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Characterization of the Interaction between Bovine Serum Albumin and Lomefloxacin by Capillary Zone Electrophoresis

Ming GUO[1], Qing Sen YU[1]*, Jian Wei YAN[2], Fei TAN[1], Guo Zheng MA[1]

[1] Department of Chemistry, Zhejiang University, Hangzhou 3100271
[2] Department of Chinese Medicine Science & Engineering,
Zhejiang University, Hangzhou 3100271

Abstract: Three capillary zone electrophoresis (CZE) methods of the frontal analysis (FA), vacancy peak (VP) and simplified Hummel-Dreyer (SHD) were applied to investigate interaction between bovine serum albumin (BSA) and lomefloxacin, the experimental condition was established after a large number of tests. Based on the site-binding model, the binding parameters were measured according to the site model by Scatchard.

Keywords: CZE, the site model, BSA, lomefloxacin.

The drugs can be bound to serum albumins with a high affinity. This binding interaction results in a non-covalent protein-drug complex formation. Lomefloxacin (LMFX) is a newer fluoroquinolone with effective broad-spectrum antimicrobial activity [1]. The clinic therapy and adverse effect of the drug has a good relationship to the concentration of the protein-drug complex, so the study for binding phenomena from different view will be important for interpretation of the metabolism and transported process[2,3].

Capillary electrophoresis offers advantage for study non-covalent interactions. Recently, several CZE methods have been used to characterize the binding interaction [4-6]. To our knowledge, the exploration should be extended. In this paper, we investigated the binding interaction between BSA and LMFX by the FA, VP and SHD, respectively; the binding parameters were fitted according to the site-binding model by Scatchard equation[5,7].

Experimental

BSA and lomefloxacin were used from Huamei Biotechnological (Shanghai). All reagents were of analytical grade and double distilled water was used throughout. The running buffer was 0.0335 mol·L^{-1} phosphate buffer (pH=7.4). For the CZE experiments, a P/ACE MDQ system (Beckman, Fullerton, CA, USA) with UV detector was used. The lengths of capillary were 67/56 cm, respectively. The different condition of three methods for each

* E-mail: tdky@hotmail.com

run could refer to the literatures[4-6], the detection wavelength, were 214 nm and 254 nm.

In order to calculate properly the binding parameters of LMFX, a series of concentration of BSA-LMFX isotherm for three methods were desired. In all studies the BSA concentration was kept constant at 2.5×10^{-5} mol·L^{-1} and the LMFX concentration varied from 0.2×10^{-4} mol· L^{-1} to 9×10^{-4} mol· L^{-1}.

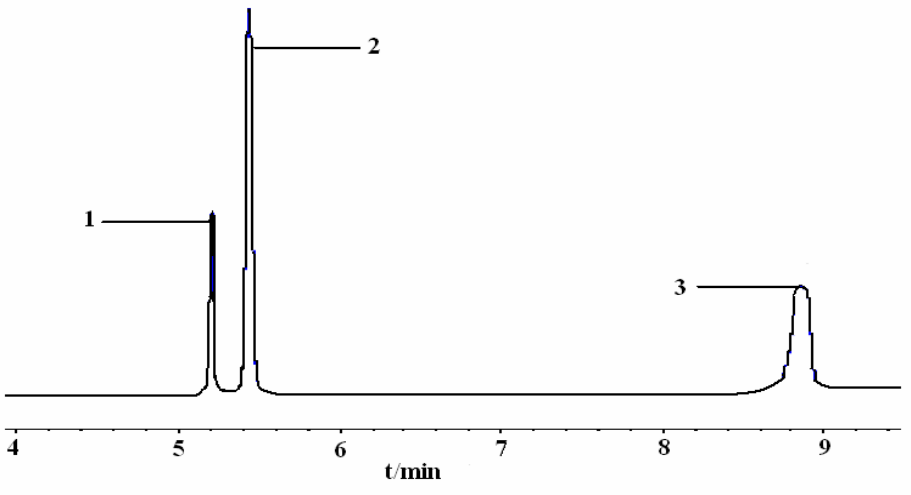
Results and Discussion

During the explorative experiments to lomefloxacin-binding research using CZE, the concentration of free drug could be obtained from the calibration curve for the FA (r=0.9951) and VP (r=0.9991). For the calculation of the bound fraction with the SHD, the formula, given by Pinkerton and Koeplinger[8] was applied. Then, the binding equation described by Scatchard [5,7] could be used to estimate the binding constant (K) and the binding site (n) for three methods.

The electropherogram of the test solutes in running buffer is shown in **Figure 1**, monitored at 214 nm, could be obtained, when drug, BSA and marker (methanol) all can be responded to detector.

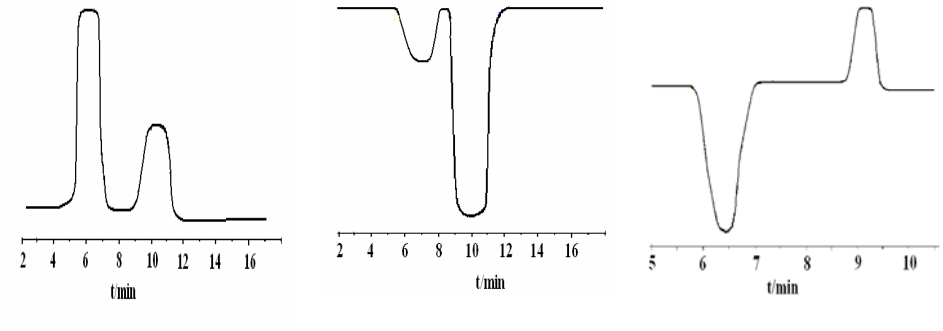
It can be seen that the BSA is not completely homogeneous and it is reasonable to assume that the size and charge on the protein are not significantly altered by the presence of adsorbed drug molecules. This means that the protein and protein-drug complex will have the same electrophoretic mobility. From **Figure 1** we can see that the electrophoretic mobility of protein (BSA) is larger than that of the drug.

Figure 1 Electropherogram of the test solutes (15KV, 25℃, 0.5 psi 5s, UV: 214 nm)



1) Methanol; 2) LMFX; 3) BSA.

Figure 2 Electropherogram of the capillary zone electrophoresis (UV: 254 nm)



(a) (b) (c)

(a) The frontal analysis method; (b) The vacancy peak method ; (c)The simplified Hummer-Dreyer method.

Figure 2 shows typical electropherograms at 254 nm, which the detector has only the trace of drug in the mobile phase. The results demonstrated that the LMFX has been bounded to BSA in the sample for three methods. If the ligand had no bound to the protein at all, the detector response would not have changed. Then, for the FA, one peak should be observed in the chromatographic profile, which reflects the LMFX concentration at 254 nm; For VP, the chromatographic profile should be one negative peak, the same effect as the injection of blank buffer, and the response of detector was the result of diluted sample; For SHD, the chromatographic profile should also be one negative peak, the same profile would be obtained with injection of the blank buffer.

When measuring binding parameters is used, the consideration of the kinetics of binding is important. In the absence of kinetic information, one can observe the shape of chromatographic peak. If the chromatographic peak is asymmetric, particularly fronting, one can surmise that the kinetics of dissociation may be too slow. The detailed explanation can be found from the literatures [9]. From **Figure 2**, it is sufficient to assume that "instantaneous equilibration" between BSA and LMFX is achieved throughout the running of CZE.

The non-covalent binding interaction of LMFX with BSA is less and weak compared with the covalent, so the preparation of the solutions and the measurements were performed according to a stringent protocol, the concentration of LMFX, BSA should also be selected carefully. After measurements of binding interaction between BSA and LMFX at 254 nm, the data fitting was assessed by Scatchard equation. **Table 1** shows the fitting results.

Table 1 The binding parameters for the system of lomefloxacin-BSA

Methods	Binding constant	Binding site	Correlation coefficient
The frontal analysis	9.6178×10^4	1.07	0.9779
The vacancy peak	8.7261×10^4	1.24	0.9684
The simplified Hummer-Dreyer	9.1475×10^4	0.94	0.9572

A relatively good relationship between the experiment points and the Scatchard equation shows that the interaction with LMFX bound to BSA agreed with the model of site-binding. It is also indicated that there is a stronger binding force between LMFX and BSA, and a binding site would be formed.

In this study, fluorescence spectroscopy was also used to investigate the binding interaction between LMFX and BSA, and check the result of CZE. For the experiments carried out at the literature condition [10], the binding constant of this interaction calculated from Scatchard plot was K=9.80×10^4 L·mol^{-1}, n=1.41(r=0.9993). It can be seen that the results obtain from the CZE is consistent with the fluorescence spectroscopy, although the different solution systems were introduced.

In general, in this paper, three CZE methods were applied to evaluate the binding parameters of LMFX-BSA solution system, the results demonstrate that the CZE is an availably means to study the interaction of protein-drug system, and one can expect the CZE could be applied to investigation in this field.

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