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Comparison of traditional cloud-point extraction and on-line flow-injection cloud-point extraction with a chemiluminescence method using benzo[*a*]pyrene as a marker

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Abstract In this work, using benzo(*a*)pyrene (BaP) as marker, the analytical merits of on-line flow-injection cloud-point extraction (FI CPE), including preconcentration factor, extraction efficiency, sample throughput, and analysis time were evaluated by use of peroxyoxalate chemiluminescence (CL) detection. Moreover, by detailed discussion of several preconcentration conditions for traditional and on-line FI CPE the advantages of on-line FI CPE became conspicuously apparent. When coupled with separation techniques such as high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE), on-line FI CPE–CL has much potential for analysis of low concentrations of polycyclic aromatic hydrocarbons (PAH) in environmental samples.

Keywords On-line flow-injection cloud-point extraction · Traditional cloud-point extraction · Chemiluminescence · Benzo(*a*)pyrene

Introduction

Aqueous solutions of almost all non-ionic or zwitterionic surfactants become turbid when heated to a temperature referred to as the cloud point. Addition of salting-out agents has proven to be another effective means of inducing the clouding phenomenon [1, 2]. When cloud-point extraction (CPE) is performed in aqueous solutions,

hydrophobic compounds without charges repel water, resulting in the concentration of these compounds in the small volume of the surfactant-rich phase, whereas hydrophilic components remain in the residual aqueous solution [3]. The phase separation is reversible—when the mixture is cooled to a temperature below cloud point the two phases merge to form a single phase again.

CPE has been extensively used for preconcentration/extraction of hydrophobic species from aqueous solutions [4, 5]. As an alternative to other preconcentration techniques, CPE uses a small amount of nonflammable and nonvolatile surfactant, instead of organic solvents, in solvent extractions such as liquid–liquid extraction [6]. CPE involves a series of fussy procedures, however, including equilibration, centrifugation, and removal of the surfactant-rich phase, resulting in poor reproducibility and low preconcentration factor. Widespread application of CPE is, therefore, very limited.

Flow-injection analysis (FIA) is, owing to its versatility, simplicity, and low instrumental cost, a powerful tool for automatic rapid sample pretreatment and nonchromatographic separation before determination of the analyte by a variety of detection methods [7]. On-line combination of CPE with FIA has been shown to be a promising means of analysis because of enhanced sensitivity and reproducibility, efficient removal of matrices, and high extraction efficiency. In on-line FI CPE, the equilibration and centrifugation procedures are avoided, making the entire extraction process simpler and the analysis time shorter. More importantly, the surfactant-rich phase containing the analytes can be easily removed by on-line elution rather than use of a pipette. A larger preconcentration factor can therefore be achieved by use of lower concentrations of surfactant. In recent years, on-line combination of CPE with FIA has attracted much attention. For example, Cordero and co-workers [8] were the first to recognize the advantages of combining CPE with FIA, but the cloud-point methodology was still performed off-line in their experiments. The ability to perform CPE on-line in an FIA system was been reported for the first time by Huie [9]. Paleologos [10] has reported the determination of metal

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species by on-line combination of CPE with FIA coupled with chemiluminescence detection. Analysis of mercury by on-line FI CPE has been reported by Garrido [11].

In this work we have systemically evaluated the analytical performance of on-line FI CPE, including pre-concentration factor, extraction efficiency, analysis time, and technical aspects. To overcome the strong UV-visible absorption interference from nonionic surfactants and to enhance detection sensitivity, a relatively stable peroxyoxalate chemiluminescence (CL) system was used for detection [12, 13]. We investigated 16 PAH named as priority pollutants by the EPA and found that benzo(*a*) pyrene (BaP) resulted in the highest CL intensity. We therefore used BaP as a marker.

Experimental

Apparatus

The FIA manifold for on-line FI CPE is illustrated in Fig. 1. It consisted of two peristaltic pumps (SJ-1211; Atto,

Tokyo, Japan), a six-channel injection valve (Sanuki, Japan) and a man-made CPE collection column (glass, 3.0 cm long, 0.3 cm i.d. packed with cotton). The CL intensity signal was recorded as peak height. The signal data were obtained using a F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) at the maximum emission wavelength, 415 nm. The excitation lamp was off and the emission slit width was fully opened to 20 nm during CL intensity recording. In traditional CPE a centrifuge (LG10-2.4A, Beijing Medical Centrifuge Factory, Beijing, China) was also used to hasten complete phase separation.

Chemicals

Analytical grade BaP, benzo[*k*]fluoranthene (BkF), and benzo[*ghi*]perylene (Bper) were obtained from Sigma (St Louis, MO, USA). Triton X-114 and acetonitrile (HPLC grade) were obtained from Acros (Geel, Belgium) and Siyou Biologic and Technic Company (Tianjing, China), respectively. Sodium sulfate and 30% hydrogen peroxide

Fig. 1 Schematic diagram of the on-line FI CPE system. P_1 and P_2 , peristaltic pumps; R_1 , sample solution (BaP standard and Triton X-114); R_2 , salting-out agent Na_2SO_4 ; R_3 , acetonitrile; C , collection column (effective length 1.5 cm, i.d. 3 mm); W_1 and W_2 , waste

(H₂O₂) were obtained from Beijing Chemical Company (China). Bis(2,4,6-trichlorophenyl)oxalate (TCPO) was purchased from Fluka (Lake Ronkonkoma, NY, USA). Water was deionized by means of a Milli-Q purification system with a 0.2 µm fiber filter (Barnstead, USA). Cotton was commercial absorbent cotton and made of pure cellulose (Beijing Filter Company, Beijing, China).

A stock solution (250 µg mL⁻¹) of BaP was prepared by dissolving the standard in acetonitrile and working solution was prepared by dilution with Triton X-114 solution. Triton X-114 solutions were prepared by dissolving appropriate amounts of Triton X-114 in water. Sodium sulfate solution was prepared by dissolving the salt in water and was filtered through a 0.45 µm pore-size fiber filter before use. H₂O₂ working solution was freshly prepared by diluting 30% H₂O₂ with acetonitrile. TCPO working solutions were prepared by dissolving appropriate amounts of TCPO in acetonitrile.

Procedures

Traditional cloud point extraction

Typically, a traditional CPE experiment was performed as follows. Appropriate amounts of aqueous solutions containing the analyte in the presence of Triton X-114 were prepared. Cloud-point separation was initiated by addition of appropriate amounts of sodium sulfate. The final volume of the bulk solution was 25 mL. The concentrations of sodium sulfate and Triton X-114 were 0.6 mol L⁻¹ and 1.0% (v/v), respectively. The sample solution was left to equilibrate for 10 min and then centrifuged at 3500 rpm for 20 min to achieve complete separation of the two phases. The interface between the top surfactant-rich phase and the bottom aqueous phase was very sharp. The surfactant-rich phase was carefully transferred to a glass tube by means of a pipette. Appropriate amounts of the surfactant-rich phase, TCPO, and H₂O₂ were sequentially added to a 3.0-mL quartz cell for CL measurements with the wavelength of the emission monochromator set to 415 nm.

On-line FI cloud point extraction

On-line FI CPE preconcentration of BaP was performed as follows. In the extraction stage (Fig. 1a), sample solution containing 0.05 µg mL⁻¹ BaP and 0.05% (v/v) Triton X-114 was first loaded into an FIA manifold via channel R₁. A flow rate of 2.3 mL min⁻¹ and a loading time of 90 s were used for the sample solution, resulting in loading of 3.45 mL sample solution. The sample solution from R₁ merged with the salt solution from R₂ to induce separation of the sample solution into two distinct phases—a surfactant-rich phase containing molecules of BaP solubilized within surfactant aggregates and an aqueous phase containing the salt, surfactant monomers, and other water-soluble components of the sample solution. The mixture then passed through the collection column, which enabled

the surfactant-rich phase to be trapped inside the column while the aqueous phase passed through the column. When sample loading was complete the injection valve was switched to the position shown in Fig. 1b, the elution solvent passed through the column at the rate of 1.5 mL min⁻¹, carrying the BaP and surfactant molecules into a glass tube. The amount of BaP in the surfactant-rich phase was measured by the CL method used with traditional cloud point extraction. The theoretical preconcentration factor was calculated as the ratio of the volume of the original solution to the volume of the surfactant-rich phase, which simultaneously varies with the amount of the surfactant [4, 14]. In this work, the preconcentration factor (PF) is defined by the equation: $PF = (A_2 - A_1)/A_0$, where A_0 is the CL intensity of the original sample solution (without preconcentration), A_2 and A_1 denote the CL intensities of the BaP solution after preconcentration and the blank, respectively, and A_0 and A_2 are linearly dependent on the concentration of BaP. The emission wavelength was set at the peak maximum for BaP, i.e. 415 nm.

In on-line FI CPE, A_2 and A_1 , respectively, were determined by collecting the concentrated BaP and the blank at sample-output end 1 (Fig. 1b) between 8.8 and 35.2 s after loading of sample solution into the FIA system. For the determination of A_0 , the same volume of sample solution was also collected but without the preconcentration step (without addition of salt and without the presence of the collection column).

It is well known that micelles and some inorganic cations can increase the CL intensity of analytes in the peroxyoxalate CL system [15, 16]. With this system, therefore, to evaluate enhancement of the signal because of concentration of the analyte rather than the enhancement because of the effect of the cloud-point phenomenon itself, we deducted the CL signal intensity resulting from micellar and salt enhancements by performing a blank experiment (i.e. performing CPE under the optimum conditions but without BaP solution).

Results and discussion

Selection of chemiluminescence detection

It is well known that the concentration of the surfactant containing the analytes is greatly increased after CPE, resulting in strong UV-visible and fluorescence background absorption. Application of spectrophotometry and fluorescence detection for CPE was therefore restricted. Chemiluminescence detection does not require excitation source, however, and thus can overcome these disadvantages. It has been successfully used as a detection technique after CPE [10, 17]. In this work the TCPO-H₂O₂ CL system was used for analysis of BaP after CPE. The method is based on oxidation of aryl oxalate esters by H₂O₂ in the presence of a suitable fluorescent species. The concentrations of TCPO and H₂O₂ were optimized, and 2.5 mmol L⁻¹ TCPO and 3.0 mol L⁻¹ H₂O₂ were selected for the entire experiment.

Effect of Triton X-114 concentration

The efficiency of CPE is very dependent on the concentration of surfactant. It is known that Triton X-114 has a low cloud-point temperature, results in a surfactant rich phase of high density, is commercial available at low cost, and has no electroactive groups in its molecule [18]. Triton X-114 is, therefore, often used in CPE to form the surfactant-rich phase. In this work the CL intensity of on-line FI CPE as a function of Triton X-114 concentration was investigated. It was found that the maximum CL intensity was obtained for 0.05% (v/v) Triton X-114. Larger concentrations of the surfactant led to the decreasing CL intensity, presumably because larger concentrations increase the volume of the surfactant-rich phase within the collection column, resulting in dilution of the extracted sample. The effect of the concentration of Triton X-114 in traditional CPE was also examined. It was proved that the highest CL intensity was at 0.8% (v/v). Throughput this study, however, the traditional CPE system was operated with 1.0% (v/v) Triton X-114 because lower Triton X-114 concentrations resulted in difficulty collecting the surfactant-rich phase and, subsequently, unacceptable experimental error.

The theoretical preconcentration factor is defined as the ratio of the volume of the original aqueous phase to that of the surfactant-rich phase after the phase-separation step [4, 14]. The volume of the surfactant-rich phase is proportional to the surfactant concentration in the original aqueous solution, and the preconcentration factor increases with decreasing volume of the surfactant-rich phase. From these results we can see that the optimum Triton X-114 concentration for on-line FI CPE was much lower than that for traditional CPE, which resulted in a higher theoretical preconcentration factor. In traditional CPE it is necessary to maintain the surfactant-rich volume sufficient for removal of the concentrated samples whereas in on-line FI CPE without the removal step the preconcentration factor can be improved by reducing the original surfactant concentration. Compared with traditional CPE, this characteristic of on-line FI CPE is one of the most significant advantages.

Effect of salting-out agents

On the basis of the cloud point phenomenon [1, 2] an appropriate salting-out agent acting as a cloud-point temperature (T_c)-depressing electrolyte can be used for preconcentration of hydrophobic species from an aqueous phase into a non-ionic surfactant phase (Triton X-114). Salting-out agents Na_2SO_4 , NaCl , K_3PO_4 , and NaOH (0.8 mol L^{-1} each) were compared for their ability to yield the highest CL intensity for detection of CPE concentrated BaP. It was found that Na_2SO_4 resulted in the highest CL intensity. The effect of salting-out agent concentration on CL intensity was investigated. It was found that an increase in Na_2SO_4 concentration caused a concomitant increase of CL intensity. When the Na_2SO_4 concentration was more than 0.8 mol L^{-1} , crystal particles appeared and occasional

blockage of FIA channels occurred. The optimum salt concentration of 0.8 mol L^{-1} was therefore chosen for on-line FI CPE.

In traditional CPE, addition of Na_2SO_4 to the surfactant micellar solution can often reduce the cloud point temperature. Too much Na_2SO_4 made the surfactant phase milky-white, however, which made it more difficult to separate surfactant phase accurately. In this study, the optimum concentration was selected as 0.6 mol L^{-1} .

Effect of cotton amount in on-line FI CPE column

In an on-line FI CPE column the filtering material can entrap larger size surfactant aggregates and let smaller size components pass through, including salt and water molecules, the unretained test compound, surfactant monomer, and surfactant aggregates of smaller size. Therefore, the selection of the filtering material in on-line FI CPE column is important. Huie et al. [9] investigated different filtering materials and found that cotton resulted in the best reproducibility. On the basis of previously reported results [9, 11], moreover, an inner diameter of 3 mm and a length of 3.0 cm were used for the on-line FI CPE column in this work.

The amount of cotton in collection column was optimized. Results showed that small amounts resulted in low CL intensity because of inefficient collection of surfactant-rich phase whereas larger amounts led to deterioration of the CL signal because of blockage of channels and insufficient washing-out of the retained surfactant-rich phase. In this work, 0.03 g cotton, leading to a column density of approximately 0.28 mg mm^{-3} , was sufficient for extraction.

Extraction efficiency

Extraction efficiency is the ratio of the BaP concentration retained in the collection column to the original concentration [19]. As shown in Fig. 2, as sample-loading time was increased from 10 to 140 s, extraction efficiency was gradually decreased from 97.3% to 38.8%, because cotton sorption efficiency decreased with loading time. When the sample-loading time exceeded 100 s, the extraction efficiency decreased sharply. These results showed that 100 s sample-loading time almost resulted in adsorption saturation of the cotton column, which was helpful for selecting an appropriate sample-loading time.

In traditional CPE, the extraction efficiency for BaP was estimated by determining the concentration of BaP present in the top layer and the original concentration. The results indicated that the extraction efficiency was ca 86% for a sample solution containing $0.05 \text{ } \mu\text{g mL}^{-1}$ BaP and 1.0% Triton X-114. In traditional CPE improvement of the extraction efficiency can be achieved by increasing the concentration of surfactant, although higher surfactant concentrations can result in lower preconcentration factor. For on-line FI CPE, improvement of the extraction

Fig. 2 Effect of sample-loading time on extraction efficiency. The concentrations of Triton X-114, Na_2SO_4 , and BaP were 0.05% (v/v), 0.8 mol L^{-1} , and $0.05 \text{ } \mu\text{g mL}^{-1}$, respectively. For a sample-loading time of 90 s the loading rate (flow rate of aqueous Na_2SO_4 solution) was 2.3 mL min^{-1} and the elution rate was 1.5 mL min^{-1} . The filter material was 0.03 g cotton

efficiency can be achieved by changing CPE column length and the amount of cotton packed in the collection column. This was another merit of on-line FI CPE.

Sample-loading time

To obtain the largest preconcentration factor and greatest extraction efficiency the effect of sample-loading time was examined. From the Fig. 3 we can see that the CL intensity increased with increasing sample-loading time. These results showed the feasibility of achieving higher preconcentration factor by increasing sample-loading time. Considering the extraction efficiency of the collection column and the analytical time, the sample-loading time of 90 s was chosen as the optimum condition.

Fig. 3 Effect of sample-loading time on CL intensity. All conditions as for Fig. 2

Fig. 4 Three elution curves. *Diamonds*: R_1 , sample solution (BaP standard and Triton X-114); R_2 , Na_2SO_4 solution; R_3 , acetonitrile. *Triangles*: R_1 , sample solution (BaP standard), R_2 , water; R_3 , acetonitrile; *Circles*: R_1 , sample solution (BaP standard and Triton X-114); R_2 , water; R_3 , acetonitrile; All other conditions as for Fig. 2

Elution curves

To remove analyte-containing surfactant aggregates within the filter material an appropriate solvent was used for the elution of the analytes and surfactant aggregates from the collection column by switching to the second stage (Fig. 1b). It is well known that CL efficiency can be severely quenched in the presence of water [20]. Therefore, 100% acetonitrile was used as the eluent.

Three different elution curves were obtained under different conditions, as shown in Fig. 4. When on-line FI CPE was performed (diamonds) it was found that 98% BaP could be eluted with 0.66 mL acetonitrile in 26.4 s. Without surfactant and salting-out agents in on-line FI CPE (triangles) weaker CL signals were observed, because the cotton adsorbed some of the BaP molecules. Furthermore, replacement of Na_2SO_4 by H_2O (circles) resulted in lower maximum CL intensity, probably because the BaP molecules can easily enter the Triton X-114 cavity; if CPE did not occur, however, the number of the surfactant aggregates was so low and the cavity so small that the mostly mixed solution of BaP and Triton X-114 passed through the cotton column. These results clearly showed that concentration of BaP on the cotton column was mainly a result of CPE.

Table 1 Relative CL intensities for on-line FI CPE and traditional CPE

Experiments	Relative CL intensity ^a	
	On-line FI CPE	Traditional CPE
Original solution	20.4±0.1	20.4±0.1
Blank solution	26.7±1.6	28.3±3.2
Concentrated solution	846.2±2.8	432.2±4.7

^aAll the results are means from five determinations±S.D.

Preconcentration factor

Table 1 shows the relative CL intensities for on-line and traditional CPE. It was found that the relative CL intensity of on-line FI CPE was much higher than that of traditional CPE. In this study the preconcentration factors for on-line FI CPE and traditional CPE were 40.2 and 19.8, respectively. The sample volume used in on-line FI CPE, 3.45 mL, was, moreover, a factor of 7.4 less than that used in traditional CPE. The efficiency of concentration of other PAH, for example BkF and Bper, was also compared. The results showed that the preconcentration factors for these PAH in on-line FI CPE were much higher than in traditional CPE—the values were 38.5 and 41.3, respectively.

Analytical time and some technical aspects

In traditional CPE, separation of the surfactant aggregates from the bulk aqueous solution into two distinct phases is usually accomplished by allowing the solution to stand for a given time (e.g. 30 min) and subsequently by using centrifugation to hasten this phase-separation process. The equilibration and centrifugation procedures usually require half an hour or longer, and inadequate equilibration and centrifugation can lead to reduced extraction efficiency. Importantly, after separation of the two phases, manual removal of the surfactant-rich phase with a pipette can cause undesirable experimental error.

In on-line FI CPE, without long equilibration and centrifugation procedures, the entire CPE process can be completed in three minutes by using the flow injection channels to mix the solutions and using a man-made collection column packed with cotton to separate the surfactant-rich phase from the bulk aqueous solution. In this work, when the FIA system was switched to the elution stage (Fig. 1b), approximately 98% BaP could be effectively eluted using 0.66 mL acetonitrile as eluent in 26.4 s. Therefore, on-line FI CPE can achieve a higher preconcentration factor and greater sample throughput.

Conclusions

In the work discussed in this paper, on-line and traditional CPE were compared in detail. The performance of on-line

FI CPE has been systemically discussed. The results indicate that the analytical capability of on-line FI CPE, especially in terms of preconcentration factor, extraction efficiency, and analysis time, are a considerable improvement over the traditional CPE method. On the basis of this behavior it should be possible to develop on-line FI CPE preconcentration coupled with separation techniques such as HPLC and CE.

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References

- Gu T, Galera-Gómez PA (1995) *Colloids Surf A* 104:307
- Saitoh T, Hinze WL (1991) *Anal Chem* 63:2520
- Lu C, Lin J-M (2004) *Acta Phys-Chim Sin* 20:974
- Delgado B, Pino V, Ayala JH, González V, Afonso AM (2004) *Anal Chim Acta* 518:165
- Ferrera ZS, Sanz CP, Santana CM, Rodríguez JJS (2004) *Trends Anal Chem* 23:479
- Tani H, Kamidate T, Watanabe H (1997) *J Chromatogr A* 780:229
- Wang J, Hansen EH (2003) *Trends in Anal Chem* 22:225
- Laespada MEF, Pavón JLP, Cordero BM (1993) *Analyst* 118:209
- Fang Q, Du M, Huie CW (2001) *Anal Chem* 73:3502
- Paleologos EK, Vlessidis AG, Karayannis MI, Evmiridis NP (2003) *Anal Chim Acta* 477:223
- Garrido M, Di Nezio MS, Lisa AG, Palomeque M, Band BSF (2004) *Anal Chim Acta* 502:173
- Lu C, Lin J-M, Huie CW, Yamada M (2004) *Anal Chim Acta* 510:29
- Lin J-M, Yamada M (1999) *Anal Chem* 71:1760
- Pino V, Ayala JH, Afonso AM, González V (2002) *J Chromatogr A* 949:291
- Lin J-M, Yamada M (2003) *Trends Anal Chem* 22:99
- Dan N, Lau ML, Grayeski ML (1991) *Anal Chem* 63:1766
- Horvath WJ, Huie CW (1993) *Talanta* 40:1385
- Paleologos EK, Stalikas CD, Karayanni SMT, Pilidis GA, Karayannis MI (2000) *J Anal At Spectrom* 15:287
- Horvath WJ, Huie CW (1992) *Talanta* 39:487
- Imaizumi N, Hayakawa K, Miyazaki M (1989) *Analyst* 114:161