

Article

## Spectrophotometric Determination of Zinc Using 7-(4-Nitrophenylazo)-8-Hydroxyquinoline-5-Sulfonic Acid

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Um novo método espectrofotométrico para determinação de zinco(II) é proposto usando um derivado da 8-hidroxiquinolina, o ácido 7-(4-nitrofenilazo)-8-hidroxiquinolina sulfônico (p-NIAZOXS). A reação entre o p-NIAZOXS e o zinco(II) é rápida em pH 9,2 e o complexo formado é estável por pelo menos 24 h. O método permite a determinação de zinco na faixa de 0,05 a 1,0  $\mu\text{g mL}^{-1}$  com absorvidade molar de  $3,75 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$  e limite de detecção de  $15 \text{ ng mL}^{-1}$ . O método proposto foi aplicado na determinação de zinco em produtos farmacêuticos e ligas de cobre, onde a precisão (R. S. D. < 2%) e exatidão obtidas foram satisfatórias.

A sensitive and selective spectrophotometric method is proposed for the rapid determination of zinc(II) using an 8-hydroxyquinoline derivative, 7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulfonic acid (p-NIAZOXS), as a new spectrophotometric reagent. The reaction between the p-NIAZOXS and zinc(II) is instantaneous at pH 9.2 (borax buffer) and the absorbance remains stable for over 24 h. The method allows the determination of zinc over the range of 0.05-1.0  $\mu\text{g mL}^{-1}$  with a molar absorptivity of  $3.75 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$  and features a detection limit of  $15 \text{ ng mL}^{-1}$ . The proposed method has been successfully applied to the determination of zinc in several pharmaceutical preparations and copper alloys. The precision (R.S.D. < 2%) and the accuracy obtained were satisfactory.

**Keywords:** 7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid, zinc determination

### Introduction

The 8-hydroxyquinoline (oxine) behaves as a bidentate (N,O) univalent ligand to form chelates with several metal ions. Cations with  $n$  charge and  $2n$  coordination number form the so-called "coordination saturated uncharged chelates" which are insoluble in water, but easily soluble in organic solvents<sup>1</sup>. Owing to its great ability to form metal complexes, 8-hydroxyquinoline and its derivatives have been the subject of many studies involving analytical applications<sup>2, 3</sup>. Details may be found in a review<sup>2</sup>.

In this paper, the 7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulfonic acid (p-NIAZOXS) reagent has been synthesized and its complexation reaction with zinc(II) in aqueous medium was spectrophotometrically studied as an alternative method for the zinc determination. The reagent p-NIAZOXS is easily synthesized and purified and the complexation reaction with Zn(II) at pH 9.2 showed high selectivity and good stability and sensitivity for the deter-

mination of zinc. The proposed method was evaluated for the determination of zinc in pharmaceutical preparations and in copper alloys.

Zinc compounds have biocidal activity because they precipitate and denature the bacterial proteins. For this reason it has been used in dermatology as an antiseptic and disinfectant agent, in ophthalmic solutions, mouthwashes and mineral-vitamin preparations<sup>4</sup>.

In copper alloys, there are several elements that are added to provide specific attributes for the material. Zinc is seldom present in copper as an impurity, but it is intentionally added to form a series of industrial alloys. When it is added in concentrations of 5% or less, zinc acts as a deoxidizer. On the other hand, zinc increases the density, melting point, electrical and thermal conductivity of the copper alloy, decreasing the elasticity, but the strength hardness and the coefficient of expansion are increased. The effect of zinc content on the color of the alloy is also commercially important, because many brasses are used for decorative

purposes<sup>5,6</sup>. Therefore, the determination of Zn in copper alloys is important to preview the metal characteristics.

## Experimental

### Synthesis and characterization of the reagent

The reagent was conveniently prepared by coupling 8-hydroxyquinoline-5-sulphonic acid with an equivalent quantity of p-nitrodiazobenzene chloride in alkaline solution<sup>7</sup>. An amount of 0.02 moles of p-nitroaniline and 5 mL of concentrated hydrochloric acid were dissolved in 25 mL of water, cooled to 5 °C, and diazotised with 0.02 moles of sodium nitrite dissolved in 5 mL of water. After 20 min the diazonium solution was slowly added to a solution of 0.02 moles of 8-hydroxyquinoline-5-sulphonic acid containing 5 g of sodium carbonate and 50 mL of water also cooled to 5 °C. After two hours, hydrochloric acid (1:4) was added until the solution was slightly acidic (pH 3-5).

The 7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid (p-NIAZOXS) separated as a dark red precipitate which was filtered off, washed with water, dissolved in diluted sodium hydroxide and re-precipitated with hydrochloric acid. The precipitate was washed with deionized water to eliminate the free acid until a negative chloride reaction with AgNO<sub>3</sub> was obtained. Then it was dried at 105 °C overnight.

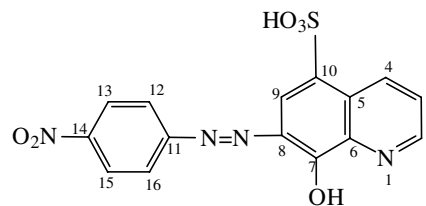
The structure of p-NIAZOXS (Fig. 1) was confirmed by means of <sup>13</sup>C-NMR, <sup>1</sup>H-NMR and IR spectrometry (Table 1 and 2). The reagent was a dark red powder, with a low solubility in water and organic solvents but very soluble in alkaline solutions. The reagent purity (97.1%) was checked by potentiometric titration with 0.100 mol L<sup>-1</sup> sodium hydroxide solution (standard solution, Merck) and gravimetric analysis of sulfur by mineralization of p-NIAZOXS with concentrated HCl, HNO<sub>3</sub> and HClO<sub>4</sub> (1:1:1 v/v). The sulfate was precipitated as BaSO<sub>4</sub>, dried, filtered and weighed<sup>8</sup>.

### Apparatus

Spectrophotometric measurements were made in a Varian Cary 1E UV-visible spectrophotometer with 1.00 cm glass cells. The pH measurements were carried out with an ANALYSER 300 pH meter. Infrared spectra were recorded on a Jasco Valor III spectrometer. <sup>13</sup>C and <sup>1</sup>H-NMR spectra were obtained with a Gemini 300 (Varian). An Applied Research Laboratories model 3410 minitorch sequential inductively coupled plasma spectrometer was used for the atomic emission spectroscopy measurements.

### Reagents

Unless otherwise stated all reagents were of analytical-reagent grade. Stock zinc(II) solution: a foil of zinc (99.9%, Aldrich) was washed with a 0.1 mol L<sup>-1</sup> nitric acid solution and then washed with pure acetone and air dried. 1.0000 g



**Figure 1.** Structure of the 7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid.

**Table 1.** <sup>13</sup>C-NMR and <sup>1</sup>H-NMR spectra of p-NIAZOXS.

Group (number)	<sup>13</sup> C-NMR δ, ppm (C atoms, peak, assignment)	<sup>1</sup> H-NMR δ, ppm (H atoms, peak, assignment)
14	157.66	-
11	157.66	-
07	147.06	-
02	146.92	8.22
10	146.58	-
8	137.10	-
4	135.99	8.66
5	129.73	-
6	128.70	-
13	125.02	8.25
15	125.02	8.25
9	123.45	8.25
12	121.24	7.87
16	121.24	7.87
3	117.14	7.49

**Table 2.** IR spectrum of p-NIAZOXS.

ν (cm <sup>-1</sup> )	Group
3699.92	Aromatic -CH stretching
3622.76	
3377.77	-OH stretching
1344.55	-NO <sub>2</sub> stretching (ArNO <sub>2</sub> )
1593.40	Aromatic -C=C- stretching
1505.62	
1098.59	-CH in-plane bending
1035.90	
1007.93	
913.41	-C-N stretching (ArNO <sub>2</sub> )
539.17	Aromatic -C=C- out-of-plane bending
470.69	
431.14	

( $\pm 0.0002$  g) of this zinc was dissolved with concentrated nitric acid and the solution was diluted to 1000 mL with deionized water. Working solutions of zinc were prepared by dilution of the stock solution with water.

**p-NIAZOXS solution:** 100 mg of p-NIAZOXS was dissolved with 250 mL of a  $0.1 \text{ mol L}^{-1}$  sodium hydroxide solution. The solution is stable, for at least, one month.

**Borax buffer solution (pH 9.2):**  $0.1 \text{ mol L}^{-1}$  sodium hydroxide solution was added dropwise to a  $0.05 \text{ mol L}^{-1}$  sodium tetraborate solution (Merck) to the required pH.

### General procedure

Into a 25 mL volumetric flask, transfer a portion containing 1.2-25  $\mu\text{g}$  of zinc(II). Add 1.0 mL of p-NIAZOXS solution and 3.0 mL of borax buffer solution. Dilute to the mark with water and mix. Then, measure the absorbance at 520 nm in a 1.0 cm cell against deionized water. The zinc content in an unknown sample was determined using a concurrently prepared calibration graph.

### Sample preparation

#### Pharmaceutical preparations

Ash ( $450^\circ\text{C}$ ) the content of one capsule for vitaminic preparation, 0.500 g of the pomade sample, 0.500 g of the powder preparation sample or 1.0 mL of ophthalmic solution. Then, dissolve the ashed sample with 5 mL of concentrated hydrochloric acid. Place the dissolved sample in a volumetric flask and make up to 500 mL. Analyze suitable aliquots of this solution for zinc as described above.

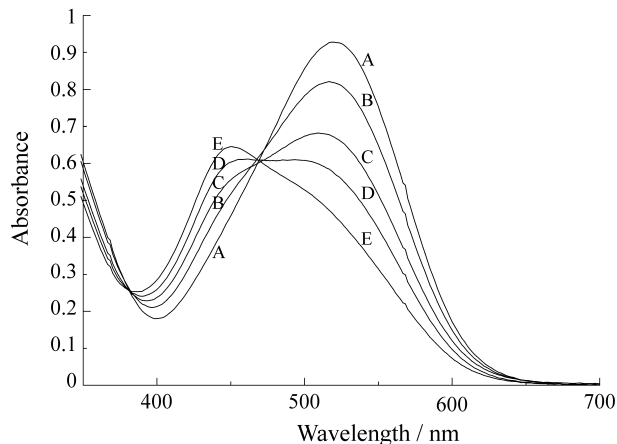
#### Copper alloys

Weigh accurately  $0.1000 \text{ g}$  ( $\pm 0.0002 \text{ g}$ ) amounts of the alloy, dissolve in 6 mL of concentrated hydrochloric acid and 1 mL of concentrated nitric acid. Heat the system up to promote the sample dissolution. Place the dissolved sample in a volumetric flask and make up to 500 mL. Previous removal of zinc by extraction from thiocyanate solutions was carried out according to the method described by Rudometkina and co-workers<sup>9</sup>. After extraction, an appropriate volume of the diluted solution was taken and analyzed as described above.

## Results and Discussion

### Absorption spectra and characteristics of the complex

The absorption spectra, in the visible region, of the p-NIAZOXS reagent and zinc-p-NIAZOXS chelate are shown in Fig. 2. At pH 9.2, the maximum absorbance of the reagent occurs at 520 nm and the zinc complex exhibits one maximum at 430 nm. As it can be seen in Fig. 2, absorption spectra show an isobestic point at 470 nm. The p-NIAZOXS reacts immediately with zinc forming an orange-yellow complex in aqueous medium (pH 9.2) and the



**Figure 2.** Spectral curves at different concentrations of Zn(II): (A)  $0 \mu\text{g mL}^{-1}$ ; (B)  $0.20 \mu\text{g mL}^{-1}$ ; (C)  $0.40 \mu\text{g mL}^{-1}$ ; (D)  $0.60 \mu\text{g mL}^{-1}$ ; (E)  $0.80 \mu\text{g mL}^{-1}$ .

absorbance reached its maximum within 5 min and remained stable, for at least, 24 h.

### Composition of zinc(II)p-NIAZOXS complex

The complex composition was determined by the molar ratio method. The results demonstrated that the complex has a molar ratio of 1:2 (zinc:p-NIAZOXS). Under the employed conditions, the minimum volume of 0.04% p-NIAZOXS solution required to guarantee total complex formation with 25  $\mu\text{g}$  of zinc was 1.0 mL, which corresponds to a molar ratio of 1:3 (zinc:p-NIAZOXS). Thus the minimum excess of p-NIAZOXS in the system must be 50%.

### Influence of pH

The absorbance of the complex zinc(II) p-NIAZOXS depends on the pH of the solution. The maximal and constant absorbance was obtained in the pH range of 8.5-10.0. The absorbance was measured at 430 and 520 nm.

The pH control was done using borax buffer. The effect of the buffer concentration on the Zn(II)p-NIAZOXS complex was studied and according to the results, no effect on the absorbance signal was observed when the buffer concentration was in the range of 0.002-0.01  $\text{mol L}^{-1}$ . A concentration of  $0.006 \text{ mol L}^{-1}$  was chosen for the procedure.

### Effect of foreign ions

The reaction selectivity was investigated by determining 12.5  $\mu\text{g}$  of zinc(II). The tolerance limit was taken as  $\pm 5\%$  change in absorbance. Copper(II), nickel(II), cobalt(II), cadmium(II), iron (III) and iron(II) ions interfered seriously at all proportions. However, selective extraction of zinc from thiocyanate solution in  $\text{HCl } 0.5 \text{ mol L}^{-1}$  medium followed by its back extraction with an ammoniacal solution solves this problem<sup>9</sup>. If iron is present in the sample, it is necessary to add NaF before the zinc extraction. The fol-

lowing cations, when present in the amounts shown in brackets (in  $\mu\text{g mL}^{-1}$ ) do not interfere: Al(III) [250], Ca(II) [250], Hg(II) [100], Mn(II) [100], Ti(IV) [300], Bi(III) [300], Sr(II) [300], V(V) [150], Mo(VI) [150] and W(VI) [150]. Up to  $200 \mu\text{g mL}^{-1}$  of sulfate, chloride, nitrate and phosphate do not interfere.

### Application

The calibration curves were made as described in the experimental procedure and good correlation coefficients were found. Beer's law is obeyed from  $0.05$  to  $1.00 \mu\text{g mL}^{-1}$  of zinc, with a molar absorptivity of  $1.58 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$  at  $430 \text{ nm}$  and with molar absorptivity of  $3.75 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$  at  $520 \text{ nm}$ . The measurements were done at  $520 \text{ nm}$  (maximum absorbance of the reagent) because at this wavelength the system is more sensitive. At  $520 \text{ nm}$  the reagent consumption is measured, in this way, water was used as blank for the measurements.

The analytical sensitivity<sup>10</sup>, the calibration sensitivity<sup>11</sup>, which is the slope of the analytical curve, the limit of detection<sup>12</sup>, and the limit of quantitation<sup>12</sup> as well as other analytical characteristics of the procedure are summarized in Table 3.

The proposed method has been applied to the determination of zinc in standard samples of copper-based alloys and in pharmaceutical preparations. Results are shown in Table 4 and Table 5. The results obtained, as the average of three determinations on each of the three copper based alloys, are compared with those certified for them and no significant difference was found at the 95% confidence level. For zinc determination in pharmaceutical preparations, the results are comparable to those obtained by sequential inductively coupled plasma atomic emission spectrometry (ICP-AES) method. The emission intensity

**Table 3.** Analytical characteristics of the proposed procedure (1-cm cell; N: number of determinations;  $\sigma$ : standard deviation).

Analytical parameters	at 520 nm
Molar absorptivity	$3.75 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$
Calibration sensitivity	$0.344 \text{ mL } \mu\text{g}^{-1}$
Analytical sensitivity ( $S_A$ )	$572.0 \text{ mL } \mu\text{g}^{-1}$
Inverse of the analytical sensitivity ( $1/S_A$ )	$1.7 \text{ ng mL}^{-1}$
Correlation coefficient	0.9996
Limit of detection ( $3\sigma$ )	$15 \text{ ng mL}^{-1}$
Limit of quantification ( $10\delta\sigma$ )	$50 \text{ ng mL}^{-1}$
Linear dynamic range	$0.05 \mu\text{g mL}^{-1}$ to $1.00 \mu\text{g mL}^{-1}$
Coefficient of variation ( $0.50 \mu\text{g mL}^{-1}$ of zinc)	0.87% (N = 10)

measurements were made under the conditions shown in Table 6.

**Table 4.** Determination of zinc in copper-base alloys.

Standard sample	certified content (%)	proposed method (%) <sup>*</sup>
CEPED** 486	2.87	$2.83 \pm 0.08$
CEPED 239	0.17	$0.17 \pm 0.02$
CEPED 1883	2.22	$2.30 \pm 0.06$

<sup>\*</sup> Average of three determinations, at 95% of confidence level.

\*\*CEPED- Centro de Pesquisa e Desenvolvimento da Bahia, Brasil.

Sample compositions. CEPED 486: Cu(85.86%), Ni(0.29%), Sn(2.81%), Zn(2.87%), Pb(5.32%), Fe(0.21%), Sb(0.13%), Al(0.05%), Si(0.02%); CEPED 239: Cu(80.12%), Sn(9.72%), Zn(0.17%), Pb(9.46%), Fe(0.015%); CEPED 1883: Cu(84.45%), Ni(1.51%), Sn(6.27%), Zn(2.22%), Pb(5.48%), Fe(0.033%), P(0.038%).

**Table 5.** Determination of zinc in pharmaceutical preparations.

Sample	Labelled contents	ICP-AES <sup>*</sup>	Proposed method <sup>*</sup>
1 <sup>a</sup>	120	$115 \pm 2$	$115 \pm 2$
2 <sup>a</sup>	-	$617 \pm 2$	$612 \pm 2$
3 <sup>b</sup>	1.47	$1.33 \pm 0.07$	$1.53 \pm 0.02$
4 <sup>c</sup>	0.068	$0.063 \pm 0.001$	$0.064 \pm 0.003$

<sup>\*</sup> Average of three determinations, at 95% of confidence level.

Sample composition. (1) Hipoglós sample (Biolab Ind. Farm. S/A)<sup>a</sup>: 20 mg boric acid, 150 mg oxid zinc ; (2) Anaseptil pó sample (Sanoli Winthrop Farmacêutica Ltda)<sup>a</sup>: 500 I.U. zinc bacitracin, 0.2 g zinc peroxide, q.s.q. 1 g zinc oxide; (3) MagnésioZ sample (Bionatura Com. Rep. Imp. Exp. Ltda)<sup>b</sup>: 490 mg Mg (aspartate, orotate and citrate), 5 mg zinc gliconate; (4) Colírio Moura Brazil sample (Hoechst Marion Roussel S/A)<sup>c</sup>: 0.3 mg zinc sulfate heptahydrate, 15 mg boric acid, 0.15 mg naphazoline hydrochloride.

<sup>a</sup> mg g<sup>-1</sup>, <sup>b</sup> mg/tablet, <sup>c</sup> mg mL<sup>-1</sup>.

**Table 6.** Operating parameters for the inductively coupled plasma spectrometer.

Incident output power	650 W
Reflected power	< 5 W
Nebulizer	Glass, Meinhard
Plasma gas flow rate	$7.5 \text{ L min}^{-1}$
Auxiliary gas flow rate	$0.8 \text{ L min}^{-1}$
Aerosol carrier gas flow rate	$0.8 \text{ L min}^{-1}$
Solution uptake rate	$2.5 \text{ mL min}^{-1}$
Wavelength	213.856 nm
Signal integration time	5 s
Integration for determination	3

## Conclusions

The method using 7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid as a new spectrophotometric reagent for zinc determination is selective, rapid and simple. The complex formed is very stable and it shows a good sensitivity in a borax buffer solution. This method was successfully applied for zinc determination in standard samples of copper-base alloys and pharmaceutical preparations and the results show a good agreement with certified values and with the results obtained by ICP-AES methods.

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