SPECTROFLUORIMETRIC ANALYSIS OF CEFOTAXIME SODIUM BY USING 4-FLUORO-7-NITROBENZOFURAZAN AS DERIVATIZATION AGENT

Daniela ŞTIRBEŢ[1], Ioana VASILESCU[2], Gabriel-Lucian RADU[3]

The aim of the present work was to develop a spectrofluorimetric method of analysis applicable to β-lactam antibiotic cefotaxime sodium from aqueous solutions. We found interest in developing a fluorimetric method, using a luminescence spectrometer. Cefotaxime sodium was analysed by derivatization with a specific fluorescent compound namely 4-fluoro-7-nitrobenzofurazan which is a fluorophore used in the detection of amines. The method developed proved to be suitable for this purpose. This method was applied in the controlled release, in phosphate buffer solutions (pH=7.4), of cefotaxime sodium from hybrids obtained by the immobilization of the antibiotic in MCM-41 nanoparticles.

Keywords: 4-fluoro-7-nitrobenzofurazan, cefotaxime sodium, cephalosporins, fluorescence, luminescence

1. Introduction

Cephalosporins are β-lactam antibiotics which are closely related in structure and in their anti-bactericidal mechanism of action to penicillin and cephamicin which are also β-lactam antibiotics. The main nucleus of cephalosporins is 7-amino cephalosporanic acid (7-ACA) which is a cephem derivative. Cephalosporins which are used for therapeutic purposes are semisynthetic products [1]. Cefotaxime sodium (structure presented in Fig. 1) is a drug of the third generation cephalosporin family widely used for the treatment of Gram-negative bacteria. It is a broad-spectrum β-lactam antibiotic and treats many kinds of infections, including those of the skin, bone, stomach, brain, blood, respiratory tract, sinuses, ears, and urinary tract [2]. A wide variety of analytical methods have been reported for the determination of cephalosporins in pure form, in pharmaceutical preparations and in biological fluids. These methods include

[1] PhD. student., Faculty of Applied Chemistry and Materials Science, Dept. of Analytical Chemistry and Environmental Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: daniela.stirbet@gmail.com

[2] PhD, National Institute of Research and Development for Biological Sciences, Bucharest, Romania

[3] Prof., Faculty of Applied Chemistry and Materials Science, Dept. of Analytical Chemistry and Environmental Engineering, University POLITEHNICA of Bucharest, Romania

spectrophotometry [3-6], fluorometry [7, 8], liquid chromatography [9-14], capillary electrophoresis [15, 16], chemiluminescence [2, 17], voltammetric [9, 18-21] and polarographic [22] methods.

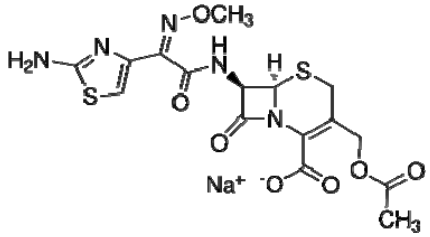
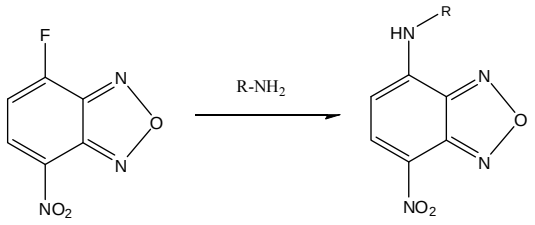


Fig. 1 Molecular structure of cefotaxime sodium

The aim of this paper was to develop a spectrofluorimetric method for the determination of cefotaxime sodium immobilized in nanoparticles as a controlled release system, by labeling the molecule using 4-fluoro-7-nitrobenzofurazan (NBD-F) as derivatization agent. Analytical techniques based on fluorescence detection are popular because of their high sensitivity and selectivity, together with the advantages of spatial and temporal resolution, but when it comes to the assay of cephalosporins, most reported methods of analysis are electrochemical and especially high performance liquid chromatographic methods, and to our knowledge just a small number of methods are available in the literature for fluorimetric determination of cefotaxime sodium or other related cephalosporin antibiotics, using fluorophores like 2-Cyanoacetamide, 1,2-naphthoquinone-4-sulfonic (NQS) or ortho-phthalaldehyde (OPA). Moreover, few use NBD-F as derivatization agent for these types of antibiotics, so we found interest in developing a fluorimetric method using this compound. NBD-F is highly reactive and can label primary and secondary amines under mild conditions. NBD-F is also a pre-labeling compound for high performance liquid chromatographic analysis of small molecules. NBD-F-labeled compounds are orange with a maximum wavelength at 470 nm [23]. The proposed derivatization reaction is presented in Fig. 2.



NBD-F fluorescent derivative

Fig. 2 Derivatization reaction of cefotaxime sodium with NBD-F

2. Experimental

Instrumentation

A Perkin-Elmer LS45 luminescence spectrometer was used with a xenon discharge lamp, excitation and emission slit of 10 nm and quartz cuvettes of 1x1 cm. The tension used was 650.

The pH of the buffer solutions was controlled by using a multiparameter CyberScan PCD 6500 with a pH glass electrode.

Chemicals

4-fluoro-7-nitrobenzofurazan and cefotaxime sodium standard (FW 477.5) used in this study were purchased from Sigma-Aldrich, Steinheim, Germany. All stock solutions and samples were prepared with ultrapure water obtained from a Barnstead EASYpure RoDi water system, having a conductivity of 17.6 MΩ-cm, and then filtered through a 0.45μm membrane filter. Methanol was of HPLC grade.

Stock solutions

A sample of cefotaxime sodium was accurately weighted (50 mg) and quantitatively transferred into a 50 mL volumetric flask and dissolved in water to produce a stock solution having a concentration of 1000 $\mu g \cdot mL^{-1}$. The standard working solution was prepared by diluting the standard stock solution to the concentration of 50 $\mu g \cdot mL^{-1}$. All solutions were kept in the refrigerator until right before the analysis.

Buffer solution

In this experiment borate buffer was used as the aqueous medium of the reaction, prepared from 19.1 g of borax in 1 L of water at pH=9.0 adjusted with a solution of 0.1M NaOH.

Derivatization agent solution

The NBD-F solution was prepared by dissolving the fluorescent compound in methanol to obtain a concentration of 120 $\mu g \cdot mL^{-1}$. This solution was kept into the refrigeration also.

Derivatization procedure

Standard working solutions (10-100 μL) were mixed with 100 μL of buffer solution and 100 μL of NBD-F solution and kept at 70°C for 15 minutes. Then the samples were rapidly cooled in ice-water to stop the derivatization process and then they were acidified with 100μL of 0.1M HCl. The addition of HCl, after cooling the mixture, has as objective to increase the fluorescence signal of the derivative products.

The obtained derivatives were extracted by using 3 times 3 mL of chloroform. All phases were mixed into a 10 mL volumetric flask and filled up with chloroform. The fluorescence intensities were read with the Perkin-Elmer luminescence spectrometer.

Sample analysis

The method was applied in the controlled release of cefotaxime sodium immobilized in MCM-41 particles.

For a better control of the release study, Franz cells were used as reacting chambers. A hybrid containing the antibiotic with a loading of 55.16% was used (the synthesis of the hybrid is the aim of another paper) at 37°C in a phosphate buffer having a pH of 7.4. The release was conducted for 7 hours, the total volume of the cell was 4 mL and the amount of hybrid was 12.4 mg. In this interval, samples of 100 µL were collected, replacing the missing volume with the same volume of fresh buffer solution. The real concentration measured will be:

$$C_{n,corrected} = C_{n,measured} + V_{sample} / V_{receptor} \quad (1)$$

where:

C_n is the sample concentration, $\mu g \cdot mL^{-1}$;

V_{sample} is the receptor chamber, 100µL;

$V_{receptor}$ is the total volume of the cell (receptor), 4mL[24]

3. Results and discussion

3.1. Optimization of chemical and physical conditions for the derivatization of cefotaxime sodium in the presence of NBD-F

Influence of NBD-F concentration

The effect of NBD-F concentration, as added volume, on the fluorescence intensity of the derivative products was studied in the range between 50 and 200 µL. The maximum fluorescence intensity was obtained when 100 µL were added. Hence, 100 µL was chosen as the optimum volume to ensure that a sufficient excess of reagent was present. The results are presented in Fig. 3.

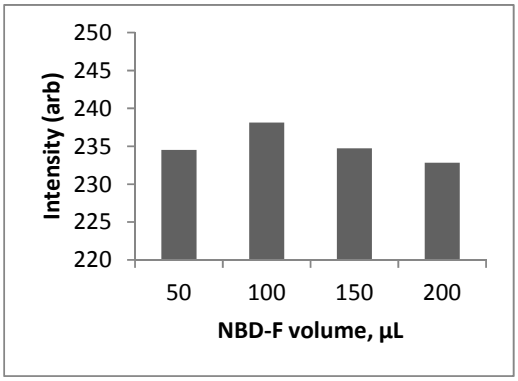


Fig. 3. Influence of NBD-F volume on the fluorescence intensity

Influence of heating time

The formation of the fluorescent products between cefotaxime sodium and NBD-F depends on the reaction time. The effect of the heating time was studied at 70°C. Identical samples were prepared (100µL of 50µg·mL^{-1} of standard solution) and were heated at different times between 1 and 20 minutes. The fluorescence intensity increases with increasing the heating time, as it can be seen in Fig. 4. At 70 °C, 15 minutes are necessary and sufficient for the formation of the fluorescent derivative. These conditions were selected as optimum.

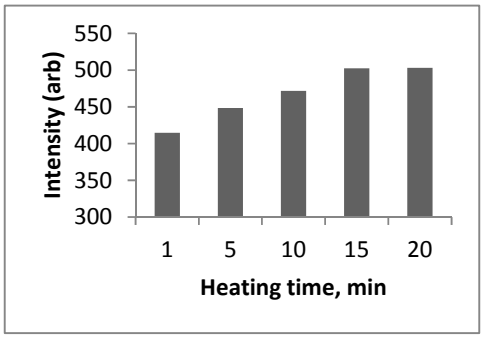


Fig. 4. Influence of heating time on fluorescence intensity

Influence of the solvent volume used for extraction

Chloroform was used as extraction solvent. For maximum extraction of the fluorescent derivative the influence of solvent volume on the fluorescence intensity was studied. Identical samples of fluorescent products were extracted using different volumes of chloroform in the range of 3 – 10 mL as shown in Fig. 5. The optimum extraction volume was 9 mL and was used in all the experiments.

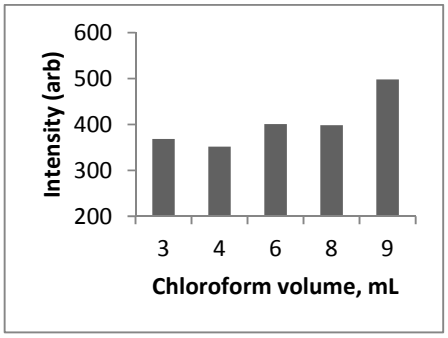


Fig. 5. Influence of chloroform on fluorescence intensity of product

Analytical parameters

Under the optimum operating conditions selected, there was a satisfactory linear relationship between fluorescence intensity and cefotaxime sodium concentrations in the range 50-500 ng·mL^{-1}. The calibration graphs were obtained by preparing samples in triplicate with increasing concentrations of each analyte. Analytical characteristics of the determination of cefotaxime sodium are summarized in Table 1.

Table 1

Analytical and statistical parameters for the fluorimetric determination of cefotaxime sodium, previous derivatization with NBD-F

	Cefotaxime sodium
Dynamic range, ng·mL^{-1}	50 - 500
Slope	0.1642
Intercept	132.94
Standard deviation of slope	0.0077
Standard deviation of intercept	3.0735
Regression standard deviation	2.784
Correlation coefficient (R^2)	0.9703
Regression coefficient (R)	0.9850

The maximum wavelengths for the excitation and emission spectra for NBD-F are 470 nm and respectively 530 nm. The spectra are shown in Fig. 6.

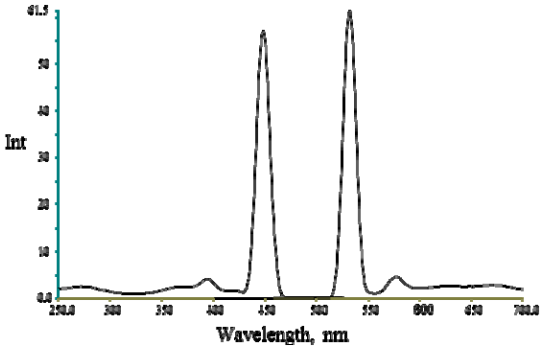


Fig. 6. Excitation and emission spectra of NBD-F

3.2. Application of the spectrofluorimetric method in the controlled release of cefotaxime sodium immobilized in MCM-41 particles.

Cefotaxime sodium was introduced in the MCM-41 material in order to study the drug adsorption and release profile in pH controlled media.

There can be differentiated two behaviors in the drug release, the first one at short times (100 min) with high delivery rates, corresponding to the cefotaxime sodium adsorbed in the outer surface and in the pores close to the surface, which can be released more easily, and the second one with slower delivery rates, corresponding to the cefotaxime sodium inside the pores, further to the canal open, this is due to the diffusion of the remaining cefotaxime sodium through the channels (Fig. 5).

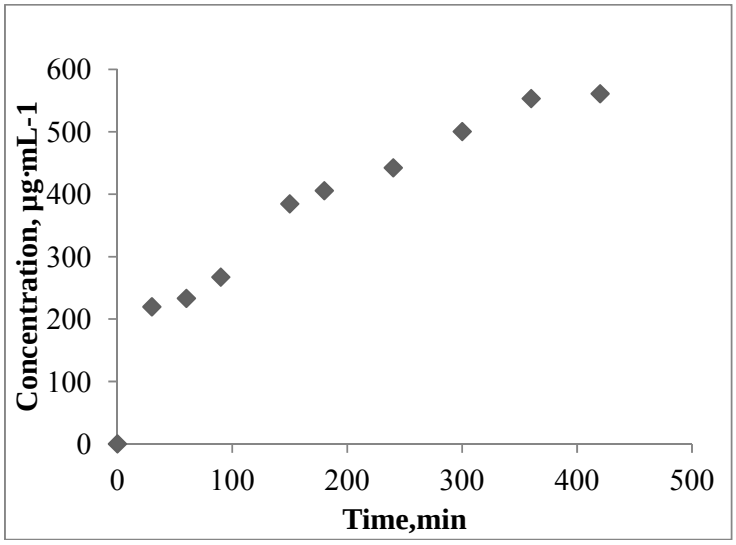


Fig. 5. Release profile for cefotaxime sodium immobilized in MCM-41

4. Conclusion

A preliminary study was conducted in order to observe the capacity for cefalosporins to form fluorescent compounds in particular conditions by using a specific fluorophore for the derivatization of the biologically active substances. Using this technique, (spectrofluorimetry) very small concentrations of analyte can be determined. The influence of different parameters was also studied.

Then, this method was applied in the controlled release studies, simulating intestinal conditions and proved to be adequate for this purpose.

In the future works it may be considered to extend the application of this method for determination of other cefalosporins with amine side groups.

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