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Automatic trace-enrichment of bisphenol A by a novel continuous flow liquid membrane extraction technique

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Bisphenol A was enriched by a novel continuous flow liquid membrane extraction technique, which is based on the combination of continuous flow liquid-liquid extraction and a supported liquid membrane. Related parameters such as flow rates, liquid membrane solvents, and pH of donor and acceptor were optimized. Using dichloromethane as liquid membrane, over 200-fold enrichment of $50 \mu\text{g L}^{-1}$ BPA was obtained after a 40-min enrichment time. The major advantages of this technique are that it provides a relatively high enrichment factor, freedom of choice and long term stability of the liquid membrane, much lower consumption of organic phase, typically 0.05 mL min^{-1} , scope for full automation, and easy on-line coupling to various detectors.

Key Words: Automatic trace-enrichment; Bisphenol A; Water samples; Continuous flow liquid-liquid extraction; Supported liquid membrane

Ms received: June 18, 2001; revised: October 17, 2001; accepted: October 22, 2001

1 Introduction

Environmental sources of Bisphenol A (BPA or 4,4'-isopropylidenediphenol) include the thermal degradation of many plastic products and the discharge of BPA manufacturing or processing plants. Recently, much attention has been focused on the compound's estrogenic properties and some authorities have reacted to these concerns by imposing a daily intake limit for BPA. The development of sensitive and selective methods for determination of BPA in environmental and food matrices is a new challenge.

So far, the analysis of BPA has been accomplished mainly by chromatographic methods such as TLC, GC, GC-MS, HPLC, and HPLC-MS. Generally speaking, samples must be pretreated before analysis by these methods. The sample pretreatment procedures applied include Soxhlet extraction [1], liquid-liquid extraction [2, 3], solid-phase extraction (SPE) [4, 5], microwave-assisted solvent extraction (MASE) [4], supercritical fluid extraction (SFE) [5], and solid-phase microextraction (SPME) [6]. Among them, the SPE procedure was the most widely used technique.

This study describes an alternative procedure for automatic preconcentration of BPA in water samples. This procedure is based on a new aqueous-organic-aqueous extraction technique that we termed continuous flow liquid membrane extraction (CFLME) [7]. CFLME is the combi-

nation of continuous flow liquid-liquid extraction (CFLLE) [8–11] and supported liquid membrane (SLM) extraction [12–14]. In this proposed procedure, BPA was first extracted into the organic phase in the CFLLE step, and then transported onto the organic liquid membrane that formed on the surface of the micro porous membrane of the SLM equipment. Finally, it passes through the liquid membrane and was trapped by the acceptor. Parameters related to CFLME were studied and an enrichment factor of over 200 was obtained after 40 min enrichment of $50 \mu\text{g L}^{-1}$ BPA. The proposed method is very stable, fully automatic and easy coupled on-line to various detectors.

2 Experimental

2.1 Apparatus

A schematic diagram of the flow system is shown in **Figure 1**. It consisted of a FIA 5020 Analyzer (Tecator, Sweden), FIA-3000 flow injection analyzer (SNK, Japan), a manually controlled 6-port valve, and a custom-made SLM device. The mixing coil (MC) and extraction coil (EC) were made of 0.5 mm ID polytetrafluoroethylene (PTFE) tubing and T-shaped three-way connectors made from PTFE were used to mix solutions or solvents.

The custom-made SLM device is the same as described elsewhere [7]. Briefly, a sheet of Fluoropore FG PTFE porous membrane (Millipore Corp., Bedford, MA.) was clamped between two custom-made PTFE blocks with grooves of 0.5 mm deep, 2.0 mm wide, and 50 mm long to form two channels (the acceptor channel and the donor channel) with the same volume of $50 \mu\text{L}$.

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Figure 1. Schematic diagram of the proposed continuous flow liquid membrane extraction system manifold. See text for details.

An SPD-M10Avp diode array detector (Shimadzu, Japan) setting at 240 nm wavelength was used as detector. Data acquisition and process were accomplished with a Class-VP Workstation (Shimadzu, Japan), which was connected with the detector with an SCL-10Avp system controller (Shimadzu, Japan).

2.2 Reagents

Bisphenol A (BPA, 97%) was purchased from Acros Organics (NJ, USA). Standard stock solution was prepared by dissolving 20 mg of standard in 100 mL of water and stored at 4°C. Working solutions were obtained daily by appropriate dilution of the stock solutions with water. All other chemicals were purchased from Beijing Chemicals Corporation and were analytical grade or better. De-ionized water was used throughout.

2.3 Procedure

A schematic diagram of the proposed system is shown in Figure 1 and the installation of the whole system was described in our previous paper [7].

As shown in Figure 1, sample solution (S) and diluted sulfuric acid (R) are introduced into the system by a peristaltic pump (P1) of the FIA 5020 Analyzer. These two solutions are mixed in a mixing coil (MC) and are segmented by organic solvent (O) delivered by the piston pump (P3) of the FIA-3000 Flow injection analyzer. BPA is first extracted into the organic phase in an extraction coil (EC). Then, by switching V1 at the sampling position, the segments of aqueous solution and organic solvent are introduced into the donor side of the SLM to form an organic liquid membrane on the surface of the micro porous membrane. The acceptor (A) stream, delivered by a peristaltic pump (P2) of the FIA 5020 Analyzer, is kept stagnant in

the acceptor channel of SLM by switching V2 at the bypass position. BPA therefore diffuses through the liquid membrane and is trapped by the alkaline acceptor. After a certain time (typically 20 min), V1 is switched to the bypass position and V2 is switched to the injection position to introduce BPA that has been enriched in the acceptor channel of the SLM to the detector (PDA), and the peak height is determined.

All the solutions were degassed by ultrasound for 30 min before use to prevent the formation of air bubbles in the flow system.

3 Results and discussion

3.1 Influence of the CFLLE related parameters

It is well known that the inner diameter and length of extraction coil, the flow rates of sample, reagent, and organic solvent, and the ratio of aqueous to organic phase significantly influence the extraction efficiency of a CFLLE system. In this study, optimization of these parameters was conducted by 20-min enrichment of 50 $\mu\text{g L}^{-1}$ BPA. A solution of 0.32 M H_2SO_4 was used to acidify the donor and a solution of 12 g L^{-1} $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ was used as acceptor.

Experiments show that the mixing coil (EC) did not affect the peak height in the range of 30–100 cm. A 30-cm long mixing coil was therefore used throughout.

The length and inner diameter of the extraction coil has a significant influence on the peak height. As small tubing diameter is helpful for extraction, 0.5 mm ID PTFE coil was adopted without optimization, while the coil length was optimized in the range of 60–360 cm. Results show that the greatest peak height was obtained when a 240-cm long extraction coil was used. Therefore, a 240-cm long extraction coil was adopted.

To decrease the dilution factor of sample in the flow system, the flow rate of sulfuric acid should be as low as possible. In this study, 0.4 mL min^{-1} of sulfuric acid, the lowest flow rate that can be obtained, was used throughout. The adopted flow rate of acceptor, which does not affect the extraction efficiency, was 0.8 mL min^{-1} .

Keeping the sample flow rate at 1.2 mL min^{-1} , the flow rate of organic phase, dichloromethane, was optimized in the range of 0.05–0.20 mL min^{-1} and the results show that the peak height decreased with increasing flow rate of dichloromethane, as expected. Though it might be helpful for attaining a greater peak height, a lower flow rate than 0.05 mL min^{-1} of dichloromethane was not studied as this is the lowest flow rate the instrument can provide. Therefore, 0.05 mL min^{-1} was used in the following studies.

Figure 2. Effect of solvents on the peak heights of 50 $\mu\text{g L}^{-1}$ BPA. Enrichment time, 20 min; acceptor, 12 g L^{-1} $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; samples were acidified with 0.5 M H_2SO_4 .

Keeping the flow rate of H_2SO_4 at 0.4 mL min^{-1} and the concentration of H_2SO_4 at 0.08 M after mixing with different flow rates of sample solutions, by using appropriate concentration of H_2SO_4 , the influence of sample flow rate was investigated from 0.4 to 2.8 mL min^{-1} . The results demonstrated that the peak height increased with increasing sample flow rate. Though the highest peak height was obtained at 2.8 mL min^{-1} , 2.0 mL min^{-1} sample flow rate was adopted as the whole flow system is more stable at this flow rate.

3.2 Solvent used as the liquid membrane

As BPA is a relatively polar compound, polar solvents should be beneficial for high enrichment rate according to the rule of "like dissolve in like". Three kinds of polar solvents were tested in this study and the results are shown in **Figure 2**, which indicates that the largest peak height was obtained when dichloromethane was used. Dichloromethane was adopted in the following studies.

3.3 Sulfuric acid concentration in the donor stream

BPA is a weak acid, which should be protonated to form an uncharged substance before extraction into organic phase. Sulfuric acid was adopted to acidify the sample solution, and its concentration was optimized by introducing different concentrations of sulfuric acid into the donor stream while keeping the flow rate at 0.4 mL min^{-1} . As shown in **Figure 3**, the greatest peak height was obtained when 0.4–0.8 M sulfuric acid was used. Therefore, 0.5 M sulfuric acid was introduced in the following studies. After

Figure 3. Effect of sulfuric acid concentration on the peak heights of 50 $\mu\text{g L}^{-1}$ BPA. Donor: 2.0 mL min^{-1} of sample solution mixed with 0.4 mL min^{-1} of H_2SO_4 , and 0.05 mL min^{-1} of dichloromethane; acceptor, 0.8 mL min^{-1} of 12 g L^{-1} $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; Enrichment time, 20 min.

mixing with the sample solution in the donor stream, this corresponds to 0.08 M H_2SO_4 .

3.4 Effect of the acceptor pH

The final step of the proposed CFLME procedure is back extraction of BPA into the acceptor solution. In order to trap BPA in the acceptor, an alkaline acceptor should be used to ionize the BPA molecule, which diffuses through the membrane into the acceptor, to prevent it from re-entering the liquid membrane. The pH of acceptor was optimized using Kolthoff buffer solutions (KH_2PO_4 – $\text{Na}_2\text{B}_4\text{O}_7$ buffer for pH 7.0–9.0, Na_2HPO_4 – NaOH buffer for pH 10.0–12.0) and 0.1 M NaOH (for pH 13.0). The results shown in **Figure 4** demonstrate that the highest peak was obtained at above pH 12.0. This result is in agreement with the theory reported by Jönsson et al. [15], i.e., the optimum pH should be $\text{pH} > \text{p}K_a + 3.3 = 9.5 + 3.3$ (the $\text{p}K_a$ value of BPA is around 9.5). A 0.1 M Na_2HPO_4 – NaOH buffer solution of pH 12.0 was adopted in the following studies.

3.5 Influence of salt effect

It is well known that the addition of salt can usually increase the extraction efficiency. To examine the effect of the addition of salt, different amounts of sodium chloride were added into the sample solution to give 0, 5, 10, 15, and 20% NaCl in the donor after mixing with sulfuric acid solution. The results shown in **Figure 5** indicate that the peak height of BPA increased significantly up to 15% NaCl and then remained unchanged. Therefore, 15% NaCl was adopted in the following studies.

Figure 4. Effect of acceptor pH on the peak heights of 50 $\mu\text{g L}^{-1}$ BPA. Donor: 2.0 mL min^{-1} of sample solution mixed with 0.4 mL min^{-1} of H_2SO_4 , and 0.05 mL min^{-1} of dichloromethane; acceptor, 0.8 mL min^{-1} of buffer with different pH; Enrichment time, 20 min.

Figure 5. Effect of NaCl concentration on the peak heights of 50 $\mu\text{g L}^{-1}$ BPA. Donor: 2.0 mL min^{-1} of sample solution mixed with 0.4 mL min^{-1} of H_2SO_4 , and 0.05 mL min^{-1} of dichloromethane; acceptor, 0.8 mL min^{-1} of 0.1 M Na_2HPO_4 -NaOH (pH12.0) buffer; Enrichment time, 20 min.

3.6 Repeatability and linearity

To examine the repeatability of the system, 50 $\mu\text{g L}^{-1}$ BPA was enriched 5 times with an enrichment time of 20 min. The RSD was 3.4%.

The linearity of the system was investigated by performing 30 min enrichment of a series solutions contains 10, 50, 100, and 200 $\mu\text{g L}^{-1}$ BPA, respectively. Experiments show that the peak height (A , mAU) and concentration (C , $\mu\text{g L}^{-1}$) were related by the regression equation $A = 4.95C - 16.1$ with a correlation coefficient of $r = 0.9986$.

Figure 6. Effect of enrichment time on the enrichment factors of 50 $\mu\text{g L}^{-1}$ BPA. Donor: 2.0 mL min^{-1} of sample solution mixed with 0.4 mL min^{-1} of H_2SO_4 , and 0.05 mL min^{-1} of dichloromethane; acceptor, 0.8 mL min^{-1} of 0.1 M Na_2HPO_4 -NaOH buffer (pH12.0).

3.7 Influence of enrichment time

During long term enrichment, the pH or buffer capacity of the acceptor chamber might change and thus affect the enrichment efficiency. Thus it is necessary to investigate the influence of enrichment time on the enrichment factor.

The enrichment factor was obtained according to

$$E_e = (H_e/C_e)/(H_i/C_i)$$

Here, H_e is the peak height determined by enriching BPA with initial concentration of C_e . H_i is the peak height obtained by direct injection of a standard BPA solution with concentration of C_i prepared in acceptor. Direct injection was conducted by replacing the SLM unit with a sampling loop of 50 μL , the same volume of the acceptor channel of the SLM device, while keeping the other conditions the same as in the enrichment process. A 1 mg L^{-1} BPA solution was directly injected as the peak height of direction of 50 $\mu\text{g L}^{-1}$ BPA was too low for quantification.

Figure 6 shows the effect of enrichment time in the range of 10–60 min, which indicates that over 200-fold concentration was obtained when samples were concentrated for 40 min or longer.

4 Conclusion

Combination of continuous flow liquid-liquid extraction and supported liquid membrane, a novel automatable technique, was developed for trace enrichment of BPA with an enrichment factor of over 200 after 40 min of enrichment.

The proposed method has some of the advantages of SLM, such as high enrichment factor, convenient automation, and easy on-line coupling to analytical instruments. Furthermore, this proposed CFLME technique has some advantages over the SLM technique. As polar organic solvents can be used as liquid membrane, this CFLME technique provides freedom of choice of liquid membrane and much higher enrichment rate than that of the original SLM. Another advantage of this CFLME procedure is the robustness of the liquid membrane, which is stable without the danger of the membrane breakthrough, such as often occurred in the original SLM procedure. This procedure needs an additional pump to deliver organic phase and the consumption of organic phase, typically 0.05 mL min^{-1} , is a little more than in the original SLM procedure but much less than in continuous flow liquid-liquid extraction.

This proposed CFLME technique is a promising sample pretreatment platform for various selective detectors. Further studies on the on-line coupling of CFLME with HPLC and CE for determination of BPA were conducted in this laboratory.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (20177026 and 29825114) and the Chinese Academy of Sciences.

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