Modified Method for Preparation of Aurintricarboxylic Acid and Prepare of it's Chromium (III) Complex: Study its Interaction with Some Human Serum Proteins.

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Abstract:

This study involves the use of a modified method for preparation of aurintricarboxylic acid (ATA) and use this acid as a ligand for preparation of complex with chromium (III) ion. The prepared complex was studied using different spectrophotometric methods including, UV-Visible spectrophotometry and IR, in addition to molar conductivity. The molar ratio of ligand-cation was found to be (1:2). The complex showed an octahedral geometric structure. The interaction between the formed complex with six blood components including serum total protein (STP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), and alkaline phosphatase (ALP) were studied. The measurement occurred before adsorption and after one hour of incubation with the complex. The quantity of the substance adsorbed on complex obtained from the difference in the concentration before and after adsorption per weight of complex. The results showed that the Cr(III)-ATA complex has surface activity and able to adsorb the serum proteins in different capacities according to the following order of quantity adsorpe:

S.AST> S.Albumin> S.T.ACP > S.T.Protein > S.ALP>S.ALT

The quantity of the blood proteins components that have the ability to release from the complex surface into one milliliter of distilled water (desorption) was estimated by adding the distilled water to the complex precipitated after adsorption process and then measurement of the solution concentration of the released blood components.The results showed that the order of the percentage of desorption of the adsorbed proteins followed the order:

S.AST > S.ALT> S.ALP> S.T.ACP > S.Albumin> S.T.Protein

These results may show the dependence of the adsorption-desorption process strength on the present functional groups on each protein.

الخلاصة:

هذه الدراسة تتضمن استخدام طريقة محورة لتحضير آرين ثلاثي الكاربوكسيل (ATA), وأستخدم هذا الحامض كليكند في تحضير معقد مع ايون الكروم الثلاثي التكافؤ. و قد تم تشخيص المعقد المحضر بواسطة مطياف الأشعة تحت الحمراء ومطياف الأشعة فوق البنفسجية,إضافة إلى قياس التوصيلية الكهربائية, وبالاعتماد على دراسة المعقد طيفياً بواسطة طريقة (النسب المولية) تمت معرفة النسبة بين الكاشف والأيون الفلزي وكانت(2:1) أي مول واحد من الكاشف إلى مولين من الأيون الفلزي,ومن خلال هذه الطريقة وطرق التشخيص الطيفي تم التوصل إلى اقتراح الشكل الهندسي للمعقد الناتج بصيغة (الثماني السطوح).من الناحية البيولوجية تم دراسة المعقد المحضر مع ست مكونات للدم وهي (البروتين الكلي للمصل (STP), الألبومين, اسبارتيت امينو ترانسفيريز(AST), الالنين امينو ترانسفيريز(ALT), أنزيم الفوسفاتيز الحامضي(ACP), إضافة إلى أنزيم الفوسفاتيز القاعدي(ALP) من خلال امتزازها على سطح المعقد المحضر. تمت دراسة كمية المادة الممتزة على سطح المعقد عند تراكيز مختلفة قبل وبعد الأمتزاز لنفس وزن من المعقد. أظهرت نتائج الأمتزاز إن المعقد له القدرة على امتزاز مكونات الدم ولكن بكميات مختلفة حسب الترتيب الأتي:

S.AST> S.Albumin> S.T.ACP > S.T.Protein > S.ALP>S.ALT

وبعد عملية الأمتزاز تم تحرير المادة الممتزه بعملية (الابتزاز) وهي إزالة مكونات بروتين الدم الممتزة على سطح المعقد بأستخدام 1 مللتر من الماء المقطر إلى راسب المعقد. أما نتائج النسب المئوية للابتزاز كانت بالترتيب الأتي:

S.AST > S.ALT> S.ALP> S.T.ACP > S.Albumin> S.T.Protein

هذه النتائج توضح بان عملية (إمتزاز- ابتزاز) تعتمد على قوة المجاميع الفعالة الموجودة في كل بروتين.

Introduction:

Aurintricarboxylic acid (ATA) is a polymeric carboxylated triphenylmethane derivative [(1)] . In cell free systems, ATA has been reported to be a nonspecific enzyme inhibitor by virtue of its polyanionic structure with effects on many systems [(1)].

Aurintricarboxylic acid (ATA) was used as an analytical reagent in the estimation of copper (II) by using silica gel as a support medium to form bidentate complex [(2)]. The binding of metal ion-ligand complexes with protein is important for preparation of different biosensors [(3)]. ATA could bonded to the different polymers including modeled aminopropyl silica gel [(2)]. A selective interaction of ATA with RNA, unlike DNA via phosphoric-ether bond (P-O-C), which was absent in the case of DNAATA complex [(4)]. This property can be tested for the protein molecules, which are polymers, to have the ability to interact with ATA complexes in the present work.

Aurintricarboxylic acid has unusual properties as a small molecule of nonimmunological origin that have important implications as anti-HIV compounds [(5)]. ATA selectively prevented the binding of specific monoclonal antibody (mAb) to the CD4 cell receptor for human immunodeficiency virus type 1 (HIV-1). Thus, ATA is a selective marker molecule for the CD4 receptor. ATA also interfered with the staining of membrane-associated HIV-1 glycoprotein by a mAb against it [(6,7)].

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 sometime this fact depends on the affinity of different substances molecules (adsorbate) towards an active sites on the surface of a solid substance (adsorbent) [(8)].

The adsorption of some blood components (mainly proteins) have been studied extensively for estimation of some biological molecules including enzymes [(9)], extraction purposes, compatibility studies, and other biological research fields [(10,11)].

Adsorbent may display alterations with respect to the amount of protein adsorbed on the surface, with individual serum proteins (albumin and fibronectin) displaying contrasting adsorption characteristics. Changes in protein adsorption corresponded to changes in cell adhesion [(12)]. These facts showed the importance of the adsorption of proteins on physical and biochemical properties of different surfaces.

The aim of the present work is to prepare ATA by a simple method by modifying the original method [(13)]. The second aim is to prepare the ATA complex with chromium (III) ion and use it as a surface active substance for the adsorption and desorption of some blood components as a possible tool for extraction of some important biochemical components.

Materials and Methods:

I- Preparation of Aurintricarboxylic acid:

20 milliliters of concentrated phosphoric acid were mixed with 2.8 grams (0.0029M) of solid potassium nitrate. When solution is complete, add 5.8 grams (0.0042M) of salicylic acid with stirring. The mixture should then be light red to brown in color. It is surrounded by an ice-salt bath. Add 1.4 milliliters of formaldehyde is slowly with extremely vigorous stirring. A bout 30 grams of crushed ice is then added, the stirring should be vigorous during the addition. The contens of the flask are stirred until the aurintricarboxylic acid has disintegrated into small pieces.

II- Preparation of the Chromium(III) Complex with Aurintricarboxylic acid:

500 milligrams of ATA (0.0011M) were mixed with 15mls of absolute ethanol and then 0.63 grams (0.0022M) of the solid salt $CrCl_3.6H_2O$ that dissolved previously with a minimum amount of absolute ethanol in a 50ml beaker. The mixture then mixed vigorously at room temperature for ten minutes. The complex then filtered and precipitated by adding a few amounts of diethyl ether as a

precipitating agent. The precipitate then dried and stored in a tightly closed container until using it in the following experiments. The formation of the complex was confirmed by different techniques including (FTIR spectrophotometer (Schimadzu 8400)), UV-Visible spectrophotometer (Schimadzu 160A), and molar conductivity (Gallankamp).

III- Adsorption process:

50 milligrams of complex was incubated with 1 milliliter of serum, mixed vigorously at room temperature for one hour. After the time elapsed the serum separated from the suspension by centrifuge at 3000g for 20 minutes to assure complete precipitation of complex particles. Serum total protein [14], albumin [15], aspartate aminotransferase (AST) (EC 2.6.1.1) [16], alanine aminotransferase (ALT) (EC 2.6.1.2) [16], acid phosphase (EC 3.1.3.2) [17,18] and alkaline phosphatase (ALP) (EC 3.1.3.1) [17,18] were measured by the routine methods in the clinical chemistry laboratories of the hospital using ready for use kits. The measurement occurred before adsorption and after one hour of incubation with complex. The quantity of the substance adsorbed on complex obtained from the difference in the concentration before and after adsorption per weight of complex. All experiments were repeated three times and the mean values were used.

IV- Principles of Serum Components Estimation:

Precision Multi-Sera Normal Human (lyophilized human sera) were obtained from Randox® Company (Cat.No.UN1557) and used after reconstitution each vial with exactly 5ml of distilled water. Randox human sera are lyophilized control sera based on human serum for use in the quality control of tests.

Colorimetric method of (Reitman and Frankel) for determination of serum AST and ALT activity according to Randox® kit.

Albumin in serum was measured by Bromocresol Green (BCG) method [15] as described in (Randox kit) leaflets. Total protein in serum was measured by Biuret method [14] according to the procedure of the as described in (Randox kit). The sera used for ALP were stabilized for at least one hour before use according to the instruction of the manufacturer. Colorimetric determination of ALP activity according to Sigma® kit manual. The sera used for total ACP were stabilized by adding one drop of 0.7M acetic acid solution to 1ml of the serum according to the instruction of the manufacturer. Colorimetric determination of total ACP activity according to Randox® kit manual depending on the Anderson *et al* (1947) method [17,18].

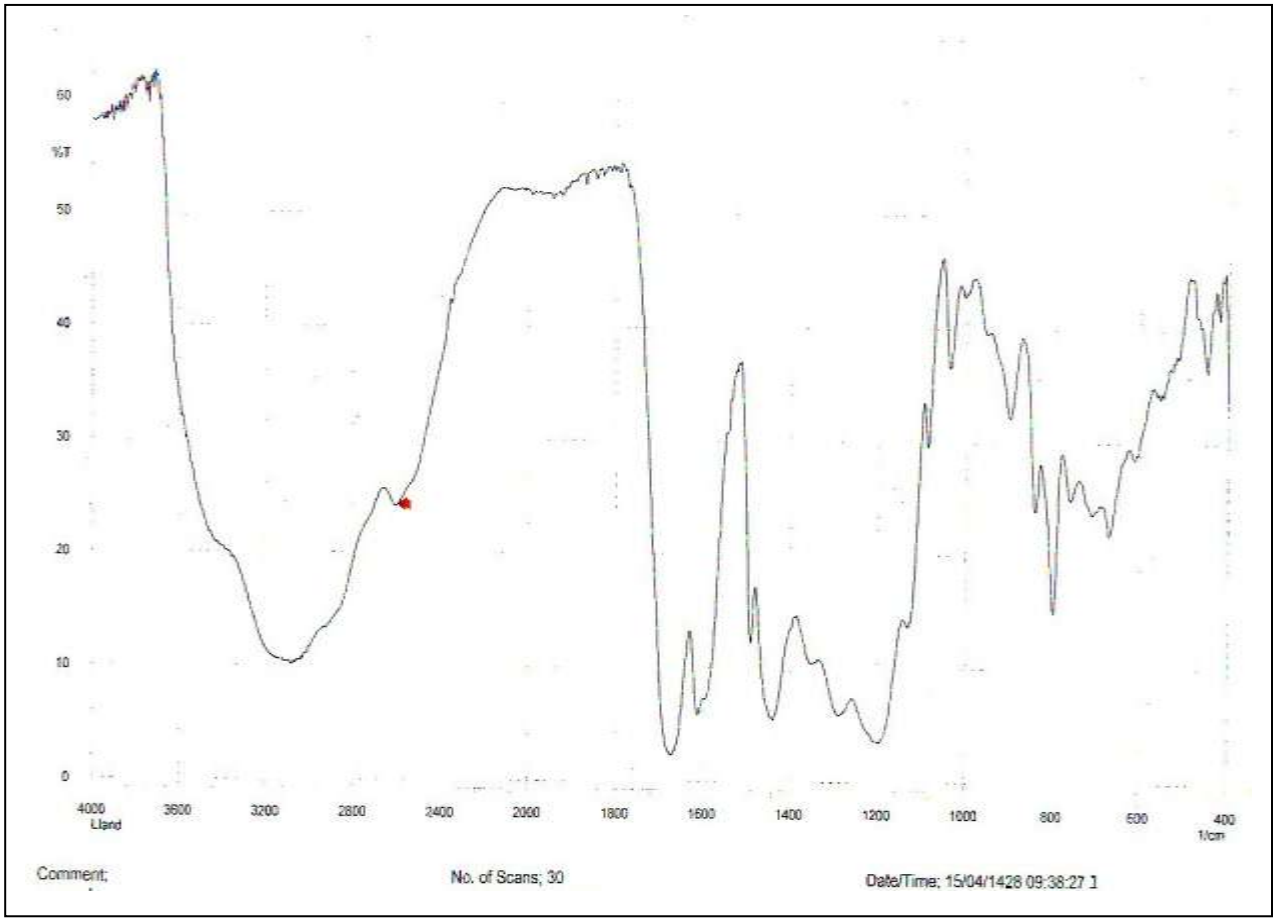
V- Desorption:

To obtain the quantity of the measured blood proteins components that have the ability to release from the complex surface into the solution (desorption) the following procedure were followed: One milliliter of distilled water was added to the complex precipitated after adsorption process. The mixture then mixed for one hour at 37.5ºC, centrifuged at 3000Xg for 20 minutes, and the blood components (Serum total protein, albumin, AST, ALT, total ACP, 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 complex.

Results and Discussion:

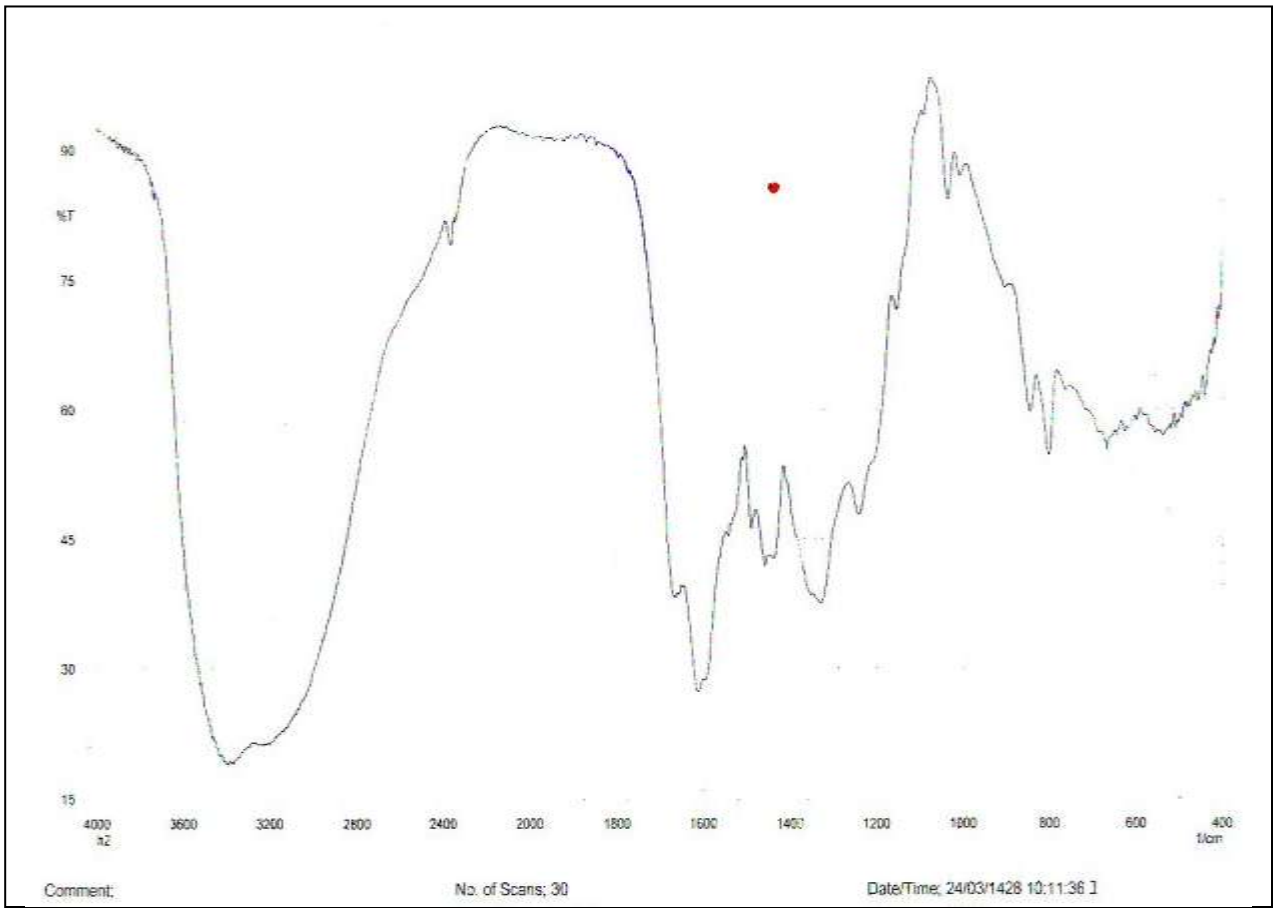
The modified method for preparation of ATA give a good yield and its easier than the prescribed original method [13]. The physical properties of the chromium(III)-ATA complex are; molecular weight =955.27g.mole $^{-1}$, melting point =>300ºC, yield=56%. The complex is insoluble in water, acetone, and dichloromethane. While its soluble in absolute ethanol, dimethylsulphoxide, and dimethylformamide.

The IR spectrum of the ligand ATA alone (Scheme (1)) showed a wide absorption peak in the range (2700-3500cm^{-1}) due to the stretch of (-OH) bond and to the hydrogen bonding in carboxyl groups [19,20]. The spectrum also showed two absorption peaks at 1440 and 1490 cm^{-1} due to the symmetric stretch of (-COOH) groups and two absorption peaks at 1610 and 1670 cm^{-1} due to the asymmetric stretch of (-COOH) groups [20].



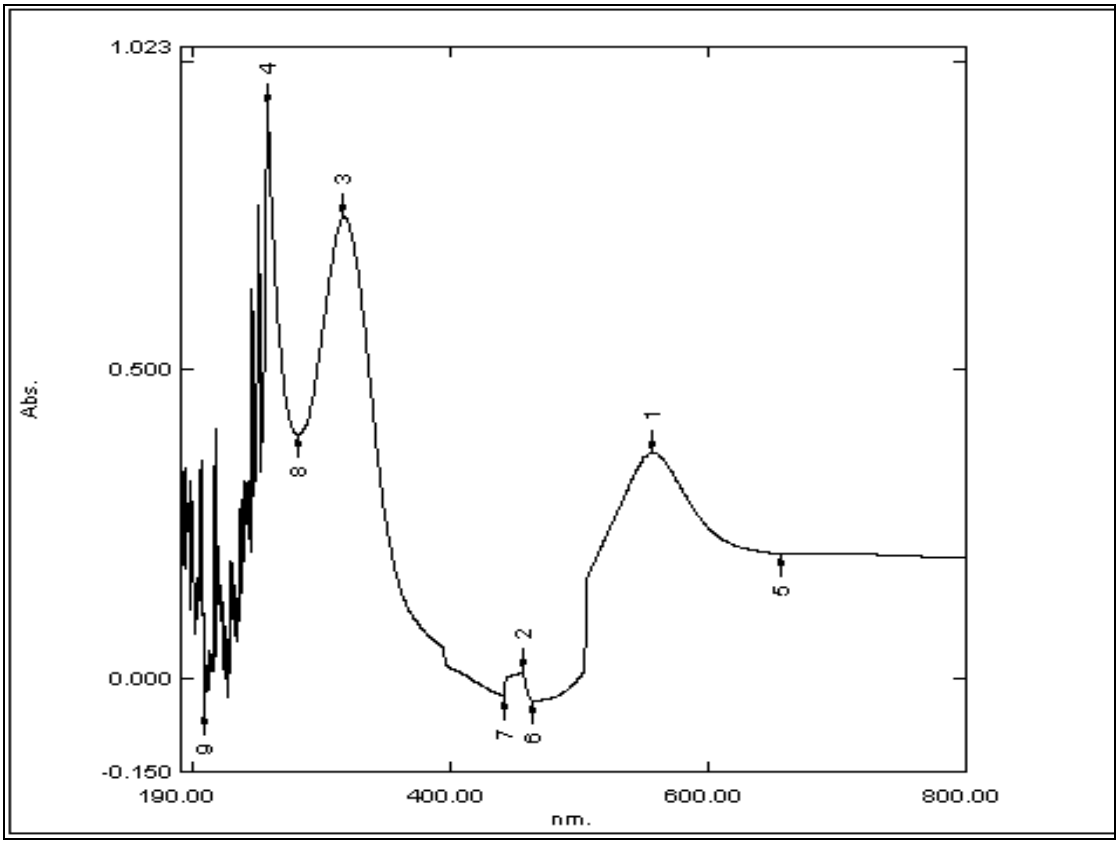
When the complexation with Cr(III) occurred (Scheme (2)), there is a change in the phenolic (-OH) bands. This band is narrowed in the complex as compared with its wide shape in the ATA alone spectrum. This fact give an evidence about the formation of coordination with the metal ion by covalent bonds via oxygen atom. Also the location of the stretch of and asymmetric stretch of (-COOH) groups at 1670 was changed (shifted to lower frequencies) [21]. There is also a shifting noticed in the (C-O) group of phenol group from 1200cm^{-1} toward 1240cm^{-1} producing another evidence about involvment of phenol group in coordination with chromium ion via oxygen group [22].

The spectrum of complex revealed a splitting of carboxyl group band into two peaks at 1430cm^{-1} and 1450 cm^{-1} formation of coordination bonds between Cr(III) ion and carboxyl groups. Two new peaks also noticed in the spectrum of the complex at (530 and 440) due to the presence of water coordination and Cr-O bond, respectively [22].



In one research [2] it was concluded that, if the unbonded contact distances between ATA atoms and surface atoms are larger than the sum of their van der Waals radii, it can be concluded that the ATA could be immobilized on that site. This approach indicates that there is no steric hindrance around the immobilized ATA. So ATA could easily form chelate with metal ions and this accounts for the high uptake of metal ions by ATA [2].

In order to obtain the type of the complexation process (number of metals ion that bind with the ligand), a mole ratio method was used to study the ratio between the ligand ATA and metal ion Cr(III). In this method, a series of different volumes solutions of the ATA ligand were mixed with different volumes of Cr(III) ions solutions to obtain a constant volume each time (5ml). The maximum wave lengths used to follow the formation of the complex is maximum wave lengths (λ_{max}=525nm). The results showed that the molar ratio of ligand:ion was (1:2). When the pure solid complex dissolved in dimethylsulphoxide at a concentration (0.001M), the UV-Visible spectrum showed two obvious peaks at 455nm and 556nm due to the electronic transformation ^4A_2g $^4T_1g(F)$ and ^4A_2g ^4T_2g in the disturbed octahedral structure of d^3 system of the Cr (III) ions [23,24] according to the following chart.



The molar conductivity results using dimethylsulphoxide as a solvent at concentration (0,003M) that the complex is not conductive (neutral non-electrolyte substance). According to these results and those obtained from FT-IR study, an octahedral geometry around Cr (III) ion can be suggested, as follow.

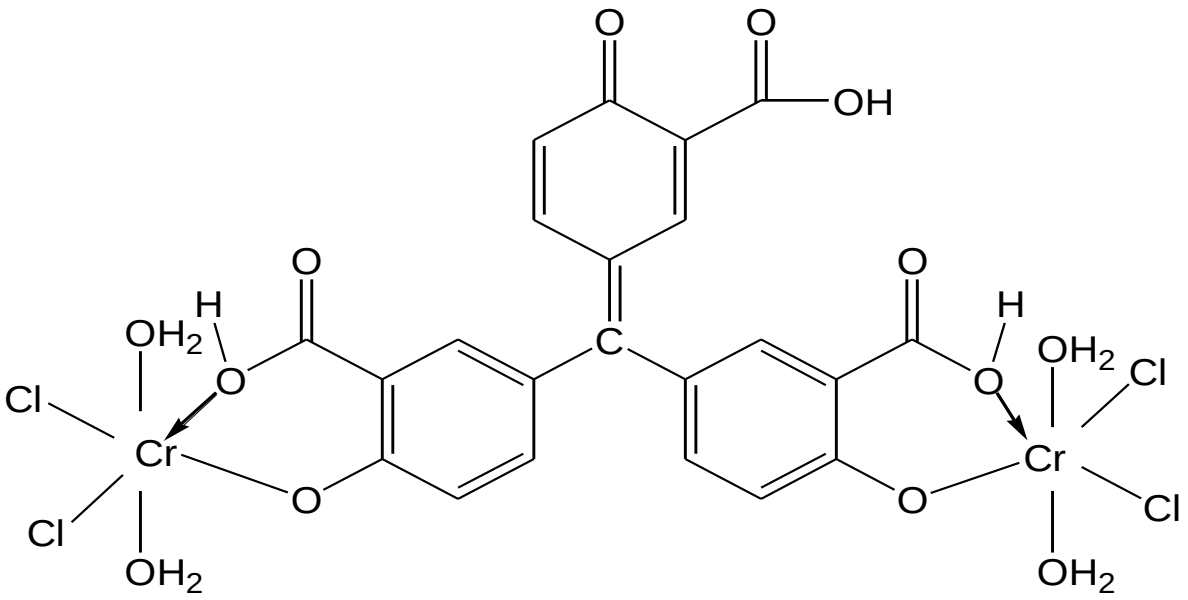


Table (1) showed the quantities adsorbed of different serum components by 50mg of solid complex particles after incubation of the complex with one milliliter of serum. The results showed that the serum total protein is the most susceptible substance for adsorption on the surface of the complex particles followed by serum albumin. Knowing that the albumin is the main components of serum proteins, albumin showed the highest adsorption capability for complex surface among the other types of serum protein. These findings were noticed in many other adsorption studies for blood components on different surfaces [25,26,27]. These results due to the large amounts of these substances in the serum. While the percentages (Figure (1)) showed that the proteins obeyed the following order in the ability to be adsorbed:

S.AST> S.Albumin> S.T.ACP > S.T.Protein > S.ALP>S.ALT

The results indicated a decrease in the concentration of all the biochemical parameters. Johnson and Arnold (1995) [28] noticed that, during adsorption, some arrangement of functional groups on the protein (e.g., charged or hydrophobic amino-acid residues or specific ligand 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 [28].

The interaction between different serum proteins and the complex may involve different forces and explanations. The possible effect of the complex on the compounds that used in the estimation of the measured serum proteins is excluded because the complex is separated from the suspension by centrifugation before any measurements as described in the material and methods section. There is also some reports on the mutual effect of adsorption of some components on the adsorption of other serum components [29].

One of the suggestions to explain the protein complex interaction is a protein adsorption process that occurred on the surface of solid surfaces which is a subject of various studies [30,31]. This phenomenon include various forces due to the huge number of functional groups on protein molecules

which may have the ability to interact physically or chemically by the groups on the complex molecules which may lead to formation of dissociable or stable conformation. The protein-surface interaction may include hydrophilic, electrostatic [32], or superposed hydrophobic interactions [33]. There is also different techniques uses ion exchange interaction between protein-surface [34].

To test these explanation, the desorption studies were carried out. These studies confirm the adsorption forces and give information about the strength of the forces between the complex and different serum proteins. Table (2) showed that the STP and albumin have the most amounts released from the surface to the solution because their high amounts of them adsorbed previously on the complex surface as noticed in Table (1). While the results showed that the order of the percentage of desorption (release) of the adsorbed proteins (Figure (2)) followed the order:

S.AST > S.ALT> S.ALP> S.T.ACP > S.Albumin> S.T.Protein

These results may show the dependence of the adsorption process strength on the present functional group on each protein. The forces included and complete adsorption studies are the future study of this work suggested that there is a specific affinity between each of serum protein type and the solid complex surface. This suggestion requires more studies that require an advanced techniques but the quantities desorbed referred to the suggested concept i.e., there is a specific adsorption that differ from other nonspecific adsorbents like activated charcoal which adsorb any substance without specificity [35]. Many enzymes were immobilized and extraceted by the adsorption process on different surfaces but using of insoluble metal ion complexes as adsorbent for enzymes is rare [36]. In general, it can be concluded that some serum components of the adsorbed substances were releasable from the surface of complex. 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 complex 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 than the introduced method after development and enhancement of this method which necessary for good separation of these substances.

The adsorbed protein conformation may affect and changed after adsorption on different types of surfaces [37]. This fact adds other evidence about the fact that the adsorbed molecule may suffer from conformational changes when they adsorbed on the surface but they return to normal shape when they release (desorbed) from the surface.

In conclusion, this preliminary study opens the door for more studies about using the newly formed water insoluble complexes in the extraction of different blood components by the adsorption-desorption phenomenon. This extraction is very important in industrial and experimental field to prepare a pure expensive vital compound from serum using simplest method that uses a new insoluble complexes surface adsorbent.

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Table (1): The quantities adsorbed of different serum components by 50mg of solid complex.

Parameter	S.Albumin	S.T.Protein	S.AST	S.ALT	S.T.ACP	S.ALP
Quantity adsorbed by 50mg of complex	33.00mg	36.80 mg	0.12U	0.05U	0.07U	0.27U

Figure (1): The percentages adsorbed of different serum components by 50mg of solid complex.

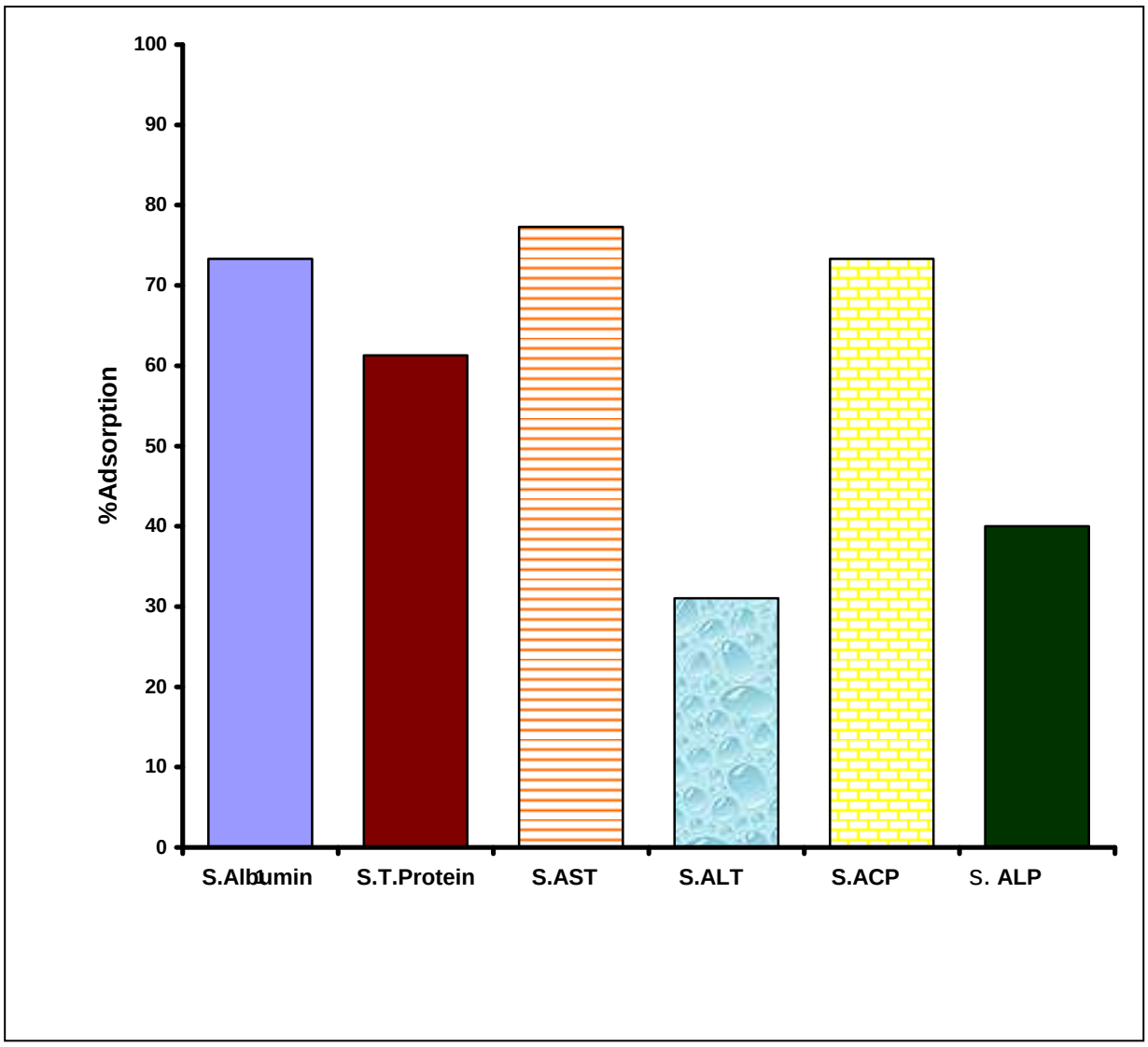

Table (2): The quantities desorbed of different serum components that adsorbed from the 50mg of complex.

Parameter	S.Albumin	S.T.Protein	S.AST	S.ALT	S.T.ACP	S.ALP
Quantity desorbed from 50mg of complex to 1ml of distilled water	0.9mg	2.7mg	0.06U	0.01U	0.01U	0.34U

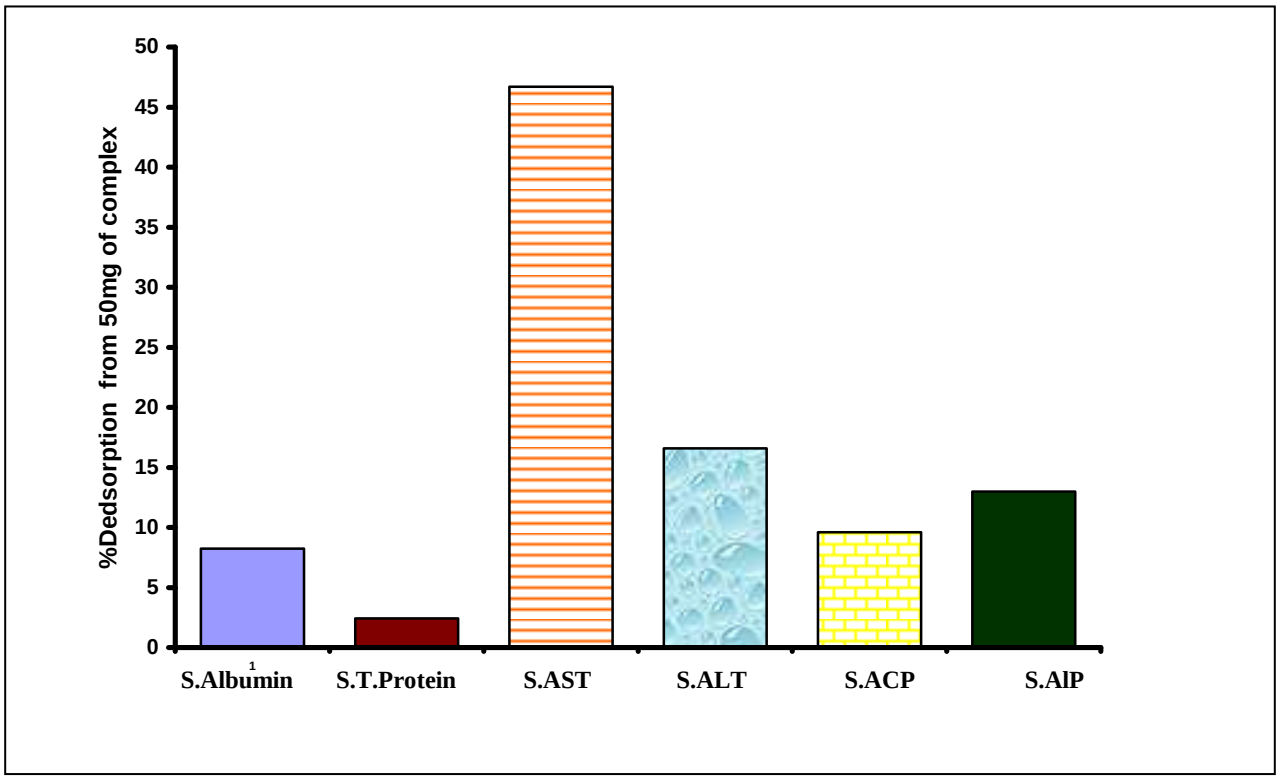

Figure (2): The percentages desorbed from the total quantities adsorbed on the solid complex to 1 milliliter of distilled water.