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Organic high ionic strength aqueous two-phase solvent system series for separation of ultra-polar compounds by spiral high-speed counter-current chromatography

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Abstract

Existing two-phase solvent systems for high-speed countercurrent chromatography cover the separation of hydrophobic to moderately polar compounds, but often fail to provide suitable partition coefficient values for highly polar compounds such as sulfonic acids, catecholamines and zwitter ions. The present paper introduces a new solvent series which can be applied for the separation of these polar compounds. It is composed of 1-butanol, ethanol, saturated ammonium sulfate and water at various volume ratios and consists of a series of 10 steps which are arranged according to the polarity of the solvent system so that the two-phase solvent system with suitable *K* values for the target compound(s) can be found in a few steps. Each solvent system gives proper volume ratio and high density difference between the two phases to provide a satisfactory level of retention of the stationary phase in the spiral column assembly. The method is validated by partition coefficient measurement of four typical polar compounds including methyl green (basic dye), tartrazine (sulfonic acid), tyrosine (zwitter ion) and epinephrine (a catecholamine), all of which show low partition coefficient values in the polar 1-butanol-water system. The capability of the method is demonstrated by separation of three catecholamines.

Keywords

Organic-high ionic aqueous two-phase solvent systems; high-speed counter-current chromatography (HSCCC); flat-twisted spiral column; sulfonic acids; zwitter ions; catecholamines

1. Introduction

Since the 1980s, high-speed counter-current chromatography (HSCCC) has been widely used for the separation of natural and synthetic products [1–6]. It uses a variety of organic-aqueous two-phase solvent systems for adjusting the partition coefficient values for the target compounds. Hydrophobic to moderately polar compounds are efficiently separated with two-phase solvent systems composed of hexane-ethyl acetate-methanol-water by

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modifying their volume ratios, whereas polar compounds such as peptides are separated by polar butanol solvent systems such as butanol-water, 1-butanol-acetic acid-water (4:1:5, v/v) and 2-butanol/aqueous trifluoroacetic acid (1:1, v/v). However, there are still a number of highly polar compounds which cannot be efficiently separated by the standard HSCCC technique with the existing polar two-phase solvent systems mainly due to their low partition coefficient values. The separation of highly polar compounds such as catecholamines [7,8] and sulfonic dyes [9] by HSCCC requires a suitable ligand (hydrophobic counter-ions) in the organic stationary phase.

The present paper introduces a new series of two-phase solvent systems composed of 1-butanol-ethanol-saturated ammonium sulfate solution-water at various volume ratios which can be used for separation of extremely polar compounds by the standard HSCCC with a spiral column. Important physical parameters such as two-phase volume ratios, settling times, difference in density between the two phases, and partition coefficient values of polar test samples are investigated, and capability of the system is demonstrated by separation of a set of test samples with high polarity.

2. Experimental

2.1. Apparatus

The present studies used a type-J coil planet centrifuge manufactured by PC Inc. Potomac, MD, USA. It holds a separation column and a counterweight symmetrically at a distance of 10 cm from the central axis of the centrifuge. The spiral tube support for the separation column was purchased from CCBiotech (Rockville, MD, USA). The spiral column was made in our laboratory by inserting a flat-twisted PTFE (polytetrafluoroethylene) tubing (Zeus Industrial Products, Orangeburg, SC, USA) of 1.6 mm ID into the tube support forming 10 spiral layers with a total capacity of about 70 ml [10]. The revolution speed of the centrifuge was regulated from 0 to 1200 rpm by a speed controller. The mobile phase was pumped with an HPLC pump (model: LC10 Av, Shimadzu Corporation, Kyoto, Japan) and the effluent was monitored by a UV detector (model: Uvicod IIS, LKB Instruments, Bromma, Sweden) and a strip-chart recorder (Pharmacia, Bromma, Sweden). Fractions were collected with a fraction collector (LKB Instruments). Partition coefficients of test samples were determined using a spectrophotometer (Thermo Spectronic, Rochester, NY, USA) at suitable wavelengths.

2.2. Reagents

Organic solvent such as 1-butanol and ethanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA), and ammonium sulfate from Sigma (St Louis, MO, USA). Test samples included methyl green (Matheson Coleman & Bell, Norwood, Cincinnati, Ohio, USA), tyrosine (United States Biochemical Corporation, Cleveland, Ohio, USA), (-)-epinephrine (Aldrich, Milwaukee, WI, USA), (±)-norepinephrine (Aldrich), DL-normetanephrine (Sigma, St. Louis, MO, USA), and DL-metanephrine (Sigma) from the companies indicated in the parentheses. Tartrazine (FD&C Yellow 5) was a gift from Dr. Adrian Weisz at the U.S. Food and Drug Administration, Silver Spring, MD, USA.

2.3. Preparation of two-phase solvent systems

First, the ammonium sulfate saturated stock solution was prepared as follows: To a large glass bottle containing water placed on a magnetic mixer ammonium sulfate was added until the solution became cloudy. The mixing was continued for a few more hours until the temperature of the solvent was returned to room temperature. Then, the solvent was left for overnight to form a clear supernatant which was used for the preparation of the following two-phase solvent systems. A series of two-phase solvent systems (1 to 10) composed of 1-

butanol-ethanol-saturated ammonium sulfate solution (ca. 700 g/liter)-water at various volume ratios are prepared by adding the solvent mixture in a separatory funnel and repeating vigorous shaking and degassing. Then, each solvent system was introduced into the graduated cylinder to measure the volume of each phase to obtain volume ratios. The settling time of each solvent system was measured by introducing 2 ml of each phase (total of 4 ml) in a capped test tube (13 mm × 100 mm) and measuring the time required to form clear two layers after vigorous shaking. The measurement was repeated 3 times to get the mean value. Density difference between the upper and lower phases was also measured by weighing 1 ml of each phase with a micro balance.

2.4. Measurement of partition coefficient (K)

In order to demonstrate the capability of the present solvent system series, a set of polar samples including ethyl green (basic dye), tartrazine (sulfonic acid), tyrosine (amino acid and zwitter ionic compound) and epinephrine (catecholamine) were selected, all of which have low K values in the conventional butanol two-phase solvent systems. K value of each test sample was determined in solvent system series from systems 0 to 10 (see Table 1) where system 0 composed of 1-butanol-water is one of the most polar conventional two-phase solvent systems used as a reference.

2.5. HSCCC separation procedure

Based on the partition coefficient of 3 catecholamine test samples solvent system No. 9 was selected, which composed of 1-butanol-ethanol-saturated ammonium sulfate-water (0.25:0.875:1:1, v/v). The separation was initiated by filling the separation column with the organic stationary phase followed by injection of the sample solution containing three catecholamines (each 2 mg) dissolved in 2 ml of the solvent consisting of equal volume of each phase. Then, the column was rotated at 800 rpm while the aqueous mobile phase was pumped into the internal head terminal of the spiral column at a flow rate of 0.5 ml/min. The effluent from the outlet of the column was continuously monitored with a UV detector at 280 nm and collected into a graduated cylinder to determine the volume of the stationary phase retained in the column.

3. Results and discussion

3.1. Organic-high ionic strength aqueous solvent series

As mentioned earlier, a set of solvent systems for the separation of highly polar and moderately polar compounds have been developed so that the partition coefficient of the target compounds were systematically determined to optimize the composition of the two-phase solvent system [11, 12]. In the present system, 10-step solvent systems shown in Table 1 provide suitable partition coefficient values for highly polar target compounds. All systems yield acceptable volume ratios between the two phases. The settling times of these solvent systems measured by a standard method [13] were 20 seconds or longer indicating that the conventional multilayer coil would fail to retain a satisfactory amount of the stationary phase in the column. However, the spiral column configuration used in the present studies permits stable retention of the stationary phase of over 50%, since it utilizes a centrifugal force gradient along the spiral channel to enhance the stationary phase retention [14, 15]. Because this centrifugal force gradient is greatest at the internal portion of the spiral, the system becomes free from the effect of the β values which restrict the retention of stationary phase in the conventional multilayer coil. The relatively large difference in density between two phases provided by high concentration of ammonium sulfate in the aqueous phase also enhances the retention of the stationary phase in this spiral system. After separation, ammonium sulfate present at a high concentration in the aqueous fractions is largely eliminated by adding methanol followed by centrifugation.

3.2. Partition coefficient values (K) of polar test samples

In order to demonstrate the utility of the present solvent series, four polar test samples were selected, all having a low K value in the 1-butanol-water system. They are methyl green (basic dye), tartrazine (sulfonic acid), tyrosine (twitter ion) and epinephrine (catecholamine), each representing the group of the compounds in the parenthesis. Figure 1 is a plot of logarithm of K values vs. the solvent system series number. K values of all four compounds steadily increase from systems 1 to 10. From this figure, the best solvent system for the separation of each compound can be easily selected, where $\text{Log } K = -/+ 0.3$ corresponds to K value between 0.5 and 2, respectively.

3.3. Separation of catecholamines by HSCCC

In Figure 2 is shown the HSCCC separation of three catecholamines with a two-phase solvent system composed of 1-butanol-ethanol-saturated ammonium sulfate-water at volume ratios of 0.25:0.875:1:1, v/v (solvent system No. 9 in Table 1). The separation was performed using the lower phase as the mobile phase at a flow rate of 0.5 ml/min at 800 rpm. Three components including DL-norepinephrine ($K = 0.21$), DL-normetanephrine ($K = 0.51$) and DL-metanephrine ($K = 1.17$) were well resolved. The retention of the stationary phase was 64 % after separation and maximum column pressure measured at the outlet of the pump was 91 psi.

4. Conclusions

A series of polar organic-aqueous solvent systems with 10 steps can be efficiently used for separation of highly polar compounds such as zwitter ions (free amino acids and peptides), sulfonic acids, and basic catecholamines with the standard HSCCC technique. All 10 systems yield acceptable volume ratio between the two phases and satisfactory levels of stationary phase retention in the spiral column assembly. Capability of the method was demonstrated by separation of catecholamines. This novel ultra-polar solvent series will greatly expand the utility of HSCCC. Application of the present solvent systems for purification of polar molecular imaging dyes for PET scan is now underway in our laboratory.

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Figure 1.
Partition coefficient values of 4 polar samples in the organic-high ionic strength aqueous solvent system series.

Figure 2.

Separation of three catecholamines by HSCCC.

Experimental conditions: Apparatus: Type-J coil planet centrifuge with 10 cm revolution radius; column: spiral tube support assembly with a flat-twisted PTFE tubing (1.6 mm ID and 70 ml capacity); Solvent system: 1-butanol-ethanol-saturated ammonium sulfate-water (0.25:0.875:1:1, v/v) (system 9); sample: DL-norepinephrine ($K = 0.21$), DL-normetanephrine ($K = 0.51$) and DL-metanephrine ($K = 1.17$) each 2 mg in a total of 2 ml consisting of equal volumes of each phase; elution mode: lower aqueous mobile phase introduced from the internal head toward the outer tail terminals of the spiral column; flow rate: 0.5 ml/min; revolution speed: 800 rpm; retention of the stationary phase: 64%; maximum column pressure: 91 psi.

Table 1

Organic/high ionic strength aqueous two-phase solvent system

	1-Butanol	Ethanol	AS*	Water	phase ratio (UP/LP)	Setting time (sec)	Density difference LP - UP g/cm ³
0	1	0	0	1	1.1	23	0.156
1	2	0	1	1	1.1	20	0.360
2	1.75	0.125	1	1	1.1	21	0.338
3	1.5	0.25	1	1	1.1	>100	0.323
4	1.25	0.375	1	1	1.1	58	0.317
5	1	0.5	1	1	1.2	>100	0.303
6	0.75	0.625	1	1	1.2	22	0.296
7	1	1	1	1	1.2	25	0.303
8	0.5	0.75	1	1	1.2	25	0.338
9	0.25	0.875	1	1	1.2	35	0.255
10	0	1	1	1	1.2	55	0.159

* AS: saturated ammonium sulfate solution