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# Continuous countercurrent salting-out extraction of 1,3-propanediol from fermentation broth in a packed column

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ABSTRACT

The possibility of continuous extraction of 1,3-propanediol in a experimental packed column was investigated using a salting-out extraction system of dipotassium phosphate/ethanol. Mass transfer of 1,3-propanediol takes place from the dispersed phase (salt-rich solution) to the continuous phase (ethanol). The influences of flow rate of dispersed phase and size of packing material on partition coefficient and recovery of 1,3-propanediol were investigated and the results were compared with those obtained in spray column and test tube. Furthermore, the influences of various system compositions on hold up of dispersed phase, mass transfer coefficient, and system stability were also studied in the column packed by stainless steel Dixon 3 × 3 mm. It was found that the packed column showed a good extraction efficiency and stability. Besides, 1,3-propanediol recovery of 90.30% was obtained during a 11 h continuous operation when the real fermentation broth was used. At the same time, 94.4% of phosphate could be recovered when 0.2 volume of anhydrous ethanol was added into the raffinate phase at pH 4.0.


## 1. Introduction

Numerous extractants have been attempted for the extraction of 1,3-propanediol from dilute aqueous solutions or real fermentation broth. Unfortunately, low partition coefficient makes traditional liquid–liquid extraction inefficient [1,2]. The appearance of salting-out extraction (SOE) (formerly also called aqueous two-phase extraction) as a novel and efficient separation technique for meeting the special need of concentration and purification of hydrophilic bio-based chemicals [3–13], as well as protein [14,15] and natural products [16–19], has attracted increasing attention. Different from traditional polymer-based aqueous two-phase extraction system (e.g. PEG-dextran or PEG-salt system), SOE has uncommon properties, such as low viscosity, low cost, easy recovery of extractant and salt, short phase separation time and easy scale up, which make it have a promising application prospect.

The validity of SOE of 2,3-butanediol has been carried out in tubes and flasks on various scales (from 10 to 1000 ml) [7,8]. Furthermore, a continuous operation would be pursued due to its high space–time yield, low cost and automated controlling [20]. Different types of equipments have been employed in continuous liquid–liquid extraction, such as mixed settler [21], Karr column [22], perforated rotating disk contactor [20,23,24], spray column, pulsed sieve plate column [25] and packed column [26,27]. Among them, packed column requires small floor space and its operation is easy. Furthermore, the packing elements can reduce back mixing, provide tortuous pathways for the two phases, and increase the contact area strengthening the mass transfer [26]. Similarity in principle to conventional liquid-liquid extraction, the single or multi-contactors and relevant techniques can be adequately applied to SOE systems (SOESs). Due to these benefits, the packed columns can be adapted as a cheaper candidate.

In this paper, the technical feasibility of separation and purification of 1,3-propanediol in a continuous mode was investigated by using a laboratory packed column. The influences of size of packing and inlet velocity of the disperse phase on the partition coefficient and recovery of 1,3-propanediol were also studied in a SOES composed of dipotassium phosphate and ethanol described previously [3]. In addition, the mass transfer coefficient and dispersed phase hold up, two important parameters in designing industrial-scale packed column, as well as system stability were measured in a column packed stainless steel Dixon 3 × 3 mm at different system compositions. Finally, the real fermentation broth was used to testify the performance of the packed column, and the salt recovery was also considered. To our knowledge, this is the first report on the continuous countercurrent extraction in a packed column using SOES.

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## Nomenclature

| | |
|---|---|
| $C_1$ | phosphate concentration in the raffinate phase, g/L |
| $C_2$ | phosphate concentration in the filtrate, g/L |
| $C_C$ | 1,3-propanediol concentration in the continuous phase, g/L |
| $C_{Di}$ | initial 1,3-propanediol concentration in the dispersed phase, g/L |
| $C_{Df}$ | final 1,3-propanediol concentration in the dispersed phase, g/L |
| $C_E$ | concentration of 1,3-propanediol in the extracted phases, g/L |
| $C_R$ | concentration of 1,3-propanediol in the raffinate phases, g/L |
| $E_D$ | hold up of dispersed phase |
| $F_C$ | flow rate of continuous phase, mL/min |
| $F_D$ | flow rate of dispersed phase, mL/min |
| $F_E$ | flow rate of extracted phase, mL/min |
| $K$ | partition coefficient |
| $K_Da$ | mass transfer coefficient, $min^{-1}$ |
| $R$ | recovery of 1,3-propanediol in the extracted phase |
| $R_2$ | recovery of phosphate |
| $V_1$ | volume of the raffinate phase, L |
| $V_2$ | volume of the filtrate, L |
| $V_C$ | volume of the continuous phase, L |
| $V_D$ | volume of the dispersed phase, L |
| $V_S$ | volume of the salting-out system, L |

## 2. Materials and methods

### 2.1. Materials

1,3-Propanediol standard was obtained from Sigma. The cellulose triacetate hollow fiber dialyzer with effective surface area of 1.5 $m^2$ and cut-off relative molecular weight of 5000 Da was manufactured by NISSHO Corp., Osaka, Japan. All the other chemicals were of analytical grade.

### 2.2. Experimental apparatus

The packed column was a glass tube with 30 mm internal diameter and 400 mm height with a nozzle in the inlet of bottom. The apparatus was performed at room temperature (about 20 °C). A schematic diagram of the experiment was shown in Fig. 1. The packing materials used in this experiment were stainless steel Dixon 3 × 3 mm and 5 × 5 mm (height × diameter). The column without packing is a spray column, and can be used as contrast.

### 2.3. Preparation of the dispersed and continuous phases

The 1, 3-propanediol fermentation broth was obtained by an anaerobic fed-batch fermentation of crude glycerol using *Klebsiella pneumonia* CGMCC 2028 [4]. It was filtrated by a hollow fiber dialyzer, and the concentration of 1,3-propanediol was 71.8 g/L in the filtrated fermentation broth (ethanol 5.34 g/L, 2,3-butandiol 6.98 g/L, residual glycerol 10.56 g/L, proteins 1.16 g/L, nucleic acids 3.29 mg/L). A simulated solution of 1,3-propanediol was prepared at a concentration of 70 g/L (not contains other components). Appropriate amount of anhydrous salt was mixed with the filtrated fermentation broth or simulated solution to give a final composition of 37.5% (w/w) dipotassium phosphate. The said salt-rich solution and anhydrous ethanol were then introduced into the packed column by means of two peristaltic pumps separately. In all the experiments the ethanol phase formed the dispersed phase with the help of a nozzle, whereas the salt-rich phase formed the continuous phase.

### 2.4. Phase diagram of SOES of dipotassium phosphate/ethanol

The phase diagram was obtained by the cloud point method at 20 °C. Dipotassium phosphate solution with various concentrations was titrated dropwise with ethanol, until the solution became turbid. The compositions of these points were noted and determined by an analytical balance. The tie-line composition was determined by analyzing the concentration of ethanol and dipotassium phosphate in the top and bottom phases after the equilibrium was achieved.

### 2.5. Experimental procedure

The continuous phase (salt-rich solution) and the dispersed phase (anhydrous ethanol) were delivered downward and upward, respectively, while the raffinate phase (exhausted salt-rich solution) and the extracted phase (1,3-propanediol-enriched ethanol solution) were withdrawn at the bottom and the top of the column, respectively (Fig. 1). Three different system compositions (on different tie-lines) were studied, and the shift of the system compositions was achieved via changing the dispersed phase flow rate. The flow rate of continuous phase was maintained constant at 3.0 ml/min, the flow rate of dispersed phase varied from 1.0 to 3.0 ml/min, while the raffinate phase was set at a proper flow rate to keep the phase interface stable. Methyl red, an alcohol-soluble indicator, was used as tracer for visualizing the phase interface. Samples were collected every 10 min for simulated solution and 30 min for filtrated fermentation broth from the raffinated and extracted phases for analyzing the concentration of 1,3-propanediol for at least 2 h and 11 h, respectively. Partition coefficient, recovery, hold up of dispersed phase and mass transfer coefficient were then calculated.

After the continuous extraction, phosphoric acid was used to adjust the pH of raffinate phase for recovery of phosphate in the form of monopotassium phosphate due to its low solubility. Anhydrous ethanol was then tested as a crystallization solvent to increase the yield of the salt.

### 2.6. Analytical methods

The concentration of ethanol and 1,3-propanediol was determined by gas chromatography (SHIMADZU GC-2010, Japan) with FID detector according to the method described before [4]. The concentration of phosphate was determined by conductivity meter (DDS-307, Leici, China).

### 2.7. Definition of process parameters

In order to determine the hold up of dispersed phase ($E_D$), the volume of the salting-out system ($V_S$) and the dispersed phase ($V_D$) were measured. This was performed by stopping of the three peristaltic pumps simultaneously, followed by removing and measuring the volume of the two phases after the steady state was achieved at the end of each test run. $E_D$ was calculated according to the equation:

$$E_D = \frac{V_D}{V_S} \quad (1)$$

The partition coefficient ($K$) was calculated according to the equation:

$$K = \frac{C_E}{C_R} \quad (2)$$

where $C_E$ and $C_R$ are the concentration of 1,3-propanediol in the extracted and raffinate phases, respectively.

The mass transfer coefficient ($K_Da$) was calculated according to the flowing expression proposed by Patil et al. [27].

$$K_Da = \frac{F_D}{V_C} \ln \frac{C_{Di} - KC_C}{C_{Df} - KC_C} \quad (3)$$

where $F_D$ is the flow rate of dispersed phase, $V_C$ is the volume of the continuous phase, $C_{Di}$ and $C_{Df}$ are the initial and final 1,3-propanediol concentration in the dispersed phase, respectively; $C_C$ is the 1,3-propanediol concentration in the continuous phase.

The recovery ($R$) of 1,3-propanediol in the extracted phase was calculated according to the following equation:

$$R = \frac{C_E \times F_E}{C_C \times F_C} \times 100\% \quad (4)$$

where $F_E$ and $F_C$ are the flow rate of extracted phase and continuous phase, respectively.

The recovery ($R_2$) of phosphate was calculated according to the equation:

$$R_2 = \left(1 - \frac{C_2 \times V_2}{C_1 \times V_1}\right) \times 100\% \quad (5)$$

where $C$ and $V$ are the phosphate concentration and volume, respectively; the subscripts 1 and 2 represent the raffinate phase and filtrate after salt recovery, respectively.

## 3. Results and discussion

### 3.1. Phase diagram of SOES of dipotassium phosphate/ethanol

Fig. 2 shows phase diagram and system compositions of dipotassium phosphate/ethanol used in this work. Tie-line 1, 2 and 3 represents the corresponding system composition when the flow rate of dispersed phase is 1, 2 and 3 ml/min, respectively.