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Binding of Naphthofuran derivatives to Bovine Serum Albumin by Distribution Experiments

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The interaction of 2-phenyl-3-*p*-(β-pyrrolidins)ethoxyphenyl naphthofuran hydrochloride with N,N-diethyl amino derivative to bovine serum albumin (BSA) at pH 7.4 was studied applying partition method. A comparision of binding constants of two types of compounds indicated stronger combination with N,N-diethyl amino derivative than that of pyrrolidine derivative. The intrinsic association constants being 1.62×10^3 with former and 1.53×10^3 for the later, respectively. The solvents used for the distribution experiments do not play any significant role deciding the extent of ligand-BSA interactions. The data indeed revealed that the value of n, K and ΔF^o was the same for bovine serum albumin either from toluene or from *n*-octyl alcohol.

Key Words: Bovine serum albumin, 2-Phenyl-3-*p*-(β-pyrrolidins)ethoxyphenyl naphthofuran.

INTRODUCTION

A considerable research work has been done on the mode of dispersion of various lipids like bile salts, phospholipids, long chain fatty acids and cholesterole in the biological constituents. Several highly water insoluble organic compounds have been proved as essential components of membrane proteins called lipoproteins. Several synthetic compounds of steroidal structures have been proved as good medicinal compounds in various fields of chemotherapy. Such compounds are derivatives of naphthofuran used as potent antifertility agents. These are reported to interact with purines and purine nucleotides[1], aromatic amino acids[2], polynucleotides[3], deoxynucleic acids[4-9]. In the case of organic molecules charge transfer interaction appears to occur between hydrophobic compound and the π-electron donors and same may be true for nucleic acids and proteins[2]. Anthracene is also reported to bind with the biological materials[10]. Goodman have studied the binding of long chain fatty acids to bovine serum albumin (BSA) by distribution between organic phase and aqueous

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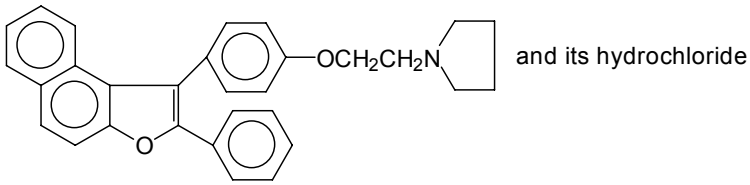
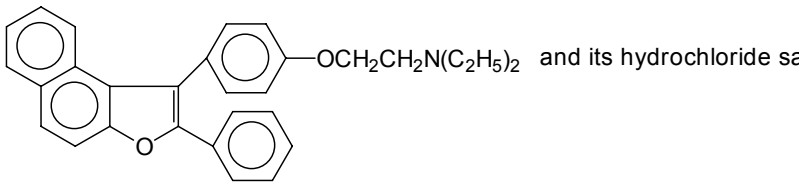
buffered solutions of protein[11]. In present studies, the binding of naphthofuran derivatives and BSA has been studied using distribution between organic and buffer phase containing the protein. The effect of pH on the binding has been critically discussed.

EXPERIMENTAL

A solution of bovine serum albumin (BSA) was prepared in double distilled water and its strength determined colorimetrically by biuret method. It was stored in a deep freez under purified toluene to check the surface denaturation.

Naphthofuran derivatives of the following types were obtained from Central Drug Research Institute, Lucknow, India.





Their stock solutions were prepared in toluence or *n*-octyl alcohol as desired.

Buffers of desired pH values were prepared from reagent grade (GR) chemicals with the help of an Elico-pH meter.

An Elico UV/Visible spectrophotometer was used for the estimation of naphthofurarn derivatives after equilibration. In UV spectra peaks at 219-220 and 330-345 μm represents the aromatic nucleus and the furan ring, respectively. For the analysis the organic and aqueous phases separately was evaporated and then extracted in ethanol, the absorbance recorded at 330 mm and concentrations were determined with the help of a calibration curve (Fig. 1).

Distribution experiments: (a) For these determinations 10 mL of organic phase (toluene or *n*-octyl alcohol) having varying amount of ligand were taken in stoppered neckless separating funels. To each of these 10 mL of the desired buffer was added. These were stopped and shaken on a mechanical shaken for 48 h to attain the equilibrium. The solutions were allowed to stand overnight and the organic and aqueous phases were

separated. Both organic and aqueous phases were estimated. (b) 10 mL of organic phase containing varying amounts of ligand were mixed with 10 mL of aqueous buffer containing a fix amount of BSA (1 g/L). These were shaken for 48 h. The concentration of organic and aqueous phase in each case was determined.

RESULTS AND DISCUSSION

The distribution coefficient of naphthofuran derivatives were determined to investigate the structure of the ligand in organic and aqueous phases after equilibration. There was no change in structure in organic and aqueous phases, respectively. While studying the effect of pH on the distribution coefficient of pure bases and their hydrochloride salts, the later exhibited a decreasing ratio of partition coefficient due to ionization of the salt to pure base. The distribution coefficient (k) was found to be 1:108 in buffer and organic phases, respectively. The binding of naphthofuran derivative to BSA is complied in Tables 1-3.

TABLE-1
BINDING CONSTANT OF BSA-DERIVATIVE I HYDROCHLORIDE AT DIFFERENT pH-VALUES

pH	n	$K \times 10^{-3}$	$\Delta F°$ (Kcal/mol)
5.60	7	1.552	-4.49
7.40	10	1.530	-4.48
10.50	8	1.744	-4.56

TABLE-2
BINDING OF (BASE) TO BSA AT pH 7.40

Medium	n	$K \times 10^{-3}$	$\Delta F°$ (Kcal/mol)
Toluene	9	1.522	-4.49
n-Octyl alcohol	9	1.530	-4.48

TABLE-3
BINDING OF N,N-DIETHYL AMINO DERIVATIVE TO BSA AT pH 7.4 (FROM TOLUENE)

Medium	n	$K \times 10^{-3}$	$\Delta F°$ (Kcal/mol)
Toluene	6	1.62	-4.52

In Tables 1-3 and Figs. 1-5, the data on the binding of naphthofuran derivates by BSA in different buffers at 30 ºC are given. Fig. 2 represents the logarithmic plots for base hydrochloride and free base, respectively. It

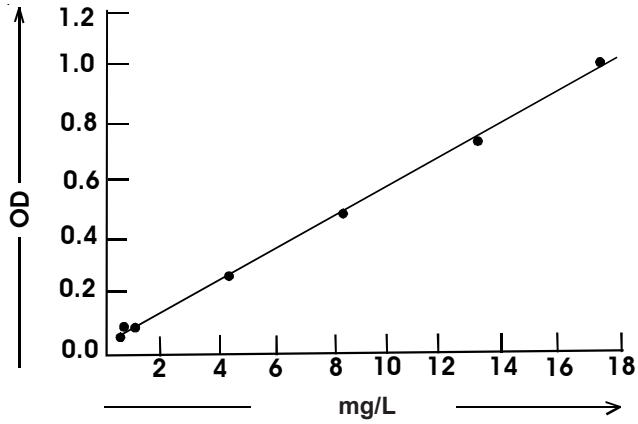


Fig. 1. Calibration curve of naphthofuran derivative at 330 nm

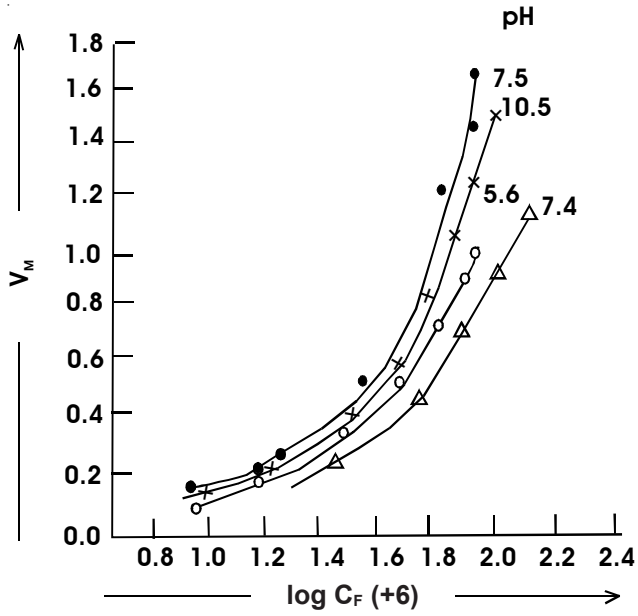


Fig. 2. V_M Plotted against log free concentration of ligand (log C_F)

has been presented as V_M, the number of naphthofuran derivatives molecule bound per mole of BSA, *vs.* the logarithm of the free equilibrium concentration of the organic ligand. The results revealed the increase of binding with rising free equilibrium concentration of the substrate. In this case no saturation limit was attained so no intrinsic association constants could be determined from the logarithmic plots. The binding at pH 7.4 was found to be maximum and least at pH 5.6 as is indicated form the positions of the isotherms. Further the binding of hydrochoride salt was

more than the free base at pH 7.4. The extent of binding was found to be highly pH dependent (Fig. 3). The V_M value start rising from pH 4.6 to 7.4 and then falls upto pH 10.5. It is also observed that the pyrrolidine derivative binds more than the diethylamino derivative (Fig. 3A and B).

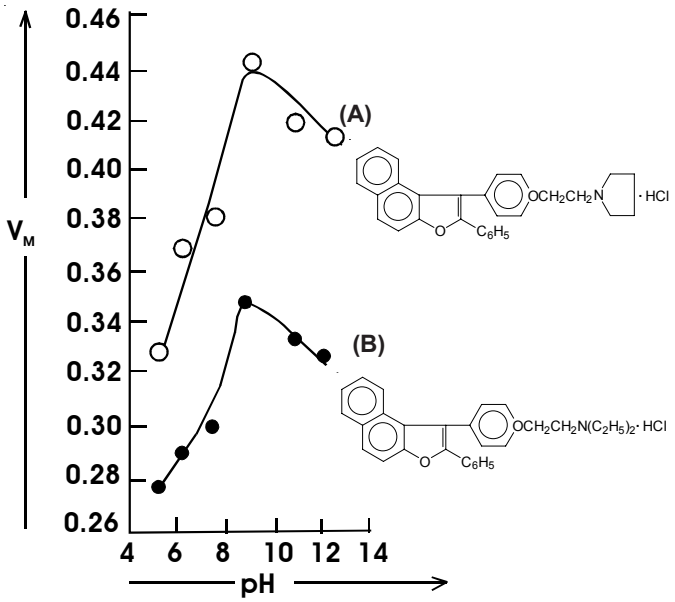


Fig. 3. V_M Plotted against pH for BSA naphthofuran derivatives systems

The experimental data were analyzed with the following equation to determine the association constant (K) and the maximum number of binding sites[12] (n),

$$\frac{1}{V_M} = \frac{1}{n} + \frac{1}{Kn} \times \frac{1}{C_F}$$

The plot of $1/V_M$ *vs.* $1/C_F$ would be linear in case of binding at a single set of equivalent non-interacting sites. The plots were linear over the range of $1/C_F$ (Figs. 4 and 5). From the linear plots the values of K and n were obtained which are given in Tables 1-3. These binding results revealed that the extent of binding decreased below and above the physiological pH *i.e.* pH 7.40. This conclusion could be correlated with the observation on optical rotation changes which are due to structural changes, within serum albumin molecule. It has been shown that a large change in the tertiary structure of the proteins occurs in the pH range of 7 to 9. Towards more basic side the decreased binding may be possible due to the deprotonation of naphthofuran derivatives as well as those of the side chain protonated nitrogen groups of the protein.

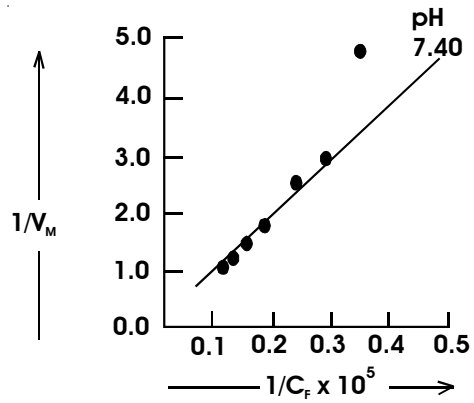


Fig. 4. Plot of $1/V_M$ *vs.* $1/C_f$ for BSA-naphthofuran derivatives system in toluene at pH 7.40

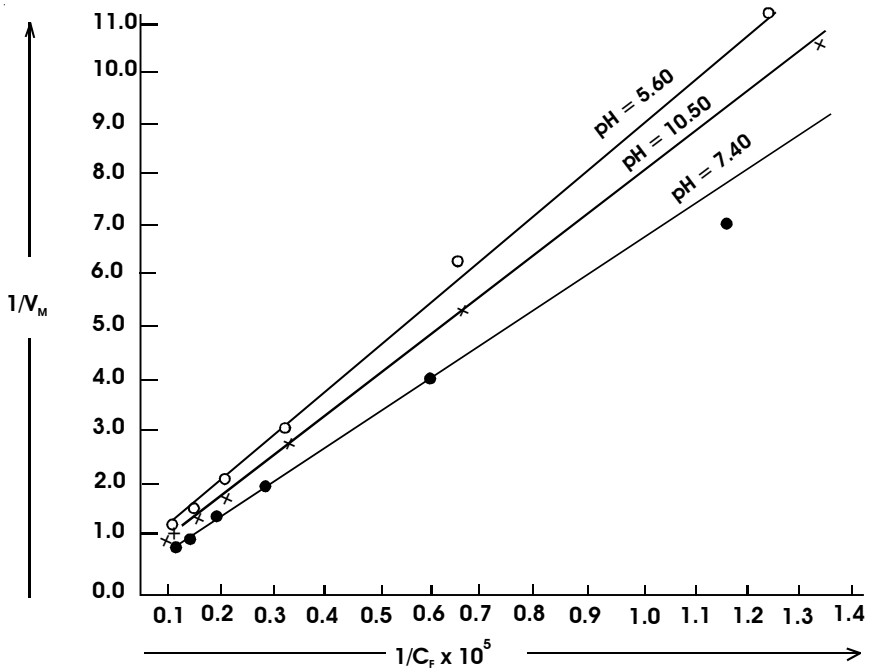


Fig. 5. Plot of $1/V_M$ *vs.* $1/C_f$ for BSA-naphthofuran derivatives in *n*-octyl alcohol at different pH values

Increases in BSA concentration decreased the binding of naphthofuran derivative, the V_M values were found to be 0.54, 0.45, 0.44, 0.43 and 0.506 at 0.724 at 0.724×10^{-5} M, 1.448, 2.896, 3.620 and 4.344×10^{-5} M BSA concentrations, respectively for pyrrolidine hydrochloride salt at pH 7.4. Klotz and Urquhart[13] have reported that the binding of methyl orange by

BSA was dependent on the protein concentration. Ray *et al.*[14] observed that one BSA protein solution bound less dodecyl sulphate and dodecanol than 0.1 per cent protein solution. Many hypotheses have been offered to explain the BSA concentration effect[15,16]. The most convincing explanation appears to be that the kinetics of equilibration may be slower in the case of concentrated protein solutions. However, in the present studies, the authors have not able to test this type of kinetics of binding and so the hypotheses.

The comparsion of binding constants of the two types of derivatives indicated that stronger combination occured with N,N-diethyl amino derivative than the pyrrolidine derivative. The intrinsic association constants being 1.62×10^3 with former and 1.53×10^3 for the later, respectively. A comparison of binding data of protonated and unprotonated ligands again pointed striking differences in the binding ability. A protonated nitrogen atom could interact with the unprotonated carboxylate residues, the binding with tyrosine groups is unlikely since these remain hydrogen bonded and only could be available after protein denaturation. The solvent used for the distribution experiments does not play any significant role in deciding the extent of organic ligand -BSA interaction. The experimental data indeed revealed that the value of n, K and ΔF° is same for BSA either from toluene or *n*-octyl alcohol *i.e.*, n = 9, $K = 0.97 \times 10^3$ and ΔF° = -4.13 kcal/mol, respectively for the ligand pyrrolidine hydrochloride.

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