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A novel technique for the separation and concentration of penicillin G from fermentation broth: Aqueous two-phase flotation

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ABSTRACT

Separation by solvent sublation has been used for the first time in an aqueous two-phase system, and a new concept, aqueous two-phase flotation (ATPF), is proposed. The new technique was used to separate and concentrate penicillin G from a fermentation broth. In an ATPF system of polyethylene glycol (PEG)/ammonium sulfate, the effects of varying solution pH, concentration of ammonium sulfate in aqueous solution, nitrogen flow rate, flotation time and initial volume of the PEG phase were investigated in detail, and the optimal conditions for ATPF were obtained. Under these optimal conditions, the separation efficiency of ATPF was more than 95%. Compared with liquid–liquid extraction, solvent sublation and aqueous two-phase extraction, ATPF is shown to be an effective way of separating and concentrating penicillin G from an aqueous phase, and also gives a reduced consumption of organic solvents.

1. Introduction

Aqueous two-phase systems are commonly used for the separation and purification of biological materials [1,2]. The most famous aqueous two-phase systems are the two-polymer systems, e.g., polyethylene glycol (PEG)/dextran, and PEG/salt systems, e.g., PEG/ammonium sulfate. Such aqueous two-phase systems are widely used for the separation of proteins [3–6], amino acids [7–9], DNA [10,11], and other biomaterials. Recently, traditional aqueous two-phase systems have been improved by incorporating random copolymers of ethylene oxide and propylene oxide (EOPO) [12–15], which can be conveniently recovered by a temperature-induced effect. Systems incorporating other surfactants, and two-phase aqueous micellar systems have been investigated in detail [16–18] and Au nanoparticles have also been used to increase the separation efficiency of proteins in an aqueous two-phase system [19]. To date, all the reported improvements in aqueous two-phase systems involve a change in their chemical composition. In fact, changing the traditional mass transfer mode can also both increase the separation efficiency and reduce the cost.

Our laboratory is interested in solvent sublation [20–22]. This is a type of adsorptive bubble separation technique in which the surface-active compounds in water are adsorbed on the bubble surfaces of an ascending gas stream and then collected in an organic layer placed on top of the water column [23,24]. Because of the soft separation process and very low loss of organic solvent, solvent sublation has been widely applied to extract surface-active compounds and hydrophobic compounds, such as surfactants [25,26], dyes [27], complex compounds [28,29], and the active components in natural products [30]. In this paper, the use of solvent sublation as the mass transfer mode in an aqueous two-phase system is described for the first time. We name this new technique, which combines solvent sublation with aqueous two-phase extraction, aqueous two-phase flotation (ATPF). The mass transfer mode of bubble adsorption can afford high separation efficiencies with very low amounts of organic solvent, and the mass transfer mode is also much more effective than the mechanical vibration employed in conventional liquid–liquid extraction.

In this work, penicillin G was selected as the target molecule, since liquid–liquid extraction is one of the most widely used separation techniques for the recovery of this material [31]. In order to solve the environmental problems [32] and the problem of the emulsion of butyl acetate [33] associated with the conventional extraction, many new techniques have been developed including aqueous two-phase extraction [34], supported liquid membrane extraction [32], the micro-filtration technique [35], emulsion liquid membrane extraction [36], the hollow-fiber membrane technique [37,38], and solvent sublation [39]. Although all these techniques have advantages over the original process, their low separation efficiencies and high costs are still major problems. The main objective of the work is to solve these problems by means of ATPF.

In the separation process of ATPF (Fig. 1), the surface-active compound penicillin G can be adsorbed on the surface of bubbles [39,40], which float to the top of the aqueous phase (a solution of penicillin G and ammonium sulfate) where they encounter a layer of PEG, in which the penicillin G dissolves. Many parameters can

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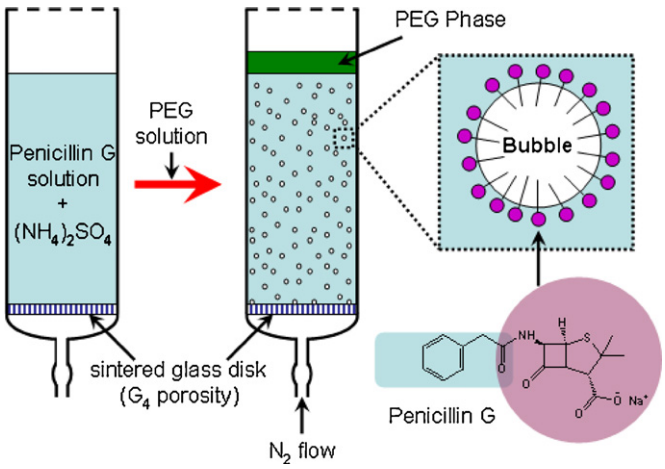

Fig. 1. Separation of penicillin G in the aqueous two-phase flotation (ATPF) system.

influence the separation process, such as solution pH, concentration of ammonium sulfate in aqueous solution, nitrogen flow rate, flotation time and the volume of the PEG phase. These parameters were first optimized in this work, and the ATPF technique was subsequently used to treat a real fermentation broth under the optimal conditions. The results obtained with ATPF are compared with other separation techniques.

2. Materials and methods

2.1. Chemicals and apparatus

The purity of penicillin G standard (China Institute of Veterinary Drug Control, China) was more than 99.4%. The purity of the crude penicillin G (Shijiazhuang Pharmaceutical Group, Zhongnuo Pharmaceutical Co., Ltd., China) was more than 94.0%. Polyethylene glycol 1000 (PEG1000) ($M_w = 1000 \pm 50$) was obtained from Fucheng Chemical Factory (Tianjin, China). Ammonium sulfate, phosphoric acid and sodium chloride were all of analytical grade (Beijing Chemical Factory, China). The methanol was of chromatographic grade (Fisher, USA), and the water was deionized water. The fermentation broth was obtained by the *Penicillium chrysogenum* fermentation method (from Dr. Zheng Wang and Prof. Tianwei Tan, Beijing University of Chemical Technology), and it mainly consisted of small quantities of glucose and inorganic salts, *P. chrysogenum* and its metabolites, and a large amount of penicillin G (more than 2.0 g/l).

A Mettler Toledo 320-S pH meter (Mettler, Switzerland) was used to determine the pH of the solution. An Agilent 1100 Series chromatograph (Agilent, USA) with a Diamonsil TM C18 Column (150 mm × 4.6 mm) was used to analyze the aqueous solution and the flotation product (PEG phase). The ATPF apparatus was similar to the one mentioned in earlier papers [29,30].

2.2. Optimization of separation parameters

According to a previous report [34], the separation efficiency of penicillin G in PEG1000 aqueous two-phase systems is higher than for other systems (e.g., PEG2000, PEG4000 and PEG6000), so PEG1000 was used in this work. Because the penicillin G concentration of the fermentation broth was more than 2.0 g/l, a model solution containing 2.5 g/l crude penicillin G was first used to optimize the ATPF parameters. Moreover, for convenience of transfer, the PEG phase contained 50% water (w/w). The ATPF parameters (concentration of ammonium sulfate in aqueous solution, pH, nitrogen flow rate, initial volume of PEG phase and flotation time) were optimized in turn. All the initial volumes of aqueous phase were 300 ml, and all separation processes were performed at room temperature.

2.3. Separation procedure with the real system

In the ATPF separation, the aqueous phase consisted of fermentation broth with a large amount of dissolved ammonium sulfate. In order to accurately obtain the concentration of ammonium sulfate, the relationship between the volume concentration and the mass concentration was determined: $w = 0.0771C + 2.6067$ ($r = 0.9990$), where C is the volume concentration (g/l) and w is the mass concentration (%). Using this equation, we can easily calculate the volume concentration of ammonium sulfate without using volumetric measurements.

A 300-ml aliquot of the fermentation broth and 126 g of ammonium sulfate were transferred into a 500-ml beaker (the volume concentration of ammonium sulfate was about 350 g/l). This solution was adjusted to pH 6.8 with HCl solution and NaOH solution, and then transferred to a flotation cell. The penicillin G was floated by bubbling nitrogen gas at a flow rate of 40 ml/min from the bottom of the cell for 40 min, and extracted into the PEG phase (the initial volume was 15.00 ml) on the surface of the sample solution. All separation processes were performed at room temperature.

2.4. Comparison experiments

In this work, ATPF was compared with liquid–liquid extraction (LLE), solvent sublation (SS) and aqueous two-phase extraction (ATPE). It is well known that penicillin G can rapidly decompose at low pH at room temperature, so the solution pH was always larger than 3.0. In the LLE experiment, 200 ml of penicillin G standard solution with pH 3.5 was extracted by 200 ml of *n*-octanol solution containing 50% butyl acetate (v/v); in the SS experiment, 200 ml of penicillin G standard solution with pH 3.5 was extracted by 10.00 ml of *n*-octanol solution containing 50% butyl acetate (v/v), and the nitrogen flow rate was 40 ml/min; in the ATPE experiment, 300 ml of penicillin G standard solution with pH 6.8 was extracted by 100 ml PEG solution containing 50% water. All separation processes were performed at room temperature.

2.5. Determination of penicillin G

Penicillin G in the flotation product (PEG phase), the aqueous phase and other systems was determined by HPLC. In the HPLC analysis, the mobile phase was a mixture of 50 mM phosphoric acid and methanol (40:60, v/v). The flow rate was 1.0 ml/min, the detection wavelength was 215 nm, and the injection volume was 10 μl. All chromatographic analyses were performed at room temperature. The calibration curve for penicillin G was obtained over the range 7.73×10^{-3}–5.18 g/l. The regression equation was $y = 428.82x - 15.37$ ($R^2 = 0.9999$), where y is the peak area and x is the concentration (g/l), and this was used to quantitatively analyze the separation efficiency and the distribution ratio.

3. Results and discussion

3.1. Optimization of ATPF parameters

In order to calculate the separation efficiency, two assumptions were made: firstly, the concentrations of penicillin G in both phases are completely homogeneous; secondly, the volume of aqueous phase is not changed before and after flotation.

The separation efficiency (E), the distribution ratio (D) of penicillin G between PEG phase and aqueous phases, and the con-