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Use of 1-Anilino-8-napthalenesulphonate as an Ion Probe for the Potentiometric Study of the Binding of Sulphonamides to Bovine Serum Albumin and Plasma

A. ANGELAKOU, G. VALSAMI, M. KOUPPARIS* AND P. MACHERAS

Department of Pharmacy and Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece*

Abstract—The binding of sulphafurazole, sulphamethizole and sulphamethoxazole to bovine serum albumin (BSA) and human plasma has been studied in-vitro by potentiometry using an electrode selective for the ion probe 1-anilino-8-napthalenesulphonate (ANS). The method requires two separate potentiometric titrations of the binder solution with ANS in the absence and in the presence of sulphonamides. ANS displaced sulphonamides from the first-class of binding sites of both binders. The binding constants for sulphonamide-BSA interactions were higher than those for sulphonamide-human plasma. The expansion of the least linear limit of the response curve of the electrode down to 10^{-7} M in the presence of BSA was also demonstrated. The reverse reaction, i.e. the displacement of the probe from the binding sites induced by the sulphonamides, was also explored. The proposed method is suitable for studying competitive binding interactions in biological specimens.

The methods of studying drug-protein interactions can be broadly classified into two groups—direct techniques (based on the monitoring of a physicochemical property of the protein or the drug upon binding) and indirect techniques (based on the measuring of the free drug after separation from the bound drug). The major advantage of the direct techniques, which are mainly spectroscopic, is associated with the low risk of interference with the drug-protein equilibrium. Among the direct techniques and especially in the field of competitive binding experiments, fluorimetry has been extensively applied. For example, the fluorescence probe 1-anilino-8-napthalenesulphonate (ANS) has been used in a number of studies in the investigation of drug interactions with proteins (Hsu et al 1974; Sato et al 1984; Gizurarson & Jensen 1989; Essassi et al 1990). However, the major objection to the fluorescence probe technique is that extensive dilution of the biological specimen is required to avoid spectral interference. Such a dilution causes disturbance of equilibrium conditions. In recent years, a novel direct technique devoid of this drawback using potentiometry with ion-selective electrodes (ISEs) has been developed and applied to binding studies (Takisawa et al 1988; Christopoulos & Diamandis 1990; Valsami et al 1990, 1991, 1992).

In this paper, we describe the development of the potentiometric ion probe technique for the study of drug-protein interactions. The technique is based on the continuous monitoring of the free ion probe in the reaction mixture of protein-drug-probe by direct potentiometry. The ion probe used was the ANS, for which a homemade indicator electrode has been constructed in our laboratory. The characteristics of this ISE of ANS and its use in the binding study of ANS to human plasma, and human and bovine serum albumin have recently been reported (Valsami et al 1991).

Correspondence: P. Macheras, Laboratory of Biopharmaceutics and Pharmacokinetics, Department of Pharmacy, University of Athens, Panepistimiopolis, Athens 15771, Greece.

Materials and Methods

Apparatus

The construction of the ANS indicator electrode and the necessary potentiometric system have been previously reported (Valsami et al 1991).

Materials

1-Anilino-8-napthalenesulphonate ammonium salt and bovine serum albumin (fraction V) were obtained from Fluka (Buchs, Switzerland). Sulphafurazole, sulphamethizole, sulphamethoxazole and sulphadiazine were kindly donated by American Cyanamid Company. Plasma was obtained from nine healthy volunteers of either sex, aged between 25 and 35 years, who had received no drug for at least 1 week before the blood collection. The plasma obtained by centrifugation at 3000 rev min^{-1} for 10 min was stored at −40°C in 5 mL glass tubes.

Reagents

All solutions used were prepared in deionized water. Other chemicals used were of analytical grade. Phosphate buffer, 0·10 M, pH 7·4 was prepared by dissolving sodium dihydrogen phosphate in water and adjusting the pH with 18 M NaOH using a pH-meter. ANS stock solution was 0·1 M with respect to ANS in phosphate buffer. More dilute working solutions were prepared by dilution of the stock solution in phosphate buffer. BSA solution was prepared to contain 22·5 g L^{-1} of BSA in phosphate buffer and stored at 4°C for no more than one week. Plasma working solution was prepared by 1:1 dilution with phosphate buffer. Mixed working solutions used as titrants (ANS-BSA, ANS-BSA-sulphonamide and ANS-sulphonamide) were prepared to contain ANS at a concentration of 0·01 M, BSA at a concentration of 22·5 g L^{-1} ($3{\cdot}36 \times 10^{-4}$ M) and sulphonamides at various concentrations (5×10^{-4}–5×10^{-3} M) in phosphate buffer.

Methods

Construction of calibration (response) curve of the ISE. Five millilitres of phosphate buffer were pipetted into the thermostated measurement cell, the indicator and reference electrodes were immersed, and after the potential was stabilized, various portions of a 0·006 M ANS working solution were added, with a 100 μL Hamilton microsyringe (concentration range covered $1{\cdot}3 \times 10^{-6}$–$2{\cdot}4 \times 10^{-3}$ M). The emf values were recorded and measured after stabilization ($\pm 0{\cdot}1$ mV) following each addition. The potential values E, were plotted against the negative logarithm of the ANS concentration, to give the calibration (response) curve of the ISE, using a least squares linear fitting program. Corrections for the change in volume after addition were performed by the program utilized for the analysis of data (Christopoulos & Diamandis 1990; Valsami et al 1991). The construction of the calibration curve is necessary on each working day.

Binding experiments (potentiometric titrations). Solutions of BSA or plasma (5·0 mL) with or without sulphonamide, were pipetted into the measurement cell and small amounts of either 0·01 M ANS solution or the mixed ANS-sulphonamide solution (both in the case of plasma), or the mixed ANS-BSA or ANS-BSA-sulphonamide solution (both in the case of BSA) were added with a 100 μL Hamilton microsyringe. By using these mixed titrant solutions, the BSA and the sulphonamide concentrations remain constant during the binding experiment, making the data analysis simpler. In the case of plasma, corrections for the dilution after each addition were performed by the program (Christopoulos & Diamandis 1990; Valsami et al 1991).

Data analysis. In all binding experiments concentrations of free ANS (F) were calculated from the calibration curve of the electrode and concentrations of bound ANS (B) from the equation:

$$B = T - F$$

where T is the total ANS molar concentration. In the binding experiments of ANS with BSA in the absence of sulphonamides, the data obtained were fitted according to the Scatchard equation:

$$B = \sum_{i=1}^{m} \frac{n_i K_i F}{1 + K_i F} P_t \qquad (1)$$

where m, n_i, K_i and P_t denote respectively the number of distinct classes of independent and non-interacting binding sites, the number of binding sites of the ith class, the ANS (intrinsic) association constant of the ith class, and the molar protein concentration. The data of binding experiments of ANS with human plasma in the absence of sulphonamides were analysed with the modified Scatchard equation (Loscher 1979; Valsami et al 1991):

$$B = \sum_{i=1}^{m} \frac{N_i K_i F}{1 + \left(\frac{K_i F}{1000\ MW}\right)} P_t \qquad (2)$$

where B and F are given as μg mL^{-1}, P_t as g L^{-1} and K_i as M^{-1}, N_i is the binding capacity of the ith class of binding sites expressed as mol g^{-1}, and MW is the molecular weight of ANS.

In the cases of ANS binding to BSA and human plasma in the presence of sulphonamides, equations 1 and 2 were also used for the analysis of data. However, apparent association constants, $(K_i)_{app}$, were estimated instead of K_i.

The parameters n_i or N_i, K_i or $(K_i)_{app}$, for all sets of experiments, were calculated by a nonlinear least-squares method of performing fitting of equations 1 and 2 to the experimental data (potential readings E – total ANS concentration T) (Christopoulos & Diamandis 1990; Valsami et al 1991).

The association constant, K_I for the binding of sulphonamides (the so-called inhibitor) to BSA and human plasma was calculated by the relation (Brodersen 1974; Essassi et al 1990):

$$K_I = \frac{K_1 - (K_1)_{app}}{(K_1)_{app}\,[I]} \qquad (3)$$

where $(K_1)_{app}$ and K_1 are the apparent association constant and the association constant of ANS, respectively, for the first binding class, and [I] is the total concentration of sulphonamide in the displacement experiment.

Results

In order to examine the ability of the ANS ISE to measure accurately low concentrations of ANS in the presence of relatively high sulphonamide concentrations, the potentio-

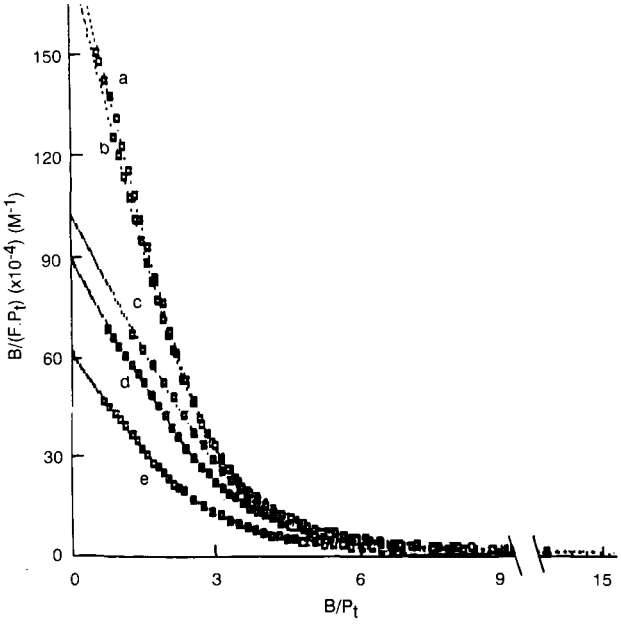

FIG. 1. Scatchard plots for the binding of ANS to BSA at 25°C and pH 7·4: in the absence of drug (a) and in the presence of 5×10^{-4} M sulphadiazine (b), sulphamethoxazole (c), sulphamethizole (d) and sulphafurazole (e). The theoretical Scatchard curves, based on the calculated binding parameters according to equation 1, are drawn over the experimental points.

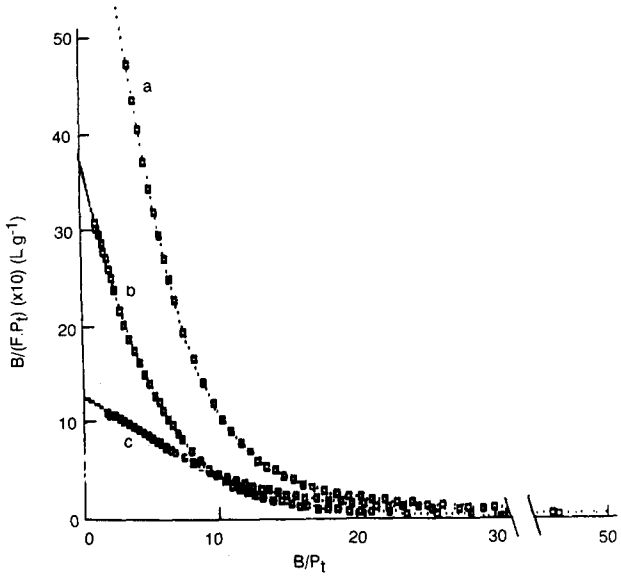

FIG. 2. Scatchard plots for the binding of ANS to human plasma at 25°C and pH 7·4: in the absence of drug (a) and in the presence of 10^{-3} M sulphamethoxazole (b) and 5×10^{-3} M sulphamethizole (c). The theoretical Scatchard curves, based on the calculated binding parameters according to equation 2, are drawn over the experimental points.

metric selectivity coefficients, $K^{pot}_{ANS.Sulph}$, were determined in the buffer used, using the so-called mixed solution method (Moody & Thomas 1970). The values obtained, 4·24–$7{\cdot}45 \times 10^{-4}$, indicate no serious interference from sulphonamides.

The Scatchard plots for the binding of ANS to BSA in the absence or in the presence of the sulphonamides are shown in Fig. 1. The shape of the curves in Fig. 1 indicate that the sulphonamides are bound only to the first class of binding sites of ANS and are displaced by the probe. Similar profiles and identical conclusions can be drawn by inspecting the Scatchard curves for the binding of ANS to human plasma in the absence or in the presence of sulphamethizole and sulphamethoxazole (Fig. 2). Parameter estimates for the binding of ANS to BSA and human plasma in the absence of drugs are summarized in Table 1. These data were obtained over a period of ten months using different ISEs (reconstructed approximately once a month). The between-run precisions are sufficient for such binding studies. The results of a typical set of experiments for the binding study of sulphamethoxazole to BSA and human plasma are summarized in Table 2. A significant decrease in the apparent association constant of the first class of binding site is caused by the presence of the drug. Within-run precisions for K_1 and $(K_1)_{app}$ were in the range 0·04–1×10^5 M^{-1}. Estimates for the association constants of the sulphonamides are given in Table 3. For comparative purposes, estimates of association constants of sulphonamides reported in the literature are quoted in Table 4 along with the estimates of the present study.

The expansion of the least linear limit of the response curve of ANS ISE in the presence of BSA was also studied.

Table 1. Estimates (±s.d.)[a] for the binding parameters of the ANS interaction to BSA[b] and human plasma[c] at 25°C.

Binding parameters[d]	BSA	Human plasma
n_1	2·84 (0·32)	—
n_2	3·16 (0·22)	—
n_3	13·7 (1·8)	—
$10^5 N_1$ (mol g^{-1})	4·1 (0·51)	1·34 (0·50)
$10^5 N_2$ (mol g^{-1})	4·8 (0·73)	2·42 (0·44)
$10^5 N_3$ (mol g^{-1})	23·1 (9·5)	11·0 (3·8)
$10^{-5} K_1$ (M^{-1})	6·5 (1·1)	3·48 (0·89)
$10^{-5} K_2$ (M^{-1})	0·21 (0·05)	0·32 (0·11)
$10^{-3} K_3$ (M^{-1})	0·54 (0·14)	0·82 (0·31)

[a] Calculated from eleven titration experiments, each one consisting of approximately 60 measurement points over ten months. [b] BSA concentration was 2·25% ($3{\cdot}36 \times 10^{-4}$ M) and was kept constant during the experiment. [c] Human plasma was diluted 1:1 with phosphate buffer 0·1 M pH 7·4. [d] n_i is the number of binding sites of the ith class of protein, N_i is the binding capacity of the ith class of binding sites expressed as mol g^{-1} and K_i is the intrinsic association constant of the ith class.

Table 2. Estimates (±s.d.)[a] for the binding parameters of ANS to BSA[b] and human plasma[c] at 25°C in the absence and presence of sulphamethoxazole[d].

Binding parameters[e]	In the absence of sulphamethoxazole		In the presence of sulphamethoxazole	
	BSA	Human plasma	BSA	Human plasma
n_1	3·2 (0·32)	—	3·2 (0·50)	—
n_2	3·2 (0·21)	—	3·6 (0·2)	—
n_3	15·7 (0·5)	—	15·9 (3·9)	—
$10^5 N_1$ (mol g^{-1})	4·5 (0·54)	0·9 (0·3)	4·7 (0·78)	0·6 (0·3)
$10^5 N_2$ (mol g^{-1})	5·1 (1·2)	2·4 (0·2)	4·7 (0·85)	2·6 (0·1)
$10^5 N_3$ (mol g^{-1})	20·7 (2·2)	7·1 (1·6)	17·3 (2·5)	8·3 (2·1)
$10^{-5} K_1$ (M^{-1})	6·5 (2·0)[f]	4·3 (0·2)	4·0 (1·5)[f]	4·2 (1·1)
$10^{-5} K_2$ (M^{-1})	0·17 (0·07)	0·40 (0·08)	0·12 (0·05)	0·4 (0·9)
$10^{-3} K_3$ (M^{-1})	0·5 (0·1)	1·06 (0·15)	0·30 (0·004)	0·8 (0·2)

[a] Calculated from three titration experiments performed on different days, each one consisting of approximately 60 measurement points. [b] BSA concentration was 2·25% ($3{\cdot}36 \times 10^{-4}$ M) and was kept constant during the experiment. [c] Human plasma was diluted 1:1 with phosphate buffer 0·1 M, pH 7·4. [d] Sulphamethoxazole concentration was 5×10^{-4} M in the case of BSA and 10^{-3} M for human plasma. [e] Binding parameters as explained in the legend of Table 1. [f] Within-run precisions ranged from 0·05 to $1{\cdot}1 \times 10^5$ M^{-1}.

Table 3. Estimates (±s.d.)[a] for the association constants of the sulphonamide interaction with BSA[b] and human plasma[c] at 25°C.

Drug[d]	Binder	Association constants ($\times 10^3$ M^{-1})
Sulphafurazole	BSA	3·7 (0·5)
	Human plasma	—[e]
Sulphamethizole	BSA	2·5 (0·3)
	Human plasma	1·6 (0·5)
Sulphamethoxazole	BSA	1·5 (0·7)
	Human plasma	0·50 (0·03)

[a] Calculated from three titration experiments each consisting of approximately 60 measurement points. [b] BSA concentration was 2·25% ($3{\cdot}36 \times 10^{-4}$ M) and was kept constant during the experiment. [c] Human plasma was diluted 1:1 with phosphate buffer 0·1 M, pH 7·4. [d] Drug concentration was 5×10^{-4} M in the case of BSA and 10^{-3} M for human plasma. [e] Not measurable.

Table 4. Estimates (±s.d.) for the association constants of the sulphonamide interaction with BSA and human plasma.

Drug and binder	Association constants (M^{-1})	References
Sulphafurazole		
BSA	$3{\cdot}7\ (0{\cdot}5) \times 10^3$	This study
	10^5	Moriguchi et al (1968)
	$1{\cdot}47 \times 10^4$	Nakagaki et al (1964)
	$9{\cdot}6 \times 10^3$	Hsu et al (1974) (Fluorescence probe technique)
Sulphamethizole		
BSA	$2{\cdot}5\ (0{\cdot}3) \times 10^3$	This study
	$2{\cdot}0 \times 10^4$	Moriguchi et al (1968)
	$5{\cdot}2 \times 10^3$	Hsu et al (1974)
Human plasma	$1{\cdot}6\ (0{\cdot}5) \times 10^3$	This study
Sulphamethoxazole		
BSA	$1{\cdot}5\ (0{\cdot}7) \times 10^3$	This study
	$1{\cdot}6 \times 10^3$	Hsu et al (1974)
Human plasma	$5{\cdot}0\ (0{\cdot}3) \times 10^2$	This study
	$4{\cdot}49 \times 10^2$ (serum)	Spring (1966)
Sulphadiazine		
BSA	—[a]	This study
	1×10^2	Moriguchi et al (1968)
	$4{\cdot}2 \times 10^2$	Hsu et al (1974)

[a]Not measurable.

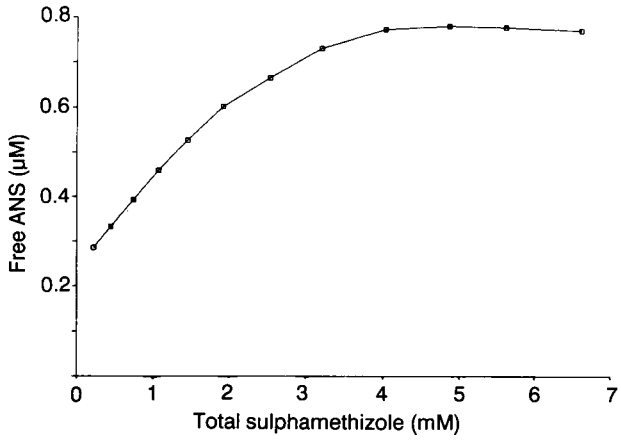

FIG. 4. Plot of the concentration of free ANS vs total sulphamethizole concentration. Experimental conditions: 3 mL BSA (2·25%)—ANS (0·2 mM) in phosphate buffer 0·10 M pH 7·4 were titrated with a mixed solution of BSA (2·25%) and sulphamethizole (0·010 M) at 25°C. Corrections of total ANS concentration due to the dilution were made.

The response curves of ANS ISE in the absence and in the presence of BSA ($3{\cdot}36 \times 10^{-4}$ M) are shown in Fig. 3. Experiments were also conducted to explore the reverse reaction, i.e. the displacement of ANS by the sulphonamides. A typical example of this reaction with sulphamethizole displacing ANS from the binding sites, is shown in Fig. 4.

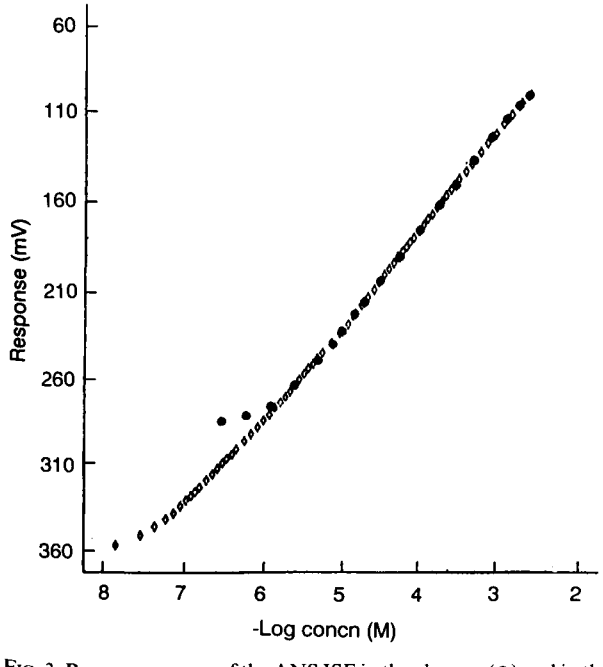

FIG. 3. Response curves of the ANS ISE in the absence (●) and in the presence (◇) of $3{\cdot}36 \times 10^{-4}$ M BSA. The corresponding equations are: $E = -37{\cdot}6\ (\pm 1{\cdot}1) - 53{\cdot}6\ (\pm 0{\cdot}2) \times pF$ (absence of BSA), $E = -36{\cdot}3\ (\pm 0{\cdot}3) - 53{\cdot}3\ (\pm 0{\cdot}1) \times pF$ (presence of BSA).

Discussion

The response time of the ISE of ANS was very short both in the absence and the presence of sulphonamides. The potential was stabilized in all studies in about 5–10 s. This observation indicates that the displacement reaction is very rapid and the ISE is capable of monitoring the free concentration of ANS in the successive equilibria.

In all studies, computer analysis of data revealed three classes of binding sites irrespective of the binder (BSA or plasma) and the absence or presence of sulphonamides (Tables 1, 2). The similarity in the estimates of the number of binding sites for each class between experiments in the absence and the presence of sulphonamides, if coupled with the variability of measurements, leads to the conclusion that the sulphonamides do not induce allosteric effects when bound to BSA or plasma proteins. Nevertheless, a significant difference exists between the estimates of the association constant and the apparent association constant of the first class of binding sites for both binders examined (Table 2). This difference gives a clear indication of the exclusive binding of sulphonamides to the high-affinity sites of BSA and plasma. This conclusion is also in accord with the shape of the Scatchard plots (Figs 1, 2). The initial, almost linear, segments of the experimental curves in the absence of sulphonamides, which correspond to the high-affinity class of binding sites, have been moved towards the y-axis exhibiting shallow slopes in the displacement experiments.

A previous binding study of sulphonamides with BSA (Hsu et al 1974) using ANS as a fluorescence probe was based on the assumption that the competitive binding is restricted only to the high-affinity class of binding sites. Our results provide conclusive evidence for the correctness of this hypothesis (Table 2). This was made possible due to the inherent advantage of ISE to study the binding phenomenon in a wider range of ANS/binder ratios compared with

fluorescence studies. It should be noted that the least linear concentration limit of the ANS ISE is approximately 10^{-6} M (Valsami et al 1991); however, an expansion of this limit down to 10^{-7} M is caused by the protein binder (Fig. 3). In the presence of BSA, the concentration of free ANS was calculated from equation 1 using the known binding parameters and a nonlinear fitting program. The expansion of the least linear limit is a well known phenomenon (Otto et al 1985) which increases the usefulness of direct potentiometry for binding studies.

Our results also demonstrate that ANS displaces sulphonamides from the first class of binding sites of human plasma (Table 2) whereas fluorescence studies are not capable of detecting interactions in plasma due to the spectral interference of the biological specimen.

The apparent association constants of ANS for the first class of binding sites, $(K_1)_{app}$, were further used to calculate the corresponding association constants of sulphonamides, K_I, to BSA and human plasma (Table 3). The binding affinity of sulphonamides was found to be higher in BSA than in plasma. The estimates for the association constants of sulphonamides to BSA agree with previously reported values (Table 4). The values found in this study are very close to those reported by Hsu et al (1974), which were determined using a similar method (fluorescence probe technique) but at low BSA concentration (0·1%). However, there are some differences with the results obtained by Moriguchi et al (1968) which may be attributed to the different methodology used. It should be noted that due to the weak binding, the binding parameters for sulphadiazine in both media and sulphafurazole in plasma were not calculated (Tables 3, 4). From equation 3 and assuming $K_1-(K_1)_{app} > SD_K$ (the maximum within run standard deviation of K is about 10^5 M^{-1}) the product $K_I[I]$ must be greater than 0·18 to allow application of this method. For typical concentrations of drugs of 5×10^{-4}, 1×10^{-3} and 5×10^{-3} M the corresponding K_I must be greater than 300, 150 and 30 M^{-1}, respectively. The concentration of the drug utilized is limited by its solubility at pH 7·4 and the possible interference with the electrode. However, in our studies, on changing the inhibitor concentration, the calculated value of K_I was found to be constant. For example, K_I for the binding of sulphamethoxazole to BSA was $1\cdot5(\pm0\cdot7)\times10^3$ and $1\cdot1(\pm0\cdot4)\times10^3$ M^{-1} for 5×10^{-4} and 1×10^{-3} M drug concentrations, respectively. In spite of the inherent advantages of ISE potentiometry, fluorimetry is a more sensitive technique capable of assessing weak binding interactions (Hsu et al 1974; Essasi et al 1990). The concentration of BSA used in the present study was relatively high (335 μM) to resemble the albumin concentration (325 μM) found in the 1:1 plasma-phosphate buffer solution. It is advisable, however, for the drug-protein interaction to be studied with a low concentration of BSA ($\leqslant$50 μM) in order to obtain accurate values for the association constants. Experiments carried out in our laboratory have shown that the sulphonamide-BSA interaction can be monitored with the ISE of ANS using BSA concentrations in the range 15–150 μM.

The reverse reaction, i.e. the titration of ANS-BSA mixture with the drug (inhibitor) under study (containing the same concentration of BSA to keep its concentration constant) is also feasible, if a concentrated stock solution of the drug can be prepared. Fig. 4 shows the increase of the concentration of free ANS in a solution of BSA-ANS after the addition of sulphamethizole. Fig. 4 in conjunction with Fig. 1 provides conclusive evidence that ANS and sulphamethizole are mutually displaced from the binding sites of BSA. Work in progress in our laboratory is aiming at a quantitative application of this procedure.

References

Brodersen, R. (1974) Competitive binding of bilirubin and drugs to human serum albumin studied by enzymatic oxidation. J. Clin. Invest. 54: 1353–1364

Christopoulos, T., Diamandis, E. (1990) Binding studies using ion-selective electrodes. Examination of the picrate-albumin interaction as a model system. Anal. Chem. 62: 360–367

Essassi, D., Zini, R., Tillement, J. P. (1990) Use of 1-anilino-8-napthalene sulfonate as a fluorescent probe in the investigation of drug interactions with human alpha-1-acid glycoprotein and serum albumin. J. Pharm. Sci. 79: 9–13

Gizurarson, S., Jensen, A. (1989) A simple flow injection analysis for studies of drug-protein binding interactions in serum. Acta Pharm. Nord. 1: 291–294

Hsu, P. L., Ma, J. K. H., Jun, H. W., Luzzi, L. A. (1974) Structure relationship for binding of sulfonamides and penicillins to bovine serum albumin by fluorescence probe technique. J. Pharm. Sci. 63: 27–31

Loscher, W. (1979) A comparative study of the protein binding of anticonvulsant drugs in serum of dog and man. J. Pharmacol. Exp. Ther. 208: 429–435

Moody, G. J., Thomas, J. D. R. (1970) Selective Ion Sensitive Electrodes. Merrow Publ. Co. Ltd, Watford

Moriguchi, I., Wada, S., Nishizawa, T. (1968) Protein binding III. Binding of sulfonamides to bovine serum albumin. Chem. Pharm. Bull. 16: 601–605

Nakagaki, M., Kuga, N., Terada, H. (1964) Physicochemical studies on the binding of chemicals with proteins. II. The mechanism of binding of several sulfonamides with serum albumin. Yakugaku Zasshi 84: 516–521

Otto, M., May, P., Murray, K., Thomas, J. D. R. (1985) Calibration of ionized calcium and magnesium with ligand mixtures for intracellular ion-selective electrode measurements. Anal. Chem. 57: 1511–1517

Sato, J., Sugiyama, Y., Hanano, M. (1984) Use of fluorescence probe 1-anilino-8-naphthalenesulfonate in predicting interindividual differences in the plasma protein binding of acidic drugs in rats. J. Pharm. Sci. 73: 519–524

Spring, P. (1966) Binding of several sulfanilamides to human blood protein bodies. Arzneim. Forsch. 16: 346–353

Takisawa, N., Hall, D., Wyn-Jones, E., Brown, P. (1988) The construction and characteristics of drug selective electrodes. Applications for the determination of complexation constants of inclusion complexes with α- and β-cyclodextrins including a kinetic study. J. Chem. Soc. Faraday Trans. 84: 3059–3070

Valsami, G., Macheras, P., Koupparis, M. (1990) Binding studies of ions with cyclodextrins using ion-selective electrodes. J. Pharm. Sci. 79: 1087–1094

Valsami, G., Macheras, P., Koupparis, M. (1991) Binding study of the fluorescent probe 1-anilino-8-napthalene sulfonate to human plasma and human bovine serum albumin using potentiometric titration. Pharm. Res. 8: 888–892

Valsami, G., Koupparis, M., Macheras, P. (1992) Complexation studies of cyclodextrins with tricyclic antidepressants using ion-selective electrodes. Pharm. Res. 9: 94–100