Adsorption of Some Blood Components on Bentonite

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Abstract

Different weights at (10, 20, 30,40,50,60, and 70milligrams) of bentonite clay were incubated with 1milliliters of serum, mixed vigorously at room temperature. Serum total protein (STP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were estimated before and after adsorption. Desorption processes were carried out by mixing one milliliter of distilled water with the clay that produced from adsorption process and measurement of the substances that released to the water.

Albumin showed the highest adsorption capability followed by STP, ALP, ALT, and AST. The desorption process revealed that there are more than one half of the quantities adsorbed on bentonite were able to be eluted by distilled water.

In conclusion, the bentonite clay has the ability to adsorb and extract of some blood component from human serum.

Keywords: Serum total protein (STP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), adsorption, desorption, bentonite.

الخلاصة

أخذت أوزان مختلفة من طين البنتونايت (10, 20, 30, 40, 50, 60, 70ملليغرام) ورجت مع واحد ملليلتر من مصل الدم عند درجة حرارة الغرفة. تم قياس تراكيز مكونات الدم الآتية قبل وبعد الامتزاز: بروتين الدم الكلي STP, الألبومين, أنزيم الانين امينوترانسفريزALT , أنزيم اسبارتيت امينوترانسفريز AST, أنزيم الفوسفاتيز القاعديALP . كما أجريت دراسة لمعرفة كمية المادة المستخلصة وذلك بمزج الطين الناتج من عملية الامتزاز مع واحد ملليلتر من الماء المقطر وقياس كمية المواد المتحررة من الطين الى الماء.

اظهر الألبومين أعلى قابلية على الامتزاز على سطح طين البنتونايت يليه ALT, ALP, STP ثم AST. في تجارب الامتزاز ظهر أن أكثر من نصف كمية المواد الممتزة تتحرر الى الماء من سطح الطين.

يمكن الاستنتاج ان لطين البنتونايت القدرة على امتزاز واستخلاص بعض المكونات الحيوية من مصل دم الانسان.

مفاتيح الكلمات: بروتين الدم الكليSTP , الألبومين, أنزيم الانين امينوترانسفريزALT , أنزيم اسبارتيت امينوترانسفريزAST , أنزيم الفوسفاتيز القاعديALP ، طين البنتونايت، الامتزاز، الابتزاز.

Introduction

The extraction of certain substances from solution on solids is one of the cheapest and easiest separation methods. It depends mainly on the adsorption phenomenon and some time this fact depends on the affinity of different substances towards certain substance that called adsorbent. Solids have the property of holding molecules at their surfaces either from the gas phase or from solution; this property is quite marked in the case of porous and finely divided materials [(1)]. The term adsorption refers to the accumulation of atoms, ions or molecules (adsorbate) on a surface of a solid substance (adsorbent) [(2)]. The adsorption of some blood components (mainly proteins) have been studied extensively for extraction purposes, compatibility studies, and other biological research fields. Auditore *et al* (2002) [(3)] reported the study of the adsorption behavior of a model protein such as human serum albumin (HSA) onto surfaces of silicon carbide and carbon thin films. It has been found that HSA tends to preferentially adsorb on Si-rich surfaces. Plasma protein adsorption onto an artificial surface is strongly influenced by the surface characteristics of materials and the fluid dynamics inside the blood pump, and it would influence subsequent platelet adhesion or activation, Polyurethane blood pumps displayed different degrees of protein adsorption and subsequent platelet adhesion on each segment [(4)].

The medical significance of some active surface materials arises from their high adsorption capability. One of the uses of these active substances is in the utilization of it as a drug carrier [(5)]. The most important application of these materials in medicine is their use as physical antidotes in the treatment of acute poisoning by toxic substances and drug over dosages [(6-7)]. Previous works showed a usefulness of bentonite as active adsorbent in medical field as antidote [(8-9)]. Bentonite used in the adsorption and as a drug carrier [(10)] and also .showed an adsorption ability of bacteria[(11)]

The results of Clark and Macias (1995)[(12)]demonstrated electrostatic membrane-protein interactions may influence the kinetics of both the adsorption and transmembrane mass transfer of plasma proteins on Hydrophobic, anionic polyacrylonitrile (PAN) and hydrophilic, uncharged cellulose triacetate (CT) membrane fragments.

Albumin (Alb) and fibrinogen adsorption from single protein solutions to the plasma-coated filters was measured by Morra (1995) [(13)]. Results illustrate the marked effects of the deposition condition on the surface composition, the surface field of forces, and the protein adsorption behavior [(13)]. One of the important application of adsorption of serum protein on diefferent surfaces is that; protein adsorption on the dialyser membrane seems to modulate the bioincompatibility parameters in a different way [(14)].

Other factors also previously studied like the effects of temperature, chemical denaturation, time of

incubation, compatibility, and protein adsorption on different surfaces [15-17]. There are also some reports on the mutual effect of adsorption of some components on the adsorption of other serum components [18].

Apparently, the structures of the attached BSA layer on the biomaterial particles play a significant role. Also, the association or equilibrium constant of the adsorption of BSA were determined and represented the isotherm curves in function of the zeta potential measurements [19].

The effects of protein size on the adsorption capacity and rate is determined for an acrylamido-based polymeric anion-exchanger. The homogeneous diffusion model was found to predict the experimentally observed trends with respect to protein concentration and boundary layer mass transfer effect [20].

It is believed that the antibody-antigen reaction technology that started with adsorption process may be useful in developing immunosensors for a variety of applications [21]. In this work, an attempt was carried out to use bentonite clay as a cheep and available adsorbent to extract different blood proteins (Serum total protein, albumin, aspartate aminotransferase (AST) (EC2.6.1.1), alanine aminotransferase (ALT) (EC2.6.1.2), and alkaline phosphatase (ALP) (EC3.1.3.1) from human serum.

Materials & Methods

(a) Clay Treatment:

The bentonite clay was collected from an open mine in Trifawi area in the western of Iraq and classified as bentonite contains about (75%) of its weight montmorillonite mineral. The analysis showed the chemical components of the bentonite expressed as weight per weight ratios are (SiO_2=54.66%, Al_2O_3=14.65%, MgO=6%, Fe_2O_3=4.88%, CaO=4.77%, SO_3= 1.2%, Na_2O=0.65%, and Loss On Ignition=12.56% in addition to other rare ions that were not analyzed. The clay was washed with excessive amounts of distilled water to remove any soluble materials, filtered and dried at 160° C for three hours and kept in an airtight container. The clay was grinded and sieved to a particle size of 75μm and then used in all adsorption experiments.

(b) Adsorption process:

Different weights (10, 20, 30, 40, 50, 60, and 70milligrams) of bentonite clay were incubated with 1milliliters of serum, mixed vigorously at room temperature. After the determined time elapsed the serum that separated from the suspension by centrifuge at 3000g for 20 minutes to assure complete precipitation of bentonite.

Serum total protein measured according to Biuret method as descried by {Henry 1974} [21A], albumin was measured by Bromocresol Green (BCG) method depending on the procedure of Iraqi Sera and Vaccines Institution kit}, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured colorimetrically according to Reitman and Frankel (1974) method [21B], and alkaline phosphatase (ALP) was measured colorimetrically depending on the manual of Sigma® kit [21C]. These measurements were carried out routinely in the clinical laboratories of the hospital. The measurement occurred before adsorption and after one hour of incubation with bentonite.

Serum concentration of each component before adsorption is found to be S.T.protein=74 g/L, S.albumin=39.4g/L, S.AST=14.8U/L, S.ALT=11.9 U/L, and S.ALP=9.6 KAU/L. The quantity of the substance that adsorbed on bentonite obtained from the difference in the concentration before and after adsorption on bentonite.

(c) Desorption: To obtain the quantity of the measured blood components that have the ability to release from the clay surface into the solution (desorption) the following procedure were followed: One milliliter of distilled water was added to the clay precipitated after adsorption process. The mixture then mixed for one hour at room temperature, centrifuged at 3000g for 20 minutes, and the blood components (Serum total protein, albumin, (AST), (ALT), and (ALP) were measured by the routine methods. The percentages of desorption were obtained by division of the quantity in solution after desorption by the quantity adsorbed on the same weight of the clay.

Results and Discussion:

The concentrations of different serum components after incubation with different amounts of bentonite after adsorption process are presented in Table (1). The results showed a decrease in the concentration of all the biochemical parameters. Albumin showed the highest adsorption capability for bentonite surface among the other types of serum protein Figure (1). These findings were noticed in many other adsorption studies for blood components on different surfaces [22-26]. Johnson (1995) [27] noticed that, during adsorption, some arrangement of functional groups on the protein (e.g. charged or hydrophobic amino-acid residues or specific ligands binding sites) interacts with complementary sites distributed on the adsorbent surface. The protein will show the highest affinity for the surface arrangements which best match its own distribution of functional sites, resulting in a distribution of binding energies[27]. The stability in the quantity adsorbed with increasing the weight of bentonite to a certain limit can be explained in the other concept of negative adsorption i.e. the clay adsorb and absorb water from the serum fluid and hence compensate the quantities adsorbed by concentration factor. Other types of proteins adsorption (Figure (2, 3, and 4)) showed a continuous increase in adsorption quantities with increasing bentonite weights except AST (Figure (5)). This behavior is probable and due mainly to the increase in the surface area of clay with increasing bentonite clay in serum fluid and high affinity of these proteins to the bentonite active sites.

Interestingly, a polymeric film selectively adsorbed albumin when compared with gamma-globulin and fibrinogen, suggesting that a selective albumin adsorption on the film is responsible for the suppression of platelet adhesion [28].

Fibrinogen, immunoglobulin G, and albumin were the predominant proteins identified on the polydimethylsiloxane surfaces with other proteins adsorbing at intermediate levels [29]. These researches confirmed our suggestion that the decrease in albumin concentration after incubation with bentonite is due mainly to the adsorption process.

Table (1): The concentrations of different serum components after incubation with different amounts of bentonite after complete of the adsorption process.

Weight of Bentonite (mg)	S.GOT (U/L)	S.GPT (U/L)	ALK (KAU/L)	S.T.PR (g/L)	S.Alb (g/L)
10	14.3	10.8	8.6	69	35.6
20	13.8	10.7	8.4	66	31.2
30	10.88	9.6	6.65	65.3	29.9
40	9.65	7.3	7.3	67	18.17
50	8.75	6.5	6.9	56	17.6
60	6.5	.6.7	6.7	53.9	18.3
70	8.1	6.4	5.8	49.7	16.32

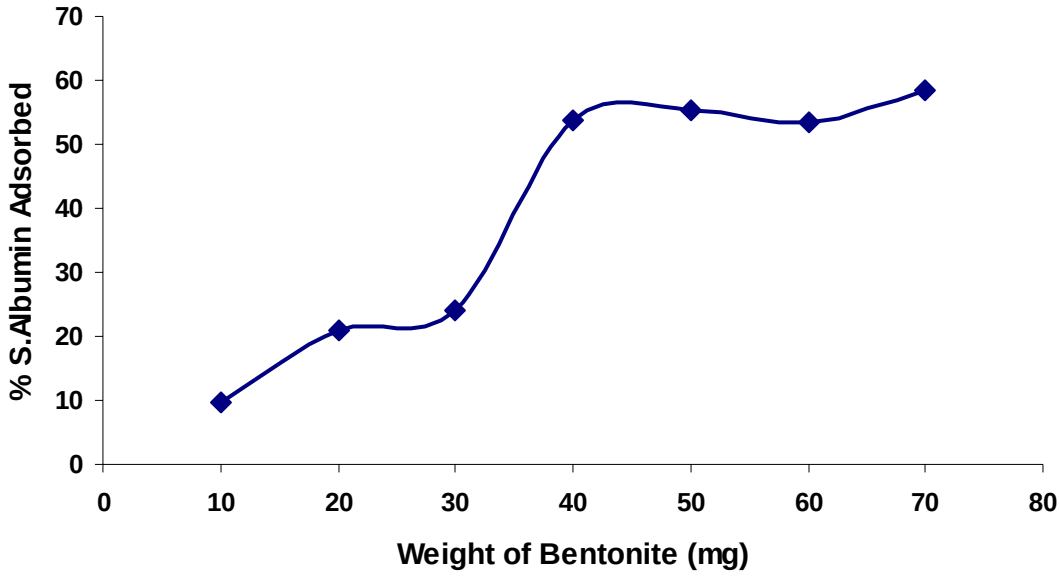

Figure (1): The Percentages of the amount of serum albumin adsorbed from the total albumin concentration on different weights of bentonite.

The adsorbed protein conformation may affect and changed after adsorption on different types of surfaces [30]. Hence it can be suggest that the shape of the adsorbed protein has the important effect on the subsequent adsorption to form multilayer adsorbate.

The adsorption kinetics of BSA and gamma-globulin on porous anion-exchange adsorbent can be very well fitted by parallel diffusion model, because the model reflects correctly the intra-particle mass transfer mechanism. In addition, for both the favorably bound proteins, the pore diffusion model fits the adsorption kinetics reasonably well. The results here indicated that the pore diffusion model can be used as a good approximate to depict protein adsorption kinetics [31-32].

The interlayer spacing of montmorillonite is very dependent on the external ionic strength [33]. Phenomenologically, strong water adsorption on surface is repressed by the addition of an electrolyte. Hence, replacing the chlorpromazine HCl molecules the water molecules on the bentonite surface active sites might occur.

In an interesting research that reflects the importance of adsorption process; the highly efficient activated carbons, with clinically feasible acidification of plasma, can remove strongly albumin-bound uraemic toxins [34].

The result of Kawamoto (1998) concluded that the excellent blood-contacting properties of polypropylene surfaces can be achieved by reducing the macromolecular entanglement and the hydrophobic interaction with proteins [35]. It was found that qualitative analysis disclosed that most of the adsorbed protein was albumin [36-37]. These results are agreed with the finding of our research.

One of the applications of the adsorption results is that, the enhanced albumin adsorption to a surface may improve surface thromboresistance [38]. Bentonite from this view may be expressed as a thromboresistant substance even this results needs more confirmation.

Experimental data show that serum albumin adsorption on anion-exchanger is endothermic for both systems which suggest that the process is entropically driven [39]. Furthermore Ishihara (1998) concluded that fewer proteins are adsorbed and their original conformation is not changed on polymer surfaces that possess a high free water fraction [40]. These findings support our results because the bentonite clay has an anionic surface and has possesses high amount of free water. Therefore the same enterpretation applied for the adsorption of blood protein on its surface.

Many researchers found unexpected factors that may affect adsorption of protein on adsorbent. Sunny and Sharma (1992) [41] noticed that the presence of pineapple juice increases the adsorption of albumin and reduces the adhesion of platelets on poly(etherurethane urea) surfaces. Hence it can be started (for the future work) to add different amount of substances to the adsorption system of our work and studied the possible effects on the adsorption process.

Other possible application of this work also is immobilization of other proteolytic enzymes on different adsorbents (supports) for analysis applications as shown in other research [42].

Extraction of some enzymes may be carried out by adsorption. Adsorption of several crude and purified cellulases on indigo particles and Avicel cellulose was studied. Much higher amounts of protein were bound to indigo than to cellulose under similar conditions. However, in general, the enzyme adsorption on indigo was less specific than the adsorption on cellulose [44]. From the result of this research bentonite can be used as a cheap adsorbent for extraction of the studied enzymes (AST, ALT, and ALP) by the same simple technique (adsorption) as noticed from the good adsorption ability (Figure 3,4,and 5)

Adsorption of other phosphatase enzyme (acid phosphatase) on different clays; goethite, kaolinite and two colloids from the soils in the presence of organic acids and phosphate was studied. This observation suggested that the impact of anions on enzyme adsorption varies with anionic type and the surface characteristics of soil

components [45]. Therefore the effect of other anions in the serum should be considered and some of the result of our work may be due to the possible effect of these anions (e.g. Phosphate, Chloride, bicarbonate…etc) on the adsorption capacity of the bentonite surface.

Table (2) showed the percentages of desorption of the measured blood biochemical parameters at different weight of clay. In general, it can be concluded that more than fifty percent of the adsorbed substances were releasable from the surface of clay. This may be due to different adsorption mechanisms and different forces included in the connection between the biochemical substances and the active sites of the clay surface and the weakest forces are more susceptible for breaking. Hence this method can be used to concentrate and extract these substances from the blood as an adjuvant tool to the electrophoresis or chromatography which are more expensive. The introduced method can be developed and enhanced to obtain good separation of these blood components.

Table (2): Desorption of different serum components from the bentonite surface after adsorption process.

Weight of the clay that the adsorbed blood component released from	% GOT released	%GPT released	%STP released	%ALP released	%Alb released
50	60.9	64.1	95.1	52.8	54.5
60	50.4	56.7	88.3	57.5	73.8
70	73.3	91.7	62.9	68.7	61.9

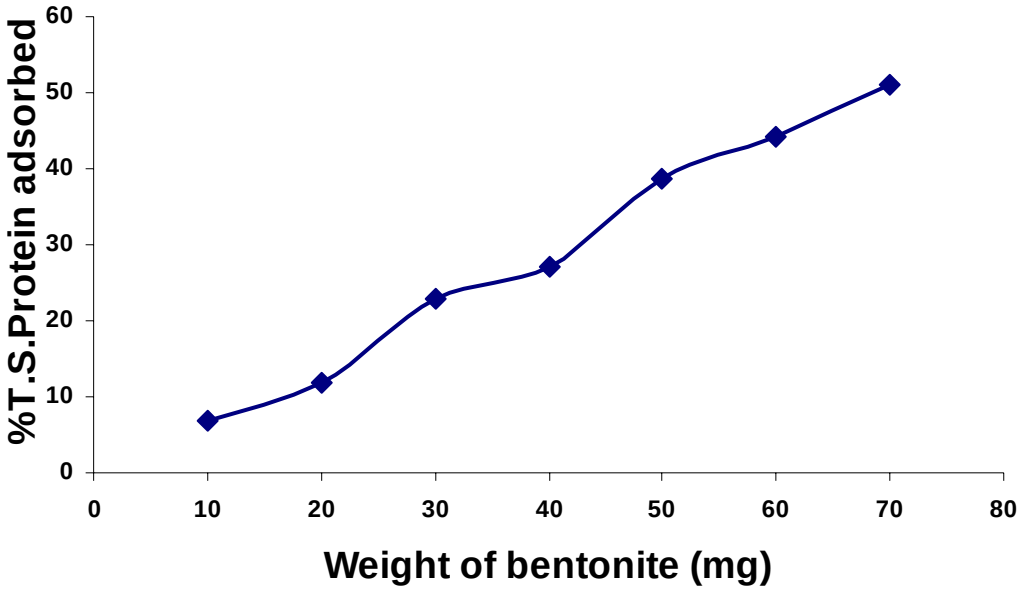

Figure (2): The percentages of the amount of total serum protein adsorbed from the total serum protein concentration on different weights of bentonite.

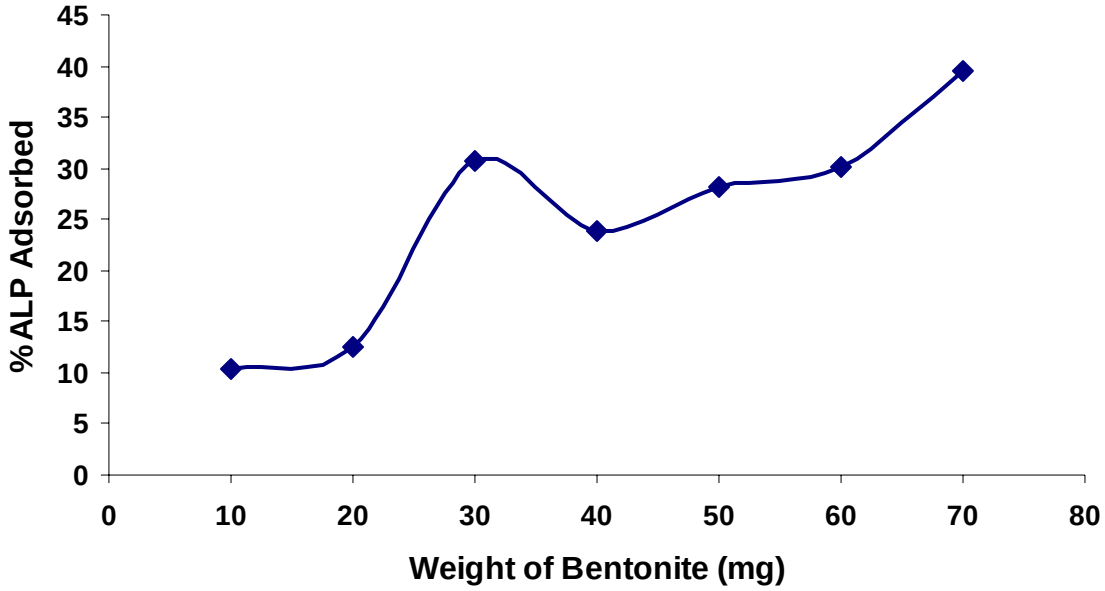

Figure (3): The percentages of the amount of serum alkaline phosphatase adsorbed from the total serum alkaline phosphatase concentration on different weights of bentonite.

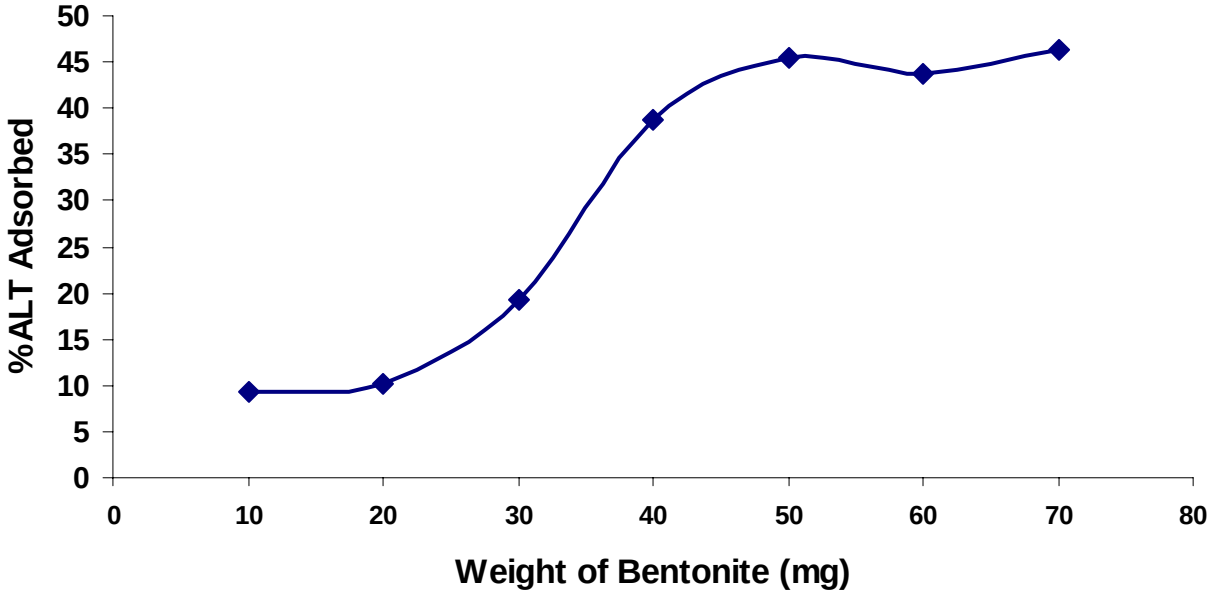

Figure (4): The percentages of the amount of serum alanine aminotransferase adsorbed from the total serum alanine aminotransferase concentration on different weights of bentonite.

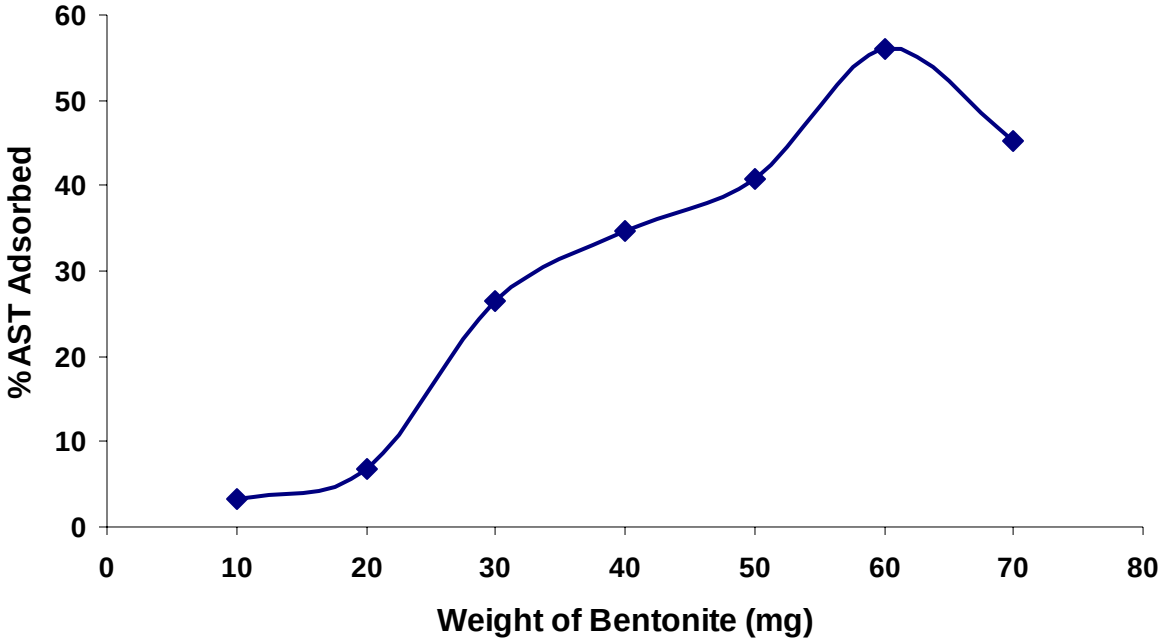

Figure (5): The percentages of the amount of serum aspartate aminotransferase adsorbed from the total serum aspartate aminotransferase concentration on different weights of bentonite.

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