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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.

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.

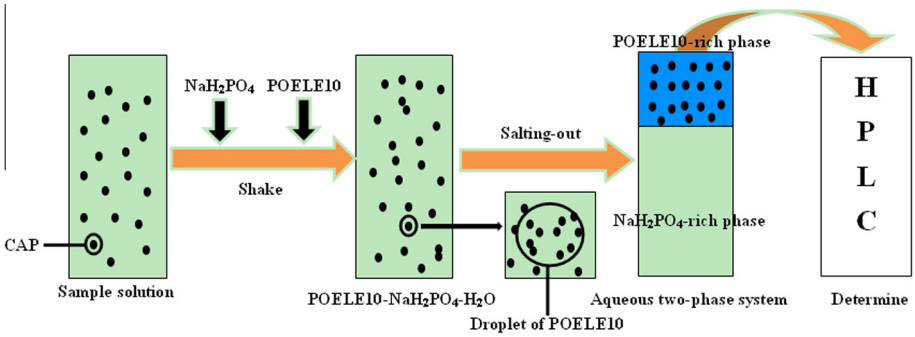

Fig. 1. The schematic diagram of separation CAP in POELE10-NaH_2PO_4 ATPS.

2.5. Statistical analysis

The response surface methodology (RSM) was used to determine the optimized condition of aqueous two-phase extraction system for the extraction and determination of trace CAP. The RSM includes the factorial designs and the regression analysis. A central composite design (CCD) with four variables at five levels was chosen for the factorial design of RSM in this experiment. The independent variables were the concentration of NaH_2PO_4 (X_1), the concentration of POELE10 (X_2), pH of system (X_3) and the temperature (X_4). The CCD was applied by using Design-Expert 7.0. The four factors and their level and ranges are shown as follow: the five level of X_1 are $-2(0.174\ g{\cdot}mL^{-1})$, $-1(0.180\ g{\cdot}mL^{-1})$, $0(0.186\ g{\cdot}mL^{-1})$, $1(0.192\ g{\cdot}mL^{-1})$, $2(0.198\ g{\cdot}mL^{-1})$; the five level of X_2 are $-2(0.021\ g{\cdot}mL^{-1})$, $-1(0.024\ g{\cdot}mL^{-1})$, $0(0.027\ g{\cdot}mL^{-1})$, $1(0.030\ g{\cdot}mL^{-1})$, $2(0.033\ g{\cdot}mL^{-1})$; the five levels of X_3 are $-2(3.0)$, $-1(3.4)$, $0(3.8)$, $1(4.2)$, $2(4.6)$; the five levels of X_4 are $-2(15\ °C)$, $-1(20\ °C)$, $0(25\ °C)$, $1(30\ °C)$, $2(35\ °C)$.

The regression analysis of experimental data was performed by the response surface regression procedure, and the second order polynomial equation (Eq. (3)) was used as analysis model.

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{ij} \beta_{ij} X_i X_j \quad (3)$$

where Y is the response, β_0, β_i, β_{ii} and β_{ij} are regression coefficient for the intercept, linear, quadratic and interaction coefficients, respectively. X_i and X_j represent the factors. The statistical significance of model was evaluated by F-test.

3. Results and discussion

3.1. The choice of phase-forming salt

The suitability of various organic ($Na_3C_6H_5O_7$ and $Na_2C_4H_4O_6$) and the inorganic salts ($(NH_4)_2HPO_4$, $ZnSO_4$, $(NH_4)_2SO_4$, Na_2WO_4 and NaH_2PO_4) for forming ATPS with POELE10 were discussed. It was concluded that the ATPS can be formed by mixing the appropriate amount of previously mentioned salts with POELE10 solution. A certain amount of CAP was added into these systems in order to discuss the extraction efficiency and enrichment factor of the ATPS containing various salts. The changes of the extraction efficiency and the enrichment factor for the various ATPSs were shown in the Fig. 2, from which it was found that POELE10-NaH_2PO_4 ATPS is obviously better than other POELE10-salts ATPSs in terms of the extraction efficiency and enrichment factor. Therefore, the NaH_2PO_4 was selected as a phase-forming salt for the aqueous two-phase extraction system.

3.2. CAP distribution in the POELE10-NaH_2PO_4 ATPS

3.2.1. Effect of NaH_2PO_4 concentration on the distribution

The concentration of NaH_2PO_4 is an important factor to influence the distribution of CAP in the POELE-NaH_2PO_4 aqueous two-phase extraction system. To illustrate this, the concentration of NaH_2PO_4 is controlled with the range from $0.162\ g{\cdot}mL^{-1}$ to $0.198\ g{\cdot}mL^{-1}$ when the other parameters are fixed. Fig. 2 showed that the extraction efficiency and the enrichment factor rises with the increase in the concentration of NaH_2PO_4. This is because the salting-out strength of system enhanced with the increasing concentration of salt, and it results that more CAP was excluded to the top phase. It was found that the changes of extraction efficiency and enrichment factor are not obvious when the concentration of NaH_2PO_4 is more than $0.198\ g{\cdot}mL^{-1}$, and meanwhile, the extraction efficiency is too low when the concentration of NaH_2PO_4 is below $0.174\ g{\cdot}mL^{-1}$. For these reasons, the concentration of NaH_2PO_4 is constrained within an ideal range from $0.174\ g{\cdot}mL^{-1}$ to $0.198\ g{\cdot}mL^{-1}$ in the subsequent multi-factor experiment.

3.2.2. Effect of concentration of POELE10 on the distribution

The relationship between the extraction efficiency and the concentration of POELE10 was shown in Fig. 2, in which the effect of concentration of POELE10 on the enrichment factor was also given. The extraction efficiency of CAP increased with the increasing concentration of POELE10, however, the enrichment factor decreased. The reason is that the volume of top phase reduced with the decreasing concentration of POELE10 in the total system. This leads to the increase in the concentration of CAP at top phase. The concentration of CAP in top phase (C_t) rises, and the concentration of CAP in total system (C_s) remains unchanged. Therefore, the enrichment factor (F) will increase with the decrease in the concentration of POELE10. In the multi-factor experiment, the range of concentration of POELE10 will be set between $0.021\ g{\cdot}mL^{-1}$ and $0.033\ g{\cdot}mL^{-1}$ for balancing extraction efficiency and enrichment factor.

3.2.3. Effect of temperature on the distribution

Besides the concentration of two phase-forming materials, temperature is another significant factor on the phase diagram of ATPS (Lu et al., 2012). Thus it has some influences on the extraction efficiency and enrichment factor. The effect of temperature on the extraction efficiency and enrichment factor was studied and their relationship was shown in Fig. 2. With the rising temperature, the extraction efficiency and enrichment factor increased. Because CAP will be inactivated when the temperature is too high or low, the temperature varies from 15 °C to 35 °C in the multi-factor experiment.

3.2.4. Effect of pH on the distribution

The extraction efficiency of CAP and enrichment factor was studied as a function of pH of whole system, and the results are shown in Fig. 2. POELE10 and NaH_2PO_4 cannot form ATPS at the pH value below 3.0. The reason is that with the lowering pH, the ability of $H_2PO_4^{-1}$ combining with H^+ strengthened and the ability of $H_2PO_4^{-1}$ dissociating H^+ decreased. All these result to that the ion strength of system weakened and the ability of competing with POELE10 for water molecules decreased. When the pH is less to a certain extent, di-hydrogen phosphate salt has no capability to seize the water that is united with POELE10. The extraction efficiency and enrichment factor increased with rising pH and they remained almost unchanged when pH is higher than 4.5. Therefore, pH of ATPS is within the range from 3.0 to 4.6 in the multi-factor experiment.

3.3. The multi-factor experiment using RSM

3.3.1. Factors affecting enrichment of CAP

Table 1 presented the details of the multi-factor experiment design that used CCD and the experimental results are also reported in Table 1. The statistical analysis of the extraction efficiency and enrichment factor using RSM was tested by the Fisher's F-test for the analysis of variance and the results were given in Table 2. The probability equals the proportion of the area under the curve of the F-distribution that lies beyond the observed F-value, which was expressed as the "Prob > F" value. The small probability values called for the rejection of the null hypothesis, in other words, the particular term was statistically significant (Ahmad, Derek, & Zulkali, 2008). When the values of "Prob > F" are less than 0.05, it indicates that the model terms are significant.

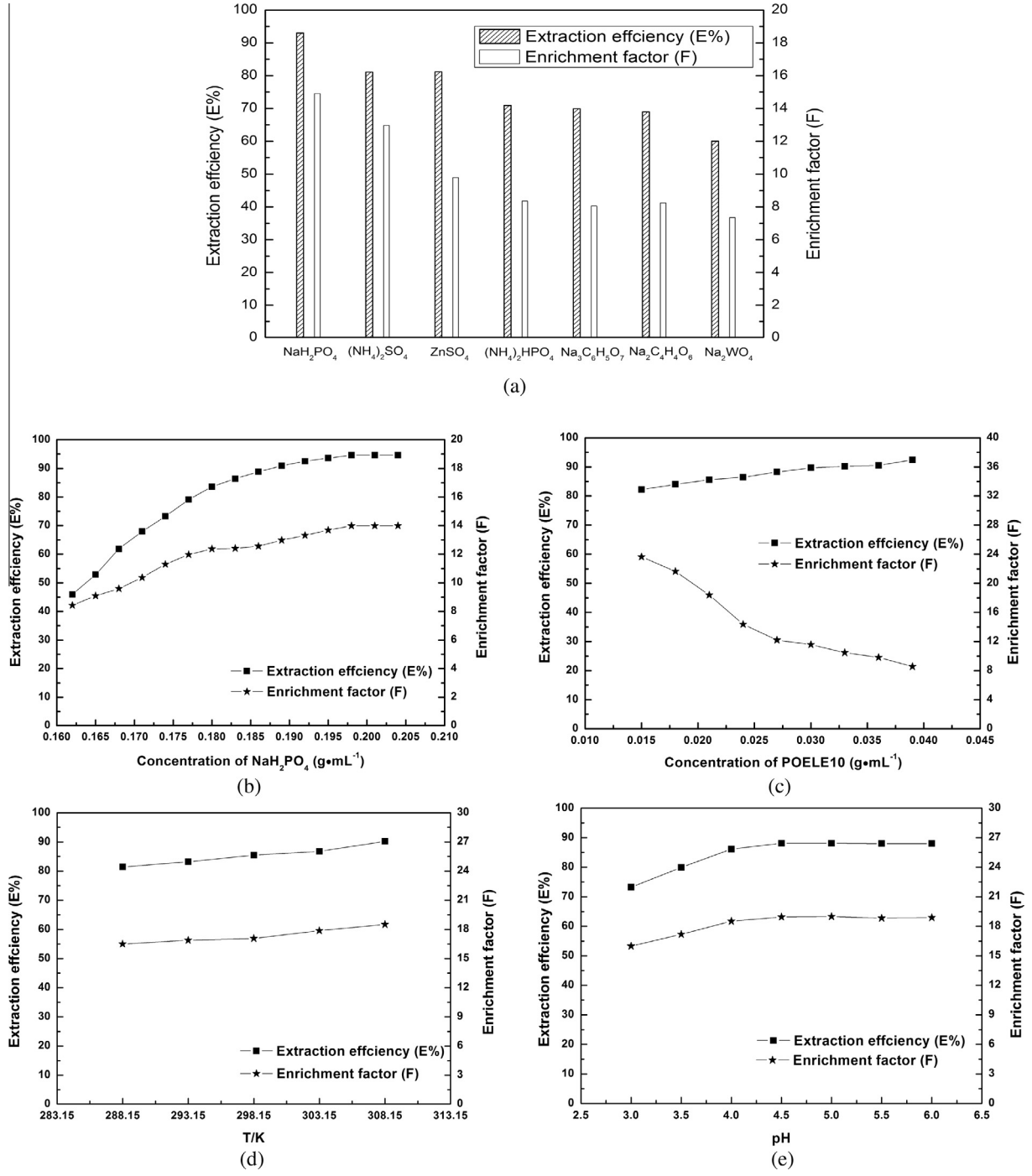

Fig. 2. The changes of the extraction efficiency and enrichment factor with (a) the different POELE10-salts ATPSs, (b) the concentration of NaH_2PO_4, (c) the concentration of POELE10, (d) the temperature and (e) the pH.

When the values are greater than 0.1, it indicates that the model terms are not significant. In this case the factor X_1, X_2, X_3, X_4 as well as the interaction of factor X_1X_3, X_1X_4, X_1^2, X_3^2 were significant in terms of affecting extraction efficiency of CAP as shown in Table 2. On the other hand, the factor X_1, X_2, X_3, X_4 as well as the interaction of factor X_2X_3, X_4^2 has an important effect on enrichment factor of CAP according to the data in Table 2. The influence of the factors on the extraction efficiency follows the sequence: the concentration of NaH_2PO_4 (X_1, $P < 0.0001$) ≈ the system temperature (X_4, $P < 0.0001$) > pH (X_3, $P = 0.0002$) > the concentration of POELE10 (X_2, $P = 0.0362$). The order of the effect of four factors on enrichment factor is: the system temperature (X_4, $P = 0.0002$) > the concentration of NaH_2PO_4 (X_1, $P = 0.0016$) > the concentration of POELE10 (X_2, $P = 0.0024$) > pH (X_3, $P = 0.0077$).

3.3.2. Regression analysis

In order to predict the outcome of enrichment of CAP, the response function (Eq. (3)) was fit by using equation regression analysis. Based on the experimental data of extraction efficiency of CAP (Table 1), the multiple regression analysis was performed, and the coefficients of the model were evaluated for its significance. We have neglected the non-significant interaction factors.

Table 1
The extraction efficiency and enrichment factor of CAP for each experiment of the CCD.

Run	X_1 NaH_2PO_4 ($g \cdot mL^{-1}$)	X_2 POELE10 ($g \cdot mL^{-1}$)	X_3 pH	X_4 Temperature (°C)	Y_1 Extraction efficiency (E%)	Y_2 Enrichment factor (F)
1	0.180	0.030	3.4	20	72.73	12.95
2	0.180	0.024	4.2	20	82.93	14.99
3	0.186	0.027	3.8	25	93.85	14.31
4	0.198	0.027	3.8	25	91.61	16.72
5	0.192	0.030	3.4	30	92.21	16.47
6	0.186	0.033	3.8	25	99.42	13.43
7	0.180	0.030	3.4	30	89.93	13.65
8	0.180	0.030	4.2	30	94.71	13.76
9	0.192	0.024	3.4	30	96.28	17.66
10	0.186	0.027	3.8	15	79.93	13.83
11	0.192	0.024	4.2	20	95.39	17.48
12	0.192	0.024	4.2	30	98.23	22.56
13	0.192	0.024	3.4	20	89.79	15.00
14	0.186	0.021	3.8	25	88.27	15.34
15	0.180	0.024	4.2	30	96.85	22.21
16	0.192	0.030	4.2	20	93.93	14.32
17	0.180	0.024	3.4	30	82.18	14.84
18	0.192	0.030	3.4	20	92.94	14.16
19	0.180	0.024	3.4	20	60.36	11.82
20	0.186	0.027	4.6	25	86.11	12.92
21	0.174	0.027	3.8	25	75.22	13.44
22	0.186	0.027	3.8	25	88.00	12.00
23	0.186	0.027	3.8	35	95.72	17.54
24	0.192	0.030	4.2	30	99.23	18.25
25	0.180	0.030	4.2	20	83.12	10.73
26	0.186	0.027	3	25	79.22	10.78

Table 2
Results of the analysis of variance (ANOVA) performed to the extraction efficiency model and enrichment factor model.

Source	Sum of squares	df	Mean square	F value	P-value Prob > F	
Extraction efficiency model	2107.683	14	150.5488	12.51779	<0.0001	Significant
X_1	682.3767	1	682.3767	56.7381	<0.0001	
X_2	63.67707	1	63.67707	5.294606	0.0362	
X_3	278.5902	1	278.5902	23.16416	0.0002	
X_4	504.4472	1	504.4472	41.94366	<0.0001	
X_1X_2	23.93537	1	23.93537	1.990172	0.1787	
X_1X_3	84.85087	1	84.85087	7.05516	0.0180	
X_1X_4	160.2039	1	160.2039	13.3206	0.0024	
X_2X_3	29.16672	1	29.16672	2.425148	0.1402	
X_2X_4	8.586596	1	8.586596	0.713956	0.4114	
X_3X_4	7.748252	1	7.748252	0.64425	0.4347	
X_1^2	115.7892	1	115.7892	9.627615	0.0073	
X_2^2	8.41432	1	8.41432	0.699632	0.4160	
X_3^2	137.7909	1	137.7909	11.45701	0.0041	
X_4^2	24.7714	1	24.7714	2.059687	0.1718	
Residual	180.4017	15	12.02678			
Lack of fit	151.9221	10	15.19221	2.667209	0.1452	Not significant
Pure error	28.47961	5	5.695921			
Cor total	2288.084	29				
Enrichment factor model	189.3004	14	13.52146	6.317906	0.0005	Significant
X_1	31.51408	1	31.51408	14.72496	0.0016	
X_2	28.37826	1	28.37826	13.25975	0.0024	
X_3	20.20265	1	20.20265	9.439695	0.0077	
X_4	52.13562	1	52.13562	24.36039	0.0002	
X_1X_2	0.661664	1	0.661664	0.309163	0.5864	
X_1X_3	0.049696	1	0.049696	0.023221	0.8809	
X_1X_4	1.21E−05	1	1.21E−05	5.67E−06	0.9981	
X_2X_3	20.4446	1	20.4446	9.552747	0.0075	
X_2X_4	4.01202	1	4.01202	1.874618	0.1911	
X_3X_4	6.979189	1	6.979189	3.261029	0.0910	
X_1^2	7.629126	1	7.629126	3.564712	0.0785	
X_2^2	3.415459	1	3.415459	1.595875	0.2258	
X_3^2	2.161903	1	2.161903	1.01015	0.3308	
X_4^2	12.63782	1	12.63782	5.905027	0.0281	
Residual	32.10271	15	2.14018			
Lack of fit	27.66494	10	2.766494	3.116994	0.1107	Not significant
Pure error	4.437761	5	0.887552			
Cor total	221.4031	29				

The final predictive equation for the extraction efficiency of CAP was obtained, and it was given bellow:

$$Y_{E\%} = 84.87 + 5.33X_1 + 1.63X_2 + 3.41X_3 + 4.58X_4 - 2.30X_1X_3 - 3.16X_1X_4 - 2.05X_1^2 - 2.24X_3^2 \quad (4)$$

For the enrichment factor of CAP, the final predictive equation with the significant terms was also obtained from the experimental data of enrichment factor of CAP in Table 1.

$$Y_F = 13.92 + 1.15X_1 - 1.09X_2 + 0.92X_3 + 1.47X_4 - 1.13X_2X_3 + 0.68X_4^2 \quad (5)$$

In the Eqs. (4) and (5), $Y_{E\%}$ represents the extraction efficiency of CAP; Y_F represents the enrichment factor of CAP, and X_1, X_2, X_3, X_4 are four factors. For the extraction efficiency of CAP, an *R*-square value of 0.9548 was obtained and it indicates a good response between the model and the experimental results. However, a lower *R*-square value of 0.9272 was obtained for the enrichment factor of CAP.

3.3.3. Response surface plot

Factors influencing the extraction efficiency and enrichment factor of CAP are shown in Fig. 3 as response surface plots. The relationship between these two responses (*E*% and *F*) and experimental levels of each variables was visualized in the 3D response surface plot, meanwhile, which provides a method to directly observe the interactions between two test variables (Qiao et al., 2009). Through the response surface plots, the interactions between two variables and their optimum ranges can be well observed. By analyzing the Fig. 3 and Table 1, the optimal value of extraction efficiency of CAP (*E*%) was 99.42%, when 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, 298.15 K, respectively. Meanwhile, the optimized condition for the enrichment factor of CAP (*F*) was 22.56, and it occurred when the concentration of NaH_2PO_4, the concentration of POELE10, pH and temperature were 0.192 g·mL^{-1}, 0.024 g·mL^{-1}, 4.2, 305.15 K, respectively.

3.4. Samples analysis

The aforementioned extraction technique was applied to separate and determine CAP in the shrimps that come from Qinhuai River, Yangtze River, Yellow Sea, respectively, under the optimal conditions as described above. After the POELE10-NaH_2PO_4 separation process, CAP in shrimp, were separated to POELE10-rich phase, and then it was determined by HPLC-UV method mentioned above. The related HPLC spectrogram was shown in Fig. 4. It was found that the recovery of CAP was 98–100.4% with a SD of 0.8–1.9%

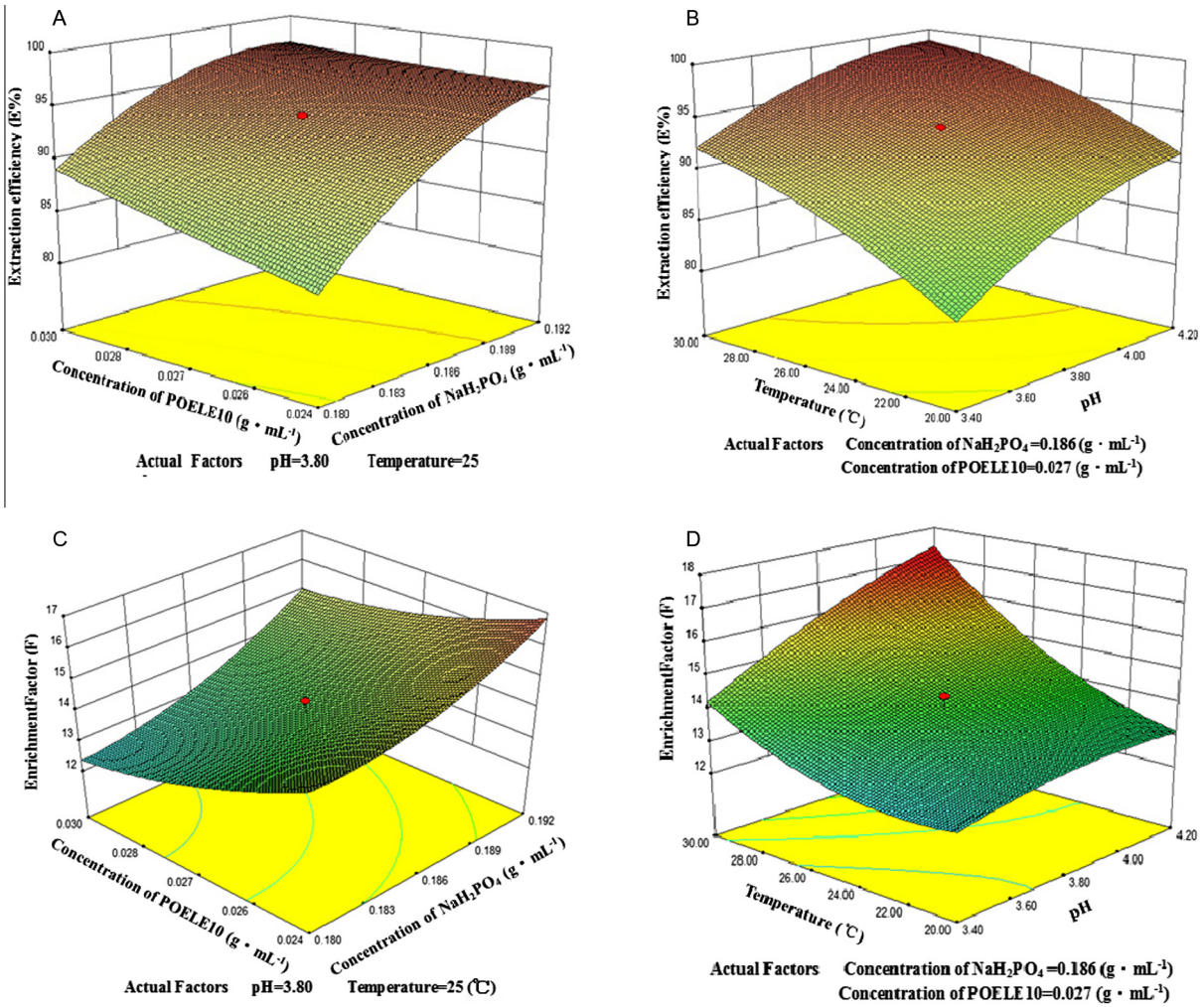

Fig. 3. The 3D response surface plot for the interactive effect of (A) concentration of NaH_2PO_4 and concentration of POELE10, (B) Temperature and pH on the extraction efficiency of CAP, while other variables are set at the fixed value; and the 3D response surface plot for the interactive effect of (C) concentration of NaH_2PO_4 and concentration of POELE10, (D) Temperature and pH on the enrichment factor of CAP, while other variables are set at the fixed value.

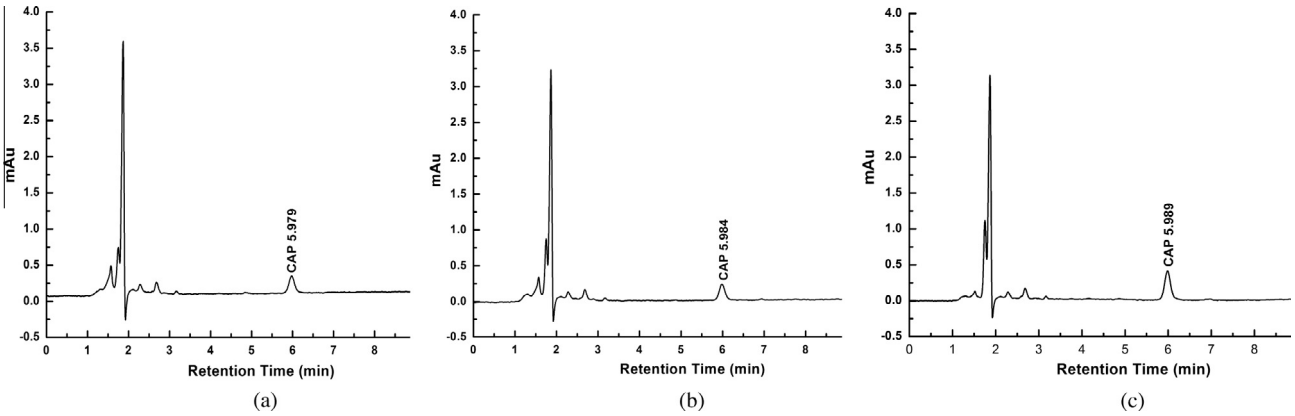

Fig. 4. HPLC chromatograms with UV detection of real sample added with 10 ng·mL^{-1} CAP after ATPS extraction. (a) Real sample: shrimp in Qinhuai River (b) real sample: shrimp in Yangtze River (c) real sample: shrimp in Yellow Sea.

when the concentration of added CAP was 10–100 ng·mL^{-1}. This extraction method was applied to the analysis of CAP in shrimp samples with the linear range of 0.5–3 μg·kg^{-1}. The limit of detection (LOD) and the limit of quantification (LOQ) are 0.8 μg·kg^{-1} and 1 μg·kg^{-1}. This illustrated that the present extraction technique has a satisfactory recovery and reproducibility for the determination of the CAP in a wide range of concentrations. Thus, this method was applied to the quantitative analysis of residual CAP in the food and environment.

3.5. Comparing with other extraction systems

Han et al. (2011) has compared the ionic liquid–salt aqueous two-phase flotation (ILATPF) with ionic liquid aqueous two-phase extraction (ILATPE), liquid–liquid extraction (LLE) and solvent sublation (SS) under the optimal conditions for the separation and enrichment of CAP. In this work, the POELE10-NaH_2PO_4 ATPE was compared with ILATPE, ILATPE, LLE, SS under the optimal conditions for the extraction efficiency of CAP. It was found that the extraction efficiency of the method ($E\%$ = 99.4%) proposed in this paper was much better than LLE ($E\%$ = 2.9%) (Han et al., 2011) and SS ($E\%$ = 53.4%) (Han et al., 2011), and it was slightly higher than ILATPF ($E\%$ = 98.5%) (Han et al., 2011) and ILATPE ($E\%$ = 95.7%) (Han et al., 2011). The enrichment factor of present method (F = 22.6) was much better than LLE (F = 2.0) (Han et al., 2011), ILATPF (F = 18.4) (Han et al., 2011) and ILATPE (F = 9) (Han et al., 2011). The enrichment factor of present method was slightly lower than SS (F = 25.0) (Han et al., 2011), but the extraction efficiency of SS is too low. Thus, the POELE10-NaH_2PO_4 ATPE well suits for the separation and enrichment of residual CAP in the food and environment.

4. Conclusions

As a new extraction system, the POELE10-NaH_2PO_4 ATPE was successfully applied to separating and purifying trace CAP in the environment and food. In this paper, the optimized conditions for the extraction efficiency of CAP ($E\%$) and enrichment factor of CAP (F) were confirmed by the multi-factor experiment that was carried out by RSM. The trace CAP in shrimp from different waters was quantitatively determined by this novel separation method with a HPLC method under the optimized conditions. This extraction method was compared with other reported techniques on the extraction efficiency of CAP ($E\%$) and enrichment factor of CAP (F), and it was found that this method was more applicable to separate and purify the residual CAP. It also gives us enlightenment for the future research on the possibility of applying of POELE10-NaH_2PO_4 ATPE to extract other trace antibiotics.

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