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Determination of caffeine content in coffee using Fourier transform infra-red spectroscopy in combination with attenuated total reflectance technique: a bioanalytical chemistry experiment for biochemists

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Abstract

In this report we describe an experiment to estimate the amount of caffeine present in common beverages with the use of Fourier transform infra-red spectroscopy (FTIR), using an attenuated total reflectance accessory as a sampling system. The absorbance band at 1655 cm^{-1} was used to estimate caffeine in standards and in the samples. The sensitivity of the technique was 5 ppm. The experiment provides a good demonstration of a bio-analytical technique that can be adopted either for a biochemistry or an analytical chemistry course.

1. Introduction

The experiment described in this article introduces the use of Fourier transform infra-red (FTIR) spectroscopy and attenuated total reflectance (ATR) techniques for the analysis of caffeine in beverage samples. The infra-red spectrum of mineral oil dispersion in a caffeine sample is one of the standard methods currently used to identify caffeine [1]. Caffeine has widespread name recognition with a controversial impact on human health, and therefore, its quantitative analysis in beverages introduces a genuine curiosity and enthusiasm among students. Biochemistry students must become familar with the use of advanced instrumentation for the analysis of biological samples. Many of the analytical courses at undergraduate level or even at graduate level in Chemistry Departments typically do not include analysis of biological samples. The set of experiments described in this article, therefore, could provide an example of analytical experiments which could be introduced in regular courses such as in the analytical chemistry laboratory, biochemistry laboratory and for instrumental methods of analysis.

Caffeine is an alkaloid present in coffee and tea, and is also present in soft drinks such as cola [2]. Chemically, coffee is one of the most complex consumables that contains many other chemicals in addition to caffeine [2,3]. It is well known to the public as the health effects of caffeine have been controversial [3–8]. Caffeine is a methylxanthine whose primary biological effect is the antagonism of the adenosine receptor [3]. The physiological consequences of caffeine intake include increased blood pressure, increased serum fatty acid levels, increased plasma catecholamine levels, increased urine production, and increased gastric acid secretion. Caffeine acts as a stimulant, and its presence in coffee, tea, soda beverages, chocolate, and many prescription and over-the-counter drugs makes it the most commonly used stimulant drug [3]. Excessive use of caffeine can be addictive, and could cause medical problems such as tumors, breast cancer, coronary disease and myocardial infarction [2,3]. However, the long-term health effects of moderate use of caffeine have not been associated with any medical problems in the absence of other risk factors such as smoking and alcohol [2,3,6].

This experiment was designed to educate students in the practice of method development using instrumental analysis of biologically relevant compounds. The goal and experimental plans of the experiment allowed students an opportunity to work as a team to complete the analysis. The team concept, although sometimes overlooked in favor of independent work by students, allows students to tackle problems from different angles and to work collectively toward a common goal. Learning the team concept along with the ability to

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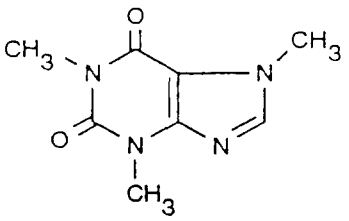
perform independent sets of experiment can become a powerful tool for the future career of students.

In addition to the use of a sophisticated instrument and a unique sampling technique (ATR), the experiment introduced several other chemical methods such as generation of standard curves, solvent extraction of a biological agent, determination of the partition coefficient, use of the Beer–Lambert law, and finally, quantitative analysis of a chemical compound.



Structure of Caffeine

2. Principles (design and approach)

The infra-red spectrum (Fig. 1) of caffeine dissolved in chloroform shows two characteristic bands at 1655 and 1700 cm^{-1}. The solvent does not have an interference band in the 1600–1700 cm^{-1} region. The band at 1655 cm^{-1} was chosen for the quantitative analysis because of its relative strength. A calibration curve for a range of standard caffeine solutions can be obtained to determine the concentration of caffeine from an extract of coffee sample.

Attenuated total reflectance (ATR), internal reflection spectroscopy (IRS), and total internal reflection (TIR) are terms used to refer to the use of an optical element to perform surface studies. Figure 2 illustrates the geometry commonly used for the generation of attenuated total reflectance and an evanescent wave [9]. A ray of light that travels the length of the light guide is totally reflected at each opposite face of the optical element. At each reflection point, it can be shown that the electric field of the ray of light (termed the evanescent wave) has an exponentially decreasing distribution outside the waveguide. This field will interact with materials at or near the crystal surface, resulting in an infra-red spectrum of the materials within a defined distance of the surface, the so-called depth of penetration, which is expressed as:

$$dp = \lambda o/2\pi n_1(\sin^2\theta - n_{21})^{1/2}$$

where λo is wavelength of radiation, θ is the angle of light incidence, and $n_{21} = n_2/n_1$; n_1 and n_2 being refractive indices of the zinc selenide crystal and sample, respectively.

Since depth of penetration remains constant for any given crystal, it can be considered as a pathlength of typical spectrophotometric cuvettes. The Beer–Lambert Law can then be applied to determine concentration based on absorbance measurements.

3. Methods

3.1. Preparation of standards

Standard solutions of caffeine containing 5, 15, 30, and 40 ppm caffeine were prepared in chloroform. Caffeine is readily soluble in chloroform. The extraction coefficient determined by extracting caffeine from 20 ppm standard aqueous caffeine solution was found to be 0.97. Another major reason for choosing chloroform as a solvent is the absence of its interference in the caffeine IR bands in the 1650–1700 cm^{-1} region.

3.2. Preparation of beverage samples

Known amounts (approximately 1.5 g) of coffee samples (powders) were weighed and dissolved in approximately 70 ml of hot distilled water, and boiled for 10 min. The solutions were diluted to 100 ml and allowed to cool down. For solutions with fine suspension, this was filtered through a Whatman No. 1 filter paper. Exactly 3 ml of an aqueous coffee solution was mixed with 3 ml of chloroform (Fisher Scientific, HPLC grade). After phase separation, 1 ml of caffeine solution in chloroform was diluted 10-fold with chloroform, and the final solution was used for FTIR analysis.

3.3. Data collection

FTIR spectra were recorded using a MIDAC (M 2000) spectrometer equipped with a liquid nitrogen cooled mercury/cadmium telluride detector and a 45° horizontal zinc selenide crystal as an ATR accessory (Pike Technologies, Madison, WI). Spectra were recorded at 4 cm^{-1} resolution with 512 co-added scans. Lab Calc™ (Galactic Industries, Salem, NH) was employed for spectral processing. In order to avoid IR signals from moisture, the instrument was purged with dry air for at least 4 h before recording any interferogram.

First, an open beam background spectrum was collected through the clean zinc selenide crystal after rinsing it with chloroform. For the collection of sample spectra, 0.5 ml of caffeine solution in chloroform was layered onto the crystal, and the solvent was allowed to dry before collecting the interferograms of the caffeine film formed on the ATR crystal. Each sample interferogram was ratioed against the background interferogram, and the resulting transmission spectrum was transformed

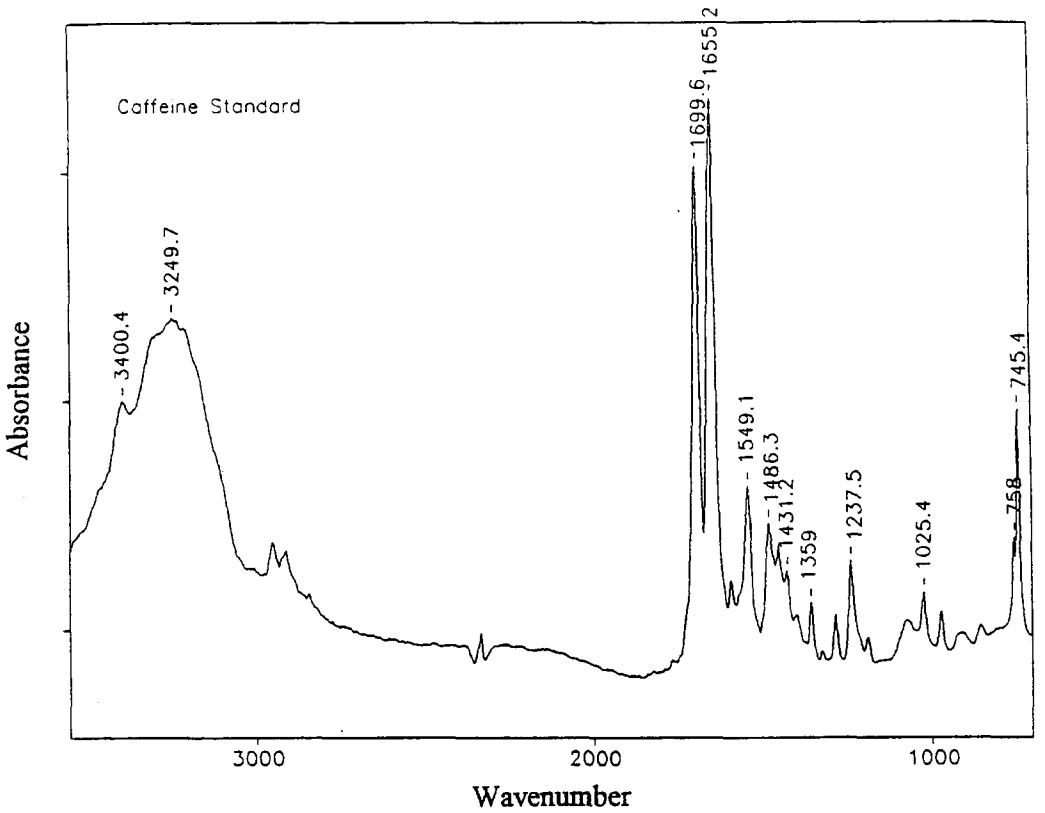


Fig. 1. FTIR spectrum of standard caffeine recorded using the ATR accessory.

into an absorption spectrum. Chloroform was used to remove the caffeine film from the zinc selenide crystal before the next sample was applied for its spectral recording.

In order to construct the standard curve, we used the absorbance at 1655 cm^{-1} (Fig. 1) band for standard caffeine samples. The concentration of caffeine in an extract of coffee was determined using the standard curve and absorbance of the extracted caffeine.

4. Results and discussion

A standard curve for absorbance vs concentration of a standard caffeine solution in chloroform is shown in Fig. 3. The infra-red spectrum of the caffeine extracted (as described in Methods) from a national brand of coffee singles is shown in Fig. 4. The concentrations of caffeine in the extracted and 10-fold diluted sample of the coffee are shown in Fig. 3. The content of caffeine was

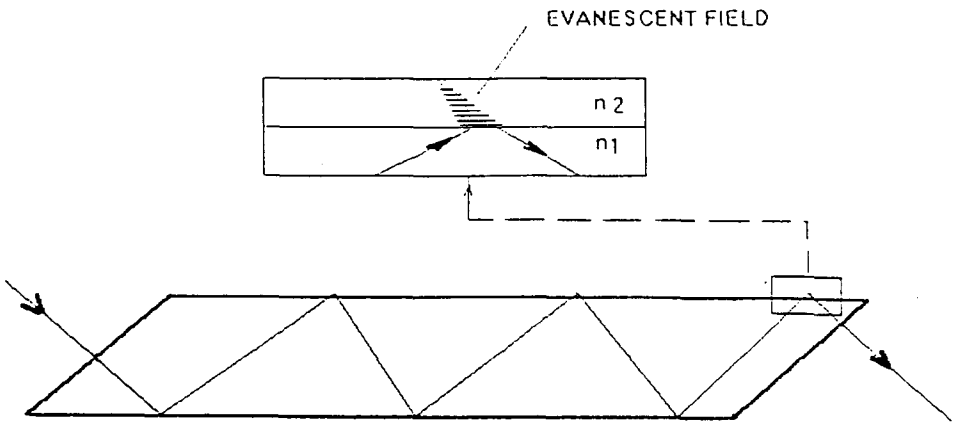


Fig. 2. Schematics of the ATR accessory showing the generation of evanescent field.

calculated first by multiplying the concentration by 10 for its dilution. Assuming an extraction coefficient of 1, we calculated the concentration of caffeine in water after extraction with chloroform. The final concentration of caffeine was thus obtained in the 100 ml water extract obtained from boiling approx. 1.5 g of coffee powder. The amount of caffeine extracted in boiled water from a given amount of coffee sample was estimated by multiplying the concentration in water by 100, and converting ppm into gram weight. Table 1 shows a sample calculation by a student for 1.497 g of national brand regular coffee. It shows 12.4 mg caffeine in 1.5 g of coffee sample. Information obtained from the manufacturer suggested 116.7 mg caffeine per single (5.4 g), suggesting the presence of 21.6 mg caffeine per g coffee. Our analysis suggested 8.3 mg caffeine per g of coffee extracted in aqueous solution. A similar analysis of decaffeinated coffee from the same national brand revealed only 0.38 mg caffeine per g of coffee, against the manufacturer's suggested amount of 0.53 mg caffeine per g of coffee.

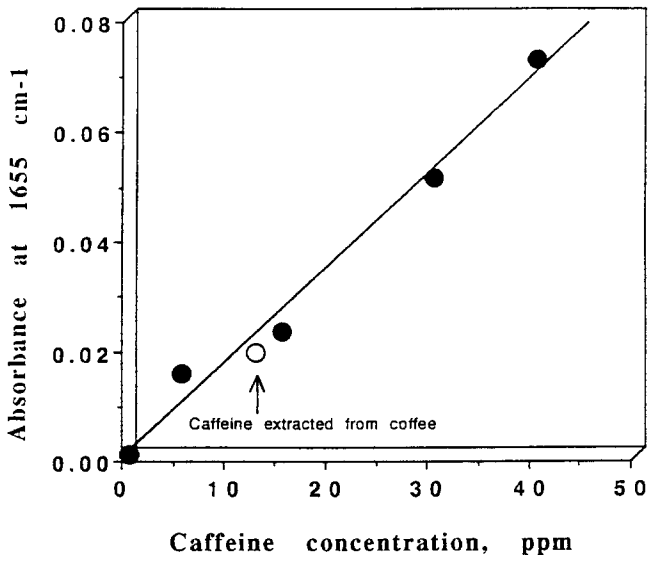


Fig. 3. Standard curve for caffeine solution as determined by absorption at 1655 cm^{-1}. The arrow indicates the concentration of caffeine extracted from a coffee sample, as described in the text.

5. Concluding remarks

The experiment provides a good introduction to the IR analysis of biomolecules. It provides the opportunity to introduce students to the concept of interferometry and Fourier transformation of interferograms. In addition, the experiment introduces a unique sampling

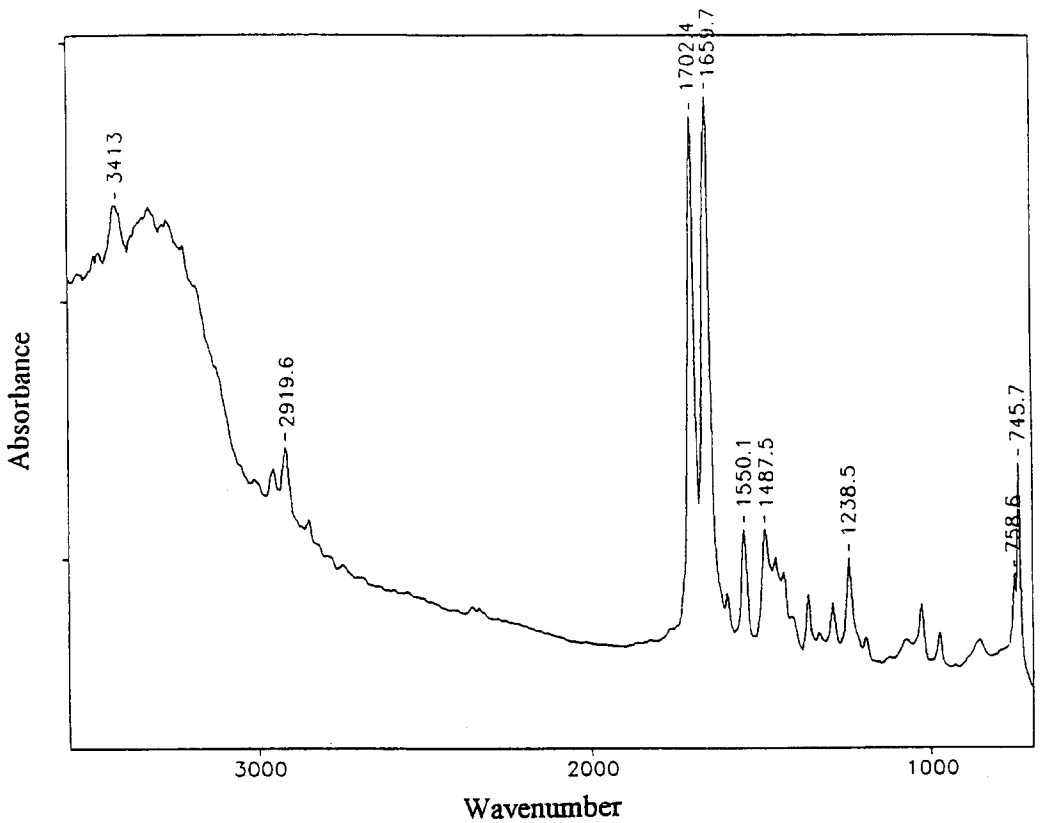


Fig. 4. FTIR spectrum of caffeine extracted from coffee sample.

Table 1
Quantitative determination of caffeine in a regular coffee sample

Amount of coffee per single	5.41 g
Mass of coffee	1.497 g
Absorbance caffeine solution at 1655 cm^{-1}	0.018678
Concentration of caffeine in chloroform extract	124.4 ppm
Concentration of caffeine in water solution	124.4 ppm
Amount of caffeine in 100 ml water solution	12.4 mg
Amount of caffeine per gram of coffee extracted in water	8.3 mg/g

method of attenuated total reflection. The experiment deals with the quantitative analysis of caffeine in coffee, a very familiar agent with strong public concern. However, it can be conveniently adopted for other beverages such as tea and soda.

Although FTIR instruments with full capability to perform experiments described in this article are currently available under US $15k, such instruments are still not routinely available for undergraduate laboratories. In such cases, this experiment can still be performed using CaF_2 or KBr windows and a dispersive IR instrument. In order to save time in recording spectra for all the standard samples, a predetermined standard curve can be provided so that students are required to record IR spectra of their samples only, and can still use the exercise to estimate caffeine using IR spectroscopy in a timely fashion.

Acknowledgements

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