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An approach to the determination of the enantiomeric excess at the extreme case by capillary electrophoresis

Hongting Xiao[a], Xia Fu[a], Shuang Liang[a], Youxin Li[b], James J. Bao[b,**], Yong Zhang[a,b,*]

[a] Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, China
[b] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China

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ABSTRACT

Capillary electrophoresis (CE) has been applied to determine the percentage of enantiomeric excess (ee%) of chiral compounds. In such assays, the quality of chiral selectors (CSs) plays vital roles in resolving the enantiomers for accurate determination of the ee%. Selecting an efficient CS is usually by trial and error, and is, if ever possible, time-consuming and costly. Here we propose a new approach by using the velocity gap mode of CE (VGCE) method, to simplify the method development process for ee% determination. With VGCE, it is still possible to measure ee% even when the CS has a weak resolving power. This is especially important at the extreme cases where one of the enantiomers is significantly higher than the other one. The key point of VGCE in this case is to fractionate the small part of the mixture containing both enantiomers from the major component of the enantiomer, which is already enantiopure. Baseline separations can be achieved between the two enantiomers for the small mixture due to less longitudinal dispersion, making it possible to determine the ee%. The feasibility of this VGCE approach was confirmed by the ee% measurements of amlodipine and ofloxacin, respectively. And the practical application of VGCE was tested by analyzing levamlodipine besylate tablet.

1. Introduction

The importance of stereochemical specificity in biological systems has attracted high attention. Two isomers of a drug may be different in bioactivity, pharmacology, pharmacokinetics and toxicology [1,2]. However, enantiomers have exactly the same physical and chemical properties in an isotropic environment. This makes it a challenge task to separate them from each other [3]. Determination of enantiomeric excess (ee%), is of special importance for the quality control of chiral synthesis and chiral pharmaceuticals. Food and Drug Administration requires, besides evaluating the effects of individual enantiomers, clarifying the purity of all chiral molecules produced [4]. The enantiomeric content of a mixture can be expressed as the following eq:

$$\text{ee\%} = \frac{a-b}{a+b} \times 100\% \quad (1)$$

where ee% is the percentage of enantiomeric excess, a is the major enantiomer, and b is the minor enantiomer. Two enantiomers are baseline separated first, then ee% is calculated according to their peak areas. CE has become an alternative separation technique in recent years [5–7]. The separation mechanism is based on the formation of diastereomeric complexes between the analytes and the chiral selector (CS) [8]. So a suitable CS is the key to achieve a chiral separation. The fact is that no CS could efficiently resolve all chiral substances. And usually one CS has good effect on one or few chiral substances but weak or no effect on the others [9]. Consequently, various CSs have been developed for chiral separations of different chiral substances. Commercially available CSs include cyclodextrins (CDs) and their derivatives, crown ether, metal complex as well as macrocyclic antibiotic [10–14]. Among them, CDs and their derivatives are the most widely adopted due to their broad enantioselectivities.

Two issues should be taken into consideration in the ee% measurements using conventional CE. Firstly, the tailing peak of the major enantiomer may overlap the peak of minor enantiomer if it elutes second, so it is desirable for the minor enantiomer to migrate faster than the major enantiomer by optimizing the experimental conditions [15]. Secondly, a higher resolving power of CS is needed to perform the ee% measurement, i.e., the conditions under which the racemate can be baseline resolved are sometimes

* Corresponding author at: Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China.
** Corresponding author.
E-mail addresses: bao@tju.edu.cn (J.J. Bao), yongz@tju.edu.cn (Y. Zhang).

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required further optimized to determine the enantiomeric purity of the drug. This is because the peak of the major enantiomer may overlap the peak of the minor enantiomer due to dispersion. Consequently, a CS with higher resolving power or at higher concentration is normally adopted to obtain baseline separations in the ee% measurements. But the most suitable CS is usually selected by trial and error, which is time-consuming and costly [16].

In order to address these problems, we propose a new method, velocity gap mode of capillary electrophoresis (VGCE), as a general solution. Since all these problems are related with dispersion, the strategy adopted in VGCE is to decrease the effect of dispersion on the enantioseparation. Based on the velocity gap (VG) theory [17], the mixed part of the enantiomers can be fractionated from enantiopure major enantiomer. The mixed part of the enantiomers contains minor enantiomer and a small amount of major enantiomer. Baseline separation of two enantiomers is then easily achieved due to less dispersion effect. The mixed part of the enantiomers is then easily baseline separated due to less longitudinal dispersion caused by the overwhelming amount of the major enantiomer. Baseline separation is achieved even for racemates that are hard to be separated by CE alone. Therefore, VGCE provides an alternative addition to perform ee% measurements, especially in the case of one enantiomer is in high excess as compared with the other one. The feasibility of the VGCE for this application was demonstrated by the ee% measurements of amlodipine and ofloxacin. Using the same experimental conditions, the measurements failed in conventional CE but achieved in VGCE.

2. Theoretical basis

The sample used in an ee% measurement is composed of the major enantiomer and the minor enantiomer and the major enantiomer is usually overwhelming in amount. During the separation process, the minor enantiomer focuses in the edge of sample zone, but no resolution is obtained due to serious zone dispersion of the major enantiomer. This makes it difficult to perform an ee% measurement in conventional CE (Fig. 1A). In VGCE, by contrast, two motion phases are formed in two independent electric circuits which function as a knife to "cut" the sample zone into two parts, i.e., the edge part and the main part. The edge part contains the minor enantiomer and a small amount of major enantiomer. The main part contains the enantiopure major enantiomer. As the two sample parts migrate from the VG interface to the detection window, two enantiomers in the edge part will easily be baseline separated due to less dispersion. Consequently, the ee% measurement is achieved based on calculating peak area of three peaks, one for the minor enantiomer and the other two for the major enantiomer (Fig. 1B).

3. Results and discussion

In order to test the feasibility of VGCE, the ee% measurement of amlodipine was carried out as an example.

3.1. Effect of longitudinal dispersion on the ee% measurement of amlodipine

One of two enantiomers is usually overloaded in the ee% measurement. Peak overlap may deteriorate the separation due to the dispersion of the major enantiomer. To confirm this point, enantioseparations of amlodipine in different ratios were carried out. The samples were prepared by mixing the same amount of *R*-amlodipine with increased amount of *S*-amlodipine. The final ratios of amlodipine (*R*/*S*) were 1:1.05, 1:5, 1:10 and 1:20, respectively. Under the optimized experimental conditions, the enantioseparations were performed in CE, adjusting $E_1 = E_2 = 200$ V/cm for all three steps. As shown in Fig. 2, only the racemic amlodipine (1:1.05) was baseline resolved. With increasing the amount of *S*-amlodipine, the resolution decreased. When the ratio of *R*-amlodipine/*S*-amlodipine reached 1:20, two peaks were almost totally overlapped. It was predictable that only one peak may be observed if the amount of *S*-amlodipine continued to increase. Our results indicated that the longitudinal dispersion caused by the major enantiomer could deteriorate the enantioseparation in the ee% measurement (Tables 1–3).

Table 1
The effect of α-CD concentration on chiral separation of amlodipine.[a]

Concentration (w/v%)	T_R (min)	T_S (min)	Resolution
1.5%	19.912	20.426	1.50
2.0%	20.671	21.231	1.65
3.0%	21.565	22.215	1.86
4.0%	24.031	24.754	1.85
5.0%	25.687	26.492	1.85
6.0%	28.786	29.591	1.82

[a] The sample is racemic amlodipine. The experimental conditions were the same as those in Fig. 1.

Table 2
Effect of buffer pH on chiral separation of amlodipine.[a]

pH	T_R (min)	T_S (min)	Resolution
2.0	24.871	25.687	1.90
2.5	20.542	21.045	1.86
3.0	20.402	20.927	1.71
4.0	17.999	18.407	1.45
5.0	11.889	12.245	1.05

[a] Racemic amlodipine. The experimental conditions were the same as those in Fig. 1.

Table 3
The repeatability of VGCE in the ee% measurement of amlodipine.[a]

Amlodipine mixture ee (%)	Measured by VGCE ee (%)	Average ee (%)	RSD (%)
1:35[b]	94.50 94.30 94.20	94.33	0.16
1:125[b]	98.20 98.40 98.40	98.33	0.12

[a] The ee values were calculated according to Eq. (1). The experimental conditions were the same as those in Fig. 3.
[b] The ratio was R-amlodipine/S-amlodipine.

3.2. Comparison of ee% measurement of amlodipine in conventional CE and VGCE

The sample was prepared by mixing *R*-amlodipine and *S*-amlodipine at the ratio of 1:100. All enantioseparations were performed under the optimized conditions. For comparison, the enantioseparation of the amlodipine mixture was first performed in conventional CE. As we expected, only one peak was observed due to the severe dispersion of *S*-amlodipine. In this case, it was impossible to calculate the ee% value (Fig. 3A). The enantioseparation was then performed in VGCE. In step 1, $E_1 = E_2 = 200$ V/cm. Step 1 lasted for 23.4 min. *R*-amlodipine existed mainly in the front edge of the sample zone because it migrated faster than *S*-amlodipine. In step 2, $E_1 = 0$ V/cm and $E_2 = 200$ V/cm. Step 2 lasted for 0.5 min. The sample zone was fractionated into two sections. The front section included *R*-amlodipine and a small amount of *S*-amlodipine. The other section was enantiopure *S*-amlodipine. In step 3, baseline separation of *R*-amlodipine and *S*-amlodipine was achieved when the sample

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