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Analytical Methods

Separation, concentration and determination of trace chloramphenicol in shrimp from different waters by using polyoxyethylene lauryl ether-salt aqueous two-phase system coupled with high-performance liquid chromatography

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ABSTRACT

Polyoxyethylene lauryl ether (POELE10)-NaH_2PO_4 aqueous two-phase extraction system (ATPES) is coupled with HPLC to analyze chloramphenicol (CAP) in aquatic product. Response surface methodology (RSM) was adopted in the multi-factor experiment to determine the optimized conditions. The extraction efficiency of CAP (*E*%) is up to 99.42% under the optimal conditions, namely, the concentration of NaH_2PO_4, the concentration of POELE10, pH and temperature were 0.186 g·mL^{-1}, 0.033 g·mL^{-1}, 3.8 and 25 °C respectively. The optimal value of enrichment factor of CAP (F) was 22.56 when the concentration of NaH_2PO_4 was 0.192 g·mL^{-1}, the concentration of POELE10 was 0.024 g/ml, pH was 4.2 and temperature was 30 °C. The limit of detection (LOD) and limit of quantification (LOQ) of this method are 0.8 μg·kg^{-1} and 1 μg·kg^{-1}, which meet the needs of determining trace or ultratrace CAP in food. The *E*% and *F* of this technique are much better than other extraction methods.

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1. Introduction

Chloramphenicol (2,2-dichloro-N-[(αR,βR)-β-hydroxy-α-hydro xymethyl-4-nitrophenethyl] acetamide, CAP, CAS 56-75-7) isolated by David Gottlieb is originally derived from the bacterium Streptomyces venezuelae. CAP was introduced into clinical use in 1947 (Gottlieb & Legator, 1953; Gikas, Kormali, Tsipi, & Tsarbopoulos, 2004). Since the 1950s, it has been extensively used in the treatment of animals all over the world due to its low cost, ready availability and excellent performance in the treatment of several infectious diseases through protein inhibition. Nevertheless, CAP is potential threats to the health of humans and animals, such as hypoplastic anemia, aplastic anemia, bone marrow depression, thrombocytopenia and granulocytopenia (Mottier, Parisod, Gremaud, Guy, & Stadler, 2003). Thus, CAP is restricted to clinical use in the treatment of serious infections. USA, the European Commission, China and some other countries have legislated the maximum residue limits of CAP (Gude, Preiss, & Rubach, 1995; Gantverg, Shishani, & Hoffman, 2003) in order to control the use of CAP in food-producing animals. However, the illegal use of CAP in livestock and aquaculture still exists due to its steady antibiosis effectiveness and low price. Therefore, it is necessary to develop a simple, rapid and asensitive method to determine the CAP in food commodities.

Nowadays, many methods are applied for detecting CAP residues in foods, such as enzymatic assay (Yamato, Sugihara, & Shimada, 1990), microbiological assay (Singer & Katz, 1985), chromatography (Forti, Campana, Simonella, Multari, & Scortichini, 2005), immunoassay (Dumont, Huet, Traynor, Elliott, & Delahaut, 2006), sensor method (Park, Kim, Adanyi, Varadi, & Kim, 2004), dispersive liquid–liquid microextraction (DLLME) (Chen, Chen, Ying, Huang, & Liao, 2009) and matrix solid-phase dispersion (MSPD) (Guo, Guan, Zhao, & Zhang, 2008). Nevertheless, these methods have some defects and insufficiencies, such as long sample-preparation time, complex analyzing procedures and expensive equipment. More importantly, it is hard to determine the residual CAP in foods by these techniques when its concentration is lower than 1.5 μg·kg^{-1}.

As a powerful green extraction technique, aqueous two-phase system (ATPS) (Albertsson, 1986, chap. 2; Zaslavsky, 1995, chap. 3; Tan et al., 2013) is more widely used to separate and extract the biological materials, such as nucleic acids (Luechau, Ling, &

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Lyddiatt, 2009), proteins (Rawdkuen, Pintathong, Chaiwut, & Benjakul, 2011; Ooi, Hii, Kamal, Ariff, & Ling, 2011; Yücekan & Önal, 2011), viruses (Luechau, Ling, & Lyddiatt, 2011), antibiotics (Li et al., 2009; Bi, Li, & Dong, 2009; Xie, Wang, Han, & Yan, 2011; Chen et al., 2014) and other biological molecules (Silva, Coimbra, Rojas, & Teixeira, 2009; Azevedo et al., 2009; Gomes, Azevedo, Aires-Barros, & Prazeres, 2009). Nowadays, the ATPSs mainly divides into the following four kinds: the polymer–polymer ATPSs (Li & Cao, 2010), polymer-salt ATPSs (Zafarani-Moattar & Hosseinpour-Hashemi, 2012), ion liquid-salt ATPSs (Han et al., 2012) and micromolecule alcohol-salt ATPSs (Lu et al., 2013; Zafarani-Moattar, Nemati-Kande, & Soleimani, 2012).

On the basis of analyzing and selecting, we found that nonionic surfactant polyoxyethylene (10) lauryl ether (POELE10, $C_{32}H_{66}O_{11}$) was consisted of the hydrophobic alkyl domain and hydrophilic polyoxyethylene tail. Because POELE10 has this feature, it was an appropriate choice to form polymer-salt ATPS. In our previously published articles (Lu et al., 2012; Lu, Han, Tan, & Yan, 2012) we have reported the phase behavior of the ATPSs composed of POELE10 and five kinds of inorganic salts at different temperatures. In this paper, we studied the extraction abilities of several POELE10-salt ATPSs for CAP, and found that the POELE10-NaH_2PO_4 ATPS is more suitable to purify the CAP.

2. Experimental

2.1. Materials

Nonionic surfactants POELE10 with a quoted purity of greater than 0.99 mass fraction was obtained from Aladdin reagent company (Shanghai, China). Inorganic salts ($(NH_4)_2HPO_4$, $ZnSO_4$, $(NH_4)_2SO_4$, NaH_2PO_4, $Na_3C_6H_5O_7$, $Na_2C_4H_4O_6$ and Na_2WO_4) were analytical grade reagents (GR, min. 99% by mass fraction), which were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The Chloramphenicol standard sample was purchased from Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All reagents were used without further purification and the water used in experiments was double distilled.

2.2. Apparatus and procedure

An analytical balance (BS124S, Beijing Sartorius Instrument Co., Ltd, China) with an uncertainty of $\pm 1.0 \times 10^{-7}$ kg was used to weigh. A digital pH meter (Shanghai LIDA Instrument Factory, China) was used to determine the pH of solution. An HPLC (Agilent 1200, Agilent, USA) equipped with ultraviolet–visible (UV) detector was employed for the qualitative and quantitative analysis of the CAP. The Agilent ChemStation software was used to control the machine and process data. The centrifuge (Anke TDL-4, Shanghai Chemical Machinery Plant Co., Ltd., China) was used for centrifuging.

The appropriate amounts of POELE10, salt, and CAP were put into the vessel from stock solutions, and water was added to 10 mL. The mixed solution was placed in the thermostat water bath after it kept stirring for 20 min. The temperature of the thermostat water bath was controlled at the constant temperature. The concentration of CAP in the top phase was determined by using HPLC after the two phases were separated. An Eclipse XDB-C18 reversed-phase column (serial no G1314B, 250 mm × 4.6 mm, 5 μm) was used for chromatographic separation at the 298.15 K column temperature. The mobile phase was consisted of water and methanol with the ratio of 55:45, and it flowed at the rate of 1.0 mL·min^{-1}. The injected volume of sample was 20 μL, and the column effluent was monitored at the wave length of 277 nm (Fig. 1).

2.3. Preparation of real samples

Shrimp from Qinhuai River, Yangtze River and Yellow Sea were purchased from the local supermarket. Shrimp was minced and placed into a 10 mL tube, and 2 mL of trichloroacetic acid (15%) was added. Then the CAP working solution was put into the tube, and water was added to 10 mL. The mixture was shaken by using homogenizer-disperser until it was thoroughly mixed. The mixture was put into centrifugal tube and kept centrifuging at 4000 rpm for 30 min. The supernatant was taken out and filtered through the microfilter with a pore size of 0.45 μm to remove the proteins. Finally, the filter liquor was stored at 4 °C.

2.4. Determination of the partition parameters of CAP

The partition and enrichment efficiency of CAP was characterized by the enrichment factor and the extraction efficiency. The enrichment factor (F) was defined at the ratio of the concentration of CAP in the top phase to that in the initial system.

$$F = \frac{C_t}{C_s} \tag{1}$$

where the C_t was the concentration of CAP in the top phase, C_s was the concentration of CAP in the initial system before the two phase was separated. The extraction efficiency (E) was calculated by the following equation:

$$E = \frac{C_t \times V_t}{m_s} \tag{2}$$

where C_t was the concentration of CAP in the top phase, V_t was the column of top phase, m_s was the total mass of CAP added in the initial system.

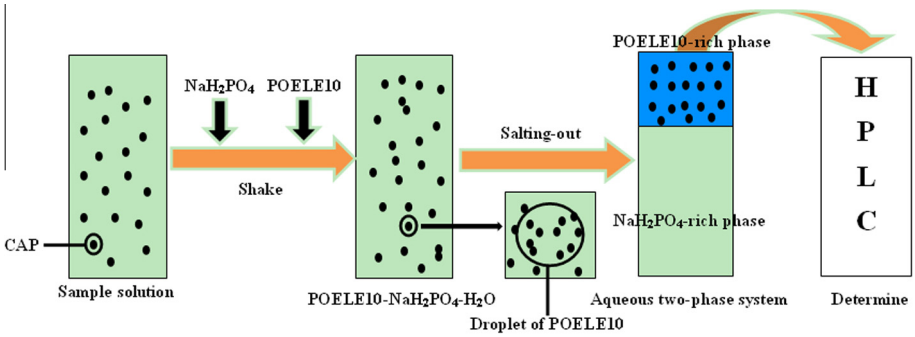

Fig. 1. The schematic diagram of separation CAP in POELE10-NaH_2PO_4 ATPS.

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