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Protein-Based Affinity Capillary Electrophoresis for Enantioseparation of Calcium Channel Blockers

Ratih Ratih[1,2], Mufarreh Asmari[1], Hermann Wätzig[1], Matthias Stein[1] Sami El Deeb[1]

[1]Institute of Medicinal and Pharmaceutical Chemistry | TU Braunschweig, 38106 Braunschweig, Germany

[2]Faculty of Pharmacy | The University of Surabaya, 60293 Surabaya, Indonesia

ratih.ratih@tu-braunschweig.de

Abstract

The development of enantiomeric separation methods has been an ongoing concern in pharmaceutical research, particularly the use of proteins as binding agents. In the circulatory system, human serum albumin presence as the most abundant transport protein which is stereoselective towards a wide range of chiral compounds. Hence, protein-based chiral capillary electrophoresis performs as a powerful technique in affinity study. In this research, the enantioselectivity and binding constants of calcium channel blockers were investigated by mobility shift-affinity capillary electrophoresis using human serum albumin as a chiral selector. For this purpose, positively charged racemic mixture of amlodipine and verapamil were selected as chiral drug models. The separation was conducted in a bare-fused silica capillary with a background electrolyte of 20 mM phosphate buffer pH 7.4, at 15 kV applied voltage and 25 ºC. As a sample of chiral compounds was introduced into capillary electrophoresis system, enantiomers bound to human serum albumin in different extents. The association constant of the enantiomers was determined using nonlinear regression as a function of total selector concentrations and effective mobilities. The difference in apparent mobility shifts of enantiomers corresponds to the resolution value of 1.0-3.9 and selectivity of 1.02-131. *R*-(+)-verapamil proved to be more strongly bound to human serum albumin compared with *S*-(-)-verapamil. The K_A value of *S*-(-)-amlodipine in the racemic mixture was found to be higher than its antipode. In addition to this, a close agreement with the *S*-(-)- enantiomer was achieved.

Introduction

The primary structure of proteins consists of chiral amino acids. Hence, protein are stereoselective by nature. Various types of proteins have been employed as chiral selectors (CS) such as serum transport proteins and glycoproteins. The enantiomeric separations are carried out based on differences in the binding strength and number of binding sites for the chiral compounds. Their ability to be employed in direct chiral separation brings simplicity in chiral capillary electrophoresis (CE) technique by free solution approach. The protein-based CS can be either introduced as a sample or added into background electrolyte (BGE) that does not require the immobilization. Mobility shift-affinity capillary electrophoresis (ms-ACE) has been applied using human serum albumin (HSA) as a biologic-related agent for assessing of non-covalent binding. The availability of HSA in the circulatory system makes it an ideal candidate for studying the enantiomer-protein interactions which are similar to the enantiorecognition in biological systems. In order to imitate the interactions, ms-ACE is applied at a physiological pH. In principle, the entire capillary is filled with buffer containing the selected ligand in various concentrations under a certain electric field. Along with electrophoretic mobility, the interactions between enantiomers and HSA will result in different mobility shifts. Thus, ms-ACE offers a powerful tool for simultaneous 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

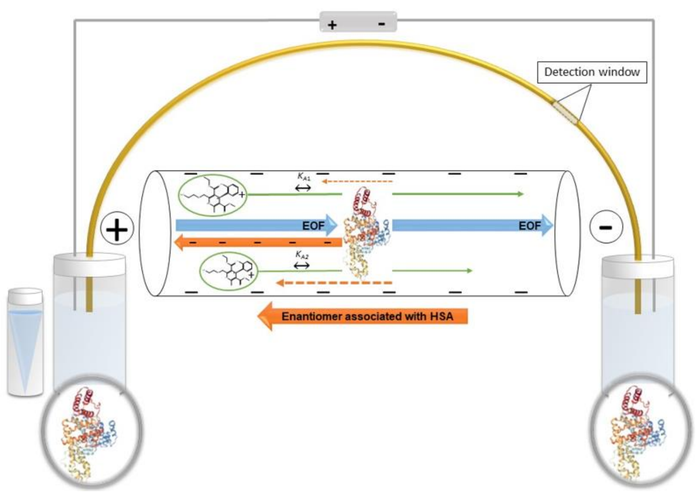


Figure 1. ms-ACE applied system

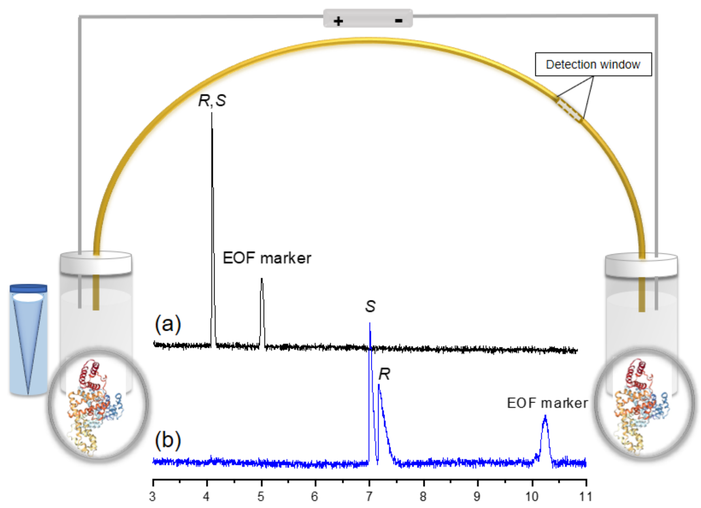


Figure 2. Separation profile with (a) net BGE; and (b) BGE containing HSA.

Rinsing (before each separation)		
Reagent	**Time [min]**	**Pressure [mbar]**
30 mM SDS	1.0	1500
Water	0.5	1500
0.1 M NaOH	1.0	1500
Water	0.5	1500
BGE	2.0	1500
Separation		
CE Parameter	**Condition**	**Pressure [mbar]**
Injection	60 sec	50
Voltage	15 kV	
Temperature	25 ºC	
Wavelengths	240 & 250 nm	

Equation 1.

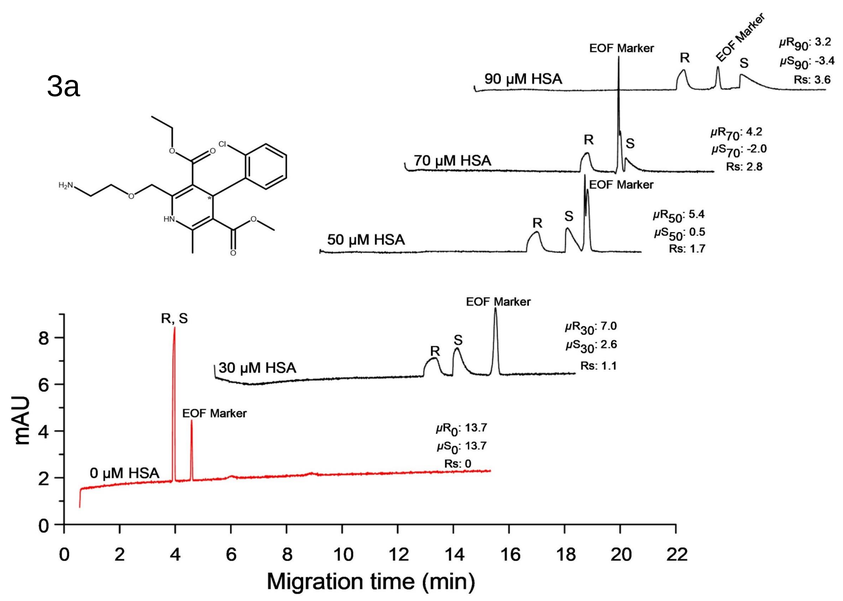
$$R_s = 1.177 \frac{(t_2 - t_1)}{(w_1 + w_2)}$$

Equation 2.

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

Results and Discussion

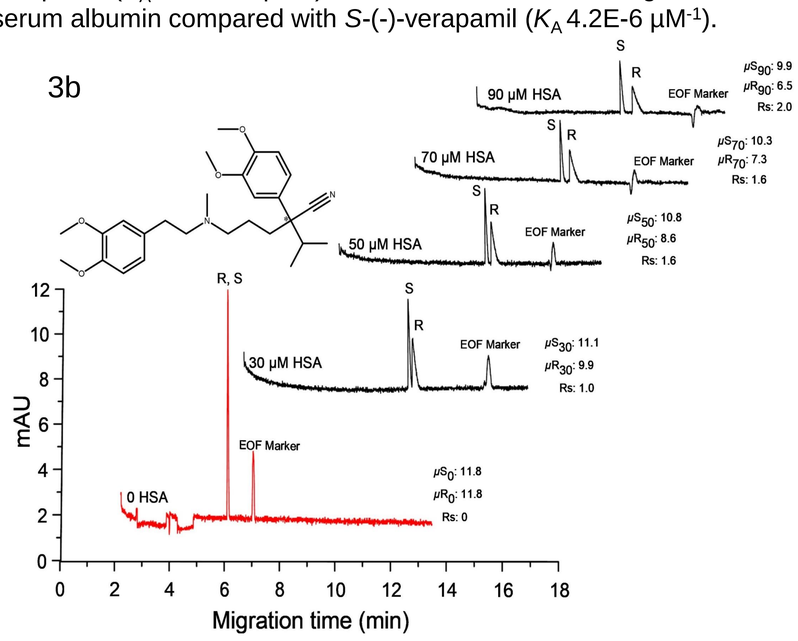
Amlodipine (AML) is a basic drug (pK_a 9.45) with a primary amine group and acquires positive charge in a pH lower than its pK_a value. As shown in figure 3a, slower migration of *S*-(-)-amlodipine indicates a stronger interactions between enantiomer and HSA, which leads to slower movement of the enantiomer towards the detector.



At higher HSA concentrations (70-110 µM), the migration time of *S*-(-)-amlodipine dramatically shifts, which also represents longer in the complex form compared to its antipode.

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 4a).

Baseline separation of verapamil (VER) enantiomers are shown in figure 3b. The enantiomers resolution gradually increases, in the migration order of *S*-(-)-verapamil followed by *R*-(+)-verapamil. *R*-(+)-verapamil (K_A 0.00184 μM^{-1}) showed to be bound stronger to human serum albumin compared with *S*-(-)-verapamil (K_A 4.2E-6 μM^{-1}).

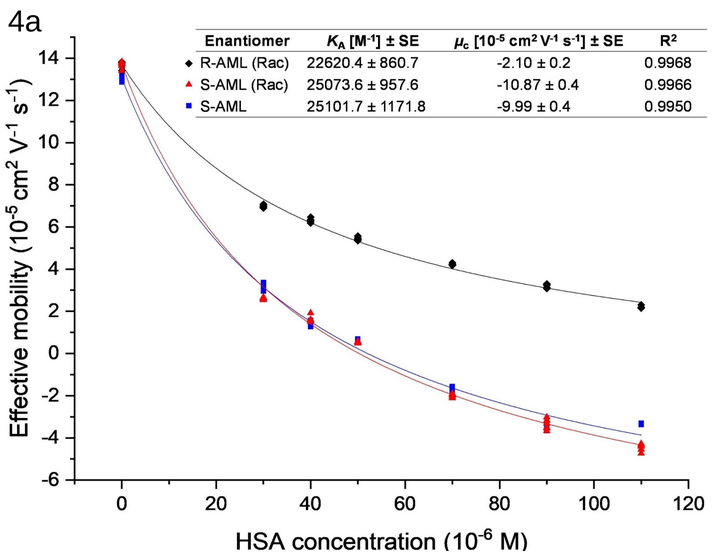


However, a reliable binding curve could not be obtained for VER with HSA concentrations of 30-110 µM. Due to the weak interactions between VER and HSA in the CE applied system, binding curve seems very straight (4b).

An insufficient binding curve potentially leads to the low accuracy of binding constants prediction. Indeed, the K_A values of VER enantiomers correspond to the apparent banding constants at a certain CE conditions.

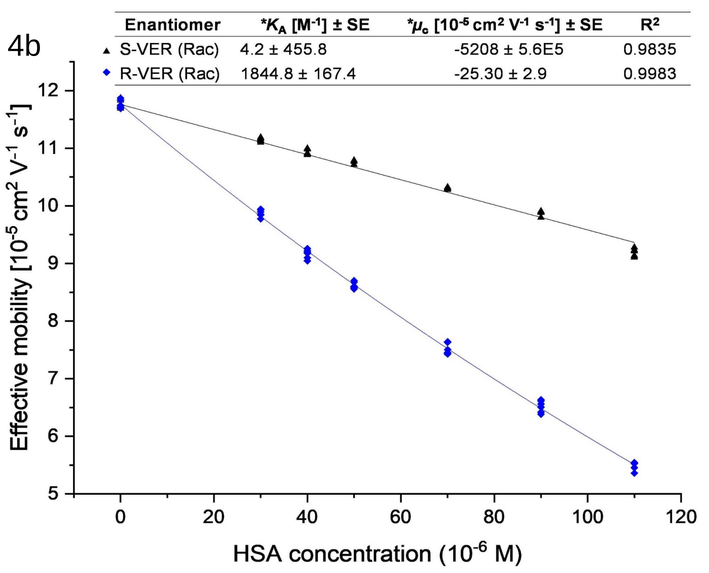
4a

Enantiomer	K_A [M^{-1}] ± SE	μ_c [10^{-5} cm^2 V^{-1} s^{-1}] ± SE	R^2
R-AML (Rac)	22620.4 ± 860.7	-2.10 ± 0.2	0.9968
S-AML (Rac)	25073.6 ± 957.6	-10.87 ± 0.4	0.9966
S-AML	25101.7 ± 1171.8	-9.99 ± 0.4	0.9950



4b

Enantiomer	*K_A [M^{-1}] ± SE	*μ_c [10^{-5} cm^2 V^{-1} s^{-1}] ± SE	R^2
S-VER (Rac)	4.2 ± 455.8	-5208 ± 5.6E5	0.9835
R-VER (Rac)	1844.8 ± 167.4	-25.30 ± 2.9	0.9983



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 in which *R*-(+)-verapamil binds stronger than *S*-(-)-verapamil.
- Strong interaction profile between enantiomer and HSA provides accurate prediction in binding constants determination.

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