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Flow injection-capillary electrophoresis frontal analysis method for the study of the interactions of a series of drugs with human serum albumin

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ABSTRACT

In this paper, a fast method for the study on the interactions of a series of drugs used in the treatment of hypertension with human serum albumin (HSA) by flow injection-capillary electrophoresis (FI-CE) was developed based on the principle of frontal analysis (FA). The binding parameters were determined by FI-CE-FA from Scatchard equation and compared with results obtained by non-CE methods and literature values. A multiple linear regression (MLR) model between the drug–protein binding constants (K) and structural descriptors of drugs was constructed. *L*-tryptophan (*L*-try) and phenylbutazone (PB) were used as displacement reagents to investigate the binding sites of a series of drugs on HSA. The binding synergism effect between drugs and the effects of many metal ions existing in human plasma on protein binding were also investigated systematically.

1. Introduction

Drug–protein interactions are determinative factors in pharmacodynamics and pharmacokinetics of drugs [1]. The unbound drug is considered to diffuse from the blood to the extravascular site of drug action and to exhibit the pharmacological activity and/or side effect [2]. In order to adjust the optimum therapeutic dose of a drug, it is necessary to know the extent of drug–protein binding. In addition, the displacement from HSA due to competitive binding of simultaneously administered drugs may lead to increased levels of free drug and thus, potentially side effects. So the effect of the displacement of drug caused by simultaneously administered exogenous compounds should be known to avoid unwanted side effects for the patient [3,4]. Human serum albumin (HSA: 35–50 mg/mL in plasma; MW: 66,500) is an important and abundant drug binding protein in plasma [5], and is responsible for the reversible binding of a wide range of drugs. Therefore, it is interesting to develop a method for the determination of drug–albumin affinity constants while providing information on the drug binding site simultaneously.

A variety of methodologies such as fluorescence spectroscopy (FLU) [6], equilibrium dialysis (ED) [7,8], HPLC [9,10], flow injection chemiluminescence or sequential injection analysis [11,12], mass spectrometry (MS) [13] and CE [4,14] have been developed to evaluate drug–protein interactions. FLU is a simple method and has advantages such as high sensitivity and small sample requirement, but it can only be applied in a limited field. Although ED is a classical reference method, it needs a long time for establishing binding equilibrium and there is a problem of membrane adsorption. The HPLC method is not widely used for evaluating drug–protein interactions because the affinity column is often very expensive. The main disadvantage of flow or sequential injection analysis is that they cannot be used for multicomponent analysis. Among these methodologies, the CE-frontal analysis (FA) method has been proven to be superior to others because of its simplicity, high speed, efficiency and versatility to study multiple equilibria [15]. The principle of CE-FA is simple. Briefly, in CE-FA a large sample plug is injected into a capillary filled with plain background electrolyte. The sample plug consists of drug, protein and drug–protein complex. Due to the difference in mobility, the free drug is separated from the protein/protein–drug complex. Binding equilibrium is maintained in the overlapping zone because the bound drug is released, leading to a plateau region. The height of the plateau region is proportional to the free drug concentration in the original sample. The free drug concentration can be determined with the aid of a calibration curve prepared by injecting samples containing drug alone of known concentration.

Although CE has the advantage of high resolution capability, the discontinuous sample introduction mode confined the sample throughput and precision. FI offers an elegant means for sample

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injection, it can be fully mechanized. Automated procedures yield higher precision and can be performed in a shorter time compared with the corresponding manual sample injection. The combined FI-CE system solves the problem of discontinuous manipulation of CE, enhances sampling frequency and improves reproducibility contrasted with traditional CE injections [16]. The developed FI-CE-FA method avoids Donnan effect, sieve effect, nonspecific adsorption and leakage through membranes comparing with the conventional methods.

In this paper, the characterization of the interaction between 11 cationic drugs used in the treatment of hypertension (3 calcium channel antagonists, 5 β-blockers and 3 diuretics) and HSA under simulative physiological conditions using the FI-CE-FA method was presented. The reliability of this method was estimated by comparing it with conventional fluorescence spectroscopy (FLU) and equilibrium dialysis combined with UV–vis spectrophotometry (ED-UV). The purpose of the work was to study the effect of co-administrated drugs on interaction of drug and protein, and to find some relationship between the structures of drugs and binding parameters. The effect of metal ions on binding constants was also investigated.

2. Materials and methods

2.1. Chemicals and reagents

Amlodipine besylate (AL) and metoprolol tartrate (Met) were obtained from Dingkang Technology Company (Shanghai, China). Atenolol (AT), carvedilol (KW), carteolol hydrochloride (KT), propranolol hydrochloride (Pro), chlortalidone (LS), indapamide (YD), hydrochlorothiazide (QL), verapamil hydrochloride (VER), diltiazem hydrochloride (Dil) and phenylbutazone (PB) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). *L*-tryptophan (*L*-try) and HSA were purchased from Sigma (St. Louis, MO, USA). NaH_2PO_4, Na_2HPO_4, NaCl, KCl, $MgCl_2$ and $ZnCl_2$ were obtained from Xi'an Chemical Reagent Plant (Xi'an, China). All chemical reagents were analytical grade. The drug–protein mixed solutions were prepared in sodium phosphate buffer (pH 7.4, ionic strength 0.17 M). All solutions were filtered through 0.45 μm pore size nylon membranes prior to use.

2.2. Apparatus

A model HPE-100 CE system with 12 kV maximum voltage (Bio-Rad, Hercules, CA, USA) was used for electrophoretic separations, which was connected to a 486 PC with a Chroma chromatography collection system (BioRad) for data acquisition and treatment. Uncoated fused-silica capillaries of 75 μm I.D., 375 μm O.D. and 43.7 cm length (40.7 cm effective length) were purchased from Yongnian Optical Fiber Factory (Baoding, Hebei, China). The UV detection was set at 275 nm for QL, 214 nm for the other drugs. All operations were controlled at 23.5 ± 0.5 °C by an ambient forced-air cooling system.

A K-1000 FI Analyzer (Hitachi, Japan) was used for transporting carrier solution and sample solution. It was equipped with a double plunger pump used for delivery of carrier solution, a 16-way auto-switching valve with three PTFE solution loops, a peristaltic pump used for delivery of sample solution to the middle PTFE sample loop (115 μL), and a running buffer plug to the first (20 μL) and the third (20 μL) PTFE solution loops. A 31-cm length, 0.5 mm I.D. PTFE tubing was used for connecting the valve to the split-flow interface (anodic reservoir). The manifold of FI-CE was shown in Fig. 1. The detailed description of the H-channel microchip and the schematic diagram of two stages of the FI system with a 16-way auto-switching valve had been given elsewhere [17].

2.3. FI procedure

During the charging stage, with the valve in the "load" position, the sample solution was pumped by the peristaltic pump to fill the middle loop (sample loop) of the 16-way auto-switching valve, and the running buffer was pumped to fill the first and the third loops (reagent loops). Simultaneously, the carrier solution, which also functioned as the running buffer, was pumped by the double plunger pump through the split-interface. When the charging stage was finished, the peristaltic pump stopped and the valve was switched automatically to the "inject" position, the buffer solution flowed through the sample and reagent loops. The sample solution in the middle loop of the 16-way auto-switching valve was sandwiched by the buffer solution and transported through the connecting conduit into the split-flow interface, and a fraction of the sample zone was introduced into the separation capillary by the electrokinetic injection. After the injection sequence, the valve returned to the "load" position, and the next cycle began. A series of samples were injected continuously without interrupting the voltage.

2.4. Determination of unbound drugs concentrations by FI-CE-FA

The standard drugs solutions (30, 50, 100, 200, 300 and 500 μM) without HSA were used to prepare calibration curves. A series of protein–drug solutions with increased concentrations of a

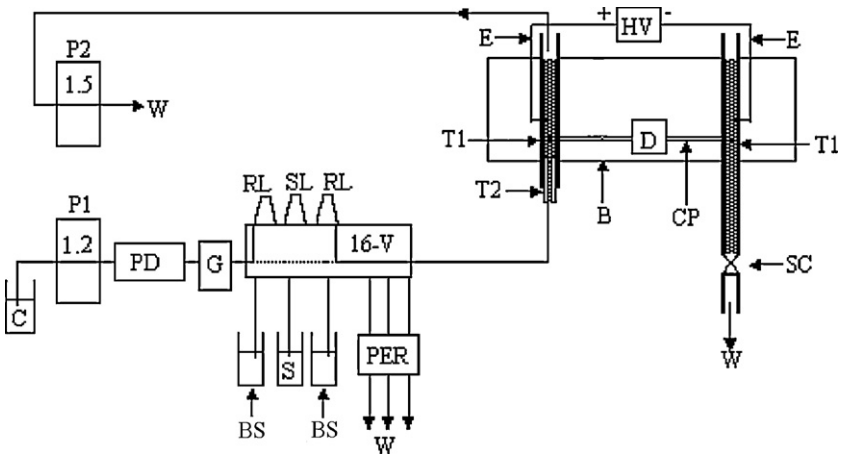


Fig. 1. Manifold for the FI-CE (not to scale). C, carrier solution; S, sample; P1 and P2, pumps; PD, pressure damper; G, pressure gauge; SL, sampling loop; RL, reagent loop; 16-V, 16-way valve; PER, peristaltic pump; B, planar plastic base; T1, Tygon tubing; T2, Tygon tubing (1.2 mL/min); CP, separation capillary column; E, platinum electrode; W, waste; HV, high voltage; D, detector; SC, screw clamp; BS, buffer solution (pH 7.4, ionic strength 0.17 M).

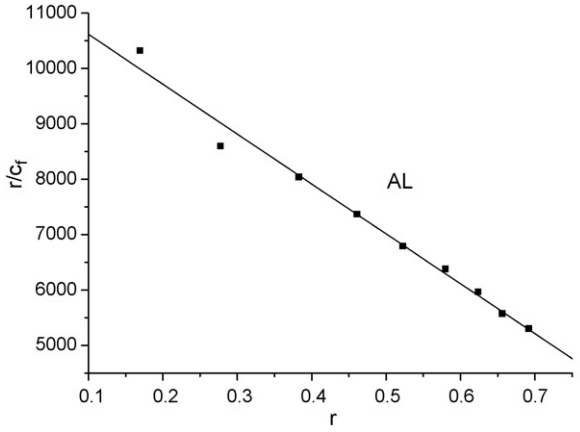


Fig. 2. Regression plots to Scatchard equation of AL drug using batch spectrofluorimetry. HSA concentration: 3.0×10^{-6} M; phosphate buffer, pH, 7.40; λ_{ex} = 282 nm, λ_{em} = 338 nm; 296 K.

drug (50–300 μM) and fixed concentrations of HSA (30 μM) were incubated at 37 °C for 2 h prior to CE analysis and were introduced electrokinetically into the capillary. The unbound drug concentration was measured from the plateau height of electropherogram.

2.5. Determination of binding parameters by FLU

FLU was operated with a RF-5301PC spectrofluorophotometer (Shimadzu, Kyoto, Japan), using 5 nm/5 nm slit widths. The excitation wavelength was set at 282 nm and the emission spectra recorded between 280 and 500 nm. An electronic thermo regulating Water-bath (NTT-2100, EYELA, Japan) was used to control the temperature. 3.0 mL of a solution containing appropriate concentration of HSA was titrated manually by successive additions of 1.0×10^{-3} M drug with trace syringes, respectively, and the fluorescence intensity was measured. Quenching data from the fluorescence titration experiments could be analyzed according to the Scatchard equation. The Scatchard plot of AL obtained by FLU was shown in Fig. 2. Because such drugs as Pro, VER, Met, AT and KW not only had intrinsic fluorescence but also had the same fluorescent location as HSA, their binding parameters had not been determined by FLU.

2.6. Determination of binding parameters by ED-UV

ED-UV was used as a reference standard method to determine the binding parameters of drugs to HSA. HSA at a concentration of 90 μM (5 mL) in phosphate buffer was dialyzed against the same buffer containing various concentrations (50–1000 μM) of drugs (120 mL) using dialysis tubing (Solarbio, USA, DM 25 mm, dialysis MW 8000–14,000). Two sectors were allowed to equilibrate for 24 h at 37 °C. The drug concentration outside the dialysis tubing represented the free concentration of drug (C_f). This concentration was determined by CARY-100UV–vis Spectrophotometer (Varian, CA, USA). The binding parameters were obtained by Scatchard equation.

2.7. QSAR studies of the interaction between HSA and drugs

The molecular structures of the drugs were drawn with ISIS DRAW software and optimized in Hyperchem program using the Polak-Ribiere algorithm until the root mean square gradient was 0.001. Each compound was represented by about 400 structural descriptors which were calculated using CODESSA software, developed by the Katritzky group [18,19]. Heuristic method in CODESSA was used for selecting the descriptors responsible for the binding constants (K) of drug–protein. Firstly, all descriptors were checked to ensure: (a) the values of each descriptor were available for each structure; and (b) there was a variation in these values. Descriptors for which values were not available for every structure in the data set in question were discarded. Descriptors having a constant value for all structures in the data set were also discarded. Thereafter all possible one-parameter regression models were tested and insignificant descriptors were removed. Secondly, the program calculated the pair correlation matrix of descriptors and further reduced the descriptor pool by eliminating highly correlated descriptors. All two-parameter regression models with remaining descriptors were subsequently developed and ranked by the regression correlation coefficient. A stepwise addition of further descriptor scales was performed to find the best multi-parameter regression models with the optimum values of statistical criteria (highest values of R^2, the leave-one-out cross-validated R^2_{cv} and the F-value).

3. Results and discussion

3.1. Adsorption and reproducibility

Protein adsorption to the bare silica wall in CE can cause problems in separation and imprecise of quantification. A lot of studies on protein adsorption had been reported for minimizing this phenomenon [5]. NaOH and SDS had been proven to be useful for minishing the adsorption of the HSA onto the capillary wall [20,21]. In order to compare the desorbing effect of NaOH and SDS, the relative standard deviations (RSDs) of plateau height, plateau area and migration time were investigated in the conditions of washing capillary using 0.1 M NaOH and 30 mM SDS, respectively. The washing pressure was 20 kPa and the flow rate of FI system was 1 mL/min. The experiment was carried out in the phosphate buffer (pH 7.4, ionic strength 0.17 M) with FI-CE-FA method, the sample used was 150 μM AL + 30 μM HSA. When capillary was rinsed using 0.1 M NaOH and 30 mM SDS after each run, respectively, the RSDs of plateau height, plateau area and migration time were 6.18, 2.63, 2.43 and 1.79, 3.00, 4.75%, respectively. The results suggested that the quantitative analysis by plateau height preferred rinsing procedure with 30 mM SDS. When plateau area was used for quantitative analysis, 0.1 M NaOH was proved to be more effective for desorbing protein adsorption. Because the unbound drug concentration was measured from the plateau height of electropherogram, 30 mM SDS was used for rinsing the capillary in this work. In order to validate the desorbing effect of SDS, we compared the RSDs of plateau heights obtained by continuous five sample injections without washing capillary with that obtained by five individual sampling (after each run, the capillary was rinsed with 30 mM SDS and run buffer each for 5 min). The RSDs were 6.30 and 1.62%, respectively. The results suggested that using SDS solution to rinse the capillary was effective to improve the repeatability and reliability of CE analysis of samples containing protein.

3.2. FI-CE-FA

Fig. 3 showed the typical electropherograms of Met of FI-CE-FA. In Fig. 3, the curve A was due to 300 μM Met solution without protein, and the curves B and C were due to the 300 μM Met + 30 μM HSA and 300 μM Met + 60 μM HSA mixed solutions. The plateau height of curve A represented the total drug concentration of Met. The plateau heights of curves B and C represented the concentrations of unbound drug. Because of protein binding, the plateau heights of curves B and C were lower than that of curve A. Furthermore, the concentration of unbound drug decreased with increase of the HSA concentration.

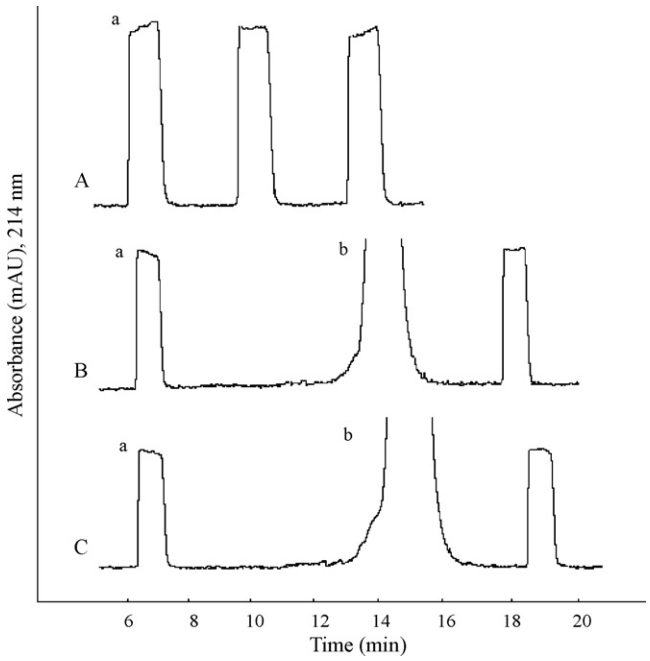


Fig. 3. Electropherograms of Met with different concentrations of HSA. (A) 300 μM Met + 0 μM HSA, continuous three sampling; (B) 300 μM Met + 30 μM HSA, continuous two sampling and the HSA peak of the second sampling was omitted; (C) 300 μM Met + 60 μM HSA, continuous two sampling and the HSA peak of the second sampling was omitted. CE parameters: capillary, 75 μm I.D., 375 μm O.D. and 43.7 cm length (40.7 cm effective length); UV detection, 214 nm; buffer, phosphate (pH 7.4, ionic strength 0.17 M); temperature, 23.5 ± 0.5 °C; running voltage, 8 kV. FI parameters: sample loop volume, 115 μL; charging time, 15 s; injecting time, 84 s; flow rate, 1.2 mL/min.

The binding parameters between protein and drug can be obtained by the following Scatchard equation:

$$r = \sum_{i=1}^{m} \frac{n_i K_i [D_f]}{1 + K_i [D_f]} \quad (1)$$

If there is only one class of binding site or one of the two binding sites is weak, the binding parameters can be obtained by the simplified Scatchard equation $r/C_f = -Kr + nK$ [22], where r is the ratio of the bound drug concentration to the protein concentration, C_f is the unbound drug concentration, K is binding constant and n is the number of binding sites on one protein molecule. The binding parameters obtained by FI-CE-FA were shown in Table 1. The drug–protein-binding percentage (PBP) was calculated according to the following equation:

$$\mathrm{PBP} = \frac{C_t - C_f}{C_t} \times 100\% \quad (2)$$

where C_t is the total concentration of the drug. The results were shown in Table 2. According to the values of n and PBP, it can be concluded that the drugs KT and Pro bind less to HSA while AT and LS hardly bind to HSA.

3.3. Comparison of results obtained by different methods

The results obtained by different methods and the literature values [5,23–27] were summarized in Table 1. As shown in Table 1, the results obtained by different methods or different laboratories were different. The reasons were as follows: (1) there was a bias in some protein–drug binding measurements among the techniques [5]. Each method measured specific parameters under certain conditions. (2) Different types of HSA or plasma used also caused a difference. Non-defatted HSA gave much higher binding constants than defatted HSA because fatty acids also bind to the drugs. (3) The different concentrations of HSA employed in different studies affected the binding parameters [15].

3.4. Relationship between drug–protein binding constants and structural parameters of drugs

The best linear model with three parameters was shown in Table 3. The logarithm values of predicted K according to the multiple linear regression (MLR) model were shown in Table 2. As shown in Table 2, the predicted values were in good agreement with the experimental values. The linear relationship (correlation coefficient $R^2 = 0.9865$, $R^2_{cv} = 0.9664$) between the predicted and the experimental K values was good.

According to the t-test values in Table 3, the most important descriptor affecting the K was a quantum chemical descriptor, maximum nucleophilic reactivity index for an O atom, which estimates the relative reactivity of the atom in the molecule for a given series of compounds and is related to the activation energy of the corresponding chemical reaction. Analysis of the results indicated that the selected molecular descriptors could describe the structural features of the compounds and could be responsible for their binding to protein. The MLR model proposed in this experiment could provide some structural features related to the drug–protein inter-

Table 1
Binding parameters of drugs to HSA obtained by different methods vs. literature valves.

Drug	K (M^{-1}), n (FI-CE-FA)	K (M^{-1}), n (FLU)	K (M^{-1}), n (ED-UV)	K (M^{-1}), n (Ref.)	Binding site[a] (FI-CE-FA)
AL	2.29×10^4, 2.81	8.86×10^3, 1.29	2.47×10^4, 1.51	–	I
Dil	1.35×10^4, 4.20	4.03×10^3, 1.52	2.83×10^3, 1.70	$(5.9 \pm 0.3) \times 10^2$, – (PACE-FA) [5]	II
VER	3.17×10^3, 2.48	–	7.97×10^3, 1.61	$(1.10 \pm 0.03) \times 10^3$, – [5] 1.8×10^3, 1.24 (CE-FA) [21]	I
Pro	1.04×10^4, 0.48	–	1.48×10^4, 0.79	$(2.2 \pm 0.2) \times 10^3$, – [5] 1.5×10^3, 0.58 [21]	II
KT	7.85×10^3, 0.59	3.49×10^3, 1.53	8.09×10^3, 0.62	–	I, II
AT	3.88×10^3, 0.16	–	2.04×10^3, 0.96	–	I, II
Met	2.38×10^3, 2.88	–	8.66×10^3, 2.15	–	III or others
KW	9.46×10^3, 1.80	–	2.81×10^3, 1.51	$(6.42 \pm 0.33) \times 10^4$ (nK)[b] (ED) [22]	I, II
QL	3.48×10^4, 1.05	6.00×10^3, 1.55	1.75×10^3, 1.83	1×10^3, 1.4 (CE-FA) [6] 1.35×10^5, 1.10 (FLU) [23]	I, II
YD	5.01×10^3, 0.77	9.41×10^3, 1.34	1.00×10^4, 1.03	$(3.6 \pm 0.3) \times 10^3$, – [5] $(2.5 \pm 0.6) \times 10^3$, 0.94 ± 0.17 (ED) [24]	I, II
LS	4.05×10^4, 0.13	7.51×10^3, 1.02	5.59×10^3, 0.62	–	II

PACE, pressure-assisted capillary electrophoresis; FLU, fluorescence; ED, equilibrium dialysis.

[a] According to Sudlow nomenclature [25].

[b] The product of binding constant and the number of binding site.

Table 2
Compounds structure, drug-protein-binding percentage (PBP) and the predicted results of *K* from the MLR regression model.

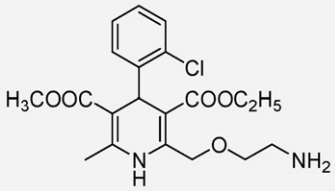
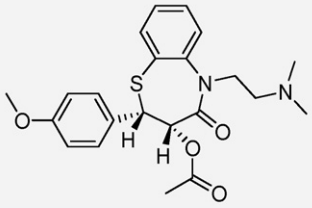
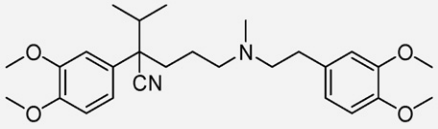
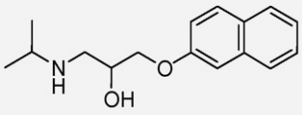
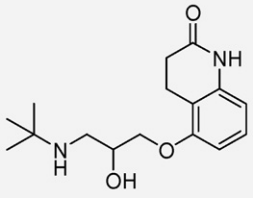
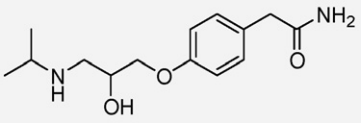
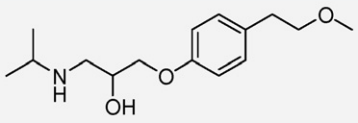
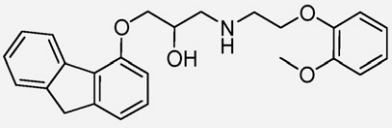
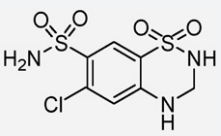
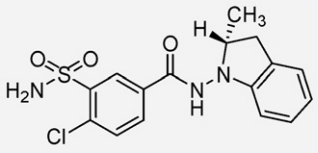
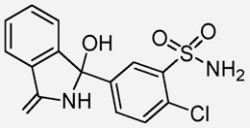
Drug	Structure	pK_a	PBP (%) (C_p:C_t = 1:5)	lg K (M^{-1}) (exp.)	lg K (M^{-1}) (pred.)
AL		8.6	24.3	4.36	4.30
Dil		7.75	54.7	4.13	4.12
VER		8.92	22.0	3.50	3.54
Pro		9.45	8.6	4.02	3.95
KT		9.24	6.0	3.89	3.92
AT		9.6	2.7	3.59	3.52
Met		9.7	12.5	3.38	3.45
KW		8.0	20.4	3.98	3.98
QL		7.9	80.5	4.54	4.56
YD		8.80	72.4	3.70	3.70

Table 2 (*Continued*)

Drug	Structure	pK_a	PBP (%) (C_p:C_t = 1:5)	lg K (M^{-1}) (exp.)	lg K (M^{-1}) (pred.)
LS		9.20	2.3	4.61	4.65

action and afford some instruction for further investigation of other drug–protein interactions.

3.5. Drug displacement studies

In order to investigate a possible binding site on protein for a given drug, drugs that are deemed to bind at particular sites and have high drug–protein-binding percentage, such as phenylbutazone (PB) (site I and 98%) and *L*-tryptophan (*L*-try) (site II and 90%) [4], may serve as guides to evaluate the binding site of given drugs by displacement experiments. Drug's binding site on a protein can be determined by the change in concentration of unbound drug after adding the coadditive (either *L*-try or PB) to the equilibrated mixture of analyte and protein. The ability of *L*-try and PB to displace QL from HSA was illustrated in Fig. 4. As shown in Fig. 4, when *L*-try or PB with varied concentrations (20–200 μM) was added into the mixed solution of QL (100 μM) and HSA (50 μM), respectively, all of the unbound concentrations of QL increased. It suggested that QL had two binding sites (i.e., site I, site II) in one HSA molecule. The binding sites of drugs obtained by FI-CE-FA were summarized in Table 1. For the drugs such as KT, AT, KW, QL and YD, which had two classes of binding sites in one HSA molecule and one of the two binding sites was weak according to the displacement experiments, their binding parameters obtained approximately from equation $r/C_f = -Kr + nK$ may have large errors compared with the real values.

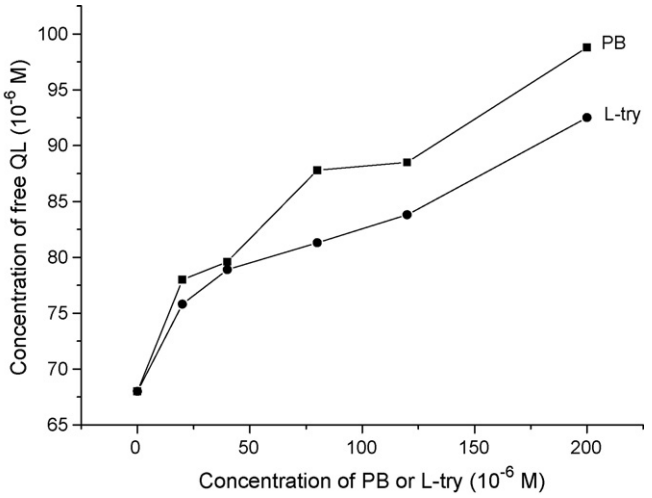


Fig. 4. Graph of free QL concentration ($C_{f,QL}$) against the concentrations of *L*-try or PB ($C_{L\text{-try}}$ or C_{PB}) added, respectively. Sample, 200 μM QL + 50 μM HSA.

3.6. Binding synergism

Simultaneous administration of drugs influences their protein binding behavior, absorption, excretion, distribution and subsequently their efficacy and toxicity. In clinic, calcium channel blockers and β-blockers are often used together with diuretics. The ratios of binding constants of drugs to HSA in the absence and presence of co-administration were listed in Table 4. From the data in Table 4, it can be seen that the competitive interaction toward protein between two drugs existed, and only when YD and Met were co-administrated, both of their binding constants increased at the same time. So co-administration of YD and Met was safe, but co-administration of other drugs should be careful.

Table 3
The linear model between structure and binding constants (K) (R^2 = 0.9865, F = 170.30, s^2 = 0.0033).

Descriptor	Coefficient	Error	*t*-Test value
Constant	−2.8527	0.5522	−5.1656
Maximum nucleophilic reactivity index for a O atom	−56.8190	3.7628	−15.1000
Minimum total interaction for a C–N bond	0.5152	0.0378	13.6176
Number of single bonds	0.0115	0.0016	−7.1422

Table 4
Influence of co-administration on binding constants of drugs (K'/K)[a].

Compound	AL	Dil	VER	Pro	KT	AT	Met	KW	QL	YD	LS
AL	–	–	–	–	–	–	–	–	1.87[b]	0.09	6.20
Dil	–	–	–	–	–	–	–	–	0.17	0.01	0.04
VER	–	–	–	–	–	–	–	–	1.53	0.45	2.04
Pro	–	–	–	–	–	–	–	–	0.004	0.002	0.005
KT	–	–	–	–	–	–	–	–	0.59	0.86	0.85
AT	–	–	–	–	–	–	–	–	1.76	0.38	0.55
Met	–	–	–	–	–	–	–	–	1.53	1.88	2.18
KW	–	–	–	–	–	–	–	–	0.50	0.84	0.66
QL	0.34	0.06	0.45	0.21	0.01	0.23	0.14	0.07	–	–	–
YD	5.14	2.80	7.20	15.80	49.85	8.55	13.13	3.14	–	–	–
LS	0.18	0.14	0.08	0.12	0.03	0.12	0.19	0.06	–	–	–

[a] K', the binding constant of A drug after adding co-administrated drug B; K, the binding constant of single A drug.
[b] The ratio of binding constant (K') of AL after adding QL to the binding constant (K) of only AL was 1.87.

Table 5
Influence of ions on binding constants of drugs.

	Cl^-	KCl	$MgCl_2$	$ZnCl_2$
$K_{QL(AL)}$[a]	1.07×10^4	3.88×10^4	2.39×10^3	1.08×10^3
$K_{QL(Pro)}$	6.58×10^3	6.86×10^3	5.24×10^2	1.86×10^3
$K_{AL(QL)}$	4.55×10^4	2.12×10^4	3.05×10^4	3.87×10^4
$K_{Pro(QL)}$	38	36	27	30

[a] The binding constant of QL in presence of AL.

3.7. Effect of metal ions on binding constants of drugs

The binding of ions to protein makes this protein become an important regulator of intercellular fluxes and pharmacokinetic behavior of many drugs [28]. As major ions, Na^+, K^+, Mg^{2+} and Zn^{2+} are distributed widely in humans and animals bodies. It has been known that Na^+ does not bind to HSA [26]. Therefore, NaCl was used to examine the probable interference of chloride ion. In order to study the effect of these metal ions on the binding constants of drugs at near-physiological conditions, these metal ions were investigated while their concentrations were near biological concentrations (Na^+: 100 mM, K^+: 5 mM, Mg^{2+}: 1 mM and Zn^{2+}: 15 μM). Two mixed solutions of AL + QL-HSA and Pro + QL-HSA were used as examples to investigate the changes in binding constants of AL, QL and Pro when above metal ions were added into these equilibrated mixed solutions. Table 5 showed the influence of the metal ions on the binding constants of drugs. The results suggested the existence of competition interaction between metal ions and drugs. Most of metal ions could decrease the binding constants of drugs. It was effective to therapy in clinic for enhancing short-term pharmacological action of drugs.

4. Concluding remarks

In this paper, the developed FI-CE-FA method was used to investigate the interactions of HSA and 11 drugs (5 β-blockers, 3 calcium channel antagonists and 3 diuretics) and was compared to non-CE methods. The results suggested that the binding parameters obtained by different methods or different laboratories had a little difference. According to the values of n and PBP, it was concluded that the drugs KT and Pro bind less to HSA while AT and LS hardly bind to HSA. The main binding site of most drugs was site I according to displacement studies. The competitive experiments suggested that simultaneous administration of drugs and metal ions can influence drug–protein binding behavior. By comparing the K values of predicted and experimental ones, it was concluded that two quantum chemical descriptors and a constitutional descriptor were responsible for the interaction of drug–protein, which afforded some instructions for the investigation of other drug–protein interactions. The adsorption studies suggested that 0.1 M NaOH was more effective for desorbing protein adsorption when plateau area was used for quantitative analysis, and the quantitative analysis by plateau height preferred rinsing procedure with 30 mM SDS. The developed method was simple and preferred for screening in the early stage of drug development.

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