Simultaneous determination of the lipophilicity and dissociation constants of dialkyl phosphinic acids by negligible depletion hollow fiber membrane-protected liquid-phase microextraction

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ABSTRACT

Determination of the physicochemical properties, especially the lipophilicity (expressed as the logarithm of distribution coefficient, log *D*) and dissociation constant (*pK*_a), is of great importance in the early stage of environmental risk assessment for an ionizable compound without these data. Currently, the log *D* and *pK*_a values of dialkyl phosphinic acids (DPAs), the environmental hydrolysates of aluminum dialkyl phosphinates (ADPs) that is one class of emerging phosphorus-containing flame retardants, are not available. In this study, the log *D* and *pK*_a values of three DPAs including methylethylphosphinic acid (MEPA), diethylphosphinic acid (DEPA) and methylcyclohexyl phosphinic acid (MHHPA), were simultaneously determined by negligible depletion hollow fiber supported liquid phase microextraction (nd-HF-LPME) followed by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS). The *pK*_a and log *D* of DPAs were determined by curve-fitting the experimental data with equations derived on the basis of the Henderson–Hasselbalch equation and compared with model calculated data. For MEPA, DEPA and MHHPA, the *pK*_a values were close and around 3, but the log *D*s were strongly pH-dependent with values from −5.01 to 1.01. The log *K*_{OW} of the neutral form (log *K*_{OW,HA}) and ionic form (log *K*_{OW,A}) were in the range of −0.67–1.02 and −3.86–−1.33, respectively. The experimentally determined *pK*_a values were highly in good agreement with ACD/*pK*_a predicted values and the measured log *K*_{OW,HA} values were closely related to KOWWIN calculated ones, suggesting ACD/*pK*_a and KOWWIN are good alternative methods to estimate *pK*_a and log *K*_{OW} of DPAs, respectively. As far as we know, this is the first report on the *pK*_a and log *D* data for DPAs, which are fundamental for the product design and evaluating the environmental behavior and effects of DPAs and ADPs.

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1. Introduction

As one class of emerging additive halogen-free phosphorus-containing flame retardants (PFRs), aluminum dialkyl phosphinates (ADPs) have grabbed a growing share of FRs market with global restrict and phase-out of many commonly used brominated FRs such as penta-bromodiphenyl ether (penta-BDE) mixtures and decabromodiphenyl ether (deca-BDE) [1–3]. ADPs have been widely used in glass fiber reinforced polyamides and polybutylene terephthalate, thermoplastics and thermosets, electrical and

electronic equipment, connectors, switches, and encapsulated electronic components [4–6]. With their growing consumption, ADPs will inevitably be discharged into environment during the manufacture, use and disposal process of ADP-containing by-products or finished items [1]. An elevated levels of ADPs, containing aluminum salts of methylethylphosphinic (AMEP), diethylphosphinic (ADEP) and methylcyclohexyl phosphinic (AMHP), were found in soil and sediment core samples collected in the vicinity of a manufacturing plant [3]. ADPs are considered as very persistent in environment due to their low solubility [1]. However, once dissolved in water, they will be rapidly hydrolyzed to the corresponding dialkylphosphinate acids (DPAs), which have a substantial solubility in water and therefore a high potentiality of mobility [3]. Although ADPs are generally considered as a class of less toxic chemicals, their chronic exposure toxicity to daphnia [4], mild hepatotoxicity and reproduction toxicity to male BALB/c mice [3,7], as well as embryotoxicity to

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zebrafish [8] have also been reported. Meanwhile, the reported toxicities are speculated to be caused by DPAs according to the fact that once dissolved in water ADPs are readily hydrolyzed to DPAs [3,8]. However, the current understanding of the environmental behavior and toxicological effects of ADPs is scarce. Therefore, further studies are urgently needed to better understand the fate, behavior and toxicity of ADPs and also their hydrosates DPAs.

Generally, the fate and behavior of a compound in the environment are largely dependent on its physicochemical properties. For an ionizable compound, in particular, the lipophilicity, usually denoted as the logarithm of its distribution coefficient between 1-octanol and water ($\log D$) and the acid ionization constant (pK_a) have been identified as fundamental parameters for predicting the uptake, bioaccumulation, metabolism and elimination in animals, absorption and translocation in plants, and sorption to and consequently mobility in soil [9–11]. Additionally, determination of pK_a and $\log D$ of an ionizable organic compound is a prerequisite for certain analytical application. However, to our knowledge, no information about pK_a and $\log D$ of DPAs are available in the literature. It is, thus, essential to determine their basic physicochemical properties, especially pK_a and $\log D$.

So far, a number of methods for separation or simultaneous determination pK_a and the 1-octanol/water partition coefficient (K_{OW}) of organic compounds have been reported, including capillary electrophoresis, potentiometric titration, spectrophotometric methods, shake-flask method, micro-extraction methods, chromatographic methods and theoretical methods such as quantitative structure activity/property relationships (QSAR/QSPR) model [9,11–24]. Currently, gradient reverse-phase high performance liquid phase chromatography (RP-HPLC) is regarded as the most commonly used indirect experimental method to simultaneously determine pK_a and K_{OW} of a compound. However, this technique is not applicable for some analytes whose hydrophilicity is fairly strong ($\log K_{OW} < 0.5$) and compounds with identical molar masses [16,25]. Amongst, negligible depletion hollow fiber membrane-supported liquid phase microextraction (nd-HF-LPME) has already proved to be a simple, reliable and suitable alternative tool for simultaneous determination of $\log D$ and pK_a of an ionizable compound [11,26]. With the exception of directly measurement by various experiments, mathematical models are also important tools used in prediction of the K_{OW} and pK_a of a compound. Among numerous prediction tools, the ACD/ pK_a DB module developed by Advanced Chemistry Development, Inc. (ACD/Labs, Toronto, Canada) was suggested to be the most accurate theoretical prediction software for pK_a of an acidic analyte [27] and several substructure approaches, like KOWWIN and AB/ $\log P$, could provide reliable $\log K_{OW}$ for simple organics [28].

Herein, the nd-HF-LPME method was used for direct simultaneous determination of $\log D$ and pK_a of three DPAs, including methylethylphosphinic acid (MEPA), diethylphosphinic acid (DEPA) and methylcyclohexyl phosphinic acid (MHPA). The experimental values were also compared with the computationally predicted values. To the best of our knowledge, this is the first report on the physicochemical properties of DPAs. It could provide better insight into their partition processes and environmental behavior.

2. Experimental

2.1. Chemicals and materials

AMEP, ADEP and AMHP with purity > 95% were kindly provided by Jiangnan University (Hubei Province, China). MEPA, DEPA and MHPA, with purity larger than 95%, 95% and 90%, respectively, were synthesized individually from corresponding aluminum dialkyl

phosphonate (ADPs) according to previously reported method [3]. The structures of the three DPAs are shown in Fig. S1. HPLC-grade methanol, 1-octanol, acetone and formic acid were, respectively, purchased from Fisher Scientific (Fair Lawn, NJ), J&K chemical Ltd. (Shanghai, China), J.T. Baker (Phillipsburg, NJ, USA) and Acros Organics (Morris Plains, NJ). Other chemicals such as ammonium hydroxide ($\text{NH}_3 \cdot \text{H}_2\text{O}$, 15 M), hydrochloric acid, sodium hydroxide, sodium dihydrogen phosphate were all provided by Sinopharm Chemical Reagent Co. (Beijing, China). Unless exceptions are noted, all other chemicals used were of analytical reagent grade. Ultra-pure water (18.3Ω) produced by a Milli-Q Gradient system (Millipore, Bedford, USA) was used for preparation of samples and solution.

The Q3/2 Accurel PP polypropylene hollow fiber membranes (600 μm inner diameter, 200 μm wall thickness and 0.2 μm pore size, ~70% porosity) was purchased from Membrane GmbH (Wuppertal, Germany).

2.2. Preparation of phosphate buffer and sampling device

The 1-octanol saturated phosphate buffer and sampling device were prepared by procedures adapted from the procedure of our previous work [11]. The details are as follows: For preparation of 1-octanol saturated phosphate buffer solution, 1000 mL of 1 mM sodium dihydrogenphosphate (NaH_2PO_4) buffer and 4 mL of 1-octanol were added into a 1000 mL separatory funnel and shaken vigorously for about 2 min, then separated statically for 12 h. The two phases were completely separated and used in the further experiments.

The Q3/2 Accurel PP polypropylene hollow fiber membranes were manually cut into a length of 5.5-cm pieces and ultrasonic treated for approximately 10 min in acetone to remove any contaminants. The hollow fibers were then taken out and placed in a fume hood for 2 h to volatilize of acetone in the fiber completely. Subsequently, the hollow fibers were two-end sealed to an ellipse-ring shape by using a heated steel stainless tweezers. To impregnate 1-octanol into fibers lumen, the ellipse-ring hollow fibers were immersed into 1-octanol saturated with water and sonicated for more than 2 h. Prior to extraction, the hollow fibers were taken out from the 1-octanol, then immediately immersed into water and shaken for about 1 min to wash out surplus 1-octanol. Generally, the prepared sampling HF device had an effective fiber length of about 5.3 cm with a sampling phase (1-octanol) of approximately 15 μL in the lumen and 18 μL in the micropores of the wall (~70% porosity) as determined by GC–MS (for details see Supplementary Materials Text S1).

2.3. nd-HF-LPME extraction procedure

Two pieces of the above prepared HF devices were put into 620 mL sample solution held in a capped flask with nearly no headspace and shaken at 200 rpm on a shaker (IKA® KS 4000 i control; IKA-Werke GmbH, Staufen, Germany) at room temperature (23.8–25.3 °C) for the prescribed time. The sample solutions were prepared by adding known amounts of DPAs standard into the 1-octane saturated 1 mM NaH_2PO_4 buffer solution with various pH values, and 1000 ng mL⁻¹ MEPA, 500 ng mL⁻¹ DEPA and 250 ng mL⁻¹ MHPA in the finally sample solutions, respectively. After the equilibrium partitioning of DPAs between 1-octanol and aqueous phases was reached, the HF sampling device was collected, and then the two sealed ends were cut open to flush the 1-octanol into a 250- μL micro glass inserts for 1.5-mL autosampler vials with a 1-mL syringe. Normally, ~15 μL of 1-octanol can be collected, of which 10 μL was injected into the ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) system for DPAs analysis. In order to eliminate the

possibility of carry-over effects, the HF-LPME devices used throughout the experiment were disposable. The pH of sample solution was determined once more at the conclusion of extraction using a pH meter (Thermo Scientific), and only this pH value was used for data processing.

2.4. UPLC–MS/MS analysis

The determination of MEPA, DEPA and MHPA in HF acceptor phase (1-octanol) was conducted on a Waters Acquity ultra-high performance liquid chromatography (UPLC) (Waters, Mildford, MA), coupled with a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA) system in the negative ion electrospray ionization mode (UPLC–(ESI[−])–MS/MS). The detailed instrumental parameters are described in the Supplementary Material (Text S2). For more method details, please refer to our previous publication [3].

To quantify the concentration of DPAs in 1-octanol of sampling device, external calibration curves (Table S1) were prepared by injecting 10-μL aliquots of standard solutions (0.01–1 μg mL^{−1}).

2.5. Theoretical considerations

2.5.1. Uptake profiles to 1-octanol

Mayer et al. [29] described in detail the kinetics of the analyte partitioning to a passive sampler and the use of a first-order one-compartment model to determine the uptake profile. Thereafter, a modified first-order one-compartment model shown as Eq. (1) has widely been used to fit the uptake profiles in equilibrium sampling by hollow fiber supported liquid membrane microextraction (HF-LPME) devices [11,30,31] in the context of linear partitioning.

$$C_{0,t} = C_{W,0} \cdot D_{OW} \cdot (1 - e^{-k \cdot t}) \quad (1)$$

where $C_{0,t}$, $C_{W,0}$ and D_{OW} are the concentration of analyte in the 1-octanol in the lumen of hollow fiber at time t , the initial concentration of analyte in sample solution and distribution coefficient, respectively; k is the analyte elimination rate constant from the 1-octanol.

The extraction equilibrium times can be estimated using the elimination rate constant k value in Eq. (1). For instance, the time required to reach 95% of equilibrium can be estimated with the following equation [29]:

$$t_{95\%} = \frac{\ln 5}{k} \quad (2)$$

For negligible depletion equilibrium extraction, the extracted amount should be kept below 5% of the amount that dissolved in sample solution to guarantee a nearly constant concentration of analytes in sample solution and minimal impacts on equilibrium between freely dissolved and bounded analytes [29]. Liu and co-workers [30–32] firstly extended the negligible depletion criterion in HF-LPME for determining dissolved concentrations of both organic compounds and metals, in which the D_{OW} obtained from Eq. (1) was used to calculate the sample depletion

$$\text{depletion\%} = \frac{D_{OW}V_0}{V_S} \times 100 \quad (3)$$

where V_0 and V_S represent the volume of 1-octanol and sample solution, respectively.

2.5.2. Distribution coefficients to 1-octanol filled in the lumen of hollow fiber

DPA extraction was carried out with a negligible depletion liquid phase microextraction (nd-LPME). As aforementioned, in an nd-LPME extraction mode, analyte distributed into acceptor (1-octanol) account for less than 5% of initial concentration in the

sample solution, thus, the analyte concentration in the aqueous sample solution can be identified as a constant. Therefore, distribution coefficients can be defined as [11]

$$D_{OW} = \frac{C_0}{C_W} \quad (4)$$

where C_0 (ng mL^{−1}) is the measured equilibrium concentration of solute in the 1-octanol phase, and C_W (ng mL^{−1}) is the solute concentration in the aqueous phase when the system is at equilibrium that equals to its initial concentration.

The K_{OW} and pK_a values of a compound can be derived from the D_{OW} values measured at various pH values [11]. Presuming that both the protonated (HA) and deprotonated (A) state of phosphorus acid in DPAs can partition to 1-octanol, the pH-dependent D_{OW} can be fitted to the following modified Henderson-Hasselbalch equation [10,11].

$$D_{OW} = \frac{K_{OW,HA} + K_{OW,A} \times 10^{(pH-pK_a)}}{1 + 10^{(pH-pK_a)}} \quad (5)$$

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

2.6. Data processing

All experiments were performed at least triplicate, and results were presented as means ± standard deviation (SD). Experimental data were fitted to models using GraphPad Prism (ver. 5.0, Graphpad Software, San Diego, CA, USA). For comparison, pK_a and log D values of the studied analytes were also calculated theoretically using the online version of ACD/Labs Algorithm Version: ACD/ pK_a DB (ver. 12.1.0.50374) and ACD/log D module (ver. 5.0.0.184, 2010–2013), respectively (<https://ilab.acdlabs.com/iLab2/index.php>) [33,34]. Additionally, comparisons were also performed between the log $K_{OW,HA}$ obtained from the fitting of experimental data and the log K_{OW} values predicted by three commonly used log K_{OW} predictors, i.e. ABlog P (Pharma Algorithms ADME Boxes ver. 2.0), ACD/log P (Phys Chem Batch ver. 5.0.0.184) and KOWWIN[®] (EPI Suite ver. 1.68). The difference between experimental data and software predicted values presented as average absolute error (AAE) were calculated as

$$AAE = \frac{\sum_{i=1}^n \text{observed} - \text{predicted}}{n} \quad (6)$$

where n is the number of analytes studied. A paired t -test with two tailed distribution for a 95% confidence interval was also performed to compare the difference between the predicted pK_a and K_{OW} values and experimental values.

3. Results and discussion

3.1. Uptake kinetics to lumen of hollow fiber

For reliable determination of partition coefficient, it is essential to ensure the equilibrium partitioning of analytes [35]. Fig. 1 shows the uptake profiles of DPAs against extraction time, which was conducted as described in experimental section with sample solutions at pH 3.0. The data fittings, calculated minimum extraction time and magnitude of sample depletion are tabulated in Table 1. The C_0 of the analytes increased and then reached a constant with the prolonged extraction time. The time needed to reach a plateau in an increasing order of MEPA < DEPA < MHPA, which more or less mirrors that the rate of exchange between 1-octanol and water decrease with increasing compound hydrophobicity [26]. The 90% and 95% extraction equilibrium time, $t_{90\%}$ and $t_{95\%}$, estimated according to Eq. (2) with the k value obtained from Eq. (1) for MEPA, DEPA and MHPA were listed in Table 1, which indicates

Fig. 1. Uptake profiles of DPAs to the 1-octanol in the lumen of hollow fiber of nd-HF-LPME equilibrium sampling devices. Eq. (1) was used to fit the experimental data. Sample solution: 1000, 500 and 250 ng mL⁻¹ of MEPA, DEPA and MHPA, respectively, were spiked in 620 mL of 1-octanol saturated 1 mM sodium dihydrogenphosphate buffer (pH was adjusted to 3.0 with 1 M HCl).

Table 1
The uptake rate constant (k), equilibrium time ($t_{90\%}$ and $t_{95\%}$) and sample depletion.^a

Compound	$k \pm SD$ (h ⁻¹)	Equilibrium time (h)		R^2	Sample depletion (%)
		$t_{90\%}$	$t_{95\%}$		
MEPA	1.774 ± 0.138	1.298	1.689	0.955	0.0006
DEPA	1.357 ± 0.109	1.697	2.208	0.958	0.0022
MHPA	0.919 ± 0.027	2.510	3.260	0.997	0.0654

^a Results of data fitting for the uptake profiles to the 1-octanol filled in the lumen of hollow fiber according to Eq. (1). R^2 is squared correlation coefficients of the fitting.

4 h of extraction time was enough to ensure partition equilibrium for all three studied analytes and adopted in the further studies.

With the D_{OW} value obtained from Eq. (1), the sample volume ($V_W = 620$ mL) and the 1-octanol volume ($V_O = 66$ μ L), the sample depletions calculated from Eq. (3), were 0.005%, 0.007% and 0.065% for MEPA, DEPA and MHPA, respectively. As the sample depletion for all the target compounds are much less than 5%, the criterion of negligible depletion [32], the C_W can be regarded as a constant during the extraction and thus the initial C_W was adopted to calculate D_{OW} directly.

3.2. Determination of K_{OW} and pK_a values of DPAs

In general, ionogenic compounds ionize in aqueous solution and exist in a distribution state of neutral and charged species, which are strongly dependent on the ionizing capability of the molecule (pK_a) and the surrounding pH [20]. Owing to neutral and charged species exhibit different polarities, the D_{OW} values of ionizable molecules are strongly pH dependent [10]. Thus, pH can markedly affect the status of ionizable compounds and influence partition of neutral and ionic forms between 1-octanol and water solution. Fig. 2 depicts the D_{OW} of MEPA, DEPA and MHPA from pH 1–10 determined by nd-HF-LPME coupled with UPLC–MS/MS. The typical sigmoidal decrease curves of D_{OW} against pH indicates the largely dependence of the D_{OW} of DPAs upon pH of the sample solution. The D_{OW} values decrease slightly with the increase of pH from 1.0 to 2.0, and decrease sharply with the further increase of pH to 3.0, and then approximate to zero when pH value is over 5.0. This is because D_{OW} is defined as the ratio of the sum of all species (neutral and ionized) partitioning into 1-octanol to that in sample solution phase at any given pH, the pH increase could accelerate the

dissociation of an acidic compound and therefore reduce its partitioning into the 1-octanol phase, as the neutral compound is more lipophilic than the charged one.

As shown in Fig. 2, the experimental data fitted well with Eq. (5) with correlation coefficients $R^2 \geq 0.9961$. As shown in Table 2, the pK_a values of MEPA, DEPA and MHPA were 3.02 ± 0.04 , 3.08 ± 0.02 and 3.17 ± 0.06 , respectively. The nonlinear regression values of $\log K_{OW}$ of the neutral form ($\log K_{OW,HA}$) and ionic form ($\log K_{OW,A}$) were also listed in Table 2, the difference between $\log K_{OW}$ of the neutral ($\log K_{OW,HA}$) and ionic forms ($\log K_{OW,A}$) varies with the compound. The lipophilicity differences ($\Delta \log K_{OW}$) between neutral species and ionized species of MEPA, DEPA and MHPA were 3.19, 2.37 and 1.79 respectively, indicating the indispensability of detection $\log K_{OW,A}$ of ionized species.

The effect of initial concentration on the measured D_{OW} was also investigated. According to previous knowledge, D_{OW} was expected to be solute concentration independent. In this work, six concentration levels (50, 100, 250, 500, 1000, 2500 ng mL⁻¹) were selected to evaluate effect of the variation of initial analytes concentration on the D_{OW} . The $\log K_{OW,HA}$ and $\log K_{OW,A}$ values kept constant in the whole range of studied concentration for three DPAs (Fig. 3), confirming that $\log D$ values were independent of the solute concentration. Due to their high hydrophilicity, however, the contents of MEPA and DEPA partitioned into 1-octanol were just slightly higher than instrumental detection limits (MEPA: 0.5 ng L⁻¹; DEPA: 0.4 ng L⁻¹) at concentration level of 50 and 100 ng mL⁻¹ at sample solution pH > 3. Hence, considering the poor partition of DPAs in 1-octanol, especially for the ionic ones, relatively higher initial concentrations (1000, 500 and 250 ng mL⁻¹ for MEPA, DEPA and MHPA, respectively) were spiked to phosphate buffer to enhance their response in the further UPLC–MS/MS analysis.

Fig. 2. The distribution coefficients (D_{ow}) of DPAs to 1-octanol in the lumen of hollow fiber measured at various sample pH. The solid curves are Eq. (5) curve-fitting output of the experimental data with the GraphPad Prism. Sample solution: MEPA, DEPA and MHPA were added into 620 mL of 1-octanol saturated 1 mM sodium dihydrogenphosphate buffer with initial concentration were 250, 500 and 1000 ng mL⁻¹, respectively.

Table 2

Comparison of DPAs pK_a and $\log K_{ow}$ obtained by curve-fitting of experimental data according to Eq. (5) with calculated by theoretical models.

Compound	pK_a		$\log K_{ow,HA}$				$\log K_{ow,A}$		R^2
	Exp. ^a	ACD/ pK_a DB ^b	Exp.	AB/ $\log P$	ACD/Labs	KOWWIN	Exp.	$\Delta \log K_{ow}$ ^c	
MEPA	3.02 ± 0.04	3.04 ± 0.5	-0.67 ± 0.01	-1.09	0.15 ± 0.56	-0.66	-3.86 ± 2.7	3.19	0.9987
DEPA	3.08 ± 0.02	3.18 ± 0.5	-0.23 ± 0.003	-0.75	0.68 ± 0.56	-0.17	-2.60 ± 2.58	2.37	0.9997
MHPA	3.17 ± 0.06	3.22 ± 0.2	1.02 ± 0.01	0.9	1.68 ± 0.56	1.11	-1.33 ± 0.77	2.35	0.9961
AAE (P -value) ^e	-	0.06 (0.113)	-	0.35 (0.097)	0.94 (0.008)	0.20 (0.157)			

^a Exp. represents the results obtained from experimental data fitting.

^b ACD/ pK_a DB represents pK_a values calculated by ACD/ pK_a DB ver. 12.1.0.50374.

^c $\Delta \log K_{ow}$, the difference between $\log K_{ow,HA}$ and $\log K_{ow,A}$.

^d R^2 , squared correlation coefficients of the experimental data fitting.

^e AAE is the average absolute errors between experimental values and the predicted values and P -value in parentheses was obtained from a paired t -test with a two-tailed distribution.

Fig. 3. The lipophilicity ($\log K_{ow,HA}$ and $\log K_{ow,A}$) – concentration profiles obtained for three DPAs.

Fig. 4. Plots of $\log D_{(\text{exp.})}$ and $\log D_{(\text{ACD})}$ against pH. $\log D_{(\text{exp.})}$ and $\log D_{(\text{ACD})}$ represent the experimental $\log D$ (solid cycles), each data point is an average of five repeats and predicted $\log D$ (open cycles) respectively.

3.3. Comparison of measured pK_a and partition coefficients with computationally calculated values

The measured pK_a values were compared with the predicted ones by ACD/ pK_a DB module, which is considered to be the most reliable pK_a theoretical calculation program [27] and the experimental $\log K_{OW,A}$ values were also compared with the predicted ones by three commonly used $\log K_{OW}$ calculation programs. The pair- t test P -values for predicted models are listed in Table 2. There were evidently no statistically significant difference between measured data and the predicted pK_a values of studied three DPAs, with a low error (AAE = 0.06) and acceptable paired t -test P -value (0.113). However, a significant difference between the $\log K_{OW,HA}$ values obtained by experiment (-0.67 ± 0.01 for MEPA, -0.23 ± 0.003 for DEPA and 1.02 ± 0.01 for MHPA) and that predicted by ACD/ $\log P$ (0.15 ± 0.56 , 0.68 ± 0.56 , 1.68 ± 0.56) was observed with a paired t -test P -value less than 0.05, showing that the $\log K_{OW,HA}$ values of DPAs predicted by ACD/ $\log P$ module are questionable. Back to the $\log K_{OW}$ values of DPAs listed in Table 2, there is no significant difference between measured $\log K_{OW,HA}$ and the predicted values by KOWWIN and AB/ $\log P$ with P values of 0.157 and 0.097, respectively. From the P -values and AAEs listed in Table 2, it can be clearly concluded that KOWWIN shows superiority in predicting $\log K_{OW}$ of DPAs with the lowest error (AAE) and the highest P -value. This is because KOWWIN was developed in a long iterative process, using simple organics for increment derivation [28].

Fig. 4 shows the profiles of lipophilicity against pH ($\log D$ vs pH) of three DPAs in the range of pH 1–10 measured by the nd-HF-LPME UPLC–MS/MS method and that predicted by ACD/ $\log D$ module. A sigmoidal decrease of DPAs' $\log D$ s with increasing sample pHs were observed. The experimental $\log D$ s of MEPA, DEPA and MHPA fell in the range of -5.01 – -0.66 , -4.81 – -0.23 and -3.08 – -1.01 respectively, showing a considerably variation in compounds. As molecules in neutral form are generally more lipophilic than their charged counterparts, so the acidic DPAs changed from nonionized lipophilic state to ionized hydrophilic state with the increase of solution pH. The curves of $\log D$ measured by experiment closely resemble to those predicted by ACD/ $\log D$ program but are slightly offset towards lower pH values, especially for stronger polar compounds like MEPA and DEPA, indicating ACD/ $\log D$ might more or

less underestimate the $\log D$ s of more polar compounds at pHs below their corresponding pK_a values. In turn, the pK_a values of DPAs could be determined by profiling $\log D$ against solution pH. As aforementioned, similar lipophilicity/pH profiles of three analytes (MEPA, DEPA and MHPA) were inverse sigmoidal with respect to $\log D$. The pK_a value of 3.02, 3.08 and 3.17 of MEPA, DEPA and MHPA, at which half of each compounds are in ionized state, lie soon after the logarithm of distribution coefficients, that is $\log D$ s, started to reduce.

4. Conclusions

The pK_a and $\log D$ values of three DPAs were simultaneously determined by nd-HF-LPME coupled with UPLC–MS/MS. For MEPA, DEPA and MHPA, the pK_a values were close and around 3, but the $\log D$ values were strongly pH-dependent with values from -5.01 to 1.01 . The $\log K_{OW}$ of the neutral form and ionic form were in the range of -0.67 – 1.02 and -3.86 – -1.33 , respectively, indicating low lipophilicity. The experimental measured pK_a values obtained here were in good agreement with values predicted by ACD/ pK_a DB module. Among the three $\log K_{OW}$ prediction programs, KOWWIN produced relative better results and thus would be an alternative method to estimate $\log K_{OW}$ of DPAs when there were no available experimental values. To our best knowledge, $\log D$ and pK_a values of MEPA, DEPA and MHPA were reported for the first time, which should be of interest to study their environmental processes and effects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2017.06.002>.

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