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Determination of zinc (II) in pharmaceuticals based on a flow-through bulk optode

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Abstract

A method based on flow injection (FI), was applied for the determination of Zn (II) using a flow-through bulk optode membrane that incorporates 1-(2-pyridylazo)-2-naphthol in a plasticized poly (vinyl chloride) membrane entrapped in a cellulose support. The calibration graph plotting the reflectance at 562 nm versus [Zn (II)] was linear in the range 0.16–3.27 mg l^{-1} (2.5×10^{-6}–5×10^{-5} M) with a detection limit of 0.10 mg l^{-1}. The variation coefficients of the sensor response for 0.33 mg l^{-1} of Zn (II) were $\pm 0.11\%$ for consecutive measurements ($n = 10$), $\pm 0.19\%$ between days ($n = 5$) and $\pm 0.22\%$ between different membranes ($n = 6$). The sensor can be readily regenerated with the same acetic/acetate carrier of pH 3.9. The FI method proposed was applied to the determination of zinc (II) in pharmaceuticals.

Keywords: Flow-through optode; Zinc determination; Pharmaceuticals

1. Introduction

The development of optical chemical sensors (optodes) as viable alternatives to other types of sensors, is of great interest [1–4] and several optodes have been successfully applied in the trace analysis of heavy metal ions and in process control, environmental and medical analysis [3–8].

The great activity seen in the field of ion-sensitive optical fibre devices and their different applications has given rise to several sensing schemes, new indicator dyes and diversified methods of immobilization. The method of immobilization and the class of matrix exert a significant effect on the performance of ion-sensitive layers [5]. One type of optode makes use of a plasticized polymeric membrane, which contains a reagent that reacts with the analyte to produce a distinctive colour change. Basic principles and techniques of optical ion sensing have been described [9,10] and the theoretical description of bulk optode membranes, which are based on the reversible mass transfer of analyte from the sample into the bulk of the sensing layer, has been elucidated by Seiler and Simon [10].

The performance of reversible optodes is probably best tested in flow injection systems (FI), and

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flow cells may be connected to fiber optics [2,11]. These configurations give them greater flexibility and automatability in addition to a wider applicability to real problem [2].

In this paper a FI method was studied for the determination of Zn (II) using a flow-through bulk optode membrane that incorporates 1-(2-pyridylazo)-2-naphthol (PAN), immobilized in a plasticized poly (vinyl chloride) (PVC) membrane, entrapped in a cellulosic support. The optode is incorporated in a flow cell in a flow configuration. The sensor layer can be regenerated rapidly and completely with the same carrier solution.

Several optodes have been described for the determination of zinc (II) and most make use of dyes immobilized on ion-exchange resins [12–17]. Lidner et al. [18], Wang et al. [19] make use of the optodes with plasticized polymeric membranes. Few optodes have been proposed for the FI determination of zinc (II) [14,16,19,20].

Zinc compounds are used in dermatology as antiseptic and disinfectant agents. Several zinc compounds are also used in the preparation of ophthalmic solutions, insulin, mouthwashes and mineral–vitamin preparations. Various methods have been used for the determination of zinc in pharmaceuticals: gravimetric [21], titrimetric [22,23], spectrophotometric [24–29] and potentiometric [30].

In this paper the proposed FI-photometric method is applied for determining Zn (II) in pharmaceuticals. To our knowledge, no method involving optodes has been described for the determination of zinc in pharmaceuticals.

2. Experimental

2.1. Apparatus

Spectroscopic measurements were made using an oriel (Stratford, USA) modular spectrophotometer. A 100-W tungsten filament lamp (Model 6333) was powered by an oriel power supply (Model 68735) at 75 W. The light from the source passed through the monochromator (Model 77250) and was focused on one leg of a bifurcated bundle of randomised glass fibbers (Oriel Model 77533). The variable slits (Model 77269) on each side of the monochromator were set at 3.16 and 1.24 mm, respectively. Light from the source at the selected 560 nm was reflected off the thin membrane, while the other leg of the bifurcated bundle carried the diffusely reflected light to the photomultiplier tube (Model 77341) powered by a photomultiplier power supply (Model 70705). The transimpedance amplifier (Model 70711) was interfaced with a personal computer via an analog-to-digital converter. Gilson Minipuls 3 (Villiers le Bel, France), peristaltic pump (Worthington, OH), omnifit injection valve (NY, USA). A home made flow-through cell designed by the authors was used [31]. Connecting tubing of 0.5 mm i.d and various end-fittings and connectors (Omnifit) were used.

2.2. Materials and reagents

PVC high molecular mass, 2-nitrophenyl octyl ether (NPOE) and tetrahydrofuran (THF) were Selectophore products from Fluka. Filter paper 235 (Albet, Barcelona, Spain). PAN. (Merck). All chemicals were of analytical reagent grade and were dissolved in doubly distilled water. The buffer solutions were 0.2 M acetate/acetic acid at pH 3.6–5.6 and 0.2 M ammonia/ammonium chloride at pH 8.2–11.0.

Standard zinc (II) solution 1×10^{-2} M, was prepared by weighing 0.2874 g of $ZnSO_4 \cdot 7H_2O$ (Merck) and dissolving in distilled water to give a volume of 100 ml and standardising with EDTA [32]. Working standard solutions of lower concentrations were prepared by suitable dilution of the stock solution with 0.2 M ammonia/ammonium chloride of pH 10.2.

Dosage form of zinc: (1) Anticongestiva Cusi ointment (Synthelabo Pharma Spain): 25 g ZnO, 25 g starch, lanolin and vaseline up to total 100 g of paste (2) Fungusol powder (Roche, Spain). 10 g ZnO, 5 g boric acid, 1 g chlorocresol and other excipients up to total 100 g of product. (3) Kalamine lotion (Synthelabo Pharma Spain): 6.25 g ZnO, 2 g glycerine and other excipients up to total 100 ml of product. (4) Pro-Crecil NT lotion (Viñas, Spain): 1.0 g zinc acetate, 1.0 g sodium hyaluronate, 0.5 g tocopherol nicotinate and other

excipients up to total 100 ml of product. (5) Gingi Kin B_5 (Kin. Spain): 0.38 zinc lactate, 0.2 g triclosan, 0.5 g provitamin B_5, 1 g xylitol and other excipients up to total 100 ml of product. (6) Ciscutil, ampoules (Medea, Spain): 0.5 g zinc sulphate, 1 g L-methionine, other excipients: water, pyridoxine chlorhydrate, parabens, sodium EDTA and perfume up to total 100 ml of product.

2.3. Optode membrane preparation

The optode membranes were prepared from a coating solution of 50 mg of PVC, 100 mg of NPOE and 4.5 mg of PAN dissolved in 3.0 ml of THF. Fifty microlitre of this coating solution was deposited on a cellulose filter paper (Albet 235). After 1–2 min the THF had evaporated and a 1.5×2.5 cm^2 piece was cut out and incorporated into the specially designed flow-through measuring cell [31]. The flow-through cell containing the optode membrane was incorporated into the flow design selected.

2.4. Measurement procedure

The manifold used in the FI method is shown in Fig. 1. To obtain the base line, a carrier stream of 0.2 M acetic/acetate buffer of pH 3.9 (buffer 1) was pumped at a flow rate of 0.4 ml min^{-1}. A volume of 90.0 μl of sample solution containing 0.16–3.33 mg l^{-1} (2.5×10^{-6}–5.0×10^{-5} M) of Zn (II) dissolved in a 0.2 M NH_3/NH_4^+ buffer solution pH 10.2 (buffer 2) was injected into the carrier containing acetic/acetate buffer of pH 3.9 and pumped at a flow rate of 0.40 ml min^{-1} through the flow cell, in which PAN was permanently immobilised. The diffuse reflectance at 562 nm was monitored.

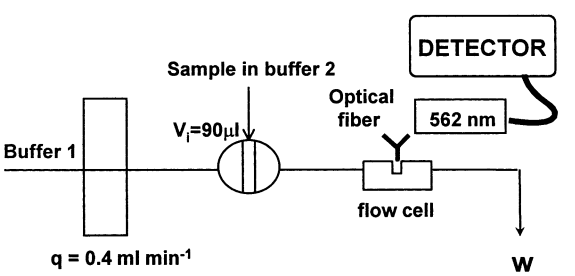


Fig. 1. Configuration used. Buffer 1: ammonia/ammonium chloride of pH 10.2; buffer 2: sodium acetate/acetic acid of pH 3.9.

2.5. Procedures for determination of zinc in pharmaceuticals

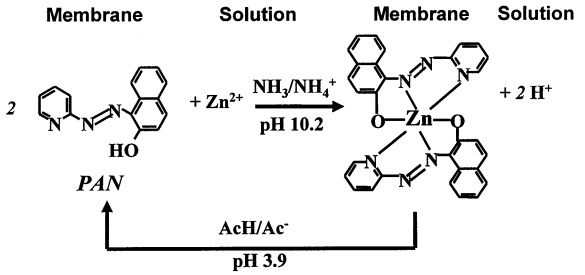
For Anticongestiva Cusi (1), Fungusol (2) and kalamine (3) portions of 0.2–0.4 g for (1) and (2) or 250–500 μl for (3) of sample were accurately weighed or measured. The samples were dissolved with 0.5 ml 2M HCl and 5.0 ml of water, heated to near boiling during several minutes and then cooled, filtered and diluted to 50.0 ml with water. Aliquots of 100 μl of these solutions were diluted to 50.0 ml with NH_3/NH_4^+ buffer solution of pH 10.2 and the recommended procedure was applied.

For Pro-Crecil NT, 1.0 ml of accurately measured sample was diluted to 10.0 ml with water. Aliquots of 100 μl of this solution were diluted to 50.0 ml with NH_3/NH_4^+ buffer solution of pH 10.2 and the recommended procedure was applied.

For Gingi Kin and Ciscutil it was previously necessary to eliminate the organic matter. For this, 5.0 ml aliquots of accurately measured product were evaporated to near dryness and digested with 10 ml of conc. H_2SO_4 and 10 ml of conc. HNO_3, heating until white fumes were evolved (5–10 min). The solution was cooled and 10 ml of water were added and boiled gently until white fumes were evolved. Finally, the solution was cooled and diluted with water to 50 ml. Aliquots of 5.0 ml of this solution were diluted with 0.02 M NaOH solution to 50.0 ml. Aliquots of 1.0 ml of this solution were diluted with NH_3/NH_4^+ buffer solution of pH 10.2–10.0 ml and the recommended procedure was applied.

3. Results and discussion

The reaction between the Zn (II) present in the aqueous phase and the optode membrane and the subsequent regeneration of the membrane are shown in Scheme 1. At pH 10.2, it may be as-



Scheme 1.

sumed that a 1:2 Zn (II)–PAN complex is formed in the optode membrane, since this was the stoichiometry found in a non-aqueous solution for the Zn (II)–PAN complex [33]. At pH 3.9 the complex is broken, the Zn (II) is eluted to the flowing solution and the PAN remains in the membrane.

3.1. Study of variables

The influence of the coating solution composition, support type, the reflectance spectrum of the sensor, instrumental and chemical variables were studied in order to select the best experimental conditions for determining zinc (II). The design of the manifold selected is shown in Fig. 1.

To choose the best composition of the coating solution, the influence of PVC, NPOE and PAN concentrations was studied by the univariate method. In all cases, the membranes were prepared by applying the general procedure described in 2.3. NPOE/PVC weight ratios of 50/100, 75/75, 100/50 and 120/30 mg/mg and amounts of PAN between 1.5 and 12 mg were tested. The highest sensitivity, the best reproducibility and the lowest regeneration times were obtained with a 2/1 NPOE/PVC ratio (100/50 mg/mg) and 4.5 mg of PAN dissolved in 3 ml of THF. The volume of THF in the coating solution affects the concentration of the other components of the solution and, consequently, the thickness of the polymeric membrane after the THF has evaporated.

Different hydrophilic and hydrophobic supports were tested to prepare the most sensitive membrane possible. The best diffusion of the coating solution and wetting were obtained using Albet 235 filter paper, which was chosen as support [31,34].

A microscopy study was made and the microphotographs showed that the plasticized PVC membrane containing PAN (yellow) or Zn (II)–PAN complex (red) becomes entrapped in the cellulose fibres of the filter paper.

Diffuse reflectance spectra of immobilised PAN before (curve 1) and after (curve 2) complexation with 0.33 mg l^{-1} of Zn (II) are shown in Fig. 2. Spectra are given as relative reflectance, that is $(R_f/R_b) \times 100$ and $(R_c/R_b) \times 100$, where R_f and R_c are the diffuse reflectance of the free and complexed forms of the immobilised indicator, respectively, and R_b is that of the blank membrane (without PAN). Greatest reflectance difference was found at 562 nm, and this wavelength was used for all subsequent measurements of reflectance.

The FI variables studied were sample volume and flow rate. The reactor length must be as short as possible in order to minimise dispersion of the sample into the eluting carrier (50 cm). The analytical signal was presented as normalized reflectance radiation intensity $R_N = R/R_{max}$, where R is the respective response signal of the detector and R_{max} is the signal of the membrane before exposure to metal ion (base line).

The volume of sample injected was varied from 40 to 240 μl, while the other variables remained

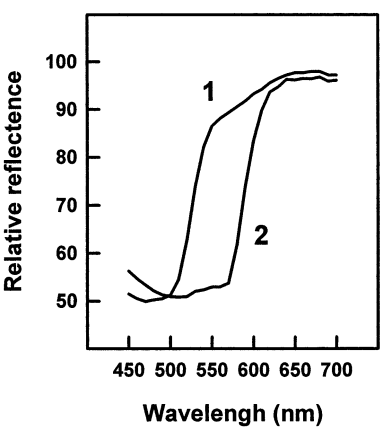


Fig. 2. Diffuse reflectance spectra of immobilised PAN before (curve 1) and after (curve 2) complexation with 0.33 mg l^{-1} Zn (II).

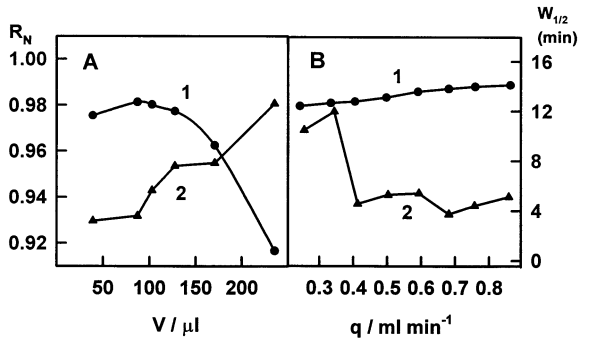


Fig. 3. Effect of sample volume (A) and flow rate (B) on the reflectance response (1) or on the peak width (2).

fixed (0.40 ml min^{-1} flow rate, 0.33 mg l^{-1} Zn (II) solution injected, pH 10.2). As can be seen in Fig. 3A(1) increasing sample volumes yielded a larger change in the reflectance signal R_N but also increased the peak width (Fig. 3A(2)), with a corresponding decrease in the sampling frequency. A sample volume of 90 µl was chosen for further experiments, since sensor recovery was very rapid with this volume.

The effect of flow rate on the sensor response was studied over the range 0.2–1.0 ml min^{-1} in the same experimental conditions described above. The results obtained are shown in Fig. 3B(1 and 2). As can be seen, the sensor response decreased when the flow rate increased while the peak width decreased considerably up to 0.4 ml min^{-1}. As a compromise a flow rate of 0.40 ml min^{-1} was selected.

The effect of the pH sample was studied in the range 8.3–10.5 in the same experimental conditions by using NH_4^+/NH_3 buffers. As can be seen in Fig. 4A(1), the response of the sensor increased when the pH increased. However, the time needed to regenerate the sensor also increased, Fig. 4A(2). A pH of 10.2 was selected for further studies as a compromise between sensitivity and sampling frequency.

3.2. Regeneration of the optode

An eluent that can also be used as carrier is best for this type of sensor since this simplifies the FI procedure. The best results were obtained using acetic/acetate buffer as carrier. To study the influence of the pH of the carrier solution on the regeneration process and on the sensor response to Zn (II), solutions of acetic/acetate buffer in the range of pH 3.5–5.6 were used. As can be seen in Fig. 4B(1), the response of the sensor increased when the pH increased. However higher pH produced longer regeneration times, and at values of pH up to 4.8, the sensor is not regenerated (the regeneration time being defined as the time taken for the sensor to reach the base line signal after the minimum reflectance has been reached). Acetic/acetate buffer of pH 3.9 was selected for further experiments, with short membrane regeneration times (2–3 min). It was observed that the optode was not damaged in this acid medium.

To study the lifetime of the membrane optode the analytical signal corresponding to 0.65 mg l^{-1} Zn (II) was recorded every 5 days over a period of 30 days. The variation coefficient obtained was $\pm 0.38\%$.

3.3. Features of the flow injection method

The reflectance response of the optode versus time in the selected experimental conditions for different Zn (II) concentrations in the range 0.16–3.27 mg l^{-1} is shown in Fig. 5. As the Zn (II) plug reached the optode the reflectance at 562 nm decreased rapidly and continued to fall as the Zn (II) zone passed through the cell, due to the

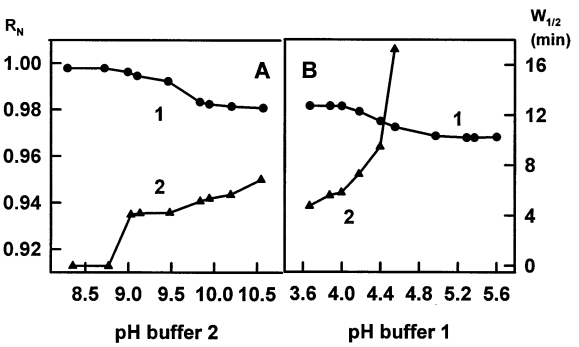


Fig. 4. Effect of pH buffer 1 solution (A) and pH buffer 2 solution (B) on the reflectance response (1) or on the peak width (2).

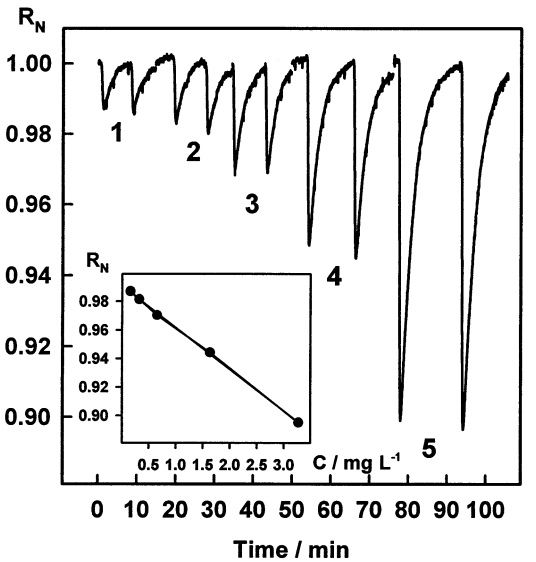


Fig. 5. Response–time curves to different Zn (II) concentrations: (1) 0.16; (2) 0.33; (3) 0.65; (4) 1.63; (5) 3.27 mg l^{-1} and the corresponding calibration graph.

formation of Zn (II)–PAN complex. Minimum reflectance was obtained at the very end of the sample zone, after which the acetic/acetate buffer contained in the carrier quickly eluted the Zn (II) from the optode, rendering the membrane ready for a new sample.

The corresponding calibration graph is shown in Fig. 5. The plot of R_N versus Zn (II) concentration is linear in the range 0.160–3.27 mg l^{-1}. The regression equation was $R_N = 0.991 \pm 8 \times 10^{-4} - 2.9 \times 10^{-2} \pm 5 \times 10^{-4}[Zn^{2+}]$ when the concentration of Zn (II) was expressed in mg l^{-1} with a correlation coefficient of 0.9994 ($n = 10$). The detection limit calculated according to Ref. [35] was 0.10 mg l^{-1} of Zn (II).

The repeatability was evaluated by performing ten determinations with the same standard solution of Zn (II). The variation coefficient of sensor response for 0.33 mg l^{-1} of Zn (II) was $\pm 0.11\%$. The reproducibility between days ($n = 5$) and between different membranes ($n = 6$) for 0.33 mg l^{-1} of Zn (II) (three determinations with each) were ± 0.19 and $\pm 0.22\%$, respectively.

The sample throughput depends on the Zn (II) concentration to be determined, since the time needed to return to the base line is concentration dependent. For a concentration of 0.33 mg l^{-1}, the sample throughput was 12 samples h^{-1}, which is within the normal range for optodes.

Potential interferences of metallic ions in the determination of Zn (II) using the proposed method were studied by adding different amounts of potentially interfering species to samples containing 0.33 mg l^{-1} Zn (II) in NH_3/NH_4^+ buffer of pH 10.2. When the assayed metallic ions precipitated in this medium, a previous filtration step was necessary. The maximum foreign ion: Zn (II) ratio assayed was 200:1. Table 1 lists the ratios of the species assayed to be tolerated. The tolerance limit was taken as the concentration causing an error of no more than $\pm 3\%$ in the determination of Zn (II). As can be seen, several metal ions do not interfere even at high concentrations. In addition to Zn (II), the sensor also produces a response to some heavy metal ions that do not precipitate at pH 10.2 (NH_3/NH_4^+) and forms complexes with PAN at this pH value. Such ions must be previously separated from Zn (II) prior to determination.

3.4. Analytical applications

The proposed sensor was successfully applied to the determination of Zn (II) in different pharmaceuticals. The results are summarized in Table 2. When different pharmaceuticals containing zinc (II) were analyzed by the proposed FI method, interference from the sample matrix posed no problems, except when the ingredients of the pharmaceuticals assayed were lactate or EDTA, which need to be eliminated, as was described in experimental.

Table 1
Interferences in the determination of 10^{-5} M Zn (II)

Species assayed	Limiting molar ratio [Species]/[Zn^{2+}]
K^+, Na^+	200[a]
Mg^{2+}, Ca^{2+}, Al^{3+}, Ag^+	50
Hg^{2+}, Pb^{2+}	10
Fe^{3+}, Cr^{3+}, Co^{2+}	0.1
Ni^{2+}, Mn^{2+}, Cd^{2+}, Cu^{2+}	0.05

[a] Maximum relation assayed.

Table 2
Determination of zinc (II) in different pharmaceuticals

Sample	Found[a]	
	Proposed method	Reference method
Anticongestiva Cusi	[b](25.16 ± 0.18)	[b](25.34 ± 0.30)
Fungusol	[b](11.03 ± 0.13)	[b](10.71 ± 0.10)
Kalamina	[c](6.16 ± 0.11)	[c](6.20 ± 0.16)
Pro-Crecil NT	[d](0.99 ± 0.02)	[d](0.98 ± 0.05)
Gingi Kin	[e](0.357 ± 0.050)	[e](0.370 ± 0.043)
Ciscutil	[f](0.468 ± 0.002)	[f](0.456 ± 0.003)

[a] Mean ± S.D. ($n = 5$).
[b] Gram of zinc oxide in 100 g of product.
[c] Gram of zinc oxide in 100 ml of product.
[d] Gram of zinc acetate in 100 ml of product.
[e] Gram zinc lactate in 100 ml of product.
[f] Gram zinc sulphate in 100 ml of product.

All the formulations were also analyzed by applying the reference method [23], the results obtained are shown in Table 2. The results obtained by the proposed and reference method were compared by applying the F-test and the paired t-test, at the 95% confidence interval. The calculated values for F (2.77 for Anticongestiva Cusi, 0.10 for Fungusol, 2.11 for Kalamina, 7.32 for Pro-Crecil, 2.63 for Ciscutil and 0.80 for Gingi Kin) and for $t = 0.27$ did not exceed the critical values of $F_{4,4} = 9.60$ and $t = 2.57$ ($n = 6$), indicating that there are no significant differences between the proposed and reference methods as regards precision and accuracy.

4. Conclusions

The optode membrane applied is easily prepared and incorporated in a FI system using a designed flow-through cell. The flow-through optode membrane described provides a simple and rapid method for the determination of zinc (II). The sensor can be regenerated readily with the same carrier solution of acetate/acetic acid buffer. It is reversible and has a long lifetime. The response of the optode was reproducible and the sensor presented a good selectivity. The method was applied to the determination of zinc (II) in pharmaceuticals.

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