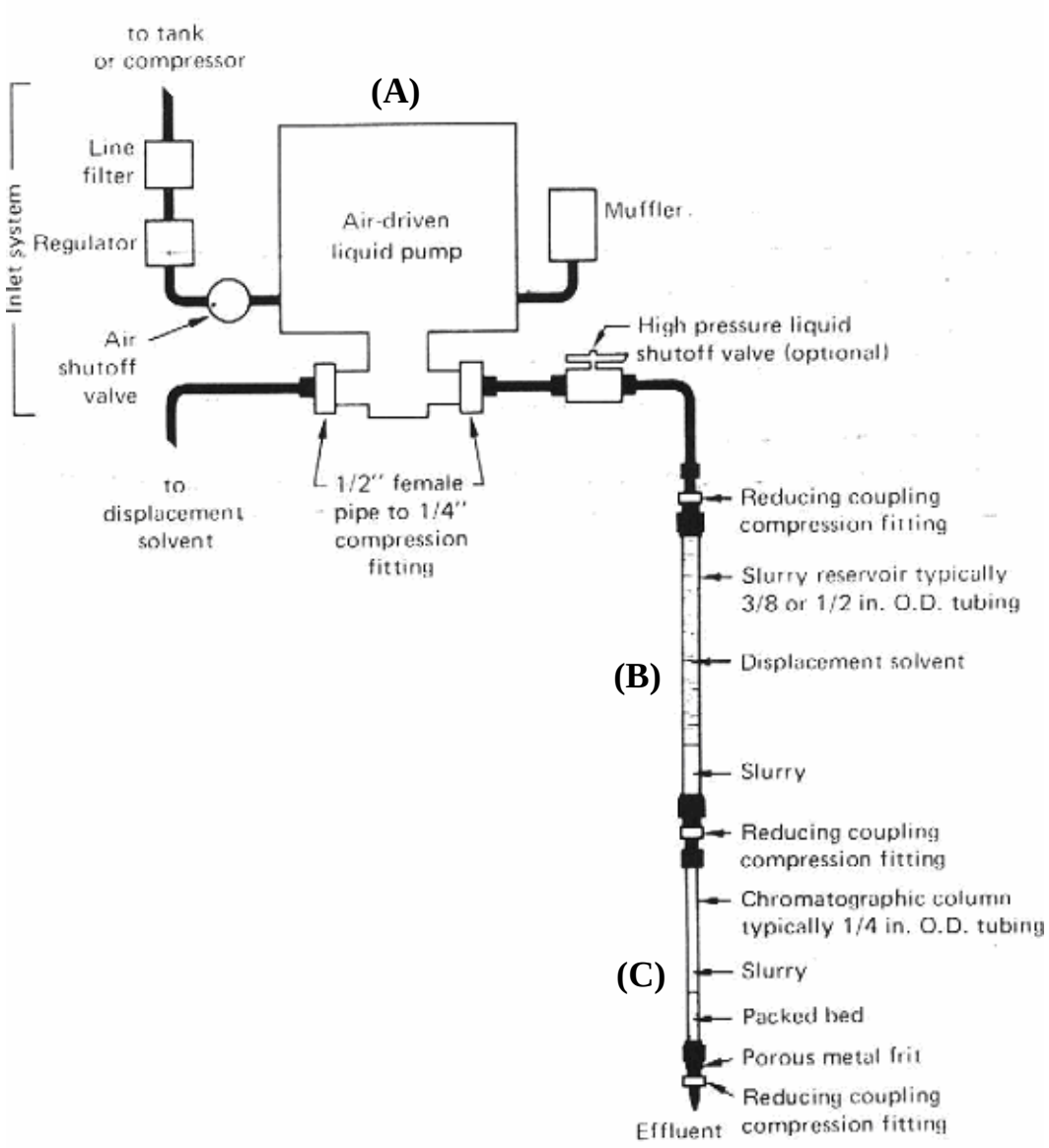


Figure (1-1) High-pressure slurry packing assembly with constant pressure pump [10].

1.5.2. Up-Flow Method: -

An alternative technique for wet filling columns is the up-flow approach, which has been described by Bristow [33]. Experience with the up-flow packing method has not extensive, but column performance results have been about equivalent to those for the down-flow procedure. The equipment used for the up-flow packing is shown in figure (1-2). In this case the slurry is pumped up into the column blank from the reservoir whose contents are continuously diluted by incoming pressurized liquid [34].

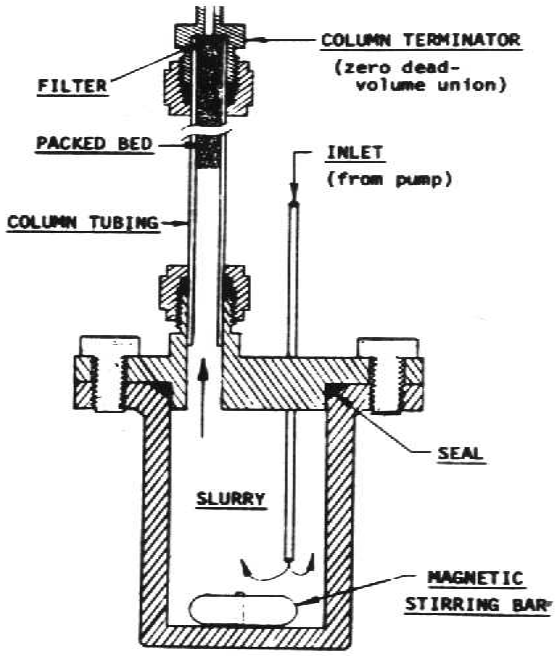


Figure (1-2) Equipment used for Up-Flow Packing System [10]

In the up-flow approach the velocity of the particles striking the forming bed must be sufficiently high and the liquid sufficiently great to prevent particles bed from falling back into the reservoir [35].

1.6.Stationary Phase: -

One of the two phases forming a chromatographic system is the stationary phase. It is the part of chromatographic system responsible for retention of the analytes, which are being carried through the system by the mobile phase. It may be a solid, a gel or a liquid; it may be distributed on a solid support. This solid support may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (bonded phase) or immobilized onto it (immobilized phase)[7]. Particularly in gas chromatography where the stationary phase is most often a liquid coated on solid support.

1.7.The Nature of the stationary phase Materials in L.C.:

A wide range of stationary phases and column packing materials have been developed over the years for liquid chromatography as shown below [30]: -

1.7.1.Immobilized material: -

A stationary phase which has been immobilized on the support particle, or on the inner wall of the column tubing, by either a physical attraction (coated stationary phase), by chemical bonding (bonded stationary phase), or by in situ polymerization (cross-linked stationary phase) after coating.

1.7.2.Coated material: -

A material in which a stationary phase is immobilized by a physical attraction to the surface of the solid support.

1.8. Bonded material: -

A stationary phase which is covalently bonded to solid support particles or to the inside wall of the column tubing. Sometime referred to as a bonded phase (material).

1.8.1. Alkyl-bonded material: -

Bonded stationary phase (material) in which the groups bonded to the surface contain an alkyl chain (usually between C_1 and C_{18}). The most widely used alkyl bonded phase materials are those derived from the surface reaction between a silica support and an appropriate organochlorosilane (or organoalkoxy) modifier. Various R groups are used, however, most common in commercial particles are those where R is octyl or octyldecylsilane (ODS).

1.8.2. Cyano- bonded material: -

Bonded stationary phase in which the group bonded to the surface contains a cyanoalkyl- (-$[CH_2]_n$-CN) group.

1.8.3. Polymeric –bonded material:-

Bonded stationary phase (material) prepared using a polyfunctional reagent which can react both with the surface of the solid support and/or with additional reagent molecules.

Currently, chemically bonded silica packing is most widely used in (HPLC). A number of advantages, including high performance, reproducibility, little irreversible adsorption and fast equilibrium with mobile phase, make alkylsilylated silica packing indispensable in reversed –phase liquid chromatography (RPLC)[31].

However, silica-based materials do have limitation. They can only be used in a relatively narrow pH ranges, between 2 and 8. Secondary effects, such as those due to silanols and metal ions in the stationary phase, produce tailing of protonated amines and carboxylic acids [(12)]. To increase the chemical stability, some silica particles that coated with a polymer layer have recently become available [(39)]. These have a longer live at higher pH range than conventional reverse- phase packing, but not as long as the organic polymer gel.

1.9.Cross-linked material: -

A stationary phase material in which the liquid phase on a solid support has been polymerized or cross-linked after coating to make it insoluble in the mobile phase.

1.10.Polymeric material:

Stationary phase materials were based on particles of a cross- linked organic polymeric material. Synthetic cross-linked organic polymer was introduced as packing in column liquid chromatography since 1960 [(40)]. The first organic polymer based packing were synthetic ion exchanger made by condensation polymerization of phenol and formaldehyde. Typical materials in this kind of stationary phase are polystyrene divinylbenzene copolymers (PS-DVB) and modified PS-DVB materials. With respect to the mechanical rigidity of the polymeric packing, cross-linking becomes an essentially means in the synthesis. Other requirements which must be met are insolubility, resistance to oxidation and reduction, and a defined, controllable, and reproducible size and pore structure [(22)].

Polymerization is performed either by condensation or addition polymerization, depending on the type of starting monomer. For cross-linking,

co-monomers such as divinylbenzene, bis-ethylene glycol methacrylate, 2,3-dibromopropanol are added [35]. The cross-linking reagent can be added to amount as much as 70 % (w/w). Macro porous copolymers are synthesized in the presence of an inert solvent, which functions as a volume modifier. Both the cross liner and the inert solvent have a substantial impact on the kinetics of the polymerization and the resulting properties of the copolymer.

As in the synthesis of silica packing, specific properties must be chosen in polymerization to manufacture polymeric packing with beads of controlled size distribution by following technical polymerization as show below.

1- Emulsion polymerization starts with a solution of a detergent to which the monomers are added. As a result, micelles swollen with the monomer are formed. After water- soluble initiator is added (for styrene as a monomer), polymerization leads to particles of exactly the same size as the swollen micelles. Emulsion polymerization processes generate particles of up to 0.5µm in one step [22].

2- Suspension polymerization is usually designed to prepare larger beads of >5µm mean particle diameter. The monomer or co-monomer solution is vigorously agitated in water in the presence of colloidal suspending agent. The colloidal agent coats the hydrophilic monomer droplets (in the case of, say, styrene or divinylbenzene). Coalescence of the droplets is prevented by the surface charge of the droplets. Adding a lipophilic catalyst or initiator starts the polymerization in the droplets and this continues until the beads are solidified in bulk. The size of the beads is thus controlled by the size of the droplets via the stirring speed [22].

A third variant in polymerization technology is the swollen emulsion polymerization pioneered by Ugelstad [42]. The procedure is performed in two

steps. First is by adding a swelling agent, which causes the sub-micrometer polymer particle to swell by large volumes of the monomer before starting the polymerization. The increase in volume can reach a factor of 1000. Second, in a consecutive step the monomer swollen beads of defined size are polymerized.

Having briefly examined the structure of organic polymer packing and the various routes in their manufacture, the most important features may be summarized as follows [(22)]: -

1- Hydrophilic as well as lipophilic organic polymer packings are synthesized with a controlled pore and surface structure depending on the type of monomer/co-monomers and the polymerization reaction. The surface structure can be attired by controlled consecutive surface reactions.

2- In accordance with the bulk composition, polymer packing is stable across almost the entire pH range, particularly under strong alkaline conditions.

3- The chemical stability is affected by oxidizing and reducing solutions.

4- Although cross-linking reactions have been optimized in as much as rigid pressure, stable particles can be manufactured, and some remaining swelling property is often noted when changing the solvent composition in HPLC.

5- As in the manufacture of silica, porosity, pore size, and surface area of polymer packing can be adjusted over a wide range, and micro-, meso-, and macro- as well as nonporous beads are synthesized reproducibly.

Pill et al. [(43)] have prepared cyclodextrin (CD) polymers from the reaction of native CD_s with a hexamethylene diisocyanate (HDI) compound in a dried DMF solution. The obtained CD polymer contained a range of (8-14)% Nitrogen due to HDI by elemental analysis.

An HPLC column was prepared using the CD polymer stationary phase with a carbamate linker by a slurry method. Separation of the phenol isomers (o-, m-,

and p-nitro phenols) was conducted using the CD polymer stationary phase by HPLC.

Sunamoto etal[44] developed new method of HPLC using packing materials modified with cross-linked poly (N-isopropylacrylamide) (PNIPAAm) hydrogel. A temperature-responsive surface was prepared by polymerization of NIPAAm in the presence of a cross-linker on the silica support. The surface properties and functions of the stationary phases change in response to the external temperature. Therefore it easily changes the interaction of a solute with the surface with a constant aqueous mobile phase. A temperature-responsive elution behavior was observed on the separation of steroid and PTH-amino acids. The method is expected to be application to separation in the pharmaceutical and biomedical fields. [44]

Yokoyama etal[45] developed a newly low-capacity cation exchange column for the separation of amino acids. A highly cross-linked macro porous polystyrene- divinylbenzene copolymer was functionalized by a sulfoacylation reaction. The exchange capacity was controllable at the acylation step. The capacity between 55 and 60 μmol/column was adequate for the practical separations in acceptable retention time. The 5-μm base polymer having average pore diameter smaller than 3nm gave satisfactory results, and those 1.5nm pore was most favorable several isocratic elution conditions at different pH values adjusted by phosphate buffer provide good separation for individual classes of amino acids, i.e., acidic, neutral, hydrophobic, and basic groups. The results provided fundamental data for constructing gradient elution system required for the simultaneous separation of protein amino acids.

The resins as matrix materials through chemical modification can be conveniently changed into various types of HPLC packing with high column efficiency, high permeability and different selectivity, for example, ion exchanger HPLC packing carrying tertiary amino groups, quaternary ammonium groups, carboxyl groups and sulfonic acid groups. The preliminary tests showed that most of the modified resins as new HPLC packing possess excellent chromatographic properties especially for the separation of biochemical substances such as amino acids[(48)].

In our team they prepared a cross linked polymer and use it as stationary phase. This polymer was prepared from reaction of mixture of trieethanolamine and glycerol with malic anhydride. The stationary phase has been packed into a stainless steel column, and used for separation of some of amino acids such as Histidine, Phenylalanine, Tryptophane, and Tyrosine. The analyzed compounds have shown characteristic retention time and different selectivity. This has aloud separation of Phenylalanine, Tryptophane, and Tyrosine with this column using 100 % hexane, at 1.5 ml/min as a mobile phase and detection wavelength of 254 nm.

Some of polyaromatic compounds such as Fluorine, Phenathrene, Anthracene, Naphthalene, and Acenaphthylene, were also analyzed by this column. These Poly aromatic compounds separated by using only distilled water as a mobile phase with flow rate of 1 ml/min[(65)].

The aim of the work:-

The aim of this work was to prepare a new cross-linked polymer HPLC stationary phase. This stationary phase was prepared based on the condensation reaction of poly acrylic acid and succinic acid di-hydrazide. An HPLC column to be packed with this stationary phase. The chromatographic performance of the new column was studied for the separation of some polyaromatic hydrocarbons and amino acid compounds.

2.1. Chemicals:-

The following chemicals and materials used in this work were obtained from different companies as listed below:-

Compounds	Supplied from
Acetonitrile	BDH
Acenaphthylene	BDH
Acrylic acid	BDH
Anthracene	BDH
Benzen	BDH
Benzoyl peroxide	Fluka
Chloroform	Hopkin and Williams
Diethyl ether	BDH
Dioxan	Fluka
DMF	Fluka
DMSO	BDH
Ethanol	BDH
Fluorine	BDH
Hexane	Merk
Histidine	Fluka
Hydrazine hydrate	Fluka
Methanol	Fluka
Naphthalene	BDH
Phenanthrene	BDH
Succinic acid	BDH
Toluene	BDH
Tryptophane	Fluka
Tyrosine	Fluka

2.2. INSTRUMENT AND EQUIPMENTS

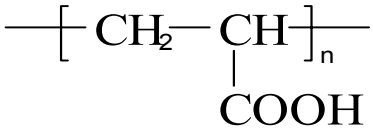
- High performance liquid chromatograph used in this work was Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP , UV-VIS detector model SPD-10AVP, a liquid delivery pump model LC-10AVP, a degasser model DGU-RA and Rheodyne manual injector model 3298 (USA), equipped with 5 mL sample loop. The HPLC system has been interfaced with computer via a Shimadzu Class-VP5 chromatography data system program supplied by the manufacture; and Epson LQ-300 printer model P852A (Japan).

● Shimadzu Fourier transforms infrared model FTIR 8300 (Kyoto, Japan) was used to measured the IR spectra of the prepared polymer.

- Melting points were recorded on hot stage Gallen kamp melting point apparatus.

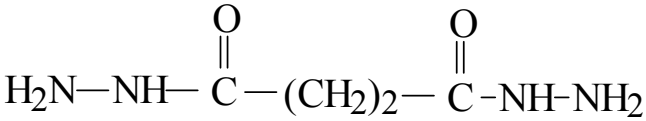
2.3. Preparation of poly (acrylic acid) in toluene

A solution of 10g of acrylic acid and 0.05 g of benzoyl peroxide dissolved in 30g of toluene was heated to the boiling point of the solvent. A very violent reaction took place, heating was discontinued for 15 minute, the reaction mixture was cooled, and the product was isolated by filtration, after drying at reduced pressure yield (75%).



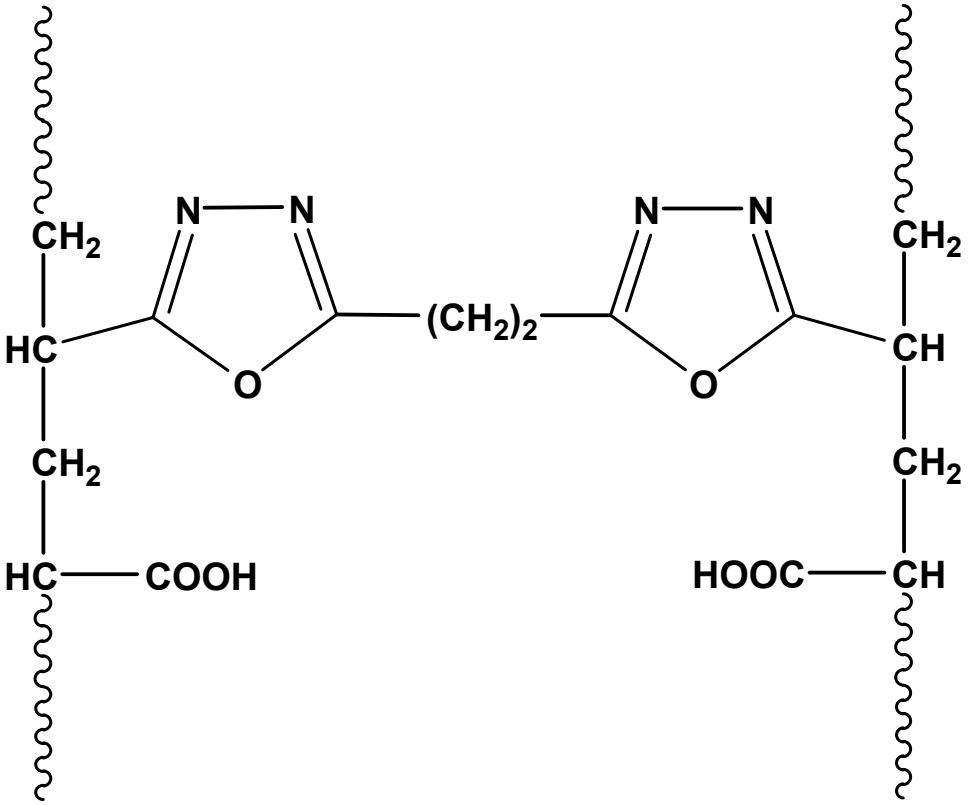
2.4. Preparation of succinic acid dihydrazide

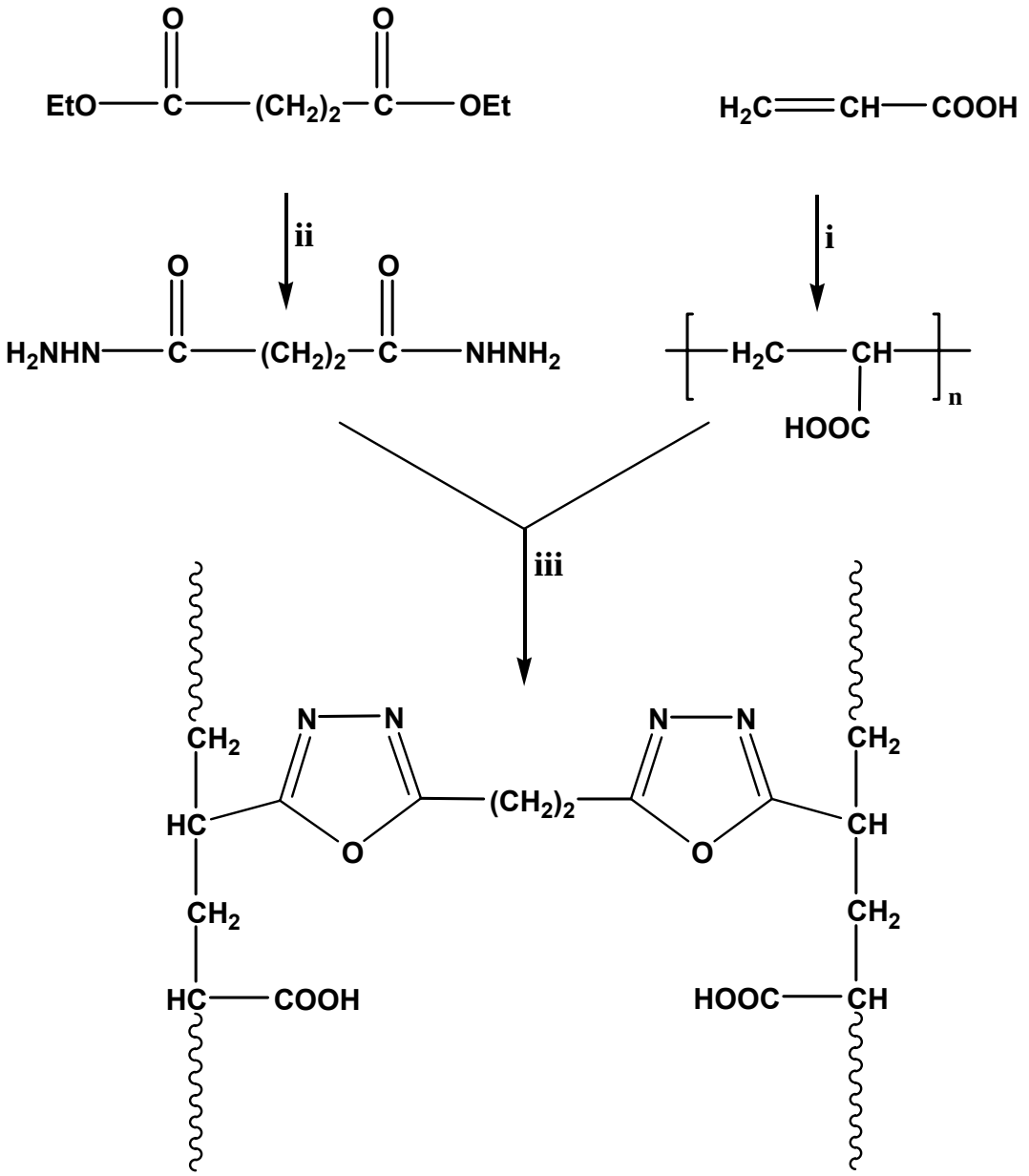
A mixture of diethyl succinate (0.06mole, 10ml) and hydrazine hydrate (0.1mole, 5ml) was refluxed for (1) hour. Ethanol (20ml) was added to the above mixture and refluxed for another (9) hours. The excess solvent was distilled off and the crude product was recrystallized from ethanol to give pure succinic acid di-hydrazide (m.p. 170-171C^0) yield (68%).



2.5. Preparation of Modified cross-linked polymer

A mixture of polyacrylic acid (0.5g) and phosphorus oxychloride (5ml) and succinic acid di-hydrazide (0.5g) was refluxed for (9) hours on water bath. The cold mixture was poured on crushed ice and made basic by adding sodium bicarbonate solution, the precipitate, was separated, filtered and washed with cold water to give the desired modified polymer as shown in scheme 1.





i) Polymerization by benzoyl peroxide.
ii) NH_2-NH_2, EtOH, reflux (9) hrs.
iii) $POCl_3$, reflux (9) hrs.

Scheme 1 preparation of cross-linked polymer:

2.6 Viscosity Measurements:-

Viscosity measurements of the polyacrylic acid solution was carried out using a suspended Ostwald viscometer type at 25^{o} C. Measurement was repeated until three consecutive reading. A Hewlett-Packared quartz thermometer measured the temperature of the water path. A constant temperature water path (Julabo Exatherm U3 electronic. Germany) with a basic control unit was used .

2.7. Polymer Swelling Determination:

Swelling measurements of the polymer was performed by placing (0.2g) of polymer samples in enough quantity of water hexane and acetone. Determination of sample weight was measured each (24) hour for (5) days until the weight become constant. The swelling was calculated using equation, which relates Δm to swelling percentage [(49)].

$$\Delta m = \frac{m_t - m_\circ}{m_\circ} \times 100$$

m_o = weight before swelling sample
m_t = weight after swelling sample

2.8 Determination the molecular weight (Mv) of Polyacrylic acid by viscometry method

Usually the molecular weight of this polymer(polyacrylic acid) is characterized by measurements of the viscosity of dilute solution; the viscosity and molecular weight are related by the Mark-Houwink equation [66].

$[\eta] = K M^{\alpha}$ ..... (1)

$[\eta]$ = is the intrinsic viscosity.

K, α = are constants dependent upon the polymer-solvent system at a particular temperature.

The intrinsic viscosity of a polymer solution was measured with on Ostwald U-tube viscometer.

Solutions were made by dissolving the polymer in a solvent [g/100ml] and the flow time of pure solvent is [t_0] and the flow time of polymer solvent is [t].

Relative viscosity [η rel.] was calculated by the equation [67]

η rel.$= t/t_0$ .....(2)

specific viscosity

η spc.$= \eta$ rel. -1 .....(3)

and intrinsic viscosity

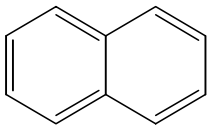
$\{ \eta \} = \eta$ spc./c .....(4)

C= concentration of polymer solution (g/100mL)

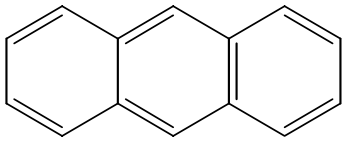
2.9. Sample preparation:

A stock solution of 100ppm of each of Tyrosine, Phenylalanine, Histidine, Tryptophane, Anthracene, Acenaphthylene, Naphthalene, Phenanthrene were prepared. The solvent used to prepare the above solutions were usually the same as the mobile phase employed for their separation. Other standard solutions of these hydrocarbons and amino acids were prepared by subsequent dilution of the stock solutions. Mixtures of two or more of the above analytes were also prepared by mixing the appropriate volumes of the stock solutions.

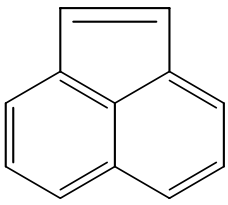
Structures of polyaromatic compounds:



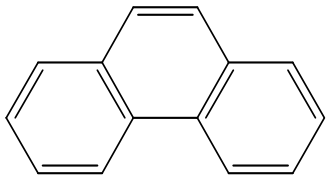
naphthalene



anthracene

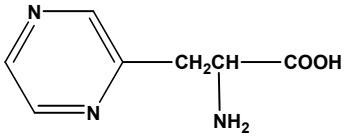


acenaphthylene

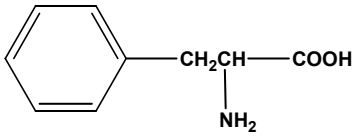


phenanthrene

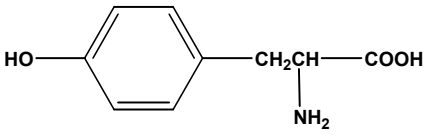
Structures of amino acid compounds:



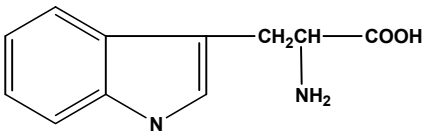
Tyrosine



Tryptophane



Phenylalanine



Histidine

2.10. Packing of the column

The blank column tube used in this work was first cleaned with 50% nitric acid and then with methanol after washing with distilled water and drying. It was packed with appropriate stationary phase as follows.

The column was packed using down-flow packing system. The first part is shimadzu pump (a) as shown in the figure (1-1), which is used instead of the air-driven pump. The packing material was dispersed in 100 ml acetonitrile and placed in the slurry reservoir (b). The column (c) was plugged with its appropriate fitting from one side and placed in the beaker for draining; the other side was connected to the slurry reservoir (b). The pump was set at the beginning to deliver 4 ml/min. the back pressure read 500 kgf/ cm^2. The flow rate was then increased by 1 ml/min increment until reaching 8 ml/min at constant period of time. The recorded pressure was 600 kgf/cm^2, 700 kgf/cm^2, 800 kgf/cm^2, 900 kgf/cm^2, respectively. After the compression process was completed, the

column was then disconnected from the system and was checked. Visually and found to be completely packed, and then connected to the HPLC system.

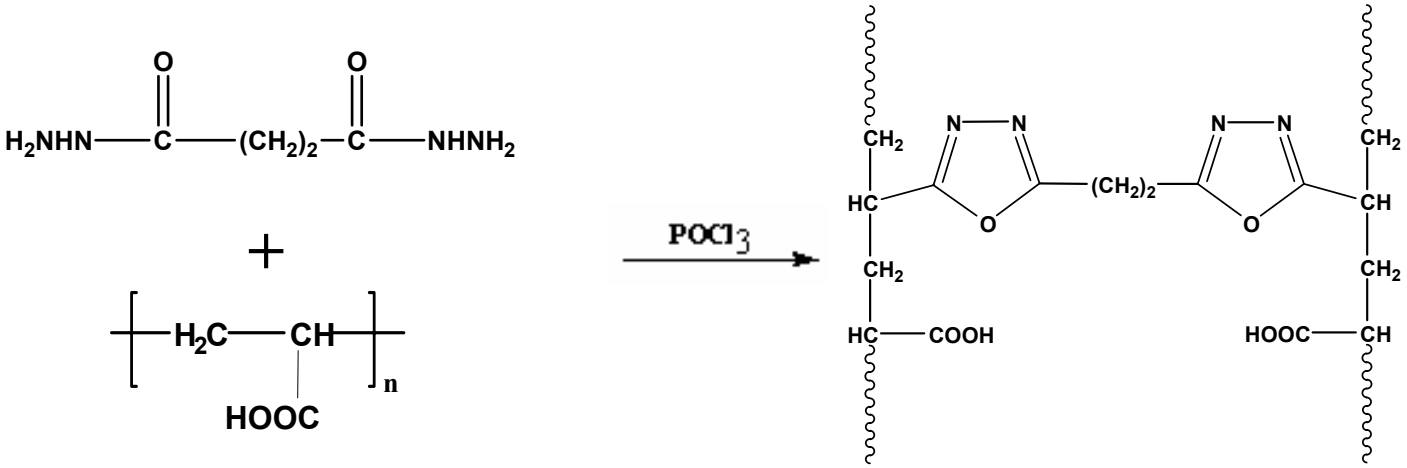
The flow rate was set at 1 ml/min, and acetonitrile was passed through the system for about two hours.

2.11. Analysis of samples:-

All the prepared standard solutions and their mixtures have been chromatographically analyzed (for at least three times) in the prepared newly packed column using different mobile phases such as hexane, acetonitrile, methanol, water or mixture of some of them at optimum flow rate of 1.0 ml/min. using UV-VIS detection at 254nm wavelength or Refractive Index detector (RI).

3.1. Preparation of cross-linked polyacrylic acid resins:-

A cross-linked polyacrylic acid polymer was synthesized (scheme I) by condensation reaction process from the reaction of polyacrylic acid with succinic acid di-hydrazide



Scheme (II). The structure of the prepared polymer.

The reaction was take place by mixing the polyacrylic acid with succinic acid dihydrazied in acetonitrile as a solvent in the presence of ($POCl_3$) as dehydrating agent . The reaction requires (9) hours to be completed. For comparison between starting material (polyacrylic acid and succinic acid di-hydrazide), the FTIR spectra for the polyacrylic acid and succinic acid dihydrazide are shown in figures (3-1) and (3-2) respectively. The spectrum, in figure (3-1), shows some distinguished peak at 1720 cm^{-1} representing the carbonyl group for polyacrylic acid. While, figure (3-2) shows the spectrum of succinic acid dihydrazide and distinguished peak at 3315.4 cm^{-1} which representing NH-NH_2 stretching vibrations (asymmetric, symmetric) and the carbonyl amide group in 1633.6 cm^{-1}.

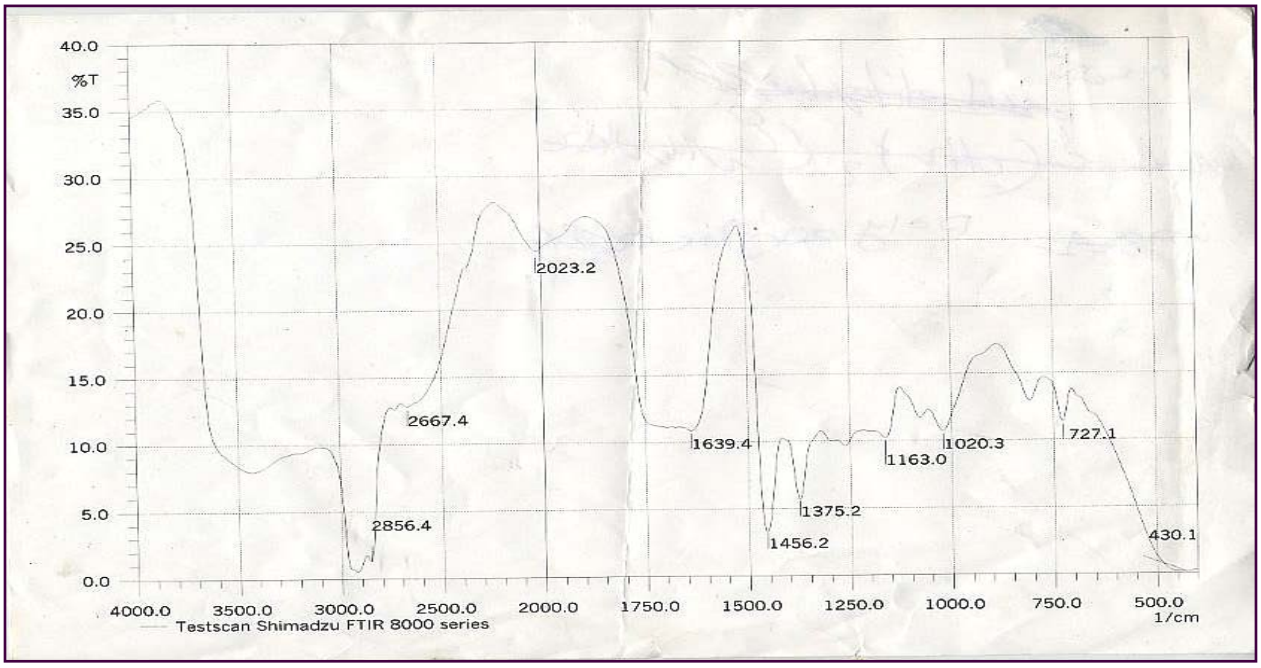


Figure (3-1): FTIR spectrum for poly-acrylic acid

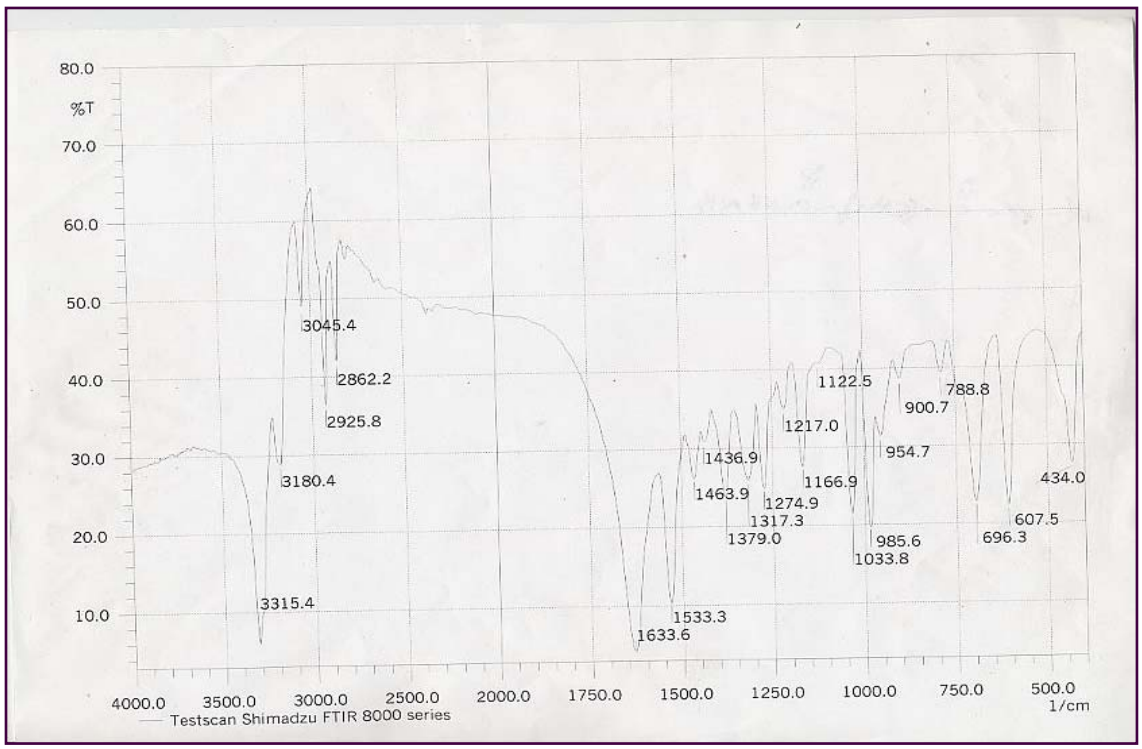


Figure (3-2): FTIR spectrum for succinic acid di-hydrazide

However, the prepared polymer (the cross-linked polymer) was characterized by FTIR spectrum as shown in figure (3-3). The spectrum exhibits disappearance of the band at (3315cm^{-1}-3180 cm^{-1}) which was attributed to the NH-NH_2 stretching vibrations (asymmetric, symmetric), and the appearance of the peak at (1630 cm^{-1}) representing the (C=N) vibration. The peaks at (3450 cm^{-1}) and (1720 cm^{-1}) represent (OH) and (C=O) vibrations respectively are for the unreacted carboxylic group (COOH) for polyacrylic acid as shown below in figure (3-3).

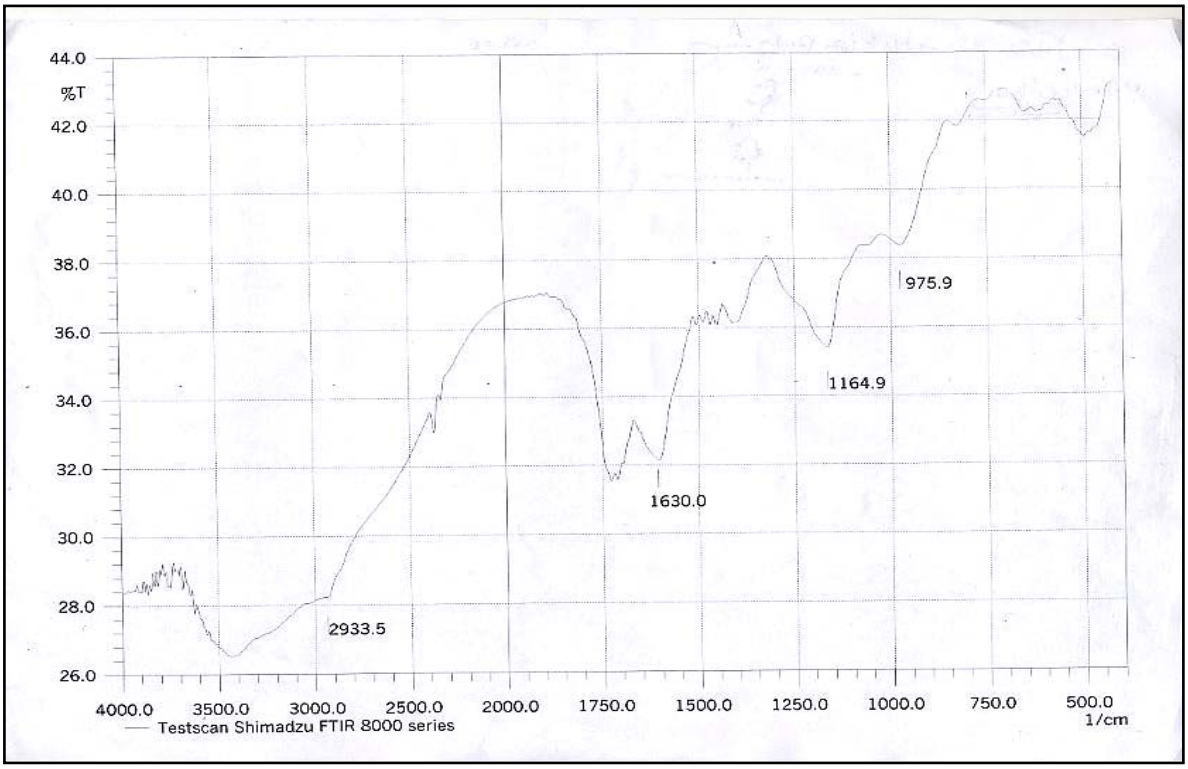


Figure (3-3) FTIR spectrum for the modified prepared polymer

3.2 Determination the molecular weight (Mv) of Polyacrylic acid by viscometry method

Usually the molecular weight of this polymer(polyacrylic acid) is characterized by measurements of the viscosity of dilute solution; the viscosity and molecular weight are related by the Mark-Houwink equation [66].

$[\eta] = K M^{\alpha}$ ..... (1)

$[\eta]$ = is the intrinsic viscosity.

K, α = are constants dependent upon the polymer-solvent system at a particular temperature.

The intrinsic viscosity of a polymer solution was measured with on Ostwald U-tube viscometer.

Solutions were made by dissolving the polymer in a solvent [g/100ml] and the flow time of pure solvent is $[t_0]$ and the flow time of polymer solvent is [t].

Molecular weight of polyacrylic acid was calculated using equation (1), for intrinsic viscosity in dioxane solution.

$\eta = 85x10^{-5} . M^{0.5}$

Table (3-1) The constants, solvent, temp and molecular weight of polyacrylic acid.

Polyacrylic acid	**solvent**	**[α]**	**[K]x 10^{-5}**	**[T] ^{o}C**	**[η]**	**[M]**
	Dioxane	0.5	85	25	0.48	318892.73

3.3. Swelling of the modified polymer:-

Swelling of the modified polymer is of considerable practical importance in considering the polymer to be used as stationary phase and its performance when packed in an HPLC column. In HPLC applications, polymer particles are packed into a stainless steel column, swelling will cause the packed bed to be tightened and subsequently will affect the flow of the mobile phase through the column producing excessive column back pressure, and cause polymer bed to fracture. The reverse process, or polymer bead contraction, can be equally undesirable in column operation. In this case, the mobile is sharply changed to produce shrinking of the polymer particles, column channeling, particularly at the column walls. This has also a significant effect on how the mobile phase flows through the column [65].

Swelling test for prepared polymeric stationary phase was performed according to ASTM procedure [63] and found to be cross-linked which explained the rigidity of the polymer. The degree of cross-linking has been measured using the solvents acetone, hexane, and water.

The results of the swelling tests are listed in Table (3-2) which indicates the swelling value for the produced polymer. These results were theoretically expected, since addition of succinic acid dihydrazied will join the polyacrylic acid chains together.

Table (3-2): Swelling percent for polymeric stationary phase.

The polymer	solvents	Swelling percentage (%)
Cross-linked polymer	Water	2
	Acetone	1.4
	Hexane	2.1

3.4. Solubility tests for cross-linked polymer:

The solubility of the cross-linked polymer has been examined using different solvents as described in Table (3-2). These results were attributed to the cross-linking of the prepared polymer, that is, the functional group (O=C-Cl) reacted with hydrazide active group of succinic acid di-hydrazide forming a rigid cross-linked polymer with an oxadiazole functional group .

Table (3-3): The solubility test for the cross linked polymer

Solvent	Solubility
Acetonitrile	**Insoluble**
Benzene	**Insoluble**
Chloroform	**Insoluble**
Dioxane	**Insoluble**
DMF	**Insoluble**
DMSO	**Insoluble**
Hexane	**Insoluble**
Methanol	**Insoluble**
Water	**Insoluble**

3.5. Column Packing:-

Column packing is not the only critical factor, but represents one of the most important aspects affecting the quality of the chromatographic system. They all agreed, however, in that stationary phase must be suspending in a liquid to give slurry. A high –pressure pump then conveys the slurry into the column at a great speed, this process prevents sedimentation, which separates the stationary phase according to size and thus impairing the separation performance. The produced polymer was grinded to fine powder and sieved using in mesh size to obtain a (38μm) average mesh size of the polymer.

In this work, the slurry was produced by mixing of polymer powder with particle size (38μm) mixed with acetonitrile and homogenized. The column was packed using down-flow packing system. HPLC pump was used instead of the air-driven pump that shown in the figure (1-1). The packing material was dispersed in 100 ml acetonitrile and placed in the slurry reservoir. The column was plugged with its appropriate fitting from one side and placed in a beaker for draining; the other side was connected to the slurry reservoir .The pump was set to deliver 4 ml/min the back pressure read 500 kgf/ cm^2. The flow rate was then increased by 1 ml/min increment until reaching 8 ml/min at constant period of time and the pressure read 900 kgf/cm^2.

3.6. Column Evaluation:-

Since this work includes packing of a new column, its performance should be tested. This has been done by evaluation the plate count(N), height equivalent to theoretical plates , HETP[H] , and also capacity factor, K' , for different chromatographic runs using the new column. The plate number of column (N) was calculated using the well-known equation [61].

$$N = 16(t_r/w)^2 \quad \text{or} \quad N = 5.54(t_r/W_{1/2})^2$$

Where t_r is retention time, (**W**) and ($W_{1/2}$) are the peak width at baseline and at half height, respectively.

The optimum flow rate was found equal to 1ml/min for the new packed from the plot of H versus the flow rate using anthracene as an analyte as shown in figure (3-4). Four polyaromatic compounds were chosen, anthracene, acenaphthylene, naphthalene and phenanthrene, to be analyzed by the new column with UV detector. Some of the measured chromatographic parameters for these analyte using are listed in Table (3-3).

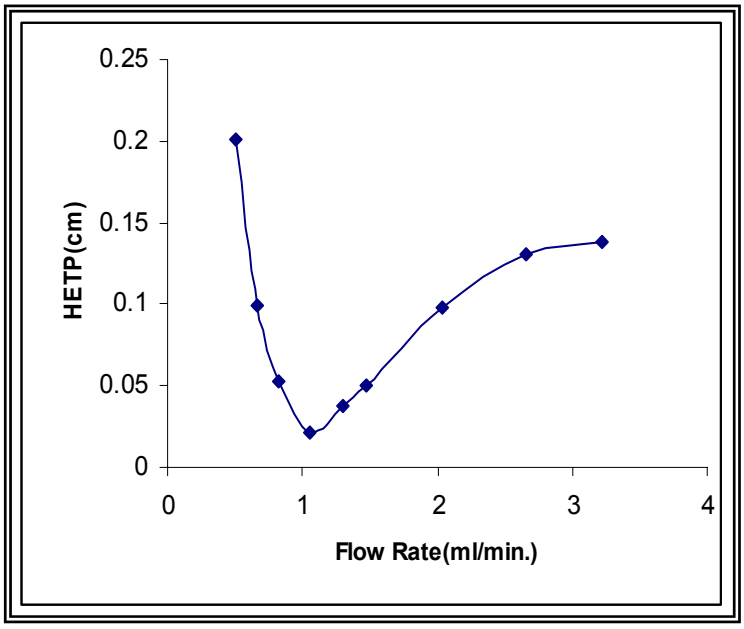


Figure (3-4). Plot of Plate height H, versus flow rate, using new polymer packed column, the mobile phase was isopropanol, 5ppm anthracene was used

Table (3-4) chromatographic parameters using polymeric packed column (25x0.4cm (i.d)). For some polyaromatic compound flow rate 1ml/min., sample loop 10µl, and detection wavelength 254nm the mobile phase used as indicated below. .

Compound name	Mobile phase	Retention time t_r /min	Capacity factor K'	Separation factor α	Peak Asymmetry at 10%
Phenanthrene	Hexane and isopropanol (40%, 60%)	2.30	1.52		1.15
Acenaphthylene		2.78	1.82	1.16	1.10
Naphthalene	Isopropanol	3.61	2.42		1.08
Anthracene		4.31	2.92	1.34	1.05

The average (N) calculated from the above analysis was (495.09). The [H] was calculated using the equation H=L/N, where L is length of the column. It was found equal to (0.03) cm for this column. The partition ratio, which is commonly called the capacity factor K', is another important term that described the interaction between solutes to be separated and the stationary and mobile phase. K' is independent on the column length and mobile phase flow rate and represents the molar ratio of the compound in the stationary phase and mobile phase. The capacity factors for polyaromatic compounds chromatographed on the polymeric packed column were ranged (1.52-2.92). These values of K' indicate good competitive interaction between these compounds with stationary phase and the mobile phase.

The column selectivity, originally called the separation factor (α) is defined as the ratio of the capacity factors of two adjacent peaks [$\alpha=K_2'/K_1'$].

Minimum value of 1.10 is required to achieve separation of the two adjacent peaks at any given experimental condition. The (α) values for polyaromatic compounds chromatographed on the polymeric packed columns were ranged from (1.16-1.34). Peak asymmetry is another important term, when the value of asymmetry larger than 1.5 or less than 0.25 means that the chromatographic system is not optimum (has low plate numbers).

The peak asymmetries for compounds chromatographed on new polymeric packed column were ranged from [1.05–1.15] with an average value of 1.095 as shown in table (3-4).

3.7. Chromatographic Analysis:-

Some polyaromatic, such as (acenaphthylene, phenanthrene, anthracene and naphthalene) were chromatographed with the new packed column, using 5µl sample loop, with flow rate 1ml/min.mobile phase were separated using a mixture of hexane and isopropanol (40%:60%) respectively ,acenaphthylene and phenanthrene, whereas anthracene and naphthalene were separated with 100% isopropanol as a mobile phase. On the other hand, these analyzed poly-aromatic compounds have given well resolved peaks as well as good detector response. The separation of a mixture of acenaphthylene and phenanthrene, anthracene and naphthalene are shown in figure (3-4) and figure (3-5), respectively. The difference between the retention times of these polyaromatic indicated good separation factors as shown previously in Table (3-4).

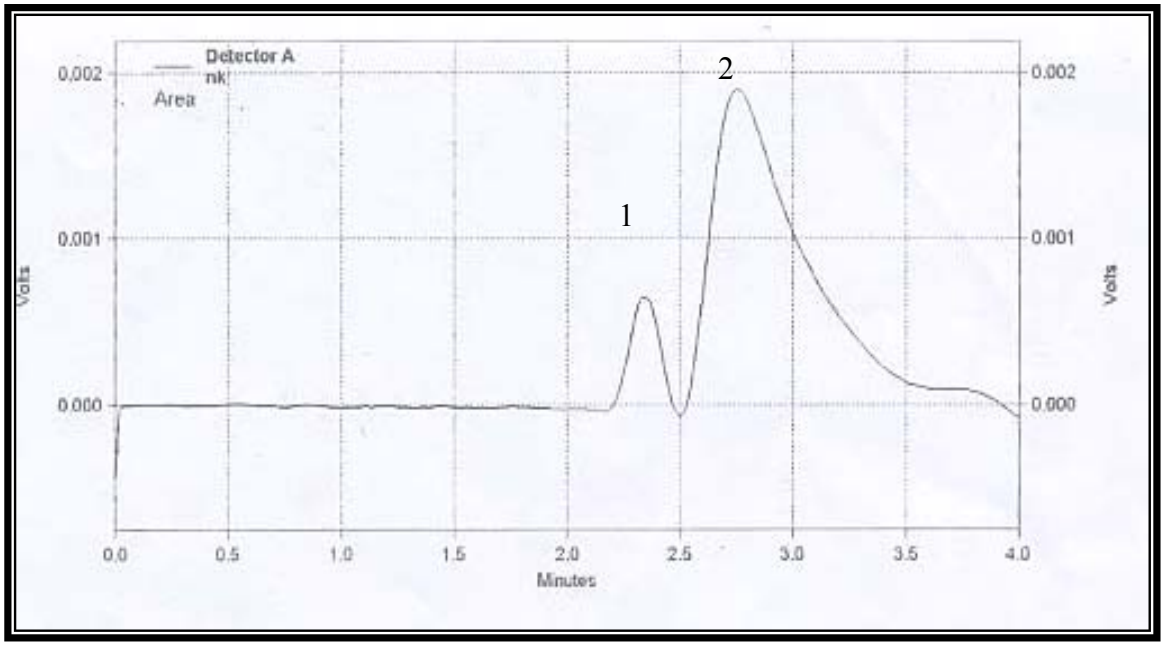


Figure (3-5). Chromatogram of polyaromatic compounds 0.5ppm phenanthrene(1) 1ppm acenaphthylene (2), with new packed column using mixture of hexane and isopropanol (40%,60%) as mobile phase, flow rate 1ml/min. detection wavelength 254nm.

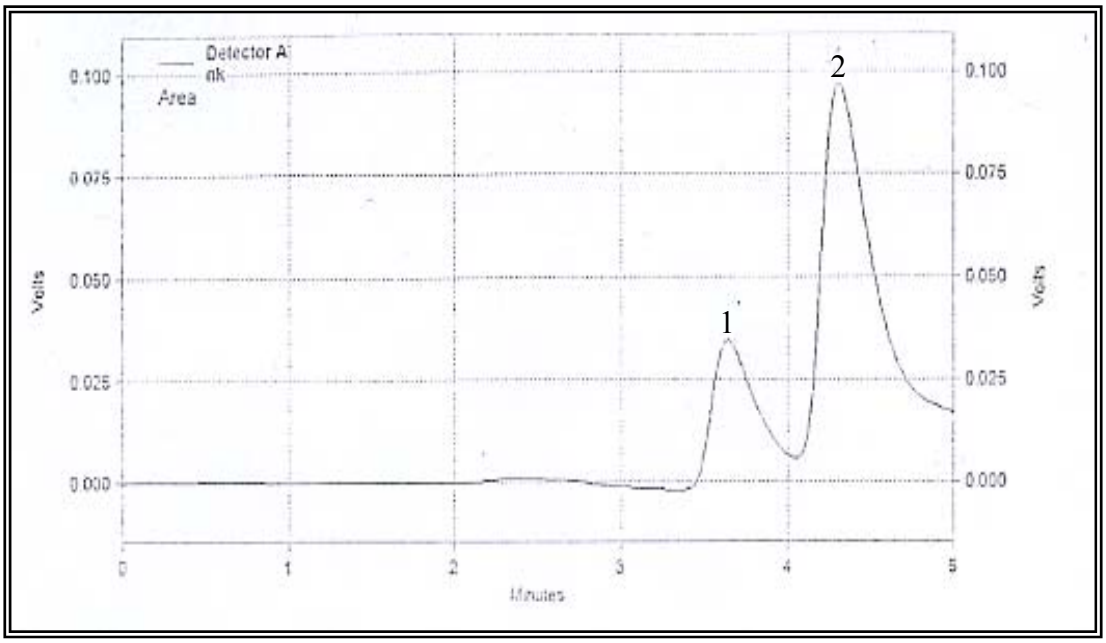


Figure (3-6). Chromatogram for the separation of 0.25ppm anthracene. (1), and 1ppm naphthalene (2). Condition were the same as in figure (6) except using 100%isopropanol as a mobile phase.

The new packed column was then tested for the analysis of some amino acids table (3-5) listed some of the chromatographic data obtained

Table (3-5) chromatographic parameters for some amino acid using polymeric packed column (25x0.4cm (i.d)). Flow rate 1ml/min., sample loop 10µl, and detection wavelength 254nm.mobile phase composition as indicated below.

Compound Name	Mobile phase	Retention time t_r /min	Capacity factor K'	Separation factor α	Peak Asymmetry at 10%
Tyrosine	phosphate buffer 10%acetonitrile at pH 6.0	6.16	4.25		2.31
Tryptophane		6.86	4.82	1.14	1.80
Phenylalanine	phosphate buffer 15%methanol at pH 6.5	4.99	3.51		1.52
Histidine		5.25	4.02	1.15	1.33
Tyrosine		6.35	4.50	1.13	1.13

These results were calculated using chromatographic runs of some amino acids analyzed with the newly column at optimum flow rate of the mobile phase.

The average (N) was calculated from the above analysis and was found to be (779.3). The (H) was calculated and found equal to (0.02) cm using these analyst.

Furthermore, some amino acids were chromatographed with the new packed column and shown in figures (3-6) and (3-7) respectively.

Figure (3-7) shows a chromatogram of two amino acids, 5ppm Tyrosine (1) and 2ppm Tryptophane (2), using phosphate buffer containing 10 % acetonitrile at pH 6.0 as a mobile phase and refractive index as detector. Figure (3-8) shows a chromatograph for the separation of three amino acids; 2ppm phenylalanine(1), 1ppm histidine (2), and 1ppm tyrosine (3) , using phosphate buffer containing 10 % methanol at pH 6.5 as a mobile phase.

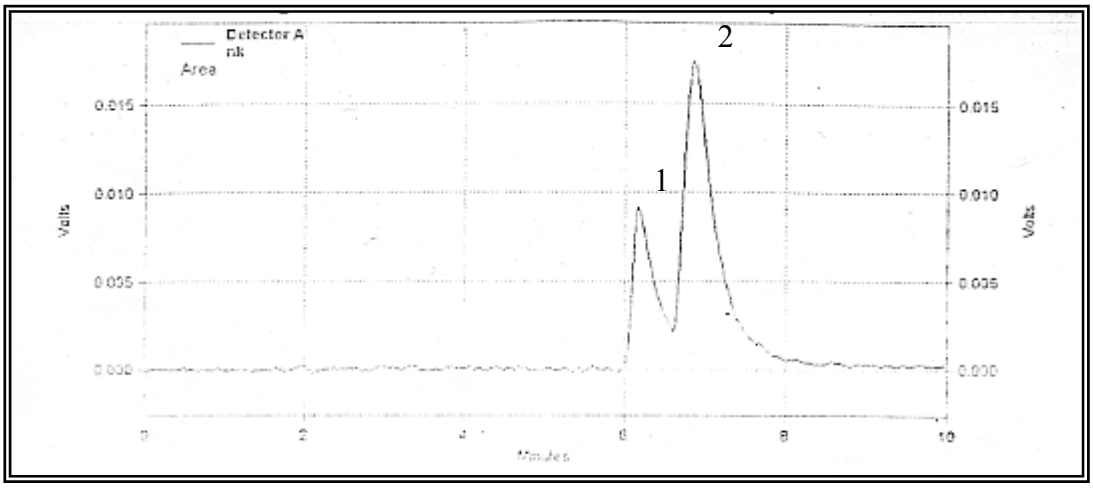


Figure.(3-7). Chromatogram of Tyrosine and tryptophane, conditions were the same as in figure (6) except using phosphate buffer pH 6.0 containing 10 % acetonitrile at as a mobile phase (1) tyrosine (1ppm), (2) tryptophane (5ppm).

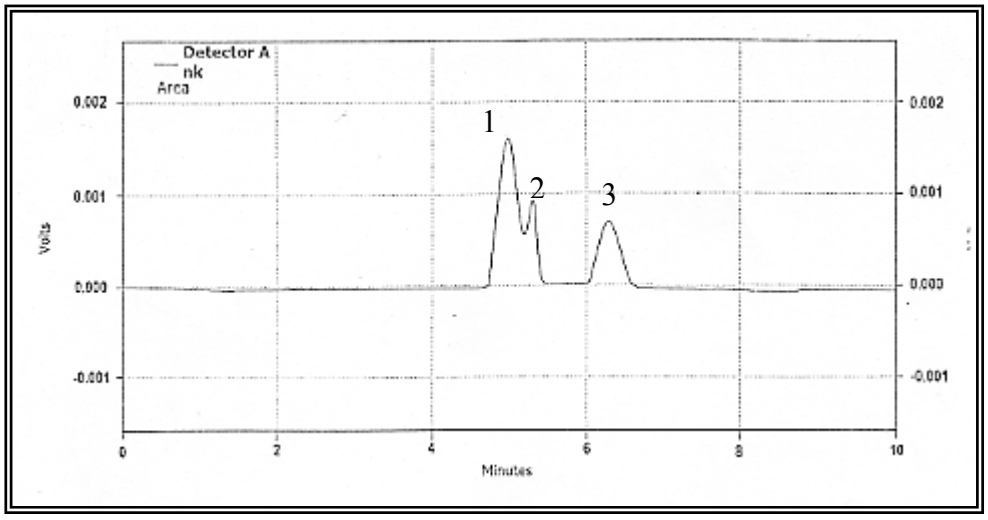


Figure (3-8). Chromatogram of phenylalanine, histidine and tyrosine, conditions were the same as in figure (6) except using phosphate buffer containing 10 % methanol at pH 6.5 as a mobile phase (1) phenylalanine (2ppm),(2) histidine (1ppm), (3) tyrosine (1ppm) with RI detector.

3.8. Quantitative Analysis:-

Calibration runs of polyaromatic compounds and amino acids on the new column are shown in figures (3-9) and (3-10), respectively. A linear dependence of the peak area on the amount injected is evident for all compounds from at least 25ppm down to the detection limit was as low as 0.05ppm for most analytes at S\N ratio of three or more.

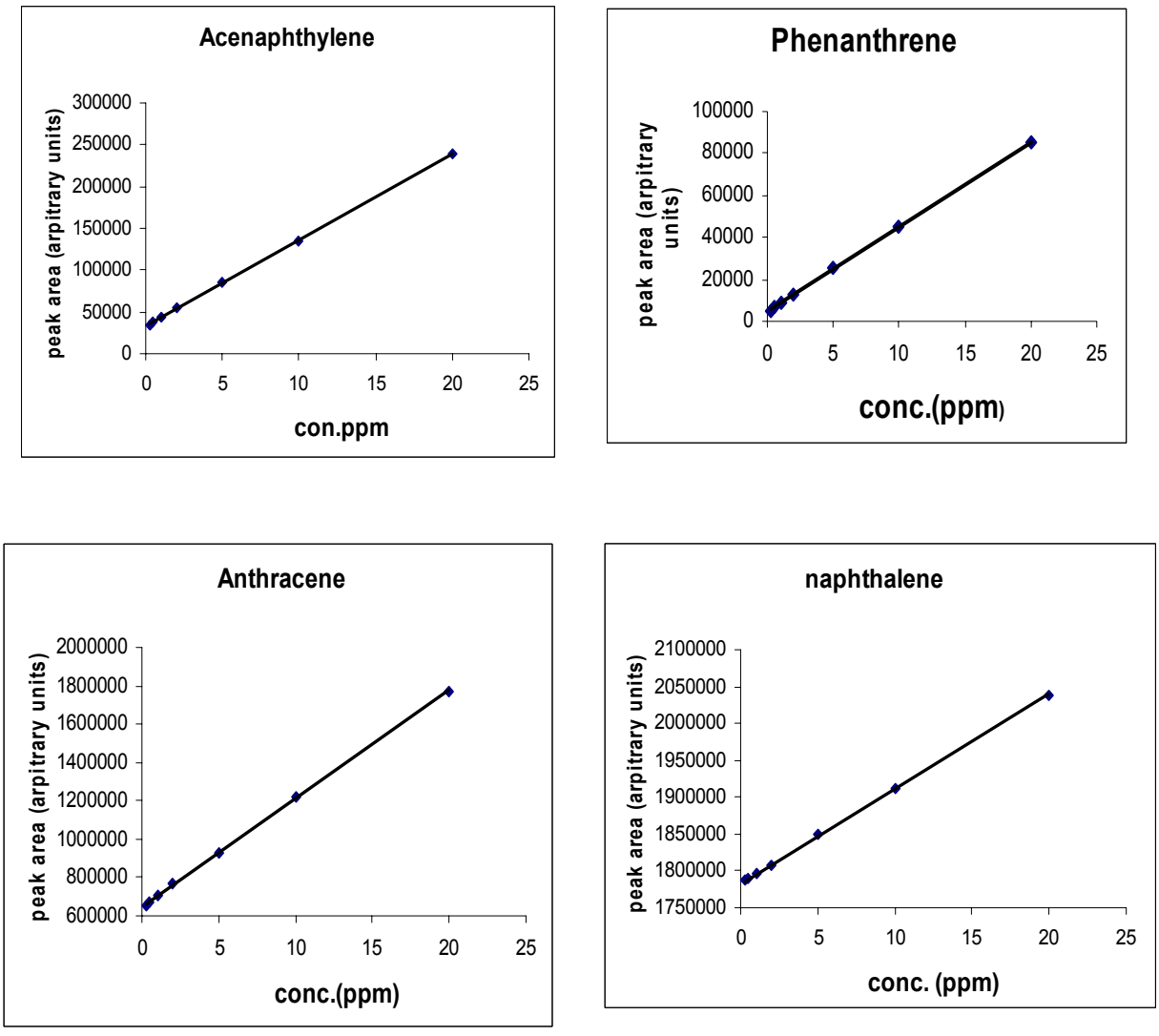
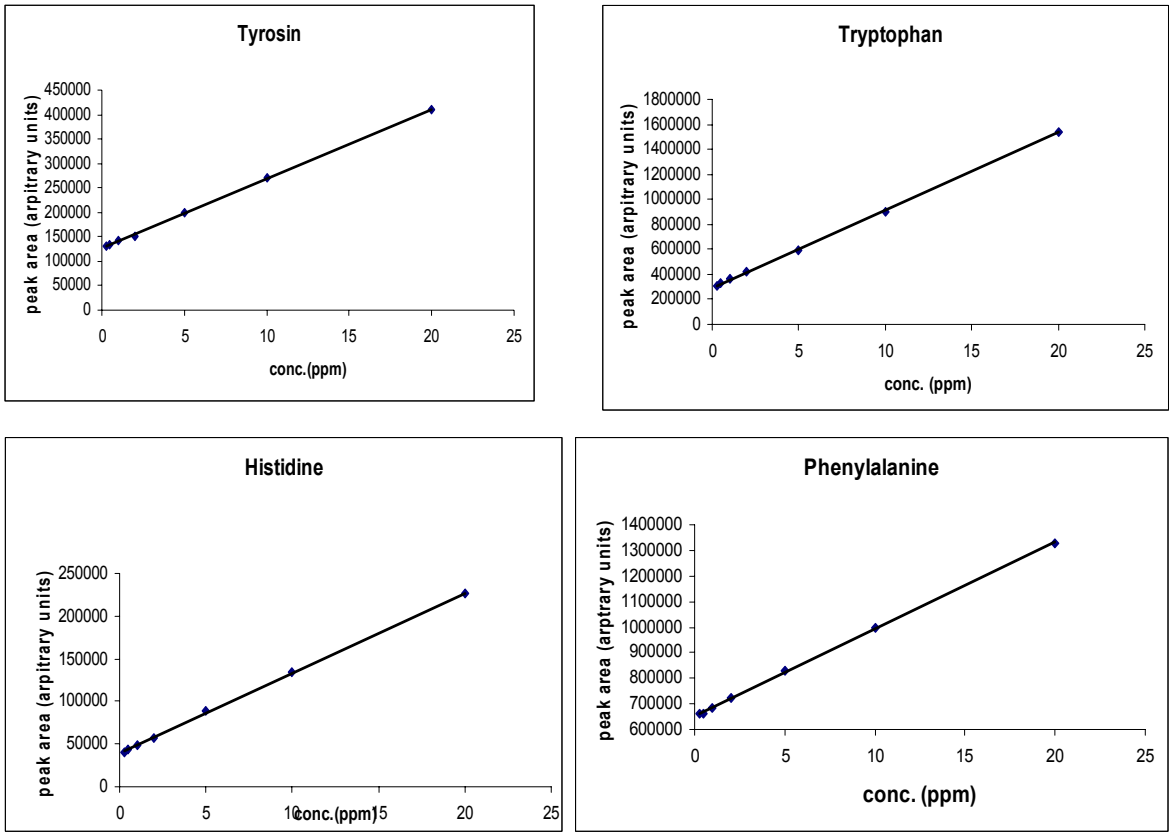


Figure (3-9): calibration curves of polyaromatic using new polymeric packed column (25x0.4cm (i.d.)). And hexane with isopropanol as mobile phase, sample loop 10µl, flow rate 1ml/min., detection wavelength 254nm.

The slope values for the linear calibration curves were ranged from (2045.4 - 11650.5) for poly aromatic compounds as show in Table (3-6). However, the slope values were ranged from (4880.3- 14250) depending upon the kind of the amino acids as listed in Table (3-7). The correlation coefficients were ranged from (0.9995) to (0.9999).

Table (3-6) linear equation , correlation coefficients R and detection limits for polyaromatic compounds using new column (25× 0.4 cm(i.d.)).using hexane mixed with isopropanol ,flow rate 1 ml/min,

Compound	Linear equation	R	Detection Limit (ppm)
Phenanthrene	Y= 40296X+4583.2	0.9998	0.10
Acenaphthylene	Y=10320X+3287.3	0.9997	0.10
Naphthalene	Y=12798X+11650.5	0.9999	0.05
Anthracene	Y=27197X+2045.4	0.9996	0.05



Figure(3-10): Calibration curves of amino acids using new polymeric packed column (25x0.4cm (i.d.)). Using phosphate buffer as mobile phase, sample loop10µl, flow rate 1ml/min., detection wavelength 254nm.

Table (3-7) linear equation , correlation coefficients R and detection limits for amino acids using new column (25× 0.4 cm(i.d.)).eluent phosphate buffer with acetonitrile and phosphate buffer with methanol ,flow rate 1 ml/min, and detection wavelength 254nm.

Compound	Linear equation	R	Detection Limit (ppm)
Tyrosine	Y=14250X+12654.6	0.9998	0.05
Tryptophane	Y=62013X+32873	0.9995	0.1
Phenylalanine	Y=4880.3X+8309.5	0.9996	0.1
Histidine	Y=7124.5X+2995.1	0.9997	0.05

Conclusion

A cross linked polymer was synthesized by condensation reaction of polyacrylic acid with succinic dihydrazide acid in the presence of $POCl_3$, at 80 C^0 . This polymer (because of its high rigidity, easy to grind and its stability in many solvents) has been tested as stationary phase for HPLC. This stationary phase was found to be suitable to separate some polyaromatic compounds and amino acids. The new column performance was tested by evaluating its chromatographic parameters. The plates number N was ranged from 259.10 to 862.89 with an average value of 449.37. The plate height (H) was ranged from 0.028 to 0.095 cm and its average value was 0.036 cm, the capacity factor was ranged from 1.520 to 4,821 and average value was 3.308, the peak asymmetry was ranged from 1.05 to 2.30 and its average value was 1.413. Polyaromatic compounds phenanthrene, acenaphthylene, anthracene and naphthalene were separated within 5 min.. The amino acids tyrosine, tryptophane, phenylalanine and histidine were separated in 10 min. Calibration curves for all analyzed compounds were linear from their detection limits to at least 25 ppm with correlation coefficient ranged from 0.9995 to 0.9999. The detection limits were ranged from 0.05 to 0.1 ppm at signal to noise ratio of three or more for most of the analyzed compounds.

Suggestions for future work.

We strongly suggest the following to more evaluate the new stationary phase and its potential to separate a wide variety of samples.

1. Using smaller stationary phase particles.
2. Carrying out an intense spectrometric and chemical analysis to better understand the active functional groups on the polymer surface texture that is affecting the chromatographic separation.
3. Improving the separation conditions for studied of polyaromatic and amino acids compounds and other kind of analytes.

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الخلاصة

تم تحضير بوليمر متشابك بواسطة بلمرة التكثيف من تفاعل (Poly acrylic acid) مع(succinic acid dihydrazid)بوجود ($POCl_3$) تم تشخيص الناتج بواسطة تحاليل الاشعة تحت الحمراء حيث ا وجد ان البوليمر الناتج من هذا المزيج يملك الصلادة العالية وسهولة الطحن. استخدم البوليمر الناتج كطور ثابت في الفصل الكروماتوغرافي السائل العالية الاداء.

تم تعبئة هذا الطور الثابت في عمود من الفولاذ المقاوم للصدأ، وتم قياس كفاءة العمود المعبأ الجديد بحساب عدد الصفيحات النظرية. الارتفاع المكافىء للصفيحات النظرية، عوامل الاستيعاب، عوامل الانتقائية، وعدم تماثل القمة بواسطة محاليل مختلفة على العمود الجديد باستخدام اطوار متحركة مختلفة النسب وسرعة جريان ثابتة.

تم تحليل الهيدروكاربوناتالاروماتية الحلقية، حيث تم فصل(Acenaphthylene و phenyalalanine)

بواسطة استخدام خليط بنسب مختلفة (hexane: 40%, isopropanol: 60%) وكذلك تم فصل (naphthalene, anthracene)باستخدام ١٠٠% (isopropanal) كطور متحرك ومعدل جريان (1ml/min) وطول موجي كاشف 254nm.

تم كذلك تحليل بعض الاحماض الامينية مثل :-

Histidine, Phenylalanin, tyrosine, and tryptophan

بواسطة هذا العمود المحضر. وكان لكل من المركبات المحللة وقت احتجاز مختلف عن الاخرى بالاضافة الى انتقائية مختلفة مما سمح بفصل (tyrosine and tryptophan) باستخدام خليط من (Phosphate buffer in PH : 6.00 , 10% acetonitrile) وتم فصل (tyrosine, Histidine, phenylalanine) باستخدام خليط من (phosphate buffer in PH : 6.50 , 10% methanale). كطور متحرك مع معدل جريان (1ml/min) باستخدام Refractive index detector.

ان المنحنيات المعيارية للمركبات المحللة كانت خطية من الحد الادنى لكشفها الى ٢٥ جزء بالمليون مع عامل ترابط يتراوح بين ٠,٩٩٩٥ ــ ٠,٩٩٩٩ وكانت حدود الكشف تتراوح من ٠,٠٥ ـ ٠,١ جزء بالمليون

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين/كلية العلوم
قسم الكيمياء

تحضير وتشخيص بوليمر جديد كطور ثابت محتمل للفصل في الكروماتوغرافيا السائل عالي الاداء

رسالة
مقدمة إلى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
نكتل عبد الحافظ حسين الدليمي
بكالوريوس ٢٠٠٢ (جامعة النهرين)

ربيع الثاني ١٤٢٦ آيار2005