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Quantitative Relationships between Protein Binding Affinities and Physico-chemical and Structural Parameters of some Aniline derivatives

NEELAM SEEDHER* AND GARIMA GOEL
Department of Chemistry, Panjab University, Chandigarh-160 014.

The binding of a series of aniline derivatives to bovine serum albumin was investigated using fluorescence spectroscopic technique. Nineteen physico-chemical and structural parameters of drugs could be divided into four groups: steric, electronic, hydrophobic and molecular connectivity parameters. In order to understand the binding mechanism and to predict binding affinity, association constants for drug-protein binding have been correlated to physico-chemical and structural parameters of drugs using statistical methods. Significant correlation could only be obtained on removing from analysis the two relatively low molecular weight drugs, benzocaine and paracetamol. Hydrophobicity of drug was not found to be a significant parameter. The size of the drug molecule and electrostatic interactions play a major role in the binding of these drugs to serum albumin. Quadratic equations gave a better fit of the data. Quantitative relationships between association constant for drug-protein binding and various statistically significant physico-chemical and structural parameters of drugs were obtained. The relationships can be useful in the prediction of association constants.

Most of the administered drugs are retained by plasma proteins, which act as major drug storage sites. The stored drug is in equilibrium with the free drug in plasma and is released as the free drug concentration falls below the therapeutic value. Thus the availability of drug at the site of action is maintained and the duration of action is prolonged[1]. The nature of forces involved in drug-protein interaction also play a significant role in drug action. Because dissociation of drug-protein complex can occur only when the driving force of dissociation is greater than the forces accounting for the binding[2].

Quantitative correlation between physico-chemical and structural properties of drugs and their protein binding affinities play an important role in understanding the nature of forces responsible for drug-protein complexation and the prediction of drug binding affinities. Most of the studies on correlation of plasma protein binding of drugs to physico-chemical parameters have been limited to hydrophobicity[3-4]. Some reports on the influence of pK_a value and solubility parameter of drug on protein binding are also available[5-6]. Recently it was shown that quantitative structure- and quantitative property-protein binding relationships are useful mathematical models to understand and predict protein binding affinities of phenothiazine derivatives and non-steroidal antiinflammatory drugs[7-9]. However, detailed studies on the role of steric, electronic, hydrophobic and structural parameters, which play a key role in such interactions, has not been explored. Aniline derivatives constitute some of the frequently used analgesic and antibacterial drugs. In the present work an attempt has been made to correlate the protein binding affinities of a series of aniline derivatives to various physico-chemical and structural parameters of drugs.

*For correspondence
E-mail: nseedher@yahoo.com

MATERIALS AND METHODS

Pure drug samples were obtained as gifts from various manufacturers. Serum albumin, bovine (BSA) was obtained from Sigma Chemical Company, St. Louis, USA. All other reagents were of analytical grade. All solutions were pre-

pared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl. BSA solutions were prepared based on molecular weight of 65000. Perkin Elmer fluorescence spectrophotometer (MPF-44B) equipped with a 150W Xenon lamp source was used.

Drug-protein binding:

Interaction of benzocaine, paracetamol, sulfacetamide sodium, sulfadiazine, sulfadoxine, sulfamethizole and trimethoprim with serum albumin was studied using fluorescence spectrophotometric technique. On the basis of the preliminary experiments, albumin concentration was kept fixed at 25 µM. Two millilitres of 25 µM BSA solution was taken in the quartz cuvette and increasing amounts of 525 µM drug solution was added. BSA concentration was kept constant (25 µM) by adding same volume of 50 µM BSA. The final drug concentration was close to 150 µM corresponding to drug:protein ratio of 6:1. Fluorescence spectra were recorded at 25° in the range 280-400 nm, keeping excitation wavelength 296 nm in each case. The absorbances of drug-protein mixtures in the concentration range employed for the experiments did not exceed 0.05 at the excitation wavelength in order to avoid inner filter effect. The drugs used in the present work did not have any intrinsic fluorescence.

Fluorescence spectroscopic data was analysed according to the method of Levine[10-11] to obtain association constants for drug-protein binding. Since the total concentration of ligand (drug) is much greater than the total acceptor (protein) concentration, the concentration of bound drug does not contribute appreciably to the total concentration of drug. Under such conditions, the free drug concentration can be essentially equated with the total drug concentration and the maximum change in fluorescence intensity, ΔF_{max} can be obtained from the intercept at $1/D_T=0$ of the conventional double reciprocal plots ($1/\Delta F$ versus $1/D_T$, where D_T is the total concentration of drug added)[12]. $1/\Delta A$ versus $1/D_T$ plots for one representative sample are shown in fig. 1. The stoichiometry of the interaction was assumed to be 1:1 so that the calculated association constants represent the weighted average of several binding sites[13]. Fraction of occupied sites on the protein molecule, $\theta = \Delta F/\Delta F_{max}$. Since a single binding site is assumed, $D_B=P_B=\theta P_T$. Concentration of free drug, $D_F=D_T-D_B=D_T-\theta P_T$. Concentration of free protein, $P_F=P_T-P_B=P_T-\theta P_T$. Moles of drug bound per mole protein, $r = D_B/P_T=\theta P_T/P_T=\theta$. P_T and D_T are the total protein and drug concentration, respectively and P_B and D_B are the bound protein and drug concentrations, respectively. The association constant, K for the binding equilibrium could be obtained from the slope of the θ/D_F versus θ plots. θ/D_F versus θ plot for sulfadiazine is shown in fig. 2.

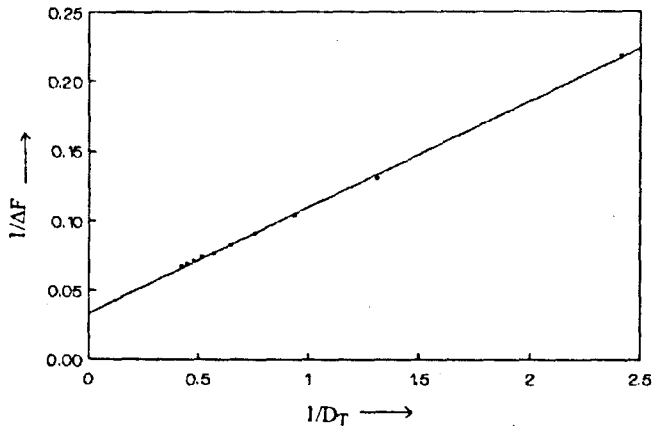

Fig. 1: $1/\Delta F$ Versus $1/D_T$ plot.

$\Delta F = F_0 - F$, is the decrease in the fluorescence intensity of BSA on the addition of sulfadiazine and D_T is the total drug concentration.

Physico-chemical and structural parameters of drugs:

Physico-chemical parameters; molecular weight (MW), melting point (MP), ionization constant (pK_a) and solubility (Sol) of various drugs were taken from Merck index[14]. Partition coefficient (log PC) was calculated using software MOLCONN-Z[15]. Conductivity of different concentrations (0 to 500 µM) of drug solutions in water were determined at 20° on a digital conductivity meter. Conductivity of various drugs at infinite dilution (K_0) was obtained from the intercepts of the linear conductivity versus drug concentration plots.

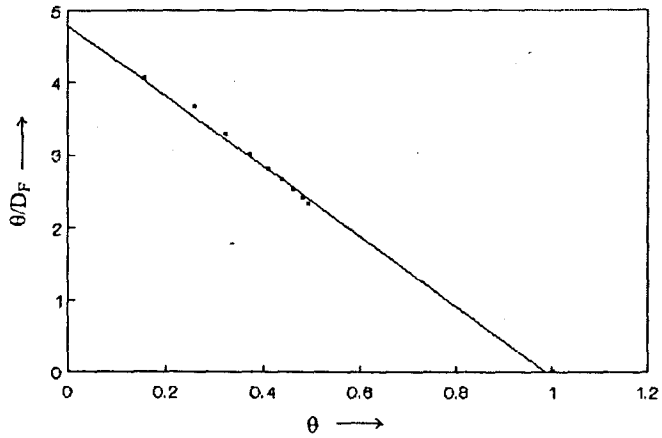

Fig. 2: θ/DF Versus θ plot.

$\theta = (F_0 - F)/F_0$, is the fraction of sulfadiazine bound and D_F is the free sulfadiazine concentration.

Thirteen structural parameters for various drug samples, Taft steric parameter (ES), van der Waal's volume (v_w), molar refractivity (MR), parachor (PR), Hammett constant for p-substitution (σ_p^m), substituent electronegativity constant (σ_x), group dipole moment for aromatic substituents (μ_{ar}), lipophobic constant for aromatic substituents (Λ_{ar}), aromatic substituent constant (π_{ar}), and zeroeth order and zeroeth order valence, first order and first order valence molecular connectivity indices ($^0\chi_{ar}$, $^0\chi^v_{ar}$, $^1\chi_{ar}$, $^1\chi^v_{ar}$,) were calculated using the tables compiled by Waterbeemd[16]. Values for various parameters were obtained by adding values for various substituents or groups in the drug molecules.

Statistical analysis:

Linear regression analysis and non-linear regression analysis was performed on the statistically significant variables using the statistical software, SPSS for windows® (SPSS Inc., Chicago, IL). The following parameters were determined: the correlation coefficient (R), the coefficient of determination (R^2), and the significance of the regression model (F).

RESULTS AND DISCUSSION

Fluorescence spectrum of BSA (280-360 nm) after excitation at 296 nm gave a single peak at ~ 340 nm due mainly to the tryptophan residues of BSA. Each BSA molecule contains two tryptophan residues. Addition of drug resulted in quenching of fluorescence of BSA in all cases. However, no noticeable shift in wavelength was observed. The fluorescence intensity continued to decrease and a saturation point could not be attained experimentally. The maximum decrease in fluorescence intensity, ΔF_{max} and the association constant for drug-protein binding were obtained by following the method outlined in the experimental section. The association constants for various samples (Table 1) are seen to vary as sulfadoxine > trimethoprim > benzocaine > sulfadiazine > sulfamethizole > sulfacetamide sodium > paracetamol.

This order could not be explained easily on the basis of molecular size, hydrophobicity or ionic character of the drugs. In order to further understand the binding behaviour and to predict drug-binding affinity, an attempt has been made in the next section to correlate the protein binding affinities of the aniline derivatives used in the present work, expressed in terms of association constants to various physico-chemical and structural parameters of drugs using statistical methods.

Various experimentally determined and theoretically calculated physico-chemical and structural parameters for

TABLE 1: ASSOCIATION CONSTANTS FOR THE BINDING OF VARIOUS ANILINE DERIVATIVES TO BOVINE SERUM ALBUMIN.

Drug Sample	Association constant (K) x 10^{-3} M^{-1}
Benzocaine	8.913
Paracetamol	1.070
Sulfacetamide sodium	1.622
Trimethoprim	9.129
Sulfadoxine	12.255
Sulfadiazine	4.862
Sulfamethizole	4.651

Association constants were determined at 25° using fluorescence spectroscopic technique[8].

the drugs used in the present work are given in Table 2. Most of the parameters could be divided into four groups: steric, electronic, hydrophobic and molecular connectivity parameters. When a drug molecule interacts with a receptor, a proper fit can occur only when a good spatial complimentarity exists between the two interacting species. Steric factors are thus important properties to describe and parametrize in terms of volume, surface area, length, width etc. Various steric parameters have been computed and applied to drug design[17-18]. Amongst steric paramaters, Taft steric parameter (Es), van der Waal's volume (V_w), molar refractivity (MR), parachor (PR) and molecular weight (MW) were used in the present work.

Electronic parameters play an important role in pharmacodynamic and pharmacokinetic events as they influence all non-covalent interactions such as ionic interactions, ion-dipole interactions, instantaneous dipole-induced dipole interactions, hydrogen bonds involved in drug-protein interaction. Electronic parameters included in the present work are hammett constant for p-substitution (σ_p^m), substituent electronegativity constant (σ_x), group dipole moment for aromatic substituents (μ_{ar}), lipophobic constant for aromatic substituents (Λ_{ar}) and ionization constant (pK_a). Hydrophobic parameters reflect binding to a hydrophobic (i.e. lipophilic) site in a receptor molecule. Aromatic substituent constants (π_{ar}) and partition coefficient (log PC) are used as hydrophobic parameters. Even molar refractivity (MR) and parachor (PR) contribute to hydrophobicity of the molecule to some extent. Molecular connectivity is the manner in which atoms are connected or branched in a molecule. It is funda-

TABLE 2: PHYSICO-CHEMICAL AND STRUCTURAL PARAMETERS FOR THE DRUGS STUDIED.

Drug Sample	Physico-chemical and structural parameter									
	MW	pK_a	K_D	MP	Sol	V_w	MR	ES	π_{ar}	σ_p^m
Benzocaine	165.19	2.80	0.400	90.0	0.400	7.65	47.22	-3.60	1.24	-0.18
Paracetamol	151.16	9.90	0.010	170.5	-	7.37	42.11	-3.50	0.32	-0.53
Sulfacetamide sodium	254.24	5.40	0.333	257.0	0.667	9.30	52.18	-4.86	-1.64	-0.71
Trimethoprim	290.32	6.60	1.270	203.0	0.040	14.18	70.33	-7.81	0.02	-1.42
Sulfadoxine	310.34	-	0.400	194.0	-	13.64	74.63	-7.06	-1.11	0.49
Sulfadiazine	250.28	6.50	-	256.0	1.600	10.82	59.92	-5.96	-1.07	1.03
Sulfamethizole	270.33	5.45	1.400	208.0	4.500	11.68	66.38	-4.22	-0.69	1.27
	PR	logPC	σ_x	μ_{ar}	Λ_{ar}	$^0\chi_{ar}$	$^0\chi^v_{ar}$	$^1\chi_{ar}$	$^1\chi^v_{ar}$	
Benzocaine	379.3	1.79	0.81	-0.35	-3.07	10.605	8.488	6.086	4.104	
Paracetamol	344.3	0.65	0.91	-5.27	-3.44	10.027	7.742	5.873	3.589	
Sulfacetamide sodium	483.3	0.52	0.83	-4.70	-7.01	13.397	9.878	7.523	4.563	
Trimethoprim	644.6	1.21	2.69	-7.28	-7.61	20.347	16.892	10.893	7.106	
Sulfadoxine	671.5	0.69	1.87	-11.08	-8.82	19.347	14.913	11.307	6.864	
Sulfadiazine	552.3	0.28	0.99	-8.42	-7.40	14.933	11.097	9.185	5.640	
Sulfamethizole	613.7	0.83	1.41	-8.53	-7.35	16.941	13.086	9.202	6.028	

Various parameters were taken from Merck Index/experimentally determined/calculated using the tables compiled by Waterbeemd[14].

mental characteristic of the structure of a molecule. Zeroeth order index, $^0\chi$ reflects general features such as the number of atoms in the molecule, while first order index $^1\chi$ is related to the molecular volume. Valence molecular connectivity index χ^v is used in molecules that have heteroatoms. In the present work molecular connectivity indices, $^0\chi_{ar}$, $^0\chi^v_{ar}$, $^1\chi_{ar}$, $^1\chi^v_{ar}$ were used.

Correlation of association constants with physico-chemical and structural parameters of drugs was done using linear and non-linear regression analysis. For correlation purpose, association constant (log K) was taken as the dependent variable and physico-chemical and structural parameters were taken as independent variables. Various statistical parameters were obtained from the bivariate correlation matrix. In general there was a poor correlation; the highest value of correlation coefficient, R being 0.658 for the steric parameter, MR. The coefficient of determination R^2, gives the fraction of variance explained by the regression equation. Since even the highly correlated parameter could explain only 43% of the variance in the data, the data could not be used for prediction of association constants. Correlation coefficients, however, improved significantly when two relatively low molecular weight drugs, benzocaine and paracetamol were removed from analysis. The values are given in Table 3. Correlation coefficients were now above 0.900 for six parameters and above 0.800 for five parameters. Molecular connectivity indices, $^1\chi_{ar}$, $^1\chi^v_{ar}$ and molar refractivity (MR) were highly correlated parameters with correlation coefficients, 0.990, 0.962 and 0.959, respectively, significant up to 0.01 level. Three other parameters. Van der Waal's volume (V_w), parachor (PR), and molecular connectivity index, $^0\chi_{ar}$ with correlation coefficients, 0.941, 0.952 and 0.908, respectively, were also significant up to 0.05 level The highest correlated parameter, $^1\chi_{ar}$ could explain 98% of variance while other parameters could explain 82-93% of

TABLE 3: CORRELATION BETWEEN ASSOCIATION CONSTANTS AND VARIOUS PHYSICO-CHEMICAL AND STRUCTURAL PARAMETERS.

Physico-chemical/ Structural Parameter	Correlation Coefficient (R)	
	Linear	Quadratic
MW	0.830	0.831
PK_a	0.754	0.754
K_0	0.261	0.770
MP	-0.805	0.840
Sol	-0.008	0.386
V_w	0.941	0.967
MR	0.959	0.961
ES	0.746	0.764
π_{ar}	0.620	0.744
σ_p^m	0.063	0.207
PR	0.952	0.952
logPC	0.462	0.472
σ_x	0.773	0.908
μ_{ar}	-0.833	0.848
Λ_{ar}	-0.821	0.997
$^0\chi_{ar}$	0.908	0.935
$^0\chi^v_{ar}$	0.859	0.922
$^1\chi_{ar}$	0.990	0.997
$^1\chi^v_{ar}$	0.962	0.969

Correlation coefficients were determined from the bivariate correlation matrix using statistical software, SPSS.

variance in the data. Quadratic equations gave a better fit of the data (Table 3).

In general, it is seen that the steric parameters are highly correlated. Electronic parameter come next in importance and hydrophobic parameters have lowest correlations. Hydrophobic parameters, π_{ar} and log PC have very low R values; 0.620 and 0.462, respectively. It may therefore, be inferred that the hydrophobicity of drug, which is considered to be an important parameter in drug-protein binding[19], is not a significant parameter in these drugs. Thus the size of the drug molecules and electrostatic interactions play a major role in the binding of these drug to serum albumin. The importance of the size of drug molecules is also apparent from the fact that the correlations improved dramatically on removing two relatively low molecular weight drug from analysis. Quantitative relationships between association constant, log K for drug-protein binding and some of the statistically significant parameters are given in Table 4. The relationship can be useful in the theoretical calculation of association constants.

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TABLE 4: QUANTITATIVE RELATIONSHIPS BETWEEN ASSOCIATION CONSTANTS AND PHYSICO-CHEMICAL/STRUCTURAL PARAMETERS OF DRUGS.

Parameter	Equation	Statistical Parametes			
		R	R^2	F	Sig. F
$^1\chi_{ar}$	Log K = 0.220 $^1\chi_{ar}$ + 1.603	0.990	0.979	142.45	0.001
$^1\chi^v_{ar}$	Log K = 0.319 $^1\chi^v_{ar}$ + 1.795	0.962	0.926	37.53	0.009
MR	Log K = 0.0366 MR + 1.352	0.959	0.919	34.16	0.010
$^1\chi_{ar}$	Log K = -0.0232($^1\chi_{ar}$)^2 + 0.6593 ($^1\chi_{ar}$) -0.4353	0.997	0.994	163.62	0.006
Λ_{ar}	Log K = -0.6015 (Λ_{ar})^2 - 10.013 (Λ_{ar}) -37.435	0.997	0.995	193.19	0.005
$^1\chi^v_{ar}$	Log K = -0.0482 ($^1\chi^v_{ar}$)^2 + 0.8837 ($^1\chi^v_{ar}$) - 0.0482	0.969	0.939	15.35	0.061

Association constant, expressed as Log K, was taken as dependent variable and physico-chemical/structural parameters were taken as independent variables. Relationships are given for parameters significant up to 0.01 level.

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