analytical chemistry

Letter

Cite This: *Anal. Chem.* 2018, 90, 2430−2433

pubs.acs.org/ac

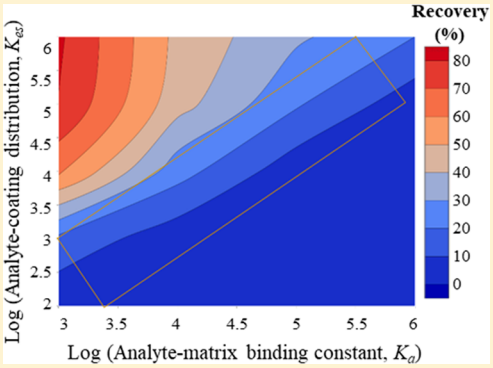
Effect of Binding Components in Complex Sample Matrices on Recovery in Direct Immersion Solid-Phase Microextraction: Friends or Foe?

Md. Nazmul Alam and Janusz Pawliszyn*

Department of Chemistry, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

Supporting Information

ABSTRACT: The development of matrix compatible coatings for solid-phase microextraction (SPME) has enabled direct extraction of analytes from complex sample matrices. The direct immersion (DI) mode of SPME when utilized in conjunction with such extraction phases facilitates extraction of a wide range of analytes from complex matrices without the incurrence of fouling or coating saturation. In this work, mathematical models and computational simulations were employed to investigate the effect of binding components present in complex samples on the recovery of small molecules varying in logP for extractions carried out using the direct immersion approach. The presented findings corroborate that the studied approach indeed enables the extraction of both polar and nonpolar analytes from complex matrices, provided a suitable sorbent is employed. Further results indicated that, in certain cases, the kinetics of extraction of a given analyte in its free form might be dependent on the desorption kinetics of their bound form from matrix components, which might lower total recoveries of analytes with high affinity for the matrix. However, the binding of analytes to matrix components also enables SPME to extract a balanced quantity of different logP analytes, facilitated by multiphase equilibria, with a single extraction device.



Today, rapid improvements in coating materials alongside the diverse device geometries offered by solid-phase microextraction (SPME) allow for application of this technology toward analysis of various complex samples, such as applications involving in vivo bioanalysis and determinations in food matrices, applications which were previously difficult to implement by directly exposing previously developed devices into complex sample matrices.[1−4] While direct immersion (DI)-SPME is often a preferable method to extract analytes both for targeted and untargeted studies due to its rapid and sensitive analysis abilities, traditionally, analyses of complex sample matrices such as food and biological matrices have been mostly confined to headspace (HS) sampling,[5] as the direct exposure of earlier SPME coatings to complex matrices was shown to lead to deterioration of the extraction phase, resulting in a lack of reproducibility, poor sensitivity, and insufficient ruggedness.[6,7] Within this context, one of the main challenges associated with DI-SPME regards the coextraction of undesirable species and matrix components, which cause poor extraction efficiency and reproducibility by fouling the extraction phase.[8,9] Extraction of undesirable high molecular weight compounds could interfere with analysis by either reducing the extraction efficiency or introducing matrix effects in the electrospray ionization source of the mass spectrometric detection system. Further, previous multiresidue analyses via DI-SPME have also been reported to be burdened by coating saturation; as traditional SPME coatings are very small in size, they can become quickly saturated with the most abundant analytes present in a given matrix, while other analytes remain unextracted.[10,11]

The recent development of matrix-compatible SPME coatings (overcoated SPME and BioSPME), where materials such as poly-dimethylsiloxane (PDMS),[8,12] Teflon, or poly-acrylonitrile are placed over an SPME extractant,[13,14] has demonstrated that, in addition to limiting matrix effects, such SPME coatings provide balanced coverage of analytes with a broad range of properties from complex sample matrices.[15,16] Indeed, while a competitive adsorption phenomenon has been reported to occur for HS ex vivo analyses of different food matrices, substantially reduced artifacts related to coating saturation were observed for analysis of complex matrices in DI batch extractions and in vivo applications.[17] In this regard, matrix components (such as suspended solids and macromolecules) present in complex matrices have been assumed to significantly bind analytes, especially nonpolar compounds, reducing their free concentration and thus their availability to be extracted by the coating.[18] However, to date, no systematic studies have been undertaken with aims to answer the question of how the presence of such matrix components helps to

Received: December 27, 2017
Accepted: January 31, 2018
Published: January 31, 2018

DOI: 10.1021/acs.analchem.7b05436
Anal. Chem. 2018, 90, 2430−2433

achieve the balanced coverage provided by matrix-compatible SPME coatings.

Our group has recently developed mathematical models for various quantification approaches employed in SPME by utilizing the commercial software COMSOL Multiphysics.[19−21] In the currently presented work, mathematical models were used to investigate how binding matrix components aid in achieving balanced coverage of analytes extracted from complex matrices via employment of single SPME devices.

RESULTS AND DISCUSSION

Balanced Coverage. SPME matrix-compatible coatings (Bio-SPME or overcoated SPME) consist of a sorbent, which can be selected from a wide variety of available materials of different chemistries, attached to a support and covered by a biocompatible protection layer made of an appropriate polymer, resulting in enrichment via a restricted access extraction process. Placing such devices in contact with complex matrices results in clean extractions, as the protection layer of the device prevents strong matrix attachments to the coating. In this scenario, balanced extraction of polar and nonpolar analytes is attained via analyte distribution in multiphase equilibria, which involves both the sorbent and various matrix components.[1] In this work, the partitioning process is modeled so as to elucidate the principles behind the balance coverage phenomenon.

Here, as analytes being extracted by a particular SPME coating encompass a wide range of physicochemical properties, they can be assumed to likewise have a wide range of coating-sample distribution constants (K_{es}). Such variations in K_{es} values in turn result in different equilibration times for these analytes. Since extraction time is kept constant in typical SPME applications, in cases where short sampling times are needed for a given application, analytes with lower K_{es} values are more likely to reach equilibrium, while analytes with higher K_{es} values may remain in the kinetic or linear extraction regime during the designated extraction time.[22] If the extraction is in the linear regime for large K_{es} analytes at a particular extraction time, the extracted amount will be independent of the K_{es} at that regime. In this condition, the extraction process will only be dependent on the diffusivity of the analytes in the sample matrix as long as the analyte concentration remains almost unaltered during the extraction period.[23] Equilibration times for samples that contain a binding matrix might also be dependent on the dissociation rate constants of analytes from the matrix−analyte complex and thereby may be longer than equilibration times for samples that do not contain binding matrix components.

In reality, many target analytes show a preference toward binding with species present in complex sample matrices. While many applications of SPME, such as negligible depletion SPME, have as a goal the nondisturbance of bound analytes present in the sample,[24] in some applications, such as bioanalysis and food applications, some of these bound analytes can be dissociated from the matrix−analyte complex due to the employment of limited sample volumes as well as high affinity coating chemistries. In such cases, the binding equilibrium (shown in Figure S1) shifts left, facilitating the extraction of larger amounts of free-form analytes by the coating, resulting in a clean "via free form" extraction process.

Previous experimental data demonstrated that direct immersion of bio-SPME coatings in complex biological sample matrices enabled extractive advantages not otherwise attainable when such SPME devices are deployed in samples without a binding matrix present.[8,25] The most noticeable advantage is the ability of such coatings to extract a wide range of analytes from complex matrices without manifestation of displacement or saturation effects. Reyes-Garcés et al. carried out extractions of 25 chemicals consisting of a wide range of polarities via an SPME coating made of hydrophilic−lipophilic balance (HLB) particles.[18] Here, a comparison of the extraction profiles of two of these analytes, stanozolol (LogP 3.81) and morphine (LogP 0.89), in phosphate buffer saline (PBS) and plasma is shown in Figure S2. PBS is a solution that mimics the pH of human blood and does not contain a matrix component, while plasma contains a binding matrix. As can be gathered from the extraction time profiles of these analytes in PBS, stanozolol shows higher affinity for the coating as compared to morphine. As seen in Figure S2, a decrease in the extracted amount of stanozolol in PBS was observed to occur after 50 min of extraction, a phenomenon assumed to occur due to displacement by other analytes present in the sample that have higher affinity for the coating.

Conversely, the extraction of analytes from blood plasma samples followed a pattern distinct from that of extraction from PBS, where extracted amounts remained constant after equilibrium was reached for morphine. The much lower amounts of Stanozolol extracted from plasma were attributed to its competitive binding to matrix components. These results have shown that the presence of binding matrix components facilitates application of SPME technologies in areas of analysis where a wide range of analytes need to be analyzed for both qualitative and quantitative determinations. Here, with the help of mathematical models and computational simulations, we sought to answer the question of how these binding matrix components aid in achieving balanced coverage of analytes extracted from complex matrices via employment of single SPME devices.

In the currently presented work, the mutual effect of the two equilibrium constants, K_{es} and binding constant of analyte with matrix components, K_a, on the recovery of a wide range of analytes was studied with a mathematical model, where analytes with extreme K_a values were considered. As shown in Figure S3, for analytes with low binding constants, for example K_a = 1000, analyte recovery can be increased up to exhaustive extraction by using a coating with higher affinity for the analyte (high K_{es} value). Although K_{es} and K_a values are similar for analytes extracted by coatings with hydrophobic chemistries such as PDMS and C-18, the chemistries of ionic or mixed mode coatings (e.g., HLB) might yield K_{es} values that differ from corresponding K_a values of a given analyte. If the K_{es} of an analyte is very high, while its K_a is sufficiently low, the analyte can then be exhaustively extracted with the SPME device. As demonstrated in Figure S3a, exhaustive extraction requires longer extraction times as compared to the equilibrium times that can be achieved when K_{es} values are lower. This observed increased time accounts for the time required to pull all analytes off the bound matrix, as well as for the time needed for the diffusion of said analytes through the sample media to take place. On the other hand, if the analyte has a very high K_a value, most of the analytes remain in their bound form in the sample matrix; in such cases, it is unlikely that exhaustive extraction will occur for this analyte, even if its K_{es} value is very high. Due to the strong binding of analytes with matrix components, analyte recovery remains very low, as shown in Figure S3b.

The lower recoveries of high K_a analytes might also contribute to lower extraction rates if the rate of desorption

DOI: 10.1021/acs.analchem.7b05436
Anal. Chem. 2018, 90, 2430−2433

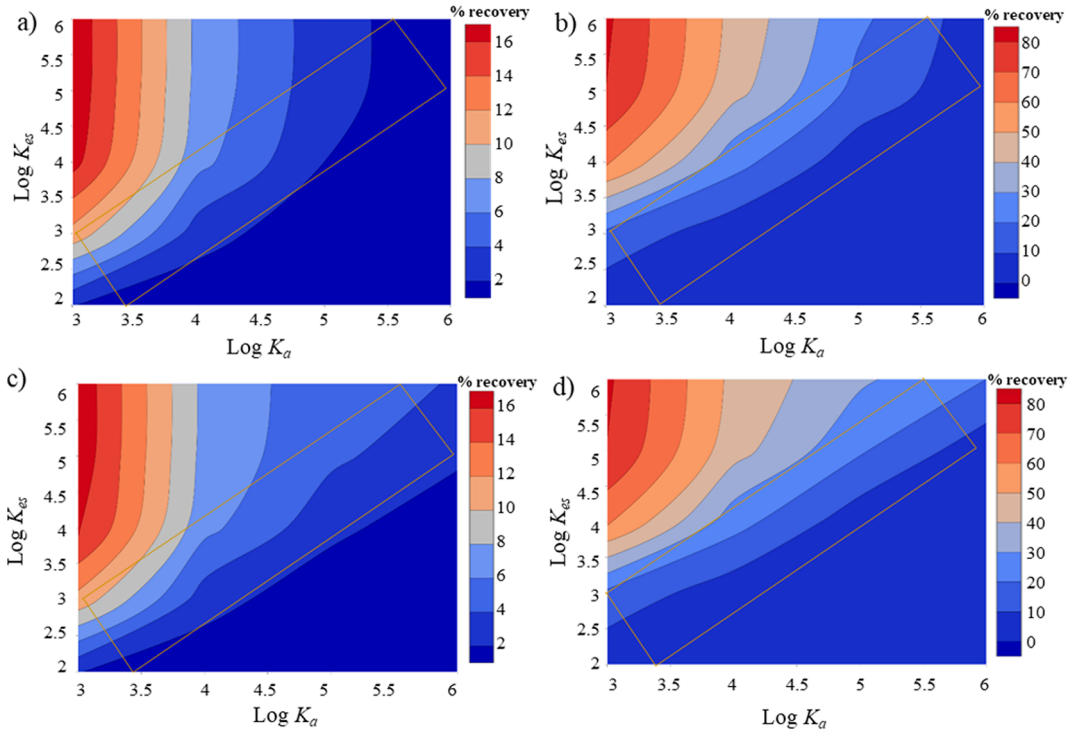


Figure 1. Effect of coating-sample distribution constant (K_{es}) on analyte recovery for varying analyte−matrix binding constants (K_a) at two different extreme cases. The first case assumes the desorption rate constant of bound analyte, k_r, changes as K_a is changed, at short extraction times of 5 min (a) and 500 min (b). In the second scenario, the k_r is assumed to be fixed at 1 s^{-1} for (c) 5 min of extraction time (d) and 50 min of extraction. Initial concentration of matrix components was kept constant at 30 ng/L.

of analytes from the bound matrix is slow enough to control the kinetics. Analytes with high binding affinities to the matrix (high K_a) also tend to exhibit strong binding with the matrix, a process which is governed by the lower desorption rate constant, k_r. As shown in Figure S4, variations in k_r values were shown to correspondingly affect analyte recovery rates. It can then be surmised that analytes with very high K_a values will yield low recoveries and slower extraction rates due to slower desorption from the analyte−matrix complex (i.e., lower k_r).

Computational simulation results corresponding to recoveries of analytes with a wide range of K_{es} and K_a values at three different extraction times have been plotted as color maps in Figure 1. In general, recovery was observed to increase as K_{es} values increased for analytes with lower K_a values. This observation was true for all extraction time regimes. For the 5 min extraction (Figure 1a), the recoveries of analytes with Log K_a values of more than 4.5 were less than 2%; such compounds had low free concentrations due to their binding with matrix components, while lower Log K_a compounds yielded higher recoveries due to their lower matrix binding affinities, despite the fact that their affinities for the extraction phase were lower than those of high Log K_a compounds. In this scenario, recoveries are almost independent of K_{es} values, owing to the fact that extraction occurs in the linear diffusion-controlled regime during the experimental extraction time. As extraction time increases (Figure 1b), the recoveries of analytes with higher K_a values also increase, while lower Log K_a recoveries remain constant once equilibrium is reached for these compounds. Therefore, analytes with very low matrix-binding affinities can be extracted rapidly according to their affinities for the coating (K_{es}), while high matrix-binding analytes can also be extracted in sufficient quantities for detection through utilization of longer extraction times. The lower recoveries of hydrophobic analytes within practical extraction times, caused by the presence of binding matrix components affecting their free concentrations in the matrix, hinders the displacement of hydrophilic analytes by hydrophobic analytes in the coating. This interplay between K_{es} and K_a values during the extraction process results in the *balanced coverage* feature of direct matrix compatible SPME. Expected analyte coverage is shown in the rectangular box in Figure 1 for general purpose coatings of a hydrophobic nature. Results shown on the two extreme corners of the plots, where recoveries are either extremely high or low, are only correspondingly possible for specific binding of analytes by coatings or matrix components.

Additionally, the coating chemistries typically employed in SPME of organic compounds (even polar coatings) have higher affinities toward hydrophobic analytes, which show higher binding affinities toward binding matrix components as compared to hydrophilic analytes. The high matrix-binding affinities of hydrophobic analytes result in lower free concentrations of said analytes in the sample matrix, which in turn restrict their levels of recovery by the coating. Since the value of the desorption rate constant, k_r, is unknown for most analytes,[26] in Figure 1c,d, the desorption rate was assumed to be fast, with k_r = 1 s^{-1} for all studied values of K_a. Thus, equilibrium is reached faster as compared to cases where the extraction rate is controlled by desorption rate from the matrix. Here, hydrophobic analytes achieved equilibration at 50 min of extraction (Figure 1d), whereas some hydrophobic analytes in the case of lower k_r were still in their linear regime of extraction at even 500 min (Figure 1b). Such a finding would indicate that, in cases where analytes are bound very strongly or irreversibly with matrix components, the extracted amounts of such compounds achievable via SPME might be too low for instrumental detection. Nevertheless, the currently presented

DOI: 10.1021/acs.analchem.7b05436
Anal. Chem. 2018, 90, 2430−2433

model data indicates that employment of longer extraction times would allow for sufficient method sensitivity toward even highly matrix-bound analytes.

CONCLUSIONS

The emerging biocompatible coating chemistries of SPME devices have enabled the employment of direct immersion SPME for analysis of complex samples, both for targeted and untargeted determinations. In this communication, experimental data and mathematical models were used to illustrate the effect of binding matrix components on analyte extraction by SPME devices employing matrix-compatible coatings. Previous experimental studies have demonstrated that a single extraction device is capable of attaining enrichment of a wide range of analytes from a complex sample, while substantially reducing matrix effect in mass spectrometry determinations. The interplay between the partitioning of analytes in a multiphase system and the desorption kinetics facilitates the balanced coverage of physicochemically varying compounds provided that suitable coating chemistries, of high enough affinity for the targeted range of analytes, are employed. The dependence of the extracted amount of analyte on the distribution constants and/or desorption kinetics allows for measurements of these parameters in situ and in vivo, which leads to better characterization of the investigated system. Although analyte displacement can be an issue for matrix-free solutions such as PBS, or in cases where sufficiently long extraction times are enabled, the binding of nonpolar compounds to matrices in complex samples lowers their free concentration greatly, reducing the ability of lipophilic compounds to overwhelm the coating capacity. This phenomenon results in substantial reduction of matrix effects when performing quantification with electrospray ionization as extract does not contain overwhelming amounts of phospholipids and ions are not extracted at all by the extraction phase. It should be emphasized that the achieved balance coverage phenomenon described in this communication is limited to hydrophobic-based extractions in aqueous media. In a scenario where an ion-exchange coating was to be directly exposed to an aqueous complex matrix, saturation of the extraction phase would be expected to occur rapidly due to high free concentration of ionic species. However, a similar phenomenon to the one described in this work may be observed for extraction of ionic species present as residue in hydrophobic matrices such as oils. It is possible that an ion-exchange resin covered by a protection layer, which does not allow ions to penetrate through the barrier for extraction from aqueous matrices, may also be successfully employed in such cases. The balanced coverage feature not only facilitates untargeted analyses for applications such as metabolomics, for instance, but also targeted determinations as well, since any small molecule capable of penetrating the protection layer and being effectively adsorbed by the selected sorbent would be effectively extracted. The developed strategies can be extended to other microextraction technologies such as LPME.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b05436.

Schematic representation of the model domain and effect of analyte properties on recovery (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: janusz@uwaterloo.ca.

ORCID

Md. Nazmul Alam: 0000-0002-6442-3569

Janusz Pawliszyn: 0000-0002-9975-5811

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Natural Sciences and Engineering Research Council of Canada-Industrial Research Chairs program (NSERC-IRC) for providing the funding of this project.

REFERENCES

(1) Bojko, B.; Cudjoe, E.; Gomez-Rios, G. A.; Gorynski, K.; Jiang, R.; Reyes-Garces, N.; Risticevic, S.; Silva, E. A.; Togunde, O.; Vuckovic, D.; Pawliszyn, J. *Anal. Chim. Acta* **2012**, *750*, 132−151.

(2) Souza-Silva, É. A.; Gionfriddo, E.; Pawliszyn, J. *TrAC, Trends Anal. Chem.* **2015**, *71*, 236−248.

(3) Souza-Silva, É. A.; Jiang, R.; Rodríguez-Lafuente, A.; Gionfriddo, E.; Pawliszyn, J. *TrAC, Trends Anal. Chem.* **2015**, *71*, 224−235.

(4) Piri-Moghadam, H.; Alam, M. N.; Pawliszyn, J. *Anal. Chim. Acta* **2017**, *984*, 42−65.

(5) Gionfriddo, E.; Souza-Silva, E. A.; Pawliszyn, J. *Anal. Chem.* **2015**, *87* (16), 8448−8456.

(6) Vas, G.; Vékey, K. *J. Mass Spectrom.* **2004**, *39* (3), 233−254.

(7) Page, B. D.; Lacroix, G. *J. Chromatogr. A* **1993**, *648* (1), 199−211.

(8) Risticevic, S.; Pawliszyn, J. *Anal. Chem.* **2013**, *85* (19), 8987−8995.

(9) Vuckovic, D.; Pawliszyn, J. *Anal. Chem.* **2011**, *83* (6), 1944−1954.

(10) Contini, M.; Esti, M. *Food Chem.* **2006**, *94* (1), 143−150.

(11) Górecki, J.; Yu, X.; Pawliszyn, J. *Analyst* **1999**, *124*, 643−649.

(12) Souza-Silva, É. A.; Gionfriddo, E.; Shirey, R.; Sidisky, L.; Pawliszyn, J. *Anal. Chim. Acta* **2016**, *920*, 54−62.

(13) Mirnaghi, F. S.; Mousavi, F.; Rocha, S. M.; Pawliszyn, J. *J. Chromatogr. A* **2013**, *1276*, 12−19.

(14) Mousavi, F.; Gionfriddo, E.; Carasek, E.; Souza-Silva, E. A.; Pawliszyn, J. *Metabolomics* **2016**, *12* (11), 169.

(15) Gomez-Rios, G. A.; Reyes-Garces, N.; Bojko, B.; Pawliszyn, J. *Anal. Chem.* **2016**, *88* (2), 1259−1265.

(16) Gionfriddo, E.; Boyacı, E.; Pawliszyn, J. *Anal. Chem.* **2017**, *89* (7), 4046−4054.

(17) Gionfriddo, E.; Souza-Silva, E. A.; Pawliszyn, J. *Anal. Chem.* **2015**, *87*, 8448−8456.

(18) Reyes-Garcés, N.; Bojko, B.; Pawliszyn, J. *J. Chromatogr. A* **2014**, *1374*, 40−49.

(19) Alam, M. N.; Ricardez-Sandoval, L.; Pawliszyn, J. *Anal. Chem.* **2015**, *87* (19), 9846−9854.

(20) Alam, M. N.; Ricardez-Sandoval, L.; Pawliszyn, J. *Ind. Eng. Chem. Res.* **2017**, *56* (13), 3679−3686.

(21) Henneberger, L.; Goss, K.-U.; Endo, S. *Environ. Sci. Technol.* **2016**, *50* (10), 5119−5126.

(22) Alam, M. N.; Ricardez-Sandoval, L.; Pawliszyn, J. *Anal. Chem.* **2015**, *87* (19), 9846−9854.

(23) Alam, M. N.; Ricardez-Sandoval, L.; Pawliszyn, J. *Ind. Eng. Chem. Res.* **2017**, *56* (13), 3679−3686.

(24) Bridle, H. L.; Heringa, M. B.; Schäfer, A. I. *Nat. Protoc.* **2016**, *11* (8), 1328−1344.

(25) Peltenburg, H.; Bosman, I. J.; Hermens, J. L. M. *J. Pharm. Biomed. Anal.* **2015**, *115*, 534−542.

(26) Nilsson, L. B. *Bioanalysis* **2013**, *5* (24), 3033−3050.

DOI: 10.1021/acs.analchem.7b05436
Anal. Chem. 2018, 90, 2430−2433