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Short communication

A fluorescence optosensor for analyzing naphazoline in pharmaceutical preparations Comparison with other sensors

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Abstract

We have developed an optical sensor for determining and quantifying naphazoline (NPZ) based on its inherent fluorescence property. We have placed a non-ionic-exchanger solid support (Amberlite XAD-7) in a flow cell in the light path of the excitation beam and the fluorescence signal for NPZ is continuously monitored at $\lambda_{exc/nm} = 294/306$ nm. The response time for this sensor is acceptably fast, 80 s, obtaining a detection limit of 2.6 ng mL^{-1} with standard deviations of 2.0% at 125 ng mL^{-1}. This device has been satisfactorily applied to two commercial formulations and its selectivity has been demonstrated with an interference study. The advantages have been compared with the only published sensor for determining NPZ in pharmaceutical preparations and with other analytical methods in the literature.

Keywords: Naphazoline; Fluorescence optosensor; Comparative study

1. Introduction

Naphazoline,2-(1-naphthylmethyl)-2-imidazoline (NPZ), is a relatively long-lasting action vasoconstrictor, which acts on the alpha receptors of the smooth vascular muscle [1]. There are several analytical methods to determine NPZ; the majority are photometric [2,3] and chromatographic methods: thin layer chromatography [4], gas chromatography [5] and high performance liquid chromatography [6,7]. Capillary electrophoresis [8–10] and atomic absorption and emission [11] methods have also been published. NPZ presents intrinsic fluorescence and phosphorescence emission, thus different luminescence methods have been developed for its determination [12–15]. All of them present too many complications for routine lab use. The photometric methods are not selective and sensitive enough, the separative methods are expensive and require too much time and the luminescence methods are manual and require the work of an analyst. For these reasons, the research efforts devoted to flow-through optosensors in pharmaceutical analysis are very promising because they link the advantages of solid phase spectroscopy (SPS) with the intrinsic ones from flow injection analysis (FIA) i.e. higher sensitivity and selectivity, higher speed analysis, less consumption of reagents and all the main FIA characteristics. Flow-through optosensors produce very simple and inexpensive analytical procedures with remarkable analytical features, mainly sensitivity and selectivity, compared to the respective conventional spectroscopic procedures [16].

In the literature, it is possible to find many applications of fluorescence optosensing for analysing drugs [17–19]. Our research group has published in 2004, the first optosensor based on the native phosphorescence of a compound for determining NPZ in pharmaceutical preparations [20], obtaining a detection limit of 9.4 ng mL^{-1} with 2.3% of relative

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standard deviation at 400 ng mL^{-1} concentration level.In this work, we present a simpler, cheaper, more sensitive and easier to manufacture sensor for determining NPZ in pharmaceutical preparations. To demonstrate its advantages we have compared our fluorescence optosensor with the sensor for determining NPZ and with other methods, showing the best characteristics to use in a control or a routine laboratory.

2. Experimental

2.1. Reagents

Naphazoline (Sigma Spain) was used without further purification. Working solutions were daily prepared by suitable dilution of the stock solution (30 mg L^{-1}). The strongly basic anion-exchanger resins Dowex 1x2-100, 1x4-100 and 1x8-100, Denom Sephadex-QAE A-25 and Deae Sephadex A-25, the strongly acid cation-exchanger resins Dowex 50wx2-100, 50wx4-100 and 50wx8-100, Sephadex-SP C-25 and Denom Sephadex-CM C-25 and the non-ionic resins Amberlite XAD 2, XAD 4 and XAD 7 and Silica Gel Davisil and Merck (Sigma Spain) were sieved and then tested at five-grain size (40–63, 63–80, 80–120, 120–160 and >160 μm), except all Sephadex resins which were sieved until a minimum size of 120 μm.

2.2. Flow set-up and instrumentation

The same optosensing FIA manifold which was proposed by Fernández-Sánchez et al. [16] is used. A Hellma Model 176.052-QS flow-through cell of 25 μL volume was packed with the corresponding solid support and placed in the conventional sample compartment of the spectrometer (Aminco Bowman Series 2 luminescence spectrometer). Two rotary valves (Sepulco 5020) were used for sample introduction and for elution of the retained NPZ. PTFE tubing (0.8 mm i.d.) and fittings were used for connecting the flow-through cell, the rotary valves and the carrier solution reservoirs. A Gilson Minipuls-3 peristaltic pump was used to generate the flow stream.

The optosensor response was taken to be the difference between fluorescent emissions and background signals and all the measurements were repeated three times to obtain the error.

2.3. General procedure

Two milliliter of sample was injected through the first valve into a channel of 10 mM glycine/NaOH buffer solution at pH 8 into the carrier stream. In this medium, at a 2.5 mL min^{-1} flow-rate, the NPZ is retained in the flow cell on Amberlite XAD 7. After fluorescent measurement ($\lambda_{exc/nm}$ = 294/326 nm, detector voltage of 750 V, slit width of 4 nm for excitation and emission), 250 μL of regenerative solution (acetone) was injected through the second valve to strip the analyte retained on the solid phase, before proceeding with the next sample.

2.4. Procedure for analysing pharmaceutical preparations

Two different commercial products were analysed: Colirio Alfa (Pfizer Consumer Healthcare, Barcelona, Spain) with a nominal content of 300 μg mL^{-1} of NPZ and also containing copper sulphate, trisodium citrate, potassium chrome alum, boric acid, camphor, methyl *p*-hydroxybenzoate, sodium hydroxide and sodium chloride, without indication of their concentration; and Euboral Oftálmico (Laboratorio Reig Jofré, Barcelona, Spain) with a nominal content of 10 mg g^{-1} of NPZ and also containing sodium tetraborate (970 mg g^{-1}) and methyl *p*-hydroxybenzoate (20 mg g^{-1}).

A portion of the two products was dissolved in doubly distilled water. Aliquots of this solution were treated as indicated under Section 2.3.

3. Results and discussion

3.1. Selection of the sensor phase and regenerative solution

The strongly basic anion and cation-exchanger resins and non-ionic resins commented in Section 2.1 (reagents) were studied. Only some of them were useful to retain the analyte (see Table 1) and the best difference between noise and fluorescence signals was obtained using Amberlite XAD7.

The effect of the grain size of the selected solid support was also tested. All of the grain sizes produce similar responses but the measured error decreases from 40–63 to 80–120 and increases from 80–120 to >160. Thus, 80–120 μm was chosen because it produces adequate optosensor responses with the lowest error.

Finally, studies of optimum regenerative solution were carried out to transform the system into a reusable one. Different acids and base solutions (2 and 6 M NaOH and 6 M HCl, HNO_3 and H_2SO_4) and organic solvents (methanol, ethanol, acetonitrile, dimethylformamide and acetone) were tested. The optimum regenerative solution was acetone (250 μL).

Table 1
Selection of solid support

Resin[a]	Optosensor response	
	pH 4.0	pH 10.0
Amberlite XAD 7	**1.318**	**1.469**
Silica Gel "Merck"	0.131	0.793
Silica Gel "Davisil"	0.495	1.057
SP-Sephadex	0.983	0.957
QAE-Sephadex	0.283	0.300

The bold numbers means they are the maximum signal obtained using Amberlite XAD7.

[a] Only the resins which interact with NPZ.

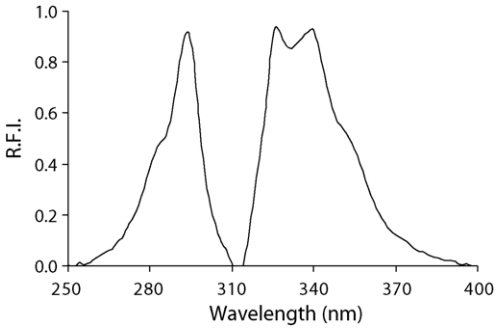

Fig. 1. Excitation and emission fluorescence spectra of naphazoline on Amberlite XAD-7, [NPZ] = 200 ng mL^{-1}, detector voltage 750 V, slit width$_{exc/nm}$ 4/4 nm and resolution 5 s.

3.2. Luminescence properties on solid surface

The fluorescence excitation and emission spectra of NPZ on Amberlite XAD7 are shown in Fig. 1. The maximum fluorescence emission is obtained when it is excited at 294 nm and the emission fluorescence is collected at 326 nm. These wavelengths are very similar to the corresponding ones in solution (290/328 nm).

3.3. Optimization of chemical variables

The chemical variables, which affect fluorescence signals, are the pH and the type and concentration of buffer solution. pH affects the dissociation of the compounds, and consequently the retention on the solid support and the fluorescence intensity emission.

Different pHs ranging from 2 to 12 (fixed with HCl and/or NaOH) were studied (see Fig. 2a), choosing pH 8.0 as optimum value.

Different pH 8.0 buffer solutions (glycine/NaOH, borax/HCl and $H_2PO_4^-$/HPO_4^{2-}) added only in the sample, only in the carrier and in both solutions were tested. The best optosensor response was obtained with glycine/NaOH buffer solution in the sample and the carrier solutions.

Different concentrations of buffer solutions were also tested (see Fig. 2b). An increase in the buffer concentration produces a decrease in the optosensor response but also diminishes the error. Thus, the selected buffer concentration was 10 mM.

3.4. Optimization of flow injection parameters

The retention of NPZ on the solid support changes with the carrier flow-rate from 0.5 to 3 mL min^{-1}. An increase in the flow-rate decreases the luminescence signal up to 1.5 mL min^{-1} and then when the flow-rate increases the optosensor response is constant but decreases the response time. An optimum value of 2.5 mL min^{-1} was chosen because it shows an adequate fluorescence signal with the lowest response time.

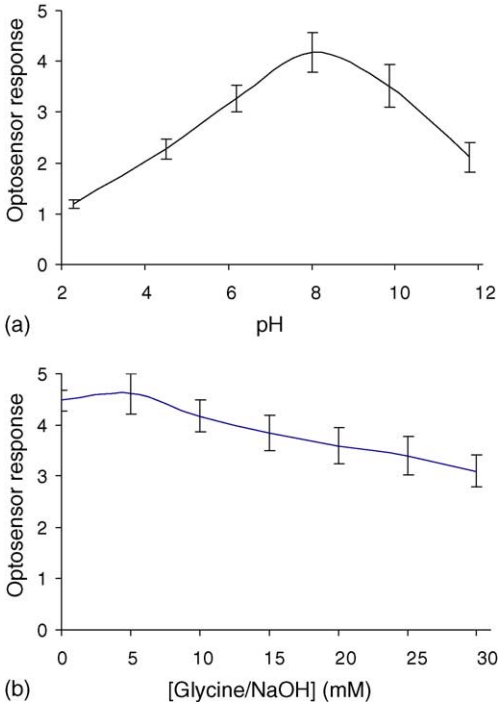

Fig. 2. Optimisation of chemical variables (a) pH and (b) concentration of glycine/NaOH buffer solution in the sample and the carrier solutions. [NPZ] = 200 ng mL^{-1}, $\lambda_{exc/nm}$ = 290/328 nm, detector voltage 750 V, slit width$_{exc/nm}$ 4/4 nm and resolution 5 s.

The injection volume of sample considerably affects the optosensor response and the response time. An increase in the injection volume significantly increases the luminescence signal (resulting in a plateau when injection volume is greater than 3 mL) and also increases the response time. In order to have a non-excessive response time and an acceptable signal, 2 mL was chosen as optimum injection volume.

3.5. Analytical performance characteristics

A standard calibration linear graph was prepared according to recommended procedures. The calibration line was fitted by least-square regression for NPZ·$I = 0.4147 + 0.0202C$; where C is the concentration in ng mL^{-1} of NPZ and I is the optosensor response. The correlation coefficient (r) was 0.9970.

The linear dynamic range and detection and quantification limits were determined using the IUPAC methods and the precision was expressed as relative standard deviation. All of these analytical characteristics are summarised in Table 2.

3.6. Interference study

To demonstrate the selectivity of the proposed optosensor, the emission spectrum of the medicines were compared to the emission spectrum of the pure analyte and both spectra are practically the same. A standard addition method of calibration was also carried out demonstrating that the optosensor

Table 2
Analytical parameters

Parameter	Value
Linear range (ng mL^{-1})	2.6–225.0
Detection limit (ng mL^{-1})	2.6
Quantification limit (ng mL^{-1})	8.7
R.S.D. (%)	2.0[a]

[a] At 125 ng mL^{-1} level.

for determining NPZ in pharmaceutical preparations is very selective.

3.7. Analytical applications

Following the procedure for analysing medicines, two pharmaceutical preparations (Colirio Alfa and Euboral Oftálmico) were analysed. Table 3 shows the experimental results.

The method was also validated with a standard addition method of calibration. To check the similarity of the slopes, a Student's *t*-test was used [21]. The statistic for the slope was calculated using the fluorescence optosensor 0.164 for Colirio Alfa and 1.386 for Euboral Oftálmico while the statistic for the slopes tabulated are 2.756 at 29 d.f. and $\alpha = 0.01$. Therefore, the slopes are essentially the same.

3.8. Comparison between both developed devices

In this section, the performance characteristics of the proposed optosensor will be compared with the published methods for determining NPZ.

3.8.1. Figures of merit

Table 4 shows the HAI-RTP method which presents the best detection and quantification limits but is followed very closely by the fluorescence sensor. All of the methods offer similar precision and as far as the LDR is concerned, the proposed sensor has the lowest range and this is the only disadvantage it presents compared to the other methods.

3.8.2. Time and cost per analysis

The fastest separative method takes at least 10 min to determine NPZ and its cost is always higher than luminescence methods. The off-line photometry, fluorimetry and phosphorimetry methods take practically the same time but they are normally longer than on-line (flow) methods. Therefore, the fastest and cheapest methods are RTF- and RTP-sensors. RTP-sensor takes 100 s for analysing one sample while RTF-sensor takes only 80 s.

Table 3
Comparative study of recoveries in pharmaceutical samples by RTF- and RTP-sensors

Pharmaceutical preparations	Sensor	Direct determination		Standard addition method determination
		Recovery percentage (%)	R.S.D. (%)[a]	Recovery percentage (%)
Colirio Alfa	RTF	99.2	6.8	106.9
	RTP[b]	104.9	9.4	121.2
Euboral Oftalmico	RTF	100.1	1.5	107.8
	RTP[b]	101.4	8.9	80.9

[a] For seven replicates.
[b] Reference [20].

Table 4
Figures of merit for different analytical methods for NPZ in pharmaceutical preparations

Method	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	LDR (ng mL^{-1})	RSD (%)[a]	Reference
Photometry	20×10^9	60×10^9	0–1000×10^9	–	[3]
HPLC	20×10^3	60×10^3	–	–	[6]
CZE	4700.0	15800.0	0–60000	1.1	[8]
MEKC	50.0	150.0	0–32300	2.2	[9]
MEKC-PLS	30.0	90.0	0–39900	2.5	[10]
Atomic emission	980.0	2940.0	0–14760	1.5	[11]
Fluorescence	100.0	300.0	0–3000	–	[12]
Fluorescence-CD	15.0	45.0	–	–	[13]
MS-RTP	4.9	16.3	0–1000	2.1	[14]
HAI-RTP	1.7	5.6	0–1000	1.1	[15]
RTP-sensor	9.4	31.2	0–1000	2.3	[20]
RTF-sensor	2.6	8.7	0–225	2.0	This work

Abbreviations: LOD: limit of detection; LOQ: limit of quantification; LDR: linear dynamic range; RSD: relative standard deviation; CZE: capillary zone electrophoresis; MEKC: Micellar electrokinetic chromatography; CD: cyclodextrine; RTF: room temperature fluorescence.
[a] In an average concentration level in the calibration curve.

3.8.3. Portability, potential for miniaturization and cost of manufactured

On the one hand, the fluorescence optosensor, which needs a continuous lamp and a photomultiplier as detector, presents a higher potential for its miniaturization and lower manufacturing costs than the phosphorescence sensor, which needs a pulsed lamp and an electronic table to program the decay time and the gate time into the detector. On the other hand, the phosphorescence optosensor presents the largest Stoke's shift, 230 nm, whilst for the fluorescence sensor it is only 32 nm.

3.8.4. Applicability

The RTF- and RTP-sensors were applied to determine NPZ in the same pharmaceutical preparations. Table 3 shows the results obtained with both sensors. It shows that the RTF has the best recovery percentage in the direct and standard addition determinations and the reproducibility of the RTP-sensor is worse than the fluorescence sensor.

4. Conclusions

The first fluorescence optosensor for the on-line determination of NPZ is presented offering excellent analytical parameters, such as sensitivity, selectivity, versatility, applicability and ease of use.

This fluorescence-based optosensor, developed for the determination of NPZ, has been compared with the published methods to determine NPZ and it has been proved to be accurate and suitable for NPZ detection. The optosensor is characterized by its reproducibility of the baseline after each single measurement cycle and it responds rapidly.

For the two kinds of commercial products tested, the statistical calculations of the assay results showed satisfactory precision of the luminescence optosensor proposed with no significant differences between the declared and experimental results. The proposed method can be recommended for the routine determination of NPZ in formulations, as it is rapid and simple, and the results obtained showed good precision.

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