A fluorescent complex of caffeine and ellagic acid

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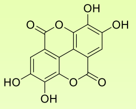
Introduction

The rapid determination of caffeine (CAF) in coffee and other beverages is definitely important for the entire coffee production chain. For this reason, the literature is rich in examples of rapid analytical methodologies and sensors for the analysis of caffeine and other xanthines [1]. The most widely employed analytical techniques for the determination of CAF content in different products include high-performance liquid chromatography (HPLC), ultraviolet–visible (UV/Vis) spectroscopy, thin-layer chromatography (TLC), and ion chromatography [2].

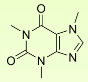
Fluorescence is helpful in the development of rapid systems, especially if it is possible to exploit a label-free approach in which it is not necessary to conjugate the analyte to a fluorescent label [3].

Ellagic acid (EA) is a natural polyphenolic compound, which is widely distributed in the plant kingdom. As most of polyphenolic compounds it exerts strong antioxidant activity through participation in redox reactions. The particular molecular design of EA also contributes to its remarkable fluorescence properties, which contribute to its broad applicability in fluorescence probes, bioimaging, and biosensor technologies [4], [5].

Due to the structure of EA and CAF we decided to study their interactions in order to establish the potential applicability of EA in fluorimetric sensing of caffeine.



Ellagic acid



Caffeine

Materials/Methods

1: Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy was deployed to execute a screening ^{1}HNMR titration, which probes the interactions between CAF and EA in DMSO as the solvent, at a concentration of 4 mM for caffeine while EA concentration was in the range 0-20 mM. The shift of imidazole proton of CAF at 7.90 ppm was studied upon titration with EA.

2: Fluorescence spectroscopy

Fluorescence spectroscopy was employed to check the effect of interaction between EA and CAF on the emission spectra. A first analysis was carried out in methanol while better results were obtained in DMSO. A standard solution of 13.5 μM EA was titrated with increasing amount of CAF to provide Stern-Volmer plot and obtain data about the quenching process. A calibration curve was subsequently developed to quantify the CAF contents in standard solutions in DMSO and obtain the analytical parameters. Finally, matrix effects were studied on water samples from green coffee.

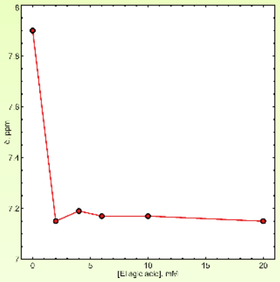
3: High Performance Liquid Chromatography (HPLC)

HPLC was employed to quantify the CAF content in Arabica green coffee bean samples, as an established HPLC method for CAF determination was already available using a Thermo Acclaim C18 5μm 120 A° 2.1 × 150 mm column with a flow of 0.25 mL/min. The eluent was a gradient mixture of water (containing 0.05% of Trifluoroacetic acid (TFA)) and acetonitrile (containing 0.05% of TFA). The results obtained from HPLC were then compared with those derived from fluorescence spectroscopy to evaluate the accuracy and efficiency of the fluorescence-based quantification method.

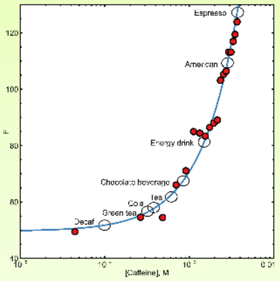
Results

^{1}H NMR titration and preliminary fluorimetric evaluation

^{1}H NMR titrations were performed initially to elucidate the preferential interactions between CAF and EA and significant shift of the imidazole proton was observed as shown in **Graph 1a**, thus justifying its selection for fluorometric analysis. A preliminary fluorometric titration (**Graph 1b**) revealed that a defined molar solution of EA in methanol, exhibited a moderate fluorescence upon excitation at 350 nm [1] and it demonstrated a marked augmentation of its emission profile in the presence of CAF, in a range of concentrations comprising the expected values in beverages. Unfortunately, a major problem was observed while attempting to replicate the measurements due to an instability of the system.



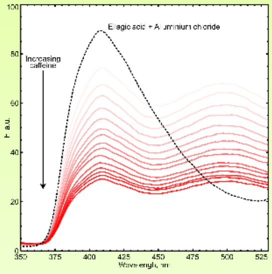
Graph 1a: ^{1}H NMR titration of EA with CAF



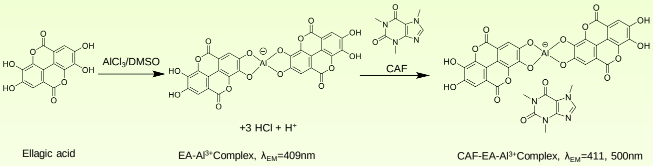
Graph 1b: CAF content in different beverages

Fluorometric Titration of Ellagic Acid and $AlCl_3$ with Caffeine

To overcome this problem, we decided to exploit the fluorescence of EA in the presence of a Lewis acid, $AlCl_3$ dissolved in a polar aprotic solvent as DMSO[6], to evaluate the effect of CAF on the emissions from the EA-Al^{3+} complex in DMSO. The fluorometric titration unveiled that a 13.5 μM solution of EA and 27 μM of $AlCl_3$ in DMSO, presented a pronounced fluorescence at 409 nm upon excitation at 270 nm. The findings revealed a substantial quenching of EA-Al^{3+}complex emission, a phenomenon that exhibited a consistent reduction in the emission with the progressive introduction of CAF aliquots coupled with a discernible bathochromic shift as shown in **Graph 2**. At the same time a new emission band can be observed at about 500 nm. Even this band was quenched by further addition of caffeine.



Graph 2: Fluorometric Titration of EA-Al^{3+} complex vs CAF



Ellagic acid

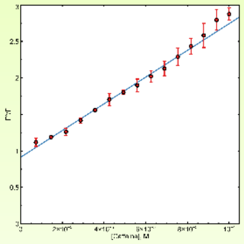
EA-Al^{3+}Complex, λ_{EM}=409nm

CAF-EA-Al^{3+}Complex, λ_{EM}=411, 500nm

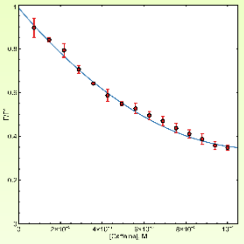
Method analysis

A Stern-Volmer plot obtained at 409 nm is reported in **Graph 3a**. The resulting K_{SV} is 18300 L·mol^{-1}. This value suggests the occurrence of a static quenching phenomenon, however, deviations from linearity at high concentrations seem to reveal also other effects as inner filter which may operate also at 500 nm. Nevertheless, the fluorescence decrease can be used to quantify caffeine in a micromolar range (**Graph 3b**). A second order polynomial equation can be used to calculate the concentration of caffeine from the fluorescence quenching in standard samples.

$$\frac{F}{F^{\circ}} = 4.88 \times 10^{7}[caffeine]^{2} - 11200\,[caffeine] + 0.98$$



Graph 3a: Stern-Volmer plot



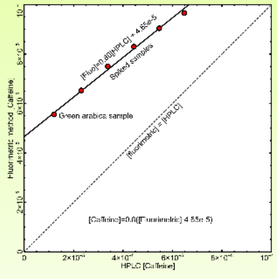
Graph 3b: Calibration curve

LOD(μM)	3.8
LOQ(μM)	11.4
Precision %	3.8

The accuracy has been preliminary evaluated and results within ± 10% in the dynamic range.

Matrix effect

The novel method introduced in this study indicates significantly higher sensitivity compared to the preliminary analysis, and exhibits a dynamic range, approximately two orders of magnitude lower than the first approach. This allows dilution of the samples to reduce matrix effects, which were nevertheless observed when CAF quantification in Arabica green coffee bean sample was performed using both fluorescence and HPLC analyses to assess the difference. A clear matrix effect was observed during the analysis of authentic coffee extracts in aqueous solution with fluorescence, although, it can be easily corrected through standard caffeine spiking, as shown in **Graph 4**.



Graph 4:Matrix effect and its correction

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Conclusion

In summation, this preliminary analysis of the interactions between EA and CAF might harbor a prospective boon towards a rapid and cheap analytical systems capable of quantifying CAF in beverages and energy drinks. We are currently working on the full validation of the system on samples from different origins. The implementation of such fluorescence technologies into customized portable devices is also considered in future developments.

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