ELSEVIER

Journal of Chromatography A, 879 (2000) 121–128

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Micelle-mediated extraction for concentrating hydrophobic organic compounds

Tohru Saitoh[a,*], Toshiyuki Matsudo[b], Chiyo Matsubara[b]

[a]*Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan*

[b]*Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan*

Received 20 October 1999; received in revised form 21 February 2000; accepted 16 March 2000

Abstract

An extraction method based on polymer-induced phase separation of aqueous micellar solutions of octyl-β-D-thioglucoside (OTG) was assessed for concentrating hydrophobic analytes. Various hydrophobic compounds such as polycyclicaromatic hydrocarbons, alkylbenzenes, alkylphenols, chlorobenzenes, chlorophenols, phthalic esters, pesticides, and steroid hormones could be efficiently concentrated into a small volume of surfactant-rich phase, while hydrophilic matrix components remained in the bulk aqueous phase. The surfactant-rich phase containing concentrated OTG could be directly introduced into the hygro-organic mobile phase of high-performance liquid chromatography with ultra-violet photometric detection. The application of this method greatly enhanced the signal intensity in the chromatogram while reducing the interference of matrix components.

Keywords: Micelle-mediated extraction; Extraction methods; Octylthioglucoside; Polynuclear aromatic hydrocarbons; Benzenes; Phenols; Phthalic esters; Pesticides; Steroid hormones

1. Introduction

Work has been carried out on the detection of trace analytes such as polycyclicaromatic hydrocarbons, alkylbenzenes, alkylphenols, chlorobenzenes, chlorophenols, phthalic esters, pesticides, and steroid hormones present in environmental or clinical samples. The analytes cited here are hydrophobic and tend to bioaccumulate in the stores of animals and humans. Thus, their monitoring is important for clarifying environmental pollution, disease conditions, or metabolic state of the compounds. Methods for determining these compounds at trace levels in matrix components generally include solvent extraction for sample cleaning prior to analysis. However, solvent extraction usually uses considerable amounts of organic solvents which often influence human health and cause environmental pollution.

An extraction methodology, the so-called 'cloud-point extraction', based on the temperature-induced phase separation of aqueous micellar solutions of polyoxyethylene-type nonionic surfactants, may be a good alternative for concentrating hydrophobic analytes [1–8]. Many hydrophobic compounds are efficiently incorporated into the surfactant-rich phase separated from the aqueous surfactant solution with

*Corresponding author. Tel.: +81-52-789-3579; fax: +81-52-789-3241.

E-mail address: saitoh@numse.nagoya-u.ac.jp (T. Saitoh)

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00345-9

elimination of hydrophilic matrix components to the bulk aqueous phase. Because of the extremely small volume fraction of the surfactant-rich phase, the analytes can be highly concentrated, thus allowing great enhancement in the sensitivity of chromatographic analysis. However, the appearance of a large number of peaks because of the ultra-violet (UV) absorption of Triton X-114 or PONPE-7.5, which are mostly used for cloud-point extraction, limits the subsequent detection method [3–8]. Recent reports of the estrogen-like properties of alkylphenols, generated from these nonionic surfactants by the sewage treatment process [9,10], is also a reason for rejecting the temperature-induced phase separation method.

The use of alkylglucoside surfactants instead of polyoxyethylene-type surfactants may solve these problems. An aqueous solution of octyl-β-D-thioglucoside (OTG) can be separated into bulk aqueous and surfactant-rich phases by adding an appropriate water-soluble polymer such as polyethylene glycol (PEG) or dextran derivatives. Many hydrophobic compounds including hydrophobic metal chelates [11] and integral membrane proteins [12,13] were effectively incorporated into the OTG-rich phase. Since OTG has little UV absorption around 254 nm, the presence of the concentrated surfactant would not hinder UV detection of analytes. In this study, extraction based on polymer-induced phase separation of OTG was examined for potential use in concentrating hydrophobic organic compounds in environmental and biological samples prior to HPLC analysis.

2. Experimental

2.1. Chemicals

The surfactant, OTG, was obtained from Dojindo Lab. (Kumamoto, Japan) and used as a 20% (w/v) aqueous solution. Triton X-114 and its reduced form were from Aldrich (Milwaukee, WI, USA). Polyethylene glycol (PEG, average molecular mass 7500) was from Wako (Tokyo, Japan). Dextran T-500 and DEAE dextran were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Analytes and other chemicals used were from Tokyo Kasei (Tokyo, Japan). Water was prepared using a Milli-Q SP reagent water system (Millipore, Milford, MA, USA).

2.2. Extraction by polymer-induced phase separation

A 750 μL volume of aqueous solution containing analytes was placed in a 1.5 mL centrifuge tube followed by 100 μL of 20.0% (w/v) OTG solution and then 150 μL of 20.0% (w/v) water-soluble polymer aqueous solution. Typical concentrations of OTG and polymer were adjusted to 2.0% (w/v) and 3.0% (w/v), respectively. The solution was well mixed and centrifuged at 3500 rpm for 5 min. All procedures were performed at 4°C to minimize analyte degeneration.

2.3. HPLC analysis

A 20 μL aliquot of surfactant-rich phase was collected with a 1700 Hamilton gas-tight microsyringe having a PTFE seal and directly injected into the HPLC system consisting of a Jasco (Tokyo, Japan) unit with a Model LCSS system station, Model PU-980 pump, Model UV-970 UV–Vis detector, and a Model 807-IT integrator. A 250 mm×4.6 mm Inertsil ODS column (GL Science, Tokyo, Japan) connected to a 10 mm×4.0 mm guard column was used for analyte separation. Aqueous acetonitrile solutions were used as the mobile phase. UV absorbance at 254 nm (for aromatic hydrocarbons) or 280 nm (for some steroid hormones, phthalic esters, and bisphenol A) was monitored.

2.4. Determination of OTG

A 1 mL aliquot of mobile phase was fractionated into a glass tube. After the solvent was evaporated under nitrogen, OTG was determined by the anthrone method [14].

3. Results and discussion

3.1. Conditions for phase separation

Water-soluble polymers can induce phase sepa-

ration of aqueous micellar solutions of OTG and, accordingly, the phase separation is influenced by the particular polymer used and its concentration. Thus, the recovery of an analyte into the surfactant-rich phase would be influenced by the polymer concentration. As shown in Fig. 1, the recoveries of some steroid hormones were dependent on polymer concentration up to 3% (w/v). The results for the anthrone method indicate that considerable amounts of OTG micelles are still present in the bulk aqueous phase at low polymer concentration. Insufficient extraction of these compounds can be ascribed to their solubilization to the micelles remaining in the aqueous phase. At higher concentration, the extent of extraction was essentially unaffected. However, the solutions containing a highly concentrated water-soluble polymer become viscous, thus resulting in difficulty for rapid phase separation. The polymer concentration of 3% (w/v) was the most adequate.

3.2. Compatibility with the HPLC system

Fig. 2A illustrates the features of a chromatogram when the surfactant-rich phase of Triton X-114, the most widely used in cloud-point extraction, is injected into the aqueous acetonitrile mobile phase. The very large peaks in the chromatogram would hinder analyte peaks in UV detection mode. The use of the reduced form of Triton X-114, having a cyclohexane moiety instead of a benzene ring, was also insufficient for eliminating such peaks.

On the other hand, only a small solvent front peak, which rarely interferes with the detection of analytes, appeared (Fig. 2B). OTG possessing no aromatic moieties has a very weak ultra-violet absorption around 280 nm and would be the reason for this. As shown in Fig. 2B, the elution of OTG from the column was observed at the solvent front. Negligible interaction of the concentrated OTG with the stationary phase is essential for preventing reduction in the reproducibility in analyte retention due to covering by surfactant molecules on the ODS stationary phase. OTG thus appears superior for surfactant-mediated extraction prior to HPLC analysis.

3.3. Extraction of hydrophobic compounds

Extraction data were obtained for various com-

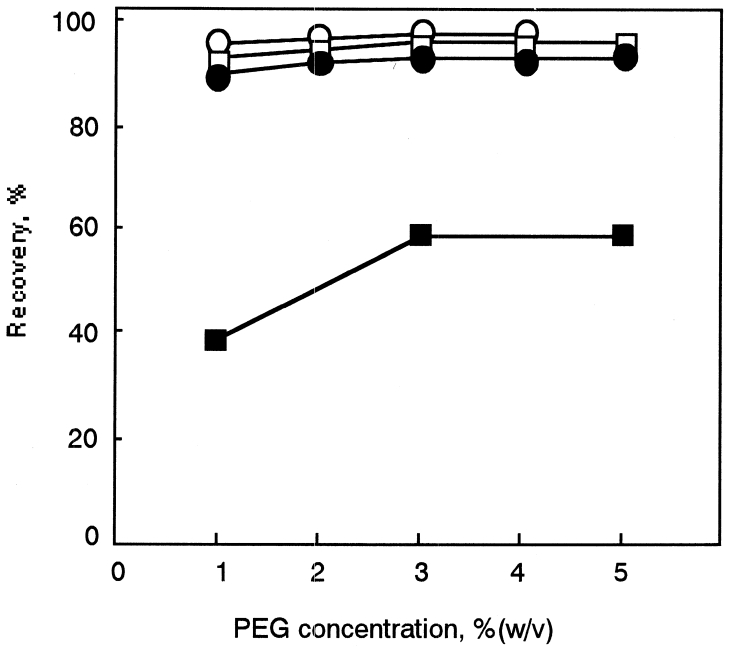

Fig. 1. Recovery of some steroid hormones as a function of PEG concentration. 2.00% (w/v) OTG; pH 7.4 (10 m*M* Tris–HCl); 4°C; compounds: (○) progesteron, (■) β-estradiol, (□) estron, (●) cortisone.

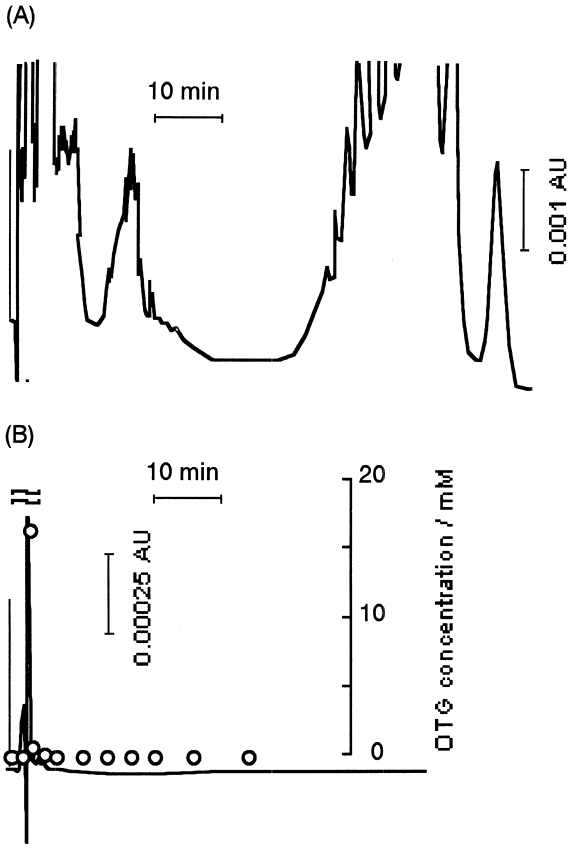

Fig. 2. Chromatograms obtained by the injection of 20 μL surfactant-rich phase. Mobile phase, 70% (v/v) aqueous acetonitrile; flow-rate, 1.00 mL/min; detection wavelength, 280 nm. (A) Triton X-114 surfactant-rich phase. (B) OTG surfactant-rich phase. (○) OTG concentration in the respective 0.5 mL fraction of the mobile phase.

pounds to confirm the applicability of the present method for concentrating analytes. Table 1 lists the recovery and concentration factor for polyaromatic hydrocarbons, alkylbenzenes, alkylphenols, chlorobenzenes, chlorophenols, phthalic esters, pesticides, and steroid hormones in the surfactant-rich phase when conducting extraction under the conditions specified in the Experimental section. Highly hydrophobic compounds such as polyaromatic hydrocarbons, phthalic esters, and pesticides were predominantly incorporated into the surfactant-rich phase. With the volume fraction of the surfactant-rich phase being quite small (0.038), compounds in the surfactant-rich phase were efficiently concentrated, although less hydrophobic compounds possessing a smaller hydrocarbon moiety were less extractable.

The extractability of compounds would thus appear to be dependent on the hydrophobic properties of the analyte. Indeed, the extracted fraction in the surfactant-rich phase of benzene or phenol derivatives apparently increases with degree of alkyl- or chloro-substitution. Fig. 3 shows the correlation of the logarithmic distribution coefficients for the aqueous and surfactant-rich phase, $\log K_{D(OTG)}$, with the logarithmic water–octanol distribution coefficients, $\log K_{O/W}$, used extensively to demonstrate compound hydrophobic properties. In Fig. 3, $\log K_{D(OTG)}$ appears to be closely correlated with $\log K_{O/W}$ for alkylbenzenes or steroid hormones, indicating that hydrophobic interactions are required for compound incorporation into a micellar assembly. Despite the difficulty in predicting the distribution coefficients of analytes due to the heterogeneous media of the surfactant-rich phase, an approximate correlation should prove useful for designing micellar-mediated extraction.

3.4. Application to water samples

Fig. 4 shows chromatograms obtained by the direct injection of commercially available distilled water for contact lens cleaning in a polyethylene bottle (A) and that obtained by the introduction of the sample after micellar-mediated extraction (B). In contrast to chromatogram (A), some peaks were observed in chromatogram (B). The peak indicated was identified to be di(2-ethylhexyl) phthalate (DEHP), a typical plasticizer. The result of the standard addition method indicated that the concentration of DEHP was 0.4 ppm.

Fig. 5 illustrates the results of application to the concentration of pesticides in a water sample which was prepared by spiking 1 ppb of *p,p′*-DDD and *p,p′*-DDE to ground water. Although no peaks appeared in the chromatogram without concentration of analytes (Fig. 5A), n*M* detection of *p,p′*-DDD and *p,p′*-DDE (a metabolite of *p,p′*-DDT) was achieved by micellar-mediated extraction (Fig. 5B). However, some peaks, possibly representing humic substances or their decomposed products, hindered detection of the analytes, which were eluted more rapidly.

Another successful application is the concentration

Table 1
Recoveries and concentration factors of compounds with the present extraction method[a]

Compound	Recovery (%)	Concentration factor	Log $K_{D(OTG)}$
Benzene	20.5±0.5	5.4	0.79
Toluene	37.9±0.6	10.0	1.17
Ethylbenzene	52.2±1.6	13.7	1.34
n-Butylbenzene	75.3±6.2	19.8	1.86
n-Hexylbenzene	81.5±3.0	21.5	2.02
n-Octylbenzene	89.2±4.9	23.5	2.30
n-Dodecylbenzene	97.0±2.1	25.5	2.89
Naphthalene	75.6±1.3	19.9	1.87
Biphenyl	83.3±2.5	21.9	2.08
Phenanthrene	87.6±1.8	23.1	2.23
Anthracene	97.2±0.7	25.6	2.92
Fluorene	92.0±3.0	24.2	2.44
Perylene	90.7±4.0	23.9	2.37
1,2-Dichlorobenzene	62.8±3.8	16.5	1.61
1,3-Dichlorobenzene	71.3±2.9	18.8	1.78
1,4-Dichlorobenzene	47.1±0.5	12.4	1.33
1,2,4-Trichlorobenzene	69.6±1.5	18.3	1.74
1,2,4,5-Tetrachlorobenzene	93.2±0.6	24.5	2.54
Dimethylphthalate	33.3±0.7	8.8	1.08
Di(*n*-butyl) phthalate	85.3±0.5	22.5	2.14
Di(2-ethylhexyl) phthalate	95.1±3.6	25.0	2.64
4-*tert*.-Butylphenol	84.9±1.9	22.3	2.13
4-*tert*.-Octylphenol	90.5±2.5	23.8	2.36
4-Nonylphenol	95.3±2.5	25.1	2.71
Bisphenol A	91.4±2.4	24.0	2.43
Methoxychlor	85.8±2.6	22.7	2.20
p,p′-DDE	81.9 ±3.3	21.6	2.06
p,p′-DDD	93.6±3.7	24.6	2.57
Cortisone	44.3±0.3	14.8	1.30
Hydroxycortisone	57.4±1.6	15.1	1.63
Corticosterone	83.4±0.5	22.0	2.10
Estriol	72.0±0.9	19.0	1.81
β-Estradiol	94.4±0.2	24.8	2.64
Estrone	92.5±0.3	24.3	2.49
4-Androstene-3,17-dion	83.7±0.2	22.0	2.11
17α-Hydroxyprogesterone	90.8±0.2	23.9	2.40
Progesterone	97.1±0.3	25.6	2.93

[a] Two percent (w/v) OTG; 3% (w/v) PEG; Temperature, 4°C. Concentration factor was calculated from the ratio of the peak area of a solute in the chromatogram with concentration to that without concentration.

of 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in a cell culture medium containing 30% (v/v) fetal bovine serum. When the sample was injected directly into the system, huge background peaks resulting from the components in serum seriously interfered with the detection of the analyte peak (Fig. 6A). Additionally, the considerable increase of the chromatographic baseline significantly hindered the subsequent chromatographic operation. In contrast, the extraction produced a clear peak of the desired analyte with enhanced peak intensity and reduced signal of the matrix components (Fig. 6B). Although a number of peaks of uncertified compounds, probably serum components, appeared, 0.2 ppm bisphenol A spiked in the medium sample was successfully observed.

It should be noted that micelle-mediated extraction has also been used to extract hydrophobic membrane

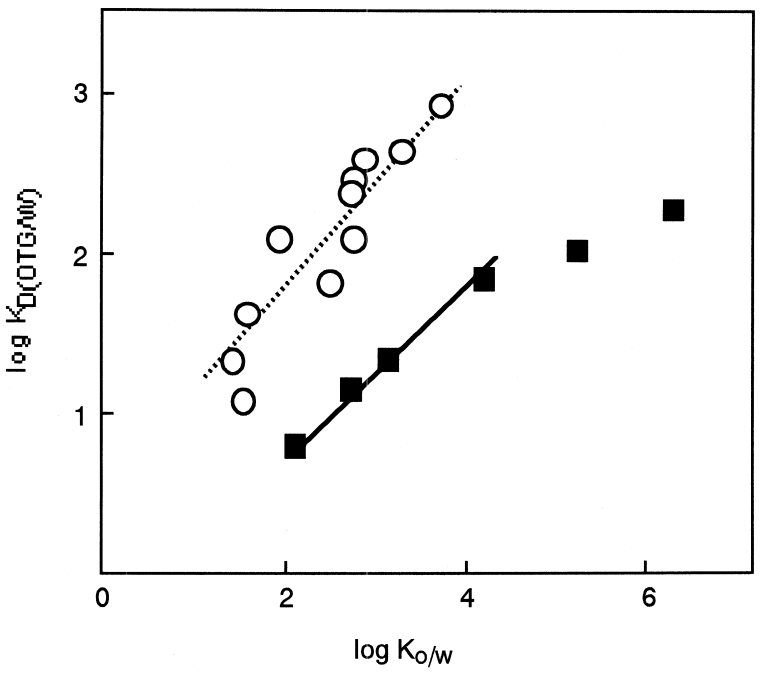

Fig. 3. Correlation of log $K_{D(OTG)}$ with log $K_{O/W}$. (○) Steroid hormone, (■) alkylbenzene.

proteins from other hydrophilic proteins [12,15,16]. Most protein components in serum are hydrophilic, and thus should be readily eliminated from the surfactant-rich phase. However, considerable amounts of adducts formed at the interface of separated phases, possibly as a result of the strong dehydrating effect of PEG to serum proteins, significantly hindered the sampling of the surfactant-rich phase. In contrast, a cationic polymer, diethylaminoethyl-dextran (DEAE-Dx), retained the serum proteins in the aqueous phase without protein precipitation. When 3% (w/v) DEAE-Dx was used for phase separation, >97% (determined by spectrophotometry with sodium bicinchoninate [17]) of protein constituents remained in the bulk aqueous phase. Such a stable solubilization of proteins in the aqueous phase may possibly be due to interaction of positively charged DEAE-Dx with negatively charged proteins.

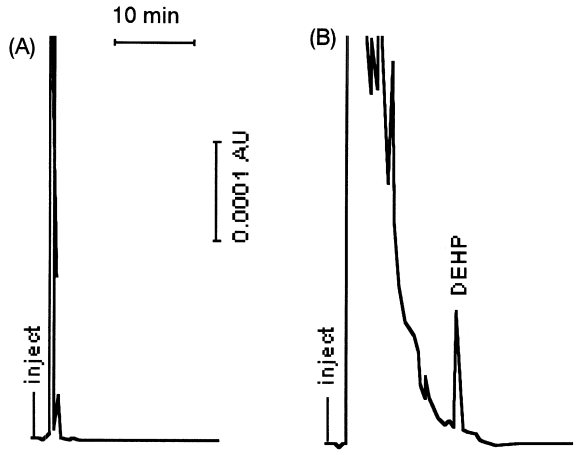

Fig. 4. Chromatograms of DEHP in distilled water. Mobile phase, 95% (v/v) acetonitrile; flow-rate, 1.00 mL/min; detection wavelength, 280 nm. (A) Before extraction, (B) after extraction.

The present method may be comparable to the extraction method based on the phase separation of aqueous micellar solutions of zwitterionic surfactants including nonyldimethylammoniopropanesulfate (C9-$APSO_4$) and decyldimethylammoniopropanesulfate (C10-$APSO_4$) [18]. Owing to the slight absorbance around 280 nm, these surfactants do not give rise to

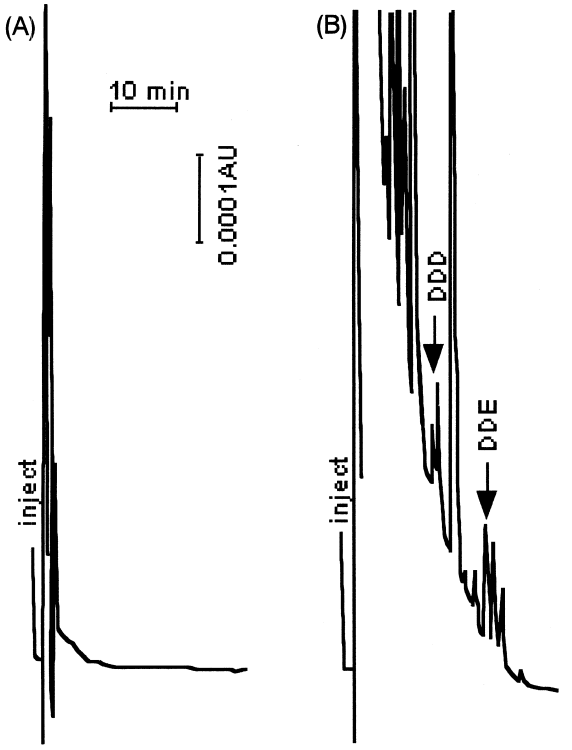

Fig. 5. Chromatograms of pesticides in ground water. Mobile phase, 70% (v/v) acetonitrile; flow-rate, 1.00 mL/min; detection wavelength, 280 nm. (A) before extraction, (B) after extraction.

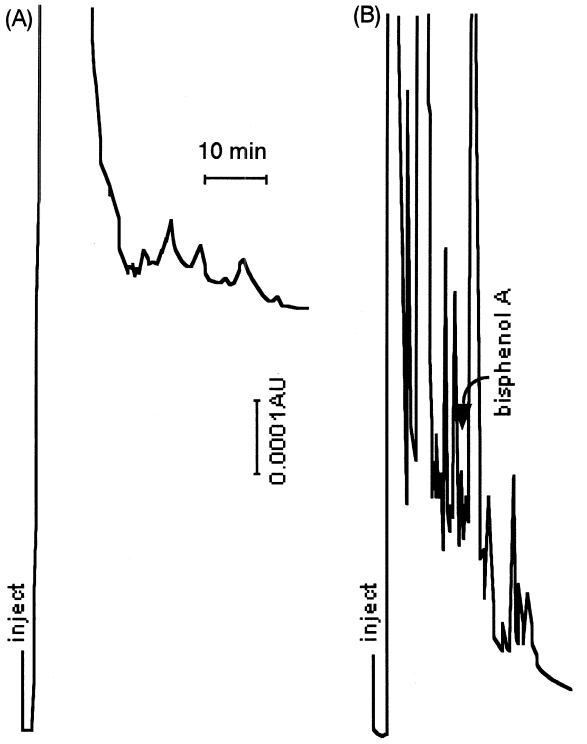

Fig. 6. Chromatograms of bisphenol A in a cell culture medium containing 30% (v/v) bovin serum. Mobile phase, 40% (v/v) acetonitrile; flow-rate, 1.00 mL/min; detection wavelength, 280 nm. (A) before extraction, (B) after extraction.

peaks in the chromatograms in the case of UV photometric detection. However, probably due to the remarkable interaction of zwitterionic surfactant molecules with proteins, the surfactant-rich phase incorporated about 30% of proteins. Thus, the use of OTG was superior for clean-up of samples containing protein constituents.

In conclusion, micelle-mediated extraction with OTG is a viable and attractive method for extracting various organic compounds in aqueous solution prior to HPLC analysis. Compared with solid-phase extraction, the present method does not require troublesome procedures for eluting analytes from an adsorbent, because the extraction phase can be directly introduced into the analytical instrument. This is particularly important for easy and rapid sample treatment. Many of the general trends described in this work for the extraction parameters and extractability as a function of the variation of the different experimental conditions and properties of the compounds should also prove to be valid for wide application of this method.

References

[1] W.L. Hinze, E. Pramauro, CRC Crit. Rev. Anal. Chem. 24 (1993) 133.
[2] H. Watanabe, H. Tanaka, Talanta 25 (1978) 585.
[3] W.J. Horvath, C.W. Huie, Talanta 39 (1992) 487.
[4] P.A. Alfred, A. Koslowski, J.M. Harris, F. Tjerneld, J. Chromatogr. 659 (1994) 289.
[5] B.M. Cordero, J.L. Pavon, C.G. Pinto, M.E.F. Laespada, Talanta 40 (1993) 1703.
[6] A. Bockenten, R. Niessner, Fresenius J. Anal. Chem. 346 (1993) 435.
[7] C.G. Pinto, J.L.P. Pavon, B.M. Cordero, Anal. Chem. 64 (1992) 2334.
[8] R.S. Sarath, R.B. John, G.P. Donald Jr., M. Li, Anal. Chem. 68 (1996) 1556.

[9] A.M. Soto, H. Justicia, J.W. Wray, C. Sonnenschein, Environ. Health Perspect. 92 (1991) 167.
[10] J.J. Lech, S.K. Lewis, L. Ren, Fundam. Appl. Toxicol. 30 (1996) 229.
[11] T. Saitoh, M. Ohba, T. Kamidate, H. Watanabe, Anal. Sci. 10 (1994) 379.
[12] T. Saitoh, H. Tani, T. Kamidate, T. Kamataki, H. Watanabe, Anal. Sci. 10 (1994) 299.
[13] H. Tani, T. Saitoh, T. Kamidate, T. Kamataki, H. Watanabe, Biotechnol. Bioeng. 56 (1997) 311.
[14] J.H. Roe, J. Biol. Chem. 208 (1954) 889.
[15] T. Saitoh, H. Tani, T. Kamidate, H. Watanabe, Trends Anal. Chem. 14 (1995) 213.
[16] H. Tani, T. Kamidate, H. Watanabe, J. Chromatogr. A 780 (1997) 229.
[17] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Anal. Biochem. 150 (1985) 76.
[18] T. Saitoh, W.L. Hinze, Anal. Chem. 63 (1991) 2520.