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Short communication

Fluorimetric SIA optosensing in pharmaceutical analysis: Determination of paracetamol

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Abstract

The coupling of sequential injection analysis (SIA) and fluorimetric solid phase transduction is here applied to the determination of paracetamol in pharmaceuticals. The reaction product between the analyte and sodium nitrite in acidic medium is inserted, after alkalinization, in the system. This product is transitorily retained on the active solid sensing phase (the anionic solid support QAE A-25) developing its native fluorescence signal, which is measured at 325/430 nm for the excitation and emission wavelengths respectively. The described system is linear within the range 6.6–80 μg ml^{-1}, with a 2 μg ml^{-1} detection limit and a 2.5% R.S.D ($n = 10$). The proposed fluorimetric SIA optosensor has been applied to the determination of paracetamol in several pharmaceutical preparations, obtaining satisfactory results.

Keywords: Optosensing; Sequential injection analysis; Pharmaceuticals; Nitrosation

1. Introduction

Sequential injection analysis (SIA) is based on the forward and reverse movement of the piston of a syringe pump, as long as a multi-position selection valve automatically controlled by a computer. The use of the computer software enables the precise sampling of chemicals into the system and propelling of the sequenced zones to the reactor and detector in a reproducible way [1,2]. The main advantages of SIA are its versatility, high sample throughput, low sample and reagent consumption and complete automation by means of appropriate software.

The coupling of SIA and solid phase spectroscopy (SPS) has been described [3] as an alternative to conventional optosensors, that is, the coupling of flow injection analysis (FIA) and SPS [4,5]. The main handicaps of conventional FIA, that is, lack of automation (the introduction of reagents into the system is manually controlled with rotary valves) and high reagent consumption (the carrier is continuously flowing through the system) are avoided by using SIA. Hence, the implementation of automatic flow methodologies, such as SIA, in SPS is an interesting research field. Here we propose a SIA–SPS methodology for the fluorimetric determination of paracetamol after appropriate derivatization reaction.

Paracetamol, PCT, (acetaminophen, *N*-acetyl-*p*-aminophenol, 4-acetamidophenol) is an extensively employed analgesic and antipyretic drug, which can be prescribed solely or with other related drugs [4–6]. Due to the importance of this drug, a high number of analytical methods have been previously described for its determination in pharmaceuticals. When the UV spectrophotometry has been used, the direct measurement of PCT is not possible due to the spectral overlapping with other compounds that usually come along in pharmaceuticals and also absorb in the UV spectral region, so different strategies have been used, such as derivatization reactions [7], partial least-squares [8], or the use of flow-through optosensing [4–6]. The determination of PCT by using fluorescence after a derivatization reaction has also been used [9–11], wasting a lot of time in the reaction process [9] or needing an extraction step to avoid interferences [11] as main handicaps. Other separations methods, such as high performance liquid chromatography [12,13], ion chromatography [14] or micellar liquid chromatography [15] with UV detection have been developed too.

The reaction between nitrite and paracetamol in acidic conditions, followed by alkalinization with sodium hydroxide is

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here applied to the determination of paracetamol in pharmaceuticals. This reaction has been previously described and used for determining PCT with UV spectrophotometric detection [7] or nitrite with fluorescence detection [16]. In the case of determining PCT [7], the obtained detection limit was very high and a proper interference study was not carried out. In this paper, we have highly improved the detection limit (in two orders of magnitude) by using fluorescence detection and the solid support in the flow-through cell and a proper interference study has been performed (acetylsalicylic acid interference, normally problematic [11] is here easily eliminated). The fluorimetric SIA optosensor here developed is based on the continuous measurement of the fluorescence signal from the oxidation product of the analyte directly retained on the solid sensing phase. The method has been satisfactorily applied to the determination of PCT in pharmaceuticals, and an additional recovery study has been also performed, obtaining excellent results.

2. Experimental

2.1. Reagents and solutions

PCT, sodium hydroxide, hydrochloride acid and sodium nitrite were purchased from Sigma–Aldrich (Alcobendas, Madrid, Spain). Stock solution of 1000 mg l^{-1} of PCT was prepared in double deionized water and was kept in the dark under refrigeration.

Sephadex QAE A-25, 40-120 μm average particle size (Sigma–Aldrich) and C_{18} bonded phase silica gel beads (Waters, Milford, USA) with 55–105 μm of average particle size were tested as sensing supports.

2.2. Instrumentation

A commercially available instrument FIAlab® 3500 system (FIAlab® Instruments, USA) with a syringe pump (syringe reservoir 5.0 ml) and an 8-port selection Cheminert valve (Valco Instrument Co., USA) was used. The manifold was equipped with fiber-optic fluorimetric detector PMT-FL (Ocean Optics, Inc., USA) with UV light source D-1000-CE. A 340 (300–380) nm primary filter and a 435 (390–510) nm secondary filter were used (Edmund Industry Optic, GmbH, Germany). The whole SIA system was controlled by the latest version of program FIAlab for Windows 5.0. Flow lines were made of 0.8 mm i.d. PTFE tubing.

A Hellma flow cell 176.752-QS (25 μl of inner volume and a light path length of 1.5 mm) was used. The cell was filled with QAE A-25 solid support micro beads, and was blocked at the outlet with glass wool to prevent displacement of the particles.

2.3. Preparation of the samples and reaction conditions

The pharmaceutical samples were chosen in several presentation ways. Two activated tablets were completely dissolved in double-distilled water, filtered and diluted to 200 ml in a volumetric flask. In the case of granular packets, one granular packet was dissolved in double distilled water by using sonication and diluted to 200 ml in a volumetric flask.

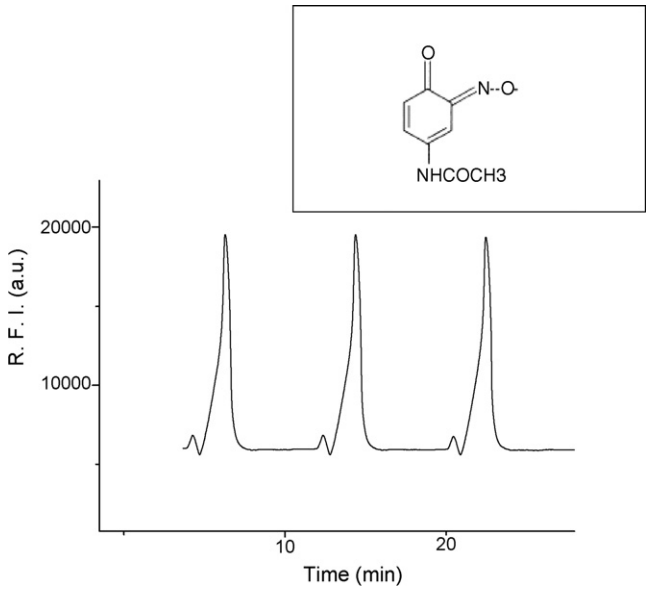

Fig. 1. Flow profile of the transient signal obtained for 10 μg ml^{-1} of PCT. Structure of the final reaction product.

The procedure for the reaction was as follows: a suitable volume of the sample (or PCT stock solution) was placed in a 25 ml volumetric flask. After that, 30 μl HCl 0.2 M and 50 μl $NaNO_2$ 1000 mg l^{-1} were added, and the flask was vigorously stirred. After 5 min, 1 ml NaOH 2 M was added and the flask was completed to volume with double distilled water. After 5 min, the reaction product is stable and the measurement can be carried out.

2.4. General SIA procedure

In the first step, 2.5 ml of the 0.2 M HCl carrier/eluting solution and 1.5 ml of sample (after the derivatization reaction took place) were aspirated by the syringe pump. The sample was pumped towards the flow-though cell at 0.9 ml min^{-1} and the transitory signal (peak height) from the reaction product from PCT was recorded. The analyte was eluted from the solid support by the carrier solution itself. Each sample was analyzed by triplicate. A typical profile of the signal is shown in Fig. 1.

3. Results and discussion

3.1. Reaction product

The reaction of PCT and nitrite has been previously described [7,16]. The first step consists of the reaction of PCT with nitrite under acidic conditions, the nitrosation of PCT occurring under these conditions. The second step is the stabilization of the reaction product, the nitroso compound, by means of using a sodium hydroxide solution. The resulting compound in this alkaline medium, which is a fluorescent stable one, is retained on the