analytical chemistry

# Bubbles in Solvent Microextraction: The Influence of Intentionally Introduced Bubbles on Extraction Efficiency

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S *Supporting Information*

ABSTRACT: Significant improvements to microdrop extractions of triazine pesticides are realized by the intentional incorporation of an air bubble into the solvent microdroplet used in this microextraction technique. The increase is attributed partly to greater droplet surface area resulting from the air bubble being incorporated into the solvent droplet as opposed to it sitting thereon and partly to thin film phenomena. The method is useful at nanogram/liter levels (LOD 0.002–0.012 μg/L, LOQ 0.007–0.039 μg/L), is precise (7–12% at 10 μg/L concentration level), and is validated against certified reference materials containing 0.5 and 5.0 μg/L analyte. It tolerates water and fruit juice as matrixes without serious matrix effects. This new development brings a simple, inexpensive, and efficient preconcentration technique to bear which rivals solid phase microextraction methods.
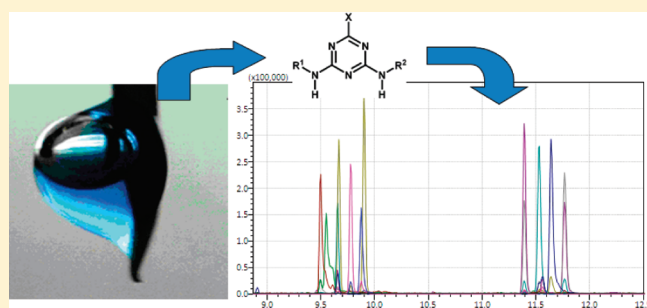
In a world where anthropogenic chemicals come to rest in unexpected places in the environment, sometimes accumulating there, it is essential to be able to detect such materials at low levels. The movement of chemicals including agricultural pesticides in the environment should be traceable, even at vanishingly low levels of presence. The extraction and preconcentration of pesticides such as triazines from various matrixes is performed by solvent- and solid phase extraction. Miniaturized methods, such as solid phase microextraction (SPME)[1] and solvent-based single-drop microextraction (SDME)[2,3] or liquid-phase microextraction,[4] have evolved as better candidates than their predecessors due to simpler handling, lower cost,[5] and the ability to use smaller samples. SDME gives detection limits of 0.02–0.4 μg/L (parts per billion, 10^{-9}) for triazines (relative standard deviation, RSD 5.6–10.9%, river water).[6] Better detection limits for triazines (0.002–0.17 μg/L, RSD 1.8–7.9%, river water) have been reported for an SPME system employing a polymethyldisiloxane-divinylbenzene cross-linked polymer fiber also with river water,[7] while detection limits in the range 0.003–0.013 μg/L (RSD 5.6–10.9%) were obtained using a hollow-fiber protected polymethyldisiloxane-divinylbenzene cross-linked polymer fiber-based SPME technique using bovine milk.[8] While the latter method meets the prescription of the United States Environmental Protection Agency (U.S. EPA) method 507 for the quantification of triazine herbicides in water bodies (requiring detection limits of 0.014–0.17 μg/L or better for several triazines), the SDME method does not meet these specifications due to higher limit of detection values. SPME techniques, while relatively costly, have been widely adopted for the preconcentration of numerous analytes, and there are many commercially available fibers.

SDME is useful in trace analysis,[9,10] but the method has notable shortfalls. Volatilization of the organic solvent, especially at relatively high ambient temperatures, causes the formation of bubbles in microdroplets, which are seen as a nuisance. Erratic results arise and efforts have been made to avoid bubble formation.[11] The relatively high ambient temperature in our laboratories also led to the formation of bubbles in microdroplets, producing similarly variable results. Of particular note, though, were the high enrichment factors we observed when the bubbles were large in relation to the droplet volume (relatively speaking) in comparison to those containing small or no bubbles. Could intentionally generated bubbles of air, incorporated within the droplet itself, provide reproducible results and secure the advantages noted in some of the exploratory experiments in a more predictable manner? The simplicity of SDME is alluring and, if successful, this effortless modification with its noteworthy effect would provide a straightforward, low cost alternative to current methods while improving on the state-of-the-art. This remarkable discovery allows the trace analysis (nanograms/liter) of a range of triazines using gas chromatography/mass spectrometry (GC/MS) in a now-validated method.

■ EXPERIMENTAL SECTION

Reagents and Standards. A 10 component triazine standard pesticide mixture [mixture TP 619: prometron (Prom), atraton

Table 1. Enrichment Factors at 10 μg/L Concentration of the 10 Component Mixture of Triazines in HPLC Grade Water with 10% (m/v) NaCl: SDME Versus BID-SDME[a]

	Prom[b]	Atro[b]	Prpz[b]	Atrz[b]	Simz[b]	Tbtz[b]	Prmy[b]	Amty[b]	Simy[b]	Tbty[b]
Reference[c]	1	1	1	1	1	1	1	1	1	1
SDME enrichment[d]	11.4	12.9	11.7	14.3	13.8	13.7	13.5	13.3	11.6	12.1
BID-SDME enrichment[d]	25.2	29.5	31.6	37.6	35.8	35.6	32.9	35.7	28.5	32.7
% RSD for BID-SDME ($n = 6$)	7.1	8.7	9.8	10.1	10.2	10.0	9.9	11.7	8.9	11.6
R^2	0.9999	0.9997	0.9999	0.9992	0.9993	0.9996	0.9998	0.9992	0.9992	0.9967
LOD (μg/L)[e]	0.003	0.007	0.002	0.012	0.011	0.008	0.008	0.011	0.012	0.010
required LOD (μg/L)[f]	0.041	0.17	0.014	0.15	0.14	0.10	0.024	0.2	0.035	0.031
LOQ (μg/L)[g]	0.010	0.022	0.0073	0.038	0.037	0.027	0.027	0.038	0.039	0.035

[a] Analytical data (R^2, LOD) are provided for the 0.05–0.5 μg/L spiking range. [b] Prom = prometron; Atro = atraton; Prpz = propazine; Atrz = atrazine; Simz = simazine; Tbtz = terbutylazine; Prmy = prometryne; Amty = ametryn; Simy = simetryne; Tbty = terbutryne. [c] "Reference" provides a normalized measure for comparison based on the direct injection of a 10 μg/L sample if the triazine mixture in chloroform. [d] Enrichment as a factor relating to direct injection of a 10 μg/L sample in chloroform. [e] Limit of detection (LOD) is calculated using standard deviation$_\text{intercept}$/slope.[13] [f] To comply with U.S. EPA method 507 detection limits,[14] except for Tbtz which is taken from WHO guidelines for drinking-water quality.[15] [g] LOQ calculated as 10× standard deviation$_\text{intercept}$/slope.

(Atro), atrazine (Atrz), propazine (Prpz), simazine (Simz), terbuthylazine (Tbtz), prometryn (Prmy), ametryn (Amty), simetryn (Simy), and terbutryn (Tbty)] with individual triazine concentrations of 500 mg/L, together with two aqueous atraton certified reference materials at concentrations of 5.0 and 0.5 μg/L obtained from Chem Service (Pennsylvania) were used. Chloroform (HPLC grade), butyl acetate (GC grade), toluene (Pestanal grade) obtained from Riedel-de Häen (Seelze, Germany) and methanol (Labscan, Dublin, Ireland) were employed throughout the study.

Equipment and Apparatus. A GC17A gas chromatograph (GC) (Shimadzu, Kyoto, Japan) with an flame ionization detector (FID) was fitted with a Zebron ZB1701 (Phenomenex, California) analytical column (30 m × 0.25 mm {internal diameter} × 0.25 μm {film thickness} dimensions) was used for milligram/liter analyses. The other column used was a mass spectrometry certified Zebron ZB35MS (Phenomenex, California) analytical GC column with the same dimensions as the ZB1701. A quadrupole QP2010 gas chromatograph coupled to a mass spectrometry (GC/MS) system (Shimadzu, Kyoto, Japan) fitted with a programmable temperature vaporizer injector was used for identification and quantitative analysis of triazine concentrations lower than 1 mg/L. Graduated 10 μL Hamilton syringes (gastight, Sigma-Aldrich, Steinheim, Germany) were used for the extraction procedures and sample injections into the analytical devices (GC/FID or GC/MS). For thermal equilibrium, a Reacti-Therm multivial compartment constant temperature bath (Pierce, Illinois) with a stirring facility was used.

GC programs were developed as shown in Supplementary Tables S-1 and S-2 in the Supporting Information). For GC/FID analyses, nitrogen (0.5 mL/min flow rate) was used as the carrier gas while for GC/MS experiments helium (1.0 mL/min flow rate) was used as the carrier gas.

The individual triazines were indentified on the GC/MS traces making use of comparative MS enabled by electron impact (EI, 70 eV) ionization to generate fragmentation spectra. The acquisition mode was changed to "selected ion monitoring" (SIM) for enhanced sensitivity during trace analyses, also using EI ionization. The following masses were selected: 200, 201, 210, 211, 213, 214 (m/z ratio used for two of the triazine species), 226, 227, and 241, respectively. These values represent the m/z ratios of signals that were characteristic of each compound as determined from the scanning mode.

Microdrop Extraction Procedure. The SDME (single drop microextraction) extraction procedure was performed following the outline set out in an earlier paper.[12] Accordingly, the organic solvent (usually CHCl$_3$, 1 μL) was drawn into the 5 μL microsyringe, the needle of which was inserted into a 2 mL GC vial with a PTFE septum containing an aqueous solution (1 mL) of the triazines. The solvent was slowly ejected into the aqueous solution to form a droplet suspended on the tip of the needle. The vial was inserted in the Reacti-Therm multivial thermal unit resting on the benchtop with the syringe clamped to a retort stand. After the required equilibration time had lapsed, the droplet was retracted into the syringe and injected into the chromatograph. The syringe was rinsed several times with pure chloroform by repetitive sucking (×5) of the solvent and dispensing it off into the waste bottle before being finally rinsed with the extracting solvent noted below to avoid sample carry-over.

For bubble-in-drop single drop microextraction (BID-SDME), CHCl$_3$ (1 μL) was drawn into the 5 μL microsyringe followed by drawing of air (0.5 μL) into the microsyringe. The contents of the syringe were introduced to the solution as described above by slowly depressing the plunger until all the solvent had been expelled from the syringe. After extraction, the solvent droplet was drawn back into the syringe with the air bubble providing the added benefit of providing a clearly visible boundary between the organic solvent and the aqueous solution.

■ RESULTS AND DISCUSSION

Various parameters applicable to the BID-SDME method were explored and optimized for triazine extractions: choice of solvent (see Figure S-1 in the Supporting Information); solvent droplet volume (see Figure S-2 in the Supporting Information); air bubble volume; addition of NaCl (see Figure S-3 in the Supporting Information); and influence of extraction time (see Figure S-4 in the Supporting Information). The conditions for optimum extraction efficiency of triazines from aqueous media are chloroform as the extracting solvent, 10% w/v NaCl added to the aqueous triazine mixture (the addition of NaCl to improve recoveries is consistent with practices involving both SDME[6] and SPME[7] extractions), a microdroplet volume of 1 μL containing a 0.5 μL bubble and an extraction time of 20 min, using static extraction (no stirring; see Figure S-4 in the Supporting
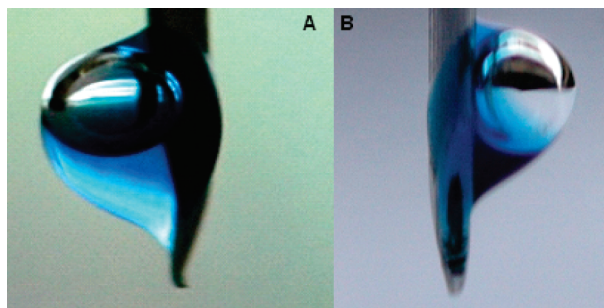
Figure 1. Photograph showing the "bubble in drop" single-drop microextraction (BID-SDME) arrangement (the solvent contains a blue dye for clarity and contrast): (a) ideal bubble size held within the micro-droplet and (b) overly large bubble causes instability and buoyancy.

Figure 2. Influence of the presence of optimum size bubbles on the extraction efficiency of triazines into 1 μL droplets of chloroform (10 μg/L triazines) using optimized conditions.

Information). After optimization, enrichment factors averaging $30\times$ over the aqueous solutions were obtained with RSD values ranging from 7 to 12% ($n = 6$) at the 10 μg/L concentration level (this concentration level was used for the optimization studies, Table 1), compared with factors averaging $13\times$ for SDME without the bubble. It is possible that the improvement in enrichment relates to increased surface area caused by the bubble being encapsulated by the droplet instead of riding on it. The method was linear in the concentration range 0.05—0.5 μg/L (50—500 ng/L; this range is useful for trace analysis; see Figures S-6 and S7 and Table S-3 in the Supporting Information) of triazines under the optimized conditions. It provides detection limit values that enable the method to be employed for the U.S. EPA method 507 for detection and quantification of triazines in water. The detection limits calculated from the calibration curves were in the low nanogram/liter (parts per trillion, 10^{-12}) range (Table 1). The accuracy of the method was validated by analyzing certified reference materials containing 5 μg/L and 0.5 μg/L of atraton, respectively ($\bar{x} = 0.50$ μg/L, SD = 0.004 μg/L, RSD = 0.8%, $\mu = 0.5 \pm 5\%$ μg/L; $\bar{x} = 5.06$ μg/L, SD = 0.07 μg/L, RSD = 1.4%, $\mu = 5.00 \pm 5\%$ μg/L; both sets of data for $n = 6$). The student's t test was applied to the results demonstrating acceptable accuracy within the 95% confidence level. The reproducibility of the method was also tested over a period of 4 days using 5 μg/L samples of the triazines (see Table S-4 in the Supporting Information) and was found to be acceptable.

The increase in efficiency in the enrichment of the triazines into the solvent droplet may arise partly due to the increased surface area of the droplet, endowed by the bubble contained therein, along with thin film effects, which are shown to improve mass transfer.[16,17] This increase in the surface area without a concomitant increase in solvent volume provides an advantage to the BID setup that cannot be otherwise realized. Increasing the size of the droplet by virtue of greater solvent volumes does not lead to the same improvement (see Figure S-2 in the Supporting Information). When bubbles are incorporated into the droplet, the procedure is simple: a given volume of solvent is drawn into the microsyringe followed by the desired amount of air. The solvent and the air bubble are slowly released into the sample by complete depression of the plunger, which deposits the droplet-encapsulated bubble onto the needle tip. This provides a setup such as that reproduced in Figure 1a, which shows an ideal bubble size (0.5 μL), while Figure 1b shows the buoyancy effect of an overly large bubble (≥ 1 μL). The effect of the bubble is clearly evident from Figure 2, in which the enrichments obtained
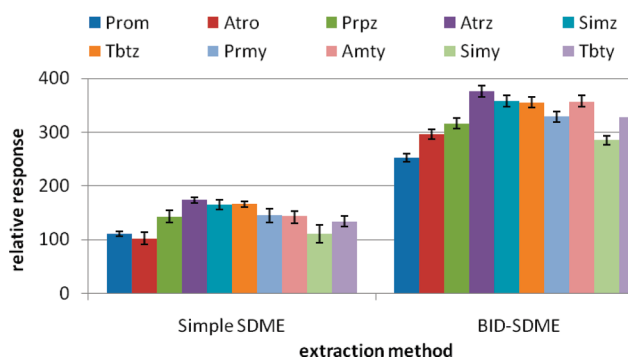
with the ideal bubble volume (0.5 μL) and without a bubble are presented. The NaCl was also found to inhibit bubble growth, which was substantial in the absence of NaCl. For a droplet volume of 1 μL, the efficiency of extraction improved with bubble sizes up to 0.5 μL. Thereafter, no further benefit was secured—the results became erratic and the droplet was often unstable, either becoming dislodged from the needle tip or suffering from excessive buoyancy, as depicted in Figure 1b.

The BID-SDME method worked well for aqueous samples constituted from HPLC grade water, water sourced from a local urban dam, and synthetic hard water,[18] which were spiked with the 10 component triazine mixture (see Figure S-8 in the Supporting Information). The recoveries and sensitivity of the method were essentially identical for all water samples and no significant matrix effect could be detected. Orange juice was also spiked with the mixture. It has been noted previously[19,20] that the recoveries of pesticides from fruit juices are characteristically low (14—108%). Pleasingly, the new method allows recoveries of triazines from spiked orange juice at levels similar to those obtained with water samples (see Figure S-9 in the Supporting Information). Our method therefore does not suffer the same negative matrix effect problems reported with SPME.[19,20]

■ CONCLUSIONS

We have demonstrated the remarkable positive effects of the deliberate inclusion of air bubbles into microdroplets destined to be used for trace sample analysis, in this case particularly with triazines. The method significantly improves upon existing pre-concentration methods, allowing detection limits in the low nanogram/liter range, % RSD values in the sub-10% range, and standard deviations that reflect the robustness and precision achievable with the method. This new development shows the accuracy of measurement requisite of any technique destined to be put to use in trace analysis of environmental pollutants. Importantly, it is easy to use and inexpensive in consumables and tolerates a variety of matrixes. Improvements will come with automation of the protocol, a matter currently enjoying our attention.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental results, discussion, processed data, graphics, and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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