



DISTRIBUTION OF CAFFEINE BETWEEN SELECTED WATER-ORGANIC SOLVENT MEDIA

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ABSTRACT

Caffeine has been recognized as a pollutant to environmental water worldwide, which may highly be due to its high consumption rate. Furthermore, caffeine has been used as a chemical tracer for the impairment of surface water and potable water with untreated domestic wastewater. The major objective of this study was to determine, which organic solvents are suitable for the liquid-liquid extraction (LLE) of caffeine from pure water by comparing their distribution coefficient (K_d). Caffeine was measured via gas chromatography-mass spectroscopy (GC-MS) without the use of any pre-concentration or reconstitution step. The method is based on direct injection after LLE and standard addition prior to analysis. Chloroform and dichloromethane were found to be primer solvents for LLE of caffeine with K_d values ranging from 9.2 to 9.9. However, chloroform may be considered more suitable for LLE as dichloromethane is more temperature labile than chloroform. No significant differences were found with the influence of temperature (20°C to 30°C) and pH (approximately 2 to 12) using chloroform as an extraction solvent. Moreover, salinity (0.01 M) had a positive effect on the LLE of caffeine with chloroform. This suits the tropical Caribbean environment of the university as caffeine measurements in environmental water should be insignificantly affected by the aforementioned abiotic factors. The non-polar solvents (benzene, diethyl ether, ethyl acetate and hexane) showed very poor extraction of caffeine from water achieving K_d values < 1.0.

Key words: Caffeine, Distribution coefficient (K_d), Solvent extraction, LLE, GC-MS.

INTRODUCTION

Many top-tier researchers have found caffeine to be omnipresent in the environmental waters worldwide¹⁻⁷. This may largely be due to its high consumption in food, beverages and medications⁸. According to statistics, the global average consumption of caffeine is between 80-400 mg per person per day⁹. Caffeine is a stimulant to the central

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nervous system, which has the ability to rejuvenate the body, increase motivation for work and restore alertness⁸. These keen characteristics of caffeine have attracted billions of consumers worldwide and has thrived the energy drink industry on a macro-economic level. In humans, approximately 0.5% to 10% of unchanged caffeine is excreted in urine and feaces¹⁻⁴. Scientists have maintained that caffeine in environmental water is primarily a result of domestic wastewater contamination^{1,5,6}. However, other notably sources of caffeine include storm water runoff, disposal of unconsumed beverages (e.g. coffee, tea and soft drinks) and medications through household pumping^{1,4,10}. Caffeine also has natural sources as it is found in a myriad of plants. More importantly, Edwards et al.⁷ maintained that caffeine in environmental water on the Caribbean island of Barbados is primarily due to anthropogenic influences as caffeine producing plants are not commonly grown on the island.

Sewage contamination of surface water and ground water is a serious problem as people can be exposed to waterborne pathogens such as *Cryptosporidium*, *Giardia* and norovirus via recreational waters (e.g. beaches) and drinking water¹¹. Caffeine has been used solely as a chemical marker to track domestic wastewater contamination of both surface water and ground water. Furthermore, it has been used in combination with other chemical markers (e.g. pharmaceuticals) and biological markers (e.g. fecal coliform)^{3,10,12-14}. Caffeine is highly soluble in water and researchers have corroborated that it is fairly stable in natural water despite the fact that is prone to biological degradation^{10,12-14}. In addition, some researchers reported that caffeine's stability in the environment is due to its resonance stability, as shown in Fig. 1¹⁵. A past study maintained that caffeine is protonated at pH less than 10.4 and the protonated form of caffeine is more water soluble and less volatile than the neutral form¹⁶. Solubility depends on the polarity of a substance. Polarity in turn depends on the electronegativity between atoms in a bond. Caffeine is partially polar. The two carbonyl groups greatly add to the molecule's polarity along with the lone pair of electrons of the nitrogen. Thus, caffeine is soluble in both water and polar organic solvents and significantly less soluble in non-polar solvents¹⁷.

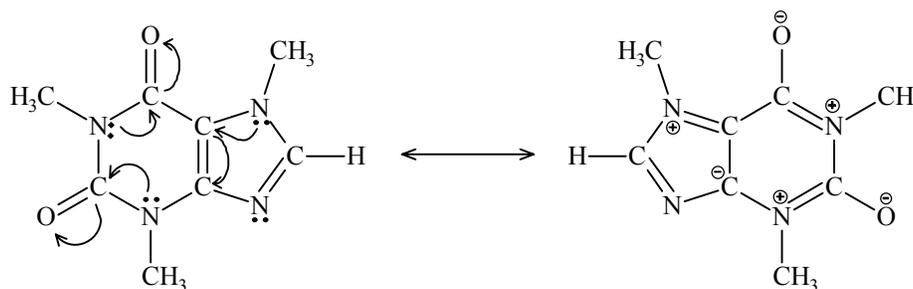


Fig. 1: One of the resonance stabilization forms of the caffeine molecule¹⁵

Caffeine has been reported to be highly soluble in chloroform and dichloromethane compared to the other investigated organic solvents, which include benzene, diethyl ether, ethyl acetate and hexane¹⁸. Moreover, caffeine has been shown to be about twice as soluble in chloroform as dichloromethane. LLE and solid phase extraction (SPE) are effective methods commonly used to extract caffeine from environmental water. LLE is the more economical method as SPE cartridges can be very costly to purchase, especially for a long-term study. Thus, it is pertinent to optimize the conditions of LLE to extract caffeine from water with high efficiency. Gas chromatography-mass spectroscopy (GC-MS) and liquid chromatography-mass spectroscopy (LC-MS) are very sensitive quantification methods commonly used today to measure contaminants of emerging concern such as caffeine and pharmaceutical compounds in water as detections can be made as low as nanograms per litre^{1-7,11,12}.

The distribution coefficient (or partition coefficient), K_d , is the ratio of the concentration of a compound (e.g. caffeine) in the two phases of a mixture of two immiscible solvents (e.g. water and chloroform) at equilibrium as shown in the equation below. S_2 represents the concentration of the substance in the organic phase and S_1 represents the concentration of the substance in the aqueous phase. K_d is generally dependent on temperature¹⁹.

$$K_d = \frac{S_2}{S_1}$$

The primary aim of this study was to assess the ability of selected organic solvents to extract caffeine from pure water by comparing their distribution coefficient. The secondary aim investigates the effect of abiotic factors (temperature, pH and salinity) on the LLE of caffeine from pure water using chloroform.

EXPERIMENTAL

Chemicals and materials

Natural caffeine 99.9% (Reagent Plus) and all solvents (Chromaslov for HPLC): benzene, chloroform, dichloromethane, diethyl ether, ethyl acetate, hexane, hydrochloric acid (HCl), methanol, sodium chloride (NaCl), sodium hydroxide (NaOH) and high purity water were purchased from Sigma Aldrich (St. Louis, MO, USA). Caffeine surrogate internal standard (purity 99% ¹³C₃-labelled, 100 µg/mL) was obtained from Cambridge Isotope Laboratories, Inc (Andover, MA, USA).

Sample preparation

Natural caffeine (100 mg/L) in pure water (25 mL) along with chloroform (10 mL) was carefully pipetted into a 50 mL volumetric flask, tightly sealed and shaken vigorously. The mixture was placed into a pre-set water bath at 15°C for 1 hr. During the 1 hr period the flask was removed at 15 mins intervals shaken vigorously and replaced into the water bath. After the time elapsed the flask was removed from the water bath and the aqueous and organic layers were carefully decanted. A microlitre syringe was used to extract the aqueous layer (20 µL) and organic layer (10 µL), which were each placed into separate amber vials along with 1 mL of methanol. Caffeine in the finally prepared samples in methanol was measured via GC-MS. All samples were prepared in duplicates and procedural blanks were run periodically to check for caffeine contamination. The same protocol outline above was used for the other investigated temperatures (20°C to 30°C), organic solvents and other variables; pH and salinity. HCl (1 M) and NaOH (1 M) were used to adjust the pH (2 to 12) of each water samples. In addition, NaCl (1 M) was used to adjust the salinity (0.01 M) of the water sample.

GC-MS analysis

Natural caffeine in pure water samples was quantified by standard addition of $^{13}\text{C}_3$ caffeine surrogate internal standard. The internal standard (1 mg/L) was added to the prepared samples prior to GC-MS analysis. Aliquots of 1 µL (Agilent Technologies 7693 autosampler) of the spiked samples were analyzed by GC-MS on an Agilent 7000A GC-MS triple quad model (USA). The samples were injected in splitless mode with the injector port temperature at 280°C. A 30 m x 0.25 mm i.d. DB-5MS (5% phenyl, 95% methylpolysiloxane) capillary column (Agilent, USA) with a 0.25 µm film thickness was used. Helium was used as the carrier gas at a constant flow of 1.2 mL/min. The column temperature program for the GC oven was as follows: initial temperature 70°C, maintained for 2 mins and then ramped at 20°C to 230°C, where it was held for 4 mins. Total run time was 10 mins. Natural caffeine (194 → 109) and its deuterated analogue (197 → 111) were characterized by multiple reaction monitoring (MRM). Both caffeine and its analogue produced a retention time of 6.2 ± 0.02 mins.

RESULTS AND DISCUSSION

According to Table 1, there was a significant difference (t-test at 95% confidence level) in the distribution of caffeine between the aqueous layer (water) and organic layer (chloroform) at 15°C. At higher temperatures caffeine may be more soluble in water thus slightly reducing the K_d . The K_d values obtained over the temperature range of 20°C to

30°C were practically the same. Thus, LLE of caffeine from water with chloroform between temperatures 20°C to 30°C should provide similar caffeine recoveries. More importantly, the extraction of caffeine from environmental waters in Barbados should be negligibly affected by our warm environment. The average atmosphere temperature in Barbados is about 27°C.

Table 1: Mean (n = 2; ± SD) K_d values obtained for LLE of caffeine from water with chloroform at various temperatures

Temperature °C	Average K_d
15	14.1 ± 0.6
20	9.8 ± 0.5
25	9.2 ± 0.9
30	10.3 ± 0.5

With reference to Table 2, there was a slight positive effect of pH on the LLE of caffeine from pure water using chloroform. However, there were no significant differences at 95% confidence between the approximate pH range of 2 to 12. Thus, similar caffeine recoveries from environmental water with chloroform are expected over the investigated pH range.

Table 2: Mean (n = 2; ± SD) K_d values obtained at various pH and salt concentration for the LLE of caffeine from water with chloroform at 25°C

pH	Average K_d
0.01 M HCl	13.8 ± 0.1
0.0001 M HCl	14.0 ± 0.2
0.0001 M NaOH	12.7 ± 0.8
0.01 M NaOH	13.9 ± 0.1
Salinity	
0.01 M NaCl	12.0 ± 0.3

The results are contrary to a previous study that reported that caffeine is protonated at pH less than 10.4 and the protonated form of caffeine is more water soluble¹⁶. In addition, the presence of salt (0.01 M) had a significant positive effect on the K_d value for the

extraction of caffeine with chloroform at 25°C (Tables 1 and 2). This may have primarily been a result of salt induced phase separation (salting out). When the salt concentration increased in the aqueous phase some of the water molecules may become attracted by the salt ions which decreases the number of water molecules available to interact with the charged part of the caffeine molecule. As a result, there is an increased demand of the caffeine molecule for the organic solvent²⁰.

The suitability of the selected organic solvents to remove caffeine from water is shown in Fig. 2. Chloroform and dichloromethane, both polar solvents, were found to be primer solvents for extracting caffeine from water. Dichloromethane ($K_d = 9.9$) was found to have a slightly higher distribution coefficient value than chloroform ($K_d = 9.2$), however; there was no significant difference between the K_d values obtained for both solvents at 25°C.

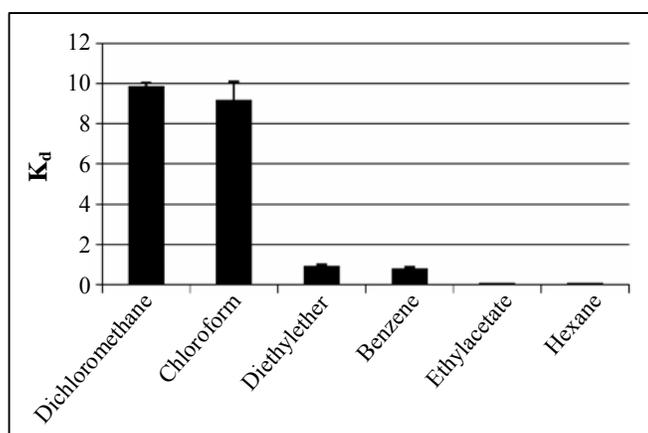


Fig. 2: Mean ($n = 2$; \pm SD) K_d values obtained for the LLE of caffeine from water using various organic solvents at 25°C

Caffeine is a partially polar molecule, as shown in Fig. 1. The two carbonyl groups along with the lone pair of electrons on the nitrogen atom increase the polarity of the molecule. Thus, caffeine exhibits good solubility in water and polar solvents¹⁷. Shalmashi and Golmohammad¹⁸ corroborated that caffeine is about 10 times more soluble in chloroform and dichloromethane than the other investigated non-polar solvents and water. The K_d values obtained for the non-polar solvents were < 1.0 . The basic principal is “like dissolve like.”

The effect of temperature, pH and salinity can also be studied for the LLE of caffeine from water using dichloromethane since its extraction results are as high as chloroform. Moreover, dichloromethane (boiling point 40°C) is more temperature labile than

chloroform (boiling point 60°C)²¹. It is vital to optimize the experimental conditions in order to achieve the highest possible recoveries of caffeine.

CONCLUSION

The high distribution coefficients (K_d) obtained for the two polar solvents, chloroform and dichloromethane, validate that they are appropriate for the LLE of caffeine from pure water at room temperature. However, chloroform may be a more suitable solvent as dichloromethane is more thermally labile. The investigated non-polar solvents showed very poor removal of caffeine from water. The influence of temperature, pH and salinity showed a slight positive effect on the LLE of caffeine with chloroform, which may make the method suitable for measuring caffeine in environmental water. In addition, GC-MS analysis was very effective in measuring caffeine in water-solvent media.

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