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Theoretical Considerations on the Use of Solid-Phase Microextraction with Complex Environmental Samples

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The equations governing the use of equilibrium solid-phase microextraction (SPME) for environmental samples with complex heterogeneous matrices were derived in terms of parameters commonly measured or estimated by environmental scientists. Parameterization of the SPME equations allowed for the a priori prediction of SPME performance as a function of analyte and sample properties as well as experimental conditions. A theoretical evaluation of SPME was performed for a broad range of realistic scenarios using calculated equilibrium partitioning parameters and the implications for practical applications were discussed. Potential pitfalls and errors in quantitative measurements were identified, and different approaches to SPME calibration were presented. The concept of an optimum minimum volume for the analysis of heterogeneous environmental samples was presented and fully developed. Data from three previous studies were used to validate the correctness of our theoretical framework; the agreement between the measured relative recoveries of a variety of hydrophobic organic chemicals and theoretical predictions was reasonable. The results of this study highlight the potential for SPME to be a valuable technique for the measurement of hydrophobic organic contaminants in complex environmental samples. The SPME technique appears to be especially well suited for samples with high solids-to-water ratios and/or large sample volumes. Examples of such applications include sediment interstitial water and in situ field measurements, respectively.

Introduction

The analytical technique of solid-phase microextraction (SPME) was first introduced more than a decade ago by Arthur and Pawliszyn (*1*) and subsequently optimized and automated (*2*). Since that time, SPME has evolved into a widely used alternative to more traditional methods for the extraction of organic compounds from aqueous and gaseous media. The SPME method does not require any solvent, and it simultaneously extracts and concentrates organic analytes in a single step. In addition, its simplicity of use, relatively short sample processing times, the variety of available stationary phases, the ability to reuse fibers, and the potential for combining derivatization with extraction of polar analytes have made SPME an attractive choice for many analytical applications (*3, 4*).

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These aspects of SPME, among others, offer distinct advantages over conventional techniques such as liquid−liquid extraction and conventional solid-phase extraction. One field in which SPME has been used extensively is environmental analytical chemistry, where it has been applied for the analysis of hydrophobic, semivolatile, and volatile organics in surface water, wastewater, sediment porewater, and air samples (*4*−*16*) and also for determining partitioning parameters such as Henry's Law constants (*17*) and octanol−water partition coefficients (*18*). Although previous studies have clearly demonstrated the effectiveness of SPME as an alternative to conventional extraction methods, several studies achieved low spiked analyte recoveries with complex environmental samples (*6, 19, 20*). Matrix interferences were recognized as the cause of poor analyte recoveries, but no detailed quantitative information was provided (*6, 19*−*21*).

In contrast to the more conventional extraction methods, SPME usually does not endeavor to extract all or even most of an analyte from a sample. It is this aspect of SPME that can make calibration problematic. Calibration in SPME is usually performed using spiked standards prepared in pure water. For typical heterogeneous environmental samples, the assumption is that an SPME fiber would come to equilibrium with only the freely dissolved analytes in the water phase or the analytes in the vapor phase, depending on the methodology used. However, the fiber in such a sample is actually in indirect interaction with every phase in the system. For example, as an analyte is depleted from the dissolved phase by sorption to the fiber, the analyte is subsequently replenished via reequilibration with the other phases in the sample. Therefore, significant errors are incurred if analyte concentrations in such a system are calculated using calibration relationships derived from standards prepared in pure water. Pawliszyn (*3*) has pointed out that it is necessary to account for matrix effects in heterogeneous environmental samples by using the standard addition method or the internal calibration technique with isotopically labeled standards. However, the standard addition method can be extremely tedious and time-consuming for a large number of samples. In addition, isotopically labeled standards are usually very expensive and not available for all analytes of interest.

The simplest and most common applications of SPME rely upon attainment of equilibrium between a sorbent-coated silica fiber and the fluid component of an aqueous sample. During an extraction, the fiber may be immersed directly into or placed in the headspace above a sample. It can be shown that the amount ultimately sorbed by the fiber is the same for either an immersion or headspace extraction (*3*). SPME has also been used under nonequilibrium conditions with an associated loss in sensitivity and additional complexities with respect to calibration (*3, 12, 22*−*24*).

To evaluate the utility of SPME for the analysis of hydrophobic organic contaminants in heterogeneous environmental samples, it is necessary to have a thorough understanding of the behavior of the analytes in such a system before and after SPME. To this end, we rederive the governing equations of equilibrium SPME in terms of the parameters commonly measured or calculated by environmental scientists and subsequently used to understand and predict partitioning behavior of chemicals in complex environmental systems. After deriving the appropriate equations, realistic parameter values are used to theoretically evaluate the efficacy of SPME for analyzing such systems. The analytical performance of SPME is predicted as a function of analyte polarity, fiber properties, and sample parameters. The

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conditions under which SPME may be effectively used for measurements of hydrophobic organic contaminants in realistic environmental samples are discussed. Finally, predictions from the derived equations are compared to previous experimental results available in the literature.

Theoretical Background

We consider an analyte partitioning among a truly dissolved phase, a solid-phase comprised of suspended solids, a colloidal phase containing dissolved organic matter (DOM), and an air phase. Mass balance requires that the total amount of the analyte in the system is equal to the sum of the amounts in the individual phases. The total amount, N_0, of the analyte in the system before SPME can be expressed as

$$N_0 = N_s^0 + N_{dom}^0 + N_w^0 + N_a^0 \quad (1)$$

where N_s^0, N_{dom}^0, N_w^0, and N_a^0 are the amounts of the analyte in the solid, DOM, aqueous (truly dissolved), and air phases, respectively. After SPME is complete, we have

$$N_0 = N_s + N_{dom} + N_w + N_a + N_f \quad (2)$$

where N_s, N_{dom}, N_w, N_a, and N_f are the amounts of the analyte in the solid, DOM, aqueous and air phases, and the SPME fiber, respectively. It is commonplace in environmental chemistry to normalize contaminant concentrations in solids to the organic carbon (OC) fraction of this phase (*25, 26*). Also, DOM is most often expressed as dissolved organic carbon (DOC) because the carbon fraction is typically what is measured. Therefore, we define that $N_s^0 = C_{oc}^0 m_{oc}$, $N_{dom}^0 = C_{doc}^0 m_{doc}$, $N_w^0 = C_w^0 V_w$, $N_a^0 = C_a^0 V_a$, $N_s = C_{oc} m_{oc}$, $N_{dom} = C_{doc} m_{doc}$, $N_w = C_w V_w$, $N_a = C_a V_a$, and $N_f = C_f V_f$, where C_{oc}^0, C_{doc}^0, C_w^0, and C_a^0 are the analyte concentrations in the solid (OC normalized), DOM (DOC normalized), aqueous, and air phases, respectively, before SPME; and C_{oc}, C_{doc}, C_w, C_a, and C_f are the analyte concentrations in the solid (OC normalized), DOM (DOC normalized), aqueous and air phases, and the SPME fiber (normalized to the polymer phase) after SPME, respectively; m_{oc} and m_{doc} are the mass of OC in the solid phase and DOC in the DOM phase, respectively; and V_w, V_a, and V_f are the volumes of the aqueous and air phases, and the sorbing fraction of the SPME fiber, respectively.

When the system is at thermodynamic equilibrium and since partitioning processes have been verified experimentally as the dominant mechanism for extraction of hydrophobic organic compounds with nonpolar SPME fiber coatings (*27*), the usual partition coefficients can be used to describe the analyte distribution in the system:

$$K_{oc} = \frac{C_{oc}^0}{C_w^0} = \frac{C_{oc}}{C_w} \quad (3)$$

$$K_{doc} = \frac{C_{doc}^0}{C_w^0} = \frac{C_{doc}}{C_w} \quad (4)$$

$$K_f = \frac{C_f}{C_w} \quad (5)$$

$$K'_H = \frac{K_H}{RT} = \frac{C_a^0}{C_w^0} = \frac{C_a^0}{C_w} \quad (6)$$

where K_{oc}, K_{doc}, and K_f are the equilibrium partition coefficients of the analyte (solid-aqueous, DOM-aqueous, and SPME fiber-aqueous). Also, K_H and K'_H are the Henry's Law constant and the dimensionless Henry's Law constant, respectively, R is the universal gas constant, and T is the absolute temperature.

In SPME experiments, the parameter actually measured is the amount of the analyte sorbed on the SPME fiber (N_f). Therefore, to determine the concentrations of analyte in the sample, it is necessary to derive a relationship between N_f and C_w^0. Combining eqs 1−6 yields

$$N_f = \frac{K_f V_f (V_w + \theta + K'_H V_a)}{K_f V_f + V_w + \theta + K'_H V_a} C_w^0 \quad (7)$$

where $\theta = K_{oc} m_{oc} + K_{doc} m_{doc}$, a matrix sorption term reflecting the effects on SPME from suspended solids and DOM. As shown later, θ is a key parameter for understanding how SPME experiments may be affected by various sample parameters.

For most of the hydrophobic organic compounds of interest for this study, e.g. polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, etc., the dimensionless Henry's Law constant is small, i.e., $K'_H \leq 0.05$ (*26*). As will be discussed in the Results and Discussion section, the amount of analyte in the headspace at equilibrium will be negligible compared to the rest of the system if the headspace volume is also minimized (e.g., less than 50% of the sample volume). Neglecting the headspace, eq 7 becomes

$$N_f = \frac{K_f V_f (V_w + \theta)}{K_f V_f + V_w + \theta} C_w^0 \quad (8)$$

Equation 8 can be used to calculate the initial dissolved phase concentrations of analytes in the sample. A similar relationship can be derived for N_f and N_0:

$$N_f = \frac{K_f V_f}{K_f V_f + V_w + \theta} N_0 \quad (9)$$

Equation 9 can be used to calculate the total concentration of analyte in a complex sample. Another useful relationship can be derived from eq 9. If the amounts of sorbing phases in the system are insignificant (i.e., $m_{oc} \approx 0$ and $m_{doc} \approx 0$), then θ approaches zero and if N'_f is defined as the amount of the analyte in the SPME fiber with insignificant amounts of sorbing phases eq 9 reduces to

$$N'_f = \frac{K_f V_f}{K_f V_f + V_w} N_0 \quad (10)$$

Equation 10 is the most basic equation in SPME and has been successfully used for analysis of volatile organic chemicals in simple sample matrices (*3*). Combining eqs 9 and 10 leads to

$$\frac{N_f}{N'_f} = 1 - \frac{\theta}{K_f V_f + V_w + \theta} \quad (11)$$

Equation 11 can be used to calculate the amount of an analyte sorbed on the SPME fiber in a complex matrix relative to the amount sorbed in a relatively "clean" sample or pure water standard of the same volume. It suggests that the presence of sorbing phases in a sample will lower the apparent recovery of the analyte, which is of course an artifact due to a lack of consideration of the partitioning behavior of the analyte within the system. The efficiency of SPME is the same for both cases, the lower amount of analyte extracted in the complex sample is due to a lower dissolved phase concentration in the presence of sorptive phases.

The matrix term θ can be generalized to include any number of heterogeneous solid and DOM phases. In gen-

eralized form θ can be expressed as

$$\theta = \sum_{i=1}^{n} {K_{oc}}^{i} {m_{oc}}^{i} + \sum_{j=1}^{n'} {K_{doc}}^{j} {m_{doc}}^{j}$$

where n and n' are the total numbers of solid and DOM phases, respectively. The unique aspect of the 11 equations derived thus far is that they provide a complete description of the partitioning of organic chemicals between the various phases of any heterogeneous environmental sample during an SPME extraction. Moreover, the governing equations are parametrized in terms of system qualities frequently and easily measured or estimated by environmental scientists. Such parameterization allows for a rapid interpretation of how changes in analyte, sample, and fiber properties will affect the performance of SPME in sample analysis. The ability to understand the complex equilibrium partitioning behavior of organic chemicals in heterogeneous aquatic systems using a small set of fundamental parameters has been previously demonstrated (*28*).

Methods

One simple way to evaluate matrix effects is to examine the variability of the SPME analyte recovery in a heterogeneous sample (N_f) under various conditions relative to that for a clean sample (N_f'), using eq 11. Realistic values for the parameters can be used in the appropriate equations to predict the performance of SPME for a given set of sample and experimental conditions. To simplify the evaluation, the heterogeneous system to be studied is assumed to be an aqueous sample consisting of pure water and two homogeneous sorbing phases, suspended solids and DOM. The effects of ionic strength and temperature will be neglected, although it is recognized that these parameters can significantly affect partitioning behavior and SPME performance (*3, 26*). The suspended solids are assumed to contain 1% OC (the fraction of OC is defined as f_{oc}, i.e., $f_{oc} = 0.01$), and the DOM phase can generally be assumed to contain about 50% DOC (the fraction of DOC is defined as f_{doc}, i.e., $f_{doc} = 0.5$) by mass (*26*). For simplicity, the density of the suspended solids and the DOM are assumed to be the same, that is $\delta_{ss} = \delta_{dom} = 1.5$ g/mL. The equilibrium partitioning parameters were calculated using correlations from the literature relating K_f, K_{oc}, and K_{doc} to the octanol−water partition coefficient (K_{ow}), i.e., $K_f = 0.123K_{ow}$ (*12*), $K_{oc} = 0.41\ K_{ow}$ (*25*), and $K_{doc} = 0.11\ K_{ow}$ (*29*).

It was necessary to specify a dissolved phase analyte concentration to evaluate the relative importance of headspace contributions to partitioning behavior in these systems. In this case, the water concentration of an analyte was estimated using a linear free energy relationship from the literature for hydrophobic organic chemicals, $\log K_{ow} = -0.85 \log {C_w}^{sat} + 0.78$ (*26*). The dissolved concentrations used for the analyses were set at half the calculated saturated water concentration. Recall that the matrix term θ contains both equilibrium constants and mass terms. The equations relating the concentrations of suspended solids (C_{ss}) and DOC (DOC) in the sample to the respective mass terms in θ are

$$C_{ss} = \frac{m_{oc}}{f_{oc} V_t} \tag{12}$$

and

$$DOC = \frac{m_{doc}}{V_t} \tag{13}$$

with

$$V_t = V_w + \frac{m_{oc}}{f_{oc}\delta_{ss}} + \frac{m_{doc}}{f_{doc}\delta_{dom}} \tag{14}$$

The total volume (V_t) was used because, strictly speaking, it is comprised of the aqueous volume and the volumes of the suspended solids and DOM. However, note that there are 6 orders of magnitude between the units typically used for suspended solids and DOC concentrations and their respective densities, δ_{ss} and δ_{dom} (i.e., mg/L vs g/mL). Therefore, unless the masses of solids and DOM are extremely large, the volumes of these phases do not contribute significantly to the overall sample volume, i.e., $V_t \approx V_w$.

Results and Discussion

Headspace Partitioning. The importance of the headspace in the sample vial as a compartment for the partitioning of organic chemicals during an SPME analysis was evaluated. Realistic system parameters were selected to predict the extent of analyte partitioning into the headspace. The results of this analysis show that even for compounds of low hydrophobicity, relatively high K_H', and allowing the headspace to equal the sample volume, only about 2.5% of the total analyte in the system will be in the headspace at equilibrium (Figure 1, Supporting Information). Most of the compounds of interest for this study, i.e., hydrophobic organic compounds, would partition extensively into the suspended solids and DOM phases and generally have much lower K_H' values, moderate to high K_{ow}'s, and low dissolved phase concentrations. These results indicate that only a very small fraction of the total amount of such compounds would be in the headspace at equilibrium. Moreover, the results validate the omission of the headspace terms from the SPME governing equations.

Variability in SPME Recoveries. It is apparent from eqs 8 and 9 that complex sample matrices may significantly impact SPME measurements as θ becomes significant relative to V_w. Such an illustration can be provided for the measurement of the truly dissolved phase concentration of an analyte. A comparison of eqs 8 and 9 reveals that ${C_w}^0 = N_0/(V_w + \theta)$. In a given sample, the matrix term (θ) in effect serves as a reservoir (sink and source) for analytes, and depending on the relative values of V_w and θ, the concentration of an analyte in the truly dissolved phase could be substantially overestimated without consideration of the matrix effects.

The ratio of the fiber-sorbed analytes N_f/N_f' in eq 11 can be thought of as the relative recovery of each compound as compared to a sample where matrix effects are insignificant. Equation 11 can be used to investigate the influence of sample, chemical, and experimental parameters on SPME relative recoveries. The relative recovery generally increases with increasing V_f value, but the trend levels off after V_f reaches certain values (Figure 1). Good relative recoveries (i.e., ≥80%) are predicted when using common SPME fibers with an 85-μm polymer coating (SPME fibers with polymer coating ranging from 7 to 100 μm are available from Supelco, Bellefonte, PA), which corresponds to an effective volume of ~0.5 μL, under the specified experimental parameters. Large SPME fiber volumes are predicted to effectively minimize the matrix interferences from suspended solids and DOM, while high K_{ow} values are expected to enhance matrix effects as more hydrophobic organic chemicals partition onto suspended solids and DOM (Figure 1). Increasing the sample volume, while maintaining the same concentrations of suspended solids and DOM, lowers the N_f/N_f' values (Figure 1a,b). The same effect is observed when the concentration of suspended solids increases while maintaining the same sample volume (Figure 1a,c). Although using large SPME fiber volumes appears to mitigate these observed matrix effects, large fiber volumes are also expected to prolong the

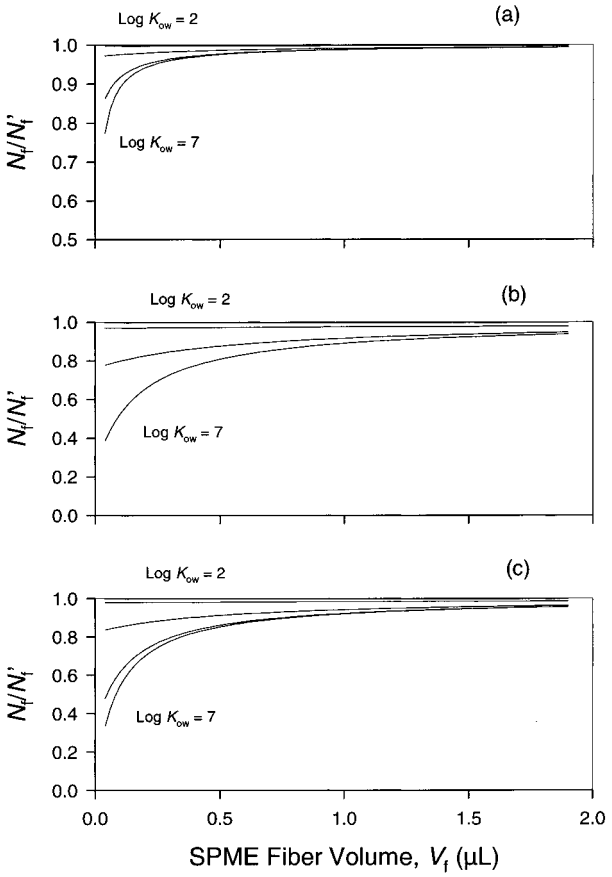

FIGURE 1. Variability of the SPME relative recovery (N_f/N_f') with SPME fiber volume (V_f). All curves corresponding to logK_{ow} = 2, 3, 4, 5, 6, and 7 were obtained from eq 11 with different values for the experimental parameters: (a) V_w = 5 mL, C_{ss} = 50 mg/L, C_{doc} = 1 mg/L, f_{oc} = 0.01, and f_{doc} = 0.5 (curves with logK_{ow} = 2, 3, and 4 overlap); (b) same as (a) except for V_w (= 50 mL) (curves with logK_{ow} = 2, 3, and 4 overlap); and (c) same as (a) except for C_{ss} (= 500 mg/L) (curves with logK_{ow} = 2 and 3 overlap).

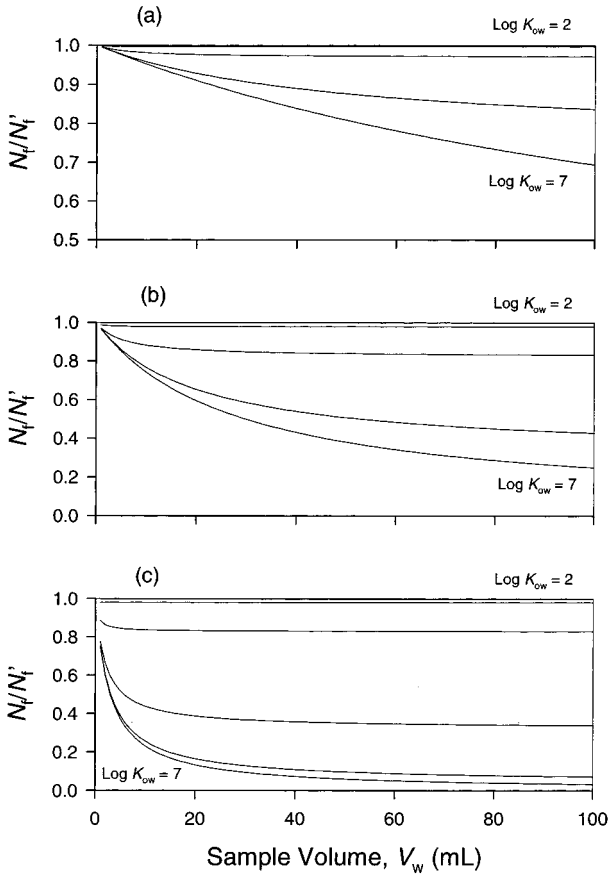

FIGURE 2. Variability of the SPME relative recovery (N_f/N_f') with sample volume (V_w). All curves corresponding to logK_{ow} = 2, 3, 4, 5, 6, and 7 were obtained from eq 11 with different values for the experimental parameters: (a) C_{ss} = 50 mg/L, C_{doc} = 1 mg/L, V_f = 0.5 μL, f_{oc} = 0.01, and f_{doc} = 0.5 (curves with logK_{ow} = 2, 3, and 4 overlap); (b) same as (a) except for C_{ss} (= 500 mg/L) (curves with logK_{ow} = 2 and 3 overlap); and (c) same as (a) except for C_{ss} (= 5000 mg/L).

time required to reach equilibrium, particularly for very hydrophobic compounds (*3*). Overall, it appears that the 85-μm SPME fiber is an appropriate choice for most applications. Therefore, in all subsequent SPME assessments, a value of 0.5 μL is selected for V_f, except where otherwise specified.

Sample volume is another parameter affecting SPME experiments. It is clear from the calculated curves (Figure 2) that SPME relative recovery decreases with increasing sample volume. The reason for this trend is not readily apparent, but the explanation can be found through a detailed analysis of the governing equation. Equation 11 can be rearranged to the following form

$$\frac{N_f}{N_f'} = \frac{K_f V_f + V_w}{K_f V_f + V_w + \theta} \tag{15}$$

and similarly, θ can be rewritten in terms of sample volume from eqs 12−14, $\theta = K_{oc} m_{oc} + K_{doc} m_{doc} = K_{oc} f_{oc} C_{ss} V_t + K_{doc} DOC V_t$ or for most environmental samples

$$\theta \approx K_{oc}\ f_{oc} C_{ss} V_w + K_{doc}\, DOC V_w \tag{16}$$

Now, combining eqs 15 and 16

$$\frac{N_f}{N_f'} = \frac{K_f V_f + V_w}{K_f V_f + V_w + V_w(K_{oc} f_{oc} C_{ss} + K_{doc} DOC)} \tag{17}$$

It is evident from eq 17 that at very small sample volumes, the water volume and matrix terms become insignificant relative to the fiber term and the ratio N_f/N_f' approaches one. As sample volume increases, the fiber term becomes increasingly less significant until the partitioning in the system is governed by the matrix term. As volume increases, eventually eq 17 reduces to

$$\frac{N_f}{N_f'} = \frac{1}{1 + K_{oc}\, f_{oc} C_{ss} + K_{doc} DOC} \tag{18}$$

Another interesting aspect of this analysis is the change in N_f/N_f' relative to analyte concentration as volume is increased (see eq 17). For any given analyte concentration, the total amount of analyte in the dissolved phase increases linearly with V_w and a slope of unity. The matrix term θ is also increasing linearly with V_w, but with a slope of $K_{oc}\, f_{oc} C_{ss} + K_{doc} DOC$. In other words, the θ term changes in magnitude more rapidly than the dissolved phase term as a function of sample volume, with a corresponding shift in the distribution of analyte among systems phases. This explains the general decrease in relative recoveries with sample volume and also why the effect becomes more pronounced at high K_{ow}'s and insignificant at low K_{ow}'s.

It is important to note that the enhanced matrix effects due to increasing aqueous phase volume does not suggest it is preferable to use small sample volumes with SPME. As discussed later, when the sample volume reaches a certain

value, the measurement of dissolved phase concentrations becomes straightforward, in that no correction for matrix effects is needed.

Also noteworthy is that the effects of suspended solids and DOM on the systems under evaluation are similar. All of the trends observed in the graphs would be exacerbated if the concentration of DOM were increased. For most of the analyses presented herein, the concentration of DOM was fixed and concentration of suspended solids was varied. This approach was selected for the evaluations since suspended solids vary over a larger range of concentrations than does DOM in natural water samples. The effect of varying suspended solids concentrations on relative recoveries is shown in Figure 2, Supporting Information.

A common feature to all of these analyses is that N_f/N_f' remains close to unity, while sample parameters vary over a wide range of values when $\log K_{ow} \leq 3$ (Figures 1 and 2 and Figure 2, Supporting Information). This emphasizes that matrix effects are only important for compounds of significant hydrophobicity. This is why SPME has been used widely and successfully for the extraction of volatile organic compounds with relatively low K_{ow} values (*8, 10, 13*).

Potential Calibration Errors. Quantitative analytical measurements require calibration. In SPME experiments, either external or internal calibration can be used. As demonstrated above, the presence of a heterogeneous matrix may significantly affect the SPME relative recovery and hence any quantitative measurement in SPME experiments. Therefore, it is important to understand how measurement errors are associated with calibration methods in SPME quantitative measurements. Unfortunately, this issue was not discussed in many previous SPME studies.

External calibration remains widely used in SPME experiments due to its simplicity. Although the response of a specific analytical detector to a specific analyte may vary, the variation may be small enough to satisfy the accuracy objectives. Alternatively, multiple experiments can be conducted to obtain a statistical average of the response. External calibration can be performed using two different approaches. In the first approach, calibration standards are prepared in clean water and analyzed by SPME to obtain response factors (RFs). Measurement errors result from the matrix difference between the calibration standard solutions and real samples. In this case, the measurement errors can be estimated using eq 11 and expressed in percent error, $\% E = 100\theta/(K_f V_f + V_w + \theta)$.

In the second approach, the RFs of target analytes are acquired by direct injection of calibration standards prepared in an appropriate organic solvent into the analytical instrument. To estimate the measurement errors stemming from matrix effects under such a scenario, the measured dissolved phase concentration of an analyte with and without any matrix interference can be compared using eq 8. When the matrix term is ignored, we can have from eq 8

$$N_f = \frac{K_f V_f V_w}{K_f V_f + V_w} C_0 \tag{19}$$

Combining eqs 8 and 19 yields

$$\frac{C_0}{C_w^0} = \left(1 - \frac{\theta}{K_f V_f + V_w + \theta}\right)\frac{(V_w + \theta)}{V_w} \tag{20}$$

Equation 20 indicates that C_0/C_w^0 is always greater than unity, i.e., the truly dissolved phase concentration of the analyte is always overestimated using this type of external calibration without consideration of matrix effects. It is possible to estimate the maximum error that can be incurred by allowing θ to become very large. As θ approaches infinity, C_0/C_w^0 approaches $1 + K_f V_f/V_w$, which is the maximum possible error using this type of external calibration.

Measurement errors with internal calibration are much more difficult to estimate as compared to external calibration. The reason for this is that the matrix effects need to be corrected for both the target analyte and internal standard in an internal calibration method. In an internal calibration approach, target analytes and internal standards are prepared in either pure organic solvent or clean water to make calibration solutions. These calibration solutions are either injected directly into the analytical instrument or extracted first by SPME and then desorbed into the analytical instrument to obtain relative response factors for the analytes.

The best internal standard calibration approach is to use isotopically substituted standards of the target analytes. However, care must be taken to allow enough time for the system to reach equilibrium prior to performing an analysis. Even so, differences in sorption behavior between recently spiked and aged field samples could still introduce substantial measurement errors (*19*). These so-called "aging effects" or slow desorption kinetics could contribute to serious measurement errors even under the best possible experimental conditions.

Optimization of Sample Volume. An approach to optimizing sample volume has been introduced by Pawliszyn (*3*). The approach is based on the fact that when the sample volume reaches a certain minimum value, the absorption of an analyte onto the SPME fiber would have little impact on the dissolved phase concentration of the analyte in the sample. After this point, the sensitivity of SPME does not increase with further increase in the sample volume. Using a similar conceptual approach, the sample volume for a heterogeneous system can be optimized. Equation 5 is rearranged to yield

$$N_f = K_f V_f C_w \tag{21}$$

where C_w is the analyte concentration in the dissolved phase after SPME. Combining eqs 8 and 21

$$\frac{C_w}{C_w^0} = \frac{V_w + \theta}{K_f V_f + V_w + \theta} \tag{22}$$

It is evident from eq 22 that if the sample volume is increased, eventually there will be a volume such that $(V_w + \theta) \gg K_f V_f$, and the ratio of the dissolved phase analyte concentration after SPME to the initial dissolved phase analyte concentration, C_w/C_w^0, will approach unity. At this point, SPME sensitivity in the heterogeneous system is maximized, and the pre-extraction concentration in the dissolved phase is reflected in the amount of the analyte sorbed to the fiber. Other investigators have recognized the existence and implications of this relationship in heterogeneous SPME and have even utilized this so-called "non-depletive extraction" mode in their research without fully developing it on a theoretical basis (*12, 30, 31*). We will fully develop the concept here by proposing that the dissolved phase volume at which $C_w \approx C_w^0$ be defined as the critical volume, V_c, and that the ratio C_w/C_w^0 be defined as the critical ratio, r_c. It is clear that the critical ratio can never be exactly unity, because the $K_f V_f$ term can never be zero. Therefore, the parameter r_c can be defined by the user to achieve any desired degree of accuracy. A mathematical expression for the critical volume may be derived from a variation of eq 22

$$r_c = \frac{V_c + \theta}{K_f V_f + V_c + \theta}$$

that can be solved for V_c

$$V_c = \alpha K_f V_f - \theta \quad (23)$$

where $\alpha = r_c/(1 - r_c)$. In eq 23, θ is also a function of V_c and other experimentally measurable parameters. The true total system volume can be obtained by combining eqs 12−14 and equating V_w to V_c:

$$V_t = \frac{V_c}{1 - \frac{C_{ss}}{\delta_{ss}} - \frac{DOC}{\delta_{dom} f_{doc}}}$$

Using the definition for θ ($= K_{oc}C_{ss}\, f_{oc}V_t + K_{doc}\, DOCV_t$)

$$\theta = (K_{oc}C_{ss}\, f_{oc} + K_{doc}\, DOC)\left[\frac{V_c}{1 - \frac{C_{ss}}{\delta_{ss}} - \frac{DOC}{\delta_{dom} f_{doc}}}\right] \quad (24)$$

Combing eqs 23 and 24 and solving for V_c

$$V_c = \frac{\alpha K_f V_f}{1 + \beta} \quad (25)$$

where

$$\beta = \frac{K_{oc}C_{ss} f_{oc} + K_{doc}DOC}{1 - \frac{C_{ss}}{\delta_{ss}} - \frac{DOC}{\delta_{dom} f_{doc}}}$$

The parameter β is composed of variables that are measurable experimentally and/or easily estimated. The expression for β may be simplified if the concentrations of suspended solids and DOC are very small compared to their densities, i.e., $C_{ss}/\delta_{ss} \ll 1$ and $DOC/\delta_{dom} f_{doc} \ll 1$. As previously mentioned, this condition is often satisfied for typical environmental samples, and thus in most cases $\beta \approx K_{oc}C_{ss}\, f_{oc} + K_{doc}DOC$.

For simplicity, the conceptual development here has been limited to a three-phase system of water, suspended solids, and DOM. However, it is straightforward to extend eq 25 to include any number of matrix phases. The variation in logV_c with logK_{ow} for $r_c = 0.99$ (1% error; Figure 3a) and 0.90 (10% error; Figure 3b) shows that V_c decreases significantly for a relatively small relaxation in the required accuracy. Also noteworthy is that the critical volume is smaller for samples with larger amounts of suspended solids after K_{ow} exceeds a certain value. Highly hydrophobic organic compounds partition extensively to the suspended solids and DOM that effectively become a large reservoir for these chemicals. As a result, the amount of an analyte partitioning onto the SPME fiber would be compensated by desorption of the analyte from this reservoir. Figure 3 also indicates that the critical volume can be very small for compounds with low K_{ow} values. This further explains why SPME has been so successful in the measurement of relatively polar and/or volatile organic chemicals.

The fact that V_c decreases with increasing amounts of solids has important implications for environmental applications. It suggests that SPME should be particularly effective for the measurement of hydrophobic organic compounds in sediment interstitial water, where extremely high solids-to-water ratios prevail. Also, the large critical volumes required for samples with low suspended solids and/or DOM concentrations suggest that SPME should be most useful for in situ field measurements. However, it remains to be demonstrated that equilibrium between SPME fibers and large media volumes (e.g., 10−100 L for water) can actually be achieved in the field. If equilibrium can be achieved, the dissolved phase concentration of the analyte

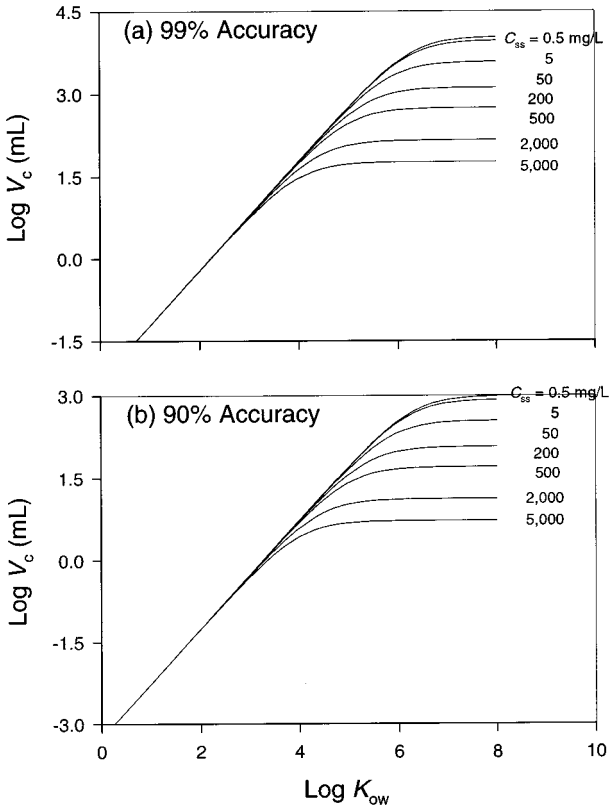

FIGURE 3. Variation of critical volume (log V_c) with log K_{ow} obtained from eq 25: (a) $\alpha = 0.99$ or 99% accuracy and (b) $\alpha = 0.90$ or 90% accuracy. $V_w = 5$ mL, $V_f = 0.5$ μL, $C_{doc} = 1$ mg/L, $f_{oc} = 0.01$, and $f_{doc} = 0.5$.

in the sample medium is approximately the same before and after SPME and can be calculated using $C_w = N_f/V_fK_f$. Such an approach can be applied to both air and water sampling.

Calibration of SPME Experimental Parameters. The key to the successful application of SPME in chemical analysis is a diligent calibration of the SPME device and sample matrix. There are two sets of parameters that need to be calibrated before actual samples may be extracted by SPME. The first set is K_fV_f, which can be calibrated in two steps. In the first step, a series of calibration standards prepared in organic solvent are injected directly into the analytical instrument. The RF of a specific analyte is the slope of the plot of A vs CV_{inj}, where A is the peak area (response) for a particular analyte, C is the concentration of the analyte in the analytical standard solution, and V_{inj} is the injection volume. Once the RF is determined, the second step is to prepare another set of standard solutions in clean water and analyze them by SPME. From eq 10 we have

$$\frac{A'}{RF} = \frac{K_fV_fV_w}{K_fV_f + V_w}C_w^0 \quad (26)$$

where A' is the peak area from the analytical instrument related to C_w^0. The slope of the plot of A'/RF vs C_w^0, $S = K_fV_fV_w/(K_fV_f + V_w)$, is therefore related to the fiber term by $K_fV_f = SV_w/(V_w\text{-}S)$. If V_f is accurately known (generally given by the manufacturer), the distribution coefficient, K_f, of the analyte can be calculated.

The other parameter that must be determined is θ. The standard addition method can be used to accomplish the task. In this method, various amounts of the target analyte are added to at least five sample replicates with their matrix being representative of the actual samples and the spiked

TABLE 1. Comparison of Experimental Results with Theoretical Predictions

analyte	log K_{ow}[a]	measured[b] (%)	predicted[c] (%)
(1) Potter and Pawliszyn (*6*)[d]			
1,4-dichlorobenzene-d_4	3.38	78 (92)	87.3
naphthalene-d_8	3.35	100 (120)	88.1
acenaphthene-d_{10}	3.92	96 (112)	66.6
phenanthrene-d_{10} (low)	4.45	34 (33)	37.1
phenanthrene-d_{10} (high)	4.63	34 (33)	29.1
chrysene-d_{12} (low)	5.61	8 (8)	5.2
chrysene-d_{12} (high)	5.80	8 (8)	3.8
(2) Chen et al. (*20*)[e]			
phorate	3.92	12.8 (±6.1)	9.4
diazinon	3.30	25.7 (±19.8)	27.7
methyl parathion	3.32	23.5 (±17.3)	26.8
ethion	5.07	2.4 (±1.5)	2.2

[a] K_{ow} values for polycyclic aromatic hydrocarbons are from Mackay et al. (*32*); high and low values indicate a range of K_{ow} values from various sources. [b] Measured as relative recoveries (%) in real samples compared to spiked clean samples. [c] Calculated using eq 11 with $K_f = 0.123K_{ow}$ (*12*), $K_{oc} = 0.41K_{ow}$ (*25*), and $K_{doc} = 0.11K_{ow}$ (*29*); other parameters are given below. [d] The numbers in the parentheses are measured values from duplicate analyses. Given parameters: $V_f \approx 0.0616$ μL and $V_t = 40$ mL. Assumed parameter values: $C_{ss} = 1460$ mg/L (*19*); $f_{oc} = 0.1$; $f_{doc} = 0.5$; $DOC = 2.5$ mg/L; and $\delta_{ss} = \delta_{dom} = 1.5$ g/mL. [e] The numbers in the parentheses are standard deviations from five measurements for each analyte. Given parameters: $C_{ss} = 250$ g/L (assuming $\delta_{ss} = \delta_{dom} = 1.0$ g/mL); $V_w = 3.75$ mL; and $V_f \approx 0.66$ μL. Assumed parameter values: $f_{oc} = 0.01$; $f_{doc} = 0.5$; and $DOC = 0.1$ mg/L.

samples are analyzed by SPME. The peak areas from the analytical instrument can be plotted against the added amounts of the analyte. The intercepts of the plot are the native amount (on the *x* axis) of the analyte in the sample replicates and the peak area (on the *y* axis) associated with the native amount, respectively. The peak area is then converted to the amount of the analyte sorbed on the SPME fiber, N_f, using the RF of the analyte obtained using external calibration as described above. Since K_fV_f has been acquired using eq 26, the value of θ can be calculated using eq 9. Alternatively, if great accuracy is not required, then the individual parameters within θ can be estimated using well-established linear free energy relationships. If it is desirable to know the individual contributions of the different sorbing phases, the suspended solids may be filtered out or removed by centrifugation, and the filtrates reanalyzed using the same standard addition method described above. This would allow determination of the value of $K_{doc}m_{doc}$. The value of $K_{oc}m_{oc}$ can be obtained by subtracting $K_{doc}m_{doc}$ from θ. Obviously, if m_{oc} and m_{doc} are accurately determined, K_{oc} and K_{doc} can be calculated accordingly.

Comparison to Experimental Studies. The introduction of the matrix term (θ) into the SPME theory provides the opportunity to account for matrix effects in SPME analysis. We conducted a comparison of the theoretical treatment presented above with available experimental data to demonstrate the validity of our approach to accounting for matrix effects. The selection of experimental data was no easy task, since many previous studies did not provide sufficient details on SPME experimental parameters. After sorting out numerous publications on SPME, we chose the experimental data from three studies by Potter and Pawliszyn (*6*), Langenfeld et al. (*19*), and Chen et al. (*20*). The experimentally measured parameters were the relative recoveries of the target analytes from the spiked field samples compared to spiked clean water (*6*, *19*, *20*), which can be directly compared to theoretical predictions from eq 11.

The agreement between the measured values from Potter and Pawliszyn (*6*) and Chen et al. (*20*) and theoretical predictions is good (Table 1). The theory not only predicts the trends of the relative recoveries for two different types of compounds but also matches the measured values quantitatively. It is remarkable that the extremely low recoveries for chrysene-d_{12} (*6*) and ethion (*20*) are well predicted by the theoretical treatment (Table 1). Comparison between the experimental data from Langenfeld et al. (*19*) and theoretical predictions is fair to good (Table 1, Supporting Information). The measured values for benz[*a*]anthracene, chrysene, and benz[*a*]pyrene are substantially larger than theoretical predictions with the samples from the Little Missouri River and wetland water (except for chrysene from the Little Missouri River samples). Another noticeable discrepancy between the experiment and the theoretical treatment is the consistently lower measured recoveries of naphthalene relative to the predicted values (Table 1, Supporting Information). Due to the success of the theoretical treatment for other compounds, we speculate that the high recoveries for benz[*a*]anthracene, chrysene, and benz[*a*]-pyrene and low recoveries for naphthalene (*19*) likely resulted from experimental factors other than the SPME process.

The above comparison indicates that low experimental recoveries of hydrophobic chemicals observed in many previous studies are unlikely to have been due to poor efficiency of the SPME methods. Instead, they probably were the result of severe matrix effects that were not properly taken into account.

Final Considerations. Two other aspects of SPME remain largely unaddressed in the theoretical considerations above, namely physical interferences with the diffusion between the SPME fiber and the sample matrix by high levels of DOM or unusual matrix properties and nonequilibrium SPME. Since absorption and desorption of an analyte with the SPME fiber are no different from a normal physical diffusion process, it is expected that physical blockage by matrix phases sorbed to the fiber surface may adversely affect the accuracy of SPME measurements. This problem may be partially resolved by applying agitation to the SPME fiber. This is the approach taken by Varian Inc. (Walnut Creek, CA) in their autosampler for GC applications, which vibrates the fiber at high frequency during the extraction/equilibration step. Another way of eliminating matrix interferences is to perform headspace SPME. However, if diffusion across the air−water interface is slow, or the target analytes have a low K_H, then equilibration times may become excessively long.

The theoretical treatment of SPME described herein is for equilibrium partitioning only. In practical applications, the time for the system to reach equilibrium may be excessively long for chemicals with high K_{ow} values. In this case, it may be desirable to conduct nonequilibrium SPME and still be able to quantify the analyte concentration using the simple proportional correlations between N_f and C_w^0 or N_0, as described in eqs 8 and 9. This issue has been addressed with the assumption of steady-state diffusion for the SPME partitioning process in the dissolved phase only (*22*, *23*). The development of the quantitative relationships between N_f and C_0 and N_0 in a complex sample matrix would be extremely involved but are a necessary prerequisite to making quantitative measurements using SPME under nonequilibrium conditions.

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Supporting Information Available

Additional information and evaluations including a glossary of terms, the importance of headspace in SPME analysis, variation in relative recoveries as a function of suspended solids, and more comparisons of theoretical predictions with

experimental results from the literature. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Arthur, C. L.; Pawliszyn, J. *Anal. Chem.* **1990**, *62*, 2145−2148.
(2) Arthur, C. L.; Killiam, L. M.; Buchholz, K. D.; Pawliszyn, J. *Anal. Chem.* **1992**, *64*, 1960−1966.
(3) Pawliszyn, J. *Solid-Phase Microextraction: Theory and Practice*; Wiley-VHC: New York, 1997.
(4) Pawliszyn, J., Ed. *Applications of Solid-Phase Microextraction*; Royal Society of Chemistry: Cambridge, UK, 1999.
(5) Louch, D.; Motlagh, S.; Pawliszyn, J. *Anal. Chem.* **1992**, *64*, 1187−1199.
(6) Potter, D. W.; Pawliszyn, J. *Environ. Sci. Technol.* **1994**, *28*, 298−305.
(7) Liu, Y.; Lee, M. L.; Hageman, K. J.; Yang, Y.; Hawthorne, S. B. *Anal. Chem.* **1997**, *69*, 5001−5005.
(8) Stahl, D. C.; Tilotta, D. C. *Environ. Sci. Technol.* **1999**, *33*, 814−819.
(9) Bernhard, M. J.; Simonich, S. L. *Environ. Toxicol. Chem.* **2000**, *19*, 1705−1710.
(10) Achten, C.; Püttmann, W. *Environ. Sci. Technol.* **2000**, *34*, 1359−1364.
(11) Magbanua, B. S., Jr.; Mitchell, D. R.; Fehniger, S. M.; Bowyer, R. L.; Grady, C. P. L., Jr. *Water Environ. Res.* **2000**, *72*, 98−104.
(12) Mayer, P.; Vaes, W. H. J.; Wijnker, F.; Legierse, K. C. H. M.; Tolls, J.; Hermens, J. L. M. *Environ. Sci. Technol.* **2000**, *34*, 5177−5183.
(13) Black, L.; Fine, D. *Environ. Sci. Technol.* **2001**, *35*, 3190−3192.
(14) Koziel, J. A.; Pawliszyn, J. *J. Air Waste Manage. Assoc.* **2001**, *51*, 173−184.
(15) Koziel, J. A.; Noah, J.; Pawliszyn, J. *Environ. Sci. Technol.* **2001**, *35*, 1481−1486.
(16) Kim, H.; Nochetto, C.; McConnell, L. L. *Anal. Chem.* **2002**, *74*, 1054−1060.
(17) Bierwagen, B. G.; Keller, A. A. *Environ. Toxicol. Chem.* **2001**, *20*, 1625−1629.
(18) Dean, J. R.; Tomlinson, W. R.; Makovskaya, V.; Cumming, R.; Hetheridge, M.; Comber, M. *Anal. Chem.* **1996**, *68*, 130−133.
(19) Langenfeld, J. J.; Hawthorne, S. B.; Miller, D. J. *Anal. Chem.* **1996**, *68*, 144−155.
(20) Chen, W.; Poon, K. F.; Lam, M. H. W. *Environ. Sci. Technol.* **1998**, *32*, 3816−3820.
(21) Grote, C.; Levsen, K. In *Applications of Solid-Phase Microextraction*; Pawliszyn, J., Ed.; Royal Society of Chemistry: Cambridge, UK, 1999; pp 169−187.
(22) Ai, J. *Anal. Chem.* **1997**, *69*, 1230−1236.
(23) Ai, J. *Anal. Chem.* **1997**, *69*, 3260−3266.
(24) Vaes, W. H. J.; Hamwijk, C.; Ramos, E. U.; Verhaar, H. J. M.; Hermens, J. L. M. *Anal. Chem.* **1996**, *68*, 4458−4462.
(25) Karickhoff, S. W. *Chemosphere* **1981**, *10*, 833−846.
(26) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*; John Wiley & Sons: New York, 1993.
(27) Poerschmann, J.; Górecki, T.; Kopinke, F.-D. *Environ. Sci. Technol.* **2000**, *34*, 3824−3830.
(28) Pankow, J. F.; McKenzie, S. W. *Environ. Sci. Technol.* **1991**, *25*, 2046−2053.
(29) Burkhard, L. P. *Environ. Sci. Technol.* **2000**, *34*, 4663−4668.
(30) Vaes, W. H. J.; Ramos, E. U.; Verhaar, H. J. M.; Seinen, W.; Hermens, J. L. M. *Anal. Chem.* **1996**, *68*, 4463−4467.
(31) Oomen, A. G.; Mayer, P.; Tolls, J. *Anal. Chem.* **2000**, *72*, 2802−2808.
(32) Mackay, D.; Shiu, W. Y.; Ma, K. C. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. Volume II. Polynuclear Aromatic Hydrocarbons, Polychlorinated Dioxins, and Dibenzofurans*; Lewis Publishers: Chelsea, 1992.

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