Thermodynamic Studies for the Interaction of Some Anticoagulant Drugs with Albumin

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Abstract

Fluorescence spectroscopy in combination with UV- vis absorption spectroscopy was employed to investigate the binding of some important anticoagulant drugs warfarin, heparin and aspirin to human serum albumin under physiological conditions. Scatchard equation provide a measure of the binding affinity between these three drugs individually and albumin. The results of thermodynamic parameters ΔG, ΔH, and ΔS at three different temperatures indicate that the hydrophobic interaction plays a major role for the interaction between each of these drugs and albumin.

Keywords: Albumin interaction, Anticoagulant drugs, thermodynamic parameters.

Introduction

The interaction between drugs and bio-macromolecules has attracted considerable attention (1). Serum albumin has been among the most intensively bio-macromolecules in terms of structure and biological properties. Important functions of serum albumin are that it contributes significantly to colloid osmotic blood pressure and aids in the transportation, distribution and metabolism of numerous endogenous and exogenous compounds. Exogenous compounds, such as drugs, can bind to albumin and then transported in the circulatory system (2). Albumin is one of the most abundant proteins in the human serum. This protein has three domains (I, II, and III) and each domain has two sub domains (A and B). Albumin molecule has a unique ability to bind with a variety of ligands such as drugs, bilirubin, fatty acids and other kinds of substances, this binding occurs at two binding sites (site 1) or Sudlow's site I and (site 2) or Sudlow's site II (3 – 5). The binding of a drug to albumin influences its metabolism, distribution, efficacy and elimination from the circulation. Thus, detailed knowledge of the binding interaction of drugs with albumin and their relative strengths is important. In this work the interaction of albumin with three different anticoagulant drugs (warfarin, heparin, and aspirin was studied. Warfarin or Coumadin ((*RS*)-4-hydroxy- 3-(3- oxo – 1 – phenylbutyl) - 2H - chromen – 2 – one) is an oral anticoagulant. Aspirin (acetyl salicylic acid) is a nonsteroidal anti-inflammatory drug (NSAID) effective in treating fever, pain, and inflammation in the body, it also prevents blood clots. Heparin is a highly sulfated glycosaminoglycan, these three drugs Fig.(1) are widely used as anticoagulant drugs that inhibits the synthesis of clotting factors, thus preventing blood clot formation. It is also important to prevent extension of clots already formed, and to minimize the risk of blood clot embolization to other vital organs such as the lungs and brain. Determination of the albumin binding of several compounds is a valuable tool for identifying and characterizing the binding forces concerned with the interaction of drugs with protein (6). Albumin binding influences the fate of drugs in the body. Only the unbound or free drug diffuses through capillary walls, reaches the site of drug action. The drug-albumin complex and the study of its thermodynamic and kinetic parameters may be considered as a model for gaining general fundamental insights into drug–protein binding (7-11).

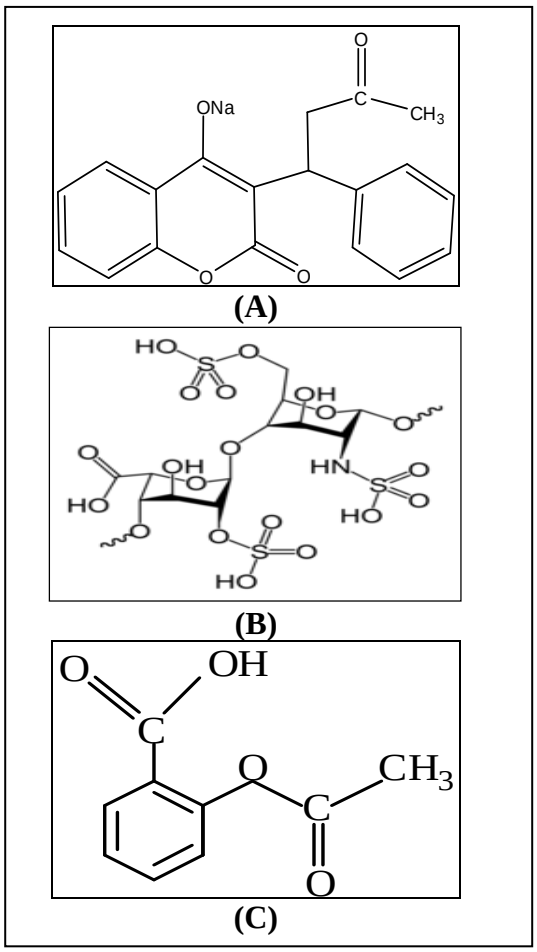


Fig. (1) Chemical structures of: A) Sodium warfarin. B) Heparin. C) Aspirin.

Experimental

Materials

Serum albumin was purchased from Merk Chemical Company, Germany. Sodium warfarin was purchased from Orion pharmaceutica, Espoo, Finland. Acetylsalicylic acid from BDH, Ltd., Poole England. Heparin from Hopkins & Williams chemical company, England. The solutions of albumin, amino acids, and drugs were prepared by the use of Michaelis phosphate buffer of pH 7.4 as a solvent. All other materials were of analytical reagent grade and distilled water was used throughout.

Absorption spectroscopy

The UV measurements of serum albumin were recorded on a UV – 1800, Shimadzu, UV Spectrophotometer using a 1cm path length cell. Absorbance value of albumin in the presence and absence of each drug were made in the range of 200-600 nm. albumin concentration was fixed at $3x10^{-6}$M while the concentrations of the drugs were varied from (3 - 25) x 10^{-6} M.

Fluorescence spectroscopy

Fluorescence measurements were made on a spectrofluorometer model SL 174 Elico. The Fluorescence quenching of serum albumin at increasing molar ratio of each drug to serum albumin was recorded in the wave length range 300-600nm after exciting the albumin solution at 295 nm, using 20nm/20nm as slit widths and sensitivity 950. Albumin concentration was fixed at $6*10^{-6}$M and the concentration of each drug was varied from $(6–24)*10^{-6}$M. To evaluate the effect of temperature at albumin-drug interaction, fluorescence was recorded at three different temperatures (290, 300, and 310K) by using an thermostatic water bath and an INV 25 incubator in order to maintain the temperature constant.

Stoichiometric analysis

The stoichiometry of interaction of each of the following drugs (sodium warfarin, aspirin and heparin) with serum albumin were determined by continuous variation method (Job's method) (12). Job method was applied by placing 1 to 9 ml of $6*10^{-6}$M solution of albumin in to a series of 10 ml flask, this was followed by placing 9 to 1 ml of $6*10^{-6}$M of the drug used, and the absorbance was measured at the maximum wave length of each drug.

FT-IR spectroscopic measurements

Infrared spectra of albumin and (1:1) albumin-drug complexes were recorded on a Shimadzu FTIR-8400S spectrophotometer on KBr disks.

Results and Discussion

Absorption spectroscopy

The UV–Vis absorption studies were preformed to ascertain the complexation of albumin with the studied drugs. The UV absorbance showed an increase with the increase in drug concentration, also a shift (blue – shift) in peak could be observed on the interaction of albumin with these drugs and the existence of the equilibrium as:

Drug + Albumin ↔ Albumin – drug complex
.....(1)

These two changes are indication of a complex formation between albumin and drug (13, 14).

Stoichiometric analysis

The stoichiometry of the complexes of albumin with the drugs was calculated by the method of continuous variations. The coordination number n could be calculated from the plot of absorbance against the mole fraction of drug. As it is evident from the figure 2 the Job's plot (continuous variation plots), implies that the stoichiometric ratio n of albumin – drug at 298K and pH 7.4 is 1:1.

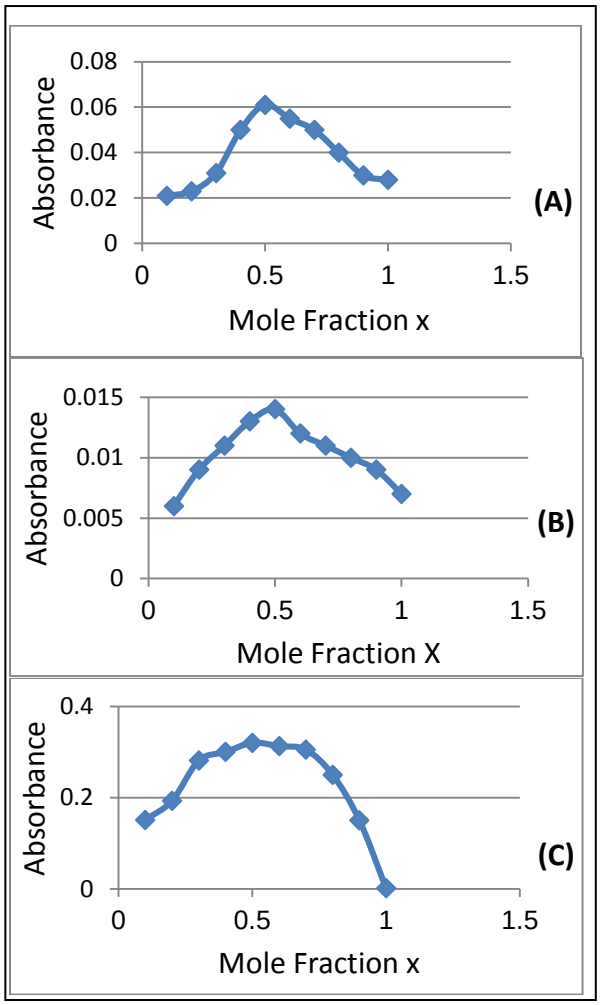


Fig.(2) Continuous variation plots of:
(A) warfarin - albumin,
(B) heparin - albumin and
(C) aspirin - albumin.

The stability constant can also be calculated from these curves, and the overall stability is given by (15, 16):

$[A]_{eq} + [D]_{eq} = [AD_n]_{eq}$

$K_{eq} = [AD_n\ complex]_{eq} / [A]_{eq}[D]_{eq}$ ..... (2)

Knowing the formula of the complexes which were (1:1), the concentration of the complexes at equilibrium was calculated as:

$[AD_n]_{eq}$ = Absorbance $_{(max)}$ / ε l .....(3)

The molar absorptivity of the complex were calculated by recording the absorbance of a series concentrations of 1:1 complex and plotting the absorbance against concentration which given a straight line with a slope equals to ε, the obtained molar absorptivity ε for these complexes were illustrated in Table (1).

Table (1)
The molar absorptivity of the albumin – drug complexes.

Complex	*Molar absorpivity ε($cm^{-1}.l.mol^{-1}$.)*
Albumin-warfarin	192300
Albumin-heparin	234900
Albumin-aspirin	265500

The equilibrium constants calculated by this method were determined in three different temperatures (298, 300, and 310) K as shown in Table (2).

Table (2)
Equilibrium constant of albumin – drug complexes at different temperatures calculated from continues variation method.

Temperature (K)	*K_{eq} albumin– warfarin complex (M^{-1})*	*K_{eq} albumin– heparin complex (M^{-1})*	*K_{eq} albumin– aspirin complex (M^{-1})*
298	1.8×10^5	39×10^5	3.9×10^5
300	3.3×10^5	88×10^5	5.1×10^5
310	5.9×10^5	145×10^5	10.7×10^5

Table (2) shows the dependence of equilibrium constant with temperature, it increase with increase in temperature for the three complexes of albumin and drugs, that means the stability of the complex increase with temperature increase that means the bond between them becomes stronger.

Thermodynamic parameters and binding mode

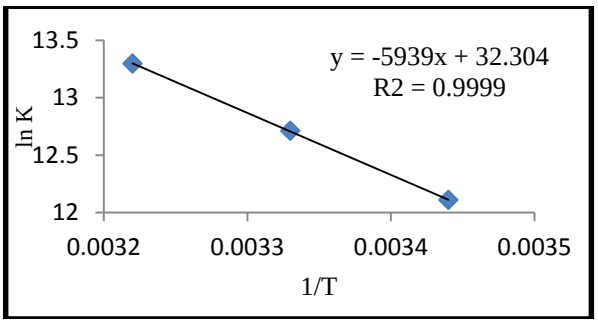
Considering the dependence of the equilibrium constant (binding constant) on temperature, thermodynamic processes were considered to be responsible for these interactions. Therefore, the thermodynamic parameters dependent on temperature were analyzed in order to characterize the acting forces between albumin and the drugs. The acting forces between a small molecule and a macromolecule mainly include hydrogen bonds, van der Waals forces, electrostatic forces and hydrophobic interaction forces. The thermodynamic parameters, enthalpy change ($\Delta H°$), entropy change ($\Delta S°$), and free energy change ($\Delta G°$) are the main evidences to determine the binding mode. The thermodynamic parameters were evaluated using the van't Hoff equation (17 – 19).

$$\ln K = - \Delta H°/RT + \Delta S°/R \quad \text{..... (4)}$$
$$\Delta G° = \Delta H° - T\Delta S° \quad \text{..... (5)}$$

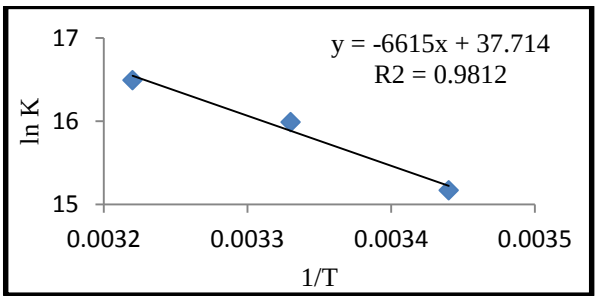
K and R are the binding constant and gas constant respectively. The results obtained are shown in Fig.(3) and Table (3).

Table (3)
Thermodynamic parameters for albumin – drug systems.

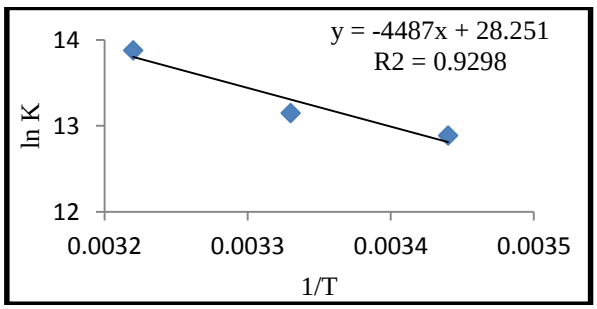
complex	*Temp. (K)*	*ΔG° (KJ.mol^{-1})*	*ΔH° (KJ.mol^{-1})*	*ΔS° (KJ.mol^{-1})*
Albumin- warfarin	290	-29.195	49.377	69.5
	300	-31.705	49.377	58.9
	310	-34.269	49.377	48.7
Albumin - heparin	290	-36.576	54.997	63.5
	300	-39.897	54.997	50.3
	310	-42.509	54.997	40.2
Albumin - aspirin	290	-31.081	37.311	21.4
	300	-32.798	37.311	15.0
	310	-35.769	37.311	4.9



(A)



(B)



(C)

Fig. (3) Van't Hoff plot for warfarin – albumin complex (A), heparin-albumin complex (B), and aspirin – albumin complex (C).

The negative values of Gibbs free energy ΔG° refers to the spontaneous interaction between albumin and drugs, in the direction of equilibrium and increase with increase in temperature. The results obtained suggest that the process is entropically driven. The positive entropy change ΔS° occurs because the water molecules that are arranged in an orderly fashion around the ligand and albumin acquire a more random configuration as a result of hydrophobic interactions, also ΔS° decrease with increase in temperature; that is, the two molecules (albumin and drug) are subject to more physical restraint as the bond between them becomes stronger. The positive enthalpy and entropy changes also refer to the type of interaction between these two molecules which are hydrophobic association and electrostatic interaction (20, 21).

Fluorescence spectroscopy

A significant shift in albumin fluorescence spectra was observed upon the interaction of albumin with drugs, especially at higher drug concentration. Fig.(4) fluorescence spectra can also be used to determine the extent of binding

using Schachard plot (22–26), the plot of r / [D] versus r should gives a straight line.

$$r / [D] = n.k_a - k_a r \quad \text{..... (6)}$$
$$r = [DP] / [P]_{total} \quad \text{..... (7)}$$

k_a = binding constant.

The binding constant of albumin and each one of the drugs were determined at three different temperatures as shown in Table (4) and Fig.(5).

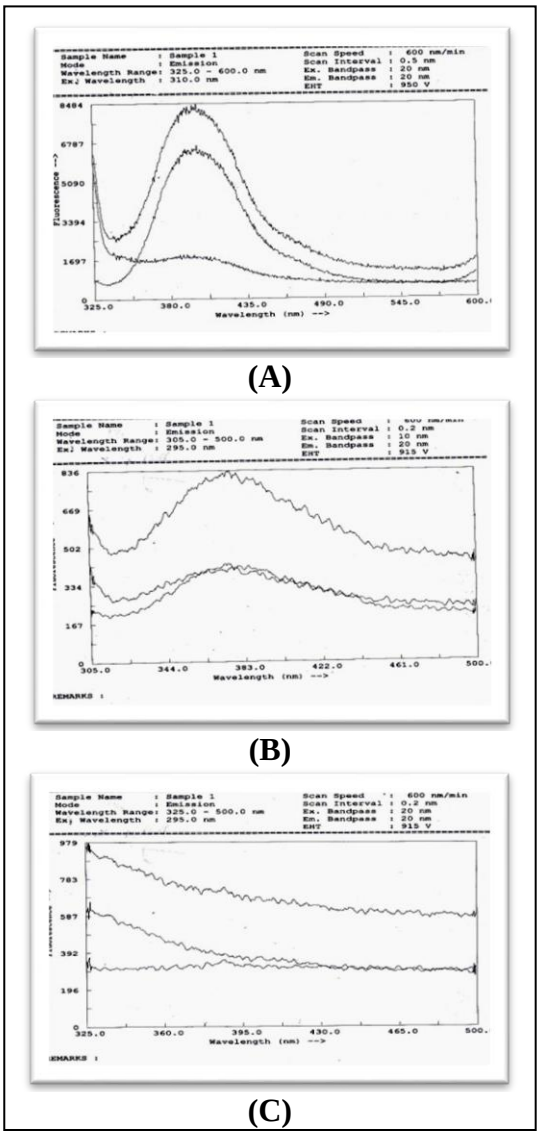


Fig.(4): Effect of drug concentration on fluorescence spectrum of albumin ($3x10^{-6}$, $9x10^{-6}$, $18x10^{-6}$ M): (A) albumin + aspirin (B) albumin + warfarin. And (C) albumin + heparin.

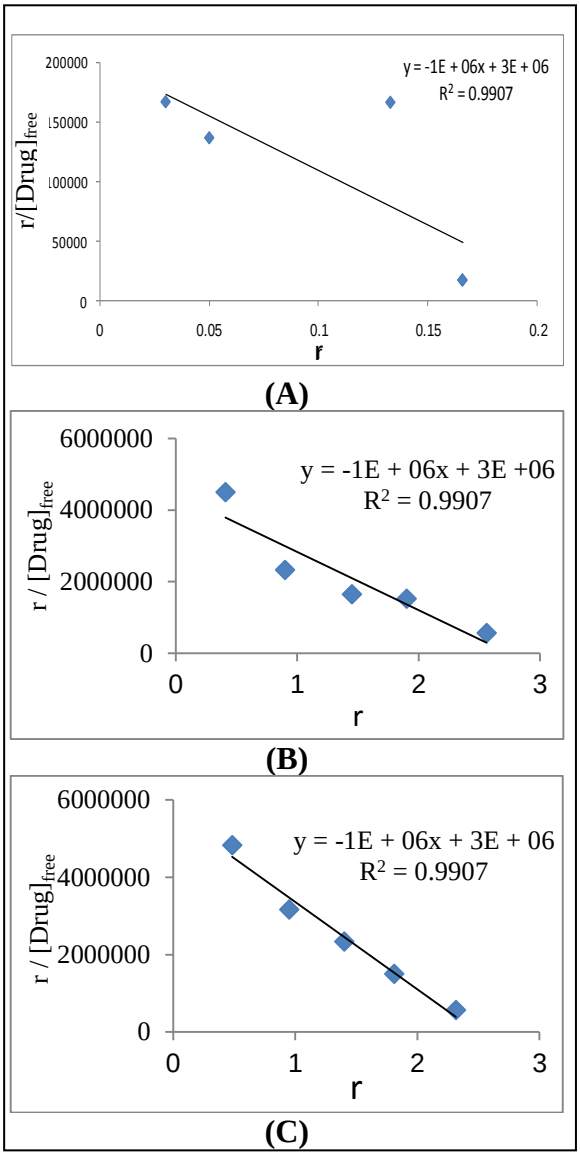


Fig.(5) Schachard plot for (A) Warfarin. (B) Heparin. (C) Aspirin complexes with albumin at 290K.

Table (4)
Temperature effect on binding constant (k_b) of albumin – drug complexes.

T(K)	*warfarin–albumin k_b ($l.mol^{-1}$)*	*aspirin–albumin k_b($l.mol^{-1}$)*	*heparin–albumin k_b ($l.mol^{-1}$)*
290	$1x10^6$	$1x10^6$	$1x10^6$
300	$2x10^6$	$2x10^6$	$2.5x10^6$
310	$1x10^7$	$3x10^6$	$3.03x10^6$

It was found that the binding constant increased with the increase in temperature, resulting in the stabilization of the albumin – drug complex.

FTIR measurements

To understand the structural alteration in albumin and gain a view of physicochemical properties of albumin after addition of drug, FTIR spectroscopic measurements were performed on albumin and albumin–drug mixture. Infrared spectra of albumin exhibit an amide bonds, and gives rise to a band in the region between approximately 1600 and 1700 cm^{-1} (27), which has a relation with the secondary structure of protein, comparing the spectrum of albumin and albumin–drug complexes, there is no difference at this band (1684 cm^{-1}), which is another evidence that the interaction between albumin and these drugs is a hydrophobic and electrostatic interaction (36).

Conclusion

The interaction of some anticoagulant drugs with albumin have been investigated in vitro under simulated physiological conditions (pH 7.4) using different optical techniques, absorption spectroscopy, fluorescence spectroscopy and FT – IR spectroscopy. The shift in λ_{max} and a change in absorbance indicate the complex formation with a stoichiometry of (1:1). The thermodynamic analysis suggested that these drugs could bind with albumin trough the hydrophobic and electrostatic interaction. The binding study of drugs with albumin is of great importance in understanding chemico-biological interaction for drug design, pharmacy, pharmacology and biochemistry.

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الخلاصة

باستخدام عدد من الطرق الطيفية، مطيافية الأشعة فوق البنفسجية، والاشعة تحت الحمراء والفلورة تم دراسة تآثر بعض الادوية المضادة للتخثر (الوارفرين والاسبرين والهيبارين) مع بروتين مصل الدم (الالبومين). تكوين المعقدات الجزيئية بين الدواء والالبومين اثبت بدلالة الازاحة في اعلى قمة امتصاص وكذلك تغير الامتصاصية. كم تم حساب نسبة الاتحاد بتطبيق طريقة التغاير المستمر. المتغيرات الثرموديناميكية، التغير في طاقة جبس الحرة، والانثالبي والانتروبي وثابت الاستقرار للمعقد حسبت عند ثلاث درجات حرارية وتبين منها بان قوى الترابط بين الالبومين والدواء تغلب عليها القوى الهيدروفوبية والالكتروستاتيكية.