

**A NOVEL APPROACH FOR THE INTEGRATION OF ORGANIC
EXTRACTION, GREEN CHEMISTRY, AND INFRARED SPECTROSCOPY
CONCEPTS IN UNDERGRADUATE ORGANIC CHEMISTRY
LABORATORY**

Victoria Andre, Hiba Ahmad, and Khaliq Ahmed*

*Lynn University, College of Arts and Sciences, 3601 North Military Trail Boca Raton FL,
33431, USA*

Corresponding Email: kahmed@lynn.edu

ABSTRACT

In almost all undergraduate organic chemistry laboratory curricula, students are taught the concepts of organic extraction and infrared spectroscopy through some appropriate experiments. In the case of organic extraction, a typical experiment, found in most organic chemistry laboratory manuals, involves the extraction of caffeine from tea. Furthermore, it is common for students to perform infrared spectroscopy in a separate experiment, allowing the student to gain a better understanding of the unique vibrations of distinct functional groups. In this short communication, we report the integration of these laboratory activities into a single activity, which teaches the concepts of green chemistry, organic extraction, and analytical infrared spectroscopy. [*African Journal of Chemical Education—AJCE 13(1), January 2023*]

INTRODUCTION

Caffeine is a naturally occurring substance in many plants. Examples include tea leaves (*Camellia sinensis*); coffee beans (*Coffea arabica*); cocoa (*Theobroma cacao*); shrub holly (*Ilex glabra*); tree holly (*Ilex cassine*); and guarana (*Paullinia cupana*) [1]. As an alkaloid, caffeine is both a stimulant and a diuretic. It can be consumed in over-the-counter drugs as well as various beverages including coffee, tea, cocoa, and many carbonated drinks.

Caffeine analysis is vital for quality and quantity control purposes. For instance, in order to meet regulatory standards, beverage industries must guarantee a certain maximum caffeine level in decaffeinated drinks [2].

Since there are many different natural products in caffeine-containing plants, the isolation of the desired compound, caffeine, can be achieved by techniques such as liquid-liquid extraction, high-pressure liquid chromatography (HPLC), and recrystallization. Such procedures require large quantities of toxic organic solvents, such as dichloromethane, and generate large quantities of organic hazardous waste [2, 3, 4]. This poses a health risk to the students in the lab setting and also has the potential of emitting toxic chemicals into the environment.

In this short communication, we demonstrate that attenuated total reflection infrared spectroscopy (ATR-IR) can be successfully utilized for the estimation of caffeine levels in tea. This technique is rapid, reagent-free, cost-effective and most importantly it does not produce any hazardous organic waste [6].

EXPERIMENTAL

The caffeine content of a dozen tea samples was investigated. As presented in Table 1, five of these were imported from Sri Lanka; six were purchased at a local grocery store in South Florida, however, originated in either China or Brazil; and one was obtained on the Lynn University campus.

Table 1. Names, types, and origins of the studied tea samples.

Name	Type of tea	Origin
Ruhunu	Black	Sri Lanka
Uva	Black	Sri Lanka
Dimbula	Black	Sri Lanka
Matale	Black	Sri Lanka
Nuwarheleya	Black	Sri Lanka
Ti Kuan Yin	Oolong	China
Gunpowder	Green	China
Jasmine	Green	China
Sow Mee	White tea	China
Mate	Holly Family	Brazil
Mate Leao	Holly Family	Brazil
Holly Tree	Holly Family	Lynn University Campus

Each tea sample, in the amount of 5.0 grams, was boiled in 100 milliliters of water for 15 minutes, using a Cimarac 2 Thermolyne hotplate, and then allowed to cool to room temperature.

Thereafter, 5 microliters of the water extract were used for infrared spectroscopic measurements. The remaining water extract for each tea was transferred to a separatory funnel and 15 milliliters of dichloromethane was added to extract caffeine. Subsequently, the dichloromethane layer from the separatory funnel was transferred into a flask and the solvent was allowed to evaporate under a fume hood in order to obtain solid caffeine crystals. The caffeine crystals were then weighed to obtain the amount of caffeine in 5 grams of tea leaves. The purity of these caffeine crystals was analyzed by determining their melting points using Electrothermal, Mel-Temp® apparatus and 1.1-1.2 Id x 100mm melting point tubes.

Infrared spectroscopy analysis was performed using a vector 33 spectrometer from Bruker® (Billerica, MA). Infrared spectra were recorded in the attenuated total reflection (ATR) mode by using Miracle® single reflection attenuated total reflection cell from PIKE technologies (Madison, WI). Globar source, KBr beam splitter, open channel, and DTGS detector were utilized in the infrared measurements. All spectra were recorded at a 4-cm^{-1} resolution and 128 scans were averaged to obtain each spectrum.

The infrared calibration was established by utilizing the Quant1 method from Bruker®.

RESULTS AND DISCUSSION

The infrared spectra of pure water and caffeine water extract are shown in Figure 1. The major peaks in these spectra arise from OH stretching 3300 cm^{-1} and OH bending (1640 cm^{-1})

vibrations. The two spectra appear identical as the infrared absorptions of the various functional groups of caffeine are very weak.

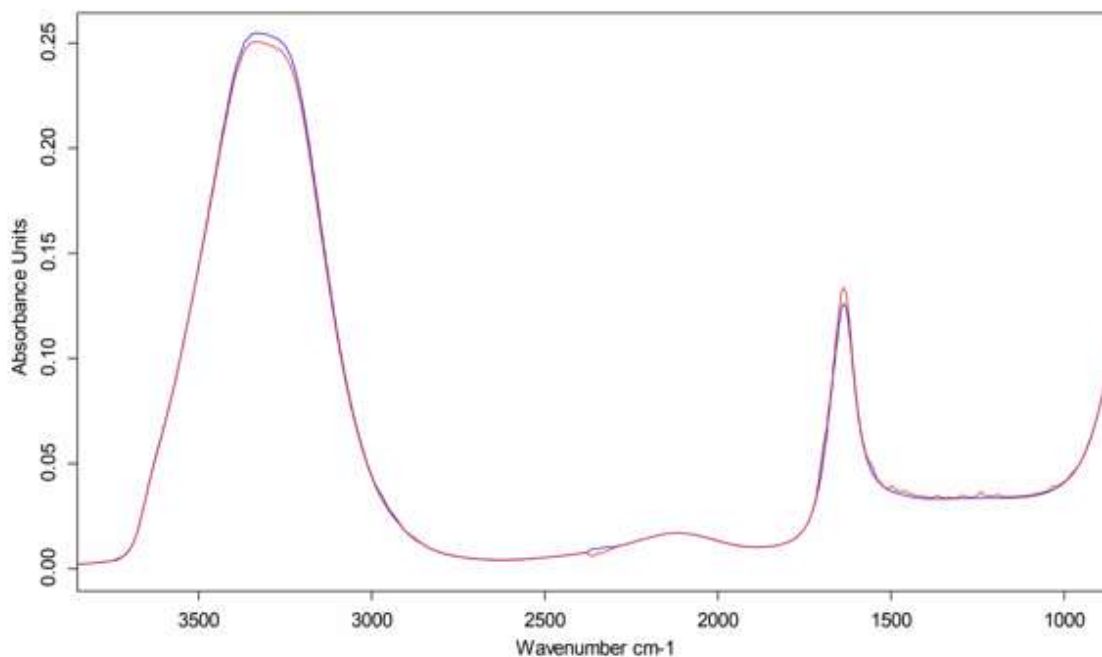


Figure 1: Comparison of the infrared spectra of five microliters samples of pure water and boiled tea in water in the region of 4000-900 wavenumbers.

Figure 2 displays an expansion of the $1550\text{-}1000\text{ cm}^{-1}$ region of the spectra of Figure 1, in order to view these caffeine infrared peaks. The identity of these caffeine peaks was confirmed through the measurement of the spectrum of pure commercial caffeine which is shown in Figure 3. The spectrum of pure caffeine has been overlaid by the spectrum of extracted caffeine to verify the success of the extraction with dichloromethane.

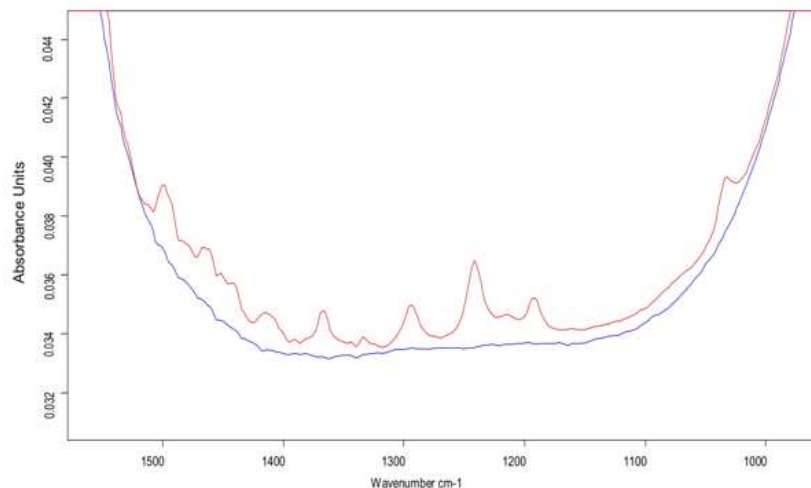


Figure 2: Comparison of the infrared spectra of pure water (lower spectrum) and boiled tea in water (upper spectrum) in the region of 1550-1000 wavenumbers.

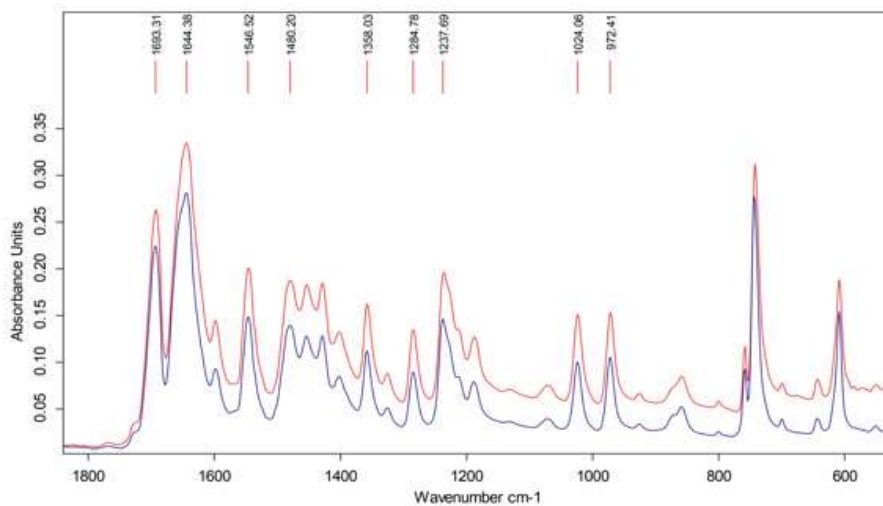


Figure 3: Comparison of Infrared spectra of extracted caffeine (lower spectrum) and commercial caffeine (upper spectrum) in the region of 1800-500 wavenumbers.

To obtain the calibration plot seven samples were randomly chosen from Table 1. The infrared spectra of caffeine water extract of the selected samples in the region of $1550\text{-}1000\text{ cm}^{-1}$ along with the associated caffeine values (mg/gram) determined through dichloromethane extraction were subjected to Bruker's Quant1 method. The resultant calibration plot is shown in Figure 4. The correlation coefficient of 0.98 indicated a nearly perfect linear relationship between the spectral features and caffeine content.

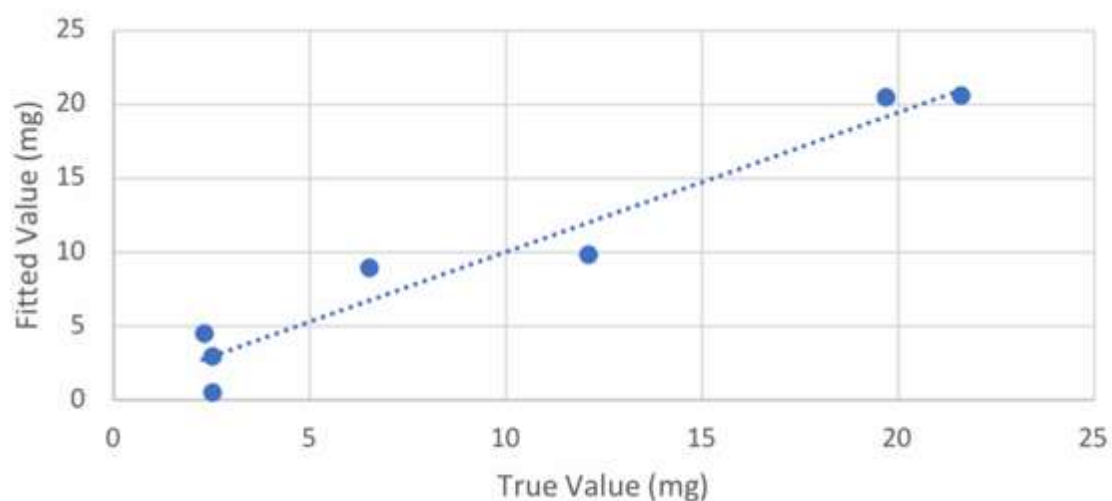


Figure 4: Infrared calibration plot for the caffeine content in representative tea samples.

The calibration of Figure 4 was used to predict the amount of caffeine in the five tea samples of Table 1 that were not used to establish the calibration. The data of Table 2 shows an excellent match between the actual and predicted caffeine values in the test samples.

Table 2: Prediction of Caffeine Content in Representative Tea Samples Based upon the Infrared Calibration.

Test Sample	Actual Value (mg/g)	FT-IR Predicted Value (mg/g)
Ruhunu	6.70	6.50
Sow Mee	8.92	9.31
Mate	5.10	5.86
Mate Leao	14.20	13.74
Holly Tree	12.90	15.61

In summary, the caffeine content of tea samples can be successfully determined through infrared spectroscopy without the use of any organic solvent.

CONCLUSION

The study demonstrates that *attenuated total reflection infrared (ATR-IR) spectroscopy* can be successfully utilized for the estimation of caffeine content in tea [5]. Infrared methodology is

rapid and eliminates the use of organic solvents. The proposed methodology can be utilized to integrate the concepts of organic extraction, green chemistry, and infrared spectroscopy in the undergraduate level organic chemistry laboratory.

REFERENCES

1. Kato M, Mizuno K, Crozier A, Fujimura T, Ashihara H. Caffeine synthase gene from tea leaves. *Nature*. **2000** 406(6799):956-957. DOI:10.1038/35023072
2. Adam D.J.; Mainwaring J.; Quigley M. N. Extraction of Caffeine from Beverage Plants. *Journal of Chemical Education* **1996** 73 (12), 1171 DOI: 10.1021/ed073p1171
3. Janusz Pawliszyn, Min J. Yang, and Maureen L. Orton Quantitative Determination of Caffeine in beverages Using a Combined SPME-GC/MS Method. *Journal of Chemical Education* **1997** 74 (9), 1130 DOI: 10.1021/ed074p1130
4. Murray S. D.; Hansen P. J. The Extraction of Caffeine from Tea. *Journal of Chemical Education* **1995** 72 (9), 851 DOI: 10.1021/ed072p851
5. Schulz H, Engelhardt UH, Wegent A, Drews H, Lapczynski S. Application of near-infrared reflectance spectroscopy to the simultaneous prediction of alkaloids and phenolic substances in green tea leaves. *J Agric Food Chem*. **1999** 47(12):5064-5067. DOI:10.1021/jf9813743