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Thermodynamic models to elucidate the enantioseparation of drugs with two stereogenic centers by micellar electrokinetic chromatography

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ABSTRACT

An equilibrium model depicting the simultaneous protonation of chiral drugs and partitioning of protonated ions and neutral molecules into chiral micelles in micellar electrokinetic chromatography (MEKC) has been introduced. It was used for the prediction and elucidation of complex changes in migration order patterns with experimental conditions in the enantioseparation of drugs with two stereogenic centers. Palonosetron hydrochloride (PALO), a weakly basic drug with two stereogenic centers, was selected as a model drug. Its four stereoisomers were separated by MEKC using sodium cholate (SC) as chiral selector and surfactant. Based on the equilibrium model, equations were derived for a calculation of the effective mobility and migration time of each stereoisomer at a certain pH. The migration times of four stereoisomers at different pHs were calculated and then the migration order patterns were constructed with derived equations. The results were in accord with the experiment. And the contribution of each mechanism to the separation and its influence on the migration order pattern was analyzed separately by introducing virtual isomers, i.e., hypothetical stereoisomers with only one parameter changed relative to a real PALO stereoisomer. A thermodynamic model for a judgment of the correlation of interactions between two stereogenic centers of stereoisomers and chiral selector was also proposed. According to this model, the interactions of two stereogenic centers of PALO stereoisomers in both neutral molecules and protonated ions with chiral selector are not independent, so the chiral recognition in each pair of enantiomers as well as the recognition for diastereomers is not simply the algebraic sum of the contributions of two stereogenic centers due to their correlation.

1. Introduction

Many chiral separation methods by chromatography and capillary electrophoresis (CE) for the resolution of one chiral center racemates are reported every year. However, the enantiomeric resolution of racemates having two stereogenic centers is still a challenging job. Only a few papers are available on this issue, although some drugs with two stereogenic centers are of high medicinal values [1–4]. Chiral drugs with two stereogenic centers have four stereoisomers belonging respectively to two pairs of enantiomers. Their separation by CE involves chiral separation in each pair of enantiomers and achiral separation between diastereomers. And two separation mechanisms, chromatographic effect from the affinity difference of stereoisomers for chiral selector and electrophoretic effect from the mobility difference of two enantiomeric pairs in water phase, work independently [5–7]. Furthermore, ionizable drugs of weak acid or base can dissociate or be protonated at a certain pH and coexist in both neutral molecules and ions, which generally have different binding constants with chiral selector. The pH of separation media affects the ionization degree of drugs while the chiral selector concentration affects the binding strength of drugs in both neutral molecules and ions with chiral selector. They cause not only the changes in resolution of adjacent peaks but also complex changes in migration order pattern of four stereoisomers. Therefore, mathematical models of electromigration are important for the optimization of experiment conditions when a new method is developed.

Wren and Rowe introduced the first model to calculate the mobility difference of enantiomers based on complex formation between analytes and chiral selectors [8,9]. However, this model

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did not consider the dissociation or protonation of analytes as a function of the pH. This work was done subsequently by Vigh and co-workers, who introduced models considering the simultaneous ionization and competing binding of the charged and uncharged analytes with chiral selectors for a calculation of the effective mobility and peak resolutions of enantiomers [10–12]. Based on these models, the reversals of enantiomer migration order for various enantiomeric analytes caused by the change of pH and chiral selector concentration were investigated by many groups [13–16]. The reversals of enantiomer migration order caused by the change in enantiomeric affinity pattern towards the chiral selectors as well as those being ascribed to different electrophoretic mobilities of chiral selectors and analyte-selector complexes were summarized by B. Chankvetadze in an early review [17]. And different cases of pH-dependent and selector concentration-dependent reversals of migration order were reviewed by Scriba et al. [18,19]. Recently, Dubský, et al. developed a more generalized model of electromigration applies to any cases where an analyte undergoes multiple equilibria among its various free forms and each of this forms complexes with the mixture of a number of chiral selectors, by introducing the overall complexation constant, the overall mobility of the free analyte and the overall mobility of the analyte-selector complex, which play the roles of the respective parameters in the electromigration model of Wren and Row [20,21]. Nerveless in all these works, the chiral selectors are either cyclodextrins (CDs) or other ligands which are dissolved in the background electrolyte (BGE) and form only 1:1 complexes with analytes by weak chemical interactions. Although these kinds of separation are also termed as electrokinetic chromatography (EKC) by some authors, there are not two immiscible phases present and thus the separation is not based on the phase distribution equilibria. Affinity capillary electrophoresis (ACE) may be a more preferable name for this family of methods [21,22].

Micellar electrokinetic chromatography (MEKC), another important mode of CE, is also widely used for the enantioseparation. Although the enantioseparation by MEKC is also a result of the combination of chromatographic principle and electrophoretic principle [23–26], similar to that by enantioselective CE using CDs as chiral selectors (ACE), it has some different characteristics. Firstly, here the chromatographic principle is really based on the distribution equilibria between immiscible phases; it will certainly give some features different from those in ACE: (1) The binding stoichiometry between analytes and chiral micelles is not necessarily 1:1; (2) The affinity of analytes to the micelles is characterized by retention factor, the ratio of the amount of analytes in micelles to that in continuous phase, which has a more complex relationship with chiral selector concentration, compared to the complexation constants [27,28]. In addition, due to the fact that the mass and charge of micelles are much bigger than those of the analyte molecules or ions, the entering of analyte would have little influence on the mobility of micelles, different from the complexation between analyte and selector in ACE. This gives rise to different implications of the electrophoretic effect in ACE and MEKC. The former refers to different electrophoretic mobilities of analyte-selector complexes while the latter means different electrophoretic mobilities of free analytes. Therefore, different eletromigration models are needed to describe the enentioseparation by MEKC. Yet very few works have been reported on this field by now, in contrast to the extensive and in-depth researches on the electromigration models in ACE. J. P. Foley has introduced a resolution equation applicable for chiral separations by MEKC, but the ionization of analytes and competing partitioning of charged and uncharged analytes to the micelles were neglected [29].

In our previous works [5–7], a weakly basic drug with two chiral centers, palonosetron hydrochloride (PALO), was separated by MEKC using sodium cholate (SC) as chiral selector and surfactant. The effect of micelle solution pH, SC concentration and the existence of low concentration sodium dodecyl sulfate in BGE on the separation and the migration order of four stereoisomers, i.e. PALO (3aS, 2S), (3aR, 2R), (3aS, 2R) and (3aR, 2S), was discussed by using the existed electromigration model of MEKC based on the portioning of analytes between micelles and continuous phase. Simultaneous protonation of analytes was not considered, so that the retention factors obtained are just pH-dependent overall retentions, i.e. the weighted average of protonated ions and neutral molecules. In the present work, an equilibrium model depicting the protonation of analytes and the partitioning of protonated ions and neutral molecules between the water phase and micelles was introduced. It was used to elucidate the migration order patterns at different pHs or chiral selector concentrations and the contributions of different effects to the separation more precisely.

In the enantioseparation of drugs with two stereogenic centers, both stereogenic centers can interact with chiral selector and contribute in varying degrees to the chiral recognition as well as the recognition of diastereomers. And the interactions of two stereogenic centers with chiral selector may have some correlations or be independent each other. A thermodynamic model for a judgment of the existence of this type of correlations was also proposed in this work.

2. Material and methods

2.1. Chemicals and reagents

The four enantiomerically pure PALO stereoisomers were purchased from J&K Scientific Ltd (Beijing, China). Sodium cholate (SC) was purchased from Serva Feinbiochemica (Heidelberg, Germany). Other chemicals used were of analytical regent grade and were used without further purification.

2.2. Preparation of separation media (BGE) and sample solutions

Micelle solutions for MEKC were prepared by dissolving appropriate quantities of SC surfactant and sodium tetraborate buffer in distilled water to the desired volume in a flask. The solution was sonicated while covered for 15 min to form a transparent micelle solution. BGE solutions for the measurement of the dissociation constant (K_a) of protonated PALO stereoisomers were prepared by dissolving sodium tetraborate in distilled water to the same concentration as micelle solutions. And sodium acetate with the same concentration as SC in micelle solutions was added to correct the difference in ionic strength. The pH of solutions was adjusted to desired values with 1.0 M HCl or NaOH under the monitoring of a PHS-3C pH meter (Shanghai Precision & Scientific Instrument Co., China).

The sample solutions were prepared by dissolving appropriate quantities of each enantiomerically pure PALO steroisomer mixedly in corresponding background electrolyte (BGE) to a concentration of 0.1 mg mL^{-1}. A small amount of dimethylsulfoxide (DMSO) and 1-phenyldocecane were added as markers of electroosmotic flow (EOF) and micelles, respectively. For the measurement of K_a, sample solutions just containing PALO (3aS, 2S) and (3aS, 2R) were prepared; each of them represents a pair of enantiomers in achiral media. And only EOF marker was added.

All solutions were filtered through a 0.45 μm filter prior to use.

2.3. Electrophoresis experiments

A TH-3100 capillary electrophoresis system equipped with a UV detector (Tianhui Institute of Separation Science, Hebei, China) was employed for all the CE experiments. The detection wavelength was 214 nm. An uncoated fused silica capillary of id 50 μm × od 365 μm