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Theory of Supercritical Fluid Extraction via the Discovery Approach

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Abstract: Supercritical fluid extraction (SFE) is a technique for sample preparation that is quickly replacing Soxhlet extraction and ultrasonic extraction. This manuscript introduces two real-world applications of SFE that serve as the basis of undergraduate laboratory experiments: the analysis of caffeine in ground coffee and nicotine in cigarette tobacco. The extracts are analyzed with gas chromatography–mass spectrometry (GC–MS). The experiments are presented in a discovery fashion that enables students to develop a theoretical understanding of the role of intermolecular forces in the extraction process. The focus on theoretical aspects of SFE makes these experiments appropriate for analytical and physical chemistry labs.

Introduction

Soxhlet extraction and ultrasonic extraction are traditional methods of extraction. In each of these methods, a liquid is used to remove the analyte from a solid matrix. With supercritical fluid extraction (SFE), a supercritical fluid is used to remove the analyte from a solid matrix [1]. The most commonly used solvent for SFE is carbon dioxide, which has a critical temperature of 32 °C and a critical pressure of 72 atm [2]. The equipment for SFE consists of an oven, which is used to heat the solvent to a temperature above the critical temperature, and a pump, which is used to pressurize the solvent to a pressure that exceeds the critical pressure. The supercritical solvent then passes over the sample, which is housed in an extraction cell. The solvent removes the analyte from the matrix and transports the analyte to a collection device at the end of the system. A piece of capillary tubing at the end of the system enables high pressures to be achieved within the system. If the solvent is a gas at room temperature and pressure (e.g., CO_2), then the solvent converts to the gas phase as it escapes through the capillary tubing and the analyte can be collected by a variety of means, the most common of which is bubbling the effluent into an appropriate liquid solvent.

There are several key advantages offered by SFE over traditional methods of extraction, including faster extraction times, less contamination (allowing for lower limits of detection), less solvent disposal problems, and the ability to extract thermally unstable compounds due to the low critical temperature of CO_2. As pointed out by Phelps et al. [3], even though there has been considerable activity in SFE in recent years, little attention has been paid to incorporating SFE into the undergraduate curriculum.

By examining the extraction recovery of nicotine from cigarette tobacco and caffeine from ground coffee as presented below, students should discover elements of the current theory of supercritical fluid extraction. According to current theory, the extraction of a compound from a solid matrix is understood as a two-step process: 1) the desorption of the analyte from the surface (dependent on the extraction temperature as well as analyte–matrix–solvent interactions), and 2) the solvation of the analyte by the solvent molecules [4–7]. The partitioning of the analyte from the matrix into the supercritical fluid is poorly understood at present, particularly given the heterogeneous nature of complex solids. As stated by Hawthorne et al., “the unfortunate truth is that high solubility in the extraction fluid is generally not a sufficient condition to yield high extraction efficiencies...and such results clearly demonstrate that additional factors for real-world samples must be considered” [4]. The solvent must be able to “overcome the interactions between the analyte and the matrix to affect a favorable partitioning into the supercritical fluid” [4]. From a study of well-characterized model adsorbents, Hsieh et al. concluded that the ability of the solvent to compete with the analyte for active sites in the matrix was a significant factor in the partitioning of the analyte from the matrix to the solvent [6].

Snow et al. published the very first experimental application of SFE for educational use [8], which described the analysis of fat in candy bar samples using SFE to extract the fat, and gravimetry to quantify the amount of fat that was extracted and collected [8]. The experiments presented below are intended to introduce the reader to two different applications of SFE to complex samples that can serve as the basis of undergraduate laboratories: caffeine in ground coffee and nicotine in cigarette tobacco. These two samples are used to develop a theoretical understanding of SFE by comparing the extraction recovery of the analytes with two different solvent systems: pure CO_2 and CO_2 with added methanol. The focus on the theoretical aspects of the relatively new analytical technique of SFE makes these experiments appropriate for analytical and physical chemistry labs.

Experimental

The instrument used for SFE was the Supelco SFE-400. SFE-grade CO_2 [CAS no. 124-38-9] was purchased from Scott Specialty Gases. The pressure in the SFE instrument was 4000 psi, the oven temperature was 130 °C, and the restrictor temperature was 130 °C for the caffeine extractions and 180 °C for the nicotine extractions (the higher restrictor temperature was used in the nicotine extractions due to frequent clogging of the restrictor at the lower temperature). Samples of approximately 0.2 g were accurately weighed and placed in the extraction cells. A small piece of filter paper was used to line the bottom of the extraction cell in order to prevent clogging the frits in the cell. A time of one hour was used for each extraction. For the CO_2/CH_3OH extractions, CH_3OH [CAS no. 67-56-1] was pipetted directly on the samples [5] so that the sample was saturated with the CH_3OH. A 0.2-g sample required about 0.5 mL of CH_3OH. The

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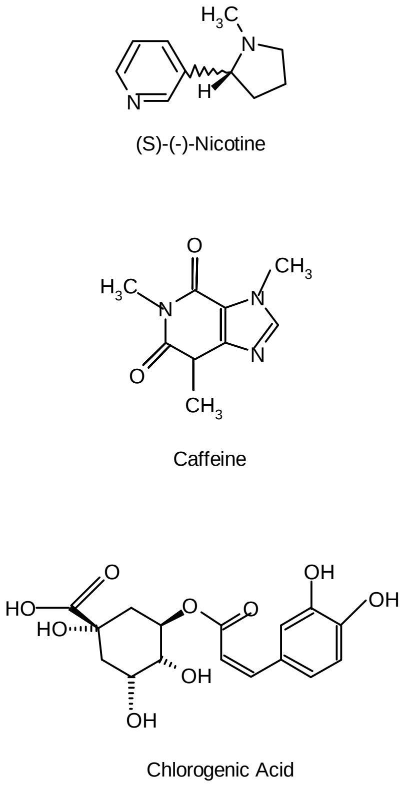

(S)-(-)-Nicotine

Caffeine

Chlorogenic Acid

Figure 1. Chemical structures of caffeine, nicotine, and chlorogenic acid.

Table 1. Recoveries of Caffeine from Coffee Grounds and Nicotine from Cigarette Tobacco using Supercritical CO_2 and CO_2/CH_3OH

Analyte	CO_2	CO_2/CH_3OH [a]
Nicotine	6 ± 1	10 ± 2
Caffeine	3.0 ± 0.1	3.4 ± 0.2

[a] Units of mg of analyte per gram of sample; quadruplicate analyses.

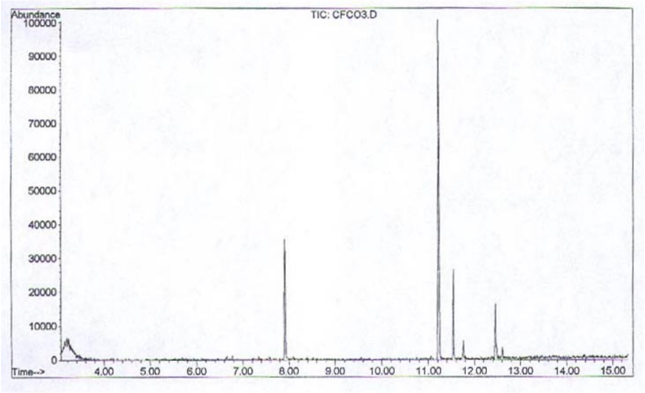

Figure 2. Total ion chromatogram of SFe/CO_2 extract of caffeine (11.2 min) from coffee grounds.

CH_3OH was added to the cells before the cells were closed for the extraction. The extracts were collected “off-line” in CH_3OH in Supelco collection vessels that were furnished with the instrument. After the extraction was complete, the extracts were transferred to 25-mL volumetric flasks, allowed to warm to room temperature, and diluted to the mark for subsequent GC-MS analysis.

The temperature ramp for the Hewlett Packard 5972 Gas Chromatograph–Mass Selective Detector (equipped with a J&W Scientific column: DB-5MS, 30 m, 0.25 mm i.d., 0.25 μm film thickness) was 70 °C for 2 minutes, 20 °C/minute to 275 °C, and then 275 °C for 3 minutes. The injector temperature was 275 °C and the detector temperature was 280 °C. One-μL quantities of the extracts were injected. Calibration curves were constructed using solutions of solid caffeine (Aldrich, 99%, [CAS no. 58-08-2]) in CH_3OH (6.10–610 ppm) and liquid nicotine (Aldrich, 98%, [CAS no. 54-11-5]) in CH_3OH (0.404–1010 ppm).

Several safety measures were used for this work. The extractor was equipped with pressure relief devices that vent the CO_2 to atmosphere if necessary. Chemical-resistant gloves were worn when preparing and handling the methanolic caffeine and nicotine solutions.

Results and Discussion

Students were given detailed instructions regarding the operation of the SFE instrument and the GC–MS. Prior to performing the experiments, students were presented with the structures of nicotine, caffeine, methanol, carbon dioxide, and chlorogenic acid (see Figure 1). Students were informed that both nicotine and caffeine are thought to be hydrogen-bonded to a carboxylic acid known as chlorogenic acid within the coffee and tobacco matrices [11, 12]. Students were then asked to experimentally compare the recovery of the two analytes by using two different solvent systems (pure CO_2 and CO_2 with added methanol).

See Figures 2–5 for sample chromatograms. The total mg of caffeine per gram of ground coffee and the total mg of nicotine per gram of tobacco were calculated using the calibration curves, the masses of the samples, and the total volumes of the extracts (recognizing that 1 μL of the sample was injected). Typical student data are presented in Table 1. The CO_2/CH_3OH solvent system yielded higher recoveries of the analytes in each case. In order to determine whether there was a significant difference in the means, a t test was performed on each the above comparisons. The tabulated t-value for 6 degrees of freedom at 95% confidence level is 2.45 [11]. For the nicotine extractions, the calculated value of t was 2.68; for the caffeine extractions, the calculated value of t was 2.81. In each case, the calculated value of t was greater than the tabulated value of t, indicating that there was a significant difference in the means at the 95% confidence level. Therefore, the data indicate that the CO_2/CH_3OH solvent system was able to extract significantly higher quantities of the analytes than pure CO_2.

Given the structures of the compounds (see Figure 1), students should be able to explain the data by recognizing that CH_3OH molecules form strong hydrogen bonds with caffeine and nicotine molecules, whereas the intermolecular forces between CO_2 and the two analytes are weaker London or dispersion forces. Therefore, students should reason that caffeine and nicotine are more soluble in the CO_2/CH_3OH mixture than in the pure CO_2. This portion of the theory is fairly easy for students to discover. The role of the competition between the analyte molecules and the solvent molecules for the matrix (chlorogenic acid) is more difficult for the students to formulate. The professor can give students some assistance by pointing out the final step in the extraction process is the solvation of the analyte by the solvent molecules. The first step in the extraction process involves the desorption of the analyte molecules from the matrix. Hopefully, students will get to the point that they realize that the CO_2/CH_3OH combination should be more effective at competing with the analyte for

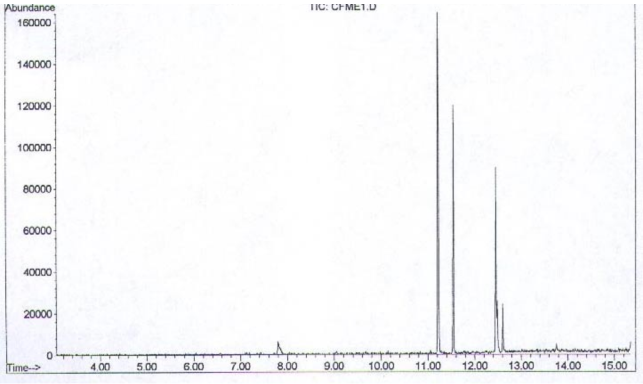

Figure 3. Total ion chromatogram of SFe/CO_2/CH_3OH extract of caffeine (11.2 min) from coffee grounds.

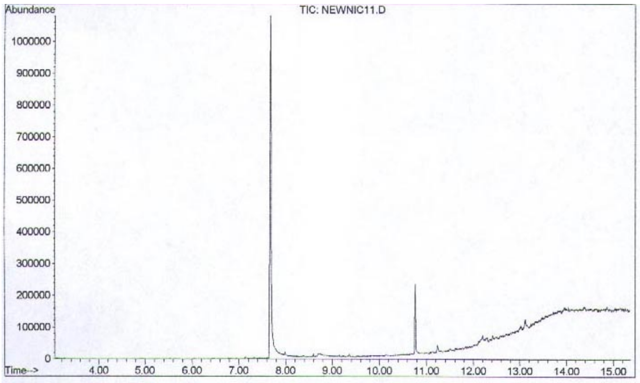

Figure 4. Total ion chromatogram of SFe/CO_2 extract of nicotine (7.7 min) from cigarette tobacco.

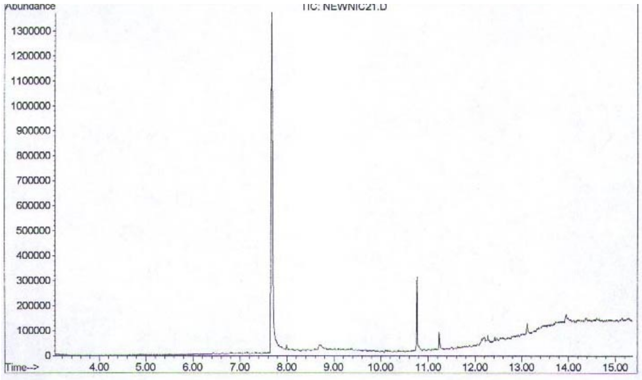

Figure 5. Total ion chromatogram of SFe/CO_2/CH_3OH extract of nicotine (7.7 min) from cigarette tobacco.

active adsorption sites within the matrices due to the ability of CH_3OH to hydrogen bond with chlorogenic acid.

Conclusion

These experiments incorporate the relatively new method of SFE into the undergraduate laboratory curriculum. These labs expand the use of existing GC-MS instrumentation with the analyses of two complex samples and introduce students to the role of intermolecular forces in the current theoretical understanding of the extraction process.

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References

1. Hawthorne, S. B. *Anal. Chem.* **1990,** *62,* 633A–642A.
2. Majors, R. E. *LC/GC* **1990,** *9,* 78–86.
3. Phelps, C. L.; Smart, N. G.; Wai, C. M. *J. Chem. Educ.* **1996,** *73,* 1163–1168.
4. Hawthorne, S. B.; Miller, D. J.; Burford, M. D.; Langenfeld, J. J.; Eckert-Tilotta, S.; Louie, P. K. *J. Chromatogr.* **1993,** *642,* 301–317.
5. Langenfeld, J. J.; Hawthorne, S. B.; Miller, D. J.; Pawliszyn, J. *Anal. Chem.* **1993,** *65,* 338–344.
6. Hsieh, Y.-Z.; Chang, A.-C. *Anal. Chim. Acta* **1997,** *342,* 41–50.
7. Pawliszyn, J. *J. Chromatogr. Sci.* **1993,** *31,* 31–37.
8. Snow, N. H.; Dunn, M.; Patel, S. *J. Chem. Educ.* **1997,** *74,* 1108–1111.
9. Rincon, J.; De Lucas, A.; Garcia, M. A.; Garcia, A.; Alvarez, A.; Carnicar, A. *Sep. Sci. Tech.* **1998,** *33,* 411–423.
10. McHugh, M. In *Biotechnology and Food Process Engineering;* Schwartzberg, H. G.; Rao, M. A., Eds.; Marcel Dekker: New York, 1990; p 208.
11. Harris, D. C. *Quantitative Chemical Analysis,* 4th ed.; W. H. Freeman: New York, 1995; p 62.