http://dx.doi.org/10.5935/0103-5053.20130132

J. Braz. Chem. Soc., Vol. 24, No. 6, 1027-1033, 2013.
Printed in Brazil - ©2013 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Separation and Preconcentration of Dioxin in Blood Samples by Nano-baskets of Calixarene and Inclusion Emulsion Membranes

Bahram Mokhtari and Kobra Pourabdollah*

Department of Chemical Engineering, Shahreza Branch, Islamic Azad University, Shahreza, Iran

Inclusão-separação e pre-concentração de dioxina em amostras de sangue são descritas pelo processo de emulsão de nano membrana líquida de inclusão-facilitada. A novidade deste estudo está na aplicação de nano-bastões de calixareno assim como membranas líquidas de emulsão na pre-concentração seletiva e eficiente da dioxina. Para este fim, parâmetros de extração-inclusão de quatro derivados de *p-tert*-calix[4]areno com sulfonamidas sintetizados previamente foram investigados. As análises por cromatografia gasosa revelaram que sob condições otimizadas de operação, a pre-concentração da dioxina foi melhorada e o método atingiu menores limites de detecção.

Inclusion-separation and preconcentration of dioxin from blood samples were reported by the inclusion-facilitated emulsion liquid membrane process. The novelty of this study is the application of nano-baskets of calixarene as well as emulsion liquid membranes in the selective and efficient preconcentration of dioxin. For this aim, inclusion-extraction parameters of four *p-tert*-calix[4]arene derivatives sulfonamide moieties previously synthesized were investigated. Analysis by a gas chromatograph revealed that under the optimized operating condition, the preconcentration of dioxin was improved and the method achieved lower limit of detections.

Keywords: nano-basket, dioxin, inclusion, calixarene, emulsion liquid membrane

Introduction

Prior to industrialization, low concentrations of dioxins were formed in nature owing to geological processes and natural combustion. Today, concentrations of dioxins are found in all humans, with higher levels commonly found in persons living in more industrialized areas. The most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), became well known as a contaminant of agent orange, a herbicide used in the Vietnam War.[1]

The United States Environmental Protection Agency (US EPA) inventory of sources of dioxin-like compounds is the most comprehensive review of the sources and releases of dioxins.[2-8] Dioxins are produced in small concentrations when organic material is burned in the presence of chlorine, whether the chlorine is present as chloride ions or as organochlorine compounds, so they are widely produced in many contexts. The general population takes up dioxins almost exclusively from ingestion of food, specifically through the consumption of fish, meat and dairy products. This happens since dioxins are fat-soluble and readily climb the food chain.[9,10] Dioxins are also present in typical cigarette smoke.[11] The estimated elimination half-life for highly chlorinated dioxins (4-8 chlorine atoms) in humans ranges from 5 to 13 years.[12] The accredited methods for sample collection, clean up and analysis of dioxins are presented in Table 1.

Emulsion liquid membrane (ELM) was invented by Li[16] in 1968 and is known as one of the most promising separation methods for trace extraction of metal contaminants[17-19] and hydrocarbons[20,21] owing to the high mass transfer rate, high selectivity, low solvent inventory and low equipment cost. Frankenfeld *et al.*[22] reported that ELM could be up to 40% cheaper than that of other solvent extraction methods. This process combines both extraction and stripping stages to perform a simultaneous purification and concentration.

In this study, calixarene nano-baskets were used as bi-functional surfactant/carrier in the ELM process. By the method of one-at-a-time, the ELM process for selective extraction of dioxins was optimized. The process factors such as calixarene type and concentration (as surfactant and

*e-mail: pourabdollah@iaush.ac.ir

Table 1. Accredited methods for sample collection, clean up and analysis of dioxins

Source	Method number	Method description
USA[13]	EPA 1613B: 1994	high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS)
	EPA 1668A: 2003	HRGC/HRMS
	EPA 8290A: 2007	HRGC/HRMS
Europe[14]	EN 1948-1: 2006	stationary source emissions; sampling of polychlorinated dibenzodioxins (PCDDS)
	EN 1948-2: 2006	stationary source emissions; extraction and clean-up
	EN 1948-3: 2006	stationary source emissions; identification and quantitation, sample collection and clean up.
Japan[15]	JSA JIS K 0311: 2005	method for determination of dioxins in stationary source emissions
	JSA JIS K 0312: 2005	method for determination of dioxins in industrial water and waste water limits

carrier), phase ratio (the volume of stripping solution to the volume of membrane) and treat ratio (the volume of emulsion phase to the volume of feed phase), mixing speed and solute concentration in feed phase were investigated and optimized.

Experimental

Chemicals and reagents

The liquid membrane consists of a diluent (as membrane matrix) and a calixarene (as surfactant and carrier, simultaneously). The calixarenes were synthesized as described below. Carbon tetrachloride (CCl_4) for high-performance liquid chromatography (HPLC) was gifted from Biosolve B. V. (Valkenswaard, The Netherlands). Doubly distilled water (DDW) with a specific resistivity of 18 MΩ cm, from a Milli-Q water purification system (Millipore, Bedford, MA), was used. Chlorobenzene (C_6H_5Cl), tetrachloroethylene (C_2Cl_4) and *n*-decane ($C_{10}H_{22}$) were purchased from Fluka and Sigma-Aldrich, respectively. Anhydrous sodium sulfate ($(NH_4)_2CO_3$), acetone and hexane were purchased from Merck (Darmstadt, Germany).

The experiments were carried out using four derivatives of *p-tert*-calix[4]arene: di-[*N*-(phenyl)sulfonyl carboxamide], *p-tert*-calix[4]arene di-[*N*-(*para*-hydroxy phenyl)sulfonyl carboxamide], *p-tert*-calix[4]arene di-[*N*-(*para*-nitro phenyl)sulfonyl carboxamide] and *p-tert*-calix[4]arene di-[*N*-(*para*-methyl phenyl)sulfonyl carboxamide]. The synthesis procedures were previously published.[23] The chemical structure of calixarene scaffolds **01**-**04** used in the experiments are presented in Figure 1.

Preparation and characterization of ELMs

The specific amounts (1, 3, 4, 5 and 10 wt.%) of calixarenes (**01**, **02**, **03** and **04**) were dissolved in 25 mL of each diluent ($C_{10}H_{22}$, C_6H_5Cl, C_2Cl_4 and CCl_4) and thus membrane solutions were prepared. $(NH_4)_2CO_3$ solution (25 mL, 0.5 mol L^{-1}) was used as stripping solution. In 100 mL beaker, stripping solution was added dropwise to the stirred membrane solution and the two-phase system was stirred continuously for 30 min at mixing speed of 1500 rpm by a variable speed mixer equipped with a turbine-type Teflon impeller. The mixture of the membrane and the stripping solution were emulsified.

The method was evaluated by characterizing size, size distribution and stability of the emulsions. Size and size distribution of water-in-oil (w/o) droplets were obtained by optical microscopy (Mettler FP). The digital format of captured micrographs was analyzed by means of an image

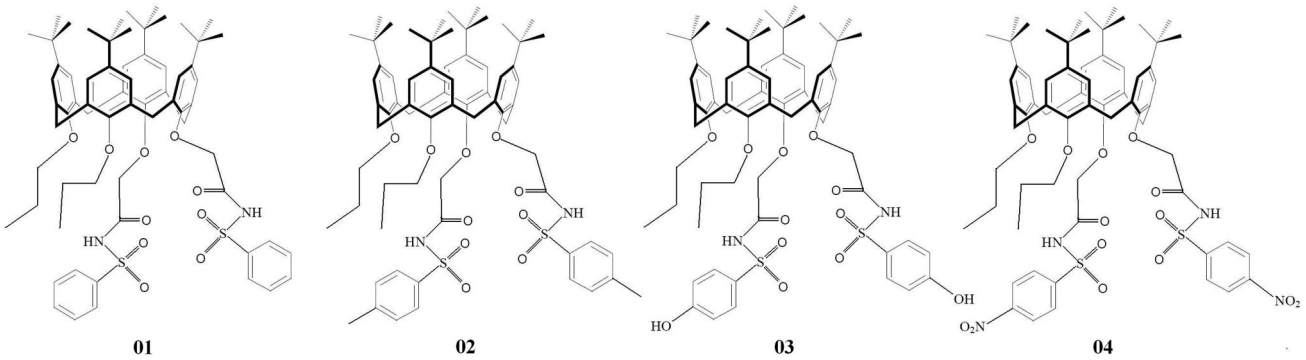

Figure 1. Chemical structures of derivatives **01**-**04**.

analyzer software (Digital Micrograph TM, Gatan Inc.). Using a Neubauer camera, the volume of analyzed samples was controlled. By size distribution changes at constant times, the stability of w/o droplets was monitored and evaluated by image analyses from photographs obtained during the diafiltration experiments.

Sample preparation and ELM experiments

Plasma samples were accurately weighed to 5 g and mixed with 4 g Isolute (International Sorbent Technology Ltd., Hengoed, Mid Glamorgan, UK). Then, they were extracted with acetone:hexane (1:4, v v^{-1}, 150 °C) for 10 min. The extracts were concentrated to dryness treated with anhydrous sodium sulfate, and the lipid contents were gravimetrically determined. The lipids were emulsified in DDW and were used as the feed solution.

In 500 mL beaker, the prepared ELM was added to some volumes of feed solution, while a variable speed mixer equipped with a turbine-type impeller stirred them at 500 rpm during 30 min. The speed of the mixer was regulated by a voltage regulator. To determine the important variables governing the permeation and separation of dioxin, calixarene type and concentration, the phase and the treat ratios, membrane diluent type, mixing speed and initial solute concentration in the feed phase were varied to observe their effects on the separation. The samples were taken from the stirred cell periodically during the course of the run. The feed phase of the samples was separated from the emulsions by filtration using a filter paper. The emulsion was demulsified by freezing. The concentration of dioxin was analyzed using gas chromatography.

Analytical instruments

Hewlett Packard (HP) model 5890 gas chromatograph equipped with a flame ionization detector (FID) was used. In order to confirm peak identities, a HP model 6890 gas chromatograph, equipped with a mass selective detector, was used. A cross-linked polyethylene glycol column (HP-INNOWax) with 30 m length, 1.0 μm film thickness and 0.53 mm i.d. was used. Based upon the best results of error and trials, the oven temperature was programmed as four distinct methods. Figure 2 shows the gas chromatograms of different trials. Figure 2a corresponds to the above-mentioned conditions except that the oven temperature program was: 50 °C (0 min), raised at 20 °C min^{-1} to 280 °C (10 min), reduced at 50 °C min^{-1} to 180 °C (1 min) and raised again at 5 °C min^{-1} to 2800 °C (5 min). Figure 2b corresponds to the above-mentioned conditions except that the oven temperature program was: 50 °C (0 min), raised at 20 °C min^{-1} to 250 °C (5 min), reduced at 50 °C min^{-1} to

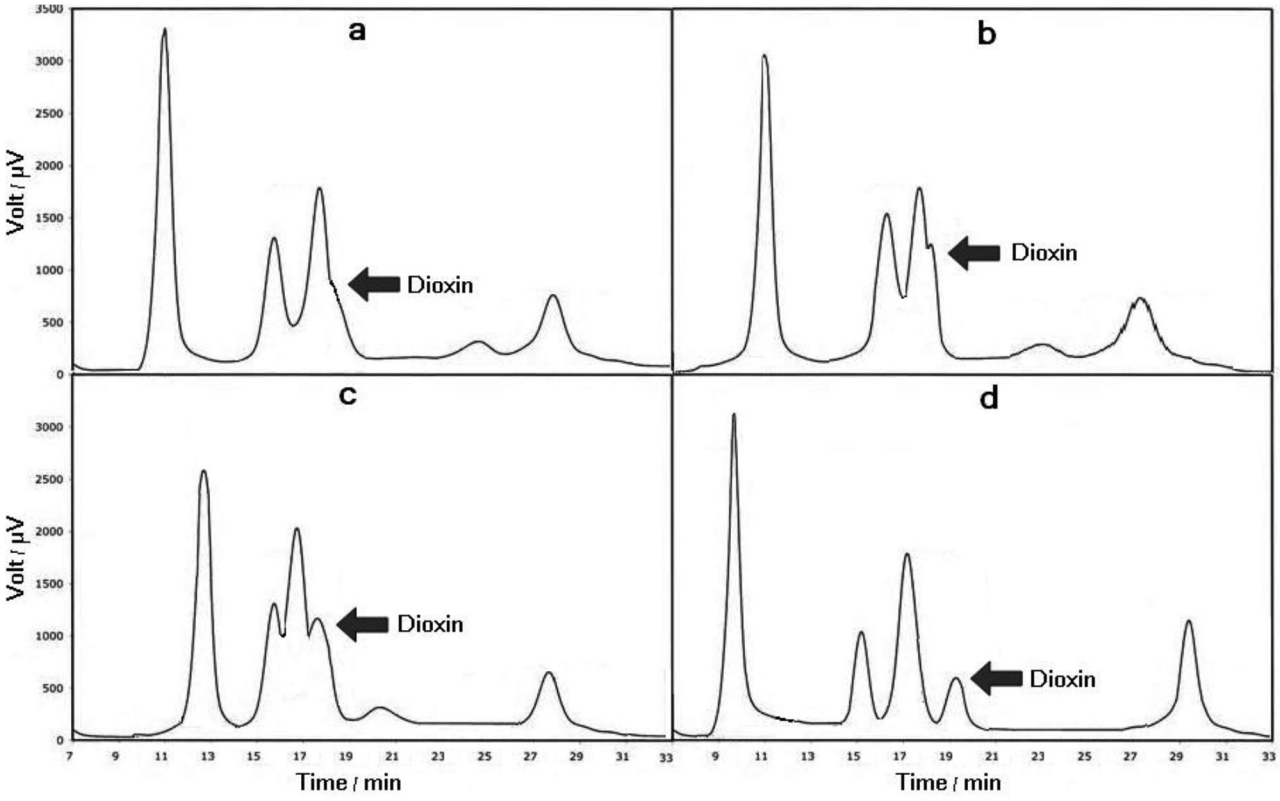

Figure 2. Gas chromatograms of different trials **a**-**d**.

150 °C (1 min) and raised again at 5 °C min-1 to 250 °C (5 min). Figure 2c corresponds to the above-mentioned conditions except that the oven temperature program was: 50 °C (0 min), raised at 10 °C min^{-1} to 200 °C (5 min), reduced at 50 °C min^{-1} to 200 °C (1 min) and raised again at 10 °C min^{-1} to 250 °C (5 min). Figure 2d (the best conditions) corresponds to the above-mentioned conditions except that the oven temperature program was: 80 °C (0 min), raised at 20 °C min^{-1} to 320 °C (5 min), reduced at 70 °C min^{-1} to 180 °C (1 min) and raised again at 5 °C min^{-1} to 320 °C (5 min). This thermal program was used for further experiments.

The temperatures of injector and FID detector were set to 290 and 300 °C, respectively. The splitless operating mode, with purge valve open after 1 min of operation, was used, while the column head-pressure, the linear velocity and flow rate of gas were 2.0 psi, 20 cm s^{-1} and 3.0 mL min^{-1}, respectively.

Results and Discussion

In several studies, it was shown that calixarenes are appropriate carriers for extraction of chlorinated aromatics in the organic phase. At the basic internal interface of the membrane phase, dioxin was stripped by the internal agent and transformed into a new species that could not reversibly penetrate the membrane. The reversible reactions at both interfaces of the membrane phase with non-ionizable calixarenes as surfactant/carrier in an ELM system are depicted in equation 1.

$$Dioxin_n + Calix \longleftrightarrow Dioxin : Calix \quad (1)$$

where Calix means the calixarene scaffold in the molecular form, and Dioxin:Calix represents the calixarene complex with dioxin.

In the acidic solutions, di-ionizable calixarenes are present in their molecular state. On the other hand, they are hydrolyzed in alkaline solutions. The ionic form includes the cationic species, while the molecular form cannot capture them. After that, the new uncharged complex diffuses throughout the organic membrane. To the natural or acidic feed phase side, the calixarene complex dissociates as an uncharged calixarene molecule, diffuses into the organic membrane again. This transportation is repeated during the extraction until the chemical potentials on both sides are equal. Figure 3 depicts the mechanism of facilitated transport of dioxin in ELM process.

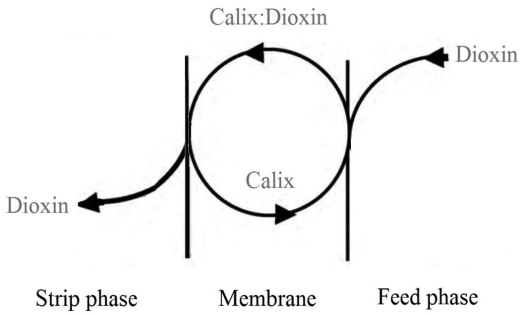

Figure 3. Facilitated transport mechanism of dioxin in ELM.

The blood matrix was used as feed-phase, behind the emulsion membrane. Selective transportation of dioxin, caused by calixarene carriers, prevents the diffusion of matrix ingredients from feed-phase to strip-phase. On the other hand, the emulsion membranes allowed the dioxin to be transferred from the blood matrix, outer side, to the strip-phase, inner side. The process concentrated the dioxin in the strip-phase. This kind of dioxin pre-concentration enhances the figures of merit (such as limit of detection) in analytical measurements.

The optimum conditions for the extraction of dioxin were determined by the method of one-at-a-time. Table 2 presents all conditions tested as well as the optimum conditions in bold type. The optimization methodologies are discussed below.

Effect of calixarene type

Type of calixarene is the most important factor that influences the selectivity of an inclusion-ELM system, and

Table 2. Experimental and optimum conditions for the extraction of dioxin

Calixarene type	01	02	**03**	04	–
Calixarene concentration / wt.%	1	3	**4**	5	10
Phase ratio	0.4	0.6	**0.8**	1.0	1.2
Treat ratio	0.1	0.2	**0.3**	0.4	–
Membrane type	**$C_{10}H_{22}$**	C_6H_5Cl	C_2Cl_4	CCl_4	–
Stirring rate / rpm	100	200	**300**	400	500
Solute concentration in feed / (pg g^{-1})	0.1	**1.0**	10	–	–

The boldface items were obtained and used as the optimum conditions.

can often be used in related liquid-liquid extractions. The effect of calixarene type on the extraction efficiency of dioxin was studied in the ELM process and the results are in Figure 4. According to the results, although calixarene **03** gives a higher rate of extraction in the first 10 min compared to calixarenes **01**, **02** and **04**, it gradually deteriorates with time. These results indicate that derivative **03** was more favorable than derivatives **01**, **02** and **04** as emulsifier/carrier. Therefore, derivative **03** was selected among other scaffolds.

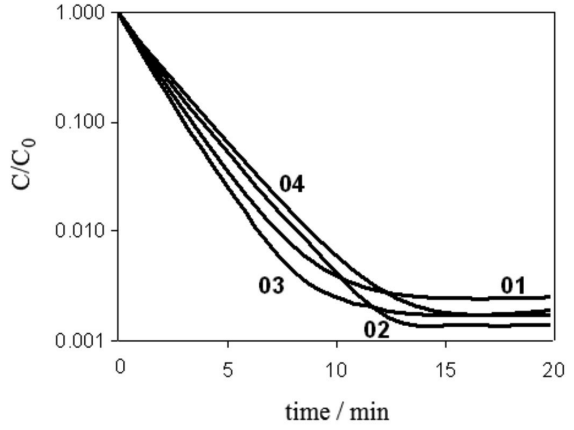

Figure 4. Effect of calixarene type on the extraction efficiency of dioxin in the ELM process.

Effect of calixarene concentration

Extraction of dioxin was enhanced by increasing the calixarene concentration from 1-5%. However, further increase of 5-10% hardly affected the extraction performance. According to Figure 5, further increase of calixarene concentration decreases the efficiency of the extraction. Increasing the calixarene concentration up to 5% increases the stability of ELMs. This leads to decrease of the break-up rate. Therefore, the extraction performance of solutes is increased. Further increase of calixarene concentration leads to decrease of the capturing rate and stripping reaction because the dioxin remains in the complex form (in the membrane). This affects the final recovery of the ELM process.

Excess of calixarenes tends to increase the interface resistance and the viscosity of membrane. Thus increasing from 5% enhances the emulsion stability, however, decreases the mass transfer. Hence, there is an optimum in the concentration of calixarenes around 4%. The excess of calixarene concentration leads to osmotic swelling and membrane breakdown. Hence, the concentration of 4% was accepted as optimum concentration. Another criterion is the financial impact of calixarenes on the ELM process cost as they are the most expensive, thus lower concentrations are preferred.

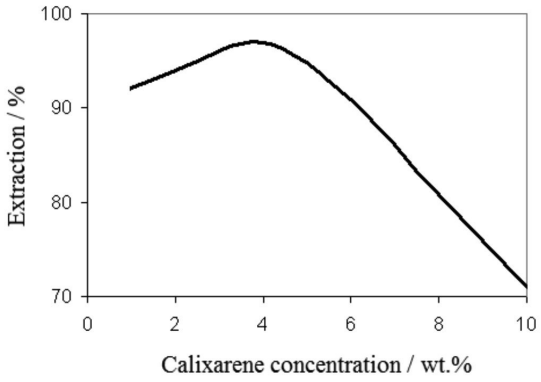

Figure 5. Effect of calixarene concentration on the extraction efficiency of dioxin in the ELM process.

Effect of phase ratio (strip phase volume/membrane volume)

The phase ratio is defined as the volume of stripping solution to the volume of membrane. Figure 6 shows the effect of phase ratio on the extraction of dioxin, in which it increases with the raise of phase ratio up to 4:5. At this level, the maximum extractions yields were observed. By increasing the strip phase volume, the film thickness of the emulsion was reduced owing to the strip phase dispersion in the membrane by mixing. This favored extractions resulting in the dioxin extraction increase. Increasing the strip phase volume beyond 4:5 caused the instability of the globules.

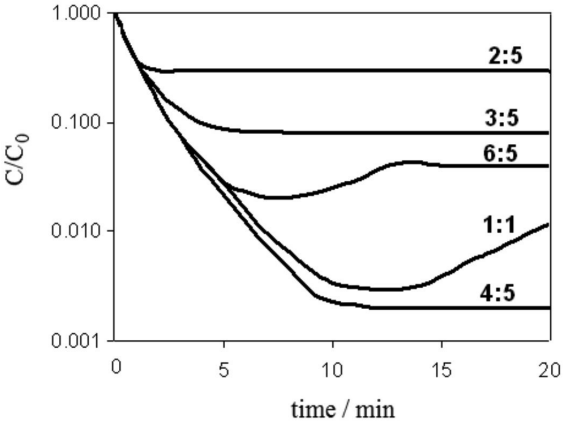

Figure 6. Effect of phase ratio on the extraction efficiency of dioxin in the ELM process.

Effect of treat ratio (feed volume/emulsion volume)

The treatment ratio, defined as the volume ratio of the emulsion phase to the feed phase, plays an important role in the determination of the ELM process efficiency. By increasing the amount of emulsion in the feed phase, the number of available droplets and interfacial surface area *per* volume unit of the feed solution increase. This leads to mass transfer increase of the solutes from the feed to the

membrane. Increasing the treat ratio slightly increased the emulsion droplet size and reduced the interfacial surface area. The increment in the size of droplets was counter-balanced by their amount reduction. The results are depicted in Figure 7, in which the extraction efficiency was improved by increasing the treat ratio from 0.1 to 0.3. Beyond 0.3, the further increase in the ratio caused the instability of globules and less extraction efficiency.

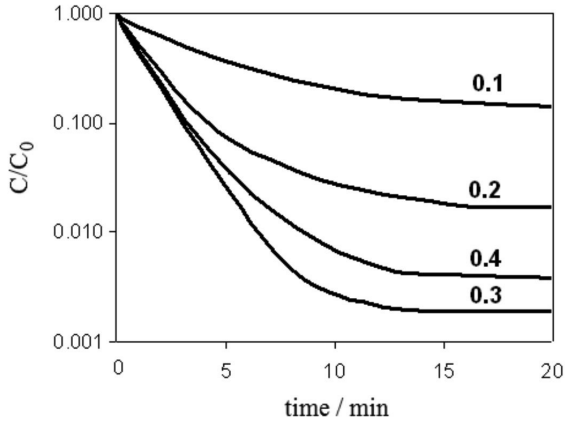

Figure 7. Effect of treat ratio on the extraction efficiency of dioxin in the ELM process.

Effect of membrane type

One of the most crucial tasks in all types of ELM processes is the choice of the membrane phase. The interactions of membrane toward the carrier as well as its viscosity are two main parameters that are controlled by choosing the membrane type. The membrane phase viscosity determines the transport rate of carrier or solutes and the residence or contact time of the emulsion with the feed phase. It is important to note that residence time is system specific and varies for each organic phase under the given conditions. In this work, the effect of four organic phases on the extraction performance was investigated. Carbon tetrachloride, chlorobenzene, tetrachloroethylene and *n*-decane were the choices. The results are presented in Figure 8. According to the results, *n*-decane was selected as the best diluent in the following experiments.

Comparison with other methods

Owing to evaluate this novel approach, its advantages and disadvantages were compared with other methods for dioxin analysis. For this aim, the present method was compared with some accredited methods (for sample collection, clean up and analysis of dioxins), which are presented in Table 1 including EPA 1613B, 1668A and

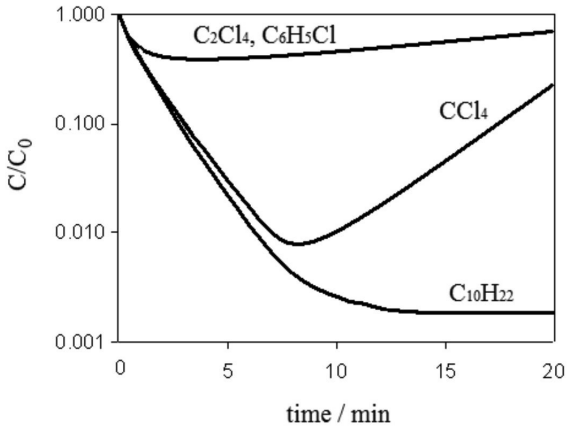

Figure 8. Effect of diluent (membrane) type on the extraction efficiency of dioxin in the ELM process.

8290A methods;[13] European Standards EN 1948 method;[14] Japanese Standards Association (JSA) Japanese Industrial Standards (JIS) K 0311 and K 0312 methods.[15]

The limits of detection (LOD) for TCDD using EPA 1613B, 8290A and 1668A methods were determined as 4.4 pg L^{-1}, 6 ng L^{-1} and 50 μg L^{-1}, respectively; while the LOD for the present method was determined to be 1.0 pg L^{-1}. Concerning the contamination and interferences, the natural lipid content of blood matrix can interfere in the analysis of TCDD. The lipid contents of different species and portions of tissue can vary widely. Lipids are soluble to varying degrees in various organic solvents and may be present in sufficient quantity to overwhelm the column chromatographic clean up procedures used for clean up of sample extracts. Hence, lipids must be removed by the lipid removal procedures. While the present method does not need such purifications since they remain in the membrane without coming to strip phase. Another issue that should be explained is the filtration of particles. EPA Methods 1613B is based upon such filtration processes, while it is not necessary in the present approach since the particles do not affect the extraction performance nor the emulsion stability.

In comparison to EN 1948 method (filter/cooler method), the present method showed similar advantages including lower limit of detection, no contaminants and avoiding filtration. Moreover, difficult conditions of high temperature as well as complicated apparatuses were eliminated in the present approach. Eventually, most common researches have reported the limit of detections in the order of 10 pg L^{-1}.[24]

Conclusion

Dioxin in blood plasma was recovered by an ELM process using calixarene nano-baskets. Hence, derivatives

of *p-tert*-calix[4]arene bearing different sulfonamide moieties (as both the extractant and the demulsifier) were investigated in ELM to extract and concentrate dioxin from the feed solutions. From this work, the following conclusions can be drawn.

The optimum conditions of the inclusion ELM process were determined experimentally and tabulated in Table 2. The best stirring speed was determined to be 300 rpm and the increase from 300 to 500 rpm resulted in deterioration of emulsion stability and the efficiency of inclusion-extractions. The optimum conditions of both the phase and the treat ratios were determined to be 0.8 and 0.3, respectively. At the optimum conditions, the extraction of dioxin was achieved with an efficiency of about 98.0-99.0% from the basic solution (ammonium carbonate, 0.4 mol L^{-1}) within 10-20 min.

Acknowledgements

This work was supported by the Islamic Azad University (Shahreza Branch) and the Iran Nanotechnology Initiative Council.

References

1. Schecter, A.; Birnbaum, L.; Ryan, J. J.; Constable, J. D.; *Environ. Res*. **2006**, *101*, 419.
2. U.S. EPA. *An Inventory of Sources and Environmental Releases of Dioxin-Like Compounds in the U.S. for the Years 1987, 1995, and 2000*. U.S. Environmental Protection Agency, Washington, DC, EPA/600/P-03/002F, 2006.
3. U.S. EPA. *An Exploratory Study: Assessment of Modeled Dioxin Exposure in Ceramic Art Studios*. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-06/044F, 2008.
4. U.S. EPA. *Pilot Survey of Levels of Polychlorinated Dibenzo-P-Dioxins (PCDDs), Polychlorinated Dibenzofurans (PCDFs), Polychlorinated Biphenyls (PCB) and Mercury in Rural Soils of the U.S*. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-05/043F, 2007.
5. U.S. EPA. *Health Effects Assessment for 2,3,7,8-Tetrachlorodibenzo-P-Dioxin*. U.S. Environmental Protection Agency, Washington, D.C., EPA/540/1-86/044 (NTIS PB86134558), 1984.
6. U.S. EPA. *Health Assessment Document for Polychlorinated Dibenzo-P-Dioxins*. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/8-84/014F (NTIS PB86122546), 1985.
7. Mukerjee, D.; Ris, C.; Schaum. J.; *Health Risk Assessment Approach for 2,3,7,8-Tetrachlorodibenzo-P-Dioxin*. U.S. Environmental Protection Agency, Washington, DC, EPA/600/8-85/013 (NTIS PB86-122546/AS), 1985.
8. U.S. EPA. *EPA Regulations Related to Dioxin*. U.S. Environmental Protection Agency, Washington, DC, 2003.
9. Schecter, A.; Cramer, P.; Boggess, K.; *J. Toxicol. Environ. Health, Part A* **2001**, *63*, 1.
10. Liem, A. K.; Furst, P.; Rappe, C.; *Food Addit. Contam*. **2000**, *17*, 241.
11. Ball, M.; Paepke, O.; Lis, A.; *Beitr. Tabakforsch. Int*. **1990**, *14*, 393.
12. Milbrath, M. O.; Wenger, Y.; Chang, C.; Emond, C.; Garabrant, D.; Gillespie, B. W.; Jolliet, O.; *Environ. Health Perspect.* **2009**, *117*, 417.
13. http://www.caslab.com/EPA-Methods/PDF/1613.pdf accessed in April, 2012.
14. http://www.caslab.com/EPA-Methods/PDF/1668a.pdf accessed in April, 2012.
15. http://www.caslab.com/EPA-Methods/PDF/EPA-Method-8290A.pdf accessed in April, 2012.
16. Li, N. N.; *US pat. 3410794* **1968**.
17. Kumbasar, R. A.; Sahin, I.; *J. Membr. Sci*. **2008**, *164*, 712.
18. Chakraborty, M.; Bhattacharya, C.; Datta, S.; *Colloids Surf., A* **2003**, *224*, 65.
19. San Román, O. M. F.; Corvalán, S. M.; Eliceche, A. M.; *Ind. Eng. Chem. Res*. **2003**, *42*, 5891.
20. Correia, P. F.; de Carvalho, J. M. R.; *J. Membr. Sci*. **2003**, *225*, 41.
21. Park, Y.; Skelland, A. H. P.; Forney, L. J.; Kim, J. H.; *Water Res*. **2006**, *40*, 1763.
22. Frankenfeld, J. W.; Chan, R. P.; Li, N. N.; *Sep. Sci. Technol.* **1981**, *16*, 385.
23. Mokhtari, B.; Pourabdollah, K.; *J. Coord. Chem.* **2011**, *64*, 4029.
24. Schecter, A.; Ryan, J. J.; Päpke, O.; *Chemosphere* **1994**, *29*, 2361.

Submitted: April 18, 2012
Published online: June 4, 2013