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Determination of Log *P* by Dispersive Liquid/Liquid Microextraction Coupled with Derivatized Magnetic Nanoparticles Predispersed in 1-Octanol Phase

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A new direct method for log *P* determination by dispersive liquid/liquid microextraction (DLLME) coupled with derivatized magnetic nanoparticles (DMNPs) predispersed in 1-octanol phase is discussed. First, the aim of DMNPs predispersed into 1-octanol phase was to provide the magnetic force when an ultrastrong magnet was used to separate the two phases. Second, the interaction of 1-octanol with inner DMNPs nuclei prevented emulsion formation in the DLLME process. Moreover, interruption of absorption of DMNPs due to the partition equilibrium of the model compound was negligible. The equilibrium of model compound between the two phases was reached in less than 3 min. The two phases were separated quickly by a super magnet because model compounds in the two phases did not interfere with each other. Fourteen model compounds of varied log *P* values were measured using this method. The log *P* values fall in the range of 0.6 to 4.8, which are in agreement with the published results. This method is a rapid, efficient and facile method for direct measurement of log *P* values.

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Introduction

A molecule's lipophilicity, as measured by the log *P* value, is of primary importance and greatly influences overall bioavailability. It is closely associated with the absorption,[1,2] distribution, metabolism,[3] excretion, and toxicity (ADMET) properties of the drug compound such as blood-brain-barrier penetration[4] and clearance.[5-7] In environmental science, the log *P* values have proven useful in situations seemingly unrelated to toxicity, *i.e*. assessing bioaccumulation and measuring soil-water or sediment-water partition coefficients.[8-10] Therefore, it is very important to develop a rapid method to determine log *P* values.

Both direct and indirect methods for log *P* determination have been developed. By far, the most common direct methods for log *P* are the shake flask and modified shake flask methods.[11,12] The shake flask methods can accurately measure the broadest range of solutes including both neutral and charged compounds. However, these methods are labor-intensive and suffer from requiring a long analysis time. They consume large amounts of materials and organic solvents such as 1-octanol, which potentially cause environmental pollution. Furthermore, the formation of emulsion in the shaking process results in the difficulty in the complete separation of the two phases. Consequently, large errors are expected for organic compounds with high partition coefficients. Recently, a method using hollow fiber membrane solvent microextaction (HFMSM) coupled with HPLC has been developed for direct determination of log *P*.[13] This accurate, simple and economical method has a high upper limit of log *P* range. However, since the 1-octanol phase in the hollow fiber tube acts as a stationary phase in the extraction process, the equilibrium time can be as long as 1 h under stirring condition. Conversely, indirect chromatographic methods for estimating log *P*, *i.e*. thin-layer chromatography,[14,15] reverse-phase column chromatography,[16,17] and microemulsion electrokinetic chromatography,[18-20] are faster and easier to automate than traditional shake flask methods. Unlike the direct methods, the structure of the compound must be known before analysis and these methods can only produce acceptable estimates for compounds with similar functional groups, or belonging to homologous series.

Very recently, a timesaving and high-throughput liquid phase microextraction method termed as dispersive liquid/liquid microextraction (DLLME) has been developed.[21-26] An organic extractant and disperser solvent are quickly injected into an aqueous sample to form an emulsified solution. Since the extractant is highly dispersed into the aqueous phase, extraction can be achieved within a few seconds. However, it is inconvenient to retrieve the organic phase after the extraction. Typically, this problem is circumvented by using an organic extractant denser than water, such as chloroform. A two-step extraction technique based on a new combined approach of DLLME and micro-solid phase extraction (μ-SPE) was also proposed.[27] This method permits the use of a solvent with a density lower than water, *i.e*. 1-octanol, in the DLLME mode. However, it is not suitable for log *P* determination. The reasons are as follows. First, even after separation of the two phases by a super magnet, part of the 1-octanol phase is still left in the aqueous layer, which causes significant errors for compounds of

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high log *P* values. Second, in the μ-SPE step, the adsorption of the model compound in the aqueous phase by the derivatized magnetic nanoparticles (DMNPs) interferes with its equilibrium between the 1-octanol phase and the water phase.

In this study, the DMNPs were evenly predispersed in the octanol phase before the extraction. The DMNPs act as a magnet medium for the phase separation by a super magnet. The interaction of the 1-octanol with the inner DMNPs nuclei prevents the emulsion formation in the DLLME process. As a result, a more complete phase separation can be achieved. Moreover, the DMNPs adsorption of the model compounds, as mentioned before, can be minimized as the DMNPs are surrounded by a thick 1-octanol layer. The DLLME coupled with the hydrophobic DMNPs predispersed in the 1-octanol phase is a rapid, efficient and facile method for direct measurement of log *P* values.

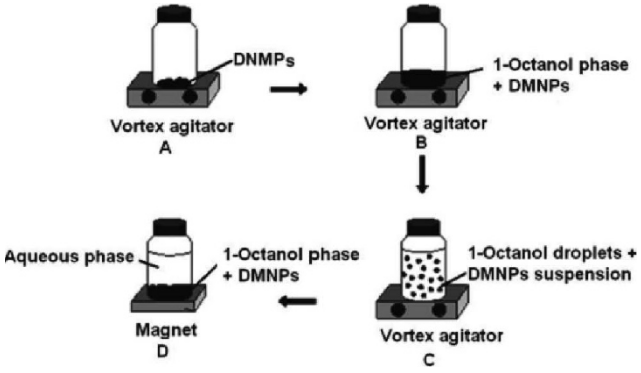

Fig. 1 The procedure for determination of log *P* by the DLLME coupled with DMNPs predispersed in 1-octanol phase method.

Experimental

Chemicals and reagents

The analytical grade model compounds used in the experiment were bought from Sinopharm Chemical Reagent Co. Ltd. (Nanjing, China). They include the following: aniline, benzyl alcohol, 4-methoxyphenol, phenol, nitrobenzene, benzene, toluene, ethylbenzene, naphthalene, anthracene, dibenzyl, bromophenol blue, hydrocortisone, and dexamethasone acetate. Analytical grade iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and sodium acetate were purchased from Xuchanghuasheng Chemical Trade Co. (Xuchang, China). Ethylene glycol and polyethylene glycol (average M_W = 20000) were purchased from Guangdong Guanghua Chemical Factory Co. (Guangzhou, China). 3-Chlorpropyl-triethoxysilane was purchased from Sigma-Aldrich (St. Louis, MO). Anhydrous benzene and anhydrous ethanol, xylene, and methyl orange were bought from Tianjin Kemel Chemical Reagent Co. (Tianjin, China), HPLC grade methanol and acetonitrile were obtained from Tianjin Kemel Chemical Co. (Tianjin, China). Analytical grade 1-octanol was bought from Sinopharm Chemical Reagent Co. Ltd. (Nanjing, China). Freshly distilled water was used in all experiments.

Synthesis of Fe_3O_4 nanoparticles and DMNPs

Fe_3O_4 nanoparticles were synthesized as follows:[28] $FeCl_3{\cdot}6H_2O$ (1.35 g, 5 mmol) was dissolved in ethylene glycol (40 mL) to form a clear solution, followed by the addition of NaAc (3.6 g) and polyethylene glycol (1.0 g). The mixture was stirred vigorously for 30 min and then sealed in a 50-mL teflonlined stainless-steel autoclave. The autoclave was heated to 200°C and maintained this temperature for 10 h. Then it was cooled to room temperature. The black products were washed several times with anhydrous ethanol and dried at 160°C for 2 h.

Derivatization of the Fe_3O_4 magnetic nanoparticles was carried out in the following fashion:[27] 0.2 g of dried Fe_3O_4 magnetic nanoparticles were added into a 20-mL vial containing 10 mL of anhydrous benzene. The vial was sealed with a butyl rubber stopper. The mixture was swirled under a nitrogen atmosphere for 10 min, followed by the injection of 1 mL 3-chlorpropyl-triethoxysilane. The resulting mixture was sonicated for 10 min and then transferred into an autoclave to react at 110°C for 10 h. After the reaction, the surface modified nanoparticles were washed with benzene, followed by methanol. Finally, they were dried at 105°C for 2 h before use.

Characterization of the DMNPs

Scanning electron microscopy (SEM, Zeiss, EVO LS-15, German) was used to characterize the size distribution of the DMNPs.

Powder X-ray diffraction (XRD, XPert Pro MPD, Holland) was used to investigate the internal array of the composite. The diffraction scan range was 5 – 80°, and the tube current was 40 mA.

Elemental analysis (Thermo Electron Corp., Flash EA, Thermo Finnigan, Italy) of DMNPs was done to determine the carbon content and hydrogen content bound on the surface of the Fe_3O_4 magnetic nanoparticles.

Microscopy of the 1-octanol phase with DMNPs in the DLLME process

DMNPs were first predispersed into the 1-octanol phase, followed by vortexing at 3000 rpm for the DLLME process. The 1-octanol phase with the DMNPs was photographed by a microscope (XSP-BM-2CA, Shanghai, China). Red colored methyl orange was dissolved in the 1-octanol phase to obtain clear images.

Sample preparation

1-Octanol and water (1000 mL each) were mixed and shaken for 10 min; the mixture was left undisturbed for 24 h, followed by the separation with a separatory funnel. Thus, the aqueous phase used for measurement was saturated with 1-octanol, and the 1-octanol phase was saturated with water. Model compounds were prepared at appropriate concentrations in water or in dimethyl sulfoxide (DMSO) as stock solutions, and then diluted with 1-octanol-saturated aqueous solution.

HPLC and GC parameter settings

Angilent 1200 (Angilent, German) with a UV detector was used to analyze the concentrations of the model compounds in the aqueous phase and 1-octanol phase. A C_{18} reverse phase column of 250 mm × 4.6 mm and 5 μm was used. The mobile phases were methanol and water mixtures at different proportions. All HPLC mobile phases were filtered through 0.22 μm Nylon membrane before use.

Shimadzu GC 2010 (Shimadzu, Japan) with a FID detector was used to measure the 1-octanol in the aqueous phase. The following parameters were employed: 60 m × 0.2 mm × 0.25 μm DB-5 column (Angilent, German); column temperature, 130°C; nitrogen carrier gas flow rate, 1 mL/min; split inject temperature, 180°C; split ratio, 30:1; FID temperature, 200°C.

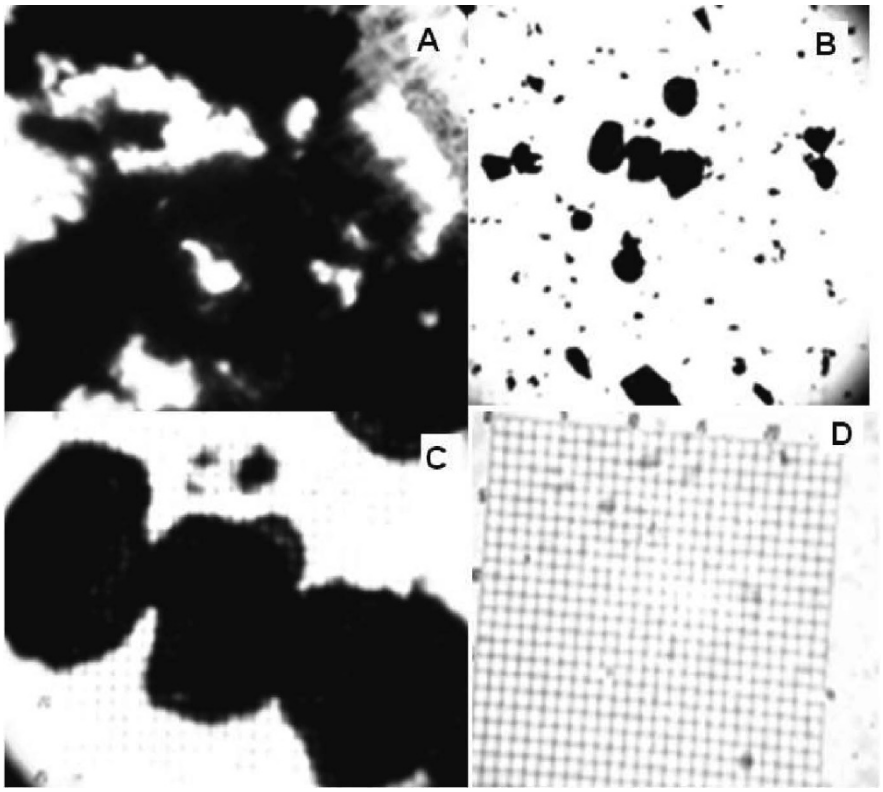

Fig. 2 The microscopic images of: (A) 1-octanol + DMNPs suspension, the magnification 10 × 100, (B) 1-octanol + DMNPs droplets in the DLLME process, the magnification 10 × 40, (C) part of 1-octanol + DMNPs droplets in the DLLME process, the magnification 10 × 100, (D) the upper surface of the aqueous phase after separation, the magnification 10 × 100.

Log P determination

A simple and inexpensive experiment setup was designed for measuring log *P* values of organic compounds by DLLME coupled with DMNPs predispersed in the 1-octanol phase, as shown in Fig. 1 and elaborated below.

For hydrophilic compounds (log *P* in the range of 0 – 3.5), 10 mg of DMNPs and 20 μL (17 mg) of water-saturated 1-octanol solution were added to a vial (Fig. 1A). The mixture was swirled on a vortex agitator at 3000 rpm for 1 min to ensure even dispersion of the DMNPs (Fig. 1B). One milliliter of the model compound solution was added to this vial, followed by agitation at 3000 rpm for 3 min (Fig. 1C). A super magnet was held next to the bottom of the vial to retrieve the 1-octanol phase with the DMNPs (Fig. 1D). After the removal of the magnet, the 1-octanol phase with DMNPs was washed with two aliquots of 100 μL acetonitrile for 2 min under sonication. The above two solutions were collected and diluted with acetonitrile to appropriate concentrations. The amounts of the model compound in the two phases were analyzed by HPLC and the log *P* values were calculated.

For hydrophobic compounds (log *P* higher than 3.5), more than 1 mL model compound was added to increase its concentration in the aqueous phase. Even with 10 mL of the model compound (anthracene), its concentration in the aqueous phase was still not detectable by HPLC with a UV detector. In order to detect trace amount of anthracene in the aqueous phase, we performed a second DLLME coupled with DMNPs predispersed in the 1-octanol phase to concentrate the anthracene.

Results and Discussion

Characterization of the DMNPs

DMNPs were spherical and have a very narrow diameter distribution centered around 285 nm (Fig. S1, Supporting Information). All the different peaks at (220), (311), (400), (422), (511), and (440) could be attributed to the inverse cubic spinel structure of Fe_3O_4 (JCPDS card file, No. 85-1436) (Fig. S2, Supporting Information). Elemental analysis indicated that the carbon content of DMNPs was 2.76% and that the hydrogen content of DMNPs was 0.46% (Table S1, Supporting Information). This suggested that 3-chlorpropyl-triethoxysilane was bound on the surfaces of Fe_3O_4 nanoparticles.

Characterization of the 1-octanol and DMNPs mixture in the DLLME coupled with DMNPs predispersed into the 1-octanol phase process

The volume of 1-octanol (20 μL about 17 mg) was much larger than that of the 3-chlorpropyl-triethoxysilane bound on Fe_3O_4. All nanoparticles were stable in the 1-octanol phase due to their hydrophobic properties. As shown in Fig. 2A, the DMNPs were surrounded by 1-octanol phase. Figures 2B and 2C show the images of the 1-octanol droplets with DMNPs nuclei in the DLLME process. The 1-octanol phase with DMNPs nuclei was divided into many microdroplets dispersed in the aqueous phase. Most droplets were not spherical, but had irregular shapes. This indicated that the surface tension of a droplet was not even, which was caused by the interaction between DMNPs with 1-octanol. The clear interface between

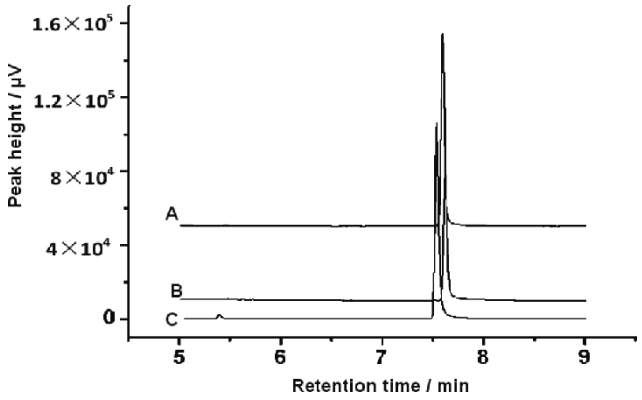

Fig. 3 Gas chromatograms of 1-octanol: (A) in water saturated with 1-octanol, (B) in aqueous phase after direct DMNPs microextraction completion, (C) in aqueous phase after the completion of microextraction using DMNPs + 5 μL of 1-octanol

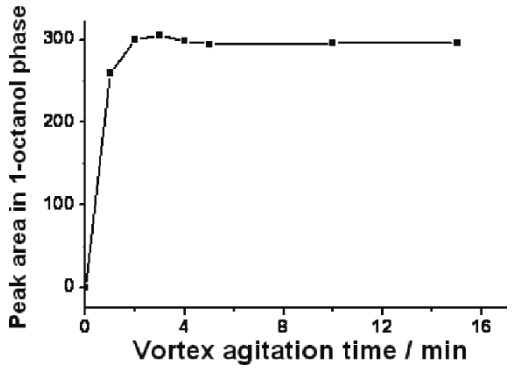

Fig. 4 Time course of equilibrium of 1 mL of 30 μg mL^{-1} benzene between the 1-octanol and aqueous phases.

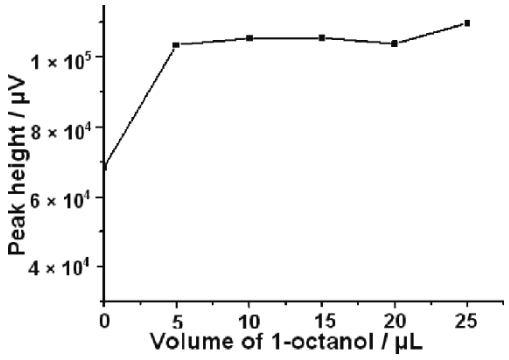

Fig. 5 The effect of volume of 1-octanol used on the peak heights of 1-octanol in the aqueous phases after DLLME and separation of two phase processes.

Table 1 The effects of benzene concentrations on log *P*

Concentration/μg mL^{-1}	Test 1	Test 2	Test 3	Average
15	2.33	2.30	2.31	2.31
20	2.15	2.20	2.11	2.15
25	2.17	2.13	2.17	2.15
30	2.15	2.16	2.17	2.16
40	2.20	2.17	2.18	2.18
50	2.19	2.16	2.16	2.17
70	2.16	2.17	2.13	2.15
80	2.14	2.15	2.17	2.15
90	2.08	2.12	2.11	2.10

droplets of the 1-octanol phase and the aqueous phase indicated that there was no emulsion formation. The average size of big droplets of 1-octanol with DMNPs was about 25 μm and the average size of small ones was about 2 μm. Swirling leads to the coagulation of the microdroplets to form bigger droplets, thanks to the hydrophobic properties of the 1-octanol. Figure 2D shows the picture of the upper surface of aqueous phases in the vial after separation by a super magnet. The picture shows that the aqueous and 1-octanol phases were completely separated and there were almost no 1-octanol droplets left in the aqueous phase.

Comparison of the DLLME coupled with DMNPs predispersed into 1-octanol phase method with the direct dispersive DMNPs microextraction method

The 3-chlorpropyl-triethoxysilane layer on the surfaces of the Fe_3O_4 magnetic nanoparticles can cause the adsorption of the model compound from the aqueous phase, which may interrupt the equilibrium of model compound between the 1-octanol and the aqueous phases. The extraction efficient of this technique was compared to that of the direct dispersive DMNPs microextraction. The extraction efficiencies of the two techniques were compared and the results are shown in Fig. S3 (Supporting Information). It can be concluded that the DMNPs could adsorb the model compound (anthracene) directly from the aqueous phase. However, the extraction efficiency of this method was much higher than that of the direct dispersive DMNPs microextraction method. The reasons are as follows. First, the hydrophobilty of 1-octanol was stronger than that of 3-chlorpropyl-triethoxysilane. Second, the volume of 1-octanol was far more than that of 3-chlorpropyl-triethoxysilane bound on these nanoparticles. Deducing from the pictures of 1-octanol droplets with DMNPs in the extraction process, one can conclude that the DMNPs were surrounded by much thicker layers of 1-octanol phase. This decreases the chances of interactions between the model compound and the DMNPs in the microextraction process and the absorption of the model compound by DMNPs. Figure 3B shows that the gas chromatographic peak height of 1-octanol in the aqueous phase, after direct DMNPs extraction, was lower than that of 1-octanol in water saturated with 1-octanol (Fig. 3A). This indicated that DMNPs can adsorb part of 1-octanol from the water phase saturated with 1-octanol. In a vial, we added 10 mg DMNPs to the vial and add only 5 μL of 1-octanol on the DMNPs. After extraction, the peak height of 1-octanol in the aqueous phase (Fig. 3C) was almost equal to that of 1-octanol in water saturated with 1-octanol (Fig. 3A). This indicates that DMNPs lost the ability for adsorption of more 1-octanol from the water phase saturated with 1-octanol because the surface of the DMNPs was surrounded by 1-octanol phase layer. The DMNPs also lost their ability to adsorb other model compounds. Thus, the interruption with the equilibrium of test compound between two phases by DMNPs adsorption was negligible.

Time course of the equilibrium

The time to reach equilibrium is used as a criterion for determining the length of the test. Since the 1-octanol phase with DMNPs was divided into many microdroplets with DMNPs nuclei, the surface areas and contact areas between the two

Table 2 Log *P* values obtained by DLLME coupled with DMNPs predispersed into 1-octanol phase

Model compound	Concentration/μg mL^{-1}	Log *P*				
		Test 1	Test 2	Test 3	Average	Literature value
Aniline	30, 70, 90	0.94	0.96	0.93	0.95	0.92,[29] 0.94[30]
Benzyl alcohol	30, 70, 90	1.08	1.11	1.09	1.09	1.10[31]
4-Methoxyphenol	30, 70, 90	1.28	1.31	1.33	1.31	1.3[31]
Phenol	30, 70, 90	1.67	1.64	1.63	1.64	1.6,[32] 1.62[33]
Nitrobenzene	40, 50, 80	1.87	1.89	1.91	1.89	1.9[31]
Benzene	40, 50, 80	2.20	2.16	2.14	2.15	2.19,[30] 2.13[34]
Toluene	40, 50, 80	2.67	2.63	2.66	2.65	2.7[31]
Ethylbenzene	30, 40, 70	3.23	3.32	3.30	3.28	3.15[35]
Naphthalene	5, 10, 20	3.42	3.54	3.48	3.48	3.40,[29] 3.51[36]
Anthracene	0.8, 1.0, 1.5	4.64	4.71	4.63	4.66	4.68,[37] 4.63[14]
Dibenzyl	0.8, 1.0, 1.5	4.81	4.84	4.76	4.80	4.8[31]
Bromophenol blue	0.8, 1.0, 1.5	4.73	4.69	4.74	4.72	4.88[13]
Hydrocortisone	6.0, 15, 20	1.64	1.60	1.65	1.63	1.71,[36] 1.45[38]
Dexamethasone acetate	10, 20, 25	2.32	2.35	2.36	2.34	2.91[39]

phases increased dramatically. This decreases the time for reaching the equilibrium dramatically. The vortex agitator speed 3000 rpm also increased the mass transfer. A spiked water sample containing 1 mL of 30 μg mL^{-1} of benzene was used to test the equilibrium time. The time course of the equilibrium (Fig. 4) shows that the concentrations of benzene increased quickly before 2 min and leveled off after 3 min. The time for extraction process and separation process was not more than 5 min. Thus, this method is a very rapid direct method for the determination of log *P*.

The effect of volume of the 1-octanol phase on the determination of log P

The volume of the 1-octanol phase affects the equilibrium of test compound between the two phases. Figure 5 shows that the peak height of 1-octanol was almost equal to that of 1-octanol in the aqueous phase saturated with 1-octanol when the volume of 1-octanol used was in the range of 5 – 20 μL. This indicates that the DMNPs did not directly adsorb more 1-octanol from the aqueous phase. There was almost no 1-octanol left in the aqueous phase after the separation of the two phases. When the volume was more than 25 μL, the peak height of 1-octanol was more than that of 1-octanol in the aqueous phase saturated with 1-octanol. This indicates that part of the 1-octanol phase was left in the aqueous phase after phase separation. In addition, the volume of 1-octanol phase also affects the mass transfer. The larger the volume of the 1-octanol phase, the more droplets formed in the microextraction process, and the bigger the transfer area became. Thus, the mass transfer increased when the volume of 1-octanol phase was increased. In this study, the volume of 1-octanol phase was optimized to be 20 μL.

The effects of model compound concentrations on determination of log P

The volume of the aqueous phase was far more than that of the 1-octanol phase. Thus, 1-octanol–water partition coefficient must be determined for dilute solutions. The concentrations of the model compound were controlled in a range. For example, benzene was controlled in the range of 15 – 90 μg mL^{-1}. The log *P* values determined are listed in Table 1. The data obtained demonstrate that the log *P* values are slightly higher when the concentration of benzene was of 15 μg mL^{-1}. When the concentrations of benzene are in the range of 20 – 80 μg mL^{-1}, the log *P* values determined are almost equal. When the concentration benzene is about 90 μg mL^{-1}, log *P* values determined are slightly lower. For model compounds of lower log *P* values, the solubility are more than those of the model compounds of higher log *P* values. Therefore, the upper concentration range for lower log *P* values of test compounds is higher than that of the higher log *P* values of test compounds.

In the log *P* values of 0.9 – 4.8, the results obtained using this method, as tabulated in Table 2, are consistent with those in the literature. This also demonstrates that the rapid direct method can be used to determine log *P* accurately.

Conclusions

A rapid, novel direct method for log *P* determination was developed by DLLME coupled with DMNPs predispersed in 1-octanol phase. Accurate and consistent log *P* values were obtained over a wide range. Only 5 min was required. This method can be widely used in medicinal chemistry, drug design, and environmental science. Furthermore, this method has the potential to be used to determine log *P* values of some compounds by just one liquid/liquid microextraction. The easiness of phase separation after the microextraction process makes it possible to automate this method for high throughput analysis.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci.

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