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RESEARCH ARTICLE

Adsorption Effect of the Modified Chitosan-Methionine Beads on Human Serum Lipids Profile and Total Protein

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Abstract

This research is concerned with one of the most important applications of surface chemistry which used in medical, physical and biochemistry.Place of conducting the research University, Faculty and Department. The research is based on the adsorption of lipid profile and total protein from human serum on the surface of modified chitosan- methionine which are associated with two different types of cross linkers substances: glutaraldehyde with two different concentration (3.13,6.25%) to prepared beads type (A,B) respectively, and ethylene glycol diglycidylether (EGDE) to prepared beads type (C). The formed beads were characterized by using Infrared (IR) spectroscopy to confirm the cross linking reaction and using Scanning Electron Microscopy (SEM) to describe the surface of the beads that used in this research. UV-Vis spectrophotometer was used to determine the concentration of the adsorbents before and after the treatment. As for adsorption of lipid profile (total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C) and total protein (TP)), it was observed that the adsorption of adsorbents on prepared beads type(A,B and C) followed the order (type C > type A > type B), while in case of (high density lipoprotein (HDL-C)) , it does not adsorbed or adsorbed very little in some cases on the surface of prepared beads.

Keywords: *Adsorption, Adsorbent, Chitosan, Methionine, Glutaraldehyde, Ethylene glycol diglycidyl ether, Lipid profile and total protein.*

Introduction

Polysaccharides as a type of naturalistic macromolecules, which have tendency to be bioactive material and characterized by the possibility of producing it by genetically processed in different ways by using agricultural wastes or wastes hard crustaceans. So one of them is chitosan, which is derived from chitin, and it has many applications in food, nutrition, biotechnology, materials science, pharmaceuticals, environmental protection and agriculture (Reference).

The net cationicity and the existence of various functionalities in the particle make chitosan a desirable biomolecule [1]. Chitosan are direct polysaccharides, contained two monomeric units to be specific N-acetyl-2-amino-2-deoxy-d-glucose (N-acetylated groups) and 2-amino-2-deoxy-d-glucose leftover (N-deacetylated gatherings, amino groups). Chitosan contain a few number of N-acetyl-2-amino-2-deoxy-d-glucose and consequently it is dissolvable in acidic solvents [2].

It is notable that chitosan is an extraordinary sorbent because of the rich amino (– NH2) and additionally hydroxyl (–OH) groups on chitosan chains avail in as conjunction sites [3], Because of that its importance in many applications related to the adsorption process in base catalysis, physical treatment for patients who suffer from hyperlipidemia & obesity, controlled drug release, as binding sites for bioactive molecules and CO2 capture, on the other hand it is non-toxic, inexpensive, hydrophilic, biocompatible, and biodegradable [4], so the adsorption is a standout amongst the most major procedures in a wide scope of modern physicochemical activities, for an adsorption system, adsorbent with high adsorption limit and fast adsorption kinetics is extremely basic [5].

The relationship between concentration of lipid and arteriosclerosis has been definitively diagnosed, over two decades ago many clinical trials were documented and recorded which indicated that the benefit of

reducing lipid level including (cholesterol, triglyceride and low density lipoprotein) on the heart and blood vessels (Reference). Hyperlipidemia is one of the disorders caused by several factors such as hereditary , smoking, diabetes, hypertension and bad eating habits, Increases the levels and concentration of harmful lipid in the blood lead to occurrence cardiovascular and metabolic disorders such as atherosclerosis, obesity and metabolic syndrome which lead to earlier death [6].

The identification and treatment patients who resist dietary and drug therapy is very important as it has been found that there is a significant reduction in the rates of patients and deaths[7]. Lipoprotein is a low density lipoprotein (LDL) produced in the liver, and comprises of a lipid center encompassed by apolipoprotein B100 particle connected through a single disulfide bond to a vast glycoprotein, known as apolipoprotein [8].

High lipoprotein values (≥90%) are associated with increased risk of myocardial infarction more than doubled [9], Increment in the levels of serum LDL can be quicken sedimentation of cholesterol while HDL cause the adverse cholesterol relocate and go about as hindering atherosclerosis factor [10]. The purpose of the present study is to find out the effect of using modified chitosan-methionine beads, different types and various concentration of cross linkers on adsorption percentage of lipid profile and total protein by studying its *in vitro*.

Experimental

Materials

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

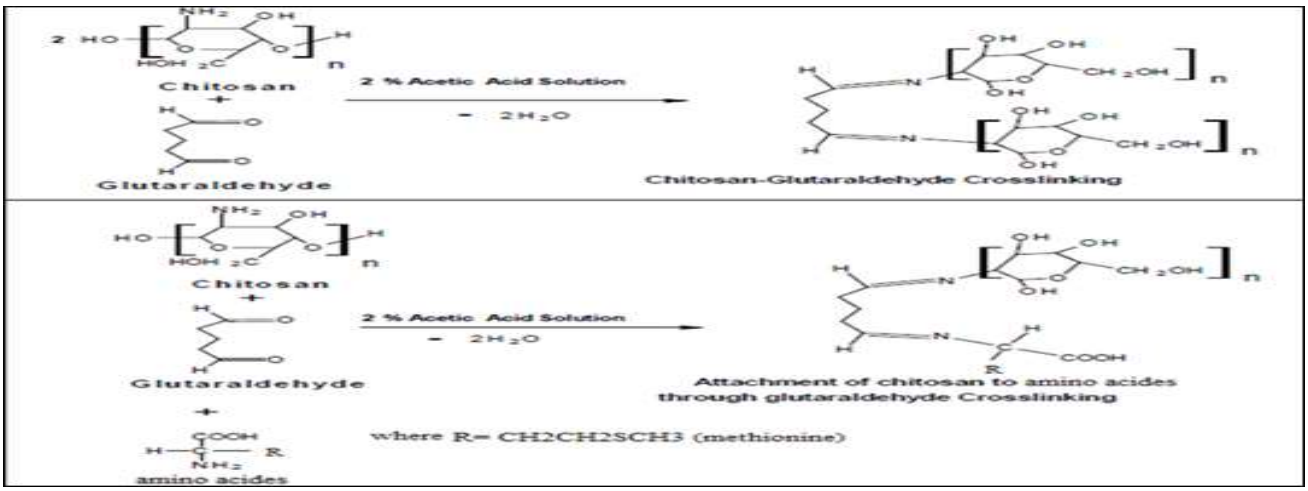
Surface Preparations (Rani M. *et al* 2011)

The pure chitosan powder was mixed with methionine; this mixture was dissolved with (2%) acetic acid, stirred for three consecutive hours at room temperature to form a homogeneous mixture. The mixture was dropped by using a syringe with diameter equal to (0.56 mm) in Noah-methanol solution (1:20 w/w) and under continuous stirrer. The formed beads were washed with hot water (50 C°) and then with cold water (25 C°) [11], the composition of the prepared beads A &B were summarized in Table (1).

Table 1: the composition of the prepared beads A&B

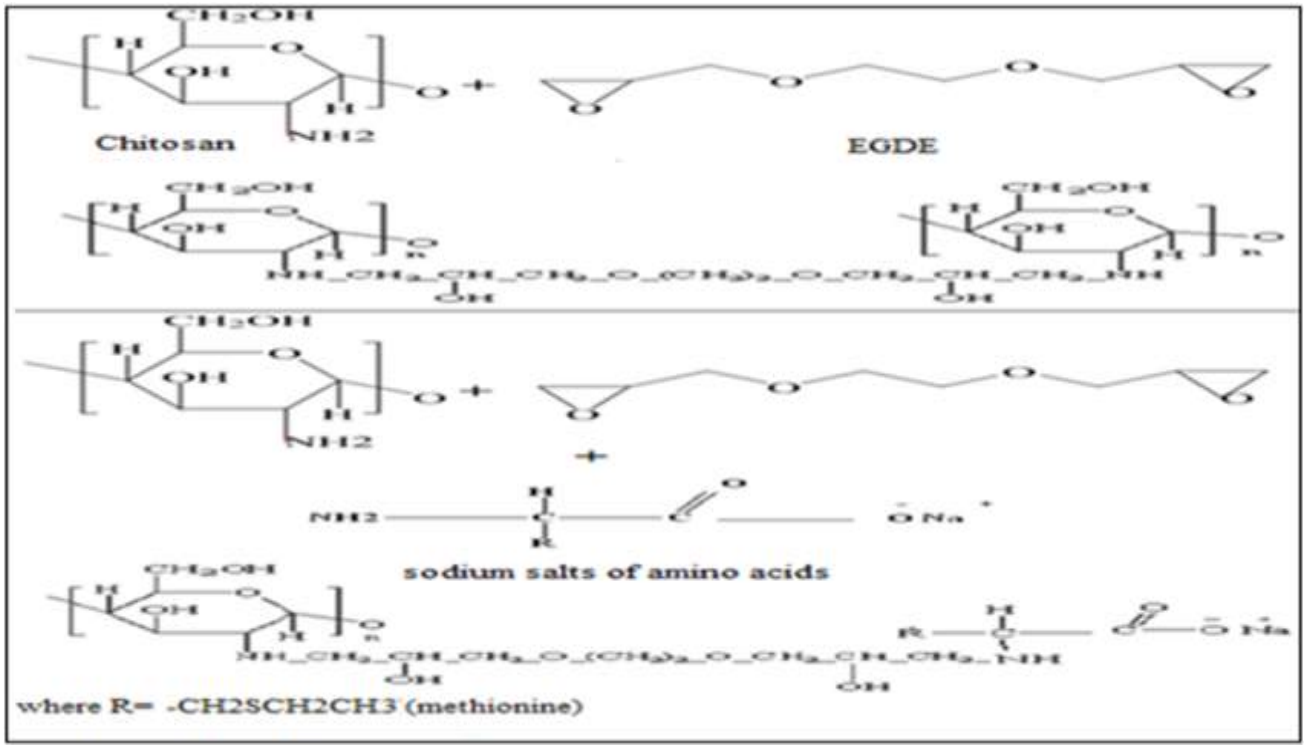
Beads Type	Chitosan (g)	Amino Acids (g)	Acetic Acids (mL)	Glutaraldehyde (%)
A	1.0	0.5	40	3.13
B	1.0	0.5	40	6.25

In order to obtain the cross linked; these beads were treated with glutaraldehyde for 10 minutes at (50 C°). Finally, the formed beads were washed with hot water (50 C°) and cold water (25 C°) respectively; the resulting beads were dried for 30 min at 55 C°, and preserved at dry and clean conditions [11]. The resulting cross linked chitosan-methionine beads commonly named interpenetrating polymer network (IPN) as shown in Scheme (1) (Reference of Scheme).



Scheme1: Show possible cross linking mechanisms of chitosan with amino acids by using glutaraldehyde to synthesis Semi-IPN beads type A&B [12]

The cross linking of Chitosan with methionine using Ethylene glycol di glycidyl ether (EGDE) - beads type (C) was done by dissolve (1g) chitosan with 19 ml of (3%) acetic acid. The formed solution of chitosan was dropped in 8% NaOH using a syringe with diameter equal to (0.56 mm). The formed chitosan beads were washed with large quantities of distilled water. To activate beads, EGDE was used to form the epoxy group, thus, (15 ml 0.6 N) of NaOH containing (30 mg) of (sodium borohydride) with (15 mL) EGDE was mixed with these beads for (8 hours) at (25C°). The formed epoxidized beads were washed with distilled water to remove non-reactive materials. To bind methionine with activated beads, (10 ml) of methionine was mixed with (5 ml 2N) of Noah to form the corresponding salt. This mixture was dissolved with 20 ml of carbonate buffer (pH 10.5), then added to the deoxidized beads for (24 hours) at (65 C°) for activation as shown in Scheme (2).



Scheme 2: Show possible cross linking mechanisms of chitosan with amino acids by using ethylene glycol diglycidylether (EGDE) to synthesis beads type C [13]

The formed beads were then washed with large amounts of distilled water and (1N NaCl) to remove carbonates and non-reactive substances. The beads were preserved for further use in a cold atmosphere and by using a solution of (0.15 NaCl) [13]

Characterization

Infrared Spectra (IR) for the Beads

The chemical structure of synthesized beads was studied by using FTIR (what do you mean FTIR) .shimadzu.8400s.

Scanning Electron Microscopy (SEM)

The shape and surface morphology of the synthesized beads were investigated by using the scanner electron microscope (SEM) Angstrom. AlS230, the images of (SEM) were taken after surrounding the beads by atmosphere of nitrogen gas and coating it with a thin layer of gold.

In vitro Adsorption Tests and Determination Adsorption Percentages

One ml of serum were taken from the patients who suffering from hyperlipidemia , added to (0.5g) of synthesized dry beads (A, B, C) each type used separately, and stirred for (3 hours) continuously by using thermostatic shaker water bath at 37C° to reach equilibrium. The concentration of adsorbents (cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, total protein) and adsorption percentage were calculated before and after treatment and during certain periods of time by using commercial test kits that supplied by (Linear, Spain). Adsorption percentage was calculated by using the following equation [13].

- Percentage of adsorption (%) = (C2 _ C1) / C1 × 100

Where C2 and C1 are the concentrations of parameter before and after adsorption (treatment) respectively.

Statistical Analysis

By using graph pad prism version 6 for all statistical analyses significance was estimated by student's t-test (unpaired). P value <0.05 were considered statistically significant.

Results and Discussion

Characterization of prepared beads

FTIR for the Beads (Fourier-transform *infrared* spectroscopy)

The chemical structure of the prepared beads that prepared by the reaction of chitosan with amino acid (methionine) and that diluted by acetic acid and cross linked with glutaraldehyde and ethylene glycol diglycidylether (EGDE) have been characterized by FTIR. In general the vibration bands of synthesized beads type (A and B) showed absorption bands (1530)cm^{-1} due to stretching and bending vibration of (C=N) bond and imine respectively, and for synthesized beads type (C) also show absorption bands (1692) cm-1 due to bending vibration of (NH) bond, and show absorption bands (1745) cm-1 due to stretching vibration of (C=O) bond, and that a good evidence that the reaction was occurred, as shown in Figure (1,2), other bands are listed in Table (2).

Table 2: Show vibration bands of prepared compounds

V (CH) Alph .	*v*(OH)	*v*(C=N)	*v*(C-O)	*v*(C=O)	*v*(C-N)	*v*(OH) bending	Others
2962,2886	3471	1530	1060	1705	1371	926	-
2955,2922	3271	-	1143	1745	1321	937	1692(NH) bending

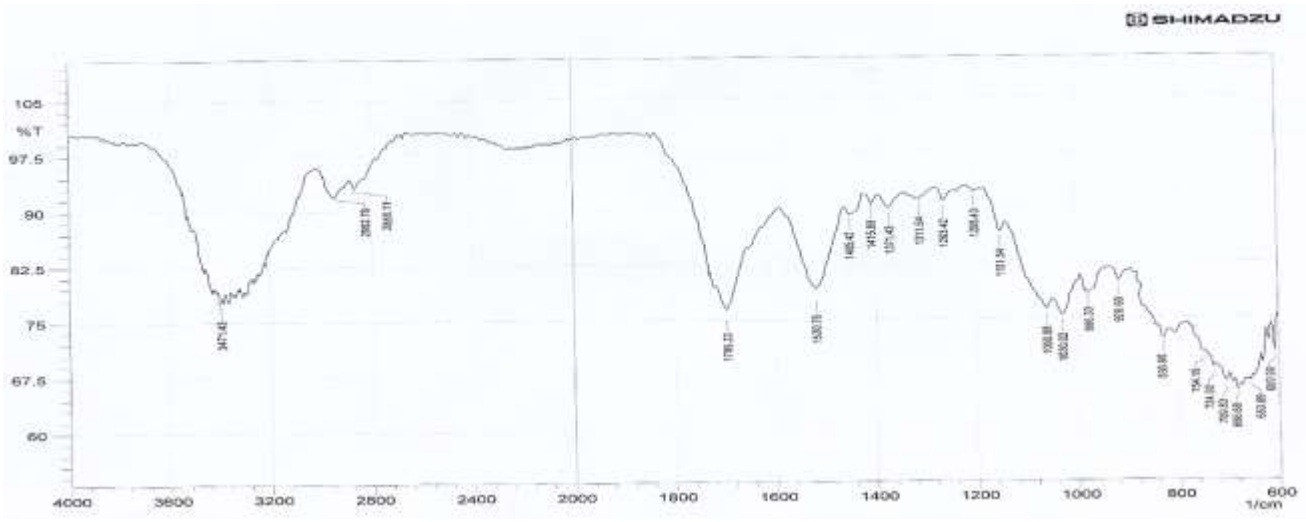


Figure 1: Vibration spectra of chitosan-methionine beads cross linking by glutaraldehyde

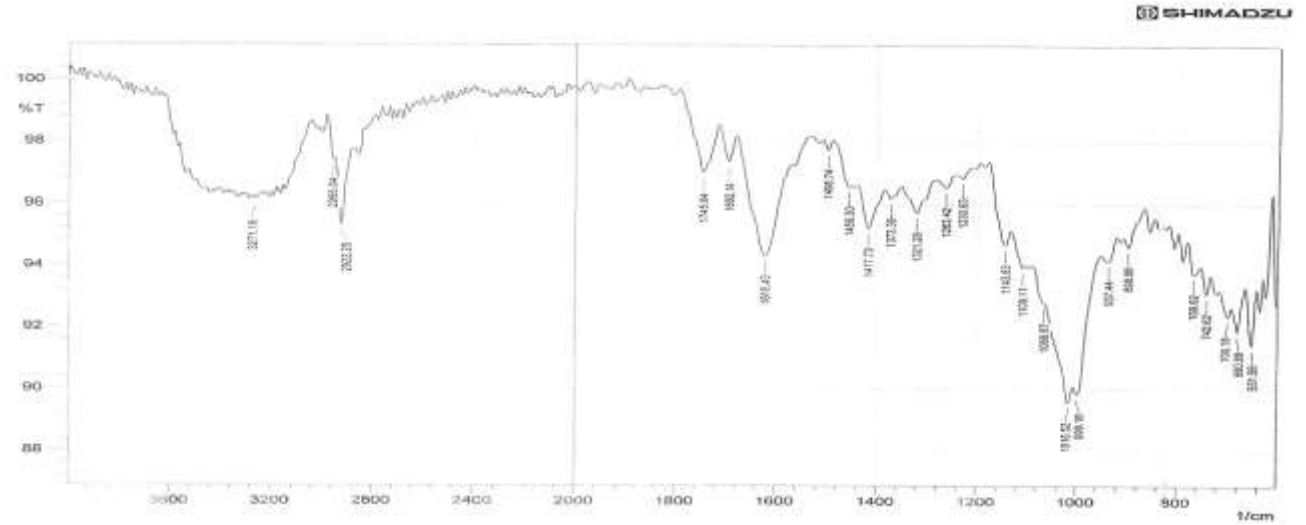


Figure 2: Vibration spectra of chitosan-methionine beads cross linking by ethylene glycol diglycidylether

Scanning Electron Microscopy analysis (SEM)

The surface morphology of prepared beads was studied by scanner electron microscope, The attached images showed that the shape of the prepared beads was spherical or oval and this is due to the difference in density between (chitosan and NaOH) solution, While the results showed that the surface of the beads was rough, curled and folded in the case of low concentration of glutaraldehyde in type (A) as shown in (P1,P2,P3), but in the case of increased concentration of glutaraldehyde in type (B), it was observed that the surface of the beads became smoother with increased concentration of glutaraldehyde which is due to the convergence chains from each other as shown in (P4,P5,P6). In the case of type (C) of beads, it is observed that the surface is more coarse and wrinkled than other type (A, B) as shown in (P7, P8, P9, p10).

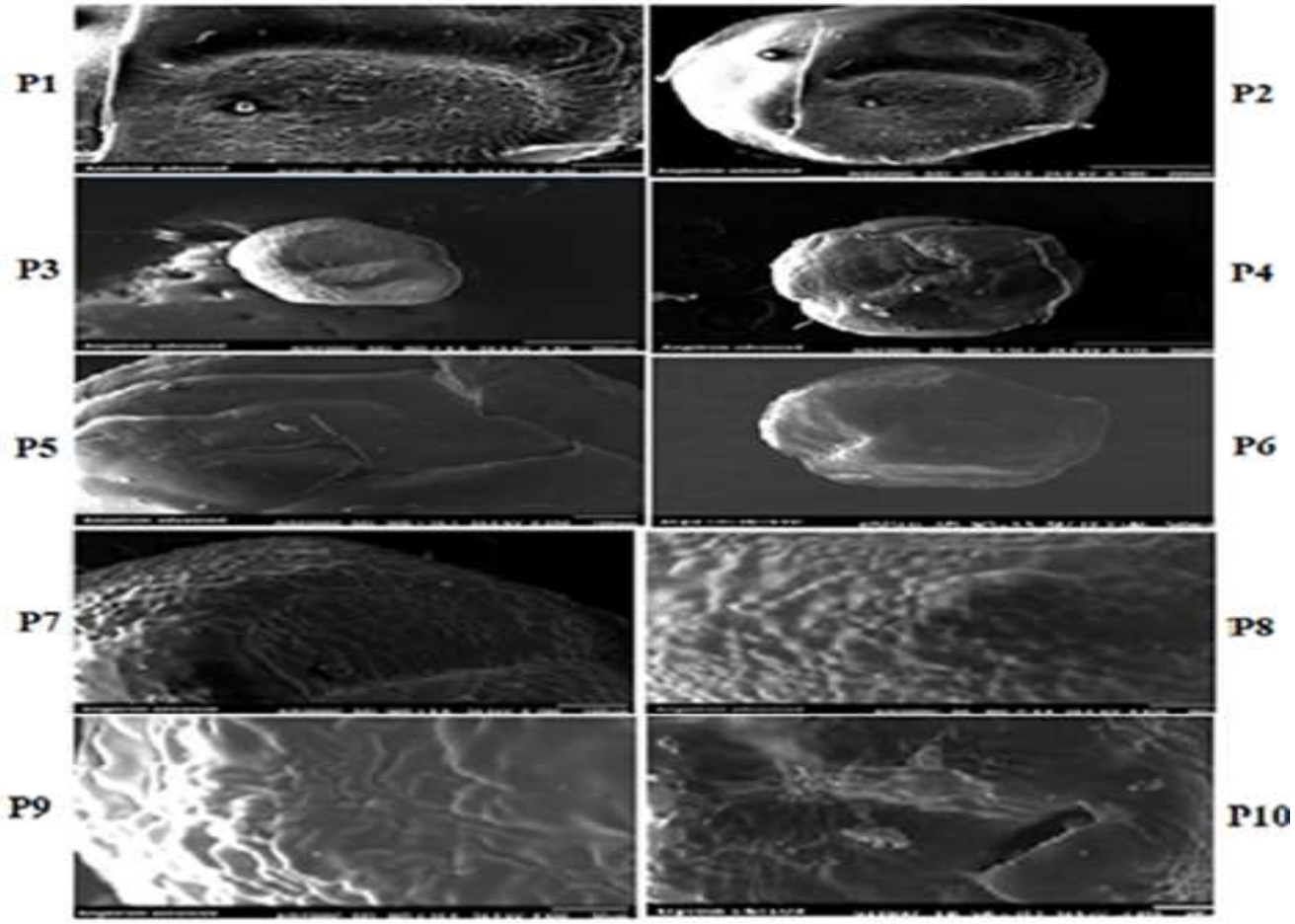


Figure 3: Show scanning Electron Microscopy (SEM) photographs of cross linked beads (p1-p10) and surface morphology

Adsorption Percentage of Lipid Profile and Total Protein

The adsorption percentages of the serum (lipid profile and total protein) were studied through using type (A, B and C) of prepared beads. In this study methionine was used as a ligand which is the most important part in adsorption process of lipid profile and total protein. This ligand may chose based on hydrophobicity, electrostatic, *Van Der Waals* interactions, and/or hydrogen bond (_NH2 and _OH) interactions [13]. It was observed through the results that type C of prepared beads was more adsorptive beads than other kinds of beads, therefore the adsorption capacity follows the following order C > A > B

Effect the Concentration of Glutaraldehyde on Adsorption Percentage of Beads (type A&B)

Different concentrations of glutaraldehyde (3.13 and 6.25%) were used as cross linker in type (A and B) of beads respectively, it was observed that the adsorption percentage decreased by increasing the concentration of glutaraldehyde as shown in Figure (4), and this may due to the strength of chemical composition of type (B) and that result of increasing the concentration of glutaraldehyde and a decrease in the number (_OH, _NH2 groups) [14].

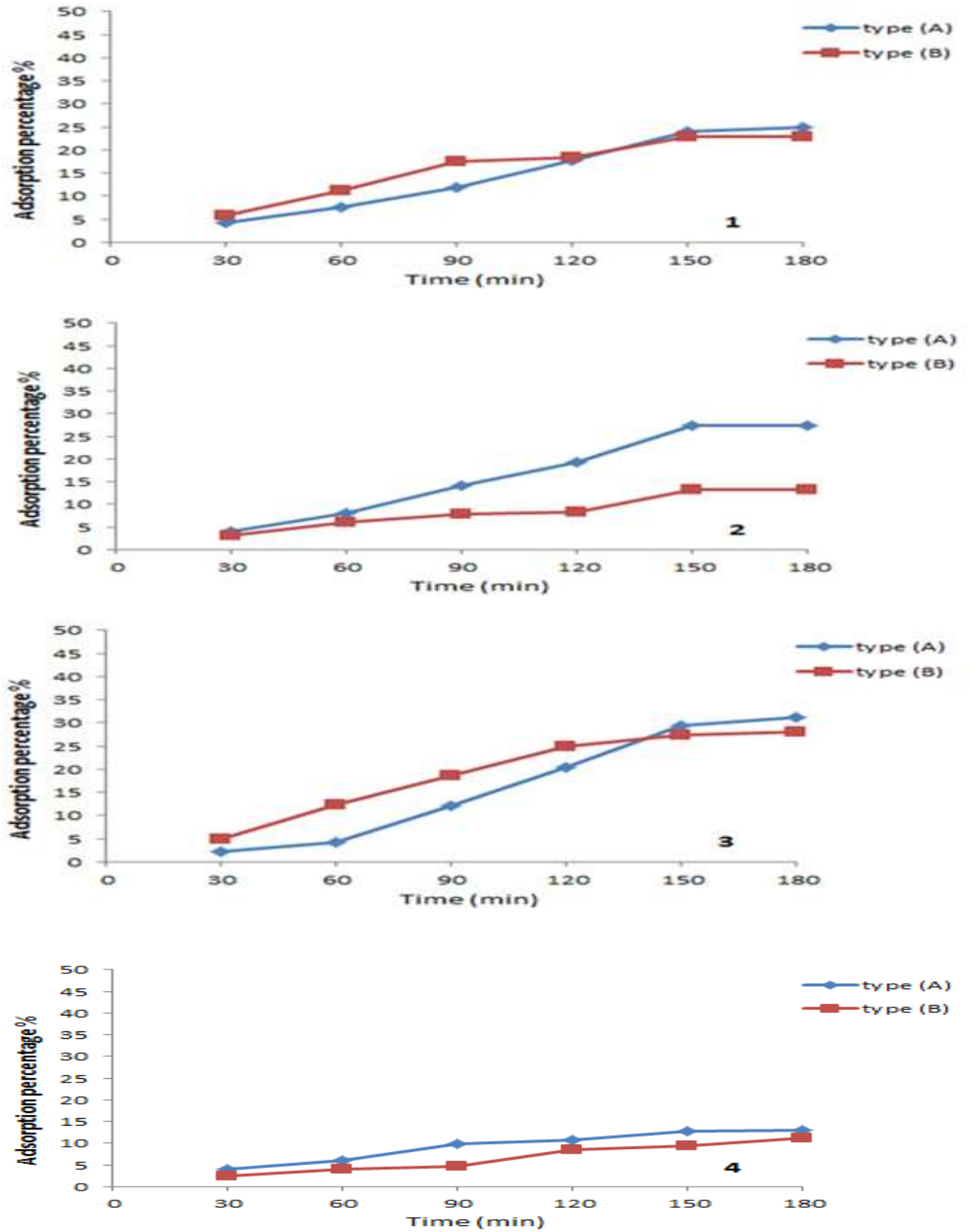


Figure 4: Show the effect of different type of beads (type A&B) (methionine) on adsorption percentage in every 30 min in 37C° for: 1= TC, 2=TG, 3=LDL, 4=TP

Effect of using Ethylene glycol diglycidylether (EGDE) on adsorption percentage in (type C)

Ethylene glycol diglycidylether (EGDE) has been used as a cross linker in type (C) of prepared beads it was observed it has a high susceptibility to adsorption of lipid profile and total protein compared with type (A, B) because of chemical composition of the beads that contain groups carrying positive and negative charges [13], which provide greater potential for adsorption through electrostatic interference [15]. This was observed through adsorption results of type (A, C) for comparison, as shown in Figure (5).

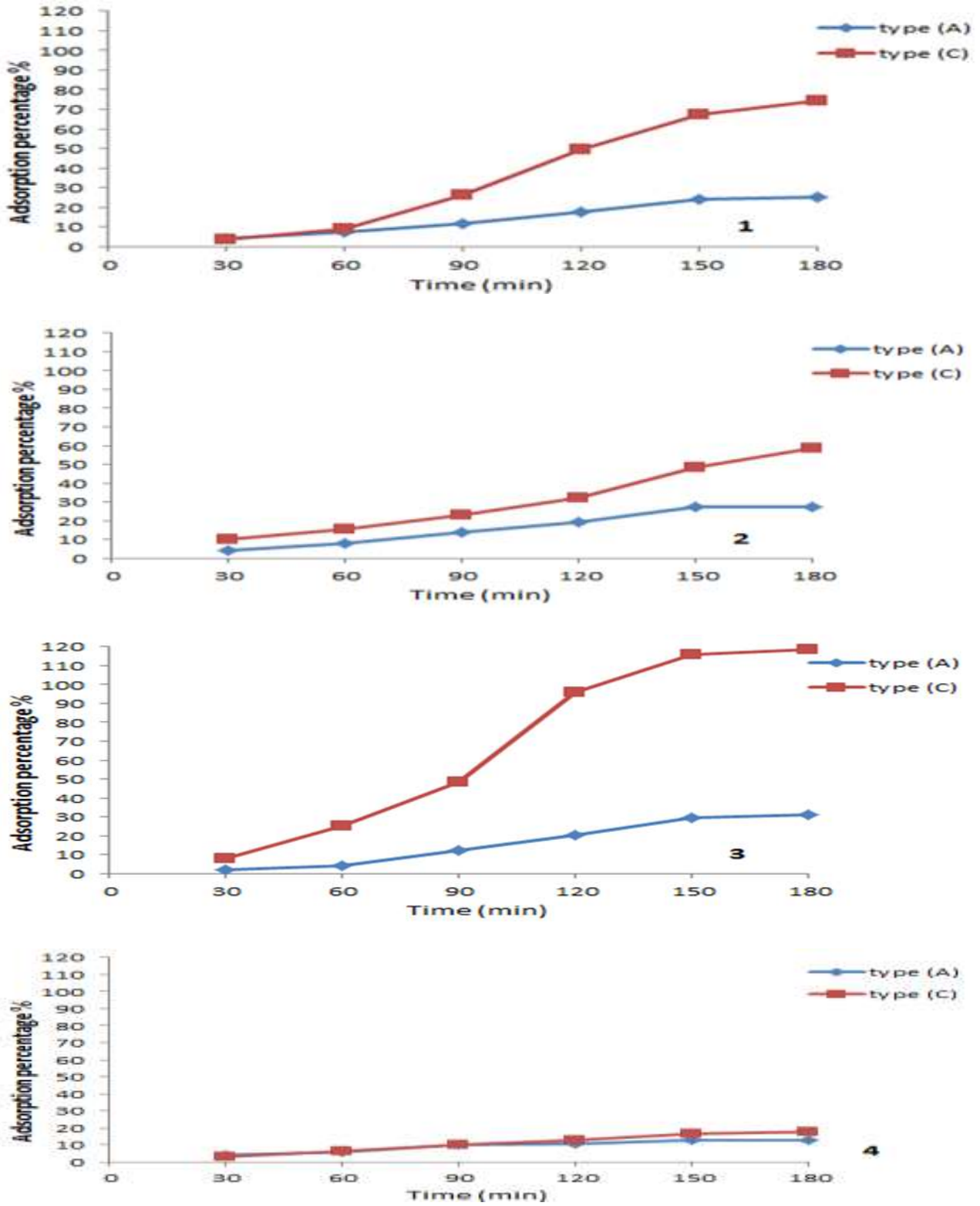


Figure 5: Show the comparison of using ethylene glycol diglycidylether (EGDE) in (type C) and using glutaraldehyde in (type A) of beads on adsorption percentage in evry 30min for : 1= TC, 2=TG , 3= LDL, 4=TP

Effect of Adsorption time on Adsorption Percentage in (type A, B and C)

It is clear from previous results that the adsorption percentage of TC, TG ,LDL-C and total protein (TP) on the surface of prepared beads type (A, B and C) gradually increases from 30 to 150 OR 180 minutes and try to stabilize after that, as shown in Figure (6). It can be seen, that HDL particles adsorbed slightly or 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 early stages of the adsorption process, after that adsorption of LDL molecules happen when adsorption continuing and this may due to competitive adsorption of LDL until equilibrium [13].

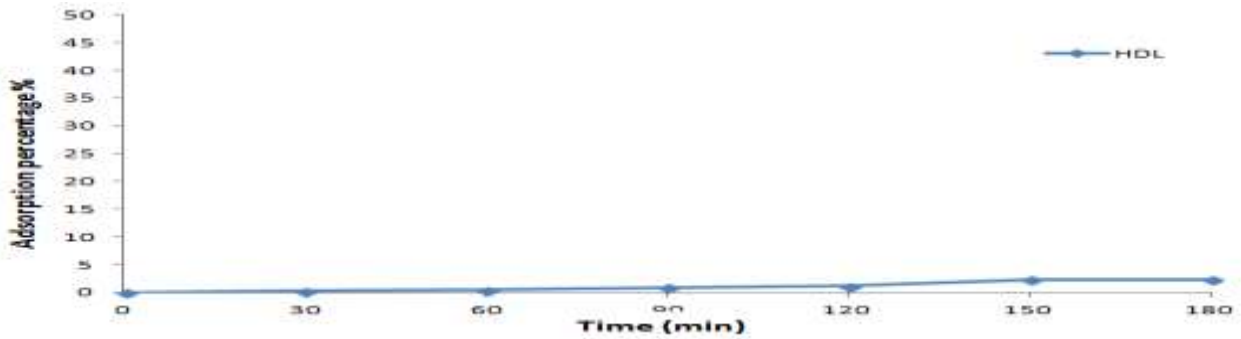


Figure 6: Show the adsorption percentage for HDL in some cases every 30min

In Vitro Study the Adsorption of Human Serum (TC, TG, HDL, S.LDL and TP) on all Type of the 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 serum "TC & TP" after adsorption process with all types of beads (A,B and C), the level of TG showed significant decrease ($p \leq 0.05$) only with beads (type C),the level of HDL showed non – significant decrease ($p \geq 0.05$) in all types of beads except with the beads (type B), finally , the level of S-VLDL showed significant decrease ($p \leq 0.05$) with all types of beads.

Table 3: Show statistical value for adsorption lipid profile and total protein on prepared beads type (A,B and C)

Type of 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
Type A	untreated	30	238.5 ± 7.98	304 ± 31.96	39.23 ± 1.807	138.4 ± 10.11	7.079 ± 0.0796
	treated	30	177.5 ± 8.058	248.3 ± 30.4	37.87 ± 1.839	90.83 ± 9.762	6.255 ± 0.0937
	P value		<0.0001*	0.2117**	0.5980**	0.0013*	<0.0001*
Type B	untreated	30	221.1 ± 10.09	244.5 ± 18.91	43.53 ± 2.05	128.3 ± 9.504	6.82 ± 0.07306
	treated	30	169.1 ± 9.63	200.1 ± 18.03	36.27 ± 1.635	102.4 ± 8.449	6.243 ± 0.07108
	P value		0.0011*	0.0944**	0.0075*	0.0460*	<0.0001*
Type C	untreated	30	235.2 ± 7.652	311.4 ± 34.01	37.8 ± 1.306	135.5 ± 9.984	6.894 ± 0.07711
	treated	30	136.6 ± 6.916	237 ± 45.55	38 ± 1.273	55.97 ± 7.964	5.891 ± 0.0873
	P value		<0.0001*	0.4379*	0.9130**	<0.0001*	<0.0001*

The results above indicated that all types of adsorbent which used in this study could remove TC, TG,SLDL and TP from human serum without substantially affecting HDL. In similar study by Ning-Ning Cao YU, et al [16], they synthesized cholesterol-modified dextran-agar bead and studied it's *in vitro* effect as adsorption, they found that it could remove TC, LDL, TG and albumin from human plasma, and they found that the adsorbent has a higher selectivity for LDL than HDL.

Handan Yavuz YU, et al [17], prepared adsorbent using a modified polymerization of suspension of poly (2-hydroxyethyl methacrylate-methacryloyl amidophenyl alanine) beads that attached with anti-low density lipoprotein antibody (anti-LDL antibody), and studied it's *in vitro* effect as adsorption, they found that it could remove TC from human plasma .

Conclusion

The modified chitosan beads were prepared by using cross linking technique, these beads was activated with two different activators (glutaraldehyde to prepare type (A&B) and EGDE to prepare type (C)) to obtain the beads with good adsorption potential and these activated beads was bonded with methionine.

The adsorbent beads showed good adsorption capacity for (TC, TG, LDL-C and TP) without substantially affecting HDL in general. It was found that type (C) of synthesized beads has a higher adsorption potential than other kinds type (A and B), as for the beads type (A and B) that activated with different concentration of glutaraldehyde the adsorption percentage increases with decreasing the concentration of glutaraldehyde, therefore the adsorption percentage followed order (C>A>B).

It was found that sufficient time needed to complete the adsorption process for (TC, TG, LDL-C and TP) on the synthesized beads was 3 hours. 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," after adsorbed with all types of beads (A,B and C), finally the level of HDL showed non – significant decrease ($p \geq 0.05$) in all types of beads.

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