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Kinetic Calibration for Automated Headspace Liquid-Phase Microextraction

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The kinetics of the absorption and desorption of analytes for headspace liquid-phase micoextraction (HS-LPME) were studied. It was found that the desorption of analytes from the extraction phase into the sample matrix is isotropic to the absorption of the analytes from the sample matrix into the extraction phase under the same conditions. This therefore allows for the calibration of absorption using desorption. Calibration was accomplished by exposing the extraction phase, which contained a standard, to the sample matrix. The information from the desorption of the standard, such as time constant α, could be directly used to estimate the concentration of the target analyte in the sample matrix. This new kinetic calibration method for headspace LPME was successfully used to correct the matrix effects in the BTEX analysis of an orange juice sample. In this study, the headspace LPME techniques were successfully fully automated, for both static and dynamic methods, with the CTC CombiPal autosampler. All operations of headspace LPME, including sample transfer and agitation, filling of extraction solvent, exposing the solvent in the headspace, withdrawing the solvent to syringe and introducing the extraction phase into injector, were autoperformed by the CTC autosampler. The fully automated headspace LPME technique is more convenient and improved the precision and sensitivity of the method. This automated dynamic headspace LPME technique can be also used to obtain the distribution coefficient between the sample matrix (aqueous or another solution) and the extraction phase (1-octanol or another solvent). The distribution coefficient between 1-octanol and orange juice, at 25 °C, was obtained with this technique.

Recently, liquid-phase microextraction (LPME), or solvent microextraction, has been developed as a novel sample preparation and enrichment technique for chromatography and electrophoresis. LPME is fast, simple, and inexpensive and combines extraction, concentration, and sample introduction in one step; thus, several LPME techniques have been developed and applied to environmental and biological analysis.[1−4] To date, the developed methods for LPME, which is similar to solid-phase microextraction (SPME),[5] include direct-immersed LPME (DI-LPME),[6−8] headspace LPME (HS-LPME),[9−11] and hollow fiber-protected LPME (HF-LPME).[12−14]

In DI-LPME or single-drop microextraction, a drop of a water-immiscible solvent is suspended at the tip of either a Teflon rod or a microsyringe needle and directly immersed into a stirred sample solution.[6−8] The analytes are extracted from the aqueous sample into the single-drop solvent. After extraction, the microdrop solvent is retracted into the needle and then injected directly into a gas chromatography (GC) system. The DI-LPME has been also reported in dynamic LPME systems.[8] In dynamic DI-LPME, 1−2 μL of solvent is withdrawn into a 10-μL microsyringe and 3−5 μL of aqueous sample is subsequently filled into the microsyringe. After a few seconds, the sample is expelled and the needle is filled with a new aliquot of the sample. This procedure is repeated several times, and finally, the organic solvent is injected into a GC system. Because the single-drop DI-LPME method is not very robust, and droplets may be lost from the needle tip of the syringe during extraction, the hollow fiber-protected LPME and headspace LPME methods were developed. HF-LPME uses a hollow fiber to stabilize and protect the organic drop to enhance the extraction efficiency.[12−14] HS-LPME extracts volatile analytes, by placing a microdrop of organic solvent, suspended at the tip of microsyringe, into the headspace of the sample solution.[9−11] Subsequently, dynamic HF-LPME and HS-LPME methods have been published,[10,14] and the dynamic process can be performed automatically.[15−17]

The external calibration method can be used for LPME when the matrix is simple, such as air or clean water, and the distribution coefficients are similar to a pure matrix. Internal standardization and standard addition are important calibration

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approaches that are very effective for complex matrixes. They compensate for the additional capacity or activity of the sample matrix. However, such approaches require the delivery of a standard. This is incompatible in some sampling situations, such as with on-site or in vivo investigations. This approach is also not practical for conventional exhaustive extraction techniques, since the extraction parameters are designed to facilitate complete removal of the analytes from the matrix.[18]

To address these needs, a new kinetic calibration technique of SPME has been developed in Pawliszyn's group.[18−21] The kinetic calibration is accomplished by exposing a SPME fiber, preloaded with a standard, to the sample matrix, during which the desorption of standard and the absorption of analytes occur simultaneously. When the standard is an isotopically labeled analogue of the target analyte, the information from the desorption process, such as time constant a, can be directly used to estimate the concentration of the target analyte.[18] The new developed calibration technique has been successfully used in on-site and in-vial investigations.[18,20,22]

Similar to SPME, LPME is also an equilibrium extraction technique rather than an exhaustive extraction technique. It means that a substantial portion of the analytes still remain in the matrix even after the extraction process is completed. This presents an opportunity for quantification of the LPME method, by implementing the kinetic calibration approach.

In this study, the headspace LPME techniques were successfully fully automated, for both static and dynamic methods, with a CTC CombiPal autosampler. The automated HS-LPME techniques were used to investigate the kinetics of the absorption and desorption processes of HS-LPME, and the isotropic nature of the desorption and absorption processes was demonstrated. The developed kinetic calibration method for HS-LPME was successfully used to correct the matrix effects in the BTEX analysis of an orange juice sample.

THEORETICAL CONSIDERATIONS

For three-phase headspace LPME in a vial, the amount of analyte in the system will remain the same before and after extraction. At equilibrium, this mass balance equation can therefore be expressed by eq 1,

$$n_T = n_e + n_h + n_s \tag{1}$$

where n_T is the total number of moles of the analyte in the system and n_e, n_h, and n_s are the amount of analyte in the extraction phase, headspace, and sample at equilibrium, respectively. Thus, eq 1 leads to eq 2,[5]

$$n_e = \frac{K_{es}V_e}{K_{es}V_e + K_{hs}V_h + V_s} n_T \tag{2}$$

where V_e, V_h, and V_s are the volume of the extraction phase, headspace, and sample, respectively. K_{es} and K_{hs} are the extraction phase/sample and headspace/sample distribution coefficients, respectively. Equation 2 can be expressed by eq 3,

$$n_e = \frac{K_{es}V_eV_s}{K_{es}V_e + K_{hs}V_h + V_s} C_0 \tag{3}$$

where C_0 is the initial concentration of the analyte in the sample. According to eq 3, if n_e, the amount of analyte in the liquid extraction phase at equilibrium, was obtained, then C_0, the initial analyte concentration in the sample, can be calculated with K_{es} and K_{hs}.

The kinetic process for the absorption of headspace LPME can be described with eq 4,[23]

$$n/n_e = 1 - \exp(at) \tag{4}$$

where n is the amount of analyte in the extraction phase at time t and a is a constant that is dependent on the volumes of the extraction phase, headspace, and sample, mass-transfer coefficients, distribution coefficients, and the surface area of the extraction phase. The kinetic process of the desorption of the analyte (internal standard) from the extraction phase is defined by eq 5,[18]

$$q = q_0 \frac{V_s}{K_{es}V_e + V_s}[1 - \exp(-at)] \tag{5}$$

where q is the amount of standard lost from the extraction phase at time t and q_0 is the amount of preadded standard in the extraction phase. Let $Q = q_0 - q$, and Q is the amount of the standard remaining in the extraction phase after exposure of the extraction phase to the sample matrix for the sampling time, t. Thus, for the in-vial desorption process, eq 5 can be expressed by eq 6,[20]

$$\frac{Q - q_e}{q_0 - q_e} = \exp(-at) \tag{6}$$

where q_e is the amount of standard remaining in the extraction phase at equilibrium. If the desorption and absorption processes occur under the same experimental conditions, the constant a should be the same/similar for the same compounds/similar compounds. According to eqs 4 and 6, there are two methods for obtaining n_e, the amount of analyte in the extraction phase at equilibrium. One approach involves using the target analytes as standards preadded in the extraction phase to obtain a desorption time profile. $\ln(Q - q_e/q_0 - q_e)$ should change linearly with the desorption time and the slope is $-a$, according to eq 6. The constant a can be used in eq 4, if the absorption process occurred under the same conditions, and n_e can then be obtained. For this kinetic calibration method, the desorption process and absorption process are performed separately. The second kinetic calibration method involves performing the desorption and absorption processes simultaneously. Deuterated compounds or compounds with similar physicochemical properties could be used as the

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internal standard, and the desorption and absorption time profile would then be obtained at the same time. Because the constant a should be similar for both the desorption and the absorption, eq 4 and eq 6 can be combined to

$$\frac{n}{n_e} + \frac{Q - q_e}{q_0 - q_e} = 1 \qquad (7)$$

and the constant a is therefore not needed.

EXPERIMENTAL SECTION

Chemicals and Supplies. All chemicals were of analytical grade. Benzene, benzene-d_6, toluene, toluene-d_8, ethylbenzene, *o*-xylene, and 1-octanol (HPLC, 99+%) were from Sigma-Aldrich (Mississaga, ON, Canada). HPLC grade methanol was purchased from BDH (Toronto, ON, Canada). Hamilton model 701N 10-μL syringes (26s gauge, no. 2 point style bevel tip) were purchased from Hamilton (Reno, NV). The 10-mL screw vials with magnetic crimp caps and PTFE-coated silicone septa (Supelco, Oakville, ON, Canada) were used for the automated analysis. Water was purified with a Nanopure filter (Barnstead, Dubuque, IA). Ultrahigh-purity helium was purchased from Praxair (Kitchener, ON, Canada). The orange juice sample was purchased from a local supermarket.

Instrument. A Saturn 3800 GC/2000 ITMS system fitted with a SPB-5 column (30 m, 0.25-mm i.d., 0.25-μm film thickness) (Supelco, Mississauga, ON, Canada) was used for the analysis of BTEX (benzene, toluene, ethylbenzene, *o*-xylene). Helium was used as the carrier gas and was set at 2 mL/min. The column temperature was maintained at 80 °C for 1 min and then programmed to increase by 20 °C/min to 120 °C, then programmed to increase by 50 °C/min to 250 °C, and then held for 4.4 min. The total run time was 10 min. An i.d. 2-mm liner packed with glass wool was used for the 1079 injector. The injector was set to 250 °C with a split ratio of 10:1. The MS system was operated in the electron ionization (EI) mode and tuned to perfluorotributylamine. The EI was set to turn on at 1 min and turn off at 3 min (before the elution of the solvent). A mass scan from m/z 40 to 120 was used, and quantification was performed using m/z 78 for benzene, m/z 84 for benzene-d_6, m/z 98 for toluene-d_8, and m/z 91 for toluene, ethylbenzene, and *o*-xylene.

Extraction and Desorption Procedure. All of the extraction procedures were performed with a CTC CombiPal autosampler (Zwingen, Switzerland) using the associated Cycle Composer software (ver 1.4.0). An 870 μg/mL stock solution of BTEX was prepared in methanol. The 870 ng/mL standard solutions of BTEX were prepared daily by spiking the stock solution in pure water with a CTC autosampler. To avoid the effect of time delay between spiking and sampling, the solution was spiked prior to capping and the spike was added below the level of the water in the vial.[20]

The extraction of the analytes in the sample and the desorption of the internal standard were performed in a 10-mL vial that contained 3 mL of 870 ng/mL BTEX aqueous solution for the determination of the absorption profile or 3 mL of pure water for the determination of the desorption profile. The single-drop extraction phase was 1 μL of 1-octanol, for the determination of the absorption profile, or 1 μL of 1-octanol containing 870 μg/mL internal standard (BTEX or benzene-d_6 and toluene-d_8), for the determination of the desorption profile.

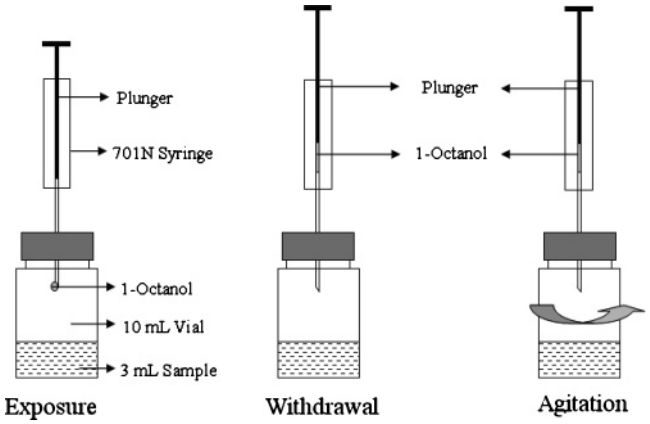

Figure 1. Schematic diagram of the automated dynamic HS-LPME procedure.

For static HS-LPME, the 10-mL sample vial was transferred from the sample tray to the vortex agitator with a temperature controller, shaken for 2 min at 500 rpm, and then the Hamilton 701 10-μL syringe, filled with 1 μL of 1-octanol, pierced the septum and slowly exposed the 1 μL of 1-octanol (0.1 μL/s) in the headspace of the sample vial. After different extraction times, the 1 μL of 1-octanol was slowly withdrawn into the barrel (0.1 μL/s) and introduced into the GC/MS for analysis.

For dynamic HS-LPME, the sample vial was also shaken for 2 min at 500 rpm in the vortex agitator, and then the Hamilton 701 10-μL syringe, filled with 1 μL of 1-octanol, pierced the septum, the plunger was slowly depressed to expose the 1 μL of 1-octanol (0.1 μL/s) in the headspace of sample vial, and then the 1 μL of 1-octanol was slowly withdrawn (0.1 μL/s) to the barrel of the syringe. Subsequently, the sample vial was shaken 10 s at 500 rpm, and the 1 μL of 1-octanol was exposed and withdrawn again (Figure 1). This exposure−withdrawal−agitation procedure was repeated different times before the 1 μL of 1-octanol was introduced to GC/MS. (Caution: since the needle of the microsyringe will be shaken with sample vial when performing the dynamic operation, the dynamic program must to be carefully set, otherwise the needle of syringe will be easy damaged. The syringe can be used repeatedly, for at least a few hundred experiments, if the program was optimized.)

Quantification. The solvent for the standard solution preparation will affect the sample transfer into the GC column, especially if the standard solution is introduced into a high-temperature injector.[24] In this study, it was found that the BTEX peak areas for a 1-μL 870 μg/mL methanolic solution, compared with the BTEX peak areas for a 1-μL 870 μg/mL 1-octanolic solution, were just ~50%. To avoid the effect of solvent on the quantification, the standard solutions for calibration also were prepared with 1-octanol. Good precision (RSD < 5%) and linearity (R^2 > 0.999) were obtained for the calibration curves by using a 1-octanolic standard solution.

RESULTS AND DISCUSSION

Kinetics of Static HS-LPME. The first challenge of the study was to demonstrate that the desorption of analytes from the

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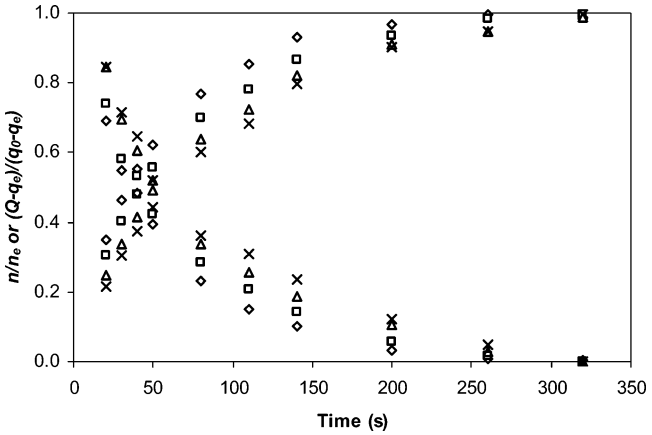

Figure 2. Absorption and desorption time profile of BTEX at 25 °C for the automated static HS-LPME procedure. ◇, benzene; □, toluene; △, ethylbenzene; ×, *o*-xylene.

extraction phase into the sampling matrix is isotropic to the absorption of the analytes into the extraction phase from the sample matrix under the same conditions. For this reason, the kinetics of absorption and desorption for static HS-LPME were studied.

Figure 2 presents the values of $(Q - q_e)/(q_0 - q_e)$ calculated from the resulting desorption time profile and the values of n/n_e calculated from the resulting absorption time profile at 25 °C. The sum of $(Q - q_e)/(q_0 - q_e)$ and n/n_e at any time is close to 1 for any BTEX component. Comparing the time constant a of the absorption and the desorption clearly shows the isotropism of absorption and desorption, as the time constant a should be the same for both the absorption and the desorption. Figure 3 illustrates the desorption and adorption time profiles at 25 °C where $\ln(Q - q_e)/(q_0 - q_e)$ or $\ln(n/n_e)$ is used as the *y*-axis, because $\ln(Q - q_e)/(q_0 - q_e)$ and $\ln(n/n_e)$ should change linearly with desorption/absorption time and the slope is $-a$, according to eqs 4 and 6. Table 1 presents the time constant a of absorption and desorption for BTEX at 25 and 50 °C. The results show that the time constant a of absorption is very close to the time constant a of desorption for all BTEX components, at 25 and 50 °C. It demonstrates that the desorption of analytes from the extraction phase into the sampling matrix is isotropic to the absorption of the analytes into the extraction phase from the sample matrix under the same experimental conditions, and a change in the temperature will not affect the isotropism. The results also show that the time constant a will increase when the temperature is increased, which demonstrates that a higher temperature will result in a shorter equilibrium time. But the increase in temperature sometimes is undesirable due to the corresponding reduction in the distribution coefficient, and the amount of analytes absorbed by the extraction phase will also decrease at equilibrium. In this study, only ~60% of the analytes were absorbed by the extraction phase at 50 °C, compared with at 25 °C, during the automated static HS-LPME procedure.

The desorption time profiles of deuterated benzene (d_6) and deuterated toluene (d_8) and the absorption time profiles of benzene and toluene were determined simultaneous. The deuterated benzene and toluene were added to a 1-octanol (870 μg/mL) solution, and then the 1-octanol was exposed to the headspace of the sample solution for different time periods. Figure 4 presents

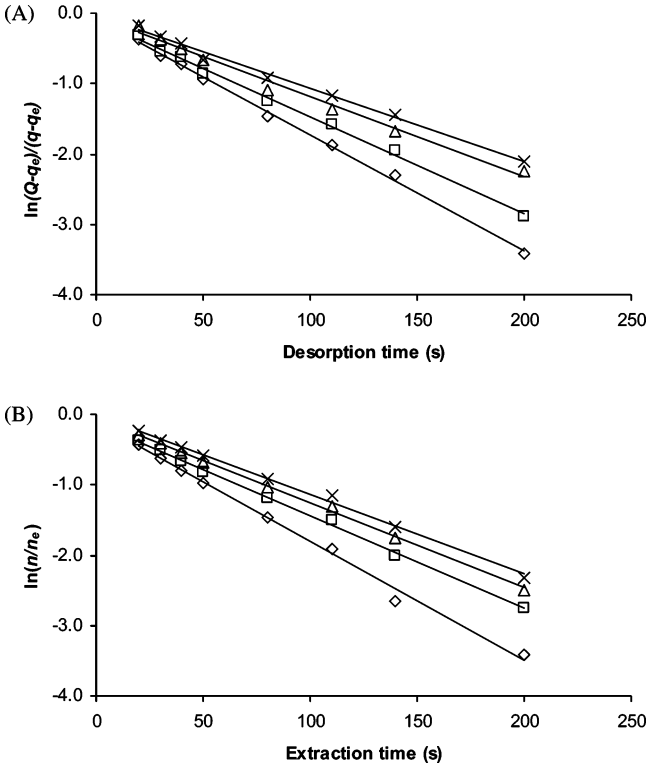

Figure 3. Desorption (A) and absorption (B) time profile of $\ln(Q - q_e)/(q_0 - q_{e-}) - t$ or $\ln(n/n_e) - t$ at 25 °C for the automated static HS-LPME procedure. ◇, benzene; □, toluene; △, ethylbenzene; ×, *o*-xylene.

the values of $(Q - q_e)/(q_0 - q_e)$ calculated from the resulting desorption time profiles of deuterated benzene and toluene, and the values of n/n_e calculated from the resulting absorption time profiles of benzene and toluene. The sum of $(Q - q_e)/(q_0 - q_e)$ and n/n_e is close to 1 (the results range from 1 to 1.2). The sum of $(Q - q_e)/(q_0 - q_e)$ and n/n_e is higher than 1 due to the slight differences of the physicochemical properties between benzene-d_6 and benzene and toluene-d_8 and toluene.

Although the isotropism of the absorption and desorption process for static HS-LPME was demonstrated, there is still a problem associated with using the kinetic method to calibrate the static HS-LPME procedure: when n_e, the amount of analyte absorbed by the extraction phase at equilibrium, K_{ow}, the distribution coefficient between 1-octanol and water, and K_{aw}, the distribution coefficient between the headspace (air) and water (Henry's law constant), are used to estimate the concentration of analytes in the sample, and the relative recovery is ~50% (Table 2). This low recovery is due to the static nature of the sample and the extraction phase. Under the static condition, the mass transfer of analytes in the liquid phases, for both 1-octanol and water, is very slow and therefore only part of the system reached equilibrium. Although the absorption and desorption is isotropic for static HS-LPME, using the equilibrium method for the calibration is unsuitable. Therefore, the static HS-LPME method must be modified to ensure that the whole system, including the sample solution and the extraction phase, can reach an equilibrium.

Kinetics of Dynamic HS-LPME. To ensure that the whole system can reach equilibrium, a fully automated dynamic HS-LPME method was developed. This method can renew the

Table 1. Time Constant *a* for the Absorption and Desorption of BETX for the HS-LPME Method

		time constant a (R^2)			
		benzene	toluene	ethylbenzene	*o*-xylene
static	absorption (25 °C)	0.0165 (0.998)	0.0138 (0.995)	0.0114 (0.988)	0.0104 (0.993)
	desorption (25 °C)	0.0169 (0.995)	0.0132 (0.998)	0.0117 (0.998)	0.0113 (0.996)
	absorption (50 °C)	0.0335 (0.998)	0.0239 (0.994)	0.0236 (0.991)	0.0215 (0.995)
	desorption (50 °C)	0.0325 (0.998)	0.0250 (0.998)	0.0238 (0.999)	0.0221 (0.996)
dynamic	absorption (25 °C)	0.146 (0.999)	0.151 (0.987)	0.144 (0.996)	0.131 (0.995)
	desorption (25 °C)	0.152 (0.999)	0.159 (0.993)	0.145 (0.994)	0.138 (0.990)

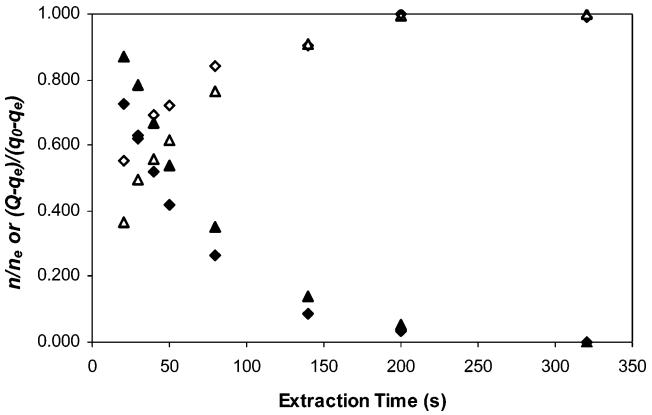

Figure 4. Desorption time profile of benzene-d_6 and toluene-d_8 and the absorption time profile of benzene and toluene at 25 °C. ◇, benzene; △, toluene; ◆, benzene-d_6 and ▲, toluene-d_8.

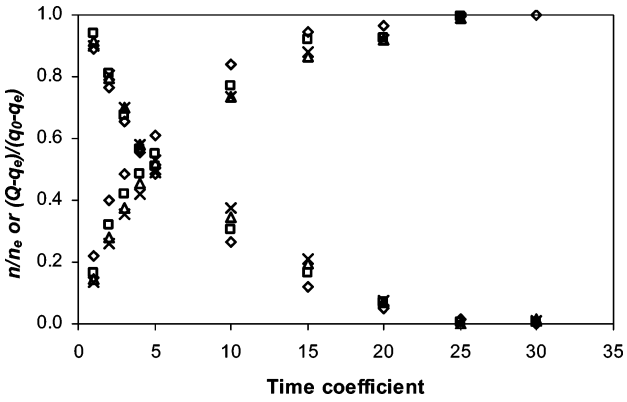

Figure 5. Absorption and desorption time profile of BTEX at 25 °C for the automated dynamic HS-LPME procedure. ◇, benzene; □, toluene; △, ethylbenzene; ×, *o*-xylene.

Table 2. Distribution Coefficient and Henry's Law Constant for BTEX at 25 °C and Calculated Recoveries of BTEX for the Automated Static and Dynamic HS-LPME Methods

	benzene	toluene	ethylbenzene	*o*-xylene
K_{ow} ($\log K_{ow}$)[a]	150 (2.18)	490 (2.69)	1380 (3.14)	1348 (3.13)
K_{aw} [b]	0.216	0.263	0.318	0.204
K_{oa} [c]	694	1863	4339	6607
K_{oj} ($\log K_{oj}$)[d]	248 (2.39)	527 (2.72)	803 (2.90)	646 (2.81)
K_{hj} [e]	0.357	0.283	0.185	0.098
relative recovery (%) (static)	51	54	48	46
relative recovery (%) (dynamic)	115	104	99	88

[a] Reference 29. [b] Reference 30. [c] K_{oa}, the distribution coefficient of BTEX between 1-octanol and air at 25 °C, calculated from $K_{ow} = K_{aw}K_{oa}$. [d] K_{oj}, the distribution coefficient between 1-octanol and orange juice. [e] K_{hj}, the distribution coefficient between the headspace and orange juice.

surface of the sample solution and the extraction phase in a fully automated process. Because the CTC autosampler is equipped with a vortex agitator, the renewal for the surface of the sample solution and the surface of extraction phase must be performed in different steps to avoid dropping of the extraction phase. It means that the extraction phase should be withdrawn into the syringe barrel when the sample solution is shaking. The automated dynamic HS-LPME procedure involves exposing the extraction phase in the headspace, withdrawing the extraction phase into the syringe barrel, and subsequent agitation of the sample (Figure 1). The surface of the extraction phase was renewed by moving the syringe plunger, and the surface of the sample solution was renewed by agitation. The test results of the new method show that the volume of the extraction phase was constant after 50 repetitions of the procedure. These findings demonstrate that the new fully automated dynamic HS-LPME is suitable for the study of equilibrium extractions.

Figure 5 illustrates the absorption and desporption time profiles of BTEX at 25 °C produced with the automated dynamic HS-LPME method. The *x*-axis is the time coefficient, which is the number of repetitions of the exposure−withdrawal−agitation procedure. The results show that, after 30 repetitions, the desorption/absorption reached equilibrium. Using n_e, the amount of analyte absorbed by the extraction phase at equilibrium, to estimate the concentration of analytes in the sample, the relative recoveries were 88−115% (Table 2). These findings demonstrate that the whole system reached equilibrium and the concentration of analyte can be estimated with eq 3.

The time constants *a* for the absorption and the desorption of BTEX at 25 °C for the automated dynamic HS-LPME are listed in Table 1. The time constants *a* for the absorption and the desorption are close for all of the BTEX components. These results demonstrate that the absorption and desorption are also isotropic for the automated dynamic HS-LPME procedure.

Application to Real-Sample Matrix. To test the kinetic calibration of the HS-LPME method, the technique was used to quantify BTEX in spiked orange juice with GC/MS.

1. Determination of 1-Octanol/Orange Juice Distribution Coefficient. The kinetic calibration method is an equilibrium standardization technique. When this method is applied to HS-LPME to estimate the concentration of analyte in a sample solution, the extraction phase/sample distribution coefficient and headspace/sample distribution coefficient should be known. When 1-octanol is used as the extraction phase, the extraction phase/

sample distribution coefficient (K_{ow}) and headspace/sample distribution coefficient (K_{aw}, Henry's law constant, if the effect of moisture in the gaseous headspace can be neglected) for most analytes can be found in references. More specifically, if the method is used for the analysis of orange juice, the distribution coefficient between 1-octanol and orange juice should be known.

In three-phase headspace LPME in a vial, the distribution coefficient between the extraction phase and the headspace (K_{eh}) and between the headspace and the sample (K_{hs}) can be considered:

$$K_{eh} = C_{eq,e}/C_{eq,h} \quad (8)$$

$$K_{hs} = C_{eq,h}/C_{eq,s} \quad (9)$$

where $C_{eq,e}$, $C_{eq,h}$, and $C_{eq,s}$ are the concentrations of target analyte in the extraction phase, headspace, and sample solution at equilibrium, respectively. The distribution coefficient between the extraction phase and sample (K_{es}) can be written as

$$K_{es} = C_{eq,e}/C_{eq,s} = K_{eh}K_{hs} \quad (10)$$

The results of the automated dynamic HS-LPME show that, by using n_e, the amount of analyte absorbed by the extraction phase at equilibrium, to estimate the concentration of analytes in the sample, the deviations are less than 15% for all of the BTEX components studied (Table 2). This illustrates that the automated dynamic HS-LPME method can be used to estimate the distribution coefficient between the extraction phase and the sample, as well as between the headspace and the sample. As K_{eh} or K_{oa}, the distribution coefficient between 1-octanol and air, can be calculated from $K_{ow} = K_{aw}K_{oa}$ (the values of K_{oa} for BTEX at 25 °C are listed in Table 2), and C_0, the initial concentration of analyte in the sample, can be fixed through two ways. The first one is spiking. When the amount of analyte added into sample is much higher than the initial amount of analyte, i.e., hundreds times, the effect of the initial amount of analyte to C_0 can be neglected. But this method will be limited if the initial concentration of analyte in the sample was very high. The second way is using standard addition method. Using standard addition method, C_0 can be calculated by eq 11 when the volume of the added standard is very small, comparing with the volumn of the sample.

$$C_0 = \frac{n_0}{V_s} = \frac{n_1 n_a}{(n_2 - n_1)V_s} \quad (11)$$

Where n_1 and n_2 are the amount of analyte absorbed by extraction phase for the sample and the sample added standard, n_a is the amount of standard added into sample, and V_s is the volumn of the sample. The amount of analyte absorbed by the extraction phase at equilibrium, n_e, can be determined by using automated dynamic HS-LPME. Then, K_{es}, the distribution coefficient between the extraction phase and the sample, as well as K_{hs}, the distribution coefficient between the headspace and the sample, can be calculated by using eqs 3 and 10.

Table 2 presents the distribution coefficient between 1-octanol and orange juice (K_{oj}), and between the headspace and orange juice (K_{hj}), by the automated dynamic HS-LPME method (The initial concentration of analyte in the sample, C_0, was obtained through spiking. The exposure−withdrawal−agitation procedure was repeated 40 times to determine n_e, because the mass transfer in orange juice was slower than in pure water and the extraction time profile shows that the procedure needed to be repeated 35 times to ensure that the system reached equilibrium.) The results show that the 1-octanol/orange juice distribution coefficient of benzene is higher than the 1-octanol/water distribution coefficient and the 1-octanol/orange juice distribution coefficients of ethylbenzene and *o*-xylene are lower than the 1-octanol/water distribution coefficient. No obvious change was found for the distribution coefficient of toluene.

Table 3. Calculated Recoveries of BTEX from Orange Juice, with and without Kinetic Calibration

	relative recovery (%) (RSD, %; $n = 3$)	
compound	using external calibration	using kinetics calibration
benzene	121 (5.3)	99 (5.8)
toluene	97 (4.6)	94 (3.5)
ethylbenzene	73 (5.7)	91 (5.6)
o-xylene	73 (6.3)	95 (3.5)

2. Analysis of BTEX in Orange Juice. The recoveries of the 870 ng/mL BTEX spiked orange juice samples, using the external calibration approach (using standards prepared in water) and the kinetic calibration approach are given in Table 3. For the kinetic calibration approach, the exposure−withdrawal−agitation procedure was repeated five times when the determination of real sample was performed. Time constant a was determined through the desorption of BTEX (becuase the amount of the analyte preadded in the extraction phase is much higher than the initial amount of analyte in the sample, the effect of the initial amount of analyte in the sample to the process of desorption can be neglected), and then the time constant a was used to calculate n_e, the amount of analyte in the extraction phase at equilibrium, by using eq 4. The concentrations of BTEX in orange juice were calculated with K_{oj} and K_{hj}, by using eq 3. The results demonstrate that the kinetic calibration technique produces a more accurate measure of the BTEX compounds in orange juice than the external calibration technique.

CONCLUSION

The kinetics of the absorption and desorption of analytes for headspace liquid-phase micoextraction were studied. It was found that the desorption of analytes from the extraction phase into the sample matrix is isotropic to the absorption of the analytes into the extraction phase from the sample matrix under the same conditions, and this allows for the calibration of absorption using desorption. The calibration was accomplished by exposing the extraction phase, prespiked with a standard, to the sample matrix. The information from the desorption of the standard, including the time constant a, could be directly used to estimate the concentration of the target analyte in the sample matrix.

The advantages of this new LPME calibration technique are obvious. First, this kinetic calibration technique efficiently avoids the interferences of the matrix effects. The kinetic calibration method for headspace LPME was successfully used to correct

for the matrix effects in the BTEX analysis of an orange juice sample. In addition, This new LPME kinetic calibration technique could also be used for on-site or field sampling or in combination with other solvent extraction field sampling techniques, such as solvent-filled devices,[25,26] semipermeable membrane devices,[27] and passive in situ concentration/extraction samplers.[28] Because the sample volume is large enough for field sampling, the q_e is equal to zero, and eq 7 is transformed to[18]

$$n/n_e + Q/q_0 = 1 \tag{12}$$

The sum of Q/q_0 and n/n_e should be 1, and therefore, the only parameters that need to be obtained are the amount of the internal standard that remains in the extraction phase and the amount of target analyte that was extracted by the extraction phase. Thus, the prespiked internal standard extraction phase was exposed in the field for a certain time and analyzed. The concentration of the target analyte in the field can be obtained according to eqs 12 and 13.[18]

$$C_0 = n_e/K_{es}V_e \tag{13}$$

The advantages of using this kinetic calibration method for field sampling are obvious: It makes the operation of field sampling and the calculation of the concentration of the target analytes more convenient, especially using 1-octanol as the extraction phase, since the distribution coefficients between 1-octanol and air or water can be obtained for most compounds. This approach will reduce the sampling time since it is a nonequilibrium sampling technique. In addition, the effects of the sampling environment, including the ambient temperature, flow rate, etc., can also be ignored when using this kinetic calibration method, since the desorption of the internal standard and the absorption of the target analyte will remain isotropic under the same experimental conditions.

In this study, headspace LPME techniques were successfully automated by using a CTC CombiPal autosampler for both static and dynamic methods. All steps of the headspace LPME method, including sample transfer and agitation, filling of the extraction solvent, exposure of the solvent in the headspace, withdrawal of the solvent in to the syringe, and introduction of the extraction phase into the injector, were automated by the CTC autosampler. The fully automated headspace LPME technique is more convenient, improves the precision and sensitivity, and can also be used for direct immersion LPME.

The automated dynamic headspace LPME technique can be also used to obtain the distribution coefficient between the sample matrix (aqueous or another solution) and the extraction phase (1-octanol or another solvent). The distribution coefficient between 1-octanol and orange juice of BTEX at 25 °C was obtained in this study with this method. Because the CTC CombiPal autosampler is equipped with a temperature-controlled agitator, the distribution coefficient of the solvent/sample at different temperatures can be easily obtained for various target analytes. Thus, the automated dynamic headspace LPME technique can be used to obtain the data of ΔH, the molar change in enthalpy of the analyte, when it moves from the sample to the extraction phase, according to

$$K_{es}(T_2) = K_{es}(T_1)\ \exp\frac{-\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{14}$$

where $K_{es}(T_1)$ and $K_{es}(T_2)$ are the extraction phase/sample distribution coefficient of the analyte when both extraction phase and sample are at temperature T_1 and T_2 and R is the gas constant.

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