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Caffeine determination by flow injection analysis employing Bovine Serum Albumin as a fluorophore☆

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ABSTRACT

A new methodology for caffeine determination was developed based on the quenching effect on fluorescent emission of the molecule of Bovine Serum Albumin at $\lambda_{em} = 338$ nm ($\lambda_{ex} = 280$). A flow injection disposition was designed improving significantly the sampling rate to 60 samples/h using potassium dihydrophosphate $5 \cdot 10^{-3}$ mol L^{-1} buffer (pH 6.8) as carrier and flow rate of 1.5 mL min^{-1}. The experimental and instrumental conditions that influence on analytical quality parameters were systematically investigated, as consider: buffer nature and concentration, fluorophore nature and concentration, and carrier flow rate. The proposal is simple, fast, inexpensive and precise, with a linear range from $6.68 \cdot 10^{-6}$ to $4.0 \cdot 10^{-3}$ mol L^{-1} and SD of 0.0668, under optimized conditions. Methodology sensibility and selectivity allowed a variety of sample analyses. It was successfully applied to caffeine quantification in energy drinks, dietary supplements and sliming infusion samples without previous treatment.

1. Introduction

Caffeine (CF) is an alkaloid of methylxanthines family, the most frequently ingested substance with pharmacological activity in the world [1]. Among its numerous physiological effects, it can be mentioned the stimulation of central nervous system, cardiac muscle, respiratory system and diuresis [2–5].

In the wild, it is found in more than 60 plants such as tea leaves, coffee beans, kola nuts and cocoa pods; CF has been part of many cultures for centuries and actually there are many new energy products, such as waffles, sunflower seeds, jelly beans, syrup, and bottled water. It also occurs in several other foodstuffs such as prescription medications, diuretics, and pain relievers. Also, more recently, CF has been added to some alcoholic beverages introduced to the marketplace.

CF is use in sports world as stimulant, enhancing the physical performance as well as mental aptitude. This substance can improve athlete's endurance in sports where long-term stamina is needed [6,7]; sports include cycling, running, and even soccer. CF can decrease fatigue in athletes and has been forbidden in sport by Word Anti-Doping Agency's (WADA) Monitoring Program [8].

With high doses unpleasant short-term side effects are exhibit, including palpitations, gastrointestinal disturbances, anxiety, tremor, increased blood pressure and insomnia [9–12]. In spite of numerous publications on the long-term consequences of CF consumption on human health, no clear picture has emerged, with reports of both protective and deleterious effects. However, there are severe concerns about unfavorable influences of CF on young children and pregnant women, including the risk of fetal death and miscarriage [13,14].

Because numerous adverse effects occurred, it results essential having CF monitoring methodologies. Several analytical methods had been proposed for the determination of CF in different samples and quality control of products including titrimetric spectrophotometry, polarography, GC and HPLC [15–18].

In other order of things, Bovine Serum Albumin (BSA) is one of the longest known and, probably, the most studied protein. It's conformed by 583 amino acids with 66 493 Da molecular weight. BSA has numerous applications, both in clinical medicine and basic research [19]. Because of the presence of Trp, Tyr and Phe residues, the BSA has a characteristic fluorescence spectrum. Fluorescence spectroscopy is a sensitive methodology chosen for studies of protein stability, hydrodynamics, kinetics, or ligand binding [20,21]. Many of these applications employed quenching phenomena. BSA binding property with several compounds enables the indirect study of many pharmacological drugs like CF [22,23].

Flow injection analysis (FIA) is well-established sample handling methodology, based on the injection of samples or standards in a continuous carrier stream, which provides the mechanization of different steps in the quantitative analysis [24]. Coupling the fluorescence determination with a flow injection analysis, multiple advantages are gained. Sampling times are usually just few minutes, furthermore, the small

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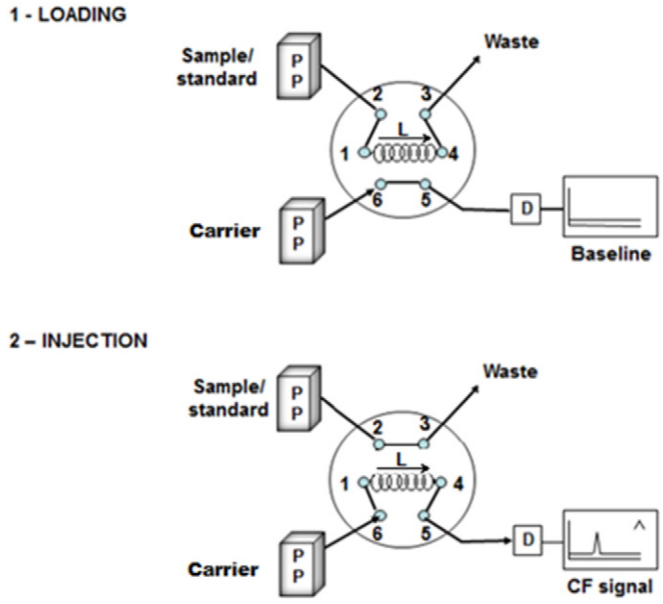

Fig. 1. Schematic representation of FIA system designed for caffeine determination by the developed methodology.

internal diameter of the PVC pumping tubes minimizes the amount of reagents consumed and the volume of waste generated. FIA is robust in wide range of operating parameters which could be optimized in order to achieve successful analyses.

On this work, a simple, fast and inexpensive on line methodology for CF determination with fluorescence detection is proposed, employing BSA as a fluorophore. Experimental parameters that impact quality of analytical results have been optimized in order to apply the new methodology to the analyte determination in different energy supplement samples and slim tea.

2. Experimental

2.1. Reagents and materials

CF stock solution (Sigma-Aldrich, St. Louis, USA) $1 \cdot 10^{-2}$ mol L^{-1} was prepared by dissolution of the appropriate amount in methanol. Further dissolutions were weekly prepared in ultrapure water.

Stock of $1 \cdot 10^{-2}$ mol L^{-1} solution BSA (Fedesa, San Luis, Argentina) was weekly prepared in ultrapure water and storage al 278–283 K.

Potassium dihydrophosphate (Biopack, Buenos Aires, Argentina) buffer solution $1 \cdot 10^{-2}$ mol L^{-1} and sodium tetraborate (Biopack, Buenos Aires, Argentina) $1 \cdot 10^{-2}$ mol L^{-1} solutions were prepared dissolving the appropriate amount in ultrapure water. Acetic acid (Mallinckrodt Chemical Works, St. Louis, USA) $1 \cdot 10^{-2}$ mol L^{-1} buffer solution was prepared diluting in ultrapure water the appropriate volume.

The pH was adjusted to the desired value, by adding NaOH solution (Merck, Buenos Aires, Argentina) using a pH meter.

All used reagent were analytical grade.

2.2. Apparatus

Spectrofluorimetric measurements were made using a spectrofluorometer (Shimadzu RF-5301 PC) equipped with a 150 W Xenon lamp. In batch studies 1.00 cm quartz cells were used and 120 μL flow cell unit (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan) for the flow measurements.

The propulsion system consisted in two peristaltic pumps (Gilson Minipuls 3) with PVC pumping tubes. A Rheodyne model 5041 six-port two-way rotary valve (Rohnert Park, CA) was employed.

A combined glass electrode and a pH meter Orion Expandable Ion Analyzer (Orion Research, Cambridge, MA, USA, Model EA940) was used for pH adjustments.

2.3. Studied samples

A total of three recipients of the same brand for each CF containing products were acquired as a strategy of randomized sampling. The whole of the contents of each product was homogenized and reserved for sample preparation.

Adequate volume or weight of each sample, containing from 1.1 to $9.7 \cdot 10^{-3}$ g L^{-1} was dissolved in ultrapure water and diluted to 25 mL in a volumetric flask.

In the slimming infusion case, 200 mL of boiling ultrapure water was poured over a tea bag and let it sits for 5 min. A dilution 1:2 was carried out.

All the solutions were reserved for CF determination applying general procedure.

2.4. General procedure

In order to perform the calibration curve, an estipulate volume of CF standard solution containing 1.1 to $9.7 \cdot 10^{-3}$ g L^{-1} was placed in a volumetric flask and 200 μL of BSA $1 \cdot 10^{-5}$ mol L^{-1} was added. The whole mixture was made up to 10 mL with ultrapure water.

Standard/samples were injected into a flowing buffer stream conformed by phosphate $5 \cdot 10^{-3}$ mol L^{-1} pH 6.8. The continuous flow diagram is shown in Fig 1. In the "loading" position, the carrier was impulse by the first peristaltic pump (PP) to the fluorescence detector generating the baseline at $\lambda_{em} = 338$ nm ($\lambda_{ex} = 280$); while the second peristaltic pump aspirate the sample charging the sample loop (L) in the injection valve located among 1 and 4 positions. The loading was carried out for 30 s, enough time to fill L with sample/standard. In the "injection position" carrier flowed through the L and carried the analyte to the detector. The second PP is turned off.

3. Results and discussion

In order to use the molecular fluorescence to monitor CF, a variety of different fluorophores such as 8-hydroxyquinoline, dithizone, chromazurol, rhodamine B and BSA were assayed. No variation on native fluorescent response of mentioned fluorophores was observed on studied experimental conditions in the presence of analyte CF, with the exception of BSA that it showed quenching effect on fluorophore emission. So, this fluorophore was selected to follow the optimization studies.

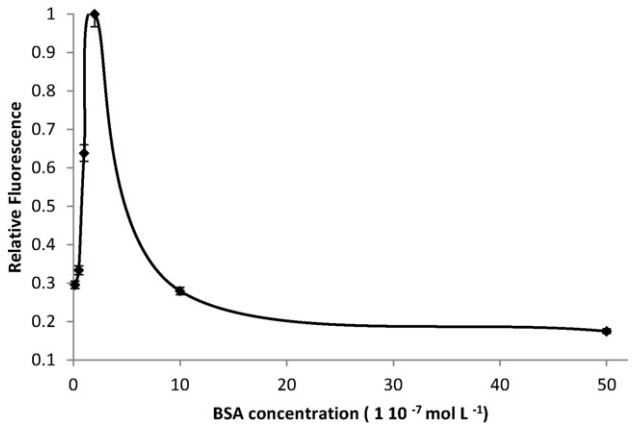

Fig. 2. Study of optimal fluorophore concentration.