1 ICE Concentration Linked with Extractive Stirrer (ICECLES)

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10 Nujud Maslamani, Erica Manandhar, Danielle K. Geremia, and Brian A. Logue*

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19 Department of Chemistry and Biochemistry
20 South Dakota State University
21 Avera Health and Science, Box 2202
22 Brookings, SD 57007
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41 *Corresponding Author.
42 Email: brian.logue@sdstate.edu
43 Phone: (605) 688-6698 Fax: (605) 688-6364

Abstract

Trace and ultra-trace analysis can be difficult to achieve, especially for polar, more volatile, and/or thermally unstable analytes. A novel technique, coined ICE Concentration Linked with Extractive Stirrer (ICECLES), may help address this problem. The implementation of ICECLES described here, combines stir bar sorptive extraction (SBSE) with freeze concentration (FC), to extract analytes from aqueous solution by slowly freezing water to concentrate analytes into a polydimethylsiloxane (PDMS) coated stir bar. Five test probe molecules with a range of log K_{ow} , 2-butanol, benzyl alcohol, benzaldehyde, dimethyl trisulfide and bromobenzene were prepared from aqueous solutions using ICECLES. Thermal desorption gas–chromatography mass–spectrometry was then used to quantify these analytes. Parameters affecting the performance of ICECLES were evaluated, such as the initial concentration, stir speed and freeze rate. Extraction at low speeds resulted in higher extraction efficiency. However, the freeze rate and initial concentrations had a minor effect on ICECLES extraction efficiency. ICECLES produced signal enhancements of up to 474x SBSE, indicating much higher extraction efficiencies than SBSE alone. ICECLES also provided lower LODs and excellent reproducibility. ICECLES performed well when used to analyze multiple pesticides and breakdown products in environmental surface waters. Overall, the ICECLES technique was excellent at preparing aqueous samples for trace analysis and shows promise as a novel analytical sample preparation technology.

Key Words: Freeze Concentration, Stir Bar Sorptive Extraction (SBSE), trace analysis, sample preparation

1. Introduction

Sample preparation is an extremely important step in most analyses, especially when trace or ultra-trace analysis is necessary (trace and ultratrace analysis is defined here as ppb and as low ppb to ppt, respectively). Analytical sample preparation serves two important primary functions: preconcentration and isolation of analytes from matrix interferences. Several advanced sample preparation techniques that serve these two functions have been introduced in recent years, such as solid phase extraction [1], solid phase microextraction [2], and stir bar sorptive extraction (SBSE) [3].

SBSE is a solvent-free sample preparation technique first reported in 1999 by Baltussen et al [3]. SBSE has since been used in many different applications areas, including environmental analysis [4], food analysis [5], and biomedical analysis [5, 6]. In SBSE, analytes are generally extracted from a solvent into a sorbent phase bound to a stir bar. The amount of solute sorbed into the stir bar is governed by its affinity for the stir bar coating, typically polydimethylsiloxane (PDMS), as quantified by the partition coefficient of the solute between the sorbent phase and the aqueous phase. After extraction, the analytes associated with the SBSE stir bar can be either thermally desorbed [4] or back-extracted into a liquid solvent[4, 6] for analysis. Because PDMS is a hydrophobic phase, only highly nonpolar analytes ($\log K_{ow} > 2.5$) are efficiently extracted [4]. In some studies, SBSE has produced LODs as low as 0.01 ng/L (ppt) [4, 7] for highly non-polar compounds (i.e., $\log K_{ows}$ greater than 5).

Another, less well-known, sample preparation technique is called freeze concentration (FC). FC is a process where solutes are concentrated in the liquid fraction of a

90 solvent as it is slowly (and progressively) frozen. The fundamental concept responsible for
91 freeze concentration is freezing point depression, where solvent volumes with lower solute
92 concentration are frozen first because of their higher freezing points. This leaves the
93 remaining solution more concentrated. FC has been suggested for use in the food
94 processing industry for almost a century to concentrate fruit juice, for ice beer production
95 and coffee extraction [8-12]. It has also been suggested as a means of industrial wastewater
96 and petroleum waste remediation [12-15], purification of drinking water or groundwater
97 [16], and the production of fresh water from seawater [17, 18]. A common implementation
98 of FC occurs with a sample vessel lowered into cold bath with an overhead stirrer rapidly
99 mixing the solution in the vessel [19]. An ice crystal forms from the bottom of the vessel and
100 grows toward the top as the vessel is lowered into the cold bath [19].

101 FC was suggested as a sample preparation technique for the analysis of trace
102 components in aqueous solutions as early as 1961 [20-26], but has not been widely
103 accepted as a routine sample preparation technique. FC has advantages compared to other
104 concentration methods, including extended operational duration and relatively low cost
105 [18]. FC can also be beneficial for certain analytes that may be difficult to concentrate by
106 other means. For example, volatile and/or thermally unstable analytes are more likely to be
107 retained in the remaining solvent as compared to heat treatment [27-29]. FC also has a
108 number of disadvantages, including duration, extremely low selectivity [15], and complex
109 equipment is sometimes necessary [30].

110 FC and SBSE appear to be highly complementary. SBSE provides selectivity, the
111 vigorous stirring necessary for FC, and the analytes are collected in a medium readily

amenable for analysis. FC provides a highly concentrate solution as the solvent is frozen, greatly increasing the extraction efficiency as compared to SBSE alone. Moreover, operation at low temperatures may allow analysis of compounds with higher volatility and thermally labile components. Reported for the first time here, FC and SBSE were combined into a technique called ICE Concentration Linked with Extractive Stirrer (ICECLES) and ICECLES was evaluated for a variety of probe analytes with varying K_{ow} s. Furthermore, multiple pesticides and pesticide breakdown products were analyzed from environmental surface waters using ICECLES.

2. Material and Methods

2.1 Materials

All reagents were of LC-MS grade unless otherwise specified. Benzaldehyde (C_7H_6O), 2-butanol ($C_4H_{10}O$), dimethyl trisulfide (DMTS, $C_2H_6S_3$, $\geq 98\%$), and bromobenzene (C_6H_5Br) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Benzyl alcohol (C_7H_8O) and methanol (MeOH, 99%) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Pure standards of deethylatrazine (DEA), deisopropylatrazine (DIA), metribuzin (MTB) and metamitron (MTT) were purchased from Dr. Ehrenstorfer GmbH (Augsburs, Germany). Atrazine in acetone was purchased from Sigma-Aldrich (St. Louis, MO, USA). Purified water was obtained from a water PRO PS polisher (Labconco, Kansas City, KS, USA) at a resistivity of 18.2 M Ω .cm.

For the probe molecules besides bromobenzene and DMTS, stock solutions (100 mM) were prepared in 2 mL of purified water and stored at room temperature for not more

than two weeks. Due to the low solubility of bromobenzene and DMTS, they were initially diluted in methanol to 100 mM and 400 mM and stored at room temperature and 4 °C, respectively. For triazine pesticides and breakdown products, stock solutions except for atrazine, were prepared by directly diluting in water. Because of its low solubility in water, atrazine was prepared by diluting in water from a received standard in acetone. After preparation, stock solutions of MTT (8.6 mM), MTB (5.58 mM), DIA (3.58 mM), DEA (17 mM) and ATR (4.5 mM) were stored at 4 °C. All test solutions were prepared by serially diluting the stock solutions with purified water to the desired concentration for individual experiments. Note: If the sample solution contained significant amounts of methanol ($\geq 0.2\%$), the uniformity of the ice produced during FC was disrupted and the effectiveness of FC decreased. Therefore, wherever possible, methanol was not used in preparing stock solutions and standards.

2.2 ICECLES Apparatus

A schematic of the ICECLES sample preparation apparatus used for this study is shown in Figure 1. The ICECLES apparatus contains five main parts: 1) a circulating chiller, 2) a double-walled (or “jacketed”) beaker, 3) a magnetic stir plate, 4) a sorptive stir bar, and 5) a glass sample vial (24 mL). The chiller used in this study was an LS-Series Compact Chiller purchased from Cole–Parmer (LS5IM11A110C; Bunker Court Vernon Hills, IL, US). The magnetic stir plate (18 cm x 18 cm) was purchased from Thermo Scientific (SP195025; Hanover park, IL, USA). The double-walled beaker and 24 mL vials (with 23 mm diameter) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Sorptive stir bars for SBSE (Twister™, 10 mm) were coated with a 0.5 mm thick polydimethylsiloxane (PDMS) layer

(Gerstel, Linthicum, MD, USA). Note: It was important to ensure the vials used for both SBSE and ICECLES did not have a rounded bottom, because the stir bar would collide with the vial wall in rounded bottom vials and break when stirring at the high speeds necessary for ICECLES.

A double-walled glass beaker was placed in the center of a magnetic stir plate. The circulating chiller was connected with the double-walled beaker by silicone tubing and a cooled mixture of 50:50 ethylene glycol and water (typically -7 °C) was circulated through the beaker. A small amount of methanol (1 mL) was carefully dispensed using a pipette into to the bottom of the double-walled beaker to form a thin layer of solvent, which ensured maximum thermal contact between the bottom of the sample vial and the bottom of the beaker. User safety was ensured by the use of protective gloves, goggles, and lab coats. Methanol could be replaced with a more benign solvent, like isopropanol, to further ensure the safety the user.

Figure 1. Schematic of ICECLES apparatus used for the current study. A PDMS-coated stir bar (10 mm length and 0.5 mm film thickness) was used to stir the solution in a 24 mL glass vial (85 mm height x 23 mm diameter). Coolant (50:50 ethylene glycol and water) was continuously circulated through a 100 mL double-walled beaker (inner diameter: 4.7 cm; outer diameter: 8 cm; inner height: 7.3 cm; outer height 8.5 cm).

2.3 ICECLES Sample Preparation

To a glass vial (24 mL), a water sample (10 mL) containing the analyte of interest at the desired concentration and a sorptive stir bar were added. The vial was capped and placed into the double-walled beaker, coolant was circulated, and the stir plate was set to the desired stir rate. The aqueous sample began freezing progressively from the bottom of the vial steadily upwards until the entire aqueous sample was frozen. The SBSE stir bar remained stirring on top of the ice throughout this process. After extraction, the stir bar was magnetically removed from the vial with a clean Teflon-coated stir bar. The stir bar was

then placed into a thermal desorption (TD) tube and analyzed via TD-GCMS. Analyses were completed in triplicate for each analyte. One disadvantage of the implementation of ICECLES used for this study is that the sample volume was limited to 10 mL. This is because the stir bar moved away from the magnetic field of the stir plate as the ice front moved towards the top of the vial. As the volume increased over 10 mL, the stir plate was no longer able to efficiently spin the stir bar. Although it is possible to analyze larger sample volumes by ICECLES, a re-designed apparatus would have to be utilized. (Note: a larger volume ICECLES apparatus is currently being developed in our lab.)

2.4 Optimization of ICECLES Experimental Parameters

Optimization of ICECLES for extraction efficiency was accomplished by varying the stir speed and coolant temperature using benzaldehyde (100 nM) as a representative analyte. In order to investigate the effect of stir speed on extraction efficiency, multiple stir speeds (1200, 1400 and 1740 rpm) were evaluated. Coolant temperatures were also varied (-3, -5, -7 and -9 °C) to evaluate the effect of the freeze rate. The effect of initial analyte concentration was evaluated by varying the benzaldehyde concentration between 100 and 3000 nM (10.6 and 318 ppb, respectively). The use of a single analyte to optimize the sample preparation conditions for the specific apparatus used is warranted because freezing point depression, the underlying phenomenon responsible for freeze concentration, depends only on the properties of the solvent when evaluating non-ionic compounds.

2.5 Gas Chromatography – Mass Spectroscopy

Prepared stir bars were analyzed using an Agilent Technologies 7890A gas

chromatograph and a 5975C inert XL electron ionization (EI)/chemical ionization (CI) mass selective detector (MSD) with Triple-Axis Detector and Multipurpose-Sampler (MPS). For all analyses, following ICECLES, stir bars were thermally desorbed in splitless mode into a cooled injection system (CIS) liner (empty deactivated notched glass liner). Thermal desorption was initiated at 60 °C, (held for 0.20 min), increased linearly at 720 °C /min to 250 °C, and then held constant at that temperature for 1 min. During this process, the analyte was collected in the CIS at 30 °C. The analyte was transferred from the CIS to the GC column by maintaining the CIS temperature at 30 °C for 0.20 min and then linearly heating to 250 °C at a rate of 12 °C/s. The compounds were separated on a HP-5MS capillary column (30 m x 250 µm x 0.25 µm) with nitrogen as the carrier gas at a flow rate of 1 mL/min and a pressure of 5.565 psi. A separate optimized GCMS method was utilized for each analyte to allow evaluation of ICECLES performance free of artifacts potentially introduced when using a GCMS method to analyze all analytes simultaneously (e.g., potentially higher LODs for SBSE). The temperature gradients for the GC separations were optimized for individual probe analytes, triazine pesticides, and atrazine breakdown products (DEA and DIA), and are reported in Table 1. In addition to the individual methods, a single TD-GCMS method was developed for simultaneous analysis of all pesticides and breakdown products (see Table 1). Using the simultaneous method all five of these analytes could be analyzed within a 10-minute chromatographic runtime. For all analytes, the MS source and MS quadrupole temperatures were 280 °C and 150 °C, respectively. Electron ionization (EI) was used as the ionization mode at 70 eV. Selected ion monitoring was used for detection with identification and quantification ions as reported in Table 1.

Table 1. Important GC-MS parameters for the probe molecules and triazine pesticides used in this study to evaluate by ICECLES sample preparation.

Compound Type	Compound name	GC parameters			Ion Mass (m/z)	
		Temperature Gradient ^a	Run Time (min)	Elution Time (min)	Quantification	Identification
Probe Molecules	2-butanol	30 °C (2 min) → 150 °C (20 °C/min; 0 min)	5.0	0.6	45	59
	Benzyl Alcohol	30 °C (1 min) → 180 °C (90 °C/min; 1 min) → 250 °C (120 °C/min; 1 min)	5.2	3.4	91	108
	Benzaldehyde	30 °C (1 min) → 120 °C (30 °C/min; 0 min) → 250 °C (120 °C/min; 1 min)	6.1	3.8	79	107
	Dimethyltrisulfide	30 °C (1 min) → 250 °C (120 °C/min; 1 min)	3.8	2.8	94	126
	Bromobenzene	30 °C (1 min) → 180 °C (90 °C/min; 3 min)	5.6	2.7	157	77
Triazine Pesticides and Breakdown Products (Individual methods)	Atrazine (ATR)	30 °C (0.5 min) → 160 °C (30 °C/min; 0 min) → 280 °C (120 °C/min; 3 min)	8.8	7.0	200	215
	Atrazine de-ethyl (DEA)	30 °C (0.5 min) → 160 °C (30 °C/min; 0 min) → 280 °C (120 °C/min; 2 min)	7.8	6.8	188	172
	Atrazine de-isopropyl (DIA)	30 °C (1 min) → 120 °C (30 °C/min; 0 min) → 250 °C (120 °C/min; 1 min)	8.1	6.8	158	173
	Metribuzin (MTB)	30 °C (1 min) → 170 °C (90 °C/min; 1 min) → 280 °C (120 °C/min; 5 min)	9.4	6.1	214	198
	Metamitron (MTT)	30 °C (1 min) → 160 °C (60 °C/min; 0 min) → 280 °C (120 °C/min; 5 min)	8.6	6.9	104	202
Triazine pesticides (simultaneous method)	All tested Triazine pesticides	30 °C (1 min) → 160 °C (60 °C/min; 1 min) → 280 °C (120 °C/min; 5 min)	10	See Figure 5	See above	See above

^aTemperature gradient and hold time are presented in parenthesis as “(temperature gradient; hold time)” if a temperature gradient is applicable or as “(hold time)”.

2.6 Calibration, Limits of Quantification, Signal Enhancement and Limit of Detection

The calibration behavior of benzaldehyde was tested to determine the linear behavior of ICECLES along with the upper limit of quantification (ULOQ). The ULOQ was selected using precision and accuracy inclusion criteria: calibrators with a precision $\geq$ 15% (as measured by relative standard deviation (RSD)), and/or an accuracy of over 100 $\pm$ 15% as compared to the nominal concentration calculated from the calibration curve were excluded from the linear range. Aqueous calibration standards were prepared in the range of 40-5000 nM from a 100 mM stock solution. Each calibration standard was analyzed in triplicate within a 24-hour period. QC standards (N=5) were prepared at three different concentrations, 75 nM (low QC standard), 300 nM (medium QC standard) and 1500 (high QC standard). The QC standards were analyzed in quintuplicate during one day using the ICECLES sample preparation process described above. The accuracy of each QC was calculated as a percent difference from the nominal concentration. The precision was calculated as a percent relative standard deviation (% RSD) of the calculated concentration for each QC standard.

The lower limit of quantification for SBSE and ICECLES (benzaldehyde only) was determined by evaluating multiple concentrations of each analyte and selecting the concentration that reproducibly produced a signal to noise ratio of 10, with noise measured as the peak-to-peak noise of the blank over the elution period of the respective analyte. Utilizing the LLOQ for SBSE, ICECLES was performed in triplicate and the signal enhancement (SE) was calculated by dividing the average peak area of

ICECLES by the respective average of peak area of SBSE for each analyte. For both SBSE and ICECLES, the LOD was defined as the lowest analyte concentration that reproducibly produced a signal-to-noise ratio of 3. The limit of detection (LOD) for both ICECLES and SBSE was estimated by the analysis of multiple concentrations of each compound below their LLOQ. The LOD enhancement (f_{LOD}) was calculated by dividing the LOD for SBSE by the LOD for ICECLES under the same conditions, aside from freezing the solvent (i.e., SBSE was completed on the same standard solution as ICECLES, but was performed in a vial directly placed on a stir plate and stirred for the entire ICECLES sample preparation time).

2.7 Application of ICECLES

After establishing the optimized sample preparation procedures, the ICECLES technique was tested on local surface water for presence of triazine pesticides and the breakdown products of atrazine (DEA and DIA). Surface water samples were collected from the Big Sioux River, Lake Preston, and Lake Oakwood with GPS coordinates: 44.340669, -96.892864; 44.3743748, -97.3520191; and 44.4265977, -96.9594141, respectively. The glass bottles for sample collection were first prepared by soaking in water, methanol and soap for approximately 19 hours. They were then rinsed in deionized water, and oven dried for approximately 4 hours. One bottle was filled with deionized water and left in the fridge for approximately 18 hours and three ICECLES analyses were performed to ensure bottle cleanliness. Samples were collected wearing new nitrile powder-free gloves at each sample site. The individual sampling the surface water waded out to about knee-depth (if possible) and bottles were placed directly

282 under the surface of the water in an upstream direction and the bottle was completely
283 filled before capping. The bottles were then transported to the research lab on ice in an
284 insulated cooler and stored in the refrigerator until used. The samples were prepared as
285 mentioned in Section 2.3.

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287 **3. Results and Discussion**

288 **3.1 Sample Preparation with ICECLES**

289 ICECLES is a simple and easy sample preparation technique well-suited for trace
290 analysis of water samples. In this study, ICECLES was implemented as the combination
291 of FC and SBSE. For SBSE, a sorptive stir bar is added to a sample and stirred. Analytes
292 partition into the stir bar, thereby concentrating them in a small volume. Since SBSE is
293 typically performed using PDMS-coated stir bars, only highly hydrophobic compounds
294 are efficiently extracted (i.e., a $\log K_{ow} > 3$ allows $\geq 90\%$ extraction of the analyte in the
295 stir bar)[31-33]. For ICECLES, a sorbent-coated stir bar is added to an aqueous sample
296 and stirred, just as with SBSE, but the sample is frozen from the bottom of the vial to the
297 top. Because of freezing point depression, as the solution is frozen, microvolumes of
298 solution with lower solute concentration are frozen first. This concentrates the analyte
299 in the remaining solution, which adds concentration stress to the equilibrium between
300 the analyte in the solution and in the stir bar. This equilibrium shift increases the
301 concentration of the analyte in the stir bar as the solution is frozen. Somewhat
302 surprisingly, if the freezing is controlled and the stir rate is vigorous, the stir bar remains

303 stirring on top of a relatively flat ice layer. This allows easy removal of the stir bar from
304 the top of the frozen sample and the ability to freeze concentrate the entire sample.

305 A striking visual example of ICECLES is shown in Figure 2, where a purple dye,
306 methyl violet, was used to simulate an analyte. The capital letters in Figure 2 represent
307 the different stages of ICECLES in a schematic depiction, whereas the corresponding
308 lower-case letters show images of ICECLES sample preparation of an aqueous solution of
309 methyl violet (the position of the stir bar in Figure 2a-c is indicated by the white arrow).
310 The start of ICECLES is shown in Figure 2A and 2a, where a stir bar is added to an
311 aqueous solution with analyte, just as with SBSE. After some time, half the aqueous
312 solution is frozen and the methyl violet is concentrated in the remaining solution and
313 the stir bar (Figure 2B and 2b). Essentially pure water forms the frozen solid as indicated
314 by the nearly clear ice in Figure 2b. At the end of ICECLES sample preparation (Figure 2C
315 and 2c), the methyl violet is concentrated in the stir bar and the last layer of frozen
316 water. The stir bar remains on the top of the ice for easy removal and subsequent
317 analysis.

Figure 2. ICECLES technique performed on a solution of methyl violet before (A and a), during (B and b), and after (C and c) ICECLES. The intensity of the blue color in the schematic (A-C) indicates the location of the analyte at each stage of ICECLES. In images b and c, the methyl violet is being clearly concentrated in the aqueous solution, with almost clear ice freezing in the bottom portion of the vial (indicating purity of the ice). The location of the extractive stir bar in each image is indicated by a white arrow.

3.2 Effect of Stir Speed and Freeze Rate

The experimental parameters important to the current implementation of ICECLES were stir speed and freeze rate. According to most studies of FC, higher stirring velocities result in more efficient concentration of analytes [17-19]. In contrast, one study found that two different stir speeds (250 and 400 rpm) had no effect on FC when evaluating the removal of methylene blue from water [27]. Although it was expected that higher stir speeds would result in greater f_{LOD} s and signal enhancements in the current study, we found that extraction at lower speeds resulted in higher signal

enhancements (12.3 for 1200 rpm, 11.4 for 1400 rpm and 10.6 for 1740 rpm). Careful observation identified that vigorous stirring of the last of 500 μL of solution produced splashing, with higher stir speeds resulting in more splashing. It is likely that increased splashing decreased the efficiency of ICECLES during freezing of the last milliliter of the sample solution. It is likely that the final volume of solution is quite important to the overall extraction efficiency because the concentration stress is greatest as this part of the solution is frozen. Therefore, disruption of uniform stirring as the final volume of solution is frozen likely decreased the extraction efficiency.

The effect of freeze rate on the extraction efficiency was also evaluated by varying the coolant temperature during ICECLES. Most previous studies of FC have shown that increasing the freeze rate decreases the extraction efficiency [17, 18, 27]. For example, in the FC of methylene blue from water, researchers found that more efficient concentration of methylene blue occurred at higher temperatures ($-6\text{ }^{\circ}\text{C}$ verses $-24\text{ }^{\circ}\text{C}$) [27]. Similarly, Fujioka [18] and Miyawaki [17], found that extraction at lower freeze rates resulted in more concentrated solutions. Conversely, Gao et al. [15] found that FC for organic contaminant removal from petroleum effluent was not affected by the freezing temperature ($-10\text{ }^{\circ}\text{C}$ and $-25\text{ }^{\circ}\text{C}$).

For the current study, variation in coolant temperature produced only minor differences in signal enhancement. Figure 3 shows the SBSE and ICECLES signals for side-by-side analysis of benzaldehyde for multiple freezing rates with no observable trend. Complete freezing was accomplished in 12, 6, 4, and 3 hours for -3 , -5 , -7 and $-9\text{ }^{\circ}\text{C}$, respectively. Although freezing at $-9\text{ }^{\circ}\text{C}$ produced the shortest analysis time and

357 resulted in the highest SE, it resulted in lower signals for both ICECLES and SBSE. This is
358 likely because the contact time of the stir bar and the sample is reduced, which may not
359 allow time for the analyte to fully reach equilibrium between the stir bar and the
360 aqueous solution. Although the optimized ICECLES freeze rate required 3.5-4 hours to
361 complete the extraction, the sample preparation time can be reduced if some signal can
362 be sacrificed (Figure 3). For comparison, typical extraction times for SBSE range from 30
363 min to four hours [34].

364
365
366 **Figure 3.** Effect of different coolant temperatures (i.e. freeze rates) on ICECLES for
367 aqueous solutions of 100 nM benzaldehyde. SBSE was evaluated using the same sample
368 preparation time as ICECLES. The sample preparation times were approximately 12, 6, 4,
369 and 3 hours for -3, -5, -7 and -9 °C, respectively. PDMS-coated stir bars (10 mm length
370 and 0.5 mm film thickness) and 10 mL sample volumes were used for all analyses.
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3.3 Effect of Initial Analyte Concentration

The initial concentration of the analyte has been previously shown to affect the efficiency of FC. For example, when investigating FC of glucose in a model solution, Miyawaki et al. [17] found that lower initial concentrations of solutes resulted in more efficient FC, and Fujioka et al. [18] found a similar result for desalination of ice. In contrast, when using FC for organic contaminant removal from a petroleum refinery secondary effluent, Gao et al. [15] reported that the initial concentration of the sample had no significant effect on the extraction efficiency.

For this study, we found that the initial concentration of benzaldehyde had minimal effect on ICECLES extraction efficiency and no trend was observed. The benzaldehyde concentrations tested (100, 200, 400, 600, 800, 2000 and 3000 nM) produced SEs of 9.5, 13, 12.8, 9.1, 10.4, 11.3 and 14.8, respectively.

3.4 Linear range, accuracy and precision

To determine the calibration behavior of compounds prepared via ICECLES, a benzaldehyde calibration curve using eight calibrators was constructed in the range of 40-5000 nM. Analysis of the calibration standards, both weighted ($1/x$ and $1/x^2$) and unweighted, indicated that a $1/x$ weighted fit was best able to describe the calibration data. The calibration curve was clearly linear and produced a correlation coefficient (R^2) of >0.999 . Accuracy and precision were determined by analysis of a low, medium, and high QCs (75, 300, 1500 nM, respectively). The accuracy was $100 \pm 16\%$ and the precision was $\leq 16\%$ RSD for the QCs (Table 2). The precision of the QCs and all calibrators was between 5-7%, except for the high QC (16% RSD). Therefore, a more

396 realistic precision is likely in the range of 5-7% RSD for the implementation of ICECLES
 397 used in the current study.

398

399 **Table 2.** The accuracy and precision of benzaldehyde QCs prepared via ICECLES.

Concentration (nM)	Precision (%RSD)	Accuracy
75	2.2%	100±3%
300	5.1%	100±10%
1500	16.0%	100±15%

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403 **3.5 Comparison of ICECLES and SBSE**

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405 Following optimization and evaluation of concentration effects, multiple probe

406 analytes, triazine pesticides, and atrazine derivatives with varying K_{ow} s were

407 investigated using both SBSE and ICECLES. Reported in Table 3, are the log K_{ow} s [35-37],

408 LODs (SBSE and ICECLES), LLOQs (SBSE only), SEs and ratios of LODs (f_{LOD}) for each

409 analyte tested. Figure 4 shows the SE and f_{LOD} plotted as function of log K_{ow} . As observed

410 in Table 3 and Figure 4, ICECLES produced improved LODs and generated significant SEs

411 compared to SBSE for each analyte. ICECLES sample preparation was efficient at

412 extracting analytes at low concentrations, and performed better than SBSE for all

413 analytes. Hydrophobicity of an analyte (as quantified by log K_{ow}) was the most important

414 property in determining the signal enhancement and extraction efficiency. Among the

415 tested probe molecules, the compound with the lowest log K_{ow} , 2-butanol (log K_{ow} =

416 0.6), produced the largest signal enhancement of 474, whereas, bromobenzene, which

417 had the highest log K_{ow} (log K_{ow} = 2.9), produced a SE of only 1.2. Similarly, metamitron

418 (log K_{ow} 0.83), with the lowest log K_{ow} among tested triazine pesticides, generated a SE

419 of 227, while, atrazine ($\log K_{ow} = 2.4$) produced SE of 2.5. These observations can be
420 explained given that approximately 90% of an analyte with a $\log K_{ow}$ of 3 can be
421 extracted with SBSE alone, leaving little to gain using ICECLES on compounds with $\log$
422 K_{ows} near or above this value. Considering this, one of the main advantages of the
423 ICECLES over the SBSE is that ICECLES performs better than SBSE for compounds that are
424 more hydrophilic.

425 There is also a limit to the utility of ICECLES for lower $\log K_{ow}$ compounds (at least
426 for PDMS-coated stir bars), as indicated by the increase in the absolute value of the LOD
427 as the SE and f_{LOD} are greatly increasing. The increase in the LOD of hydrophilic
428 compounds is caused by the unfavorable environment of the PDMS-coated stir bar for
429 hydrophilic compounds. Therefore, as the $\log K_{ow}$ decreases, at some point the analyte
430 will not sufficiently partition into the stir bar, regardless of the concentration in the
431 aqueous phase.

432 Figure 5 shows chromatograms for simultaneous analysis of triazine pesticides
433 prepared using ICECLES and SBSE. The effectiveness of ICECLES for all five pesticides is
434 seen by the enhanced signal strengths for all analytes.

Figure 4. The performance of ICECLES relative to SBSE for ten compounds, probe molecules (red) and triazine pesticides and breakdown products (blue), with $\log K_{ow}$ s ranging from 0.6 – 2.9. The behavior of signal enhancement (SE; closed symbols) and f_{LOD} (open symbols) are similar, with ICECLES performing better relative to SBSE as the K_{ow} decreases. PDMS-coated stir bars (10 mm length and 0.5 mm film thickness), 10 mL sample volumes, and a coolant temperature of -7°C were used for all analytes.

444 **Table 3.** Comparison of SBSE and ICECLES for probe molecules and triazine pesticides with a range of K_{ow} s.

Analyte type	Analytes	log K_{ow}	LLOQ for SBSE (nM)	LOD (nM)		SE	f_{LOD}
				SBSE	ICECLES		
Probe Molecules	2-butanol	0.61	140000	42000	250	474.9	168
	Benzyl Alcohol	1.1	3300	1000	40	146.4	25
	Benzaldehyde	1.48	333	100	20	12.3	5
	Dimethyl trisulfide	1.78	23	7	3	3.7	2.3
	Bromobenzene	2.9	2	0.6	0.4	1.2	1.5
Triazine Pesticides and Breakdown Products	Metamitron (MTT)	0.83	250000	60000	1000	227	60
	Deisopropyl atrazine (DIA)	1.2	150000	50000	500	137	100
	Deethyl Atrazine (DEA)	1.51	6500	2000	50	16	40
	Metribuzin (MTB)	1.71	830	250	40	8	6.65
	Atrazine (ATR)	2.4	200	80	20	2.5	4

445

446

Figure 5. Chromatograms of triazine pesticides prepared via ICECLES (upper trace) and SBSE (lower trace). Chromatographic peaks are labeled as 1) de-ethylatrazine, 2) de-isopropylatrazine, 3) atrazine, 4) metribuzin and 5) metamitron.

3.6 Application of ICECLES

The applicability of ICECLES to more realistic samples was verified by its ability to detect triazine pesticides and breakdown products in a number of lakes and rivers located near Brookings, South Dakota. Triazines are a class of pesticides extensively used in the United States for weed control [38, 39]. Atrazine, a type of triazine, is a known carcinogen and is regulated by the Environmental Protection Agency (EPA) at 200 ppb in drinking water [40]. Because triazine pesticides are of concern, even at trace

460 levels, their determination from environmental and drinking water sources requires
461 advanced sample preparation techniques, especially for the more polar compounds in
462 this class. As shown in Figure 6, atrazine was detected in water samples from all three
463 water bodies sampled (Big Sioux River, Lake Oakwood, and Lake Preston). Extracted ion
464 chromatograms of m/z 200, as plotted in Figure 6, show sharp peaks of atrazine eluting
465 at ~6.8 min. Apart from ATR, DIA was prominently detected in Big Sioux River, but was
466 below the method LOD in Oakwood Lake and Lake Preston. DEA, MTB, and MTT were
467 not detected in any of the water samples.

468

469 **Figure 6.** ICECLES analysis of the environmental surface waters for atrazine. Lake
470 Preston (top), Oakwood Lake (middle), and the Big Sioux River (bottom) were prepared

471 via ICECLES and analyzed. Atrazine was detected as a sharp peak eluting at 6.8 min in
472 the extracted ion chromatograms (200 m/z) for each body of water tested.
473

474 **4. Conclusion and Future work**

475 Presented for the first time in this study, the highly complementary nature of FC
476 and SBSE were combined to produce a novel analytical sample preparation technique
477 called ICECLES, which is well-suited for trace analysis of water samples. ICECLES proved
478 to be an effective sample preparation method for trace analysis, performing better
479 relative to SBSE, especially for lower log K_{ow} compounds. As compared to SBSE, a
480 maximum signal enhancement of around 500 times was observed for the lowest log K_{ow}
481 analyte tested (0.61), and 1.2 times for an analyte with a log K_{ow} approaching 3.
482 Moreover, ICECLES can be easily implemented and should be well-suited for analysis of
483 trace-level concentrations, thermally labile compounds, more volatile compounds, and
484 simultaneous analysis of compounds with a wide range of hydrophobicities. ICECLES was
485 shown to perform well for the analysis of surface waters for atrazine. Although the
486 implementation of ICECLES used for this study was shown to perform very well, the
487 limited sample volume and long sample preparation times are areas for future
488 improvement. We are currently evaluating the use of temperature gradients to reduce
489 the ICECLES run times. Furthermore, a larger volume apparatus is being developed.
490 Regardless, the ability of ICECLES to detect compounds, especially more polar
491 compounds that cannot be readily analyzed via other techniques, demonstrates the
492 utility of ICECLES for a number of analyses (e.g., flavor analysis).

493

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501

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