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Spectrophotometric flow injection system for determination of Zn^{2+} in ophthalmic formulations using Alizarin red S

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Abstract: A spectrophotometric flow injection method for the determination of Zn(II) in ophthalmic formulations was developed. In this work, Zn(II) ion was complexed with Alizarin red *S* in borate buffer solution (pH 9.0) and the chromophore produced was monitored at 520 nm. The analytical curve was linear in the Zn(II) concentration range from 6.05 x 10^{-6} to 1.50 x 10^{-4} mol L^{-1} with a detection limit of 3.60 x 10^{-6} mol L^{-1}. Recoveries ranged from 96.3 to 105 % and a relative standard deviation of 1.2 % (n = 10) for 5.5x10^{-5} mol L^{-1} Zn(II) reference solution were obtained. The sampling rate was 60 h^{-1} and the results obtained of Zn(II) in ophthalmic products using this procedure are in close agreement with those obtained using a comparative spectrophotometric procedure at 95 % confidence level.

Keywords: Flow injection analysis; Zinc(II); Alizarin red *S*; spectrophotometry; ophthalmic formulations.

Introduction

Zinc compounds have bactericidal activity since they can precipitate and denature several bacterial proteins. For this reason, they have been employed as antiseptic and disinfectant agents in pharmaceutical products such as creams, ointments and eye drops [1,2]. The antibacterial activity is enhanced in the mixture with quaternary ammonium compounds. Thus, the determination of Zn(II) in pharmaceutical preparations is an important analytical task.

The USP XXIII Pharmacopoeia recommends the determination of Zn(II) by atomic absorption spectrometry (AAS) or by an extractive spectrophotometric method [3].

Several spectrophotometric procedures reported in the literature for the determination of Zn(II) are summarized by Bhalotra *et al.*[4] and a previous solvent extraction step is required in other spectrophotometric methods [5-9]. Electroanalytical methods such as potentiometry [10-12], potenciometric stripping analysis [13,14] and anodic redissolution stripping voltammetry also were described [15].

Several flow injection methods have been proposed employing different detections such as optosensor [16-19], anodic stripping voltammetry [20], diode array [21] and electrothermal atomic absorption spectrometry [22]. A multicommutation flow procedure [23] and a sequential injection analysis system [20] were described for the determination of Zn(II) in pharmaceutical formulations

The development of automated flow procedures are of the great interest to quality control laboratories due the high number of analysis and possibility of data acquisition.

In this work we present a simple flow injection method for determining Zn(II) in pharmaceutical solutions based on the formation of a water

soluble complex between Alizarin red S (ALZ) and Zn^{2+} [24].

Experimental

Apparatus

A model 8452A Hewlett-Packard (Boise, ID, USA) UV-visible spectrophotometer was used in a comparative spectrophotometric method for Zn^{2+} in the ophthalmic formulations samples [8].

A peristaltic pump supplied with Tygon® pump tubing was used for the propulsion of the solutions. The manifold was constructed with polyethylene tubing (0.8 mm i.d.). The solutions were injected using an injector-commutator 2-3-2 made of Perspex® [25]. The detection was performed using a spectrophotometer (Femto, Model 435, São Paulo) equipped with a glass flow-cell (optical path, 10 mm). The absorbance was recorded using a two-channel strip-charter recorder Model 1202-0000 Cole Parmer (Chicago, IL, USA) connected to spectrophotometer.

Reagents and solutions

All reagents used were of analytical grade and water from a Millipore (USA) Milli-Q system was used throughout. Zinc sulphate heptahydrate and Borax were obtained from Mallinckrodt. Red Alizarin red S was purchase from Vetec® (São Paulo, Brazil).

The $1.60x10^{-2}$ mol L^{-1} borate buffer solution (pH 9.0) was prepared by dissolving 1.55 g of $Na_2B_4O_7 \cdot 10H_2O$ with desionized water and completing the volume in a 250 mL calibrated flask.

The 0.100 mol L^{-1} Zn^{2+} stock solution was prepared by dissolving 2.874 g $Zn(SO_4).7H_2O$ with desionized water in 100 mL calibrated flask. This solution was standardized volumetrically as described elsewhere [26]. Working solutions containing concentration range of Zn^{2+} between $6.05x10^{-6}$ to $1.50x10^{-4}$ mol L^{-1} were prepared by the appropriated dilution of the stock solution with desionized water and the volume was completed in 25 mL calibrated flask.

The $1.00x10^{-2}$ mol L^{-1} Alizarin Red S standard solution was prepared by dissolving 900 mg of monosodium salt (C.I. 58005) with $1.6x10^{-2}$ mol L^{-1} borate buffer solution (pH 9.0) in a 250.0 mL calibrated flask and the volume was completed with the same buffer solution. The $3.00x10^{-4}$ mol L^{-1} Alizarin Red S working solution was prepared by diluting 3.0 mL in the 100.0 mL calibrated flask and the volume was completed with the same $1.6x10^{-2}$ mol L^{-1} buffer solution (pH 9.0).

Flow injection system

A schematic diagram of the flow manifold in injection position is shown in Figure 1. The carrier stream (H_2O) merges downstream with the Alizarin red S solution where both are pumped at 1.2 mL min^{-1} generating a stable baseline. When a 250 µL (50 cm) sample volume containing Zn^{2+} reference or sample solution was injected, occur the formation of the soluble complex between Alizarin red *S* and Zn^{2+} in the reactor coil (150 cm), which was monitored spectrophotometrically at 520 nm. The analytical signal (absorbance) was proportional to Zn(II) concentration in the injected solution.

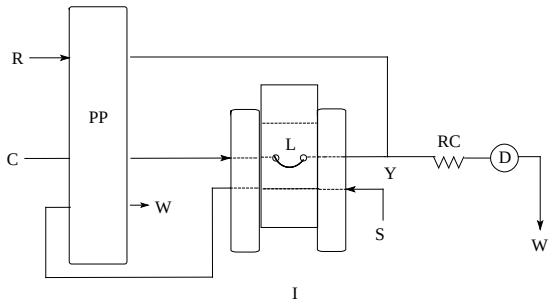

Figure 1. Flow injection manifold for the determination of Zn^{2+} in pharmaceutical solutions. PP represents peristaltic pump; I: injector-commutator, D: spectrophotometer (520 nm); C: Carrier (H_2O: 1.2 mL min^{-1}); R: Alizarin Red *S* solution ($3.0x10^{-4}$ mol L^{-1}: 1.2 mL min^{-1}); L: sample loop (250 µL); Y: confluence point; RC: reactor coil length; S: samples or references solutions. All experiments were done under ambient temperature. The length between the injector-commutator and confluence Y was 5 cm. The arrow indicates the stream direction.

Preparation of pharmaceutical samples

Three Brazilian pharmaceutical formulations containing Zn(II) such as Moura Brasil® (Aventis Pharma LTDA.), Lerin® (Allergan Produtos Farmacêuticos LTDA) and Zincolok®(Allergan Produtos Farmacêuticos Ltda) were analyzed using a proposed flow injection procedure. Eye drops formulations were appropriately diluted with desionized water to obtain a concentration of *ca.* 5.0 x 10^{-5} mol L^{-1} of Zn(II) in the diluted solution of samples. The results obtained by the proposed flow injection method were compared with the results obtained by a spectrophotometric batch procedure described by Manouri *et al.* [8].

Results and discussion

The proposed flow injection procedure for the determination of Zn^{2+} was based on the formation of complex with Alizarin red S in borate buffer (pH 9.0).

The optimization of chemical and flow injection parameters were performed using a univariate method in order to achieve a best compromise between the peak height, sample throughput, reproducibility and baseline stability.

Chemical parameters

To establish the best conditions for the Zn(II) determination, some chemical parameters such as carrier solution and Alizarin Red S concentration were investigated. The optimized values studied and selected were showed in the Table 1.

Initially, the effect of the carrier solution on the analytical signal was studied using 0.01 mol L^{-1} buffer solutions (acetate and borate buffers) in the pH range from 7.1 to 9.8. The buffer solution that promoted the highest analytical signals was 0.01 mol L^{-1} borate buffer at pH 9.0. Thus, the 1.6x10^{-2} mol L^{-1} borate buffer at pH 9.0 was selected for further experiments.

The effect of Alizarin red S solution on the analytical signal was studied between 7.5x10^{-5} and 4.7x10^{-4} mol L^{-1} using a 1.6x10^{-2} mol L^{-1} borate buffer (pH 9.0). The analytical signal increased with the increases of Alizarin red *S* concentration up to 4.7x10^{-4} mol L^{-1}. The highest concentrations of reagent promotes intense oscillation of baseline with severe increase of the washing time. The increase of washing time to highest concentrations was due the impregnation of Zn^{2+}-Alizarin complex in the flow cell. Thus, considering the best compromise between height peaks and repeatability, the 3.0x10^{-4} mol L^{-1} Alizarin Red *S* solution was selected for further experiments.

Flow injection parameters

To determine the optimum flow procedure parameters, the sample volume, carrier and reagent flow rate and reactor coil length were investigated. The parameters range studied and selected are shown in Table 1. The effect of sample volume from 100 to 350 μL (50 to 70 cm) on the analytical signal was evaluated by injection of 6.0 x 10^{-4} mol L^{-1} Zn^{2+} solution in 0.01 mol L^{-1} borate buffer (pH 9.0). The analytical signal increased with the increasing of sample volumes up to 250 μL above which it remained constant. Thus, the volume of 250 μL was selected as optimum.

Table 1. Optimized flow injections and chemical parameters.

Parameter	Studied range	Selected value
Sample loop length /μL	100 to 350	250
ALZ concentration / mol L^{-1}	7.5 x 10^{-5} to 4.7 x 10^{-4}	3.0 x 10^{-4}
ALZ flow rate / mL min^{-1}	0.7 to 1.7	1.2
Carrier flow rate / mL min^{-1}	0.7 to 1.7	1.2
pH buffer	7.1 to 9.8	9.0
Reactor coil length / cm	50 to 230	150

ALZ = Alizarin Red S.

The effect of the Alizarin red *S* flow rate and the carrier flow rate on the analytical signal were studied from 0.7 to 1.7 mL min^{-1} each one. The flow rates of 1.2 mL min^{-1} were selected to each channels because promotes the higher analytical signals.

The influence of tubular coiled reactor length on the absorbance was also evaluated in the range from 50 to 230 cm using a $4.0x10^{-5}$ mol L^{-1} Zn^{2+} reference solution. The analytical signal increased gradually with increase of length up to 230 cm. To the highest reactor lengths, the baseline was achieved slowly due the impregnation of complex on the glass flow cell. This impregnation was not observed up to 150 cm reactor length. Thus, the 150 cm reactor coil length was chosen, taking account height of the analytical signal and analytical frequency.

Recoveries and interferences studies

The recovery study was examined by adding Zn^{2+} reference solution at three levels (1.33, 2.66 and 4.00 mg L^{-1}) to the samples solution containing *ca.* $2.0x10^{-5}$ mol L^{-1} Zn^{2+} concentration. The results of Zn^{2+} determination obtained of theses solutions were compared with the results of reference solutions without addition of the sample solutions. Recoveries from 96.3 to 105 % of Zn^{2+} from three pharmaceutical formulations were obtained using the optimized flow procedure. The results are shown in the Table 2 and suggests no significant matrix effect in the samples studied.

The interference of some compounds commonly founded in eye drop formulations was studied with the proposed flow procedure. Chloride sodium, benzalkonium chloride, citric acid, $CuSO_4$ and EDTA were tested. In this study, aliquots of solutions containing the interferences at three concentration levels were added to $5.0x10^{-5}$ mol L^{-1} Zn^{2+} reference solution. The results obtained in Zn^{2+} determination in theses solutions were compared with those obtained of the reference solutions without interference added. The tolerated concentration was that promote the signal variation of ±5 %. In these group of substances studied, only the benzalkonium chloride causes severe interference on the response of proposed procedure at same concentration that Zn(II) standard solution. The cationic surfactant, such as benzalkonium chloride promotes a hyperchromic shift of the complex spectrum [27]. This benzalkonium chloride concentration was not founded in the analyzed commercial formulations. To others substances, no interference was observed in the response of flow system procedure in the presence of 10-fold excess of these studied substances. Cu^{2+} causes severe interference due the formation of stable complex with Alizarin red S with considerable overlapping of the spectra of their of Zn(II)-Alizarin complex [24]. The use of thiosulphate and thiourea as the masking reagent for Cu^{2+} do not eliminated the Cu^{2+} interference in the Zn^{2+} determination, because the Cu(II)-Alizarin complex is much more stable.

Table 2. Study of the recovery experiments

Samples	Zn^{2+} (mg L^{-1})		Recovery (%)
	Added	Found[a]	
A	1.33	1.38 ± 0.04	104
	2.66	2.81 ± 0.03	105
	4.00	4.18 ± 0.02	104
B	1.33	1.31 ± 0.04	98.5
	2.66	2.64 ± 0.02	99.2
	4.00	3.97 ± 0.06	99.2
C	1.33	1.34 ± 0.03	101
	2.66	2.68 ± 0.02	101
	4.00	3.98 ± 0.05	99.2

[a] n=3, mean ± standard deviation.

Analytical curve and applications

In the optimized conditions, the analytical curve was linear in the concentration range from $6.0x10^{-6}$ to $1.5x10^{-4}$ mol L^{-1} (Abs = 0.013 + 4 508.2 x *C*, r = 0,999; where Abs is the absorbance and *C* is the Zn^{2+} concentration in mol L^{-1}). The relative standard deviation (R.S.D.) for a solution containing $5.5x10^{-5}$ mol L^{-1} Zn^{2+} of 1.2% (n = 10) and the detection limit of $3.60x10^{-6}$ mol L^{-1} were obtained (three times blank standard deviation/ slope of analytical curve). The sampling rate of 60 h^{-1} was attained. The relative standard deviation of 3.5% (n=6) for the slope of analytical curve to Zn^{2+} determination was obtained at different working days.

The proposed flow procedure was applied to determine Zn^{2+} in eye drops under conditions fixed and shown in the Table 1. The results obtained in the determination of Zn^{2+} employing the proposed procedure are presented in Table 3. The results obtained using the proposed flow procedure are in good agreement with the results obtained by batch procedure [8] at 95% confidence level (*t*-paired test) [28].

Conclusions

The flow injection procedure proposed was applied to the determination of Zn^{2+} contained in eye drop solutions. The automatized procedure is simple, precise and has sensitive enough for the analysis of a variety of ophthalmic solutions with high sample throughput. Thus, the developed flow injection procedure is very suitable for routine analytical applications.

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Sistema de análise por injeção em fluxo com detecção espectrofotométrica para a determinação de Zn^{2+} em colírios empregando vermelho de Alizarina S como reagente.

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Table 3. Determination of Zn^{2+} in commercial formulations by using flow procedure and comparative batch procedure [29]

Samples	Zn^{2+} (mg mL^{-1})		Relative error (%)
	Comparative	Proposed flow procedure	
A	0.069 ± 0.003	0.071 ± 0.001	2,9
B	0.602 ± 0.003	0.585 ± 0.002	-2.8
C	0.126 ± 0.002	0.129 ± 0.001	2.4

n=3, mean ± standard deviation; Re_1, relative error between proposed flow procedure *vs.* comparative method. Sample composition: zinc sulphate or zinc phenolsulphonate, sodium borate, acid boric, potassium chloride, citric acid, disodium edetate dehydrated, Thimerosa

Resumo: Um método de análise por injeção em fluxo para determinação de Zn^{2+} em colírios foi descrito. Neste trabalho, o íon Zn^{2+} foi complexado com vermelho de Alizarina S em tampão borato (pH 9,0) e o cromóforo formado foi monitorado espectrofotometricamente em 520 nm. A curva analítica foi linear no intervalo de concentração de Zn^{2+} de 6,05 x 10^{-6} a 1,50 x 10^{-4} mol L^{-1}, com um limite de detecção de 3,60 x 10^{-6} mol L^{-1}. As recuperações variaram de 96,3 a 105% e o desvio padrão relativo foi de 1,2 % (n = 10) para uma solução padrão de Zn^{2+} de 5,5x10^{-5} mol L^{-1}. A freqüência analítica de 60 h^{-1} foi obtida. Os resultados obtidos para determinação de Zn^{2+} em colírios empregando-se o procedimento proposto estão em concordância com os resultados obtidos empregando-se um procedimento comparativo a um nível de confiança de 95%.

Palavras-chave: Análise por injeção em fluxo; zinco; vermelho de Alizarina S; espectrofotometria; colírio.

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