DEVELOPMENT OF MICRODEVICES FOR DETERMINATION OF CAFFEINE IN LAB-MADE SEWAGE

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Abstract: *The large consume of products that contains caffeine has been caused negative effects for aquatic environments. This emergent contaminant is found in a concentration level between 10 µg L^{-1} to 300 mg L^{-1} in wastewater. The high-performance liquid chromatography shows itself as an excellent analytical technique to determine this analyte, however it is necessary sophisticated equipment, sample preparation steps which takes time and large volume of solvents. This study aims to develop low cost and miniaturized systems to determine caffeine in wastewater by luminescence reaction. A 3D printer was used to develop a miniaturized and portable equipment. Image acquisition was done using a smartphone in order to capture the luminescence obtained by the caffeine and determine its concentration. The images were processed in grayscale and it allowed to obtain an analytical curve correlating signal in function of the caffeine concentration.*

Key-Words*: caffeine; HPTS; 3D printer; fluorimeter*

Introduction: The disposal of micro pollutants and drugs has been affected aquatic ecosystems disturbing the live equilibrium in these environments. The high consumption of caffeine-containing products in their compositions has effectively contributed to the alarming concentrations of this drug in wastewater [1] [2]. The great concentrations of this compound reach such levels that can create risks to human health [3]. Caffeine is present in diverse products of industrial segments by its stimulating properties that do not cause apparent harmful side effects. Studies of zebrafish behavior in presence of caffeine are reported by Cachat showing the disturbing effects its swimming performance [4]. Caffeine is present in wastewater at concentrations ranging from µg L^{-1} to mg L^{-1}. HPLC is a technique of choice for analysis of emerging contaminants. Although, it is expensive technique, also is not portable and consumes great volumes of solvents. Another requirement is the need of specialized and trained operator to analyze the samples. Sample preparation procedure is essential in complex samples which could be time-consuming and generates harmful chemical residues. A luminescence reaction originates from a complex between caffeine and dye 8-Hydroxypyrene-1,3,6-trisulfonate (HPTS), which presents quenching of fluorescence as analytical signal (λ_{EX} = 460 nm λ_{EM} = 509 nm) is described by Rochat et al. [5]. The dye`s species became excited and the fluorescence phenomenon can be observed and captured by camera. The image acquisition by a smartphone camera and data processing by a software has been applied for different purposes[6]. Smartphone usage permits a versatile platform with low cost and capacity to realize different operations with simple modifications. Based on this, this study aims to develop a miniaturized method able to determinate caffeine in complex samples such as lab-made sewage (mimics a domestic sewage). A simple method is purposed, without sample preparation in order to obtain a high analytical frequency. Smartphone was applied as a fluorimeter measuring the intensity of signal for each sample in different caffeine concentrations.

Experimental:

Reaction: The reaction was tested in volumetric flask of 5 mL and 1 mL PBS 100 mM, pH 6.3 and 50 µM HPTS was added. A stock caffeine solution was prepared in lab-made wastewater (Cellulose 47,0 mg L^{-1}; Sucrose 98,0 mg L^{-1}; Starch 149,0 mg L^{-1}; Beef extract 262,0 mg L^{-1}; Sodium bicarbonate 370,0 mg L^{-1}; Soyben oil 79,0 µg L^{-1}; Salt solution containing NaCl, $MgCl_2$, $CaCl_2$ 1,00 mg $L^{-1}$and Detergent 1,00 mg L^{-1}) and from that the amount of 3.5 mL was added for different caffeine concentrations in a range of 200 -700 mg L^{-1}. The reaction needs 4 minutes give the product and after the fluorimetric measure can be done. The charge transfer product has fluorescence quenching (λ_{EX} 460 nm and λ_{EM} 509 nm) measured in commercial fluorimeter Shimadzu RF 1501, Fig. 1 a,b.

Device: A sample chamber was made to hold curvette glass 5 mm tube and a chamber was developed using 3D printing (3D Cloner ST printer, Ind. Schumacher Ltda., Brazil) with black acrylonitrile-butadiene-styrene (ABS),

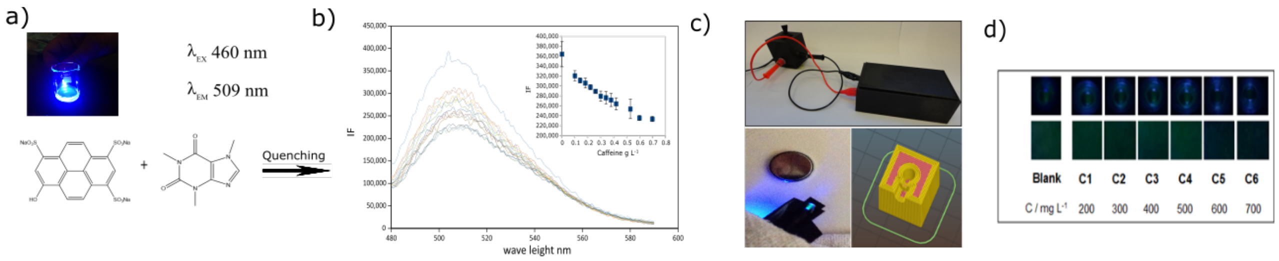
Fig. 1c. A fluorimetric detection system of detection was designed and printed by 3D technique for this purpose. The LED-based model system was calibrated and its intensity controlled by 5V energy source and the blue LED light beam was located at the side of the curvette as a light source. A small optical fiber added the light arrives to the curvette keeping its intensity. A smartphone holder was designed for smartphone model (Samsung Galaxy Win 2, camera 8.0 mpx). 50 µL HPTS was react with 50µL amount sample caffeine, after 5 minutes reaction a blue LED beam crossed the sample and image was captured by a smartphone camera, Fig 1c. The chamber design guarantees that only the fluorescence phenomenal has been detected where the smartphone was at 90° to the LED beam. Smartphone focus was locked to ensure the same region has been captured for all analytical signal acquired.

Results and discussion: The luminescence reaction between caffeine and HTPS originated a charge transference complex which presents quenching of fluorescence. The correlation between the fluorescence intensity and the caffeine concentration has shown us decreasing behavior. Linear response was obtained plotting F_0/F x caffeine concentration (mg L^{-1}) that characterizes a collisional quenching for this study, Fig 1b and Table 1. The device was designed to acquire fluorescence images using light source a commercial blue LED (λ 450 nm). The acquired images lost green light intensity when caffeine concentration is increased, Fig 1d. Analytical figures of merit are shown by Table 1. F-test shown equivalence between the device and fluorimeter methods (F_{obs} = 0,004 < $F_{tab\ 95\%}$ 2,9).

Tabela 1: The Analytical Figures of Merit.

.	Fluorimeter	Device
Sample amount	2000 µL	50 µL
Concentration Range	140-700 mg L^{-1}	200-700 mg L^{-1}
Analitycal Curve	F_0/F = 6,80 (+/- 0,34) 10^{-4} [Q] + 1,01 (+/- 0,01)	F_0/F = 2,90 10^{-3} (+/- 0,16) [Q] + 0,956 (+/- 0,0772)
R^2	0,973	0,994
Detection Limit (LOD)	118 mg L^{-1}	107 mg L^{-1}
Quantification limit (LOQ)	356 mg L^{-1}	323 mg L^{-1}
Relative Error	7,60 %	12,7 %
RSD	0,60 %	9,40 %

Figure 1: a) Representative scheme reaction between HPTS and Caffeine; b) Emission decreasing 480 – 600 nm for EX. 460 nm after added caffeine in lab made waste water; c) The fluorimetric device for reaction; d) The data from acquire image processed for each caffeine concentration.



Conclusion: The fluorescent reaction has shown efficient to determine caffeine in lab-made sewage. The analytical response was achieved by Android smartphone with the developed sample chamber. The miniaturized system has shown effective and precise compared to fluorimetric results. The present study has shown itself equivalence compared to the fluorimeter data. The system must be optimized to maximize the efficiency of capturing the radially emitted photons. Also, it is necessary to enhance image fluorescence signal acquisition and reagents concentration to reduce LOQ and other analytical parameters.

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