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Comparative studies on the interaction between Vitamins E, D, and K with Human Serum Albumin: UV-visible absorption spectroscopy.

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Abstract: The binding reaction between Vitamins E, D, and K with human serum albumin (HSA) was investigated by UV-Vis absorption spectroscopy. The results of these vitamins binding with HSA indicated that van der Waals interaction and hydrogen bonding played a major role for the vitamins-HSA association. The comparative experiments demonstrated that the primary binding sites of the vitamins are within subdomain IIIA while the binding sites of HSA are within subdomain IIA. The binding constants of vitamin E, vitamin D and vitamin K with HSA have been determined by UV-absorption spectroscopy. The values of the binding constants are calculated at room temperature and found to be: ($1.21\times10^{2}M^{-1}$) , ($6.8\times10^{1}M^{-1}$), and ($60\ M^{-1}$) for vitamin E- HSA , vitamin D- HSA and vitamin K-HSA mixtures, respectively.The effect of the vitamins (E, D, and K) on the conformation of HSA was analyzed by UV-Vis CD spectroscopy. Synchronous spectra indicated that the polarity around the tryptophan (Trp214) residues of HSA was decreased and its hydrophobicity was increased.

Keywords: Vitamin E; Vitamin D; Vitamin K, binding mode; binding constant; UV–spectroscopy.

INTRODUCTION

Vitamins are organic, low molecular weight components of the diet required by the organisms in very small amounts to perform specific cellular functions. They are not synthesized by organism, so it must be ingested with the diet or acquired in some other way[1- 2- 3.]. Hydrophilic-vitamins are nine vitamins which are soluble in water. Where Hydrophobic-vitamins are four vitamins soluble in fat, and they are released, absorbed, and transported with the fat of the diet[2].Vitamin K is a fat- soluble vitamin which

was named from the word Koagulation which is the German spelling for coagulation [4]. Vitamin K functions as a coenzyme and is involved in the synthesis of a number of proteins involved in blood clotting and bone metabolism [5-6].Compounds with vitamin K_1 activity all have a 2-methyl 1, 4-naphthoquinone ring and a side chain at the 3-position as shown in Fig. (2). In its natural form, this 3-substituent has an isoprenoid structure with varying lengths and degrees of saturation depending on the organism by which it is synthesized.Phylloquinone and menaquinones are natural forms of vitamin k_1 that are important cofactors in blood- clotting factors[7].

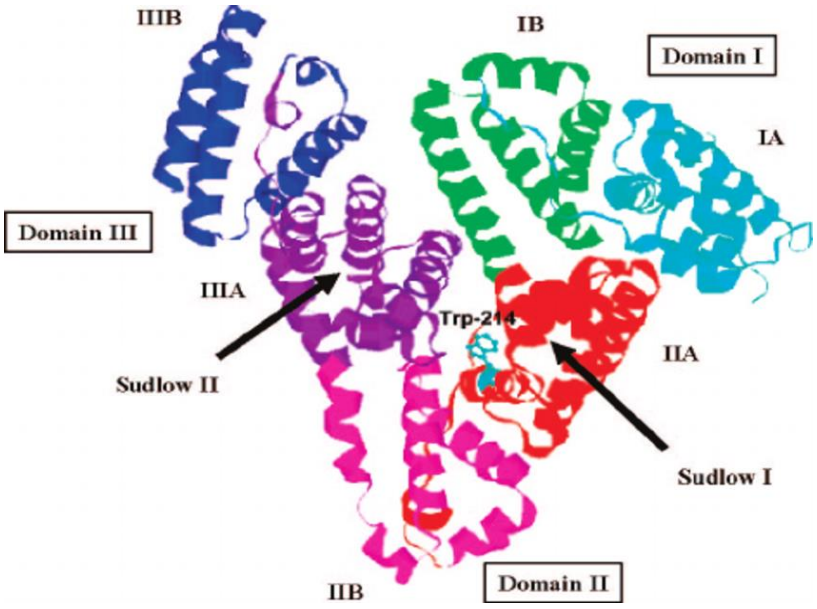


Fig. 1: The chemical Structure of Human Serum Albumin (HSA)

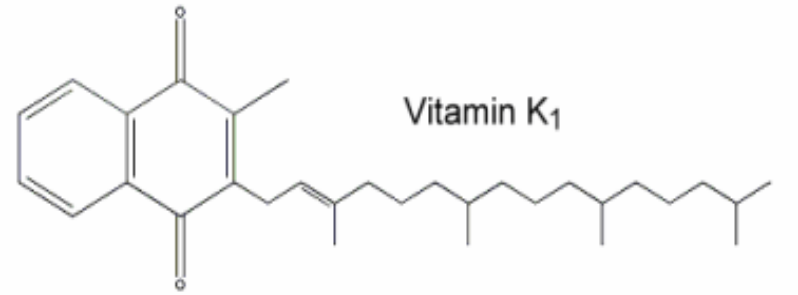


Fig. 2: Chemical Structure of vitamin K1

Phylloquinone is the predominant form of vitamin K[8] and is provided mainly by plant foods, especially leafy green vegetables, and certain legumes[9].Recently, it was demonstrated a correlation between vitamin K_1 and sphingolipids concentrations in rat brain [10]; moreover this vitamin present a protective effect on aging retina, the sparing effect being most evident in the inner plexiform layer and in the photoreceptor inner and outer segments[11] assays with vitamin K remain to be made on human. Vitamin D is one of the oldest hormones that have been made in the earliest life forms for over 750 million years. Phytoplankton, zooplankton, and most plants and animals that are exposed to sunlight have the capacity to make vitamin D. Vitamin D shown in Fig.(3) is critically important for the development, growth, and maintenance of a healthy skeleton from birth until death.

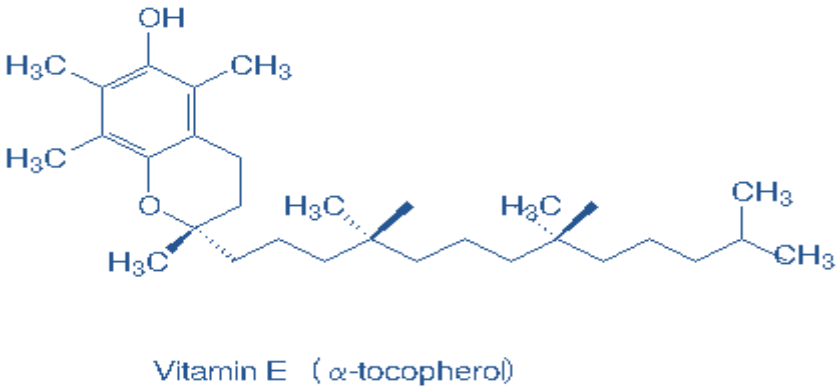
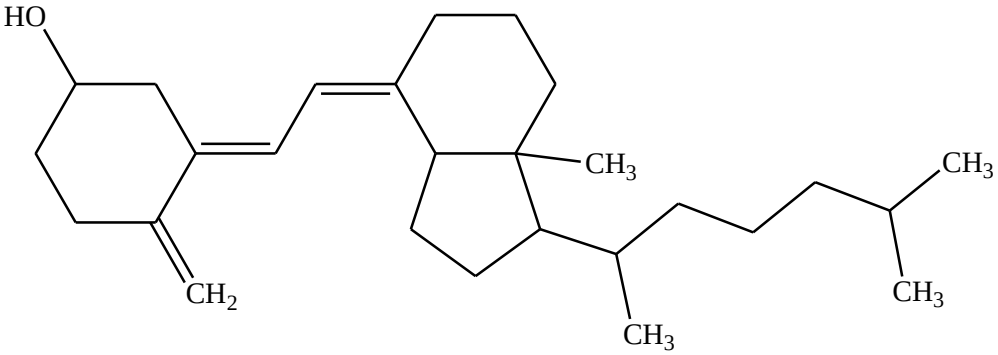


Fig. 3: Chemical Structure of cholecalciferol (E).

The major function of vitamin D is to maintain calcium homeostasis. It accomplishes this by increasing the efficiency of the intestine to absorb dietary calcium. When there is inadequate calcium in the diet to satisfy the body's calcium requirement, vitamin D communicates to the osteoblasts that signal osteoclast precursors to mature and dissolve the calcium stored in the bone.It has now been realized that Vitamin E, traditionally known as α-tocopherol, is a mixture of eight different compounds, four tocopherols and four tocotrienols, each one being designated as α, β, γ and δ forms. The two groups differ in the hydrophobic tridecyl side chain which is saturated (phytyl) in tocopherols and unsaturated having three double bonds (geranyl) in tocotrienols. Detailed reports are available on αtocopherol as shown in Fig.(4).



Structure of Vitamin D

Fig. 4: Chemical Structure of cholecalciferol (D).

However, during the last few years it has been found that all the eight forms are biologically active and perform specific functions. Clinical research has shown that mixture of tocotrienols and tocopherols offer synergistic protective action against heart ailments and cancer that is not exclusively offered by α-tocopherol. The other advantage of mixed tocopherols and tocotrienols is their role in slowing down aging. Diseases like diabetes 1 and 2, autoimmune diseases, bacterial and viral infections, Alzheimer disease, fungal (*Candida*) infections are prevented by these compounds. It helps in the maintenance of bones, muscles, eyes (vision), memory, sleep, lungs, infertility, skin and wrinkles.[12].Serum albumin is the most abundant protein constituent of blood plasma and serves as a protein storage component. HSA structure consists of three structurally-similar domains (I, II, and III), each containing two subdomains, A and B. Each subdomain has a main cavity for interaction

with ligands and, therefore, there are a total of six main cavities for interaction. The amino acid residue tryptophan (Trp) is often used for the association studies of this albumin with endogenous and exogenous molecules by fluorescence spectroscopy techniques. The HSA structure has only one Trp site, located in subdomain IIA (Trp-214), as shown in Figure (1). It is synthesized in the liver, exported as a non-glycosylated protein, and is present in the blood at about 40mg/mL (around 0.6 mM) [13]. It is the major transport protein for unesterified fatty acids, but is also capable of binding an extraordinarily diverse range of drugs, metabolites and organic compounds. The remarkable binding properties of albumin account for the central role, which can play in both the efficacy and rate of delivery of drugs. Many drugs, including anti-coagulants, tranquilizers and general anesthetics, are transported in the blood while bound to albumin (often more than 90% of drug is bound) [14]. This has stimulated a great deal of research on the interaction between small molecules and biomolecules, which are used to enucleate the transportation and distribution in the body and is very important in explaining interaction mechanisms, pharmacokinetics and toxicity of drugs. In this paper, we conducted a comparison between the interactions of vitamin E, Vitamin D and vitamin K with HSA under physiological conditions by utilizing UV absorption spectroscopy. In the meantime, the binding mode and the binding mechanism of these vitamins to HSA were discussed. Partial binding parameters of the reaction were calculated.

2. MATERIALS AND METHODS

2.1. Materials: HSA (fatty acid free), vitamin K_1 (Phylloquinone), vitamin E and vitamin D were purchased from Sigma Aldrich chemical company and used without further purifications. The data were collected using samples in the form liquid form for UV-VIS.

2.2. Preparation of stock solutions: Preparations of the thin film samples required three stock solutions as described below: HSA was dissolved in 25% ethanol in phosphate buffer Saline and at physiological (pH 6.9- 7.4), to a concentration of (80mg/ml), and used at final concentration of (40 mg/ml) in the final vitamin- HSA. Solution. Vitamins K_1, E, and D with molecular weights of (450.7 $g.mol^{-1}$, 430.71 $g.mol^{-1}$ and 384.64$g.mol^{-1}$ respectively), were dissolved in 25% ethanol in phosphate buffer Saline and, then the solutions were placed in ultrasonic water path (SIBATA AU-3T) for two days to ensure that all the amount of vitamin K_1 was completely dissolved.HSA concentrations were fixed at 40 $mg.ml^{-1}$ in all samples, and the concentration of vitamin K_1 , E, or D in the final HSA-vitamin solutions was decreased such that the molecular ratios (HSA: vitamin) are 1:20, 1:10, 1:5, 1:2, and 1:1. All samples were made by mixing equal volume from HSA to equal volume from different concentration of vitamins [15].Silicon windows (NICODOM Ltd) were used as spectroscopic cell windows, which produces a high optical transmission with little or no distortion of the transmitted signal. An amount of 40 µl of each sample of HSA –vitamin K_1 was spread on a silicon widow and an incubator was used to evaporate the solvent, to obtain a transparent thin film on the silicon window. All solutions were prepared at the same time and at room temperature 25^0c.[16].

2.3 UV-absorption spectroscopy: The absorption spectra of the measurements were performed by a Nano-Drop ND-1000 Fluorospectrometer at 25ºC. The excitation had been done at the wavelength of 210 nm and the maximum emission wavelength is at 280 nm. The excitation source comes from one of the three solid-state light emitting diodes (LED's). A 2048-element CCD array detector covering 220–750 nm, is connected by an optical fiber to the optical measurement surface. The emission spectra were recorded for free HSA (40 mg/ml) and for its complexes with vitamins, K, E and D solutions with different concentrations of HSA: Vitamins K, E and D.The solution of vitamins and

HSA were incubated for 1 h (at 25ºC) before spectroscopic measurements were taken.

3. RESULTS AND DISCUSSION

3.1. Analysis of UV-absorption spectroscopy of HSA by Vitamins K_1, E, and D: The UV absorbance intensity of HSA increased with the increasing of vitamin K_1 concentration as shown in Fig. (5). In addition, the binding of vitamin K_1 to HSA resulted in a slight shift of the HSA absorption spectrum. These results clearly indicated that an interaction and some complex formation occurred between HSA and vitamin K_1, and also indicated that the peptide strands of protein molecules extended more upon the addition of vitamin K_1 to HSA [17]. It is evident from the spectra of the pure vitamins that, the little or no absorption effect which supports that the resulted peaks are due to the interaction between the vitamin K_1 and HSA. The emission spectra were recorded for free HSA (40 mg/ml) and for its complexes with vitamin K_1 solutions with different concentrations of HSA: Vitamin K_1.The UV absorbance intensity of HSA increased with the increasing of vitamin K, Vitamin E or vitamin D concentration as shown in Fig.(5), Fig. (6) and Fig.(7)respectively . In addition, the binding of the vitamins to HSA resulted in a slight shift of the HSA absorption spectrum. These results clearly indicated that an interaction and some complex formation occurred between HSA and the two vitamins separately, and also indicated that the peptide strands of protein molecules extended more upon the addition of vitamin K, vitamin E or vitamin D to HSA [18-19]. It is evident from the spectra of the pure vitamins the little or no absorption effect which supports that the resulted peaks are due to the interaction between the vitamins and HSA. The value of the binding constant K between HSA and vitamin K_1, E, D can be determined using the data resulted from the UV-VIS spectroscopy according to the method described earlier in many published articles [17-20-21].

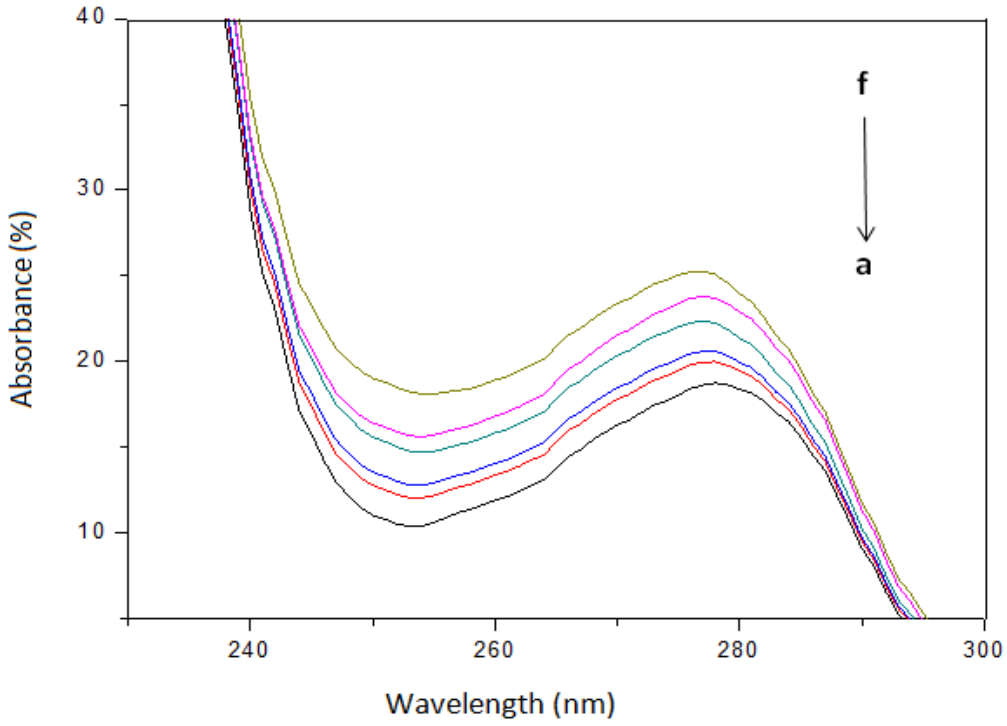


Fig. 5: UV-VIS absorbance spectra of HSA with different concentrations of Vitamin K1

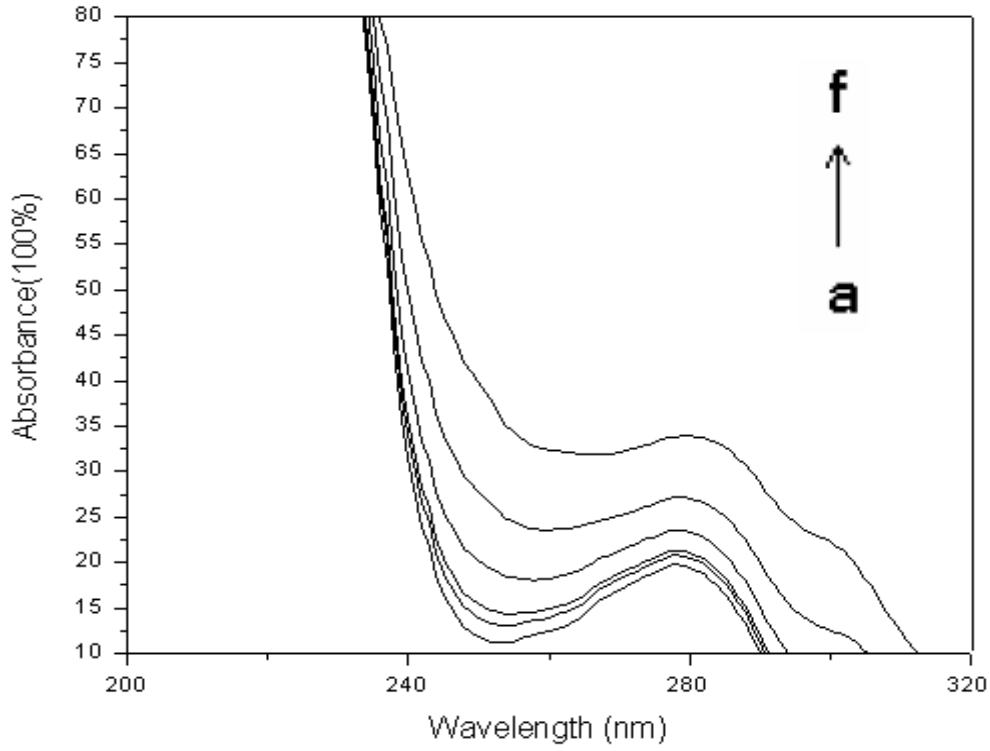


Fig. 6. UV-Absorbance spectra of HSA with different molar ratios of vitamin E

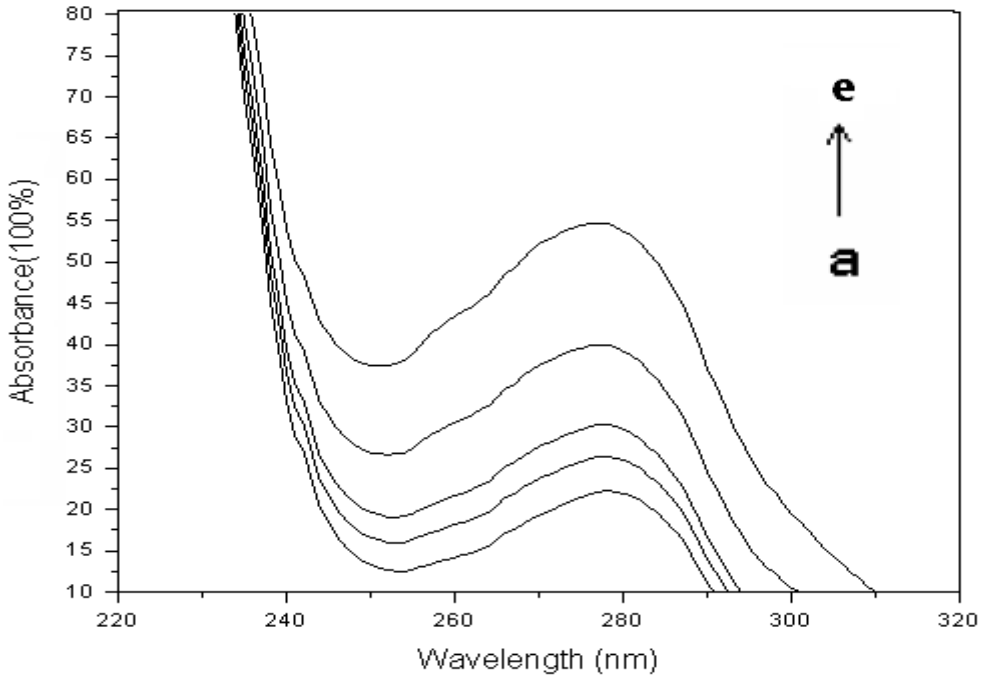


Fig. 7. UV-Absorbance spectra of HSA with different molar ratios of vitamin D.

By assuming that there is only one type of interaction between HSA and vitamin K_1, E, D in aqueous solution, which leads to establish equations (1) and (2) as follows:

HSA + vitamin $K_1, E, D \longleftrightarrow$ vitamin K_1, E, D : HSA (1)

$$K = \frac{[vita\ \min\ K_1, E, D : HSA]}{[vita\ \min\ K_1, E, D][HSA]} \quad (2)$$

The value of the binding constant K can be calculated using the following equation[22]:

$$\frac{1}{A - A_\circ} = \frac{1}{A_\infty - A_\circ} + \frac{1}{K(A_\infty - A_\circ)} \times \frac{1}{L}$$

Where $A_\circ$the initial absorption of the free protein at 280 nm is, A_∞ is the final absorption of the ligated protein, and A is the recorded absorption at different vitamin K_1, E, D concentrations (L) [22].

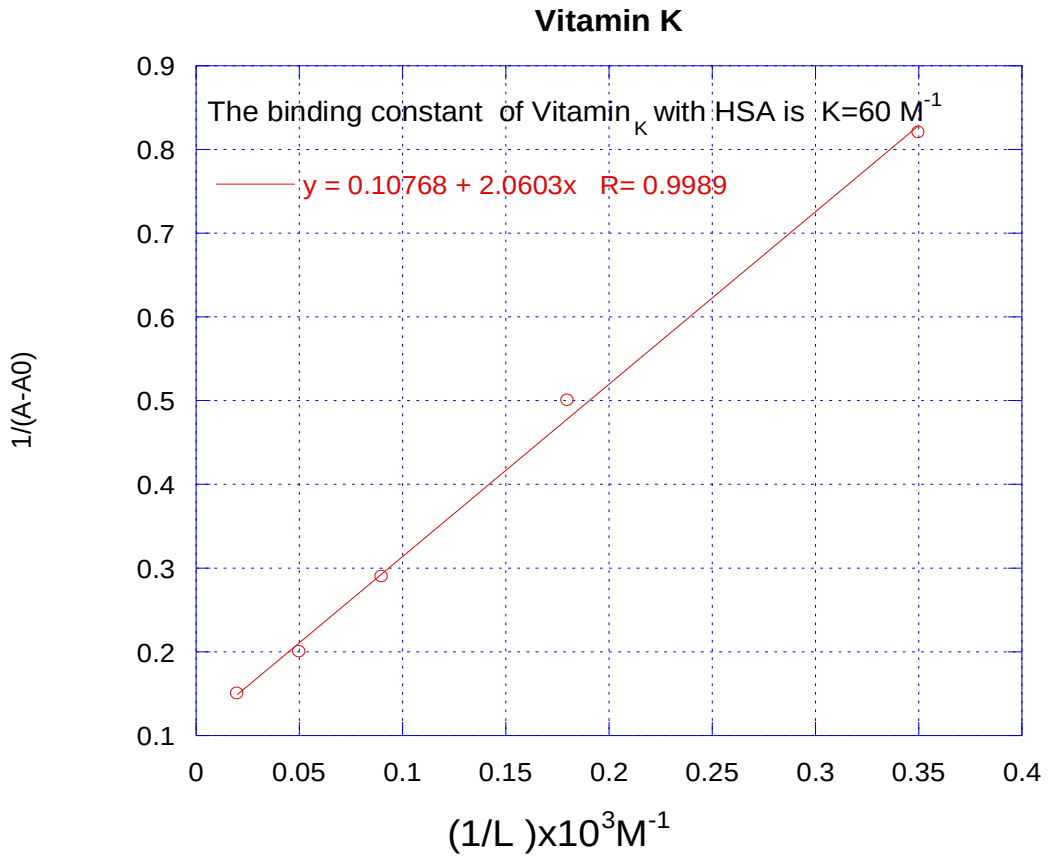


Fig.8: plot of 1/(A-Ao) vs. 1/L for HSA with different concentrations of vitamin K_1.

The double reciprocal plot of 1/($A - A_{\circ}$) versus 1/L is linear as in Fig.(8), Fig. (9) and Fig.(10) and the binding constant (K) can be estimated from the ratio of the intercept to the slope for vitamin K_1 which equals 60 M^{-1} Fig. (8), whilethe binding constant for vitamin E equals $1.21\times10^{2}M^{-1}$) Fig. (9) and for vitamin D equals ($6.8\times10^{1}M^{-1}$) Fig. (10). Figure (11) shows the reciprocal of the absorption records of different concentrations for the three vitamins as a function of the reciprocal concentration. It was obvious that the binding affinity of vitamin E to HSA is the greatest, then vitamin D which is almost the same as vitamin E is less and finally vitamin K is the smallest which indicates that the binding of vitamin E to HSA is the strongest as estimated. The value of the binding constant calculated show a weak HSA - vitamin K_1 interaction with respect to the other strong ligand–protein complexes of vitamins E and D which are much less than the binding constants of the others ligand–protein complexes that ranging from 10^5 to $10^6 M^{-1}$ [23]. The reason for the low stability of the vitamin K_1 :HSA complexes can be attributed to the presence of mainly hydrogen bonding interaction between protein donor atoms and the vitamin K_1 polar groups or an indirect vitamin K_1-protein interaction through water molecules [24]. While the reason for the low stability of the vitamin E or vitamin D-HSA complexes can be attributed to the presence of mainly hydrogen bonding interaction between protein and the vitamin E or vitamin D polar groups or an indirect vitamin - protein interaction through water molecules [25].

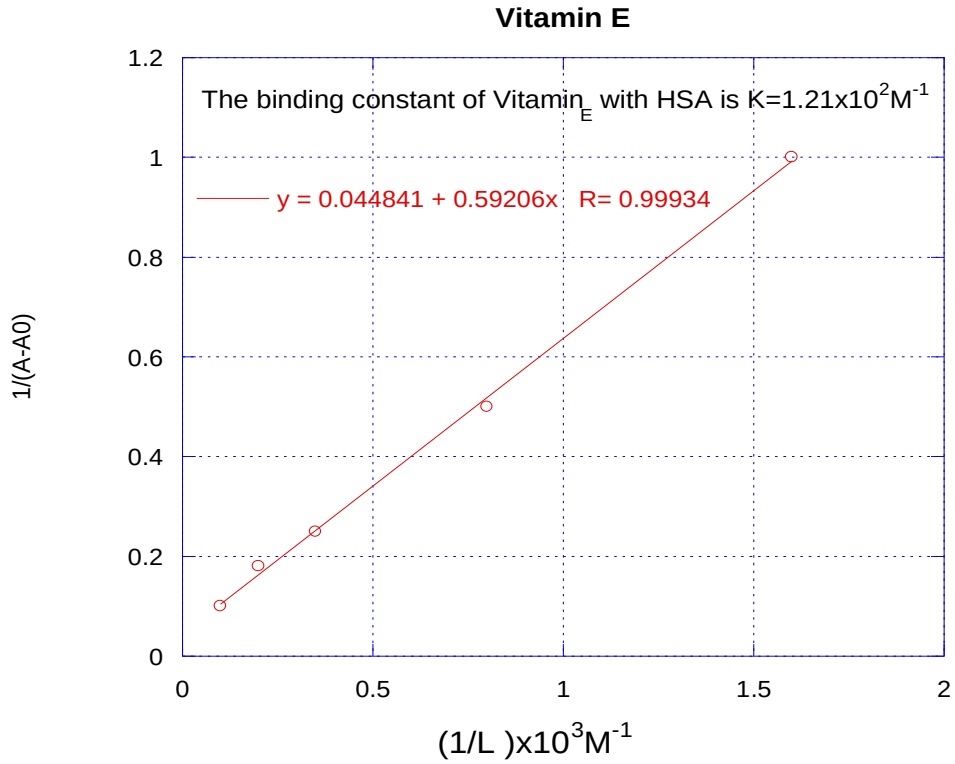


Fig.9: plot of 1/(A-Ao) vs. 1/L for HSA with different concentrations of vitamin E.

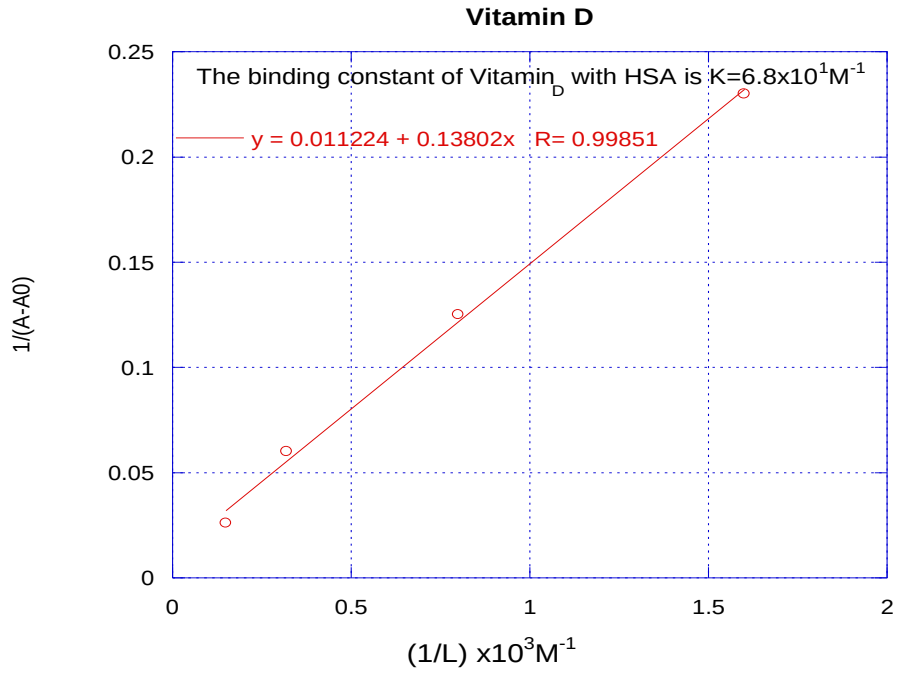


Fig.10: plot of 1/(A-Ao) vs. 1/L for HSA with different concentrations of vitamin D.

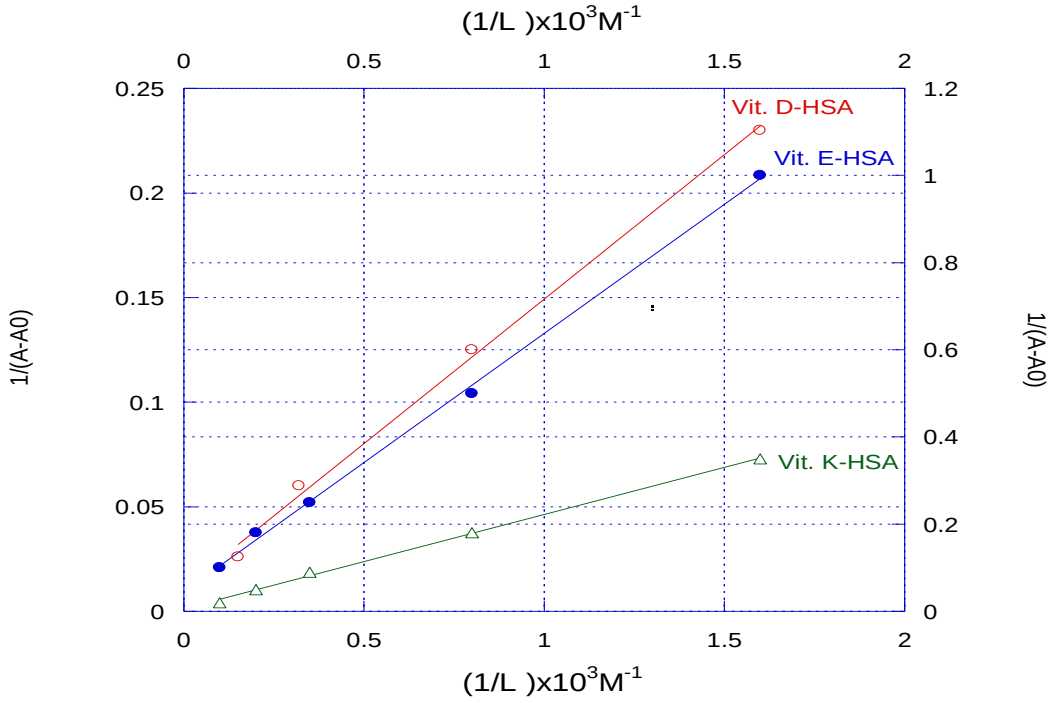


Fig. 11: plot of 1/(A-Ao) vs. 1/L for HSA with different concentrations of the three vitamins.

4. CONCLUSION

In this work, the interaction of these vitamins with HSA was investigated by means of UV-VIS spectrophotometer. The experimental results indicate a low binding affinity between these vitamins with HSA. From this study it was found that the binding affinity between vitamin k_1 - HSA is less than that between vitamin D- HSA less than that between vitamin E-HAS and therefore the dissociation constant of Vitamin k_1 –HSA> Vitamin D-HSA>Vitamin E-HSA. A high dissociation constant means low binding constant which implies that the ratio products: reactant is large. Now, if you have separate vitamins and ligands on the left, and a product on the right of an equation, a high dissociation constant, despite its name (equilibrium constant would be better) would infer great stability of the product a high affinity. The binding study of these vitamins with HSA is of great importance in pharmacy, pharmacology and biochemistry. This research can supply some important information to clinical research and provide the theoretical basis for the new vitamins designing. Furthermore studies will be a useful guide for synthesis of efficient these vitamins such as the determinations of binding sites, binding location, and thermodynamic parameters (enthalpy, free energy, entropy) at different temperatures to deduce the type of the acting force for the binding reaction between these vitamins with HAS to increase the dissociation that enable these vitamins to be delivery easily to the target. Furthermore, it is needed to investigate the effect of ions on the binding constants, because the existence of metal ions can directly influence the binding force of drug with protein and increase the dissociation constant. Thus, affecting the storage time of the drug in blood plasma and enhancing the maximum effectiveness of the drug.

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