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Application of fluorimetry to the analysis of Naproxen and its complexation with modified β-cyclodextrins

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Naproxen(NAP),(+)-6-methoxy-α-methyl-2-naphtaleneaceticacid, is a non-steroidal anti-inflammatory drug that exhibits fluorescence. Its fluorimetric characteristics have been studied. Corrected excitation and emission spectra of NAP have been obtained in different solvents at room temperature. The wavelengths of the emission maxima (nm) in the different solvents were 348 (cyclohexane), 350 (dimethylformamide), 351 (ethyl acetate, dioxane and acetonitrile), 352 (ethanol and methanol), 356 (aqueous solutions, pH 5) and 357 (0.1 N H_2SO_4 and 0.1 N NaOH). The effect of solvent on the Stokes shift allows us to calculate the increase of dipolar moment upon excitation, using the Lippert equation (Lippert, 1957). The result obtained was 1.6 ± 0.1 D. The dipolar moment of NAP in the ground state calculated by molecular modelling studies was 0.88 D.

Furthermore, the fluorescence quantum yields of NAP in different solvents have been calculated by the method proposed by Parker and Rees (1960). The values obtained for NAP in cyclohexane, aqueous solutions (pH 5), ethanol and methanol at 25°C were 0.22, 0.23, 0.27 and 0.25, respectively. All the emission spectra were obtained at an excitation wavelength of 280 nm. The solutions used were 5 and 3 μg mL^{-1} quinine sulphate (reference substance) and 0.5 μg mL^{-1} NAP.

The quantification (Miller and Miller, 1988) and detection limits (IUPAC, 1976) are two important parameters for the fluorimetric determinations of a molecule; both have been calculated in the same solvents as quantum yields. The values of the detection limits obtained operating at the longer excitation wavelength and at the corresponding emission maxima were (in ng mL^{-1}) 1.44 (cyclohexane), 4.92 (aqueous solutions, pH 5), 1.43 (ethanol) and 1.20 (methanol). The quantification limits (ng mL^{-1}) were 4.80, 16.4, 4.77 and 4.00, in the same solvents as the detection limits, respectively. The values obtained are very small, this fact indicates the possibility of determining NAP by spectrofluorimetric measurements. In order to evaluate the usefulness of the fluorimetric method to analyse NAP in pharmaceutical formulations, it has been applied to determine its content in tablets. The fluorescence intensities of the solutions (pH 2) were measured at 271 and 331 nm as excitation wavelengths and 353 nm for emission. An average recovery of 98.2% was obtained, with a standard deviation of 0.1%.

Another purpose of this work was to study the fluorimetric characteristics of the complexes between NAP and β-cyclodextrin derivatives (methyl β- and hydroxypropyl β-cyclodextrins) in solution. It is well known that many drugs form inclusion complexes with β-cyclodextrins. These complexes are usually formed in order to improve the physicochemical characteristics of the drug. In a recent paper we have employed the fluorimetric method to analyse the complex NAP–βCD in solution (Vélaz *et al.*, 1997). In order to compare the stability constant obtained in the aforementioned paper, the constants of NAP–methyl β- and NAP–hydroxypropyl β-cyclodextrin complexes have been calculated using the enhancement of fluorescence that occurs when the complex is formed. A 1:1 stoichiometry for the complexes was supposed. This study was carried out in 0.01 M sulfuric acid solutions (pH 2) because these have been found to be the best conditions for complexation (Vélaz *et al.*, 1997). The temperature used was 25°C. The concentration of NAP was kept constant at 2.0×10^{-6} M. A range of concentrations of modified βCD from 0.40×10^{-4} M up to 3.0×10^{-4} M was used. Fluorescence intensities of the solutions were measured at excitation and emission wavelengths of 331 and 353 nm (NAP–HPβCD) and 355 nm (NAP–MβCD), respectively. The experiments were repeated at least three

Table 1. Quantum yields and stability constants of complexes between NAP and βCD and derivatives in 0.01 M sulfuric acid solutions (pH 2) at 25°C

	Quantum yield	$K_s \times 10^{-3}/M^{-1}$
Naproxen	0.19	
NAP–βCD	0.28	5.73 ± 0.09
NAP–HPβCD	0.27	7.85 ± 0.18
NAP–MβCD	0.31	12.2 ± 0.12

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Table 2. Detection and quantification limits of NAP and its complexes in 0.01 M sulfuric acid solutions, at 25°C

	$\lambda_{exc.}$ (nm)	$\lambda_{em.}$ (nm)	Detection limit (ng mL^{-1})	Quantification limit (ng mL^{-1})
Naproxen	271	353	2.71	9.05
	331		4.23	14.1
NAP–βCD	271	353	1.36	4.53
	331		1.75	5.83
NAP–HPβCD	271	353	1.76	5.89
	331		1.88	6.27
NAP–MβCD	271	355	1.72	5.73
	331		1.97	6.57

times. Each value is the average of five measurements. The quantum yields of the complexes were also calculated. The results obtained are shown in Table 1 with the stability constant for the NAP–βCD complex (Vélaz *et al.*, 1997).

It can be observed that the stability constants of the complexes with βCD derivatives are greater than that with native βCD probably due to a substantial increase in the hydrophobic space inside the cavity.

Finally, the detection and quantification limits of NAP–CD complexes in sulfuric acid solutions (pH 2) were obtained; they are summarized in Table 2. It is worthy of note that the detection limits are smaller in the presence of any of the βCDs in comparison with the value of NAP alone.

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