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Simultaneous Enantiomeric Separation and Binding Constants Determination by Affinity Capillary Electrophoresis using Human Serum Albumin

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Abstract

The binding of chiral drugs to plasma proteins is potentially enantioselective and frequently exhibits different pharmacological activities. Serum albumin is one of the most important proteins in human plasma and is commonly used as protein ligand in chiral capillary electrophoresis. Furthermore, human serum albumin has been reported as a stereoselective agent for studying enantioselective interactions. The estimation of binding constant highly contributes to enantiomeric-drugs development and quality control. In this study, mobility-shift affinity capillary electrophoresis was employed for the simultaneous enantiomeric separation and determination of binding constant. The enantiomeric separation was conducted at physiological pH of phosphate buffer containing human serum albumin as background electrolyte. Accordingly, a net negative charge of protein-ligand was obtained. Therefore, positively charged racemic drugs of amlodipine and verapamil were selected as chiral model compounds. Electrophoretic parameters of resolution and effective mobility were evaluated. Subsequently, association constant was determined through nonlinear regression of effective mobilities and total ligand concentrations. In the particular separation conditions, human serum albumin in the range of 30-110 µM displayed stereoselectivity to verapamil and amlodipine. As the sample was introduced into the capillary electrophoresis system, enantiomers bound the human serum albumin to different extents. The difference in apparent mobility shifts between enantiomers corresponds to the resolution of about 1.05-3.93. Moreover, *R*-(+)-verapamil proved to be bound stronger to human serum albumin compared with *S*-(-)-verapamil. The association constant of *S*-(-)-amlodipine was found to be higher compared to its antipode when applying the racemic mixture. In addition to this, a close agreement with the *S*-(-)-enantiomer was achieved.

Introduction

Capillary electrophoresis (CE) performs as a promising method in the emerging field of enantioselective investigation. Chiral CE posses a possibility to operate in a free solution that does not require the immobilization of the chiral selector (ligand). Different modes of affinity capillary electrophoresis (ACE) have been developed using human serum albumin (HSA) as a biologic-related agent for assessing non-covalent binding assay. The availability of HSA in the circulatory system makes it an ideal candidate for studying enantiomer-protein interactions which are similar to the process drugs work in the human body. In order to imitate the enantiomeric interactions, mobility shift-ACE (ms-ACE) was applied. The common approaches that the molecular interaction under investigation must alter the size or charge in the migration pattern. In principle, the entire capillary and the run buffer vials are filled with buffer containing the selected ligand in various concentrations, under a certain electric field. Along with electrophoretic mobility, the interaction between enantiomer and HSA were obtained in different mobility shift. Thus, ms-ACE offers a simultaneous investigation of enantiomeric separation and binding constant determination

Experimental

❑ **Instrumentation**

PrinCE CEC-760 (Prince Technologies, Emmen, Netherlands).

❑ **Capillary**

Bare fused-silica capillary 50 mm id, 53.5 cm total length and 45.0 cm effective length.

❑ **Buffer**

20 mM phosphate buffer pH 7.4

❑ **EOF marker**

Acetone 2% v/v

❑ **Chiral selector**

HSA

❑ **Chiral compounds**

(*R*,*S*)–AML; (*S*)-AML; (*R*,*S*)-VER

Table 1.
Rinsing protocol and separation conditions

Rinsing (before each separation)		
Reagent	**Time [min]**	**Pressure [mbar]**
30 mM SDS	1.0	1,500
Water	0.5	1,500
0.1 M NaOH	1.0	1,500
Water	0.5	1,500
BGE	2.0	1,500
Separation		
CE Parameter	**Condition**	**Pressure [mbar]**
Injection	60 sec	50
Voltage	15 kV	
Temperature	25 °C	
Wavelengths	240 & 250 nm	

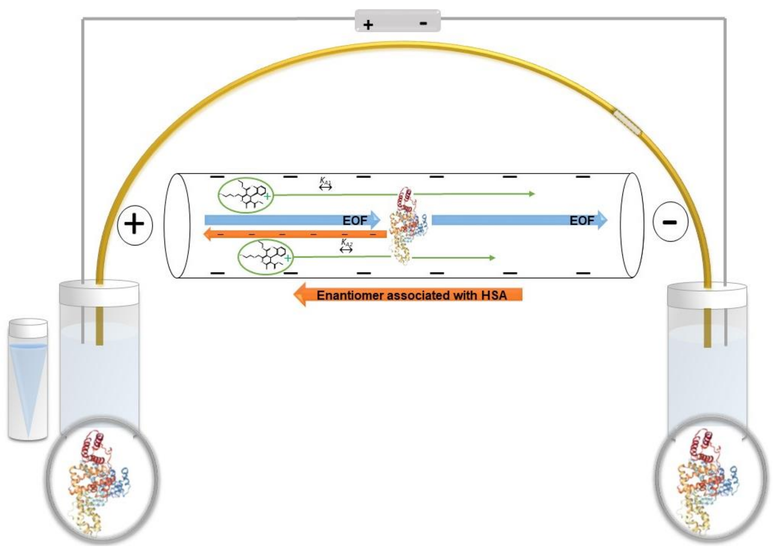
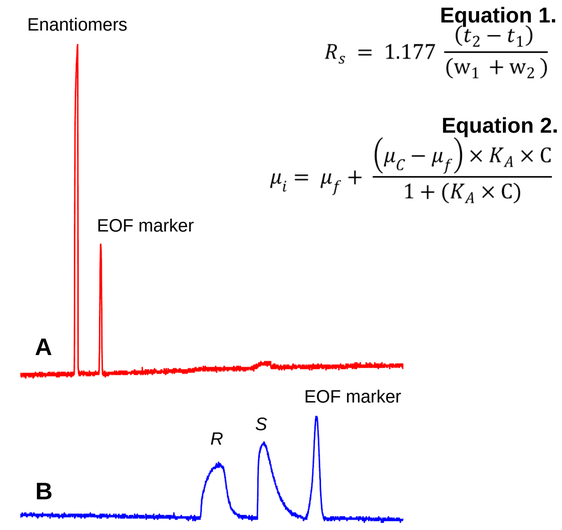


Figure 1. ms-ACE applied system



Equation 1.

$$R_s = 1.177 \frac{(t_2 - t_1)}{(w_1 + w_2)}$$

Equation 2.

$$\mu_i = \mu_f + \frac{(\mu_C - \mu_f) \times K_A \times C}{1 + (K_A \times C)}$$

Figure 2. Separation profile with (A) net BGE; and (B) BGE containing HSA.

Results and Discussion

Amlodipine is a basic drug (pKa 8.6) with a primary amine group and acquires positive charge in low pH (less than the pKa value). As shown in figure 3, slower migrating of *S*-(-)-amlodipine indicates a stronger interaction between enantiomer and HSA, which leads to slower movement of this enantiomer toward the detector.

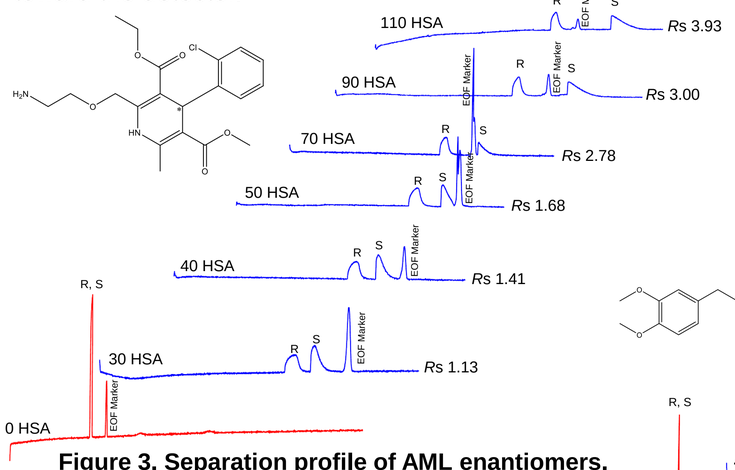


Figure 3. Separation profile of AML enantiomers.

At higher HSA concentrations (70-110 µM), *S*-(-)-amlodipine dramatically shifts to the longer migration times, which also represents changing in charge to be much more negative than its antipode.

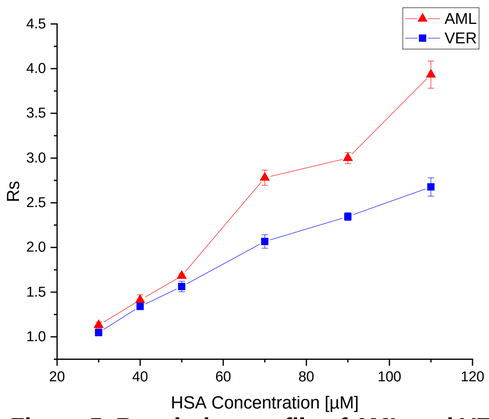


Figure 5. Resolution profile of AML and VER enantiomers.

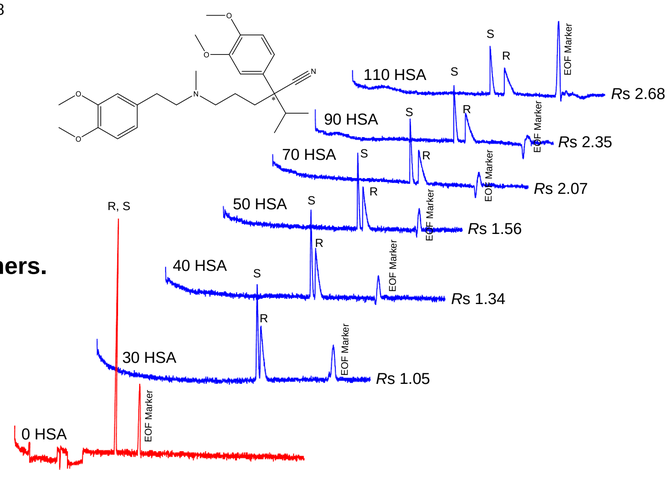


Figure 4. Separation profile of VER enantiomers.

Baseline separation of verapamil enantiomers shown in figure 4. The resolutions gradually increase, in the migration order of *S*-(-)-verapamil followed by *R*-(+)-verapamil.

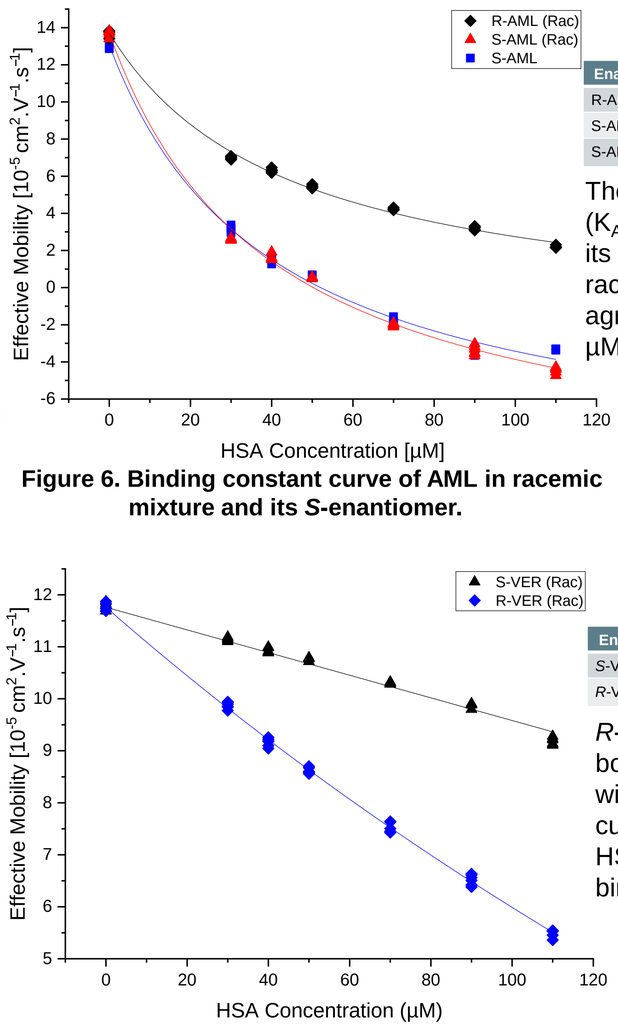


Figure 6. Binding constant curve of AML in racemic mixture and its *S*-enantiomer.

Table 2.
Binding parameters of amlodipine enantiomers

Enantiomer	K_A [µM^{-1}] ± SE	μ_c [10^{-5}cm^2.V^{-1}.S^{-1}] ± SE	r^2	Reduced chi-sqr
R-AML (Rac)	0.0226 ± 8.60E-4	-2.1022 ± 0.21	0.9968	0.0430
S-AML (Rac)	0.0250 ± 9.57E-4	-10.8757 ± 0.21	0.9966	0.1110
S-AML	0.0251 ± 11.70E-4	-9.9959 ± 0.41	0.9950	0.1459

The association constant of *S*-(-)-amlodipine (K_A 0.0250 µM^{-1}) was found to be higher compared to its antipode (K_A 0.0226 µM^{-1}) when applying the racemic mixture. In addition to this, a very close agreement with the *S*-(-)-enantiomer with K_A 0.0251 µM^{-1} was achieved

Figure 7. Binding constant curve of VER in racemic mixture.

Table 3.
Binding parameters of verapamil enantiomers

Enantiomer	KA [µM^{-1}] ± SE	μ_c [10^{-5}cm^2.V^{-1}.S^{-1}] ± SE	r^2	Reduced chi-sqr
S-VER (Rac)	6.63E-6 ± 4.56E-4	-3271.30 ± 2.25E5	0.9968	0.0430
R-VER (Rac)	0.00184 ± 1.67E-4	-25.30 ± 2.91	0.9983	0.0066

R-(+)-verapamil (K_A 0.00184 µM^{-1}) proved to be bound stronger to human serum albumin compared with *S*-(-)-verapamil (K_A 6.63E-6 µM^{-1}). The straight curve line represents a low interaction of verapamil to HSA. It also leads to the weak accuracy of the binding constants prediction.

Conclusion

- Mobility Shift-ACE has been successfully applied for simultaneous enantiomeric separation and binding constants determination of amlodipine and verapamil.
- The enantiomers of amlodipine and verapamil were selectively separated using HSA as a chiral selector through a free solution approach.
- In racemic mixture, *S*-(-)-amlodipine showed higher interaction with HSA compared to *R*-amlodipine, and a close agreement with the *S*-(-)-enantiomer was achieved.
- Verapamil exhibited interaction with HSA which *R*-(+)-verapamil bound stronger than *S*-(-)-verapamil.
- Strong interaction profile between enantiomer and HSA provided a more accurate prediction in binding constant determination.

References

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