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Fabrication Chitosan-valine Interpenetrating Polymer Network for Estimation Human Lipid Profile and Total Proteins

Ahmed Saleh*, Reem Adham and Israa Ghassan

Abstract--- *present research aimed to determine the efficiency chitosan-valine beads modification by ethylene glycol diglycidylether (EGDE) as a cross linker polymer to adsorbelipid profile and total proteins(total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C) and total protein (TP)) from human serum in patients suffering from hyperlipidemia in different contact times and temperatures. UV-Vis spectrophotometer was used to determine the concentration of the adsorption before and after adsorption. The modified beads utilizing in this research were portrayed by Scanning Electron Microscopy (SEM) to depict the surface of the beads and by Infrared (IR) spectroscopy to affirm the cross linking reaction. The results showed that the adsorption process attains equilibrium within 3 hours and the extent of adsorbate increased with increasing contact time, temperatures and concentration. The adsorption isotherms are described by means of the Langmuir and Freundlich isotherms. In vitro study it was found that there were significant decrease ($p \leq 0.05$) in the levels of serum "TC,TG,LDL-C and TP," while the level of HDL showed non – significant decrease ($p \geq 0.05$)after adsorption process. It was found that the Langmuir and Freundlich equation both fitted. Theadsorption kinetics of adsorbate was best described by the pseudo first-order reaction model. Free energy of adsorption (ΔG), enthalpy (ΔH), and entropy (ΔS) changes were calculated to predict the nature of adsorption.*

Keywords--- *Adsorption, Adsorbent, Chitosan, Valine, Ethylene Glycol Diglycidyl Ether, Lipid Profile and Total Protein.*

I. INTRODUCTION

Chitosan is classified as a family of polymers which is a linear, semi-crystalline polysaccharide composed of (1 4)-2-acetamido-2-deoxy-b-D-glucan (N-acetyl D-glucosamine) and (1 4)-2-amino-2-deoxyb-D-glucan (D-glucosamine) units as shown in figure (1). Chitosan is not widely present in nature but it can be easily produced by deacetylation chitin which acetyl groups are removed in varying degrees, the process is carried out by enzymatic hydrolysis in presence of certain enzymes or by chemical hydrolysis under basic conditions, so acetylation degree gives a picture of the balance between chitosan and chitin in the products. It is possible to distinguish between chitosan and chitin by the molar percentage when it less than 50% the product is called chitosan and becomes soluble in acidic solutions[1,2,3].

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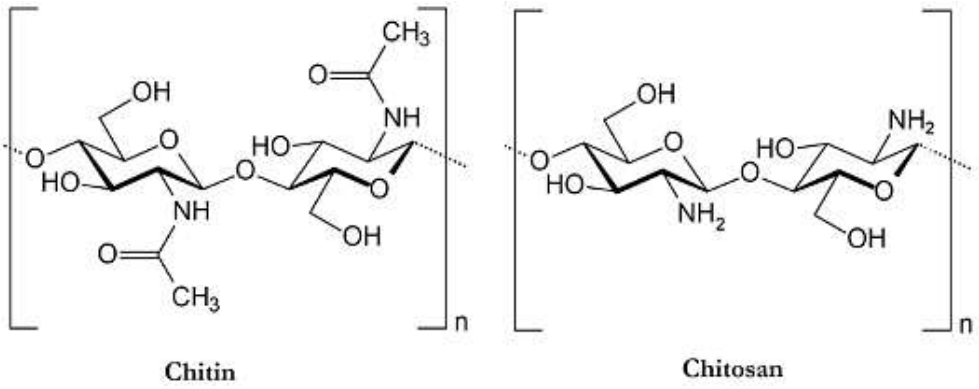

Figure 1: Chemical structure of chitin and chitosan[4]

chitosan has many important applications like wound healing, tissue engineering[5], gene delivery[6], drug delivery[7], adsorption[8], and it can be easily manufactured into various forms such as membranes, gels, nanofibrils, nanofibers[9], microparticles[10], scaffolds[11], nanoparticles[12], beads[13] and sponge-like forms[14].Because of presence of functional sites that can be modified in the chemical composition of the particles of chitosan (OH, NH) groups which in turn is a very important feature in the interactions, chitosan molecules are modified by grafting or cross-linking reactions and thus obtained derivatives of chitosan that are more effectiveness with excellent adsorption properties that could be altered to many application of surface chemistry like physical pharmacy, biochemistry, removal of heavy metals, filtration, ion exchange adsorption and membrane systems.[15].

Hypercholesterolemia is a serious clinical disorder that results from a significant reduction in the effectiveness of low density lipoprotein receptors therefore a very high cholesterol levels in the blood[16].

High cholesterol values in the blood are an important and effective factor in the heart disease, other vascular diseases and death in some cases[17],so many previous studies have shown that lowering high levels of cholesterol in patients with hypercholesterolemia would reduce the incidence of myocardial infarction[18].Therefore, low-density lipoprotein is the main carrier of cholesterol in the blood and therefore high values of low-density lipoprotein lead to the accumulation of cholesterol on the inner wall of blood vessels and thus the gradual formation of atherosclerotic plaque and thus the occurrence of cardiovascular disease[19], the principles and directions of the American College of Cardiology, American Heart Association and the European Society of Cardiology and European Atherosclerosis Society recommended to reduce the levels of cholesterol (≥193 mg/dL) in patients with cardiovascular disease[20-26].

In this work, A LDL and total protein adsorbent was obtained via quite simple reaction routes use of chitosan, an abundant natural polymer with good biocompatibility. The adsorbent demonstrated satisfactory adsorption performance and excellent blood compatibility. Some adsorption isotherm, kinetic and thermodynamic study were investigated.

II. METHODOLOGY

2.1 Materials

Chitosan powder, with a deacetylation level of around 90%and purity was ≥ 90%, was obtained from REGAL Biological Tech. Co., Ltd., Shanghai. All other reagents were of analytical grade and obtained commercially.

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2.2 Surface preparations

One gram of unadulterated chitosan powder was dissolve with 19 mL of (3%) acetic acid. solution was dropped in 8% NaOH utilizing a syringe with width equivalent to (0.56 mm). chitosan beads were washed with vast amounts of refined water. To actuate beads, EGDE was utilized to shape the epoxy group, therefore, (15 ml 0.6 N) of NaOH containing (30 mg) of (sodium borohydride) with (15 mL) EGDE was blended with these beads for (8 hours) at (25C°). The created epoxidized beads were washed with refined water to evacuate non-interactive materials. To link valine with activated beads,(10 ml) of valine was blended with (5 ml 2N) of NaOH to frame the corresponding salt. This blend was dissolved with 20 ml of carbonate buffer (pH 10.5), at this point added to the epoxidized beads for (24 hours) at (65 C°) for activation [(27)].

The created beads were then washed with a lot of refined water and (1N NaCl) to expel carbonates and non-receptive substances. The beads were saved for further use in a cool environment and by utilizing an solution of (0.15NNaCl)[(27)].

2.3. Characterization

2.3.1 Infrared spectra (FTIR) for the IPN beads

The chemical structure of synthesized beads was studied using FTIR shimadzu.8400s.

2.3.2 Scanning Electron Microscopy (SEM)

The shape and surface morphology of the prepared beads were explored by utilizing the scanner electron microscope instrument (SEM) Angstrom. AlS230, The pictures of (SEM)were taken in the wake of encompassing the beads by environment of nitrogen gas and covering it with a thin layer of gold.

2.4 In vitro adsorption tests and Determination adsorption percentages

Zero five gram of prepared beads was added to (1 ml) of serum were taken from the patients who suffering from hyperlipidemia, and stirred continuously for (3 hours)at 37C°to reach equilibrium by utilized thermostatic shaker water bath. The concentration of adsorbents (Cholesterol, Triglyceride, LDL-cholesterol, HDL-Cholesterol, Total protein) were ascertained after and before treatment by the prepared beads (adsorbent). Also adsorption percentage was calculated during specific times by utilizing commercial test kits that provided by (Linear, Spain.co).Adsorption percentage was calculated by utilized the following equation[(27)]:

- Percentage of adsorption (%) = $(C_2 \_ C_1) / C_1 \times 100$

where C_2 and C_1 are the concentrations of adsorbents before and after adsorption (treatment) respectively.

2.5 Statistical analysis

Statics implemented using graph pad prism version 6 for all statistical analyses students t-test (unpaired) at P value <0.05

2.6 Adsorption Isotherm

The adsorption isotherm for adsorbents (prepared beads) was calculated by treating diluted serum that containing different concentrations of adsorbents with prepared beads. To calculate the amount of adsorbents, the following equation was used[(28)] :

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$$Q_e = \frac{V(C_o - C_e)}{m} \quad \text{.....(1)}$$

Where :*Qe* The quantity of adsorbed (mg/g).

m: adsorbent weight (beads) (g), *Ce* : concentration at equilibrium (mg/L), $C_\circ$:Primary concentration (mg/L),*V* : solution volume (L).

2.7 Effect of Temperature and Thermodynamic parameters

The effect of temperature inthe adsorption process at different temperatures (25,37 and 40C°) was verified to find the thermodynamic functions.

The thermodynamic parameters were calculated as follows :

Adsorption enthalpy (*ΔH*) was calculated using the following equation[29].

$$ln\text{Xm} = -\frac{\Delta H}{RT} + constant \quad \text{.....(2)}$$

Where, *lnXm* the maximum amount of adsorption at a constant temperature, therefore its drawn against 1/T to give straight line with a slope equal to *–ΔH/R*.

In addition, the change in entropy can be calculated from intercept :

$$\text{Intercept} = \Delta S/R \quad \text{.....(3)}$$

Where R is the value of the gas constant (8.314 $J.mol^{-1}.k^{-1}$). T is the absolute temperature.

The change in free energy (*ΔS*) is calculated by the following equation[30]:

$$\Delta G = \Delta H - T\Delta S \quad \text{.....(4)}$$

2.8 Kinetic of Adsorption

The mechanism and outputs of the reaction were verified by using equation ofpseudo first-order and pseudo second-order[31].pseudo first-order equation can be represented as follows :

$$ln\,(qe - qt) = \ln(qe) - k_1 t \quad \text{.....(5)}$$

Where *qe*, *qt* represents the maximum adsorption at equilibrium and at t time, Where *ln* (*qe-qt*) is drawn versus t time to give a straight line, through intercept and negative slope is obtained, *qe*, k_1,andk_1 is the rate constant of first-order adsorption (min^{-1})

pseudo second-order equation can be represented as follows :

$$\frac{t}{qt} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad \text{.....(6)}$$

Where t/ *qt* is plotted against t to give a straight line where k_2 and *qe* are determined by intercept and slope, and k_2 is the rate constant of second-order adsorption (g/mg.min).

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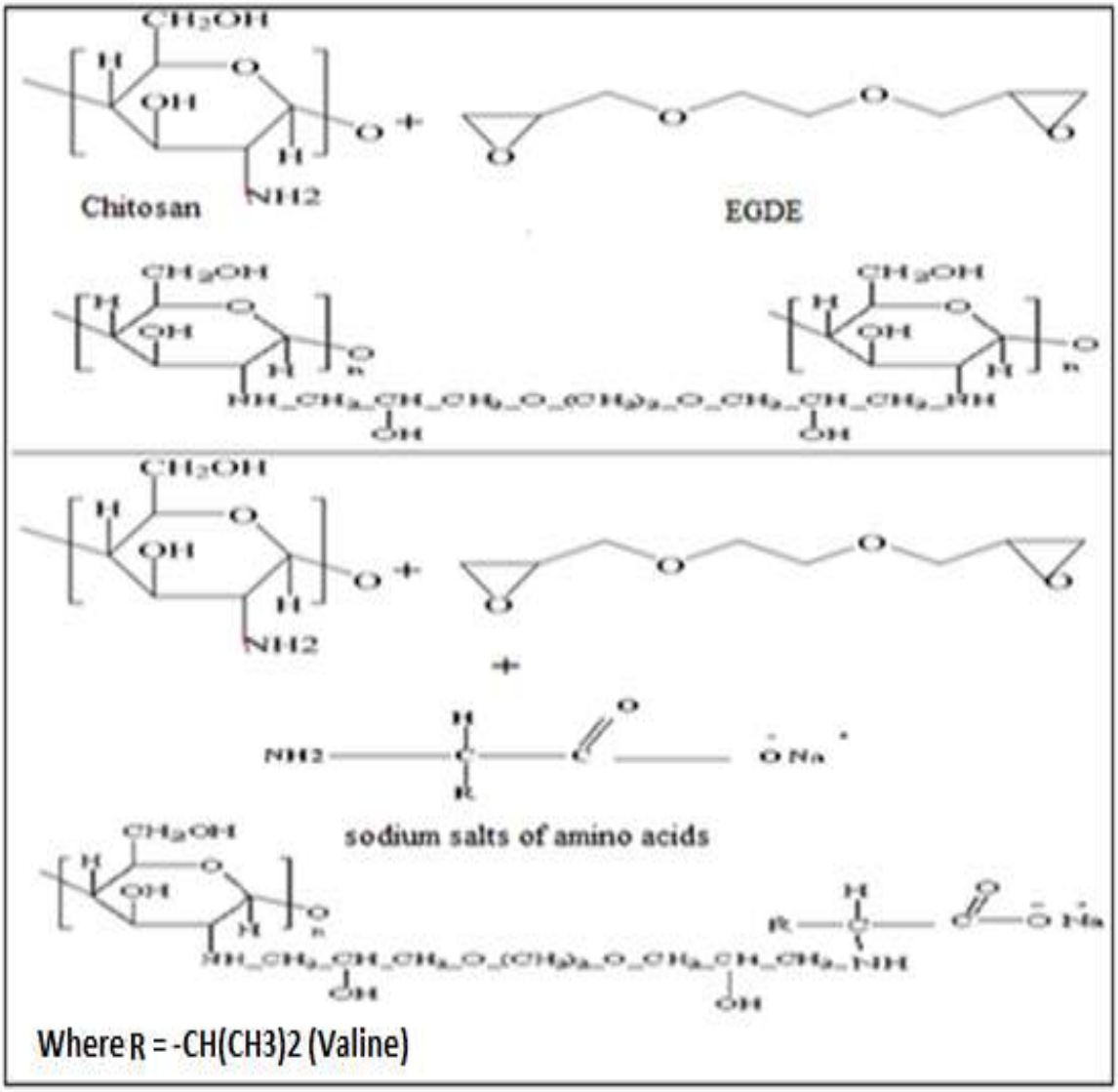
III. RESULTS AND DISCUSSION

The reaction scheme for the cross linking of chitosan with valineusing (EGDE) is shown below



Scheme 1: Show possible cross linking mechanisms of chitosan with amino acids by using ethylene glycol diglycidylether (EGDE) to synthesis beads type C[27]

3.1 Characterization of prepared beads

3.1.1 FTIR for the beads

The chemical structure of the synthesized beads that prepared by the reaction of chitosan with amino acid (valine) that diluted by acetic acid and cross linked with ethylene glycol diglycidylether (EGDE) have been characterized by FTIR. In general the vibration bands of synthesized beads showed absorption band (1604) cm^{-1}due to bending vibration of (NH) bond, and show absorption bands (1692) cm-1 due to stretching vibration of (C=O) bond, and that a good evidence that the reaction was occurred, as shown in figure (2), other bands are listed in table (1).

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Table 1: Show vibration bands of prepared beads

v(CH) Alph.	*v*(OH)	*V*(C=N)	*v*(C-O)	*v*(C=O)	*v*(C-N)	*v*(OH) Bending	Others
2903	3360	-	1153	1692	1327	937	1641(NH) bending

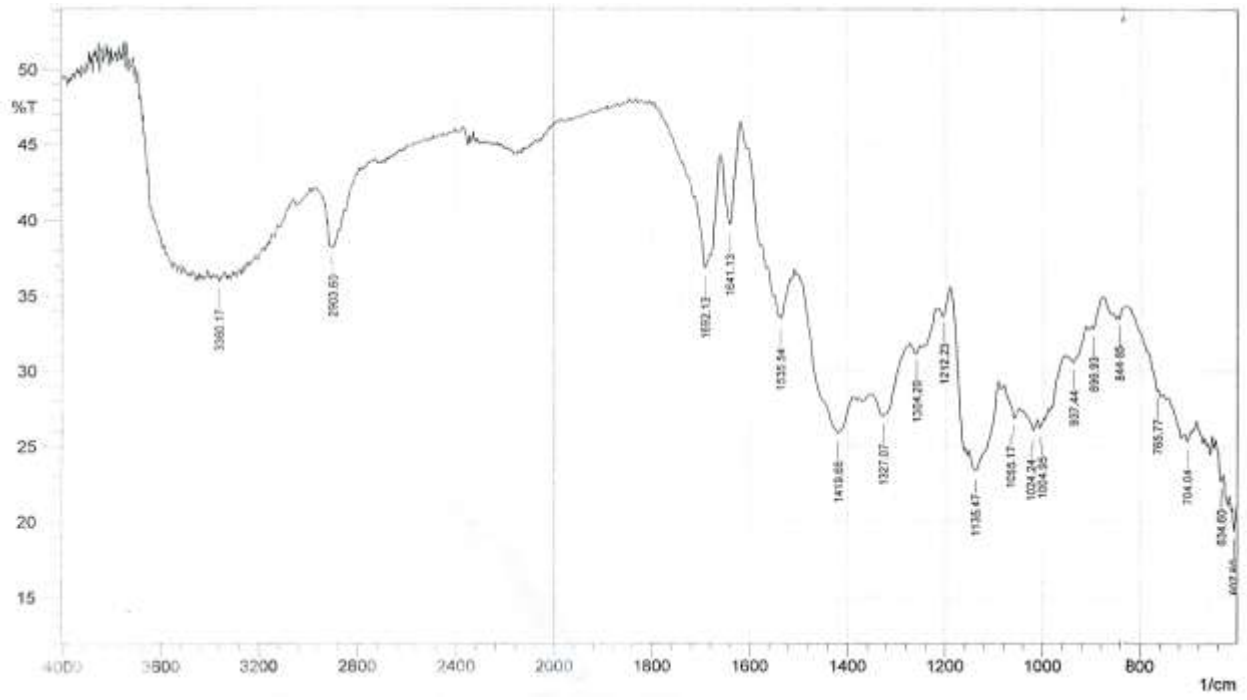

Figure 2: Vibration spectra of chitosan-valine beads cross linking by ethylene glycol diglycidylether

3.1.2. Scanning Electron Microscopy analysis (SEM)

Scanning electron microscopy was used to give a clear picture of the surface of the beads that used. The attached images showed that the prepared beads are spherical or oval in shape that because of the contrast between the concentration of beads solution and solution of NaOH-Methanol, Where the results demonstrated that the surface of the beads was rough, curled and folded.

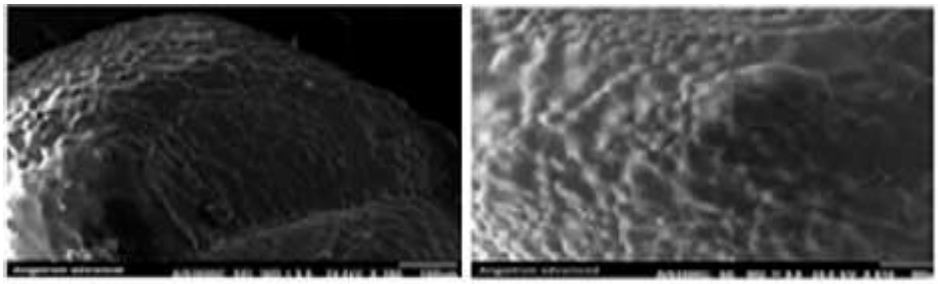

Figure 3: Scanning Electron Microscopy (SEM) photographs of cross linked beads and surface morphology

3.2. Estimation the adsorption percentage and effect of adsorption timeon the adsorption of lipid profile and total protein

The adsorption percentage of the (lipid profile and total protein) from human serum in patients who suffering from hyperlipidemia that adsorbed on the surface of prepared beads that cross linked using EGDE and valine (as a

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ligand) was studied, the results showed high adsorption capacity of prepared beads that gradually increases from 30 to 150 minutes because of chemical composition that contain groups carrying positive and negative charges[27],which provide greater potential for adsorption through electrostatic interference[32],while the HDL particles adsorbedslightlyor non-adsorbed by adsorbate because of HDL molecules are smaller (3.5–9 nm in diameter) comparing with LDL-C and its diffused inside the adsorbent thus occupy the adsorption sites prior to LDL and this is happen in the earlier stages of the adsorption process, after that adsorption of LDL molecules happened when adsorption continuing and this may due to competitive adsorption of LDL until equilibrium[27], as shown in figure (4).

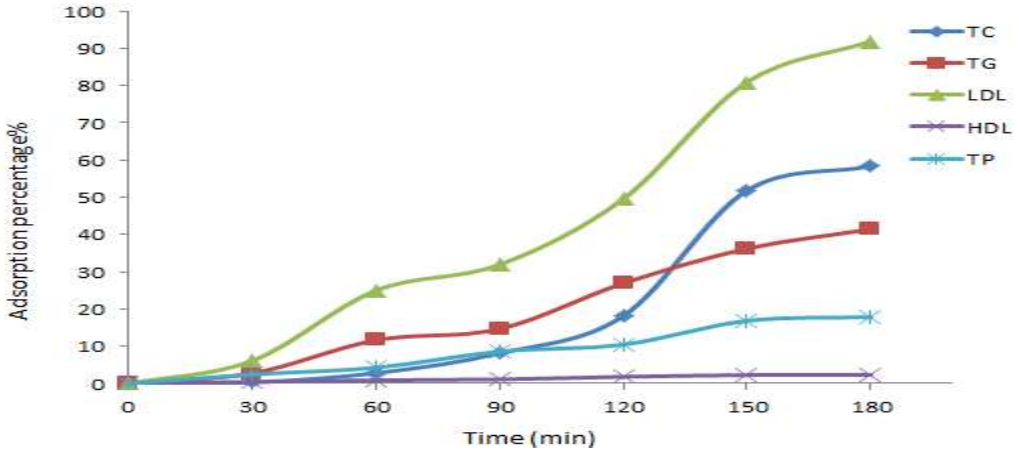

Figure 4: Adsorption percentage of lipid profile and total protein every 30 min

3.3 In vitro study the adsorption of human serum (TC, TG, HDL, S.LDL and TP) on synthesized beads

According to the results given in table (2), it was found that there were significant decrease ($p \leq 0.05$) in the levels of patiants serum "TC, TP, TG and LDL", while the level of HDL showed non – significant decrease ($p \geq 0.05$)after adsorption process.

Table 2: Statistical value for adsorption lipid profile and total protein on prepared beads

GROUP	NO.	TC MEAN ∓ SD MG /DL	TG MEAN ±SD MG/DL	HDL MEAN ±SD MG/DL	S-LDL MEAN±SD MG/DL	TP MEAN± SD G/DL
untreated	30	235.2 ± 7.652	311.4 ± 34.01	37.8 ± 1.306	135.5 ± 9.984	6.894 ± 0.0751
treated	30	150.6 ± 6.565	244.5 ± 33.13	37.57 ± 1.343	66.1 ± 8.316	5.925 ± 0.0774
P value		<0.0001*	0.1683**	0.9013**	<0.0001*	<0.0001*

The results above indicated that the adsorbent which used in this study could remove TC, TG,SLDL and TP from human serum without substantially affecting HDL.

In similar study by Shinji Yokoyama[33], prepared porous cellulose beads covalently linked with dextran sulfate, and studied its *in vitro* effect as adsorption, he found that the adsorbent has a higher selectivity for LDL and VLDL from human plasma without substantially affecting on HDL.

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Umut Selda Bayrak YU, et al[34],Prepared direct adsorption from lipoproteins (LDL) and cholesterol apheresis and combined it with lipid lowering drugs, they studied its *in vitro* effect as adsorption, they found that it could remove TC, LDL, VLDL from human serum, and They found also in-significant losses of HDL.

3.4 Adsorption of lipid profile and total protein from dilute serum on the surface of synthesis beads

synthesis beads were used to adsorb (TC, TG and TP) from diluted serum at different temperatures (25, 37 and 40 ^{o}C)Therefore, the results in figure (5)shown the concentration at equilibrium(C_e),and the quantity adsorbed on synthesis beads surface (Q_e).

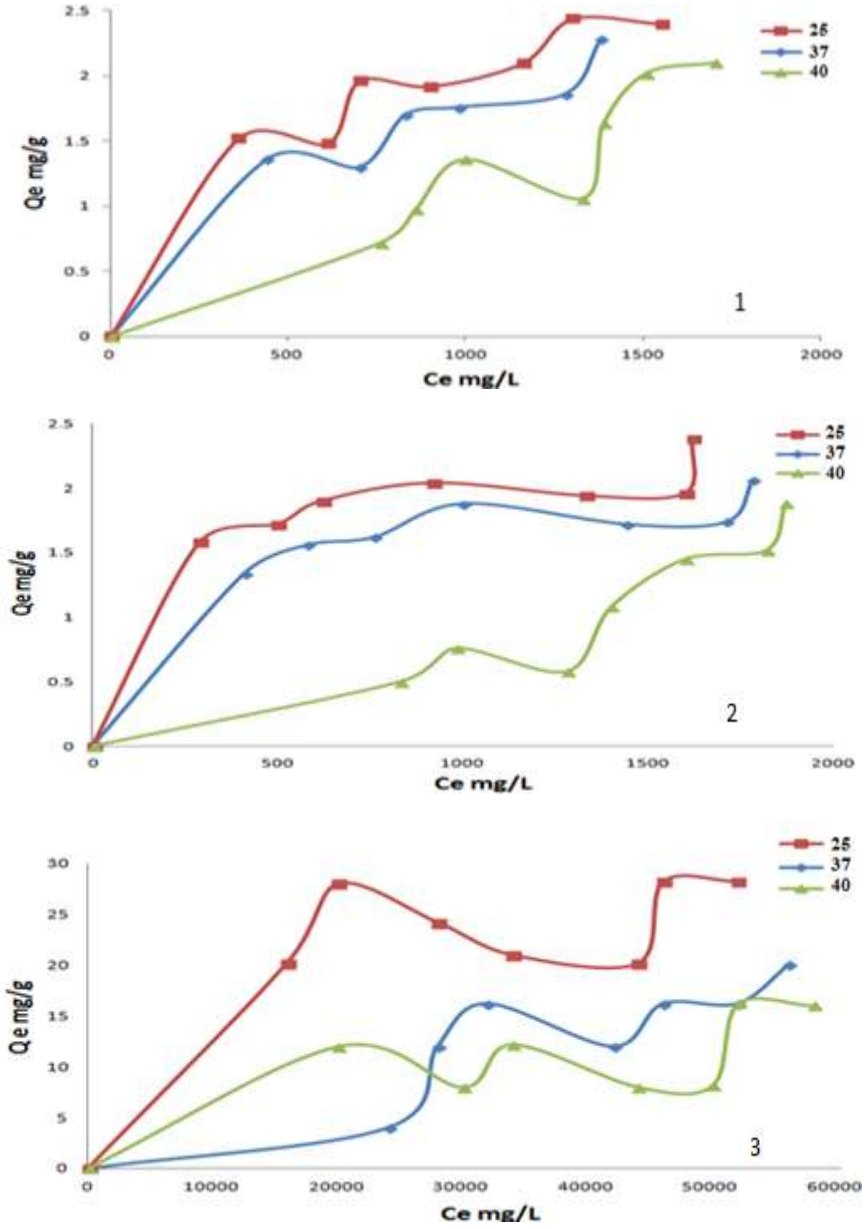

Figure 5: Show adsorption isotherms of (1=TC, 2=TG and 3=TP) on synthesisbeads surface (type C)from dilute serum at (25, 37 and 40 ^{o}C)

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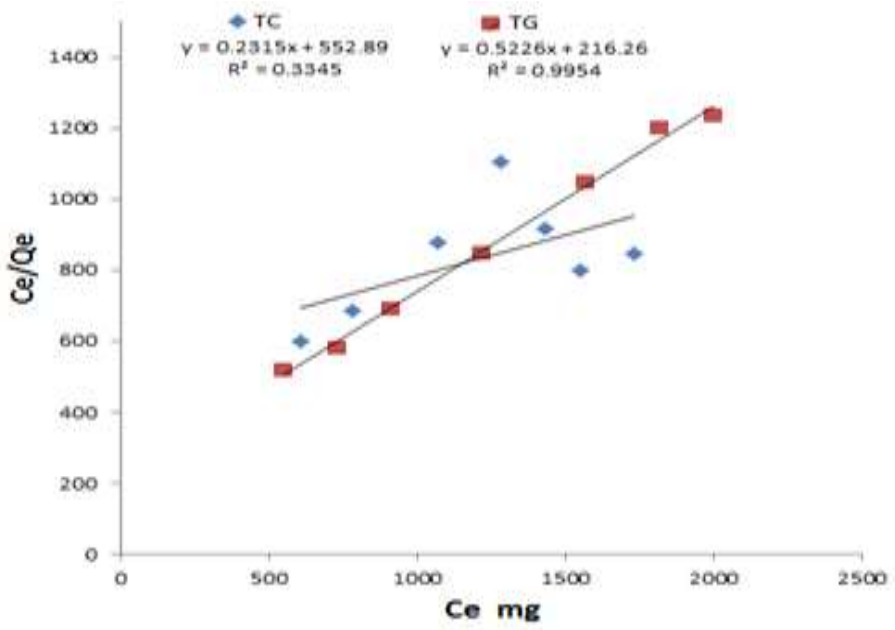
From the results given in figure (5) indicate that the amount of adsorbate(Qe) decreases by increasing the temperature[35], the amount of adsorbent (Qe) to adsorbate (TC,TG and TP) when using valine as ligand follow the order (TP> TC > TG).

3.5 Adsorption isotherms

Analysis of equilibrium data is important for developing an equation that can be used to describe the process of adsorption of (TC, TG and TP). Several isotherm equations havebeen used for the equilibrium modelling of biosorption systems. Out of these isotherm equations, two applied in this study, the Freundlich and Langmuir isotherms. The results indicated that the applicability of Freundlich isotherm for all the adsorption system under study (TC,TG, TP) as shown by the linear relationship between log *Qe* and log *Ce* and existence the constants n and *Kf*. While for the adsorption of (TG) on the modified prepared beads, it was the Langmuir model which gave the best fit line for the experimental data of the (TG) based on correlation values (R^2),as shown in table (3) and figure (6).

Table 3: isotherm parameters for adsorption (TC, TG and TP)

type of parameter	Langmuir		Freundlich		
	Qe mg/g	***R^2***	***Kf (L/mg)***	***n***	***R^2***
TC	2.04	0.334	0.002	1.113	0.829
TG	1.62	0.995	0.192	3.594	0.93
TP	20	0.356	2×10^{-7}	0.596	0.762



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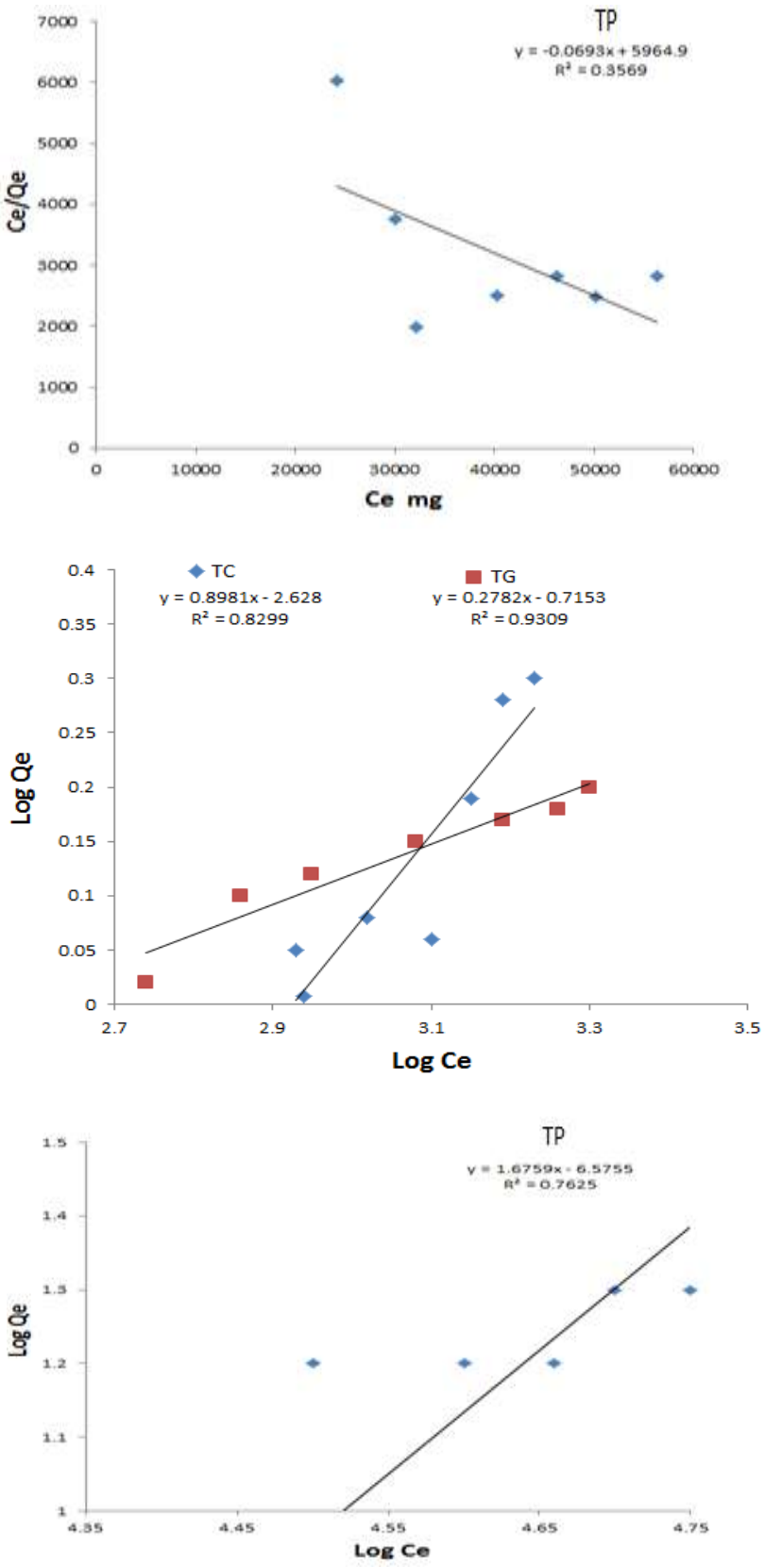

Figure 6: Langmuir and Freundlich adsorption isotherms for adsorption (TC, TG and TP)

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3.6 Effect of temperature inadsorption by modified chitosan beads surface and thermodynamic parameters

The effect of changing in temperature on adsorption of (TC, TG and TP) on prepared beads was investigated. The maximum quantities that adsorbed on prepared beads at (37C°) followed the order (TC>TG>TP). The thermodynamic functions have been were calculatedas shown in table (4) and figure (7).

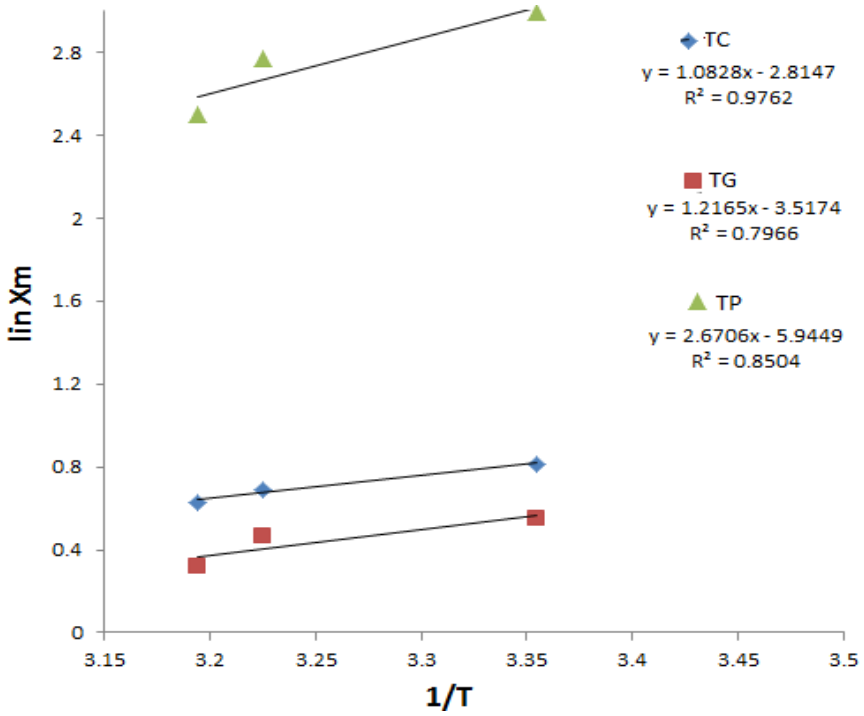

Figure 7: ($\ln X_m$) plotted against reciprocal absolute temperature for the adsorption of (TC, TG and TP)on modified chitosan beads surface

Table 4: values of thermodynamic functions for adsorption process of the (TC, TG and TP) on modified chitosan beads surface at 37°C

type of parameters	***ΔH*** **KJ.mol^{-1}**	***ΔG*** **KJ.mol^{-1}**	***ΔS*** **KJ.mol^{-1}.K^{-1}**
TC /valine beads	-8.995	-1.772	-0.0233
TG /valine beads	-10.109	-1.057	-0.0292
TP /valine beads	-22.198	-6.884	-0.0494

The adsorption of (TC, TG and TP) on the surface of prepared beads decreases by increasing the temperature and this means the interaction between the adsorbent and adsorbate does not need energy to take place. The negative value of the heat of adsorption (*ΔH*) for all adsorption system under study indicate that the interaction between these adsorbates and modified chitosan is an exothermic process[36].Through the results given, the entropy(*ΔS*)has negative values and this may due through formation of high ordered adsorbed species on the beads surface.As for

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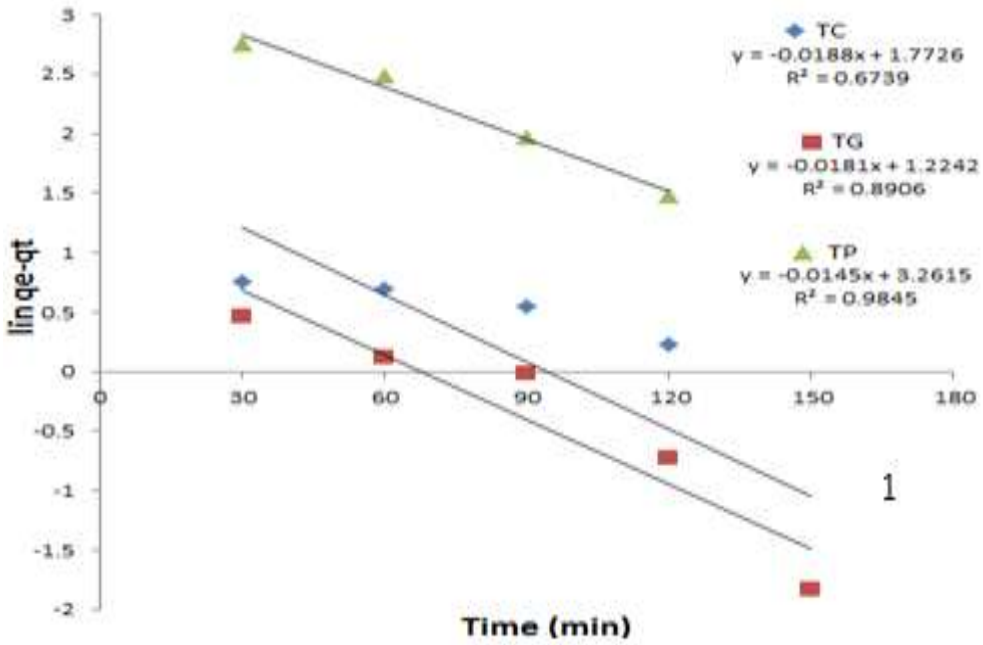
the negative value free energy change (ΔG), the adsorption process of (TC, TG and TP) on the surface of prepared beads is a spontaneous process[37].

3.7 Kinetic of Reaction

To describe adsorption kinetics of (TC,TG and TP) in the system by using pseudo first-order and pseudo second-order models,which determines the time of survival molecules that absorbed on the surface of adsorbent, so the results obtained are verified by relying on the correction coefficient (R_2)[38], and the relatively higher value of the correction coefficient indicates that the model successfully describes the adsorption kinetics for (TC,TG and TP), through the obtained results, the reactions follow the first order kinetics ($R_1^2 > R_2^2$)[39], as shown in table (5) and figure (8).

Table 5: Show kinetics Values of pseudo first-order and pseudo second-order models for a adsorption process of (TC,TG and TP) on modified chitosan beads surface at 37°C

type of parameter	pseudo first-order			pseudo second-order	
	Qe mg/g	*K(min)$^{-1}$*	R^2	*K (g/min.mg)$^{-1}$*	R^2
TC	2.16	0.018	0.673	4×10^{-6}	0.607
TG	1.74	0.018	0.890	70×10^{-7}	0.441
TP	20.2	0.014	0.984	296×10^{-8}	0.650



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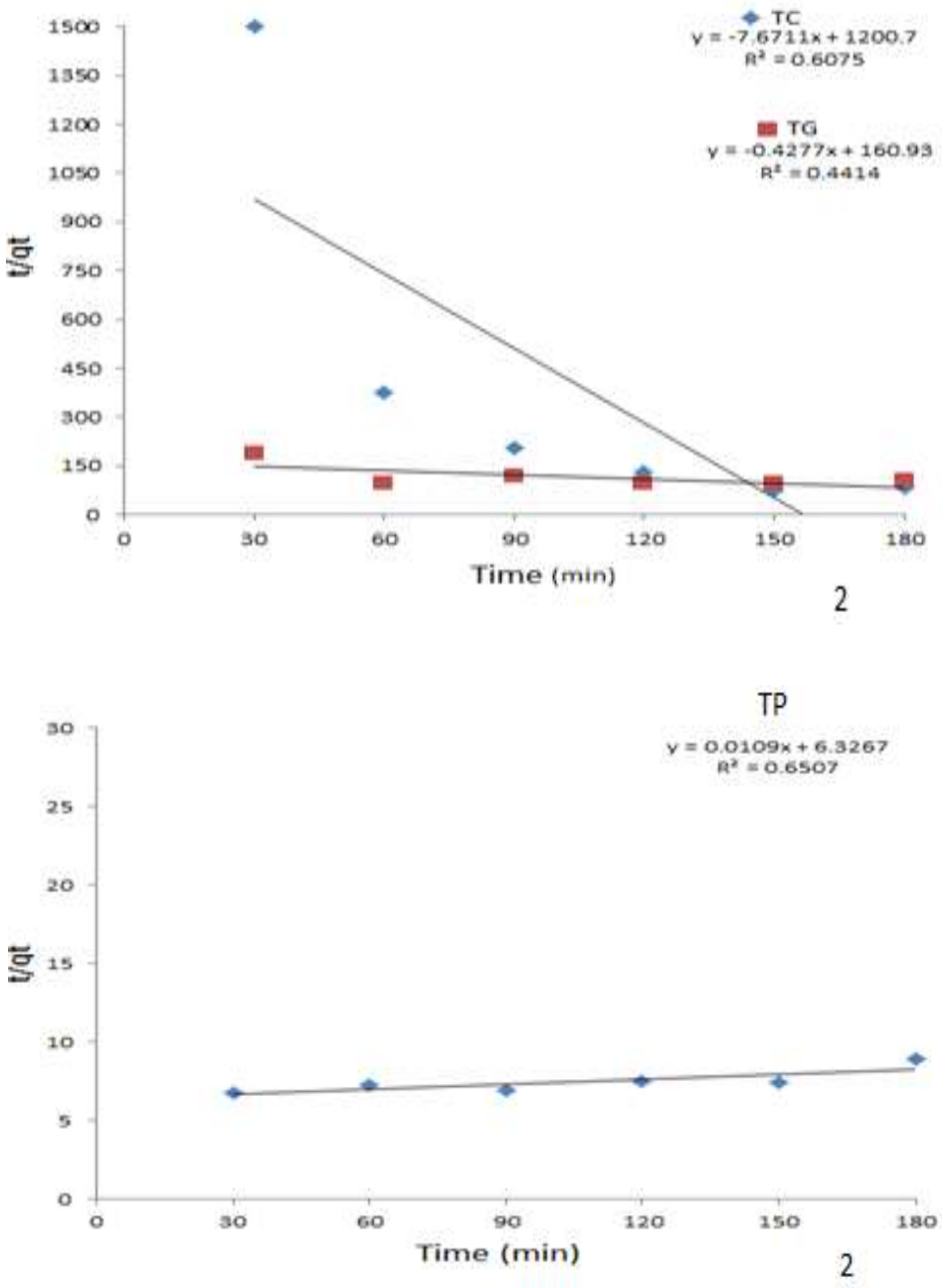

Figure 8: 1-pseudo first-order and 2-pseudo second-order for a adsorption process of (TC,TG and TP) on modified chitosan beads surface at 37°C

IV. CONCLUSION

The modified chitosan have the potential to remove (TC, TG, LDL and TP) from human serum without substantially affecting HDL, These beads were prepared by using cross linking method, these beads was activatedby EGDE as cross linker and then linked with valine to provide high adsorption capacity for the chitosan.

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