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Md. Nazmul Alam, Emir Nazdraji#, Varoon Singh, Marcos Tascon, and Janusz Pawliszyn

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Effect of Transport Parameters and Device Geometry on Extraction Kinetics and Efficiency in Direct Immersion Solid-phase Microextraction

Md. Nazmul Alam, Emir Nazdrajić, Varoon Singh, Marcos Tascon, and Janusz Pawliszyn†
Department of Chemistry, University of Waterloo, Canada, N2L 3G1
†janusz@uwaterloo.ca

Abstract

An alternative strategy to increase mass transfer entails geometry optimization of the extraction systems including design of solid-phase microextraction (SPME) probes. In this work, a computational model was employed to elucidate practical aspects such as efficiency and kinetics of extraction by employing several new geometries. Extraction of a model analyte at static conditions with the configurations, such as thin-film, fiber, coated tip, and nanoparticles, was numerically simulated to obtain an in-depth understanding of the advantages and limitations of each geometry in microextraction and exhaustive modes. The attained results associated with the equilibration time dependency on shape were in good agreement with previously reported experimental observations. They demonstrate that the mass-transfer is highly dependent on the size and shape of the coatings and increases with decrease of size of the devices particularly rapidly below 10 microns caused by radial diffusion effect. Nevertheless, extractions performed using octadecyl-functionalized magnetic nanoparticles demonstrated that higher enrichment factors are achievable with the use of a fewer number of particles in comparison to factors achieved via exhaustive extraction, where a larger number of particles must be employed confirming theoretical predictions. The conclusions reached are valid for any extraction method. The results obtained herein are very useful towards the design and optimization of future extraction technologies and approaches.

INTRODUCTION

Traditional analytical sample preparation methods based on liquid-liquid extraction (LLE) or solid phase extraction (SPE) work under the principle of exhaustive extraction, in which the main goal is to extract the majority of the analytes present in the sample matrix.[1,2] To that end, in typical exhaustive extraction workflows, a large extraction phase volume to sample phase volume ratio is employed during the sample preparation step. Conversely, solid-phase microextraction (SPME) is presented as a non-exhaustive partitioning and diffusion-controlled extractive technology that offers analyte enrichment for trace analyses,[3] while utilizing much smaller extraction phase volume to sample phase volume ratios as compared to those used in typical LLE or SPE methods.[4] Although SPME applications generally offer lower recoveries compared to exhaustive extraction methods, comparable sensitivity can be achieved by utilizing smaller extraction phase volumes, or by injecting the entire extracted amount into an instrument. In addition, several strategies can be employed to enhance the sensitivity of SPME devices for a specific target analyte or group of analytes. One such strategy consists of designing selective extraction phases for which a specific analyte or group of analytes, such as aptamers,[5,6] antibodies,[7] or molecular imprinted polymers,[3,8] will have high affinity. Another viable strategy to increase extracted amounts is the use of devices consisted of higher surface area to volume ratios, such as thin films.[9]

While SPME achieves optimum analyte enrichment when equilibrium conditions are employed for analytes with high affinity for the extraction phase, often longer equilibration times needed in SPME due to its sorption process, which is limited by diffusion through the boundary layer, remain a challenge in SPME applications. In this regard, the electrochemical research field has faced similar limitations that were addressed by modifying electrode shapes to enhance mass transfer (cylinder, disk, ring, cone, etc.).[10,11] Indeed, the introduction of ultramicroelectrodes, which offer enhanced mass transfer in comparison to the technology available at that juncture,[12] is considered to be a major breakthrough in the electrochemical field. Here, the comparative advantage of this technology is owed to its miniaturization, as a reduction in extractive device dimensions to scales comparable to the thickness of the boundary layer or the size of molecules leads to different experimental behavior *i.e.* different kinetics of extraction due to radial

diffusion.[10] However, ideas developed in the electrochemistry community regarding mass transfer enhancement have yet to be applied in the separation/extraction research field.

In SPME applications, common practices to reduce equilibrium time through enhanced mass transfer ratios include the use of thinner coatings as well as application of forced convection.[13] However, for challenging applications such as *in vivo* sampling, single cell analysis or dispersive extraction in droplets, sample agitation is not a viable option.[14,15] Additionally, analytes with high distribution constant values (K_{es}) will take a substantial amount of time to reach equilibrium, even when thin coatings are used.[16] In order to minimize equilibrium times in such cases, SPME extraction devices must thus be purposely designed to promote enhancements in the mass transport of analytes. In this regard, the development of miniaturized coating devices that utilize different geometries can certainly facilitate rapid equilibrium extractions from challenging matrices. To this end, the development of miniaturized coating geometries aimed at extractions from single cells or low microliter scale samples was recently reported in the literature.[17,18] Likewise, the development of a 100-200 μm coated-tip SPME has enabled sampling from static samples with equilibrium times as fast as 2 minutes, with researchers envisioning additional reductions in the size of these SPME probes so as to extend its applicability to other applications.[18] Certainly, attaining a better understanding of the effect of reductions in phase size and shape on the extraction process would contribute to further elucidation of the benefits of such miniaturizations. In this line, mathematical modeling has become an excellent tool to investigate the efficiency and kinetics of species detection in sensor areas prior to experimental investigations. So far, mathematical models have in fact corroborated the benefits of miniaturization of the probe geometry.[18–20] In this work, a set of new SPME geometries such as miniaturized fibers, tips, and nanoparticles are presented. In this context, the effect of transport parameters and geometry on extraction kinetics and efficiency for direct immersion and static condition is studied. Noteworthy, the enhanced mass transport together with the increasing of enrichment factors make this technology optimum in the most challenging biological applications faced nowadays such as single cell analysis, *in vivo* sampling, and extractions from very small volumes.

EXPERIMENTAL

In the current study, a theoretical investigation was carried out to elucidate the effect of the size of various SPME probes on extraction efficiency and kinetics in the case of a static, non-agitated sample system. The amount of analyte was quantified for cylindrical fibers, spherical particles, and flat membranes of different radii. Additionally, an experimental investigation was performed using magnetic nanoparticles in order to contrast the enrichment factors predicted by the model with experimental data attained for a set of model compounds extracted via different amounts of magnetic nanoparticles. In this context, both the developed theory as well as the experimental results clearly point at the benefits of miniaturization on the extraction kinetics and the enrichment factor of a given analyte. To this aim, this article intends to elucidate our current understanding of the processes pertaining to the extraction kinetics of such devices, and establish criteria for miniaturization of microextraction devices with aims of further exploiting the benefits of such approaches in novel and challenging sampling strategies.

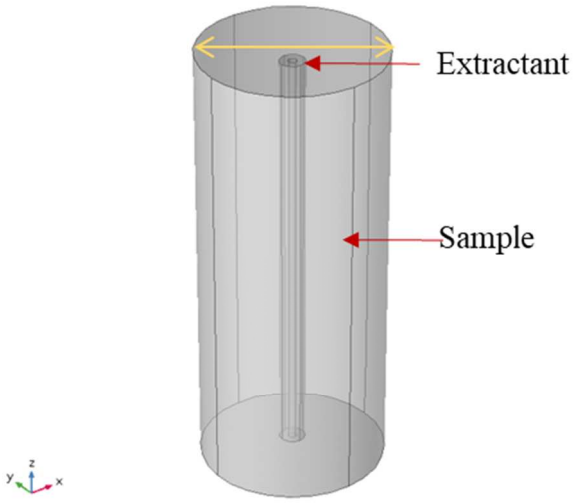

Figure 1. Schematic representation of the three-dimensional (3-D) model domains used in computational simulations of benzene extraction with fiber geometry. In addition to the fiber shape geometry, particle and thin film geometries were also used in computational simulations. For exhaustive extraction, the diameter of the sample cylinder was lowered so as to attain desired phase ratios with a fixed extractant volume.

Simulation techniques

The microextraction process of SPME is depicted schematically in Figure 1. The process of extraction is governed by a system of partial differential equations (PDEs). Similarly to our previously published models, the transport of the model analyte, benzene, from the bulk solution to the SPME device surface can be described by Eq. 1:

$$\frac{\mathrm{d}\Gamma(t)}{\mathrm{d}t} = D_A^e \nabla^2 C_A^e + k_{ads} C_A(t)\left(\Gamma_{max} - \Gamma(t)\right) - k_{des}\Gamma(t) \qquad \text{Eq. (1)}$$

where D_A^e and C_A^e are the diffusion coefficient and the concentration of analyte A at the extractant surface, respectively, and C_A is the free analyte concentration in the sample solution at time t. The maximum attainable analyte concentration on the surface is Γ_{max} (mol cm^{-2}), while the surface concentration at time t is $\Gamma(t)$. Therefore, the free active site concentration at any time t is given by Γ_{max} - $\Gamma(t)$. As the adsorption progresses, $\Gamma_{max} - \Gamma(t)$ decreases while $\Gamma(t)$ increases, until equilibrium is reached. Since a static, unstirred sample solution was considered in this simulation, the boundary condition at the extractant surface is given in terms of mass flux,

$$-e \cdot (D_A \nabla C_A) = k_{ads} C_A(t)\left(\Gamma_{max} - \Gamma(t)\right) - k_{des}\Gamma(t) \qquad \text{Eq. (2)}$$

where e is the normal unit vector to the surface of the extractant. The initial and remaining boundary conditions for Eq. (2) are as follows:

At time $t = 0$, $C_A = 0$ and $C_A^s = 0$;

For all, $t > 0$;

At the sample domain, analyte concentration was fixed at $C_A = C_A^0$;

Insulation is applied to the walls of the sample container, i.e.:

$$-\hat{n} \cdot (D_A \nabla C_A) = 0 \qquad \text{Eq. (3)}$$

The above equations were solved in COMSOL Multiphysics software via finite element analysis, utilizing two application modules: the "transport of diluted species" module, and the "reaction engineering" module. For the examples discussed in this manuscript, a diffusion

constant, D_s, of 10.2×10^{-6} cm^2/s, corresponding to the diffusion constant of benzene in water, was used.

Chemicals and materials

Cocaine, propranolol, sertraline, LC-MS grade formic acid (FA), ether, potassium chloride, sodium chloride, potassium phosphate monobasic, sodium phosphate dibasic, chloro(dimethyl)octadecylsilane, ammonium hydroxide, magnetite particles (< 50 nm), and ethanol were purchased from Sigma Aldrich. LC-MS grade methanol (MeOH) was purchased from Fisher Scientific.

Sample preparation

A stock solution of cocaine, propranolol, and sertraline was prepared in MeOH at a concentration of 5 μg mL^{-1}. The working solution used throughout the experimental portion of this work, prepared according to a previously reported procedure,[21] was consisted of 100 ng·mL^{-1} of cocaine, propranolol, and sertraline in phosphate buffer solution (PBS) at pH = 7.4. Homemade octadecyl-functionalized magnetic nanoparticles (C_{18}-MNP) dispersed in a solution of MeOH:ether (50:50, v/v) were transferred into an Eppendorf conical vial, where solvent was evaporated. Then, 40 μL of the 100 ng·mL^{-1} analyte solution was added to the tube. Extraction was carried out by dispersing the C_{18}-MNP for 30 seconds in the solution, which was the shortest extraction time that could be practically performed. Following extraction, C_{18}-MNP were collected at the bottom of an Eppendorf tube with the use of a magnet while the solution was decanted. C_{18}-MNP were washed with water (4 x 100 μL) to remove nonspecific adsorption and salts from PBS prior to desorption. Finally, 10 μL of desorption solution (MeOH with 0.1% FA) was used to desorb analytes from the extraction phase of particles. The resulting extract was then directly injected to the mass spectrometer for instrumental analysis.

MS/MS analysis

Experimental work was carried out in an MS/MS system consisted of a SCIEX API 4000 triple quadrupole mass spectrometer (Applied Biosystems, California, U.S.A.) with an electrospray ionization source. All samples were run in positive mode, with the following

transitions: cocaine [parent ion: 304.1, product ion: 282.1] propranolol [parent ion: 260.1 m/z, product ion: 116.1 m/z], and sertraline [parent ion: 306.0 m/z, product ion: 275.1 m/z].

RESULTS AND DISCUSSIONS

Effect of shape and size of extractant on mass transfer

Recent research endeavors have focused on improvements to the kinetics of analyte transfer from the bulk sample to the SPME coating. Although agitation of the sample, which can be achieved by means of stirring or vibration, remains as the easiest approach to enhance the mass transfer of analytes, application of this tactic is not feasible in many sample environments, such as *in vivo* sampling, for instance.

In this section, extractions using three basic geometries of SPME devices, namely fiber, thin film, and spherical particle, were simulated in order to further elucidate the associations between extracted amount of analyte and equilibration time with the size and geometry of the probes. First, radial variations of extractants of different shapes were carried out to calculate their corresponding overall mass flux for a static sample system, as described in the experimental section. In previously published experimental work, [17] we have demonstrated that the equilibration time of the SPME device is inversely related to the radius of the fiber. As shown in Fig. 2, an inverse relation was observed with the size dependency of the mass flux, which is in agreement with the trends obtained in previously published experimental observations. Owing to the mass-transfer limitation inherent of SPME processes, a higher flux of analytes would provide shorter equilibration times. Considering that a decrease in the size of the extractant correspondently decreases the mass transfer limitation, devices with smaller dimensions are then expected to achieve faster equilibrium times. As shown in Fig. 2, the spherical geometry (round particle) exhibits the fastest increase of flux as its radius is reduced due to the three-dimensional reduction in the size of the device. On the other hand, the fixed length of the fiber geometry is responsible for the comparatively smaller increase of flux, as the decrease in size only occurs in two dimensions. Similar observations are reported in the literature that show how utilizing a sphere geometry is advantageous over cylinder and planar geometries.[22] Similar graphs can be obtained by using exact solutions provided by Crank, which was obtained for systems involving

absorption in whole volume of the probe. However our numerical solution is closer to real situation as the thin extraction phase is used supported by the body of the device.[23]

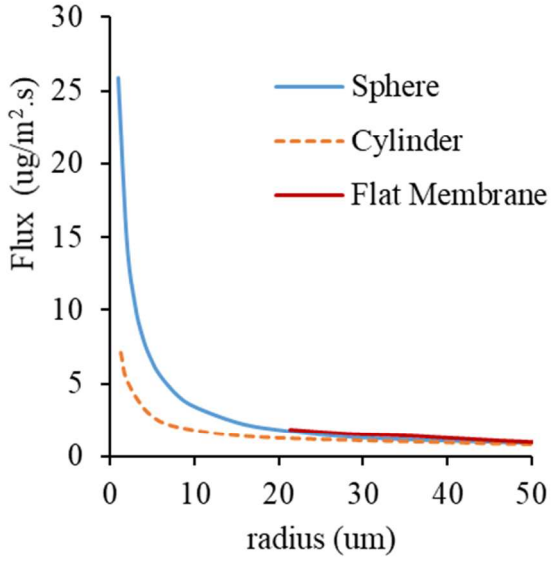

Figure 2. Effects of radius length for fiber and sphere geometries, and size of the extractant for thin membranes on the mass transfer kinetics in the linear regime of extraction (in this example the extraction time was 5 s). Membrane thickness was 1 µm, and surface reaction was considered between the bulk and the extractant. The distribution constant of the model analyte between the bulk and extraction phase (K_{es}) was 100. While the sample volume was fixed, the radius of the fiber (cylindrical) and particle (spherical) were decreased. For flat square membranes, both dimensions were decreased so as to achieve the same surface area as spherical particles. Results corresponding to flat membranes <20µm correspond to results obtained for spherical particles.

Radial diffusion: driving force for faster equilibration

As illustrated by Fig. 2, the radial diffusion phenomenon is observed only if the diameter of the probe is below approximately 30 µm. Most commercial SPME fibers have a diameter greater than 200 µm. Employment of fibers with diameters of less than 50 µm can be very challenging due to very flexible and fragile nature of the materials used to manufacture SPME devices. In this regard, previous attempts to use gold microwire or carbon microfibers as supports for SPME probes were unsuccessful, as these materials did not provide enough rigidity for their intended applications.[17] Recently, our group has introduced a miniaturized SPME fiber, namely coated-tip SPME, which employs the concept of radial diffusion to increase the uptake rate of analytes for rapid extraction of compounds from different matrices.[17] In that work, the

conical shape coated-tip consisted of a tip with a diameter of approximately 20 µm, which was increased to 50 µm at the base of the tip, and with a coating length of 500 µm. The coated tip offered better stability and convenient handling, while preventing the device from bending during sampling. The short equilibration time (up to 2 minutes) of the coated tip SPME devices can be attributed not only to its very thin coating (<5 µm), but also to the radial diffusion of analytes. As shown in Fig. 3, the effects of reductions in the diameters of two configurations of SPME, fiber and coated-tip, are compared considering both the kinetics of mass transfer and the efficiency of extraction for a given analyte.

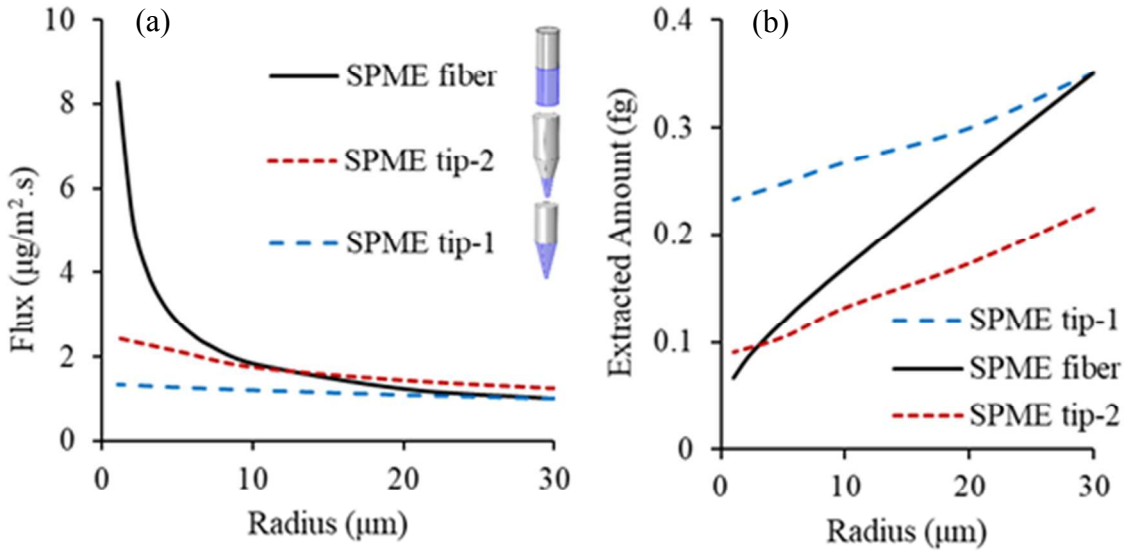

Figure 3. Effect of reduction in diameters of SPME fiber and the coated-tip on the kinetics (a) and efficiency (b) of microextraction applications. The SPME fiber was assumed to have radii from 1 – 30 µm, while the SPME Tip-1 was considered to have a top radius of 30 µm, and bottom radii that varied from 1 – 30 µm. Both extractants were of a length of 100 µm. The SPME Tip-2 was considered to have a length of 50 µm, with a 15 µm top radius and bottom radii that varied from 1 – 15 µm. The model simulation considered a surface reaction between the bulk and the extractant. Parameters used in the simulation were as follows: flow velocity 0 cm/s, molecular weight of analyte 78 $g \cdot mol^{-1}$, diffusion coefficient of the analyte 1.16 $\times 10^{-5}$ $cm^2 \cdot s^{-1}$, analyte concentration 1 $nmol \cdot mL^{-1}$, distribution coefficient 1000, time of extraction 10 s.

As shown in Fig. 3a, a decrease in the SPME fiber radius, from 30 µm to 1 µm, yielded an approximate 3-fold improvement to the mass flux. For Tip-1, an increase of the flux could

also be observed while keeping the top radius fixed and varying the bottom radius from 30 to 1 μm, although the yielded change was not as high as that observed for the fiber. However, if only the narrowest part of the tip is coated with extraction phase, as is the case of SPME Tip-2, a much higher flux could be expected. In contrast to the increase of flux that was observed by decreasing the radius of the extraction device, as shown in Fig 3b, the extracted amounts attained by the reduced devices decreased almost three-fold and one-fold for the fiber and Tip-1, respectively. In addition, for Tip-2, the extraction efficiency was even lower due to the shorter length of the coating.

Aiming to investigate further physical aspects regarding the relative effects of transport and geometric factors on the resultant equilibrium time, a comparison was carried out between concentration profiles of analytes gathered on the outside boundary of two different coating geometries with respect to extraction time. For this comparison, a fiber of 1 μm radius and a flat membrane with an area of 1000 μm^2 were considered. As shown in Fig. 4a, the concentration gradient attained by the fiber extends only to about 60 μm from the surface, with equilibrium reached within 300 seconds. On the other hand, for the flat membrane, the concentration gradient keeps increasing with time.

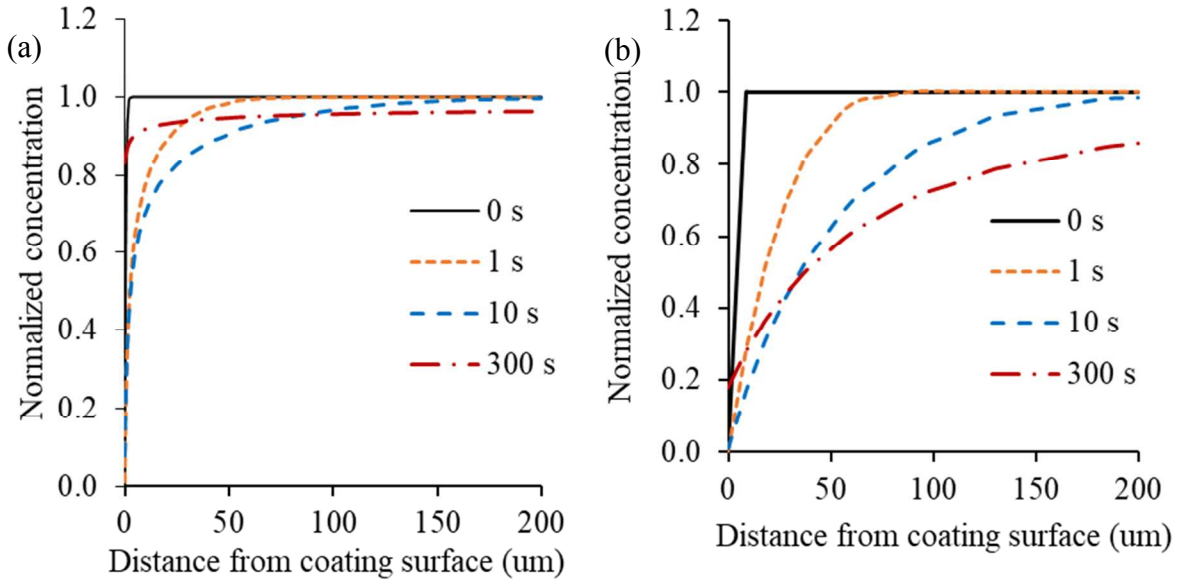

Figure 4. Analyte concentration profiles outside of coatings in the sample domain for (a) a fiber of radius 1μm and (b) a flat membrane geometry of 1000 μm^2. Simulation was carried out at static conditions with no sample agitation.

The concept of radial diffusion can be visualized by plotting total flux lines occurring at coating surfaces of two different sizes during initial stage of the extraction. As demonstrated in Fig. 5, for a flat surface of 100 μm radius, most of the flux lines are perpendicular towards the coating surface, with a very few radial flux lines occurring on the edge of the surface. In contrast, most flux lines occurring on the surface of the coating of 2 μm radius are radial-oriented. The enhanced radial flux increases the mass flux, which in turn reduces the equilibration time of devices with coatings of a size smaller than 30 μm.

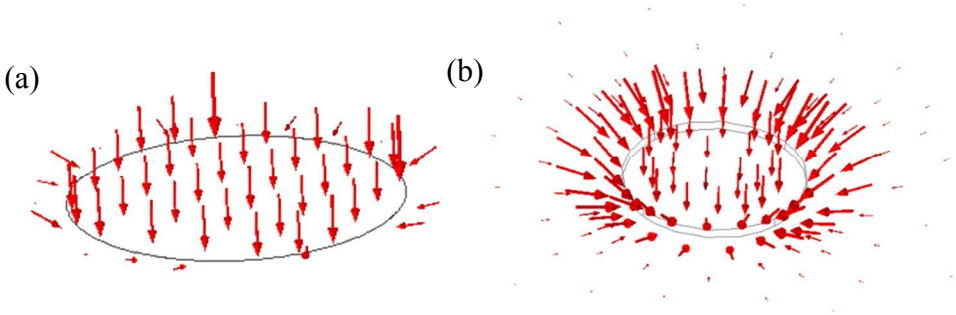

Figure 5. Simulation results of a comparison of total fluxes on circular coating surfaces of radii (a) 100 μm and (b) 2 μm. Simulation was run under static conditions with no sample agitation during initial stage of the extraction.

To improve the sensitivity of a method, enough molecules must be extracted so as to allow for instrumental detection to take place within a short time.[24,25] In cases where sensitivity is not an issue, changes to tip design, including decreasing the tip radius and using a smaller tip length, could be a viable option for applications that require fast sample analysis. While extraction efficiency is a main factor to consider in the development of exhaustive extraction procedures, in equilibrium-based extraction processes such as SPME, where recoveries are usually comparatively low, equilibration time is one of the main parameters to be considered during method development, as faster equilibria will lead to faster determinations, and thus, increase the overall speed of analysis. However, in view of the limitations imposed by the expected low recoveries attainable via SPME, reducing the device radius below the micrometer regime may not be very beneficial in some cases, as the desired sensitivity for a given compound may not be achieved. Further, from a practical point of view, it should be noted that it is quite technically challenging to prepare and operate SPME fibers with such narrow radii. On the other hand, the advantages of using small sampling device dimensions include shorter equilibration

times, leading to faster analyses, as well as reductions in sample volume requirements, a factor that proves to be quite advantageous in certain bioanalytical applications. When combined with mass spectrometry, SPME systems have recently achieved specific analyte detection at roughly pg·mL^{-1} concentrations, corresponding to just a few femtograms of analyte molecules in a microliter range sample volume.[17,26,27] In this line, the development of technologies that efficiently interface this new generation of devices to mass spectrometry, maximizing sensitivity and speed of analysis, is highly encouraged. Further, in cases where higher extracted amounts are required, a number of such small devices can be used simultaneously, either as separated items, or by connecting multiple devices to a holder, as seen in sensor array designs.[28,29]

Kinetic Aspects of Exhaustive Extraction and Microextraction

In order to compare the extraction kinetics of the microextraction approach with that of exhaustive extraction, an SPME fiber of 150 μm radius immersed in varied sample volumes containing a concentration of 1 nM of analyte was considered as model.[6] As shown in Fig. 6a, analyte concentration in the sample domain drops dramatically when the ratio of extraction phase to sample phase volume, β, is high ($\beta = V_e/V_s > 0.1$). On the other hand, for much lower values of β, the concentration of analyte in the sample domain does not change significantly, except in the boundary layer region around the sorbent, as shown in the Fig 6b.

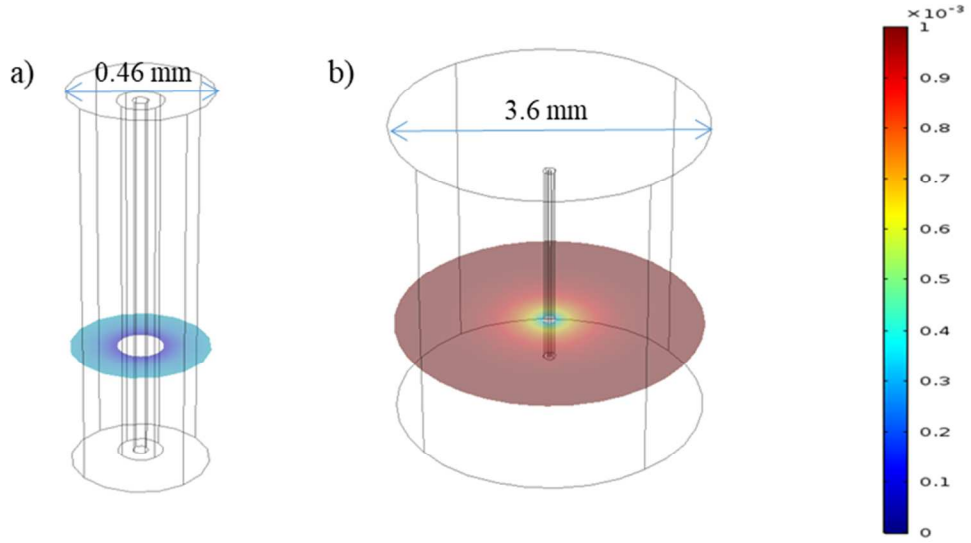

Figure 6. Change of analyte concentration in the sample domain at 0.5 minutes of extraction with (a) an extractant to sample volume ratio (β*)* of 0.1 and (b) a ratio of 0.001. For both experiments, other parameters, such as V_e and D_s, were considered fixed, while

the partition coefficient, (K_{es}), had a value of 100.

Using this model, extraction kinetics were studied for different β values. As shown in Fig. 7, exhaustive recovery occurs faster as compared to equilibrium extraction, as at lower β values, the transport of analytes from a far distance is circumvented, unlike cases where sorbents are placed in a large volume of sample. In other words, as the sample is depleted of analyte, the concentration of analytes in the extraction phase decreases correspondingly, resulting in shorter equilibrium times as lesser amounts of analyte need to reach the extraction phase via the boundary layer. While this feature might at a glance suggests that microextraction approaches are disadvantageous in comparison to exhaustive techniques, microextraction techniques allow for the use of smaller quantities of sorbents to attain comparable detection limits due to higher enrichment factors provided by these techniques, an aspect which is discussed in more detail below. It should be noted, however, that although extraction at equilibrium conditions provides maximum analyte enrichment, SPME can nonetheless be used in applications to quantify analytes at any point of extraction under the pre-equilibrium regime, provided that obtaining lower analyte sensitivities do not pose a problem for analysis. [30]

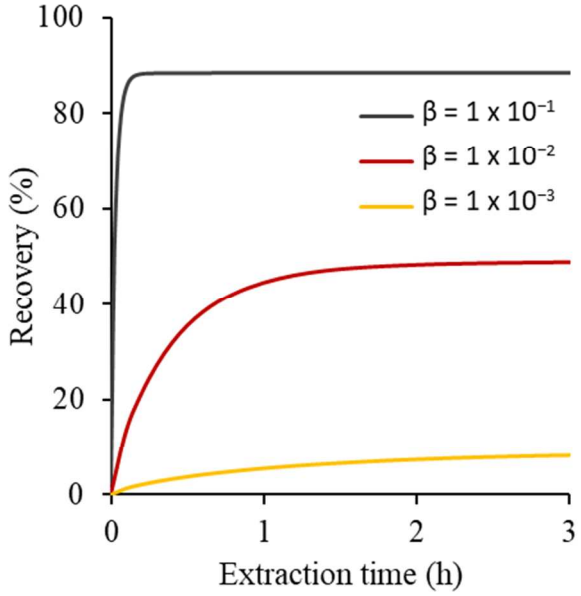

Figure 7. Computational simulation results of the dependence of the extraction time profile on variations in the extractant to sample volume ratio (β). Volume of the extractant, V_e, was assumed constant (0.6 µl) and sample volume, V_s, was varied from 6 µl to 600 µl. Extraction was considered to occur under diffusion-only conditions (static

extraction), with a partition coefficient, (K_{es}) value of 100.

Magnetic nanoparticles and SPME

A viable approach to collect a sufficient amount of analyte at a minimum time and cost entails the use of an optimum number of particles coated with a suitable extraction phase, such as magnetic particles, as extraction devices. The majority of applications to date involving the use of magnetic particles have focused on exhaustive extractions, where an excess amount of extraction phase is used.[31] In this regard, an obvious question thus arises: how can satisfactory analyte collection, in which sufficient detection limits are attained, be assured through employment of devices containing such small amounts of extractant? In order to discuss how sensitivities comparable to those attained via exhaustive extraction methods can be achieved via microextraction approaches, the differences in enrichment achieved by both approaches must first be considered. The enrichment factor is defined as the ratio of analyte concentration between the extraction phase and the matrix:

$$E_f = \frac{n_e/V_e}{n_s/V_s} \qquad \text{Eq. (4)}$$

Where the enrichment factor (E_f) is a function of the extracted amount (n_e), the volume of the extraction phase (V_e), and the concentration of analytes in the sample matrix (n_s/V_s). Eq. 4 can be rewritten as follows:

$$E_f = \frac{n_e/n_s}{\beta} \qquad \text{Eq. (5)}$$

In exhaustive extraction, β is optimized to achieve the transfer of the majority of analytes to the extraction phase, resulting in low enrichment factors as equilibrium is reached for depleted concentrations of analyte in the matrix. This is particularly true when multiresidue analysis is performed, where exhaustive extraction conditions need to be reached for all target analytes, which vary widely in affinity towards the extraction phase. In such a situation, the amount of extraction phase used needs to facilitate exhaustive extraction conditions for the lowest affinity compound, which is a much higher amount as compared to that needed for high affinity compounds, thus resulting in low enrichment factors for all target analytes. On the other hand, if the extractant volume is sufficiently small (microextraction), then the analyte distribution

constant (K_{es}) between sample and coating defines the enrichment factor for all target analytes. The main distinction between both techniques lies in their respective extraction recoveries; low for microextraction, and close to 100 % for exhaustive methods. However, despite its small extraction recovery, microextraction techniques provide higher enrichment factors, which leads to higher concentrations of analytes in the extraction phase, and therefore, similar sensitivities of overall determinations, particularly when direct introduction to an analytical instrument is employed.

Dispersive extraction approaches facilitate enhanced mass transfer due to the increase in area contact between the extraction phase and the matrix. In particular, the use of nano-sized particles caring sorbents contributes to a substantial decrease in extraction time as compared to the bulk undispersed approach, while also providing the added benefit of using a sorbent with magnetic properties, which facilitates easier handling of the sample.[32] In the experimental work presented here, extraction was performed from a water solution containing cocaine, propranolol, and sertraline. For this application, the amounts of used sorbent (C_{18}-MNP) were within the range 30-120 μg, enabling equilibrium to be reached for all analytes at 30 seconds of extraction. For every analyte, extracted amounts obtained for 30 μg of C_{18}-MNP were used to estimate the volume of the extraction phase (where the number of particles and sorbent coating thickness were known). Then, the calculated extraction phase volume was used for estimation of the distribution coefficients between the extraction phase and the sample *(*K_{es}). The estimated K_{es} values were then used to predict the enrichment factors, E_f, in the extraction phase as a function of sorbent masses. Fig. 8 illustrates changes in enrichment factors for cocaine, propranolol, and sertraline for different amounts of C_{18}-MNP sorbent. In this respect, higher E_f values in the extraction phase were obtained for analytes with higher logP values, since they have higher affinity for hydrophobic extraction phases. The more hydrophobic the compound, the more dramatic is the observed difference in enrichment between negligible depletion conditions ($E_f = K_{es}$), when fewer particles are used, and exhaustive extraction conditions ($n_s = n_e$), when more particles are used. For less hydrophobic (lower K_{es}) compounds, the change is less pronounced, although all curves approach a value of $1/\beta$ when exhaustive extraction is reached for all analytes. In other words, in multi-component determinations, exhaustive extraction applications yield the same enrichment for all target analytes, while in microextraction, the enrichment of a

given analyte is related to its affinity for the extraction phase, resulting in an approach that utilizes the full enrichment capacity of the used sorbent for all analytes under study.

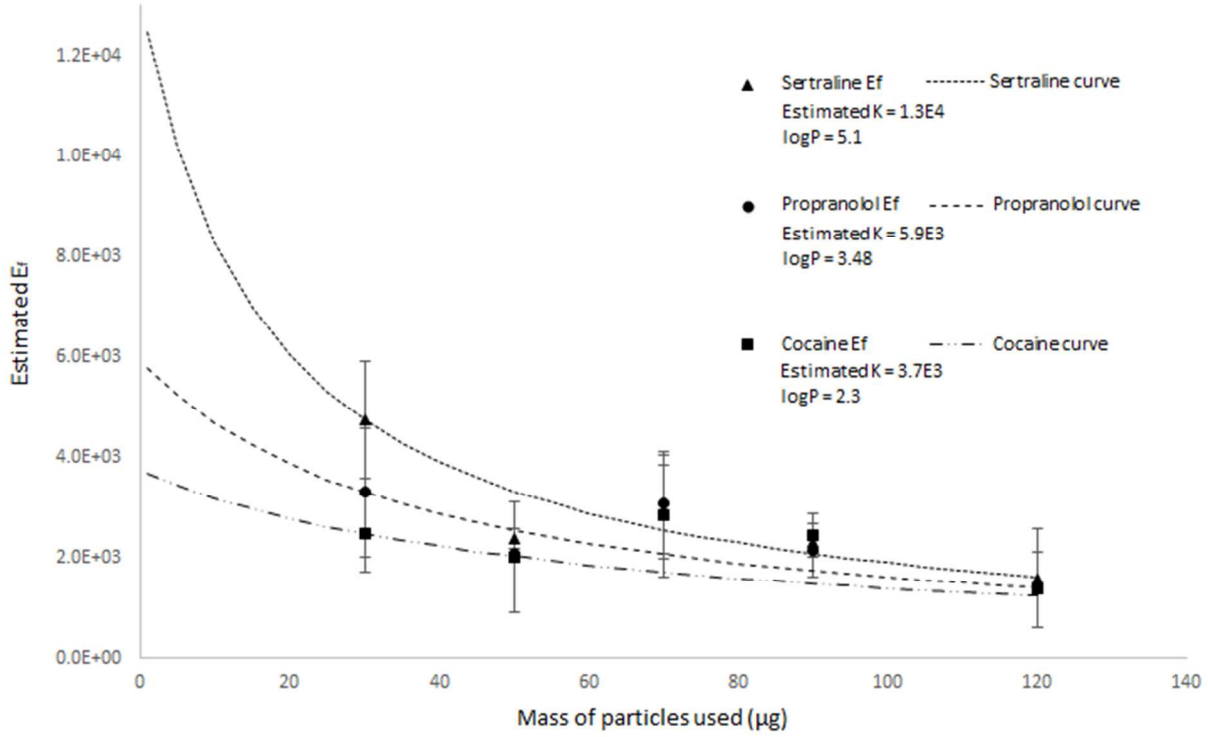

Figure 8. Relationship between enrichment factor (E_f), ratio of analyte concentration in the extraction phase at equilibrium and initially in the sample, and mass of sorbent particles, for extraction of cocaine, propranolol, and sertraline. Points on the graph represent analyte experimental results, while corresponding lines represent E_f predictions over the mass range of sorbent particles.

Although exhaustive extraction of analytes was achieved when sorbent amounts equal to or higher than 70 µg of C_{18}-MNP were employed, higher analyte enrichment values in the extraction phase were obtained when smaller amounts of C_{18}-MNP were used. In other words, addition of more sorbent in this case just "diluted" the analyte in the extraction phase. Therefore, it can be concluded that for microextraction applications such as the herein employed, a smaller amount of particle sorbent can be used to quantify an analyte, without loss of sensitivity. Hence, desirable sensitivity can be achieved with an optimum amount of sorbent particles. One of the advantages of using smaller amounts of sorbent particles includes a lower cost-per-analysis. This is especially important in high throughput applications, as well as in applications that require employment of expensive sorbent particles. In addition, employment of smaller amounts of

particles containing highly enriched analytes facilitates the design of direct introduction approaches to analytical instrumentation. Conversely, offline desorption will lead to sensitivity losses, as typically, only a portion of the desorbed analyte solution is introduced into the separation and/or detection instrumentation. The only possible advantage of using large amounts of particle is that equilibration is faster due to the larger β attainable, as illustrated in Fig. 7. However, equilibration time is comparatively much faster when small particles are used in contrast to bulk sorbent use, even in microextraction approaches such as the discussed above. In this experimental work, while equilibration was achievable below 30s, an equilibration time of 30s was selected as optimum, as this was the shortest extraction time in which we could practically perform extractions with the magnetic particles.

CONCLUSIONS

Numerical simulations along with experiments were used to examine the efficiency with which microscale sampling devices of various geometries can remove analytes from solution. The principal goal of the presented research was to explicitly examine mass transport effects on sampling with respect to various sizes and shapes of extraction devices. Care should be taken before expending considerable effort to fabricate smaller SPME devices, as decreasing the size of SPME devices may not impart all the benefits of sample analysis; for example, in instances where less sensitive detectors are used for determinations. Although unique applications certainly exist for sub-micrometer scale SPME probes, if the goal of analysis is overall sensitivity, just reducing probe radius may be misguided. The simulation results presented here suggest that optimization of device size and geometry for a given application should include an examination of the balance between the extracted amounts of analyte relative to the critical dimension of the coating. Taking the case of the coated tip SPME, if a given analyte can be extracted in a quantity that is more than sufficient for its detection, then a decrease in the coating radius may facilitate more rapid collection of analyte molecules for detection, since the coated-tip SPME allows for shorter equilibration times as compared to the fiber SPME. For most practical applications, however, larger devices are preferred, as the flux to the coating can be increased via agitation, which reduces the boundary layer, and therefore the effect of diffusion through the matrix. However, for particular applications, such as determinations in small volume

samples (e.g., single cell analysis), the smaller dimensions of such devices will be very useful, resulting in fast analyses. Finally, the sensitivity limitations inherent to smaller coating dimensions could also be overcome through utilization of multiple probes together. While such designs might complicate operation, it has been demonstrated, for instance, that efficient collection of small size devices (particles) can be accomplished with the use of magnetic particles and magnets.[31] However, it should be emphasized that the use of more particles per volume of sample may not always enhance the sensitivity of the method. The use of large amounts of particles for high throughput determinations is not only costly, but might hamper the handling of samples; therefore, particle volume should be carefully optimized during method development. As discussed in this work, an increase in the number of particles used for extraction has two effects: a negative effect, associated with a decrease in the enrichment factor attained in the extraction phase, and a positive effect, associated with a decrease in the time required to achieve equilibrium (see Figure 7). However, in cases where nano-sized particles are used as dispersive extractive devices, equilibrations for all analytes can be assumed to occur within an extremely short period of time due to radial diffusion and the dimensions of said devices, facilitating completion of extraction within seconds even when only a small number of particles are used. Therefore, it can be concluded that there are no benefits to using more particles for extraction than those required to meet the desired sensitivity and detection limits, as extraction time is not a limitation for this application.

The conclusions herein reached for extractions are also valid for the desorption step. Using dispersed particles in the desorption solvent will facilitate faster removal of target analytes from the extraction phase, as the desorption process is in fact the extraction process. Similarly, using “microdesorption” (desorption into smaller volumes of solvent) rather than exhaustive desorption results in enhancement in analyte enrichment in the desorption solvent, facilitating higher sensitivities.[33] Therefore, adjusting the desorption volume so that it corresponds to the required injection volume for a given format of extraction, rather than optimizing the volume of the solvent for complete analyte removal, is a good approach if the main objective is to obtain lowest detection limits. The above discussion does not apply to cases where the whole volume of the extraction phase is desorbed and focused in analytical instrumentation, as it is done for different formats of SPME coupled to GC.

The above discussion is not limited to solid phase extraction technologies. It is also useful in solvent extraction as well as the energy applied in liquid-liquid extraction is not only used to move the phases faster in respect to one another (better agitation), but also to produce better dispersion resulting in smaller size of solvent particles suspended in the sample. This will lead, if the particles are smaller than 10 micrometers, to additional mass transfer acceleration associated with radial diffusion. Therefore, there is an optimum point for each application depending if speed or cost saving are more important. Interestingly, estimation of such point can be done prior to the experiment by performing mathematical modeling, as discussed in this contribution. It should be emphasized that umber of approaches has been developed which create small size solvent particles, such as cloud point extraction which does not require much energy consumption but has been demonstrated to be very effective as well. Finally, the same rules and conclusions apply when the sample matrix is dispersed in the solvent. The smaller the particles, especially, if they are below 10 microns the faster removal of analytes occurs, unless the rate of extraction is controlled by dissociation rate constant for solid matrices. The similar rules as discussed above will apply to continuous extraction technologies, such as countercurrent extraction or chromatography, where reducing particles to low micron range will result not only in improvement in mass transfer in mobile phase but it will also reduce the interfacial resistance to mass transfer. We are presently investigating the enhancements for small structures at convection conditions to better understand impact of this phenomena in agitated and flow systems.

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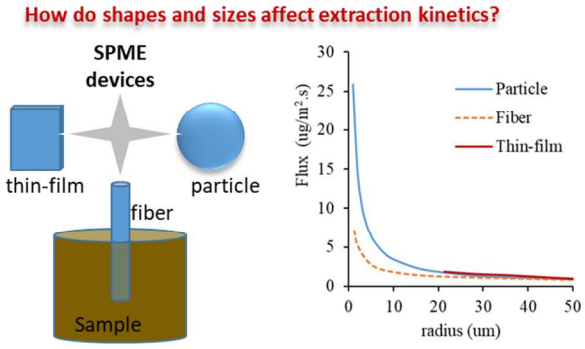
TOC


How do shapes and sizes affect extraction kinetics?
SPME devices
thin-film
particle
fiber
Sample
Flux (ug/m² s)
radius (um)
Particle
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Thin-film
0
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