Flow-injection solid-phase spectrophotometry for the determination of zinc in pharmaceutical preparations

Leonardo S.G. Teixeira, Fábio R.P. Rocha, Mauro Korn, Boaventura F. Reis, Sérgio L.C. Ferreira, Antonio C.S. Costa

* Instituto de Química, Universidade Federal da Bahia, b Departamento de Química, Universidade Federal de São Carlos, c Departamento de Ciências Exatas e da Terra, Universidade do Estado da Bahia, d Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, P.O. Box 96, 13400-970 Piracicaba SP, Brazil

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Abstract

A flow system exploiting solid-phase spectrophotometry is proposed for the determination of zinc in pharmaceutical preparations. The chromogenic reagent 1-(2-tiazolylazo)-2-naphthol (TAN) was immobilized on C18 bonded silica loaded into a homemade flow cell with 1 mm optical path and 78 mm² cross section. The flow cell was designed in such a way that the sensitivity is enhanced and the attenuation of the radiation beam due to scattering and absorption by the solid material is minimized. The flow cell was placed in the spectrophotometer so that the radiation beam was focused on the overall surface containing the adsorbing material. Reagent immobilization was performed on-line and allowed to work for at least one month. Analyte reaction, retention and detection were performed simultaneously, followed by elution with hydrochloric acid. The apparent molar absorptivity was estimated as 2.02 \times 10^5 \text{l mol}^{-1} \text{cm}^{-1} and the procedure allowed the determination of zinc in the 0.04–4.0 mg \text{l}^{-1} range with a coefficient of variation of 3.3% (n=10). A sample throughput of 45 determinations per hour and a detection limit of 10 \text{mg} \text{l}^{-1} (99.7% confidence level) were achieved. The results agreed at a 95% confidence level with those found by inductively coupled plasma atomic emission spectrometry. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Zinc is a very important metal to human health. More than 20 metalloenzymes containing this element in low concentrations have been identified. Furthermore, zinc compounds have bactericidal activity since they can precipitate and denature several bacterial proteins. For this reason, they have been employed in dermatology, mouthwashes and ophthalmic solutions as antiseptic and disinfectant agents. In addition, zinc is usually present in mineral vitamin preparations [1]. Thus, determination of zinc in pharmaceutical preparations is an important analytical task.

Flow-injection analysis (FIA) was originally designed for solution management, allowing fast and reproductive measurements in a homogeneous phase [2]. Nowadays, heterogeneous systems are quite common and an attractive approach has been the development of flow-through detectors that allow...
the integration of reaction, retention and detection steps \[2,3\]. The association of these detectors with flow systems has allowed solid-phase measurements by absorption \[4–6\], reflectance \[7\], fluorescence \[8\], photoacoustic spectroscopy \[9\] and bio- \[10\] or chemiluminescence \[11\].

The use of solid-phase spectrophotometry (SPS) has increased due to its simplicity, easy automation and low detection limits achieved \[12\]. Several advantages over batch methods are possible by coupling SPS with FIA, e.g. it is unnecessary to attain equilibrium conditions or to isolate the solid support; smaller amounts of sample and reagent are required; and measurements can be carried out at a single wavelength and with a single beam spectrophotometer \[6\]. On the other hand, the flow injection combined with solid-phase spectrophotometry (FI-SPS) permits the achievement of better performance than the flow systems that involve preconcentration and measurement of the analyte in the eluent or direct measurements in solution. Systems involving preconcentration/elution are usually designed to attain high enrichment factors \[12\]. Nevertheless, an inherent dilution is observed during the elution step, and can cause an undesirable reduction of sensitivity. This deleterious effect is more critical in systems involving conversion of the analyte after elution, due to the dilution by the reagent solutions added. The main advantage over the usual flow systems for direct measurements in solution is the increase in sensitivity \[4,5\], though selectivity enhancement and improvement in the reaction rate can also be observed \[13\]. By measurements on the solid phase, drawbacks caused by refractive index gradients (Schlieren effect) are notably reduced in comparison with measurements in solution \[12–14\].

The association of flow injection with solid-phase spectrophotometry can be carried out by placing the solid support in commercially available \[4–6,15\] or customized \[13,16\] flow cells. The approaches explored to implement FI-SPS consist in the retention of the analyte itself \[4,5\], the product of a previous chemical reaction \[6,14,16\], or in the reaction of the analyte with an immobilized chromogenic reagent, followed by the retention of the product \[15,17\].

Procedures involving FI-SPS require the periodical removal of the retained analyte from the flow cell, usually by employing an acid or a complexing solution. In this sense, a set of samples can be sequentially introduced into the carrier stream, yielding a recording with successive plateaus, followed by the periodical introduction of the eluent solution to remove the analyte \[4,18\]. However, this approach can affect the dynamic range, depending on the analyte concentration and the adsorbent amount, and can also cause memory effects \[18\]. On the other hand, some procedures employed single line manifolds, exploring the transient retention of the measured species \[5,16,17\]. The composition of the carrier stream is carefully chosen, allowing the retention of the analyte for a short time interval, just long enough to concentrate and measure the analyte, followed by elution by the carrier. Nevertheless, the most usual systems consist of the introduction of the eluent solution after the sample plateau is achieved \[6,14\]. This approach is generally more effective in removing the retained species, thereby permitting extension of the dynamic range.

In the present work, the 1-(2-tiazolylazo)-2-naphthol (TAN) was immobilized on a C\textsubscript{18} bonded silica support for the determination of zinc in pharmaceutical preparations by FI-SPS. Previous studies \[19,20\] showed that zinc(II) reacts instantaneously with TAN, in the 6.5–8.0 pH range to form a red complex. TAN can be easily immobilized on C\textsubscript{18} silica sorbent and this approach was previously adopted for on-column zinc separation/preconcentration for measurements by inductively coupled plasma atomic emission spectrometry (ICP-AES) \[20\].

2. Experimental

2.1. Reagents and solutions

All the solutions were prepared with analytical grade reagents and freshly distilled and deionized water. Zinc reference solutions (0.04–4.00 mg l\textsuperscript{-1}) were prepared by dilution of a 1000 mg l\textsuperscript{-1} stock solution. The buffer solution was 0.5 mol l\textsuperscript{-1} hexamine with pH adjusted to 6.2 with hydrochloric acid. TAN solution was prepared by dissolving 1.0 mg of 1-(2-thiazolylazo)-2-naphthol in 1 ml of ethanol and completing the volume to 100 ml with a 5% (m/v) Triton X-100 solution. Hydrochloric acid solution (0.5 mol l\textsuperscript{-1}) was prepared by appropriate dilution.
of a concentrated acid solution. TAN reagent was immobilized on-line in C_{18} bonded silica (60–100 μm) obtained from Sep-Pak cartridges (Waters) and used for zinc retention in the flow cell. Water was used as the carrier stream.

Buffered solutions (pH 6.2) were prepared containing 1 mg L^{-1} Zn^{2+} and the same amount of several foreign ions: Cr(VI), Mo(VI), V(V), Sn(IV), Sb(IV), W(IV), Ti(IV), V(IV), U(IV), Al(III), Fe(III), Cr(III), Ba(II), Be(II), Ca(II), Cd(II), Co(II), Cu(II), Fe(II), Hg(II), Mg(II), Mn(II), Ni(II), Pb(II). Solutions containing 1 mg L^{-1} Zn^{2+} and 20 mg L^{-1} Ni^{2+} or 400 mg L^{-1} NO_3^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, Cl^-, or I^- were also prepared. For masking of copper, solutions containing 5% (m/v) sodium thiosulfate and 5% (m/v) ascorbic acid were prepared.

2.2. Sample preparation

The pharmaceutical preparations analyzed and the labeled contents (amounts present in 1 g sample) were: Hipoglos pomade sample (Biolab Ind. Farm. S/A) containing 20 mg boric acid and 150 mg zinc peroxide; Ana septil sample (Sanoli Winthrop Farmacêutica Ltda) containing 500 I.U. zinc bacitracin, 0.2 g zinc peroxide, and 1.0 g zinc oxide; Ger imix sample (FDC) containing 33 mg Fe, 10 mg Zn, 1.3 mg Cu, 100 μg I, 67 mg Mg, 108 mg Ca, 83 mg P, 1.7 mg Mn, 25 mg K, 23 mg Cl, 10 μg Se, 10 μg Mo, 10 μg Cr, 3.3 μg Ni, 53 μg Si, 6.7 μg Sn and 6.7 μg V. MagnésioZ sample (Bionatura Com. Rep. Imp. Exp. Ltda) containing 700 mg Mg (aspartate, orotate and citrate), 7.1 mg zinc glycinate; Stresstabs sample (Prodome Química e Farmacêutica Ltda) containing 460 mg ascorbic acid, 2.3 mg copper(II) oxide (79.88% Cu), 19 mg calcium pantotenate, 18.4 mg zinc; Centrum sample (Lederle) containing 136 mg Ca, 2.7 mg Fe, 32.6 mg P, 102 μg I, 68 mg Mg, 10.2 mg Zn, 1.4 mg Cu, 54.4 mg K, 13.6 μg Se, 2.4 mg Mn, 88.4 μg Cr, 108.8 μg Mo, 49 mg Cl, 3.4 μg Ni, 1.4 mg Si, 6.8 μg V, 102 μg B.

A mass of 0.500 g of each vitamin formulation, pomade and powdered preparation was ashed at 430°C for 12 h and then dissolved with 5 ml of concentrated hydrochloric acid. The dissolved sample was diluted to 500 ml with water and then an appropriate aliquot was added to a 25-ml volumetric flask containing 10 ml of the hexamine buffer, 1 ml of ascorbic acid and 1 ml of thiosulfate solutions. Zinc reference solutions were processed in the same way as the samples.

2.3. Apparatus

The set up consisted of a Cary 1E spectrophotometer (Varian, Australia) with a homemade flow cell (1.0 mm optical path), and a peristaltic pump Reglo (Ismatec, Switzerland) equipped with Tygon tubes. The flow manifold Fig. 1(a) was assembled with a sliding bar commutator, employing polyethylene tubes with 0.8 mm internal diameter.

The flow cell for FI-SPS Fig. 1(b) consisted of two perspex blocks separated by a rubber strip of 1.0-mm thickness connected with screws to avoid fluid leakage. A circular hole, 10.0 mm in diameter, was made in the rubber strip to support ca. 35 mg of C_{18} beads.

2.4. Flow diagram and procedure

The chromogenic reagent was immobilized on the solid support by pumping the TAN solution through the cell at 1.5 ml min^{-1} during 2 min. Afterward, the flow cell was washed with deionized water, to remove the excess of TAN. It was found that 0.026 mmol TAN (g C_{18})^{-1} was retained on the solid support. The flow cell was inserted in the optical path of the spectrophotometer as indicated in Fig. 2. This design was employed in order to focus the optical beam on the whole surface containing the adsorbing material.

The flow diagram is shown in Fig. 1(a). The loops L_1 and L_2 are filled with HCl and sample solutions, respectively, flowing at 1.5 ml min^{-1}. The water carrier stream flows through the analytical path also at 1.5 ml min^{-1}. By sliding the commutator central bar, sample and HCl aliquots are simultaneously inserted into the analytical path, and transported by the carrier toward the flow cell. Zinc ions present in the sample react with the chromogenic reagent immobilized on the C_{18} beads and the complex is retained on the solid support. Simultaneously, spectrophotometric measurements are carried out at 590 nm. After reaction and detection, the HCl solution reaches the flow cell performing the elution of the zinc ions without removing the TAN chromogenic reagent. The coil B provides a proper time delay to avoid excessive carryover between sample and eluent zones.
3. Results and discussion

3.1. Characteristics of the flow-cell

The use of solid-phase spectrophotometry in flow systems requires an appropriate means of supporting the solid sorbent. Despite the predominant use of commercial flow cells, there are some difficulties due to the optical path length of usual flow cells and the amount of the sorbent loaded. Large amounts of solid material in the optical path can cause an excessive reduction in the intensity of radiation beam on account of absorption and scattering. On the other hand, reduced amounts of the adsorbent can affect the sensitivity and the linear response range. Considering these factors, a flow cell was designed with a relatively large cross section (78 mm²) and with a 1-mm optical path Fig. 1(b), allowing to support a relatively large amount of solid material without excessive attenuation of the radiation beam. In addition, because of the distribution of the adsorbing material over a large area, the back-pressure caused by the carrier stream was reduced. Similar flow cells can be cheaply and easily constructed making feasible the widespread use of FI-SPS.

In view of the large cross section of the flow cell, an inhomogeneous distribution of the adsorbed analyte could take place due to the occurrence of preferential
flow pathways. This drawback was overcome by placing the flow cell near the detector, as shown in Fig. 2, in order to illuminate the overall area containing the adsorbent. Fig. 3 shows the variation in the illuminated area (A) and in the coefficient of variation of the measurements (CV) by changing the position of the flow cell. When the flow cell was placed at the radiation beam focus (Fig. 2, F), the precision of the measurements diminished (ca. 16% CV). However, the coefficient of variation was improved to ca. 3%, by placing the flow cell near the detector, where the light beam is more dispersed than in the radiation focus.

3.2. Chemical variables

The TAN reagent immobilized on C18 support was very stable in neutral or acidic solutions, allowing the use of the material for at least one month while maintaining good performance. The absorption spectra of zinc-TAN complex on the C18 material and in aqueous solution showed a displacement of the absorption maximum from 582 nm in solution to 590 nm due to the adsorption of the chromogenic reagent on C18 support.

The retention of Zn(II) on C18-TAN was studied at various pH values (4.5–8.5) and no significant variation in the retention efficiency occurred in the 5.8–6.7 pH range. Thus, in further experiments, sample solutions were previously buffered at pH 6.2 with the hexamine buffer solution.

Different mineral acids were evaluated for zinc elution from the sorbent material. Hydrochloric acid was selected because it allowed proper zinc elution from C18-TAN without removing the immobilized chromogenic reagent. The effects caused by the concentration and the volume of the eluent were evaluated and it was observed that 400 µl of 0.5 mol l⁻¹ HCl was sufficient for complete zinc elution. Thus, the reversible adsorption of the analyte was assured and no significant change on zinc retention was observed even after 200 measurements.

3.3. Hydrodynamic variables

The FIA system was designed to provide the removal of the adsorbed analyte after each sample measurement to avoid saturation of the adsorbent sites. This was carried out by introducing the sample and the eluent aliquots simultaneously in an inert carrier stream (see Fig. 1(a)). The length of the coil B was varied in order to provide a suitable delay time to avoid excessive carryover between sample and eluent zones. It was found that an 80-cm coil could meet this requirement.

As the total flow rate can affect the retention efficiency and sampling rate in FI-SPS, the effect of this parameter was studied by varying the flow rate from 0.7 to 1.9 ml min⁻¹. The signal was practically unaffected by the flow rate in this range (signal variation <6%), indicating that the process of reaction/retention was fast. Flow rates >1.9 ml min⁻¹ caused fluid leakage in the joints due to the increase in back-pressure. Therefore, a flow-rate value of 1.5 ml min⁻¹ was selected as a compromise between sample throughput and system stability.

An important advantage of solid-phase spectrophotometry is the possibility of improving sensitivity by increasing the sample volume. Nevertheless, an excessive increase in the sample volume could impair the sample throughput. A linear relation (r=0.997) between the sensitivity (S, in 1 mg⁻¹) and the sample volume (V, in µl) was observed in the 175–750 µl range: S=0.00059V–0.05193. This advantage is not attained in usual flow systems, in which the increase in signal with the sample volume is linear only in a short
Another profitable feature evidenced by this study is the possibility to extend the dynamic range of the procedure by selecting different sample volumes depending on the analyte concentration. On the other hand, by increasing the sample volume, an inherent decrease in the sampling rate was also observed. Thus, a 625-µl sample volume was chosen as a compromise between sensitivity and sample throughput.

3.4. Effect of foreign ions

The effect of several species on the zinc signal was evaluated (see item 2.1). The effect of each species was considered as an interference, when the signal in the presence of the species was >5% of that obtained in the absence of the foreign ion. When present in concentrations equal that of zinc (1 mg l⁻¹), interference was observed only for Cu(II) and Co(II). Ni(II) does not interfere even when present in 20-fold excess. Zinc signal was not altered in the presence of 400 mg l⁻¹ of NO₃⁻, SO₄²⁻, CO₃²⁻, PO₄³⁻, Cl⁻, or I⁻.

For batch measurements in solution, TAN reacts with several metals and it is not very selective for zinc [21]. In the present work, the selectivity was notably improved by the combined effect of pH adjustment and the different reaction/retention rates of the analyte and the interfering species on the solid support.

Cu(II) was the main interference for the analysis of Zn in pharmaceutical preparations by the proposed procedure. However, this deleterious effect was suitably avoided by prior addition of ascorbic acid and sodium thiosulfate to the samples.

3.5. Features of the method

In the proposed procedure, Beer’s law is obeyed from 0.04 to 4.00 mg l⁻¹ Zn²⁺, according to the equation: A = 0.016 + 0.310 C (r = 0.9994). The apparent molar absorptivity was estimated as 2.02 × 10⁵ l mol⁻¹ cm⁻¹ which is ca. fivefold higher than that obtained in the analogous batch procedure [19], in which higher sample volumes (ca. 10 ml) and a cell with a larger optical path (10 mm) were employed. A valuable reduction in the reagent consumption from 1 mg per determination in the batch procedure [19] to <1 µg per determination was also achieved. The detection limit (99.7% confidence level) and CV (n = 10) were estimated as 10 µg l⁻¹ and 3.3%, respectively.

The recording of an analytical run is shown in Fig. 4, from which it can be deduced that a sample throughput of 45 determinations per hour can be achieved with good baseline stability and precision. The sampling rate can be considered high in comparison with those attained by the systems involving concentration of the analyte.

The results for zinc determination in pharmaceutical preparations by the proposed procedure are shown in Table 1. The obtained results agreed with those achieved by ICP-AES at a 95% confidence level.

4. Conclusion

The proposed procedure was suitable for the determination of zinc in pharmaceutical preparations, presenting good accuracy and precision. The association of SPS with a flow-injection system resulted in a
simple, robust, sensitive, and inexpensive analytical procedure allowing in situ analyte separation and concentration. The procedure can be also applied for other kinds of samples, since the selectivity of TAN reagent is considerably improved by the FI-SPS approach. High enrichment factors can be attained by employing large sample volumes. Since the detection is performed before elution, the dispersion process caused by the eluent does not affect the sensitivity, as occurs in the usual flow-injection systems involving preconcentration.

The geometry of the homemade flow cell provided good sensitivity and dynamic range by reducing the absorption/scattering of the radiation beam by the solid support. The flow-cell assembly at the spectrophotometer optical path allowed the achievement of higher sensitivity and precision, avoiding hindrances caused by preferential flow pathways. Measurements by solid-phase spectrophotometry with the reagent immobilized in the flow cell avoided the drawbacks caused by the low solubility of the TAN reagent and the Zn(II)–TAN complex in water. In addition, the procedure for TAN immobilization on C_{18} is inexpensive and easy to perform, permitting a considerable reduction in reagent consumption.

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