Short communication

Development of negligible depletion hollow fiber membrane-protected liquid-phase microextraction for simultaneous determination of partitioning coefficients and acid dissociation constants

Jing-fu Liu^{a,*}, Xiao-qiang Cai^{a,b}, Zi-fu Li^b, Gui-bin Jiang^a^a State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China^b Civil and Environment Engineering School, University of Science and Technology Beijing, Beijing 100083, China

ARTICLE INFO

Article history:

Received 8 December 2008

Received in revised form 21 January 2009

Accepted 21 January 2009

Available online 31 January 2009

Keywords:

Simultaneous determination

Partitioning coefficient

Acid dissociation constant

Hollow fiber membrane

Negligible depletion liquid-phase microextraction

ABSTRACT

A new method based on negligible depletion hollow fiber-protected liquid-phase microextraction coupled with high-performance liquid chromatography (HPLC) was developed for the simultaneous determination of partitioning coefficients (K_{OW}) and acid dissociation constants (pK_a), by using phenol, 4-chlorophenol and 2,4-dichlorophenol as model compounds. A 37-mm length polypropylene hollow fiber membranes (600 μ m inner diameter, 200 μ m wall-thickness, 0.2 μ m pore size, $\sim$ 70% porosity) with two-end sealed were filled with 1-octanol by ultrasonic agitation to prepare the extraction device. The extraction device was deployed in sample solutions, prepared by spiking target analytes in 1-octanol saturated aqueous solutions (500 mL), for negligible depletion extraction. After equilibrium was reached ($\sim$ 5 h), the 1-octanol in the lumen of the hollow fiber membrane was collected for HPLC determination of the target analytes. As the depletion of the analytes in aqueous samples was negligible, the distribution coefficient (D_{OW}) could be calculated based on the measured equilibrium concentration in 1-octanol (C_O) and the initial concentration (C_W) in the aqueous sample of the target analyte ($D_{OW} = C_O/C_W$). The D_{OW} values measured at various pH values were nonlinearly regressed with pH to obtain the K_{OW} and pK_a values of a compound. Results showed that the measured values of the K_{OW} and pK_a of these model compounds agreed well with literature data.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The distribution coefficient between 1-octanol and water (D_{OW}) and acid dissociation constant (pK_a) of an ionizable compound are key parameters that are related to pharmacokinetic and environmental behaviors, i.e., sorption, distribution, metabolism, elimination, and toxicity [1,2]. Since the D_{OW} of an ionizable compound is pH dependent, the pH independent partitioning coefficient of its neutral molecular form (K_{OW}) is generally reported in the literature. Though various methods have been developed for the determination of K_{OW} [3] and pK_a [4] values, alternatives for high efficient determination of these parameters are still of interests. New approaches by using magnetic nano-absorbent [5], and two-phase hollow fiber-based liquid-phase microextraction procedures with 1-octanol filled in the lumen of a hollow fiber [6] or supported in the pores of hollow fiber membrane wall [7] were adopted for the measurement of D_{OW} and K_{OW} , whereas negligible depletion solid-

phase microextraction (nd-SPME) was reported to be applicable for the measurement of pK_a [8].

Although methods for simultaneous determination of K_{OW} and pK_a are of great interest, they are sparingly studied. Reversed-phase high-performance liquid chromatography (HPLC) was recently reported to be a unique tool for the determination of both pK_a and K_{OW} of multicomponent mixtures [1]. In our previous study [9], K_{OW} and pK_a values were simultaneously determined by using hollow fiber supported liquid-phase microextraction coupled with HPLC. The extraction procedure was performed by using a sampling device with $\sim$ 0.16 μ L of 1-octanol supported in the pores of hollow fiber membrane wall, which required small sample volume to avoid sample depletion. However, this sampling device has the disadvantage of very long equilibration time (72 h), probably due to 1-octanol in the 1-octanol saturated sample solution was gradually adsorbed onto the sample device. The long equilibration time limited the application of this method as degradation of the target analytes might occur during the equilibration.

In this present study, a negligible depletion hollow fiber-protected liquid-phase microextraction (nd-HF-LPME) procedure with 1-octanol filled in the lumen of a hollow fiber was developed

* Corresponding author.

E-mail address: jfliu@rcees.ac.cn (J.-f. Liu).

for simultaneous determination of D_{OW} and pK_a . This proposed procedure markedly reduced the equilibration time, and was successfully applied to determine the K_{OW} and pK_a values of phenol, 4-chlorophenol (4-CP) and 2,4-dichlorophenol (2,4-DCP), which were used as model compounds.

2. Experimental

2.1. Reagents and materials

Phenol, 4-CP and 2,4-DCP purchased from Sigma–Aldrich (Milwaukee, WI, USA) were dissolved in HPLC-grade methanol to prepare individual standard stock solutions (2000 mg/L), respectively. Working solutions were prepared daily by appropriate dilution of the stock solution with 1-octanol saturated sodium dihydrogenphosphate buffer. HPLC-grade acetonitrile and methanol were purchased from Scharlau Chemie (Barcelona, Spain). Other chemicals such as sodium dihydrogenphosphate, hydrochloric acid, sodium hydroxide, acetone, and hydrochloric acid were obtained from Sinopharm Chemical Reagent Beijing (Beijing, China). All the chemicals used in the experiments were analytical reagent grade, and ultrapure water (18.3 M Ω cm) produced by a Barnstead Easy-Pure LF system (Dupuque, IA, USA) was used throughout.

The Q3/2 Accurel PP polypropylene hollow fiber membranes (600 μ m inner diameter, 200 μ m wall-thickness, 0.2 μ m pore size, ~70% porosity [10]) were obtained from Membrana (Wuppertal, Germany).

2.2. Pre-saturation of 1-octanol and phosphate buffer

In 1000 mL separatory funnel, 1000 mL of 1 mM sodium dihydrogenphosphate buffer and 4 mL of 1-octanol were added, and then the mixture was shaken for several minutes. After 12 h, the sodium dihydrogenphosphate buffer and 1-octanol were separated and used in the experiments.

2.3. Extraction procedure

The extraction (sampling) device for nd-HF-LPME was prepared as follows: Q3/2 Accurel PP polypropylene hollow fiber membranes were manually cut into a length of 3.7 cm and were cleaned in an ultrasonic bath for 10 min with acetone. Then, the hollow fibers were taken out and exposed to the air till the acetone in the fiber was completely volatilized. After that, the hollow fiber with two-end sealed by using a heated stainless iron tweezers was immersed in water saturated with 1-octanol and sonicated for 2 h in order to impregnate the lumen of the fiber with 1-octanol. Finally, the hollow fiber membrane was taken out and immersed into water and then shaken for about 1 min to wash out surplus 1-octanol. Generally, the prepared sampling device had an effective fiber length of ~3.5 cm with a sampling phase (1-octanol) of ~10 μ L in the lumen and ~12 μ L in the micropores of the wall. Measurement of the exact volume of 1-octanol was unnecessary as the analyte concentration in the 1-octanol phase was determined by HPLC with external calibration and that in the aqueous phase was kept constant.

Distribution of the target compounds into 1-octanol was performed at room temperature by completely immersing the prepared sampling device into 500 mL of sample solution, held in a capped flask with near zero-headspace, and stirred at a speed of 1200 rpm for prescribed time. The sample solutions were prepared by spiking 200 μ g/L each of phenol, 4-CP and 2,4-DCP into 1-octanol saturated 1 mM phosphate buffer solutions with various pH. After equilibrium distribution of the target compounds was reached between the 1-octanol and aqueous phases, the sampling device was harvested and subsequently the two sealed ends were cut open to flush the 1-octanol into a 200- μ L glass vial with a 5-mL

Fig. 1. Uptake profiles of phenols to the 1-octanol filled in the hollow fiber membrane liquid-phase extraction devices. The lines represent the fit of Eq. (1) to the data. Sample solution: 200 μ g/L each of phenol, 4-CP and 2,4-DCP spiked in 500 mL of 1-octanol saturated 1 mM sodium phosphate buffer (pH 6.0). Note that the C_0 values of phenol were multiplied 5.

syringe. Usually, 8 μ L of 1-octanol can be harvested, of which 5 μ L was manually aspirated into a HPLC microsyringe and injected into the HPLC system for the determination of analyte concentration in the 1-octanol phase (C_0). The C_0 values of each analyte were calculated with external calibration curve prepared by injecting 5 μ L aliquots of standard solutions. The used fiber was discarded and a fresh one was used for the next experiment. The pH of the sample solution was measured before and after extraction, and the pH value after extraction was used for data process.

2.4. HPLC determination

Determination of the target phenols was conducted with an Agilent 1100 HPLC system consists of a binary pump, a variable-wavelength detector set at 220 nm, a personal computer equipped with an Agilent ChemStation program for LC to process chromatographic data, and an Agilent Zorbax Eclipse XDB-C18 column (150 mm $\times$ 4.6 mm I.D., 5 μ m particles) for separation of the analytes. Separation of phenol, 4-CP and 2,4-DCP was performed by using a mixture (45:55, v/v) of acetonitrile and sodium dihydrogenphosphate buffer (10 mM, pH 3.0) as mobile phase at a flow rate of 1.0 mL/min.

2.5. Data process

All experiments were conducted at least twice and the mean values were reported. Nonlinear regression of experimental data was conducted by using Graphpad Prism (ver. 4.1, GraphPad Software, San Diego, CA, USA).

Extraction was conducted with a negligible depletion mode, i.e., extracting less than 5% of the dissolved amount of an analyte in samples. Thus, the analyte concentration in the aqueous sample solution can be regarded as a constant that equals its initial concentration (C_W), and D_{OW} can be calculated based on the measured equilibrium concentration in 1-octanol (C_0) and the initial concentration in the aqueous sample of the target analyte ($D_{OW} = C_0/C_W$). Assuming the partitioning to the sampling phase is linear, the uptake profile of analytes can be fitted to a first-order one-compartment uptake model modified from Ref [11]:

$$C_0(t) = C_W \cdot D_{OW} \cdot (1 - e^{-k \cdot t}) \quad (1)$$

with $C_0(t)$ be the analyte concentration in 1-octanol in the hollow fiber at time t , and k the elimination rate constant of an analyte from the 1-octanol in the hollow fiber.

Table 1

Results of nonlinear regression of $\log K_{OW}$ (mean $\pm$ SD), pK_a (mean $\pm$ SD) and regression coefficient (R^2) of the phenols based on fit of Eq. (2) to data obtained at different pH values. The data reported in literature were also listed for comparison.

Compound	pK_a		$\log K_{OW,HA}$		$\log K_{OW,A}$	R^2
	Measured	Literature value ^a	Measured	Literature value ^b		
Phenol	10.15 $\pm$ 0.14	9.74–10.05	1.54 $\pm$ 0.01	1.47–1.57	–0.29 $\pm$ 0.36	0.988
4-CP	9.49 $\pm$ 0.08	9.13–9.84	2.50 $\pm$ 0.01	2.35–2.53	0.93 $\pm$ 0.25	0.996
2,4-DCP	7.88 $\pm$ 0.05	7.28–7.94	3.16 $\pm$ 0.01	3.08–3.23	1.50 $\pm$ 0.22	0.997

^a Ref. [12–15].

^b Ref. [14–16].

The D_{OW} values measured at various pH can be used to calculate the K_{OW} and pK_a values of a compound. Assuming both the molecular form (HA) and deprotonated form (A) of the target analytes can partition into 1-octanol, the D_{OW} at various pH was fitted to the following equation modified from literature [8]:

$$D_{OW} = (K_{OW,HA} + K_{OW,A} \cdot 10^{pH-pK_a}) / (1 + 10^{pH-pK_a}) \quad (2)$$

where $K_{OW,HA}$ and $K_{OW,A}$ are the partitioning coefficients between 1-octanol and water of HA and A, respectively.

3. Results and discussion

3.1. Determination of the equilibration time and sample depletion

The equilibration time was determined with sample solutions at pH 6.0. The uptake profiles of phenols against extraction time are shown in Fig. 1, which indicates the C_0 of the analytes increased and then reached a constant with extraction time. The rate constant k obtained from Eq. (1) was adopted to estimate the extraction equilibration time (e.g., $t_{95\%} = \ln(0.05)/(-k)$), and results show that the 95% equilibration time for phenol, 4-CP and 2,4-DCP were 26, 98, and 298 min, respectively. Thus, 5 h of extraction time was sufficient to ensure distribution equilibrium for all the model compounds and was adopted in the following studies. Though hollow fiber membranes with thinner walls could reduce the equilibration time, they are inconvenient to handle and were not used in this study.

With the D_{OW} value obtained from Eq. (1), the sample volume ($V_W = 500$ mL) and the 1-octanol volume ($V_O = 22$ μ L), the calculated sample depletions (Depletion % = $100 \cdot D_{OW} \cdot V_O / V_W$) were 0.03%, 0.3% and 1.4% for phenol, 4-CP and 2,4-DCP, respectively. As the sample depletion for all the target compounds are less than 5%, the criterion of negligible depletion [11], the C_W can be regarded as a constant during the extraction and the initial C_W was adopted to calculate D_{OW} directly. It is noteworthy that for the hollow fiber membrane device used in this study, the 1-octanol volume in the pores of the membrane wall (~ 3.5 μ L/cm) is higher than that in the lumen (~ 2.8 μ L/cm), i.e., only 44% of the 1-octanol was effective for HPLC determination. Thus, hollow fiber membrane with thin wall is preferred to be used to prepare the extraction device. Compared to our previous sample device [9], this procedure requires large sample volume to avoid depletion of compounds due to the relatively large 1-octanol in the extraction device.

3.2. Determination of K_{OW} and pK_a values

Fig. 2 shows the measured D_{OW} values in the sample pH range of 4.4–13. The D_{OW} decreased with the increase of sample pH as expected. The nonlinear regression results including $K_{OW,HA}$, $K_{OW,A}$, and pK_a are shown in Table 1, which indicates that it is mainly the neutral molecular form of the phenols that partitioned into 1-octanol. The ratio of $K_{OW,A}$ to $K_{OW,HA}$ was 0.016 for phenol, 0.027 for 4-CP, and 0.021 for 2,4-DCP, respectively. $K_{OW,HA}$ is the K_{OW} of an ionizable compound generally reported in literature. As shown in Fig. 2 and Table 1, the experimental results fitted well with Eq. (2)

Fig. 2. Effect of sample pH on the distribution coefficients of phenols to 1-octanol (D_{OW}). The lines represent the fit of Eq. (2) to the data. Sample solution: 200 μ g/L each of phenol, 4-CP and 2,4-DCP spiked in 500 mL of 1-octanol saturated 1 mM sodium phosphate buffer with various pH. Note that the D_{OW} values of phenol and 4-CP were multiplied 10 and 2, respectively.

with correlation coefficients $R^2 > 0.98$, and the values of $\log K_{OW}$ and pK_a of these studied phenols measured by the proposed method agreed well with that reported in literature. The good agreement demonstrated the applicability of the proposed method in simultaneous determining K_{OW} and pK_a of an analyte.

The K_{OW} value of the ionized form of a compound was sparsely reported in literature. The values of $\log K_{OW,A}$ in 100 mM $K_2H_2PO_4/K_3PO_4$ buffer were reported to be –0.39 for 4-CP and –0.46 for 2,4-DCP, respectively [17]. Since the partitioning of the ionic compounds to 1-octanol depends largely on the ion-pairing with cations in solution [18], one would expect an even lower $\log K_{OW,A}$ values in the presented study as 1 mM phosphate buffer was used as the background electrolyte. The reason for this disagreement remained unknown and further study is needed.

4. Conclusion

A new method based on nd-HF-LPME coupled with HPLC was developed for the simultaneous determination of K_{OW} and pK_a . The proposed method has the merits of simplicity, low cost, and relatively short time for equilibrium extraction. Furthermore, this method can also calculate the K_{OW} of the ionized form of a compound, which is rarely reported in literature. However, the relatively large sampling phase volume (22 μ L) of this developed procedure demands a large sample volume to avoid the depletion of compounds with large K_{OW} values. For further developments the applicability of the proposed method in simultaneous determining K_{OW} and pK_a values of drugs should be investigated.

Acknowledgments

This work was supported by the High-Tech Research and Development Program of China (2007AA06Z413) and the National Natural Science Foundation of China (20877082, 20621703).

References

- [1] P. Wiczling, P. Kawczak, A. Nasal, R. Kaliszan, *Anal. Chem.* 78 (2006) 239.
- [2] S.J. Hayward, Y.D. Lei, F. Wania, *Environ. Toxicol. Chem.* 25 (2006) 2018.
- [3] A. Berthod, S. Carda-Broch, *J. Chromatogr. A* 1037 (2004) 3.
- [4] S. Babic, A.J.M. Horvat, D.M. Pavlovic, M. Kastelan-Macan, *Trends Anal. Chem.* 26 (2007) 1043.
- [5] X. Gao, C.H. Yu, K.Y. Tam, S.C. Tsang, *J. Pharm. Biomed. Anal.* 38 (2005) 197.
- [6] Y.G. Guo, J. Zhang, D.N. Liu, H.F. Fu, *Anal. Bioanal. Chem.* 386 (2006) 2193.
- [7] J.-F. Liu, X.-L. Hu, J.-F. Peng, J.Å. Jonsson, P. Mayer, G.-B. Jiang, *Anal. Chem.* 78 (2006) 8526.
- [8] H.C.H. Lutzhoft, W.H.J. Vaes, A.P. Freidig, B. Halling-Sorensen, J.L.M. Hermens, *Environ. Sci. Technol.* 34 (2000) 4989.
- [9] X.-Q. Cai, J.-F. Liu, Z.-F. Li, G.-B. Jiang, *Chin. J. Anal. Lab.* 27 (8) (2008) 5.
- [10] N. Fontanals, T. Barri, S. Bergstrom, J.Å. Jonsson, *J. Chromatogr. A* 1133 (2006) 41.
- [11] P. Mayer, J. Tolls, J.L.M. Hermens, D. Mackay, *Environ. Sci. Technol.* 37 (2003) 184A.
- [12] L. Geiser, Y. Henchoz, A. Galland, P.A. Carrupt, J.L. Veuthey, *J. Sep. Sci.* 28 (2005) 2374.
- [13] J. Han, F.M. Tao, *J. Phys. Chem. A* 110 (2006) 257.
- [14] H.J. Ulrich, A.T. Stone, *Environ. Sci. Technol.* 23 (1989) 421.
- [15] C.Y. Chen, J.H. Lin, *Chemosphere* 62 (2006) 503.
- [16] G. Ohlenbusch, F.H. Frimmel, *Chemosphere* 45 (2001) 323.
- [17] B.I. Escher, R.P. Schwarzenbach, *Environ. Sci. Technol.* 30 (1996) 260.
- [18] C.T. Jafvert, J.C. Westall, E. Grieder, R.P. Schwarzenbach, *Environ. Sci. Technol.* 24 (1990) 1795.