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Enantioselective extraction of phenylsuccinic acid in aqueous two-phase systems based on acetone and β-cyclodextrin derivative: Modeling and optimization through response surface methodology

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ABSTRACT

A novel aqueous two-phase system (ATPS) composed of β-cyclodextrin (β-CD) derivative and acetone was developed for enantioselective extraction of racemic phenylsuccinic acid (PSA). Binodal curves, tie-lines, and critical points for the investigated ATPS were determined and the experimental tie-lines data were successfully correlated by Othmer-Tobias, Bancroft, and Setschenow-type equations. ATPS containing sulfobutyl ether-β-CD (SBE-β-CD) exhibited better enantioselectivity than that using carboxymethyl-β-CD (CM-β-CD). To optimize enantioselective partitioning conditions of PSA in acetone/SBE-β-CD ATPS, three factors (PSA concentration, pH, and equilibrium temperature) were analyzed by using central composite design in response surface methodology. The calculated equilibrium constants of inclusion complexation are 1638.64 M^{-1} for SBE-β-CD-(*R*)-PSA and 835.84 M^{-1} for SBE-β-CD-(*S*)-PSA, respectively. Under the optimized conditions, the separation factor of 3.14 and high enrichment efficiency ($E_R = 98.06\%$, $E_S = 99.25\%$) were simultaneously achieved in a single step.

1. Introduction

Due to more strict regulations for pharmaceutical products, the share of drug containing only single enantiomer has grown dramatically in the past decades [1]. Two different approaches including asymmetric synthesis and enantioseparation of racemates have been well established to produce enantiopure compounds [2]. Among enantioseparation methods, enantioselective liquid-liquid extraction (ELLE) is considered to be a promising process because of its ease of scaling up [3]. Furthermore, another strength of ELLE is that a very high selectivity is not required, but with a moderate selectivity using a multistage process high purity can still be obtained. Nevertheless, the use of a large amount of organic solvent as a single phase might cause the environmental concern. As a result, there is an ongoing search for more environmentally benign extraction systems.

Aqueous two-phase systems (ATPS) can be foreseen as a greener alternative to the traditional organic solvent-water extraction system since their two phases possess a water-rich environment [4]. The utilization of ATPS in enantioseparation was first proposed by Sellergren [5], thereafter, only a limited number of studies have been reported [6–10]. The realization of chiral recognition for enantiomers in these ATPS merely relied on the introduction of auxiliary chiral selector and the enantioseparation efficiency was still unsatisfactory. Recently, chiral ionic liquid, which simultaneously serves as phase-forming agent and chiral selector, was successfully applied for enantioseparation [11,12]. Inspired by this encouraging work, we were devoted to developing the novel ATPS by employing the phase-forming agent possessing dual function as chiral selector.

To date, β-cyclodextrin (β-CD) and its derivatives have emerged to be the most widely used chiral selector for resolution of various enantiomers [13–15]. The formation of inclusion complexes and weak intermolecular forces between analytes and substituted groups cooperatively contribute to their chiral recognition ability. In addition, β-CD derivatives have an enhanced ability to be hydrated due to the existence of large amounts of hydrophilic groups, thus, potentially acting as phase-forming agent in ATPS. On the other hand, acetone is an organic solvent with excellent solvability for numerous organic substances, and miscible with water in the whole composition range. More importantly, acetone has a lower boiling point than water, which leads to the easy recov-

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ery. Herein four highly water-soluble β-CD derivatives (methyl-, hydroxypropyl-, carboxymethyl-, and sulfobutyl ether-β-CD) were employed as new phase-forming agent and chiral selector simultaneously to construct ATPS with acetone. And the potential of the proposed ATPS was evaluated using racemic phenylsuccinic acid (PSA, Fig. S1) as a target.

In the conventional optimization process, there is a variation of only one parameter at a time, keeping the other parameters at constant level, and thus, the interactions among various parameters may be ignored. Response surface methodology (RSM) [16] includes useful statistical and mathematical methods for the test of process parameters and their reciprocal interaction. By means of the empirical statistics analysis, RSM not only greatly reduces the number of experimental trials, but also generates a mathematical model to quantify the relationship between the controllable parameters and the responses. Therefore, in this work, RSM was adopted to effectively optimize the parameters for enantioselective extraction of PSA.

2. Experimental

2.1. Materials

PSA (racemate, >99%) was provided by Xiya Reagent Research Center (Shandong, China). Methyl-β-cyclodextrin (Me-β-CD, >99%, substitution degree 6.0), sulfobutyl ether-β-cyclodextrin (SBE-β-CD, >99%, substitution degree 5.0), carboxymethyl-β-cyclodextrin (CM-β-CD, >99%, substitution degree 5.0), and hydroxypropyl-β-cyclodextrin (HP-β-CD, >99%, substitution degree 5.0) were purchased from Binzhou Zhiyuan Bio-Technology Co., Ltd. (Shandong, China). Acetone was supplied by Tianjin Hengxing Chemical Preparation Co., Ltd. Organic solvent for chromatographic analysis was of HPLC grade.

2.2. Methods

2.2.1. Phase diagrams and tie-lines

Phase diagrams can be constructed for each system and are used to predict the volume and compositions of two phases. Binodal curve, which divides acetone-β-CD derivative-water system into two parts (a mixed phase system and a two-phase system), was determined by the turbidimetric titration method [17]. Firstly, β-CD derivatives were dissolved in 5 mL water to prepare stock solutions with different weight percentages (5.0–40.0 *wt* %). Acetone was then added drop-wise to the stock solution until the solution turned turbid. The temperature was fixed at 298.15 ($\pm$1) K using a bath of circulating water. The volume of the added acetone was recorded for calculating phase compositions of each cloudy point. All above steps were repeated to obtain enough cloudy points to construct a complete binodal curve. The experimental binodal curve was fitted by a logarithmic expression described as

$$[Y] = a - b\ln([X] + c) \qquad (1)$$

Where [Y] and [X] are the weight percentages of acetone and β-CD derivative, respectively. Constants *a*, *b*, and *c* are fitting parameters obtained by non-linear regression.

Tie-lines (TLs) are straight lines that join the composition values of two phases in equilibrium [18]. All points on one TL possess identical compositions in the top and bottom phases, but the relative phase volumes and phase weight are different. TLs were obtained through a gravimetric method originally described by Merchuck [19]. Briefly, a mixture at the biphasic region of each ternary system was prepared, vigorously shaken, and allowed to reach phase equilibrium. Then the top and bottom phases were separated and accurately weighed. Each individual TL was determined by the application of the lever-arm rule for the unknown values of $[Y]_T$, $[Y]_B$, $[X]_T$, and $[X]_B$ (details were provided in Supplementary Material) [20].

The tie-line length (TLL) is a numerical indicator of the composition difference between two phases, which is generally used to correlate the partitioning trends of solutes. It can be calculated through Eq. (2).

$$TLL = \sqrt{([Y]_T - [Y]_B)^2 + ([X]_T - [X]_B)^2} \qquad (2)$$

The location of critical points for the ternary systems was estimated by extrapolation of the TLs composition applying Eq. (3):

$$[Y] = f + g[X] \qquad (3)$$

2.2.2. Enantioselective partitioning experiments

The enantioselective partitioning experiments for racemic PSA were performed in 50 mL centrifuge tube. The bottom phase was prepared by dissolving β-CD derivative in 5 mL PSA aqueous solution with a fixed concentration. Before being kept in a water bath with thermostat control at 25 °C for 5 h to reach equilibrium, the prepared bottom phase and a certain volume of acetone were placed together and shaken sufficiently. The volume of each phase after phase equilibrium was recorded and the concentration of PSA enantiomers in two phases was determined by HPLC.

The partition coefficient ($K_{R/S}$) of PSA was calculated by taking into account of the concentration of enantiomer in each phase. V_r is the volume ratio between two phases. Separation factor (α) and enantiomeric excess (*ee*) of *R*-PSA in the bottom phase were used to evaluate the enantioseparation efficiency. Intrinsic inclusion selectivity α_{incl} is the ratio of inclusion complexation constants. They can be calculated according to the following equations:

$$K_{R/S} = \frac{C_{R/S,t}}{C_{R/S,b}} V_r = \frac{V_T}{V_B} \alpha = \frac{K_S}{K_R} \mathrm{ee}\% = \frac{C_{R,b} - C_{S,b}}{C_{R,b} + C_{S,b}} \alpha_{incl} = \frac{K_{(R)-PSA}}{K_{(S)-PSA}} \qquad (4)$$

Where $C_{R/S,t}$ and $C_{R/S,b}$ represent concentrations of *R*-PSA (or *S*-PSA) in the top and bottom phases after enantioselective extraction. V_T and V_B are volumes of the top and bottom phases. $K_{(R)-PSA}$ and $K_{(S)-PSA}$ are inclusion constants of *R*-PSA and *S*-PSA with β-CD derivative, respectively.

The enrichment efficiency of PSA was defined as the percentage ratio between the amount of *R*-PSA (or *S*-PSA) in the top phase and that in the total mixture, according to:

$$E_{R/S} = \frac{C_{R/S,t} V_T}{C_{R/S,t} V_T + C_{R/S,b} V_B} \times 100\% \qquad (5)$$

2.2.3. Analytical method

The quantification of PSA enantiomers was performed using Shimadzu LC-8A HPLC (Shimadzu, Japan) equipped with a SPD-M20A detector. The analytical column was Promosil C18 column (250 mm × 4.6 mm i.d., Agela Technologies, China). The mobile phase was methanol-buffer solution (H_3PO_4-0.1 mol/L NaH_2PO_4, pH = 3.5) (25:75, *v*/*v*), containing 20 mM HP-β-CD at a flow rate of 1.0 mL/min. As a cheap chiral additive, HP-β-CD also provides a better resolution for PSA enantiomers. The operating conditions were as follows: monitoring wavelength, 220 nm; injection volume, 20 μL; column temperature, 25 °C.

2.2.4. Experimental design

Typically, the experimental data obtained from the RSM are analyzed by the response surface regression procedure using a second-order polynomial equation [16,21]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \qquad (6)$$