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PREPARATION AND CHARACTERIZATION OF NEW HPLC STATIONARY PHASE

A Thesis submitted to the College of Science Al-Nahrain University in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry.

By

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Abbreviations

symbols	Meaning
LSC	**Liquid solid chromatography**
Gc	**Gas chromatography**
GSc	**Gas-solid chromatography**
GLC	**Gas-liquid chromatography**
CEC	**Capillary electro chromatography**
HPlc	**High performance liquid chromatography**
CD	**Cyclodextrin**
PLOT	**Porous layer open tubular**
CSP	**Chiral stationary phase**
N	**Plates number of column**
L	**Length of column**
H	**Plate height**
dp	**Diameter particles**
RID	**Refractive index detector**
UV	**Ultra violet**
NPC	**Normal- phase chromatography**
RPC	**Reveres –phase chromatography**
PS-DVB	**Poly styrene-divinyl benzene**
MPa	**Mega Pascal**
Meq	**Milli equivalent**
Ќ	**Capacity factor**
W	**Width of peak**
α	**Separation factor**
tR	**Retention time**
S/N	**Signal/noise**

SUMMaRY

New stationary phase for high performance liquid chromatography had been prepared by the reaction of congo red solution with amberlite anion exchange.

The F.T.I.R elemental analyses have configured the attachment of congo red on the resin as has indicated by the physical appearance of the new resin.

The resin capacities of newly prepared resin has calculated the resin capacity for the amberlite-congo red resin was found to average 7.36 meq/ gm and for the amberlite resin 3.0 meq./gm.

The new stationary phase has been packed into stainless steel column. This has been done by the special technique. The chromatographic performance of the packed column was characterized the number of plate numbers, height equivalent of a theoretical plates(HETP) capacity factors, selectivity factor and peak asymmetry were measured by analyzing different analysis on the new column using different mobile phases composition and flow rates. The parameters were measured from analysis of some amino acids such as phenyl alanine, tyrosine and tryptophan. The analysis of amino acids using amberlite-congo red column has performed with an aqueous mobile phase adjusted at pH 10. The average number of plates, N was(111.09-284.3) . The plate height H was (0.01343) cm, capacity factor, ḱ were ranged from(3.5-3.76). and peak asymmetry value was1.06. The analysis of some amino acid of the some column but with a mobile phase adjusted at pH 10 with 20% aceto nitrile, the chromatographic parameters were plate's number, N was(176.3307). plate height H was(0.128) cm, capacity factor Ḱ were ranged from(2.98-4.65).

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CHAPTER ONE

INTRODUCTION

1.1 Chromatography

"Chromatography" is a physical method of separation in which the components to be separated are distributed between two phases, one is stationary (stationary phase) while the other moves (mobile phase) in definite direction [(1)].

Chromatography is used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selectively absorbent stationary phase. Different components of the sample are carried forward at specific rates by the moving liquid phase, due to their different interactions with the stationary and mobile phases. There are a number of different kinds of chromatography, which differ in the mobile and the stationary phase used [(1)].

The discovery of chromatography is attributed to Tswett, who in 1903 was the first to separate leaf pigments on a polar solid stationary phase and to interpret this process. In the following years, chromatographic applications were mainly depends on the distribution between a solid stationary and a liquid mobile phase (Liquid Solid Chromatography, LSC).

1.2 Gas Chromatography (GC):

Most (GC) consist of four chromatographic units supported by three temperature controller and two microprocessor system, these units are gas supply, sampling, column and detector.

GC is a common type of chromatography used in analytical chromatography for separation and analyzing compounds that can be vaporized without decomposition. Typical uses of (GC) include testing the purity of a particular substance or separating the different components of a mixture [(2)].

(GC) involves two main types, gas-solid chromatography (GSC) and gas-liquid chromatography (GLC) [(3)].

There is an important parameter that affects the chromatograms obtained with (GC), which is column temperature. Typically when chromatography is obtained at constant temperature, the peak shapes and separations vary as the solute elute. At any given temperature elution is based on volatility, by which the most volatile species elute first and the solutes with the lowest volatility elute last. Thus the closer the boiling point, the closer the peaks will appear and even an overlapping may occur.

The temperature in this case has to be precisely controlled electronically. The rate at which a sample passes through the column is directly proportional to the temperature of the column. The sample moves faster through the column when the temperature is high. However, the faster a sample moves through the column, the less it interacts with the stationary phase, and the less the analytes are separated.

In general, the column temperature is selected to compromise between the length of the analysis and the level of separation. A method which holds the column at the same temperature for the entire analysis is called "isothermal." Most methods, however, increase the column temperature during the analysis, the initial temperature, rate of temperature increase (the temperature "ramp") and final temperature is called the "temperature program."

A temperature program allows analytes that elute early in the analysis to separate adequately, while shortening the time it takes for late-eluting analytes to pass through the column [(4)].

Jerome et al. [(5)], introduced a fast and reproducible isothermal gas chromatography-mass spectrometric method for validation of small ion mobility spectrometer sensor for detection of gaseous volatile organic compounds. This method utilizes an automated cyclic valving sample

technique coupled to a gas chromatography-mass spectrometer. The sampling time is reduced by operating the gas chromatography isothermally at 300℃ this approach provides rapid measurements with (15) times more data points than the conventional gas chromatography-mass spectrometer method in wich the column temperature is ramped, then cooled before the next sample can be injected. Operating the gas chromatography isothermally at 300 ˚C allowed the target analytes to pass through the gas chromatography column with minimum interaction with stationary phase.

1.3 capillary electrochromatography (CEC)

Recently, (CEC) has become a centre of attention because of its efficiency in the micro analysis of complex samples. CEC basically is a hybrid technique that combines the interaction between the sample and the stationary phase as occurs in high performance liquid chromatography (HPLC) and the high analysis effectiveness of a capillary electrophoresis (CE) system. Furthermore, reduction or avoidance of high pressure during CEC performance and the use of micro particulate stationary phase are the most significant benefits of this technique. However, limited column capacity is one of CEC drawbacks. This technique can be exploited particularly for the separation of ionic optically active biomolecules. This might be done by applying a polymer modification with a sweeping like mechanism using enantiomeric selectors such as cyclodextrin (CD).

Recently the numbers of studies that used monolithic [6,7,8] or PLOT [9,10] columns in CEC have been increased significantly. In contrast to the packed column PLOT and monolithic columns does not require high operation pressure inside the column beside moderately simple synthesis procedures. According to Ikegami et al., [11] there are two types of monolithic columns, polymer and silica based. Additionally, the same author asserted that from a number of studies it could be concluded that polymer based

columns might be more appropriate for biomolecular separation area, whereas, silica based columns could be used in the separation of low molecular weight compounds. Alternatively**,** PLOT polymer based columns are expected to be more preferable because of their unreactivity and high separateon effectiveness [(12)]. Consequently, in this project PLOT column techniques will be used to prepare chiral stationary phase (CSP) by immobilizing cyclodextrin or (R) - amines as a chiral selector on a polymer base PLOT capillary.

Class of these additives that can be used to analyze enantiomeric mixtures depending on their hydrogen bonding and hydrophobic interactions [(13)]. Additionally, Stalcup states that according to their molecular weight, cavity diameter and water solubility, there are three sub types of cyclodextrins (α-, β- and γ- cyclodextrins). Moreover, β –cyclodextrin has the lowest solubility which increases the probability of formation inner hydrogen bonding between the analyte and CD [(13)].

1.4 Liquid Chromatography

Liquid chromatography began in the early 1900s, in the form known as classical column chromatography, a glass cylinder was packed with a finely powder such as chalk, a sample was applied to the top of the column and the solvent was poured onto the column, as the solvent flows down by gravity, the components of the sample begins to move through the column at different speeds and become separated.

Another form of liquid chromatography was introduced in 1940s, called paper chromatography, in which a strip of paper is replaced the column, after the sample was spotted near the bottom of the paper strip; the paper was placed in container with solvent at the bottom. As the solvent migrated up the paper by capillary a paration took place, but in opposite direction, this open bed form of chromatography was modified by coating thin

layer of powder silica onto a glass plate which is referred on thin layer chromatography.

High performance liquid chromatography, represents the modern culmination of the development of liquid chromatography. It begins by placing samples in injection into the column and the solvent is continually pumped through the column and the separated compounds are continuously sensed by detector as they leave the column [(14)].

1.5 High Performance Liquid Chromatography (HPLC)

(HPLC) is a proven technique that has been used in laboratories worldwide over the past 30-plus years [(7)].

One of the primary drivers for the growth of this technique has been the evolution of packing materials used to effect the separation. The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). Since particle size is one of the variables, a Van Deemter curve can be used to investigate chromatographic performance [(15)].

According to the Van Deemter equation, as the particle size decreases to less than 2.5 µm, not only is there a significant gain in efficiency, but the efficiency does not reduce at increased flow rates or linear velocities. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed "Ultra Performance Liquid Chromatography", or UPLC. This can be result of using small particles size of the stationary phase as described by Van-Deemter equation.

The most important advantage of HPLC is that samples can separate much more quickly with very high efficiency. The efficiency of the chromatographic separation is usually described by the number of theoretical

plates (N). The value of (N) is related to the height equivalent to theoretical plates (H) by:

$$H = L / N$$

Where (L) is the length of the column. The efficiency of HPLC is higher due to the large number of mass transfer equilibria obtained with small values of (H).

The plate theory needs to assume that the solute during it passage through the columns, is always in equilibrium with the mobile phase and stationary phase. However this equilibrium never occurs. To take this non equilibrium condition into account, the column is considered to be divided into a numbers of cells or plates; each plate is allotted a specific length. Thus the solute will spend a finite time in each plate, the size of the cell is chosen to provides sufficient residence time for the solute to establish equilibrium with the two phases; thus the shortest the plate, the faster will equilibrium and the more plates there will be in the column.

HPLC are widely used in pharmaceutical field by calculating the concentration of the active ingredients, drugs purification and separation of an enantiomers molecules, which allows the chemist to confirm which enantiomers is presents and calculate the ratio when both are present [(16)].

1.6 Columns packing

In column chromatography, the separation phase, solid adsorbent, is placed in vertical and the mobile phase, a liquid, is flows down through the column.

The column is one of the most important components of the HPLC. Because the separation of the sample components is achieved when those components pass through the column. Analytical columns are protected by a guard column which is an essence a disposable (or sacrificial) placed on the top of the main analytical column. The guard column is the final filter both

mechanical and chemical. In addition to removing debris, it also can absorb undesirable sample components that otherwise might irreversibly bind and possibly change the stationary phase of the analytical and preparative columns.

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 [(6)]. 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. The high-pressure "wet- fill" or slurry-packing techniques were, alternatively, used for packing particles with dp <20 μm. In this technique suitable liquids were used to wet the particles and eliminate particle aggregation during packing [(17)].

1.6.1. 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 that shown in figure (1-1) is used. The slurry mixture is rapidly forced downward into the column blank with a constant pressure pump [(6)]. 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 pneumatic pump [18]. 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 [6].

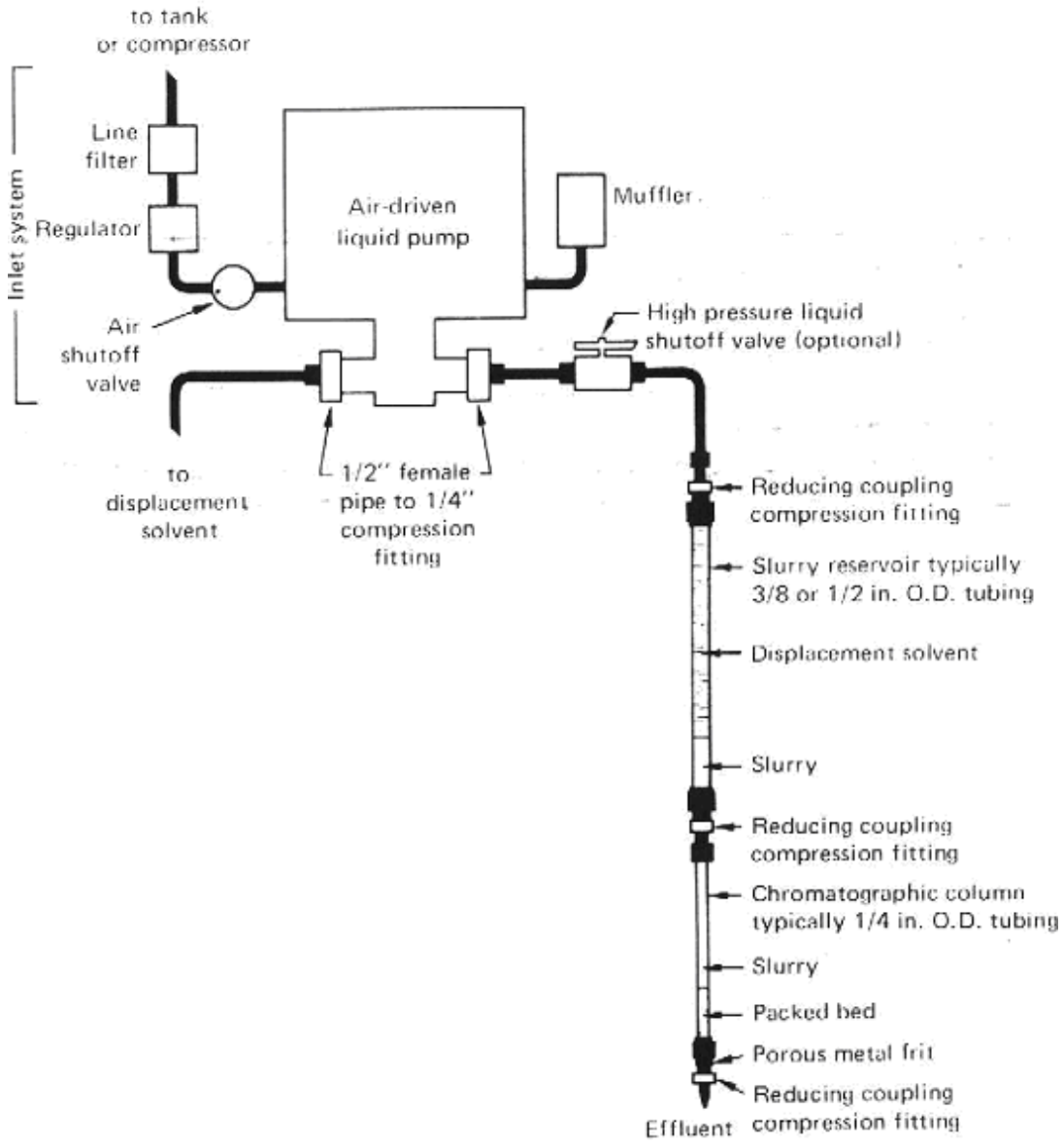

Figure (1-1) High-pressure slurry packing assembly [6].

1.6.2. Up-Flow Method

An alternative technique for wet filling columns is the up-flow approach, which has been described by Bristow [19]. 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.

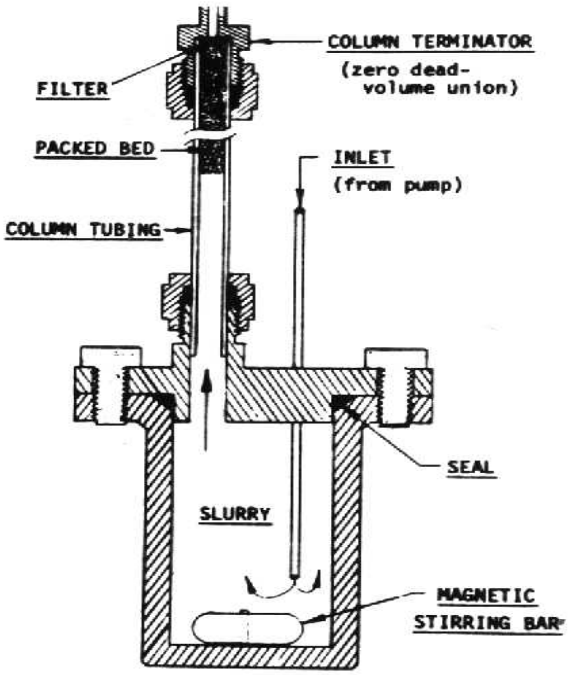

Figure (1-2) Up-Flow Packing System [11].

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 [20].

1.7 Detectors [21]

Most HPLC instruments are equipped with optical detectors. Light passes through a transparent low volume "flow cell" where the variation in light by UV absorption, fluorescent emission, or change in refractive index are monitored and integrated to display retention time and peak area.

There are many types of HPLC detectors, the most important is:

1. **Refractive Index detector (RID)**:

   The light beam is refracted from the liquid-glass interface in the detection photocell. The introduction of the sample into one cell causes light to be reflected at difference angle. The deflection of the light beam from photo resistor cause the appearance of the electrical signal, this difference between sample cell signal and reference cell signal is output to a recorder.

   (RID), are used to detect a non-UV absorbing compounds, but they are less sensitive than UV-detectors. The (RI) of an analyte is a function of its concentration. Presence of dissolved air, change in solvent composition, improper mixing and column bleed will contribute to baseline drift. Eluent pressure change of 15 psi, will cause the change of 1×10^{-6} (RI) units and 1˚C temperature variation will be equivalent to the change of 600×10^{-6} (RI) unit. Thus both of these parameters must be controlled [22].

2. **UV-Visible absorption detector**

   This detector is the most widely used in HPLC, it is relatively insensitive to temperature and flow variation. Detection limits at the nanogram level for certain types of compounds can be easily achieved. The UV-Vis detector has a linear response over a range of

5 $\times 10^{3}$M, with detection limits of 1×10^{-9} g cm^{-1} for highly absorbed compounds with large molar extinction coefficient.

3. **Fluorescence detector** :

These detectors are sensitive to compounds that are inherently fluorescent or that can be converted to fluorescent derivatives either by chemical transformation of the compound or by coupling with fluorescent reagents at specific functional groups.

1.8 High Performance Liquid Chromatography Modes

The three basic liquid chromatography modes are named according to the separation mechanisms:

1. Normal Phase LSC (Adsorption chromatography)
2. Reverse Phase LSC
3. Ion Exchange

1.8.1 Normal-phase chromatography(NPC)

Normal-phase chromatography is employed in about 20% of all HPLC separations [(23)]. It has gained an important position in preparative scale applications because it permits the use of non-aqueous mobile phases with the property to solubilize solutes of low-polarity.

Adsorption chromatography is probably the oldest type of chromatography; its utilize a mobile liquid or gaseous phase that adsorbed onto the surface of a stationary phase [(24)].

Adsorption chromatography depends upon interactions of different types between solute molecules and ligands immobilized (like silica gel or any other silica based packing) on a chromatography matrix, by which the separation is based on repeated adsorption –desorption steps.

Normal phase chromatography is not very popular with most analytical HPLC users. Poor reproducibility of the results is often cited as an argument against normal phase chromatography. One of the major reasons for the problems of reproducibility is well known but often neglected. When bare silica is used as the stationary phase, water is always adsorbed onto the silica surface and occupies sites of interaction, which are then not accessible to the analytes. Variable water content in the eluent (and also in the sample) may cause dramatic changes in the retention and separation. In order to avoid these drawbacks, it is recommended to blend the solvents from a water-saturated and a dried (by molecular sieve, sodium, etc.) portion. This gives the opportunity to keep the water content constant or to regulate it to a defined value. The latter approach may be the key to successful method development, as the separation sometimes reacts very sensitively to the concentration of water present in the mobile phase.

Dessouky et al. [(25)], have been adopted a rapid and reproducible high performance liquid chromatography method for quantitative determination of chlorphenoxamine hydrochloride (histamine receptor antagonist with anti-muscarinic), caffeine (has a stimulant activity) and 8-chlorotheophylline (a xanthine derivatives used for alleviation of motion sickness) using normal phase mode chromatography for separation and quantitative determination. This method was capable for determination of the three drugs in tablets. The mobile phase consisted of ethyl acetate : methanol (50:50 v/v), adjusted to pH 9 using triethylamine. The mobile phase was flowing at a constant flow rate of 1.0 ml min^{-1}.

Mohd et al. [(26)], developed a novel method for separation of nine vitamins-E isomers (α-, β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol) and α-tocopherol acetate using normal phase high performance liquid chromatography. The separation was optimazed by varying the eluent composition and column temperature. All isomers were separated in order of

increasing analyte polarity. The isomer with the lowest polarity were eluted first followed by the isomer with higher polarity. This developed separation method improved rapidity, show an excellent reproducibility and suitability to be used as a quantitative method in analyzing tocopherols and tocotrienols.

Britt [27], purified proteins for their activity and uses in assays by using adsorption chromatography by which the separation between proteins based on differing interaction with stationary and mobile phase.

Monroe [28], show that adsorption column chromatography can be used to separate compounds out of a mixture, by separating 50:50 mixture of ferrocence and acetylferrocence using hexane and tert-butyl methyl ether as an eluent; the less polar ferrocence was attracted to the less polar hexane, so as the hexane was passed through the stationary phase, the ferrocence moved with it and the acetylferrocence was left behind.

The percentage recovery of acetylferrocence was 82.76% and the percentage recovery of ferrocence was 72.41%.

1.8.2 Reversed-phase chromatography (RPC)

Reversed-phase chromatography is by far the most widely used technique in (HPLC) [29]. It is popular because it is applicable to most non-polar analytes and to many ionizable and ionic compounds. Most of the stationary phases used in reversed phase chromatography are hydrophobic in nature. Therefore, analytes are separated by their degree of hydrophobic interaction with the stationary phase and matrices with hydrophobic components can also be retained in a similar manner [30].

Reversed-phase chromatography can be used to separate nonpolar, polar, and ionic compounds sometimes in the same separation. The technique also can achieve a larger range of separations than all other modes combined because it enables users to manipulate the mobile phase by changing organic solvent type, solvent composition, and pH; by adding modifiers such as

surfactants, chiral reagents, competing bases, and ion-pair reagents; or by adjusting experimental conditions such as flow rate and temperature.

Reversed-phase chromatography generally is performed using octadecyldimethylsilane (C_{18}) or octyldimethylsilane (C_8) stationary phases bonded to high-purity, spherical silica gel. short-chain alkyl phases such as C_2 and C_4 and phenyl phases are used occasionally, but long-chain phases such as C_{30} are used rarely and only for specialized applications. Polymeric materials such as poly(styrene–divinylbenzene) (PS–DVB) find some use as reversed-phase media (for example, with high-pH mobile phases) but generally provide lower column efficiencies than those of silica gel–based packings. Water, usually buffered, mixed with a water-immiscible organic solvent (modifier), most commonly acetonitrile or methanol, is the preferred mobile phase. To retain most nonpolar and semi polar organic analyses, chromatographers generally begin method development by varying the water–organic solvent ratio in the 20–80% range, often using gradient elution to find the optimum separation conditions more quickly [(31)].

Sudha and Venkata [(32)], described the role of reverse phase in determination of ziprasidone hydrochloride monohydrate in bulk and pharmaceutical dosage forms. For this, a binary mixture of methanol and phosphate buffer (55:45 v/v) as a mobile phase. The resulting chromatogram show the absence of additional peaks indicates non-interference of the common excipient used in the drug, it can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of ziprasidone HCl monohydrate in pharmaceutical dosage in a short analysis time.

Idress [(33)], found a procedure that use reveres phase high performance liquid chromatography to determine hydrochlorothiazidine; a thiazide diuretics, and enalpril maleate; an antihypertensive and a vasodilator in congestive heart failure, in pharmaceutical tables with free of interferences from excipient used in tablets formulation. The mobile phase was 3.0 mM

tetrabutylammoniumhydrogen sulfate in acetonitrile/water/triethylamine (14, 85.6, 6.4 V/V) adjusted to pH 4.1 by glacial acetic acid. The result found that the recovery of both components was 97.2-101.0 % and for enalpril maleate, the recovery was 97.9-101.5%.

1.8.3 Ion-exchange chromatography:

Ion exchange chromatography is a technique based on the difference in the strength of the interaction between a sample ion and an oppositely charged functional group [(34)].

Ion exchange is basically a process of nature occurring throughout the ages. The earliest of the reference were found in the bible, which says that "Moses" succeeded in preparing drinking water from brackish water by an ion exchange technique [(35)].

Ion exchange usually used for protein purification but may be used for purification of peptides or other charged molecules [(36)]. Proteins in the mobile phase will bind through electrostatic interactions to the charged group on the column. In a mixture of proteins, positively charged proteins will bind to a resin containing negatively groups; e.g. carboxymethyl group ($-OCH_2COO-$) or sulfopropyl group ($-OCH_2CH_2CH_2SO_3-$); while the negatively charged proteins will pass through the column.

The positively charged proteins can be eluted from the column with a mobile phase containing either a gradient of increasing salt concentration or single higher salt concentration.

Yoshiteru et al. [(37)], developed a high performance anion-exchange chromatography with pulsed amperometric detection for simultaneous determination of relevant sugar alcohol, monosaccharide and monosacchride anhydrous (that comprise an important fraction of water soluble organic carbon in atmospheric aerosols). This method enables the separaton of

levoglucosan and arabitol (Levoglucosan, mannosan and galactosan, which originate from combustion of cellulose and hemicellulose,
typically comprise a large fraction of saccharidic compounds), which offer a simple, reliable and effective determination of atmospheric tracers for biomass combustion at sub-nanograms per cubic meter air concentration levels for routine analysis. The eluant was sodium hydroxide because it s indicates a good separartion relevant saccharidic compounds due to acceptable resolution value which was (1.5).

Dong et al. [(38)], investigate the separation of magnesium isotopes by ion exchange using a manganese (IV) oxide with elution chromatography. The elute was ammonium lactate. The behavior isotopes of magnesium (II) were concentrated in manganese oxide (IV) phase, while the light magnesium (II) isotopes were concentrated in the solution phase. The separation by manganese (IV) oxide is primarily electrostatic based on comparing the adsorption of cationic, anionic and uncharged organic compounds. Since at higher pH the manganese (IV) oxide has a net negative charged so it shows a high adsorption capacity for cations.

$$^{24}Mg^{2+}_{\text{Manganese Oxide}} + {}^{25}Mg^{2+}_{\text{Solution}} \rightleftharpoons {}^{24}Mg^{2+}_{\text{Solution}} + {}^{25}Mg^{2+}_{\text{Manganese Oxide}}$$

Aim of Work

The aim of this work was to prepare a new HPLC stationary phase, based on the reaction of anion exchanger resin with indicator. The properties characteristics of this new stationary phase was studied and used to packed a column. The chromatographic performance of this column was examined.

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CHAPTER TWO

EXPERIMENTAL

2.1. Chemicals

The materials and chemicals listed Table (2-1) which was obtained from different solvents has been used in this work.

Table (2-1) Chemicals and their suppliers

serial	Compounds	Supplied from	Chemical Formula	Purity%	MWT
1	Tyrosine	Merk	$C_9H_{11}NO_2$	98.9	181
2	Phenylalanine	Merk	$C_9H_{11}NO$	99.9	165
3	Tryptophane	Merk	$C_{11}H1_2N_2O$	99.9	204
4	Ethanol	GCC	C_2H_5OH	99.9	46
5	Hydrochloric acid	GCC	HCl	99.9	36
6	Sodium hydroxide	Fluka	NaOH	98	40
7	Chloroform	Hopkins &Williams	CCl_4	98.9	152
8	Nitric acid	BDH	HNO_3	99.9	63
9	Congored indicator	Merk	$C_{32}H_{22}N_6Na_2O_6S_2$	-	696.68
10	*Amberlite resin	Merk	-	-	

*Amberlite resin is strong anione exchange Amberlite resin IR-400(Cl^-).

congo red is a brownish red powder with a pH range from 3.0 (blue)- 5.0 (red), its soluble in water and ethanol, very slightly soluble in acetone and practically insoluble in ether [(31)].

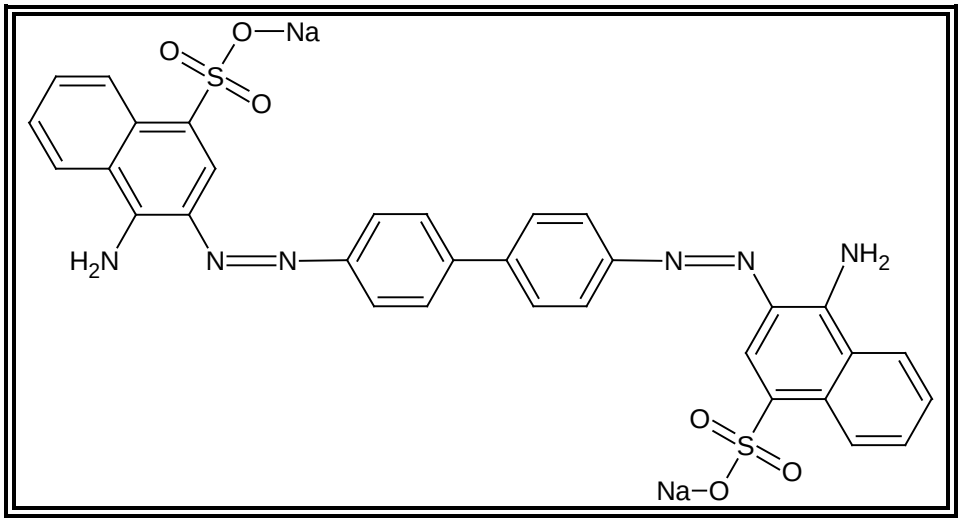


Figure (2-1): (congo red) Sodium diphenyldiazo-bis-α-naphthylamine sulfonate

2.2. Instrumental Part

High performance liquid chromatography used in this was shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10AVP, 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 5µl sample loop. The HPLC system has been interfaced with computer via a Shimadzu class-VP5 chromatography data

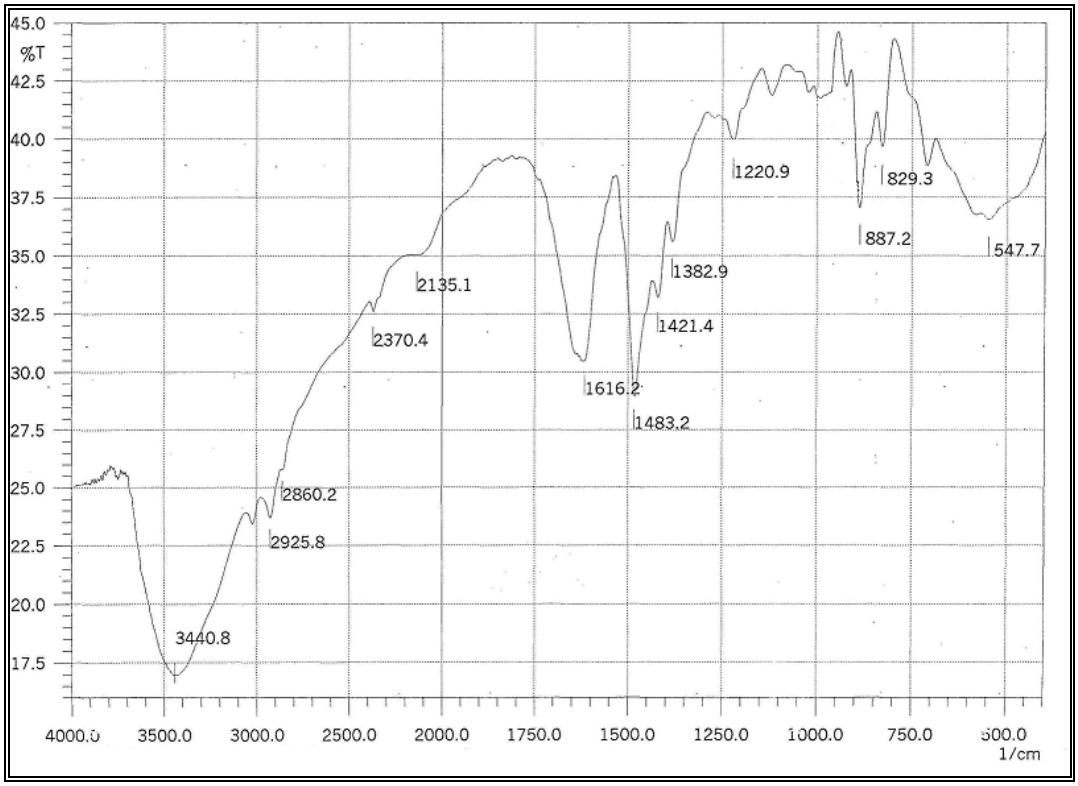
system program supplied by the manufacture; HP Deskjet D2680 {Japan), Sonicator Sonerex model Super RK 103H Mandolin (Germany), Orion expandable ion analyzer model EA 940 equipped with printer (USA), glass electrode was used to measure the pH. Shimadzu Fourier transforms infrared model FTIR 8300 (Kyoto, Japan) was used to measure the IR spectra for resins. The HPLC system has been interfaced with computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacturer. One used blank stainless steel columns of dimensions 25×0.4 cm was obtained locally.

2.3. Preparation of Stationary Phase

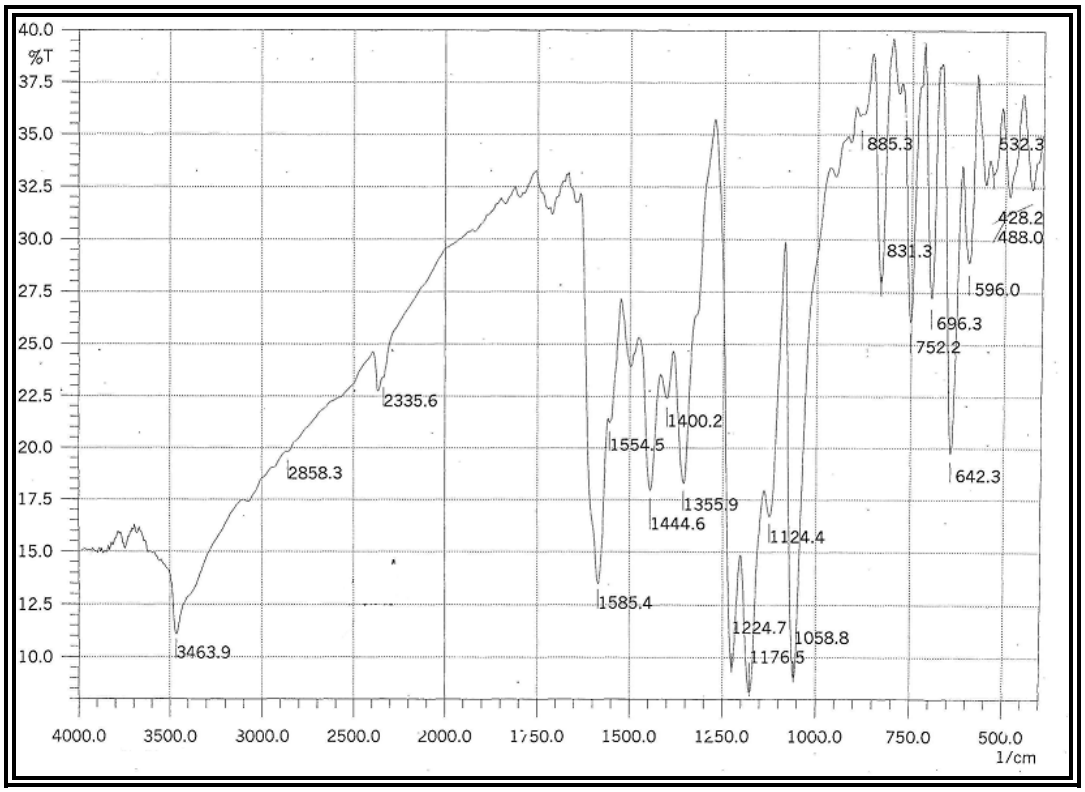
The stationary phase was prepared by rinsing (4 gm) of amberlite resin in a beaker with 100ml deionized water. cong red indicator salt solution was prepared by dissolved 0.02 g from indicator in 25 mL deionized water in a 50 mL beaker. A 10ml of 2.9×10^{-3} M cong red indicator was added gradually to the rinsed resin with continues stirring, it was noticed that the color of the resin has been changed from yellow to deep red. The contents of the beaker were led to settle for a period of time. The solution was then decanted and discarded. The resin was washed four times with deionized water, then rinsed with ethanol and then dried in the oven at 55˚C overnight and kept in desiccators for further work. A test was done by dispersing the amberlite-congo red mixture with methanol, ethanol and ether; it was noticed that sedimentation is occurred and the filtrate color was changed from red to colorless; but when chloroform and benzene was used

to disperse the stationary phase, the latter was suspended in the mixture no changing in the color.

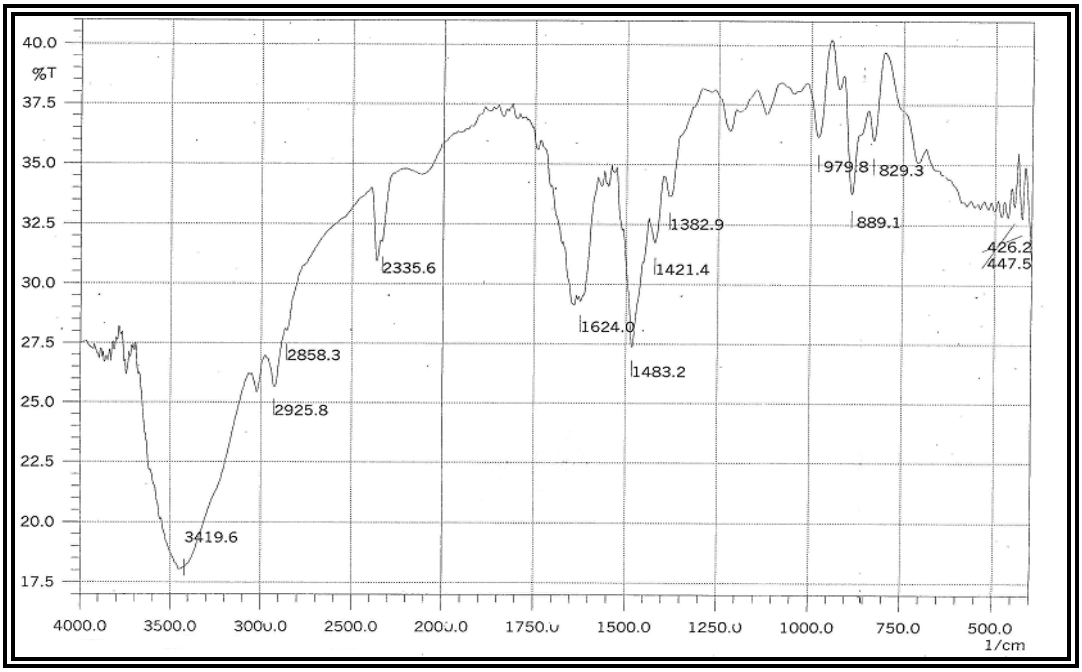
The FTIR spectrum for the resulting amberlite resin, congo red indicator and stationary phase were measured, as shown in figure (2-2).



(A)



(B)



(C)

Figure (2-2): FTIR spectrum for (A) Amberlite resin, (B) Congo red, (C) Amberlite-congo red

2.4. Measurement of Resin Capacity

The original and the prepared resin capacity were measured by potentiometer titration using pH glass electrode which calibrated using standard solution (4.001, 7.04 and 10.02).

4 grams of amberlite resin soaked in 25ml of deionized water and titrated with solution 1×10^{-3}M HCl (prepare by diluted 2mL from conc. HCl to 250mL deionized water in a volumetric flask and standrazation this solution with standard 0.1M KOH) with stirring. The pH of solution was measured after each 1 ml additional of HCl solution. The titration was stopped after reaching a constant pH values. Another four grams of amberlite resin soaked in 25ml deionized water and titrated with standard solution of 1×10^{-3}M NaOH solution (prepare by dissolved 0.14g in 250mL deionized water in a volumetric flask and standrazation this solution with 0.1 M from phosphate hydrogen phthalate) was then carried out for the resin solution after several washing with deionized water again, the pH reading was recorded until reading constant values.

The stationary phase (amberilt – congo red) was titrated similarly; an appendices lists the measured potential values for the above titrations. The equivalent points of each titration were identified from the first and second derivatives of these titrations.

2.5. Packing the Column

The blank stainless column tube used in this work was washed with water, then with 50%nitric acid followed after washing with water rinse with isopropanol and then dried. It was packed with the prepared stationary phase as follows; the column was plugged with column fitting from one side and hanged to a clamp near the open end. It was then placed in the sonicator bath filled with water. Slurry of about 3 grams resin was made with 15ml deionized water. A 1-2ml of the slurry was poured into the column using a dropper, and the sonicator turned on. After about 20 minutes the resin was settled down inside the column , and then another portion was poured in. this process was repeat until the column was visually fully filled with the resin. The column fitting was replaced on the open side and connected to the liquid delivery pump of the HPLC. The pump was set at the beginning to deliver 1ml/min distilled water. The back pressure was monitored until it reach a stable value of 1.5 MPa. The flow rate was then increased by 1ml until reaching 5ml/min at constant period of time. The recorded back pressure was (4.9-6.6-7.5-8.4-9.3) MPa respectively. After the pump was tuned off, the column fitting was opened and check if the column need another portion of the stationary phase which was added until it completely full. The packing and compression process was repeated until having a tied packed column. The column was then connected to the HPLC system. The flow rate was set at 1ml/min at first, distilled water was passed through the system for about 2 hours. The column was tested by passing 0.1 M of NaOH.

2.6. Sample Preparation

A stock solution of 50ppm of each of tyrosine, tryptophane and phenylalanine were prepared by dissolved 0.005g of amino acids in 100mL solvent in a volumetric flask in buffer phosophate and acetonitrile solution . Other standard solutions of these amino acids were prepared by subsequent dilution of the stock solutions.

2.7. Sample Analysis

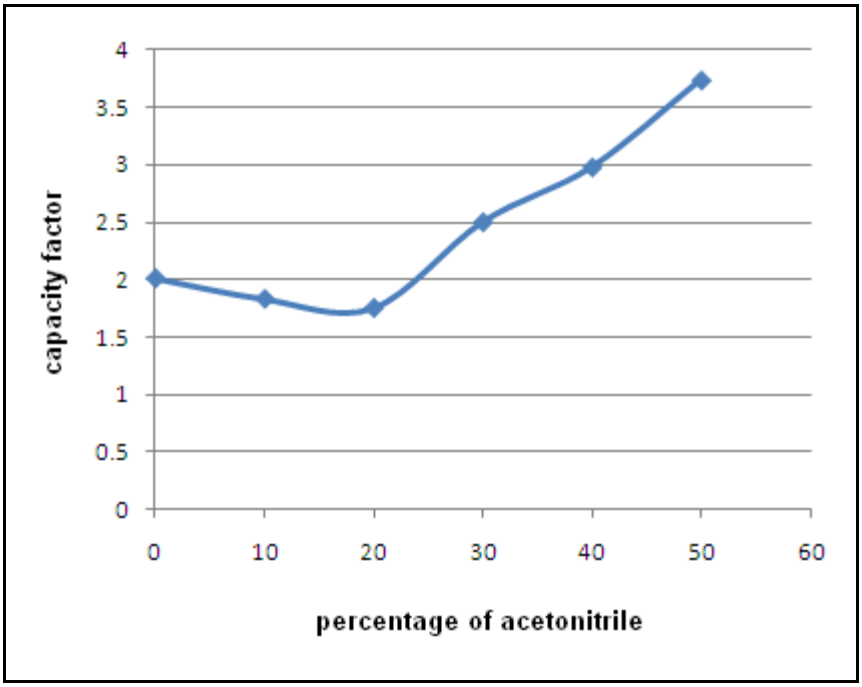
HPLC using amberlite-congo red column with different mobile phases were employed to chromatograph all prepared standard solution of amino acid.

The mobile phase at beginning of analyses was distilled water adjusted to pH 2.5 by adding a few drops of 0.1 M HCl. This mobile phase was passed through the amberlite-congo red column at 1.0 ml/min for 1 hour until achieving a stable baseline.

The amino acid, tyrosine, tryptophane and phenylalanine were chromatograph using different concentration of each sample that ranged from 0.25-50 ppm, were injected and analyzed at least three times with the above mobile phase. The same samples were analyzed after the pH of mobile phase was adjusted to buffer 2, 4, 6, 8,10 and 12.

It was found that there is no result at pH buffer (2, 4, 6, 8 and 12); while in pH buffer 10 show a good detection and separation especially when 80% buffer 10 were mixed with 20% acetonitrile for tyrosine and

tryptophane as shown in figure (2-3) and 90% buffer 10 when mixed with 10% acetonitrile for phenylalanine as shown in figure (2-3).



Figure(2-3): chart show the percentage of acetonitrile versus capacity factor for tyrosine

The retention times of each chromatographic run was recorded and averaged as listed in table (2-2).

Table (2-2) Retention times of amino acids at different pH of the mobile phase, using Amberlite-congo red column (25 × 0.4 cm), flow rate 1 ml/min, sample loop 5µl, detection wave length 270 nm and concentration 50 ppm.

pH 10 percentage buffer phosphate	Acetonitrile percentage	Retention time (minutes)		
		Tyrosine	Phenylalanine	Tryptophane
100 %	0 %	5.48	5.27	5.18
90 %	10 %	5.76	5.67	5.56
80 %	20 %	6.5	5.65	4.58
70 %	30 %	5.3	5.03	4.7
60 %	40 %	4.73	4.32	3.93
50 %	50 %	4.62	4.2	4.02

Different concentration of the studied amino acid and their mixture were analyzed after optimizing the chromatographic conditions of each group of compounds. Calibration curves of all studied analytes were constricted by plotting the calculated peak area of each analyte verses concentration. The range of the linear calibration equation, regression and detection limit was calculated.

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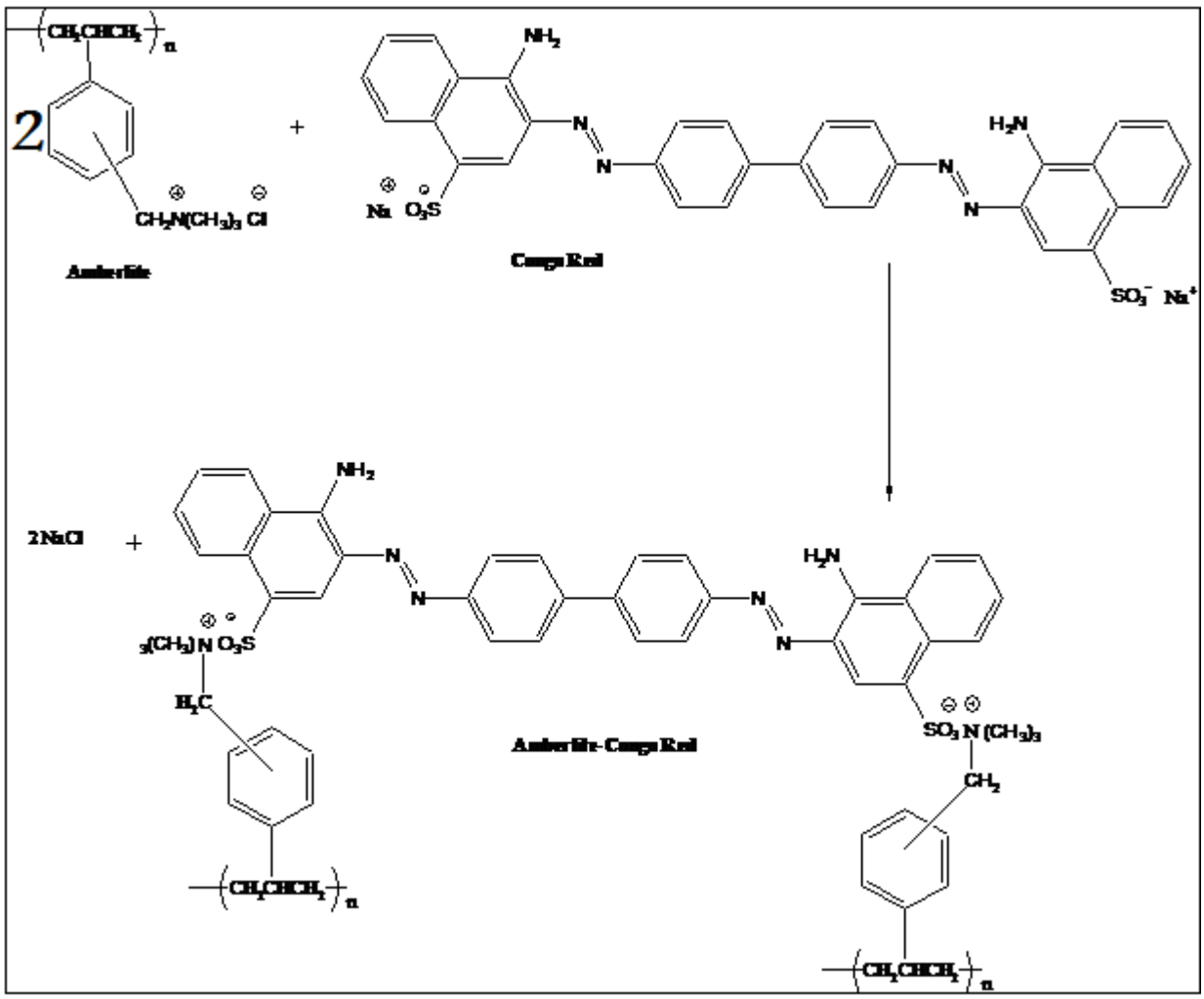
CHAPTER THREE

RESULTS AND

DISCUSSIONS

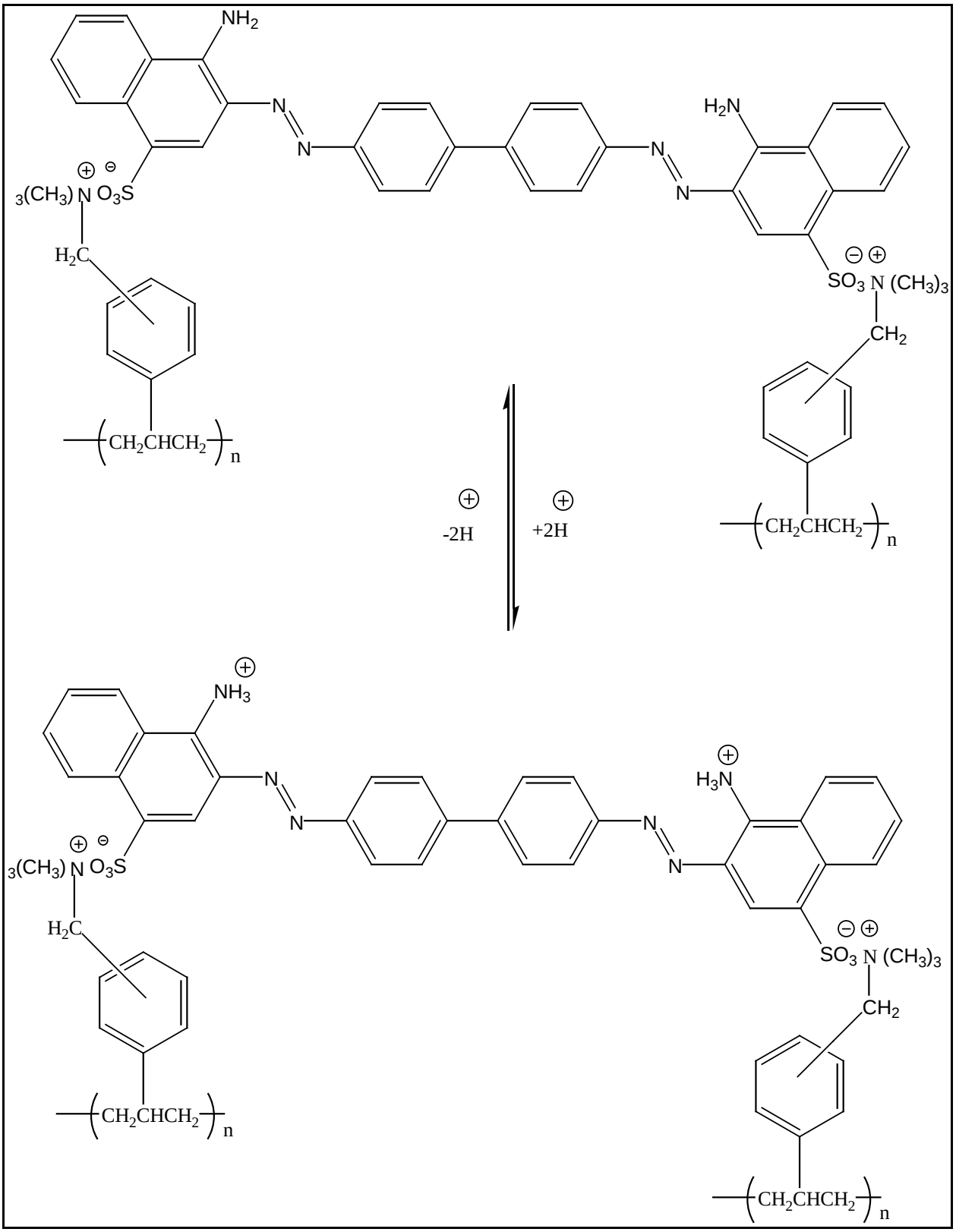
3.1 preparation of amberlite-congo red resin

Congo red was diluted with distilled water and few milliliters were added to amberlite resin, it was noticed that the color of the resin was changed from yellow to deep red. This color changed of the resin may be attributed to the reaction positively charged quaternary ammonium group of the amberlite resin with the negatively charged sulfonate group of congo red. This reaction may be represented in scheme (3-1)



Scheme (3-1): suggested reaction between Amberlite and Congo red

The new resin was washed few times with deionized water in order to remove the excess congo red from the resulted resin, a trace of pinkish red color was indicates that the resin is still holding the congo red moiety . Further addition of distilled water remove the moiety of compound and the new resin color was deep red. The addition of few milliliters of 0.1M HCl solution to this new resin has turned the color of the resin gradually from red into violet and finally to deep blue. It is, however, turned back to red coloration when few milliliters of 0.1M NaOH solution were added. This color change indicates that one or both of amine group on the congo red still having their acid-base characteristics. This equilibria process may be represented by the following suggested equation shown in the reaction scheme (3-2).



Scheme (3-2): suggested acid-base equilibria of the Amberlite and congo red

As a consequence, the new resin might act as uncharged resin in acidic solution, and posetively charged in basic solution. The chromatographic behavior of this new resin may then be influenced by changing the pH of the mobile phase.

The F.T.I.R spectrum for congo amberlite red, and amberlite-congo red new resin were shown in figure (2-2).

Figure (2-24, A) showed a (C-H) aromatic at 3440 cm-, (C-H) aliphatic stretching at 2860.2 cm-1 and bending at 1421.4 cm-1, (C=C) aromatic at 1616.2 cm-1, (C-N) at 1220.9 cm-1, (N-H) stretching at 3440.9 cm-1 and bending at 1483.2cm-1.

Figure (2-2, B) showed a (C-H) aromatic at 3050 cm^{-1}, (C=C) aromatic at 1585.4 cm^{-1}, (C-N) at 1224.7 cm^{-1}, (N-H) primary stretching at 3463.9 cm^{-1} and bending at 1444.6 cm^{-1}, para di-substitution at 831.3 cm^{-1} and free ($SO4^{-2}$) stretching at 1124.4 cm-1 and bending at 596.0 cm^{-1}.

Figure (2-2,C) showed (C-H) aliphatic stretching at 2860.2 cm^{-1} and bending at 1421.4 cm^{-1}, (C-H) aromatic at 3040 cm^{-1}, (C=C) aromatic at 1483.2 cm^{-1}, (C-N) bond at 1225 cm^{-1}, (N-H) primary stretching at 3419.6 cm^{-1} and bending at 1550 cm-1, para di-substitution at 829.3 cm^{-1}, this figure also showed the formation of (N-O) bond at 979.8 cm^{-1} and the disappearance of free ($SO4^{-2}$) stretching at 1124.4 cm^{-1} and bending at 596.0 cm^{-1}, which confirm the suggested reaction between amberlite and congo red as shown in figure (3-1) .

Total ion exchange capacity or total number of sites available for exchange is normally determined by titrations methods, this is done by converting the resin to a given ionic form by chemical regeneration techniques. The new ion may then be chemically removed from a measured of quantity of the resin and determined by any one of several analytical titrations. The total capacity is best expressed as milliequivalent per gram (meq./g) of dry resin.

The slurry of amberlite and amberlite-congo red were titrated separately with HCl solution and back titrated with NaOH solution. The first and second derivatives of these titration curves show one equivalent point for amberlit resin and two equivalent points for amberilt-congo red resin. This indicates that the both amine group of congo red still having their acid-base equillibria, but of different acid strength, which is in consistent with congo red acidic strength (pK_1=9.73 and pK_2=8.31). The equivalent points have been used to calculate the (meq./g) of these dry resins.The resin capacity for the amberlite-congo red resin was found to average (7.36 meq./g) which is approximately twice as that of the amberlite resin, (3.0 meq./g).

3.3 column packing of resin capacity

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 [(40)], however, in that stationary phase must be suspended 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 impairs the separation performance. The slurry was sonicated all the way of packing in ultrasonic bath. After the packing was completed, the column was operated under a pressure many times greater than the subsequent operating level, as recommended by several workers .

The packed column was operated at flow rate of 5ml/min with 500ml distilled water for several times, which produced a back pressure of (9.2 MPa) and this high pressure was used to prevent subsequent collapse of the packing. After the column packing was completed the column was connected to the chromatograph and the flow rate was set at 1ml/min, with distilled water for one hour. The detector wave length was set as 270 nm and the baseline was monitored at the wavelength during the period. This process has been done in order to make sure if there was any stripping of congo red bound to the resin. The baseline was stable (at zero reading) as well as the pressure of the column was constant. Visual testing of the column was made several times to check for any incomplete packing. We have found that there is no decrease or shortage in the packing material inside the column. This indicates that successful packing with this method could be possible.

3.4 column evaluation

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

$$N = 16(t_R/w_b)^2 \quad \textbf{or} \quad N = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2$$

Where $\mathbf{t_r}$ is retention time, (**W**) and ($\mathbf{W_{1/2}}$) are the peak width at baseline and half height, respectively. The $\mathbf{t_r}$, **W**, $\mathbf{W_{1/2}}$ and other subsequent parameters were calculated using the optimum flow rate. The optimum flow rate was measured by plotting (**H**) versus different flow rates. The **H** values were calculated from **H=L/N** (where **L** is the length of the column).

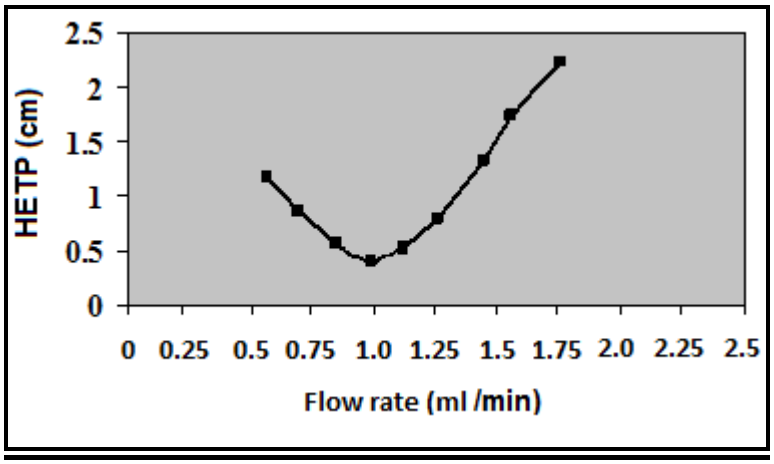


Figure (3-3) Plot of plate height H versus flow rate, using amberlite-congo red column. The mobile phase was buffer phosphate and 25 ppm phenylalanine as a sample.

The optimum flow rates were measured by analyzing phenylalanine using deionized water as a mobile phase. A plot of **H** versus flow rate has given minimum **H** near 1ml/min for the amberlite-congo red column. Some of the measured chromatographic parameters such as plate numbers **N**, plate heights **H**, capacity factor **Ќ**, separation factor **α**, and peak asymmetry for newly packed columns were then calculated using this optimum flow rate.

The mobile phase was adjusted to pH (10), the plate numbers were ranged from (111.09-284.3), with an average value of (216.276). The plate heights were ranged from (0.225-0.08) cm, with an average value of (0.01343) cm. However, these parameters were different when the mobile phase was adjusted at pH(10)+10%acetonitrile for phenylalanine and pH (10) + 20% acetonitrile for tyrosine and tryptophane, the plate numbers were ranged from (198.41-400), with an average value of (176.3307). The plate heights were ranged from (0.0625-0.1968) cm, with an average value of (0.128) cm. The above values showed that the analyte peaks obtained using acidic mobile phase were sharper than in basic medium. This indicates that the separation may be dependent on the hydrophobic interactions only and not on ion-exchange process. Since in acidic medium the resin has uncharged functional groups as described above.

Table (3-1) shows the values of retention times of the studied amino acids. These (tR) were ranged from (5.48-5.18) min for early to last eluted analyte in acidic mobile phase and ranged from (5.76-5.56) at pH (10)+10%acetonitrile and ranged from (6.5-4.58) at pH (10) + 20% acetonitrile. These variation in retention times indicates that the column interaction with the amino acid at high pH medium do not involve ion exchange process as mentioned above.

The capacity factor (Ḱ), is another important term that describes the interaction between solutes to be separated and the stationary and mobile phase. Ḱ is independent on the column length, mobile phase flow rate and represents the molar ratio of the compound in the stationary phase, to that in the mobile phase. Capacity factor between (1.5-5.0) are preferred [41]. Lower **Ḱ** value indicates no stationary phase interaction occurs and hence no chromatography. However, large **Ḱ** value is accompanied by long analysis times. The capacity factor for amino acid compounds chromatographed on the amberlite-congo red column was ranged from (3.5-3.76) in pH 10 and (3.93) in pH 10 +10%acetonitrile and (2.98-4.65) in pH 10 + 20% acetonitrile as shown in table (3-1). This indicates a good competitive interaction between these compounds with the stationary phase and the mobile phase.

Peaks asymmetry is important term, when the value of asymmetry lager than (2.5) means that the quantitative peaks area measurement will become error. The peak asymmetries for compounds chromatographed on new column were ranged from (1.01) with an average value of (1.31).

Table (3-1) retention time, capacity factor, separation factor Plate number , hight and peak asymmetry for amino acid using amberlite-congo red colum. Eluent distilled water pH=8 and PH=8+10%acetonitrile, flow rate 1 ml/min.

Compound	Mobile pase pH	Retention time, Rt	Capacity factor, Ќ	Separation factor α	Plate number N	Plate Height H(cm)	Peaks asymmet-ry
Tryptopha-ne	100%pH =10	5.18	3.5	1.022	198.41	0.126	1.21
	80%pH=10+20% acetonitrile	4.58	2.98	1.318	253.44	0.098	1.01
Tyrosine	100%pH=10	5.48	3.76	1.050	284.3	0.08	1.11
	80%pH=10+20% acetonitrile	6.5	4.65	1.183	400	0.0625	1.2
Phenylalan-ine	100%pH=10	5.27	3.58	-	111.09	0.225	1.24
	90%pH=10+10% acetonitrile	5.67	3.93	-	127.008	0.196	1.31

The separation factor (α) is defined as the ratio of the capacity factors of two adjacent peaks ($\alpha = Ќ_2 / Ќ_1$). Minimum value of (1.1) is required to achieve the separation of two adjacent peaks at any given experimental conditions. The α values for amino acids compounds chromatographed on the amberlit-congo red column were ranged from (1.31-1.38) in pH 10 and ranged from (1.14-1.16) in 90% buffer pH 10+10% acetonitrile and ranged from (1.12-1.14) in pH 10 + 20% acetonitrile. Although both condition give good separation factors, however, it may be possible to achieve better separation of these components using acidic than basic medium.

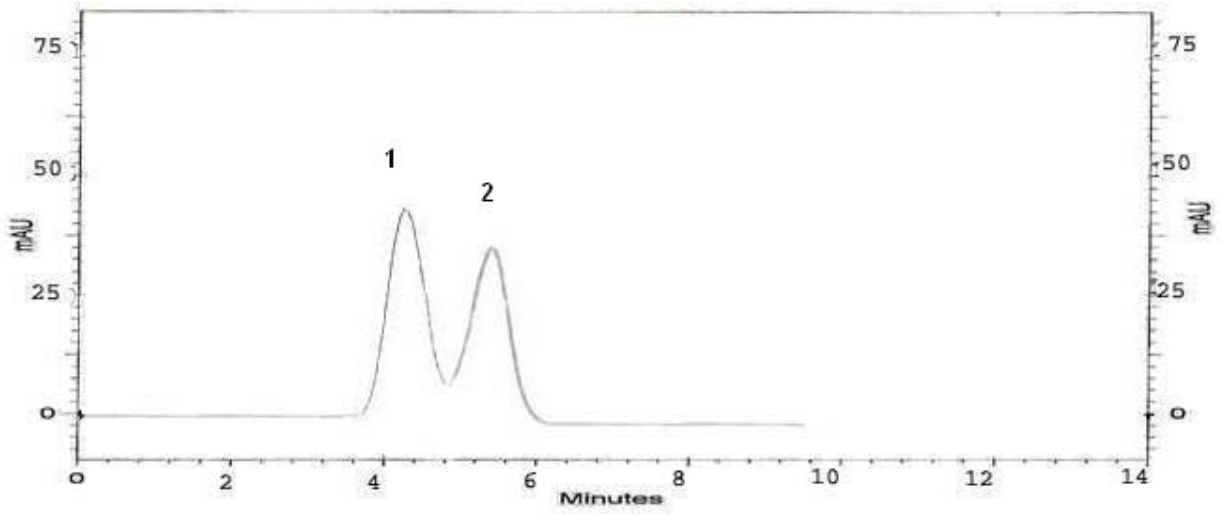


Figure (3-4): chromatogram of amino acid , (1) tryptophane 25 ppm, (2) phenylalanine 25 ppm. Using Amberlite-congo red column detection wavelength 270 nm using a mobile phase 20 % acetonitrile + 80 % buffer phosphate pH=10.

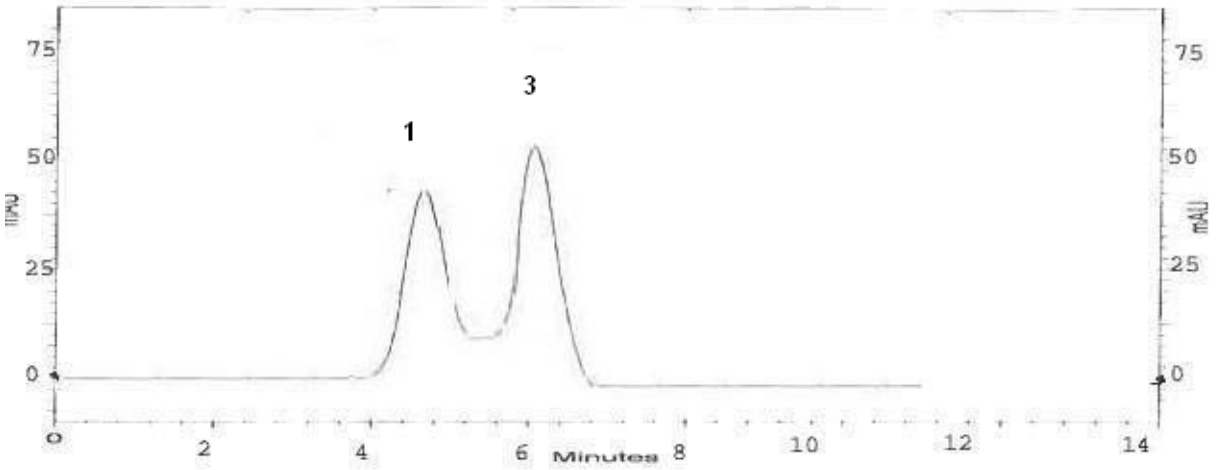


Figure (3-5): chromatogram of amino acid (1) Tryptophane 25 ppm, (3) Tyrosine 25 ppm. Using Amberlite-congo red column detection wavelength 270 nm using a mobile phase 20 % acetonitrile + 80 % buffer phosphate pH=10.

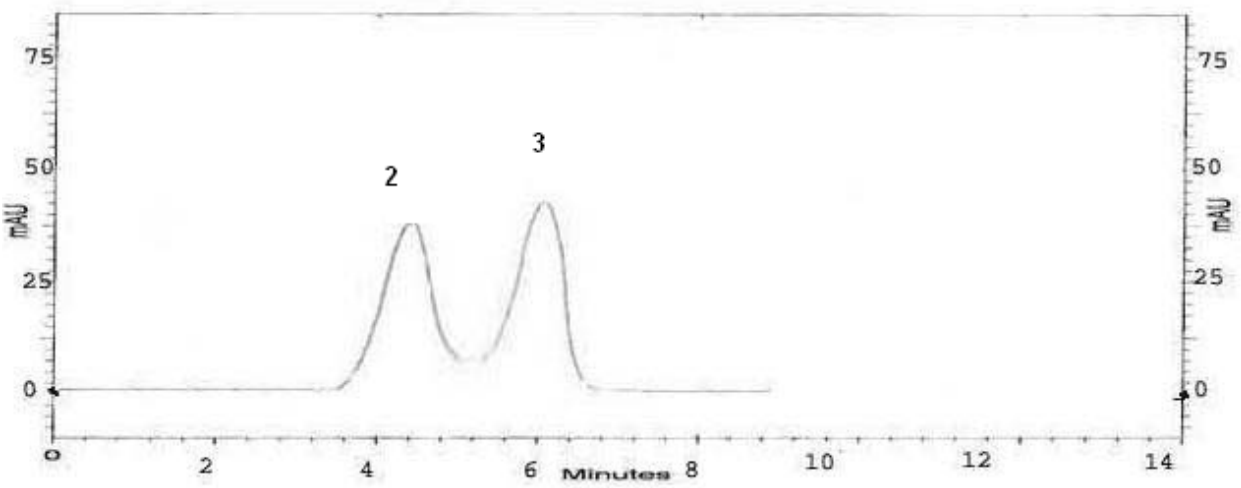


Figure (3-6): chromatogram of amino acid (2) 25 ppm phenylalanine, (3) 25 ppm tyrosine. Using Amberlite-congo red column, detection wavelength 270 nm, using amobile 20 % acetonitrile + 80 % buffer phosphate pH=10.

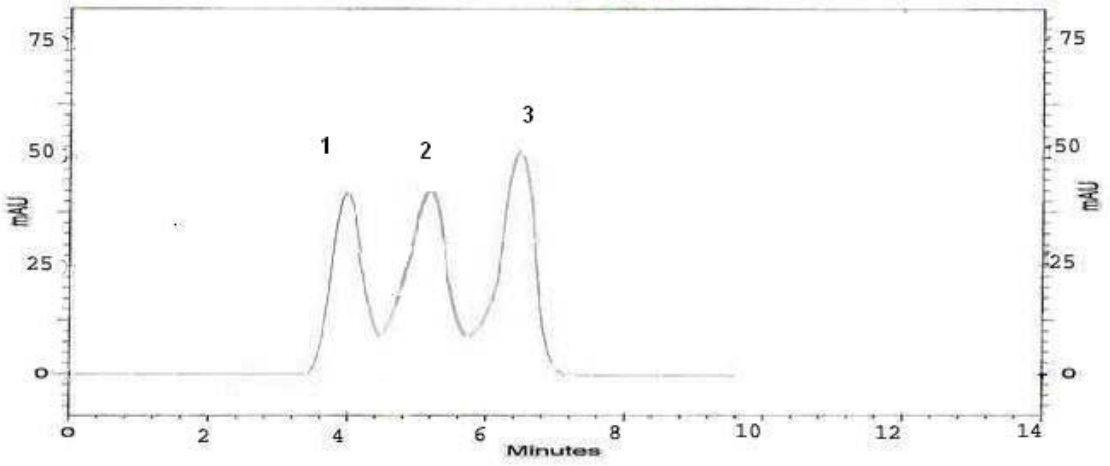


Figure (3-7): chromatogram of amino acid (1) 25ppm tryptophane (2) 25 ppm phenylalanine, (3) 25 ppm tyrosine. Using Amberlite-congo red column, detection wavelength 270 nm, using amobile 20 % acetonitrile + 80 % buffer phosphate pH=10.

Table (3-2) Resolution (R_s) of mixture, using Amberlite-Congo Red column Eluent 20% Acetonitrile + 80% buffer phosphate PH=10, flow rate 1 ml/min., and detection wavelength 270 nm

Mixture	Mobile phase	W_{b1}	W_{b2}	t_{R1}	t_{R2}	R_s
25 ppm Phenylalanine, and 25 ppm Tryptophane	%20 acetonitrile in buffer phosphate	0.8	0.9	4.58	5.67	1.67
25 ppm Tyrosine, and 25 ppm Tryptophane	%20 acetonitrile in buffer phosphate	0.8	0.7	4.58	6.5	3.49
25 ppm Phenylalanine, and 25 ppm Tyrosine	%20 acetonitrile in buffer phosphate	1.1	0.9	6.5	5.67	1.25
(1) Tryptophane 25 ppm (2) Phenylalanine, 25ppm (3) Tyrosine 25 ppm	%20 acetonitrile in buffer phosphate	0.7	0.8	4.58	5.67	(1,2) 1.72
		0.8	0.6	5.67	6.5	(2,3) 1.19

3.5 Quantitative Analysis

Calibration runs of amino acids compounds on Amberlite congo red column, in 80% buffer pH 10+20% acetonitrile for tyrosine and tryptophane as shown in

figure (3-8) and 90 % buffer pH 10+10 % acetonitrile for phenylalanine are shown in figure (3-9). A linear dependence of the peak areas on the amount injected is evidence for all compounds down to the detection limit. Amino acids has shown a linear response range extending from at least 50 ppm to the detection limit which was at low as 0.25 ppm.

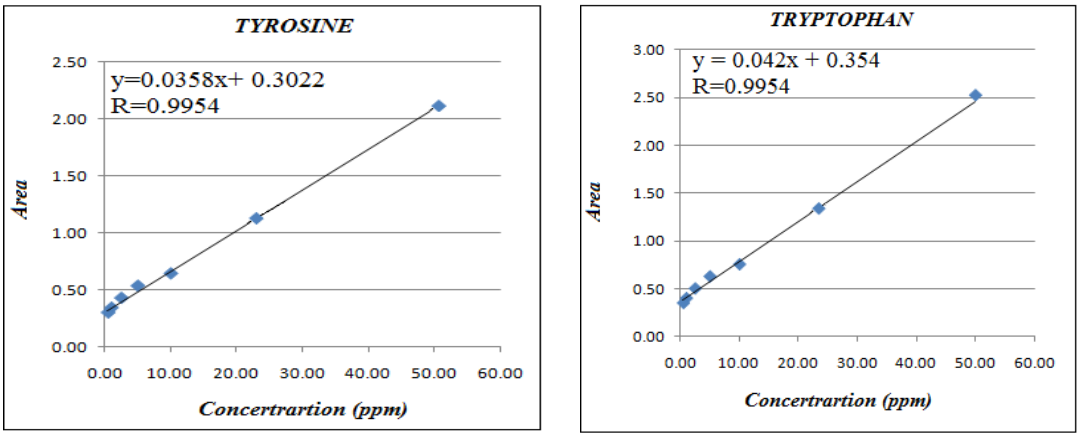


Figure (3-8): Calibration curve of amino acids using Amberlit congo red column (25 × 0.4 cm). using 80% buffer pH 10 +20 % acetonitrile as a mobile phase for tyrosine and tryptophane, flow rate 1 ml/min.

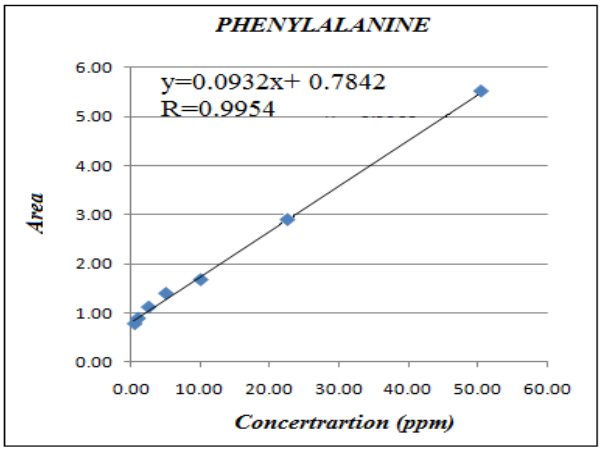


Figure (3-9): Calibration curve of amino acids using Amberlit congo red column (25 × 0.4 cm). using 90% buffer pH 10 +10 % acetonitrile as a mobile phase for phenylalanine, flow rate 1 ml/min.

The slope values for the linear calibration curves were ranged from (0.0358-0.0932) depending upon the amino acid and whether it is analyzed in 80% buffer pH 10 +20 % acetonitrile for tyrosine and tryptophan and in 90% buffer pH 10 + 10% acetontrileas shown table (3-3).

These detection limits were calculated at S / **N** ratio of 3 or more.
(Figure 3-10, 3-11, and 3-12).

Table (3-3) Linear equation and correlation coefficients (R). using Amberlite congo red column (25 × 0.4 cm). flow rate 1 ml/min.

Compound	Mobile phase	Linear equation	R	Detection limit
Tryptophan	using 80% buffer pH 10 +20 % acetonitrile	y = 0.042x + 0.354	0.9962	0.25
Tyrosin	using 80% buffer pH 10 +20 % acetonitrile	y=0.0358x+ 0.3022	0.9954	0.5
Phenylalanin	using 90% buffer pH 8 +10 % acetonitrile	y=0.0932x+ 0.7842	0.9987	0.125

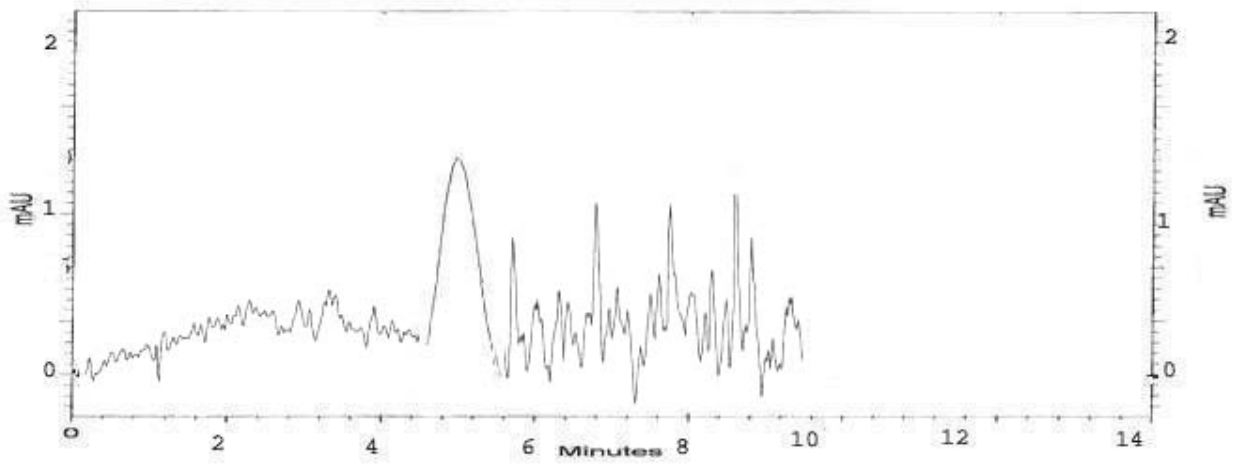


Fig. (3-10) 0.5 ppm of Tryptophane (detection limit) using Amberlite- Congo red salt column (25 x 0.4 cm (i.d.)) ,detection wavelength 270nm , flow rate 1ml/min. Eluent %20 acetonitrile in buffer phosphate

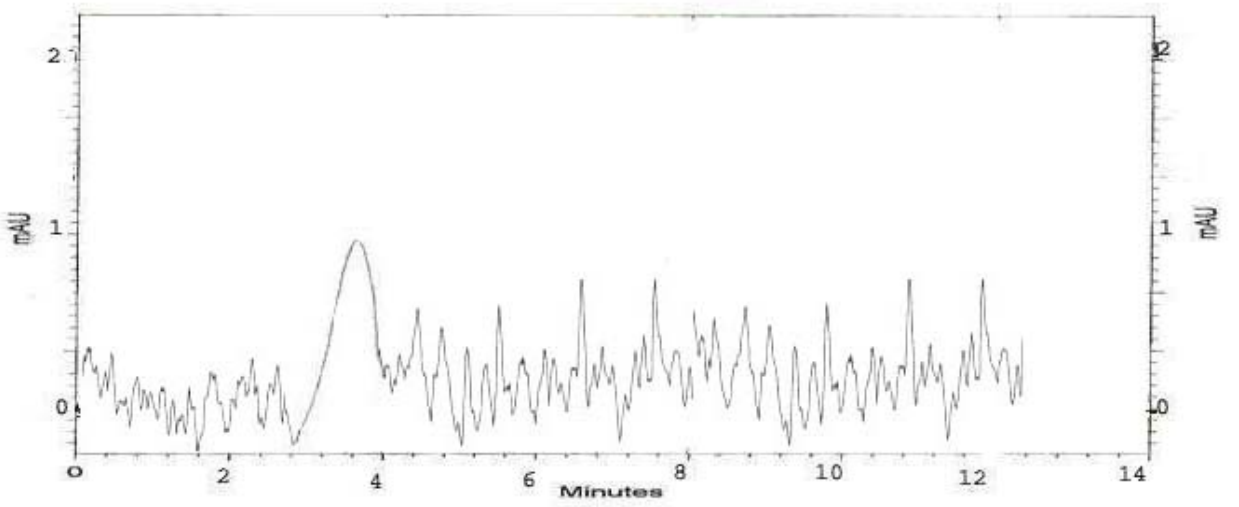


Fig. (3-11) 0.5 ppm of Tyrosine (detection limit) using Amberlite- Congo red salt column (25 x 0.4 cm (i.d.)) ,detection wavelength 270nm , flow rate 1ml/min. Eluent %20 acetonitrile in buffer phosphate

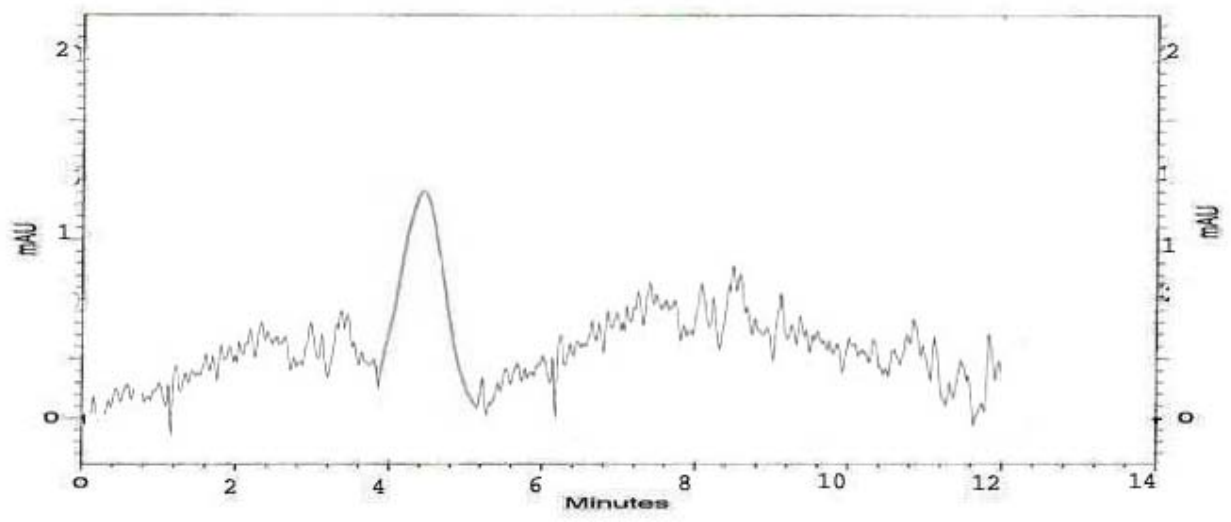


Fig. (3-12) 0.5 ppm of Phenylalanine (detection limit) using Amberlite- Congo red salt column (25 x 0.4 cm (i.d.)) ,detection wavelength 270nm , flow rate 1ml/min. Eluent %20 acetonitrile in buffer phosphate

Prepared standard mixture solutions of the analyzed amino acids were injected for at least 3 times under the same condition and their concentration were calculated by measuring the peak area of each amino acids and using their respective the linear equation. The recovery were ranged from (95.6-95.8)% . The R.S.D. were ranged from(0.0208-0.159)% . The relative errors were ranged from (-4.2 - -4.4) % as shown in Table (3-4).

Table 3-4. % R.S.D , %Recovery, and % Relative Error for the Analyzed amino acids .

Compound	Concentration injected (ppm) μ	Average Concentration Found(calculated) (ppm) $\overline{x}$	Absolut Error	Relative Error%	Rec.%
Phenylalanine	10	9.56	-0.44	-0.044	104.4
Tryptophane	10	9.57	-0.43	-0.043	104.3
Tyrosine	10	9.58	-0.42	-0.042	104.2

***average of three concentration and using the linear equation for each amino acids.**

Conclusion

New stationary phase were prepared by the reaction of Congo Red salt solution with Amberlite anion exchanger. The stability of the new attached functional group on the resin was studied against different solvents . The new prepared resin was found stable and no depletion of the Congo Red salt moity from the resin was noticed with the above materials. The new stationary phase has been packed into stainless steel column. This has been done by special technique. The resin capacity of newly prepared resin has calculated. The resin capacity for the Amberlite-Congo Red salt was found to average 7.36 meq./g which is more than the Amberlite resin, 3.0 meq./g. The chromatographic performance of the packed column was characterized. The number of plate numbers N, height equivalent of a theoretical plates H, capacity factors Ќ, selectivity factors α, Resolution R_s , and peaks asymmetry, were measured by analyzing different analyses on the new columns using different mobile phase's compositions and flow rates. These parameters were measured from analysis three amino acids Phenylalanine, Tryptophan, and Tyrosine. The analysis of amino acids using Amberlite – Congo red salt column has performed with %20 acetonitrile in buffer phosphate as mobile phase and also %100 buffer phosphate as mobile phase in flow rate 1 ml/min and detection wavelength 270 nm. This new resin at dual characteristics of both ionic and hydrophobic properties which can be used in both direction by justice the condition of separation. Calibration curves for all analyzed compound were linear from their detection limits to at least 50 ppm with correlation coefficient ranged 0.9954. The detection limits were ranged 0.5 ppm at signal to noise ratio of three or more

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الخلاصة

تم في هذا البحث تحضير طور ثابت جديد لغرض استخدامها في الفصل للكروماتوكرافي سائل عالي الاداء وذلك من خلال تفاعل راتينج التبادل الايوني السالب مع كاشف الكونكو الاحمر.

اثبتت تحاليل اشعة تحت الحمراء ارتباط الكاشف الاحمر مع الراتنج مثلما دل عليه المظهر الخارجي للراتينج الجديد وتغيير في صفاته الكروماتوكرافية.

تم قياس السعة الكروماتوكرافية للطور الثابت الجديد و وجد ان السعة للراتنج ال محضر هو (7.36meq./gm) وان السعة الكروماتوغرافية لراينج التبادل الايوني الاصلي هو (3.0 meq./gm).

تم تعبئة الطور الثابت الجديد في عمود من الفولاذ المقاوم للصدأ خاص ب (HPLC) بواسطة الطرق الخاصة بالتعبئة تم قياس كفاءة الاعمدة المعبئة بحساب عدد الصفيحات النظرية, الارتفاع المكافئ للصفيحات النظرية, عوامل الاستيعاب, عوامل الانتقائية بواسطة تحليل محاليل مختلفة على الاعمدة الجديدة باستخدام اطوار متحركة مختلفة النسب وسرع جريان مختلفة.

تم تحليل الاحماض الامينية مثل (phynalalanine, tryptophane and Tyrosine) بواسطة العمود المحضر.

في تحليل الاحماض الامينية بواسطة عمود الامبيرلايت – كونكو استخدم طور متحرك مائي (pH=10) ووجد ان عدد الصفيحات النظرية يساوي (111.09-284.3). الارتفاع االم كافئ يساوي (0.01343)سم. عوامل الاستيعاب تتراوح من .3.76-3.5). بعد تحليل الاحماض الامينية باستخدام نفس العمود ولكن باستخدام محلول مائي اخر (pH=10) باضافة 20% من الاسيتوناترايل وجد ان عدد الصفيحات النظرية يساو ي..(176.3307).الارتفاع المكافئ للصفيحات يساوي (0.128)سم. عوامل الاستيعاب تتراوح من(2.98-4.65) .

الاهداء

الشمس التي أضاءت طريقي

أمي

الى منبع علمي ومغذي عقلي

أبي

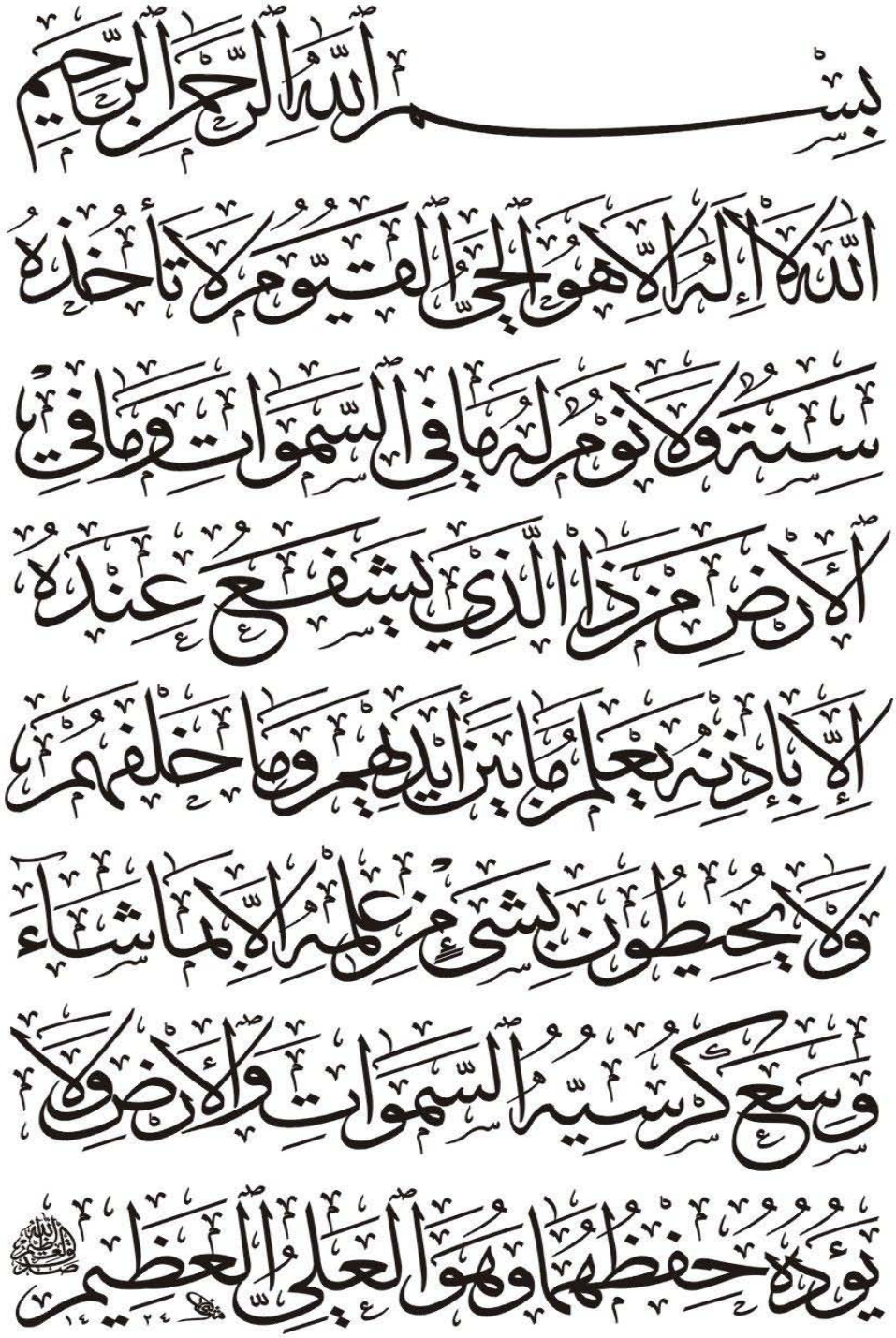
الى أحباب قلبي ومساند شدتي

أخوتي

الى كافة زملائي وصديقاتي

والى كل من له معزة في قلبي

اهدي ثمرة جهدي



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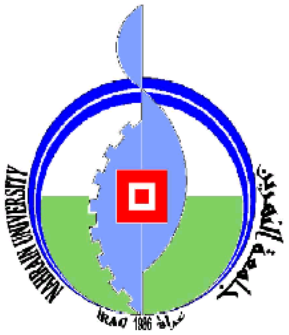

تحضير ودراسة خواص الفصل لطور ثابت جديد لتقنية HPLC

رسالة مقدمة ألى
قسم الكيمياء/كلية العلوم/جامعة النهرين
كجزء من متطلبات نيل درجة ماجستير في الفيزياء

من قبل
هاجر سعد نجم
بكالوريوس علوم كيمياء

بأشراف

د. شهباز احمد مكي

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