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Correlation between Physico-Chemical Properties and Protein Binding of Phenothiazine Derivatives

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The binding of a series of phenothiazine derivatives to bovine serum albumin was investigated using fluorescene spectroscopic technique. In an attempt to elucidate the binding mechanism and to predict binding affinity, association constants for drug-protein binding have been correlated to physico-chemical parameters by fitting linear, multiple linear and quardratic equations to the data. The rationale of including molecular weight and molecular volume terms in the quantitative structure-activity correlations has been emphasized. It has been concluded that binding primarily involves hydrophobicity and molecular size of drug cations. However, the role of electrostatic forces as measured by ionization constant, pK_a and molar conductance cannot be ignored. The association constants could be predicted with a fair degree of accuracy from physico-chemical properties of the drug molecules.

Most of the administered drugs are retained by plasma proteins which act as major drug storage sites. The nature of binding forces and the degree of protein binding has important pharmacokinetic and pharmacodynamic implications, since it is the unbound moiety that readily diffuses across biological membranes, reaches the receptor site to produce pharmacological effect and is most readily available for elimination from the body.

Phenothiazine derivatives, widely used as antipsychotic, tranquilizer and sedative drugs, are highly bound to plasma proteins[1]. The nature of binding forces have been widely investigated. Some authors[2-4] have considered the binding to be predominantly hydrophobic while others[5] have suggested the role of electrostatic interactions. Since binding usually depends on the physico-chemical properties of the drug, quantitative relationships between physico-chemical properties and binding affinities may provide an insight into the mechanism of binding. Most such studies reported in the literature pertain to correlation of hydrophobicity of drugs to association constants[6-8]. An attempt has been made to elucidate the mechanism of binding of phenothiazine compounds to serum albumin and to predict drug binding affinity from physico-chemical parameters of the drugs.

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MATERIALS AND METHODS

Bovine serum albumin (BSA, Fraction V) was obtained from Sigma Chemical Company, St. Louis, USA. Phenothiazine derivatives were obtained as gifts from various manufacturers. All other materials were of analytical grade and all solutions were prepared 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 150 W Xenon lamp source was used.

Phenothiazine-BSA interaction:

Interaction of five phenothiazine derivatives, chlorpromazine hydrochloride, promethazine hydrochloride, thioridazine hydrochloride, trifluoperazine dihydrochloride and triflupromazine hydrochloride with BSA was studied using fluorescence spectrophotometric technique. On the basis of preliminary experiments, BSA concentration was kept fixed at 24 μM and drug concentration was varied from 20 to 140 μM. Fluorescence spectra were recorded

at 37° 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. Fluorecence spectroscopic data was analysed using the Ward method[9] to obtain association constants for drug-protein binding.

Physico-chemical properties:

Molecular weight and melting point values for various drugs were taken from The Merck index[10]. Ionization constant (pK_a), partition coefficient (logP) and distribution coefficient (log D) values were taken from Franke *et al.*[11]. Parachor values were calculated from the atomic parachors and other structural features of drug molecules[12]. Van der Wall's volume, V_w was obtained from the characteristic volumes, V_x using the relationship $V_w = 0.7V_x$[13]. The characteristic volume (V_x) is an estimate of the molar volume of the drug at absolute zero and is calculated by summing individual contributions for each atom in the molecule and subtracting a contribution for each bond.

pH of 1 mM solution of drugs in water was determined at 25° on an Elico pH meter with glass and calomel electrodes. Surface tensions of 10 mM drug solutions in water were measured at 25° by drop number method using a stalagmometer[14]. All surface tension data are expressed in terms of surface pressure, SP, which is the difference between the surface tension of solvent and that of a given solution being measured[15]. Thus as surface tension is reduced, the surface pressure increases. Conductivity of 1 mM solution was determined on a digital Century conductivity meter with cell constant 0.58 cm^{-1}. Conductivity data are expressed as molar conductance, Λ_m.

Statistical Methods :

Regression of the data was carried out using SPSS for windows® (SPSS Inc., Chicago, IL). The following parameters were determined. The correlation coefficient r, coefficient of determination r^2, the significance of the regression model, F and the standard error, SE.

RESULTS AND DISCUSSION

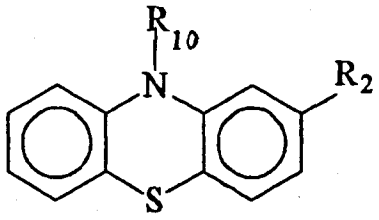
Table 1 illustrates the chemical structures of five phenothiazine derivatives used in the present study. Association constants for drug-protein interaction were determined by fluorescence spectrophotometric technique described earlier[16]. The values are recorded in Table 2. Correlation of association constant with physico-chemical properties was carried out using linear regression analysis, non-linear regression analysis and multiple linear regression analysis.

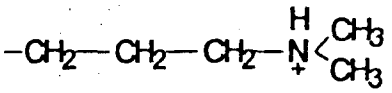
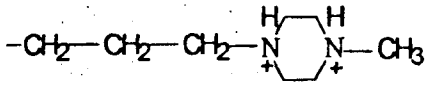
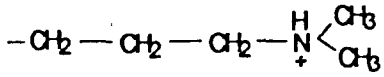
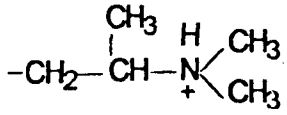
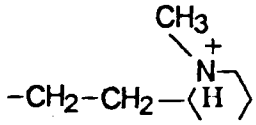
Physico-chemical parameters for various drug samples are recorded in Table 2. Correlation coefficients were obtained from the bivariate correlation matrix between association constant, logK for drug-protein binding and physico-chemical paramters of drugs. There is no correlation between association constant and melting points of drugs(r=0.022). Thus intermolecular forces in the drug molecules do not play any role in binding. Also there is practically no correlation between pH of aqueous drug solutions and association constants (r=0.356). The pK_a values which depend upon the acidity of drug cations have a small negative correlation with the protein binding parameter (r=0.507). Thus binding affinity increases with decrease in pK_a value i.e., with increase in the strength of the acid R_3NH^+. This is reasonable since at pH 7.4, protein has a net negative charge (isoelectric point =5.0) and thus it can interact with protons from drug cations. Thus electrostatic interactions play a role in such interactions. However, small correlation shows that other parameters are also involved.

There is a small positive correlation between molar conductivity of aqueous drug solutions and association constants (r=0.553). The drugs used in this study are the salts of weak bases and strong acids. Molar conductance of drug will depend upon the degree of ionization of the weak base formed on hydrolysis of drug salts and degree of hydration of the drug cations. The degree of inoization of the weak base will depend upon the stability of the cation formed on dissociation of the weak base. More stable the cation formed, more easily the base will dissociate and the stronger the base will be. The stability of cation, R_3NH^+ in this case, will depend upon the relative electron releasing affinity of -R groups attached to the N atom. Since correlation coefficient is positive, it may be inferred that more stable drug cations have higher protein binding affinity. However, the effect is minor since correlation coefficient is small. Moreover, since electric field on the surface of a sphere of radius R is proportional to $1/R^2$, smaller cations are more extensively hydrated and hence have lower conductance and lower protein binding affinity than the larger ones. This also shows that electrostatic interactions play a role in such interac-

TABLE 1 : STRUCTURES OF PHENOTHIAZINE DERIVATIVES INVESTIGATED IN THE PRESENT STUDY

Phenothiazine



Name of Drug	R_2	R_{10}
Chlopromazine hydrochloride	- Cl	
Trifluoperazine dihydrochloride	- CF_3	
Triflupromazine hydrochloride	- CF_3	
Promethazine hydrochloride	- H	
Thioridazine hydrochloride	- SCH_3	

tions. However, again small correlation shows that other parameters are also involved.

There is a moderate correlation between partition coefficient (log P) and association constant (log K) (r=0.758). An equally significant correlation exists between surface pressure, SP and log K (r=0.757). However, Franke *et al.*[11] have emphasized that the quantity relevant for the distribution behaviour of drugs under physiological conditions in the distribution coefficient (log D) at pH 7.4 rather than the octanol-water partition coefficient (log P). Significant changes in the lipophilicity of phenothiazine drugs occur during small changes in pH around pH 7.4. Our data also showed significantly improved correlation when log P was replaced by log D (r =0.891). Thus hydrophobicity is one of the major factors responsible for drug-protein binding as has been reported by other authors as well[4].

It was also found that the correlation between log P and log K improved considerably (r=0.967) when one of the samples, trifluoperazine dihydrochloride was removed from analysis. Trifluoperazine dihydrochloride contains a piperazine ring containing two basic nitrogen atoms whereas all other drugs contain only one basic nitrogen. One pK_a value of the amino group of piperazine ring is close to the physiological pH and the degree of protonation is relatively low compared with monobasic drugs. This affects the ionization of the drug[11] and hence logP values since log P is commonly defined for the uncharged form of the substance. However, the correlation between log D and log K showed no improvement in the same situation. This is because log D includes the contribution of both the charged and uncharged species to the overall distribution log D is thus a relevant parameter for monobasic as well as dibasic drugs. The pH-dependent distribution profiles of these drugs have also shown[11] that

TABLE 2 : ASSOCIATION CONSTANTS AND PHYSICO-CHEMICAL PARAMETERS OF PHENOTHIAZINE DERIVATIVES

Physico-chemical Parameter	Chlorpromazine Hydrochloride	Promethazine Hydrochloride	Thioridazine Hydrochloride	Trifluoperazine Dihydrochloride	Triflupromazine Hydrochloride
K	41550	31530	44410	51670	39080
logK	4.6186	4.4987	4.6475	4.7132	4.5920
logP	5.10	4.51	5.32	5.10	5.17
logD	3.26	2.95	3.45	4.23	3.52
log MW	2.5506	2.5063	2.6097	2.6816	2.5898
V_w	189.1	180.6	208.8	243.9	194.2
PA	956.64	908.56	1122.62	1296.31	1090.48
SP	16.275	3.967	9.233	14.974	6.379
pK	9.30	9.10	9.50	8.10	9.20
Λ_m	126	108	105	373	304
pH	4.39	5.40	6.12	3.51	3.57
MP	176.0	231.0	159.0	242.5	173.5

K = Association constant, P = Octanol-water partition coefficient, D = Distribution coefficient, MW = Molecular weight, V_w = van der Waal's volume, PA = Parachor, SP = Surface Presssure, Λ_m = Molar conductance, MP = Melting point

phenothaizines containing a piperazine ring are more lipophilic at pH 7.4 than those with a dimethyl amino group. This result is in accordance with the pharmacological potency of the phenothiazine drugs, drugs with piperazine ring have more neuroleptic power.

While the importance of lipophilicity in drug action has been well recognized[6-8], the effect of molecular size has not been appreciated fully. The rationale of including molecular weight (log MW) and van der waal's volume terms in the quantitative structure-activity correlations has been emphasized by Lien and Wang[17] and Maruyama *et al*[18]. We found a highly significant correlation between association constants and molecular weight (log MW) (r=0.928), parachor values (log PA) (r=0.874) and van der waal's volume (log V_w) (r=0.901) of drugs. Thus molecular size of the drug plays a major role in the binding of phenothiazine derivatives to serum albumin. It appears that the larger size drug molecules have large hydrophobic area which can interact with hydrophobic surface on the protein molecule.

Following quantitative relationships between association constant, logK for drug-protein binding and physico-chemical parameters of drugs have been derived using linear least square regression analysis.

$\log K = 0.193 \log P + 3.643$ (1)

$r = 0.758$, $r^2 = 0.574$, F=4.052, SE=0.059

$\log K = 0.148 \log D + 4.098$ (2)

$r = 0.891$, r^2=0.794, F=11.574, SE=0.041

$\log K = 1.111 \log MW + 1.741$ (3)

$r = 0.928$, $r^2 = 0.862$, F=18.735, SE=0.034

$\log K = 1.392 \log V_w + 1.405$ (4)

$r = 0.901$, $r^2 = 0.812$, F=12.994, SE=0.039

$\log K = 1.128 \log PA + 1.199$ (5)

$r = 0.874$, $r^2 = 0.763$, F = 9.663, SE= 0.044

$\log K = 0.011 SP + 4.501$ (6)

$r = 0.757$, $r^2 = 0.573$, F=4.020, SE=0.059

The degree of protein binding, expressed in terms of association constants, predicted from log P, log D, log MW, V_w and parachor measurements are recorded in Table 3. The values agree fairly well with the association constants obtained from fluorescmce data reported in this paper.

TABLE 3 : COMPARISON OF EXPERIMENTALLY DETERMINED ASSOCIATION CONSTANTS AND THOSE PREDICTED FROM PHYSICO-CHEMICAL PROPERTIES

Sample	Association Constant (log K)					
	Experimentally determined	values predicted from physico-chemical properties				
		log P	log D	log MW	V_w	PA
Chlorpromazine hydrochloride	4.6186	4.6273	4.5805	4.5747	4.5725	4.5606
Promethazine hydrochloride	4.4987	4.5134	4.5346	4.5255	4.5487	4.5390
Thioridazine hydrochloride	4.6475	4.6700	4.6086	4.6404	4.6276	4.6352
Trifluoperazine dihydrochloride	4.7132	4.6273	4.7240	4.7203	4.7259	4.7132
Triflupromazine hydrochloride	4.5920	4.6408	4.6190	4.6183	4.5868	4.6207

K = Association constant, P = Octanol-water partition coefficient, D = Distribution coefficient, MW = Molecular weight, V_w = van der Waal's volume, PA = Parachor

It may thus be concluded that the major factors responsible for the binding of phenothiazine derivatives to serum albumin are hydrophobicity and molecular size of drugs. However, hydrophilicity of drugs determined by pK_a and molar conductance also play a role.

Correlation between association constants and physico-chemical parameters, log P, log D, log MW, SP and pK could further be improved by fitting the following quadratic equations to the data.

$$\log K = -0.371 (\log P)^2 + 3.814 \log P - 5.150 \quad (7)$$
$r = 0.814$, $r^2 = 0.662$, $F = 1.96$, $SE = 0.065$

$$\log K = -0.093 (\log D)^2 + 0.821 \log D + 2.896 \quad (8)$$
$r = 0.922$, $r^2 = 0.850$, $F = 5.69$, $SE = 0.043$

$$\log K = -2.400 (\log MW)^2 + 13.546 \log MW - 14.386 \quad (9)$$
$r = 0.936$, $r^2 = 0.876$, $F = 7.06$, $SE = 0.039$

$$\log K = -0.279 \times 10^{-2} (SP)^2 + 0.069 SP + 4.264 \quad (10)$$
$r = 0.940$, $r^2 = 0.884$, $SE = 0.038$, $F = 7.650$

$$\log K = 0.345 (pK)^2 - 6.104 pK + 31.522 \quad (11)$$
$r = 0.918$, $r^2 = 0.843$, $SE = 0.044$, $F = 5.389$

Thus quadratic equations should give a better estimate of the association constants from these properties.

Correlation could also be improved by combining various physico-chemical parameters. Following relationships have been derived using multiple linear regression analysis.

$$\text{IOG } K = 0.068 \log P + 0.903 \text{ LOG MW} + 1.936 \quad (12)$$
$r = 0.950$, $r^2 = 0.903$, $SE = 0.035$, $F = 9.326$

$$\log K = 0.163 \log P - 1.151 \log MW + 2.348 \log V_w + 1.357 \quad (13)$$
$r = 0.982$, $r^2 = 0.964$, $SE = 0.030$, $F = 8.933$

$$\log K = 0.313 \log P - 2.198 \log MW + 2.500 \log V_w - 8.850 pK + 3.756 \quad (14)$$
$r = 1.000$, $r^2 = 1.000$

$$\log K = -0.042 \log D + 1.403 \log MW + 1.130 \quad (15)$$
$r = 0.930$, $r^2 = 0.865$, $SE = 0.041$, $F = 6.420$

$$\log K = 0.111 \log D + 0.951 \log V_w + 0.068 pK + 1.416 \quad (16)$$
$r = 0.954$, $r^2 = 0.911$, $SE = 0.047$, $F = 3.414$

$$\log K = -0.091 \log D + 5.034 \log MW - 3.578 \log PA + 2.739 \quad (17)$$
$r = 0.999$, $r^2 = 0.998$, $SE = 0.007$, $F = 170.994$

$$\log K = -0.114 \log D + 1.647 \log MW + 0.006 SP + 0.684 \quad (18)$$
$r = 1.000$, $r^2 = 0.999$, $SE = 0.004$, $F = 488.36$

$$\log K = 0.008 SP + 1.029 \log PA + 0.028 pK + 1.165 \quad (19)$$
$r = 0.998$, $r^2 = 0.997$, $SE = 0.009$, $F = 96.877$

It is seen that the correlation coefficient increases from 0.758 to 0.950 when log MW term is added to log P. It further improves to 0.982 on adding a molecular volume term (log V_w). A perfect correlation was obtained on adding a fourth term, pK value of the drug. Similarly equations 15-18 show that the correlation coefficient for log D (0.928) improves on adding other terms such as log MW, log V_w, log PA, pK and SP. Near perfect correlation could also be obtained by combining SP, log PA and pK values of drugs.