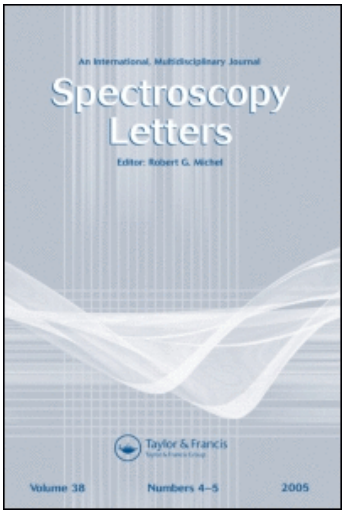

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SPECTROPHOTOMETRIC DETERMINATION OF ZINC IN PHARMACEUTICAL SAMPLES USING DI-2-PYRIDYL KETONE SALICYLOYLHYDRAZONE

Ivanise Gaubeur[a]; Madalena C. da CunhaAreias[a]; Lúcia Helena S. Ávila Terra[a]; Maria Encarnación V. Suárez-Iha[a]
[a] Instituto de Química, Universidade de São Paulo, São Paulo, Brasil

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SPECTROPHOTOMETRIC DETERMINATION OF ZINC IN PHARMACEUTICAL SAMPLES USING DI-2-PYRIDYL KETONE SALICYLOYLHYDRAZONE

Ivanise Gaubeur, Madalena C. da Cunha Areias, Lúcia Helena S. Ávila Terra, and Maria Encarnación V. Suárez-Iha*

Instituto de Química, Universidade de São Paulo - Cx. Postal 26077, São Paulo, Brasil 05513–970

ABSTRACT

A highly sensitive procedure for spectrophotometric determination of zinc has been developed. At pH 4.5, in 50% (V/V) ethanol-water medium and in the presence of di-2-pyridyl ketone salicyloylhydrazone (DPKSH), zinc forms a yellow complex which has maximum absorption at 376 nm The molar absorptivity is 4.82×10^4 L mol^{-1} cm^{-1}. The detection limit of this method is 62.1 nM for Zn(II).

The method has been applied to the spectrophotometric determination of zinc in pharmaceutical formulations and the results comply with those obtained by AAS. The proposed method is simple, rapid and accurate.

*Corresponding author. E-mail: maevsiha@quim.iq.usp.br

Key Words: Zinc determination; Spectrophotometric; DPKSH

INTRODUCTION

Zinc is essential in the human metabolism, of vital importance in biochemistry processes[1] since it represents a vital role in the operation of more than 200 enzymes, for the stabilization of DNA and transference of nervous stimulus. The human body contains 2–3 g of zinc with the largest concentration levels in the muscles, liver, kidney and bones.[2]

The recommended daily dosage of zinc for women is 12 mg and for men 15 mg. Women's dose increases to 19 mg for the pregnant or when they are breastfeeding. The main sources of zinc in foods can be found in meat and liver, seafood (especially oysters) cereals and rice, cheeses and nuts.[3] In the case of drinkable water, zinc can vary in a range between 0.06 to 7 mg dm^{-3} with an average of 1.33 mg dm^{-3}.[4] The "U.S. Environmental Protection Agency" (EPA) has established that drinkable water should not contain amounts of zinc over 5 mg dm^{-3}.

The symptoms of zinc deficiency lead to skin problems, slow healing of wounds, reduction in the senses of taste and smell, increased susceptibility to infections, decrease of fertility, retardation of children's growth, mental lethargy and loss of appetite and loss of hair.

Zinc has been used pharmaceutically in creams, ointments, eye drops, mouthwashes and others to defeat various types of infection.[5]

The combination of vitamins A, C and E + zinc has been added to human daily meals due to its free radical neutralizing properties. This specific combination can play an important role in tissue recovery from traumatic damages, cellular and tissue growth, and healing of wounds. It also contributes to healthy skin and hair and prevents LDL cholesterol from being deposited in the arteries.

A variety of methods has been developed to determine zinc,[6] and many of them are based on the formation of coordination compounds with favorable optical properties. However, in spite of these new and very sensitive methods, dithizone (diphenyl carbazone in CCl_4) is still the most widely[6] used reagent for zinc determination as the other methods offer no significant progress with respect to either sensitivity or selectivity in comparison with the dithizone method which is also a slow method because it involves several necessary steps to increase the sensitivity and mask interferences.

Hydrazones are azomethines characterized by the triatomic grouping C=N-N and find application in analytical chemistry by acting as multidentade ligands[7–17] with metals (usually from the transition group).

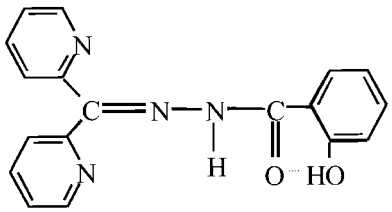

Figure 1. Structural formula of the DPKSH.

Among these hydrazones, the chelating agent di-2-pyridyl ketone salicyloylhydrazone (DPKSH) is a new and very promising reagent (Fig. 1) for spectrophotometric determination of zinc and other metals.[18] A very sensitive and fast method was developed for zinc spectrophotometric determination, using DPKSH. It is simple and does not require any long extraction period or temperature control. The evaluation was verified with the determination of zinc in pharmaceutical formulations and the results were compared to the Atomic Absorption Spectroscopy method (AAS).

EXPERIMENTAL

Instrumentation

All spectrophotometric measurements were carried out on a double-beam spectrophotometer Hitachi model U-3000 with 1 cm quartz cells. A Metrohm model 654 pH meter and a combined glass electrode were used for pH measurements.

Atomic Absorption measurements were performed on a spectrometer Perkin-Elmer model 403, under the following operation conditions: wavelengh 214 nm slit 4 and acetylene flow.

Reagents and Solutions – Analytical grade chemicals were used for this purpose. The solutions of DPKSH were prepared by dissolving the reagent in ethanol absolute.

The stock solution of zinc was prepared by dissolving 5.748 g of $ZnSO_4 \cdot 7H_2O$ in 1 L of deionized water containing the minimum amount of sulfuric acid needed to prevent hydrolysis ($pH \approx 2.5$). The solution was standardized by a standard method.[19]

Chloroacetic acid-chloroacetate mixtures (mol dm^{-3}) were used to keep the pH equal to 2.2 (2.5/0.14), 3.1 (0.75/0.46) and 3.4 (0.62/0.48). Acetic acid-acetate (mol dm^{-3}) mixtures were used for pH equal 4.0 (5.3/1.0), 4.5 (2.6/1.0), 4.9 (1.6/1.0) and 5.5 (0.05/0.35). Ammonium acetate (mol dm^{-3}) were used for pH equal 6.0 (3.2).

Synthesis of DPKSH

The synthesis of di-2-pyridyl ketone salicyloylhydrazone (DPKSH) was performed according to the procedure outlined by Garcia-Vargas et al.,[18] involving the reaction between equimolar amounts of di-2-pyridyl ketone and salicyloylhydrazide. The solid product was recrystallized three times in ethanol/water solution. After the synthesis, the melting point of the product (179–181°C), the elemental analysis and the infrared spectrum were determined, and all of the results were in excellent compliance with the data from the literature[18] (respective to DPKSH) confirming the successful synthesis.

Spectral Characteristics of DPKSH and Its Complexes with Zn(II)

The spectrum of DPKSH shows significant absorbance in the UV region and a maximum absorbance at 320 nm. The Zn(II)/DPKSH complexes show a maximum absorbance at 376 nm and a molar absorptivity of 4.82×10^4 L mol^{-1} cm^{-1}. The spectra of DPKSH and the Zn(II)/DPKSH complexes are in Fig. 2.

DPKSH has a low solubility in ethanol[18] and a much lower solubility in water. By using different ethanol percentages varying from 10 to 70%, the spectra of solutions composed by Zn(II) 1.06×10^{-5} mol dm^{-3} and DPKSH 2.39×10^{-4} mol dm^{-3} in pH = 4.0 were recorded. At 376 nm an increase as high as 40% was observed in the absorbance when the percentage of ethanol was changed from 10 to 40%. In the range of 50 to 70% the absorbance at 376 nm varied by only 5%. As sufficient high absorbance was achieved with a 50% ethanol/water solution, this condition was employed to all steps of the work.

Procedure

Certain quantities of stock zinc solution in the range of 0.2 to 13 μg, 50 μl of the buffer solution pH 4.5, 0.70 mL of DPKSH solution and 4.3 mL of ethanol absolute are transferred to a 10 mL volumetric flask. Deionized water is added to complete the volume. After this, a homogeneous part is transferred to a 1 cm spectrophotometric cell and the absorbance is measured at 376 nm against a blank prepared in the same way without zinc.

Preparation of Sample Solutions

In form of tablets/capsules the samples were firstly powdered. A (0.1 ± 0.0001)g was treated with 2 mL of 30% H_2O_2 and 0.2 mL of H_2SO_4 and heated carefully. After cooling, 2 mL of 30% H_2O_2 were added and the

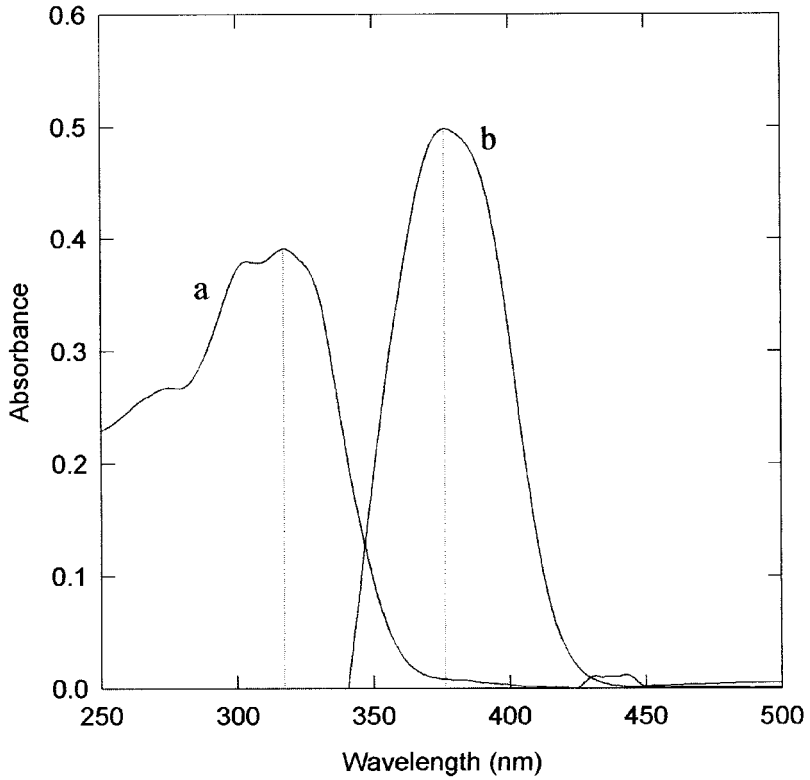

Figure 2. Spectra of DPKSH and of the complex Zn(II)/DPKSH in ethanol-water 50% (v/v), pH = 4.0 and using a 1 cm optical cell. (a) DPKSH 2.39×10^{-5} mol dm^{-3} (b) Zn(II) 1.06×10^{-5} mol dm^{-3}, DPKSH 2.39×10^{-4} mol dm^{-3}.

solution was heated. This process was repeated four times. The final solution was evaporated to 2 mL volume and filtered into a volumetric flask.[20]

Liquid formulations were diluted to the appropriate volume with deionized water.

RESULTS AND DISCUSSION

Optimization of the Analytical Parameters

The effect of the pH on the Zn(II) complexation with DPKSH was verified as the ligand can show different behaviors as a function of pH. The pH influence was available from solutions composed of Zn(II)

2.36×10^{-5} mol dm^{-3}, DPKSH 2.38×10^{-4} mol dm^{-3} and chloroacetic acid/chloroacetate buffer (2.2, 3.1 and 3.4) or acetate/acetic acid buffer (4.0, 4.5, 4.9, 5.5) and ammonium acetate (6.0) in ethanol 50%.

The different behavior of the complexes of Zn(II) with DPKSH is evident as in Fig. 3. The formation of the complex is unfavorable in the pH lower than 4.0, probably due to the protonation of one pyridine group from the ligand. When the pH is higher than 5.5 a decrease of the absorbance is observed owing to the more favorable hydrolysis of the metal in respect to the coordination with DPKSH. The most efficient DPKSH complexation was observed in the 4.0–5.5 pH range, leading to choosing 4.5 as the appropriate pH.

Different ligand/metal ratios were used to establish the highest sensitivity. Zn(II) 2.06×10^{-5} mol dm^{-3} solutions were prepared with ethanol/water 50%, pH 4.5 and several concentrations of DPKSH. The maximum and constant absorbance was reached when the ligand concentration was at least 10 times higher than the metal concentration.

The reagent addition order is essential in some methods. Eight different reagent addition orders were checked and the behavior of the complexes was followed for a period of 2 h. The Zn(II), DPKSH, buffer and ethanol order showed the highest sensitivity and stability for the complexes of Zn(II) with DPKSH. By using this addition order, the stability of a new solution was verified during a 12 h-. The color intensity was considered constant since the absorbance varied by only 0.26%.

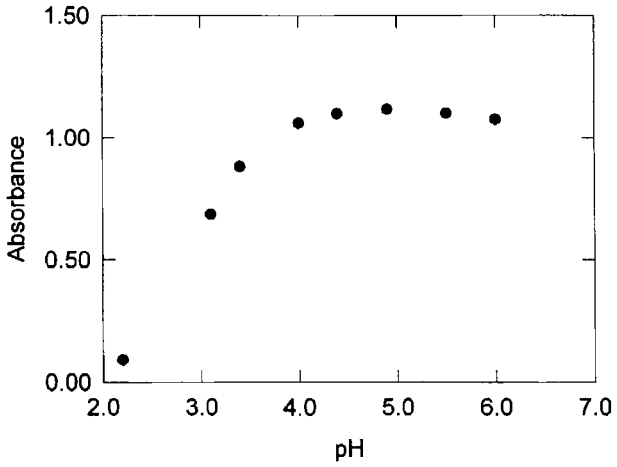

Figure 3. Absorbance at 376 nm for the complexes Zn(II)/DPKSH as a function of different pH values in ethanol 50% and using a 1 cm optical cell.

Analytical Curve

In order to obtain the analytical curve, spectrophotometric measures were carried out with several mixtures prepared according to the procedure. The linear response ranges from 0.2 to 20 μmol dm^{-3}. With a 1 cm cell, the detection limit is 62.1 nM (three times the standard deviation of the blank) zinc(II), at 376 nm. In ethanol-water the effective molar absorptivity is $(4.82 \pm 0.01)\times10^4$ L mol^{-1} cm^{-1}, at 376 nm. The logarithm stability constant for the 1:2 Zn(II)/DPKSH complex (formed in presence of DPKSH excess) in 50% ethanol is nearly 10.

The reproducibility of the absorbance measurement was investigated, and it was found that at 9.28 μM of Zn(II) the standard deviation is $\sim 2\%$.

Effect of Foreign Ions

Experiments were carried out to check the interference of some important ions (cations and anions). Table 1 summarizes the results for different ratios of species/Zn(II) concentrations. The tolerance limit (2%) was taken as three times the standard deviation (± 0.003) of the average absorbance (0.448) for tenfold measurements for solutions with no interfering species.

Table 1. Tolerance Limit to Different Species. Conditions: Zn(II) 9.28×10^{-6} mol dm^{-3}; DPKSH 2.08×10^{-4} mol dm^{-3}, EtOH 50%, pH = 4.0

Limit of Tolerance/Zn(II)	Species
≥10.000	I^-, Br^-, F^-, Cl^-, NO_3^-, NO_2^-, SCN^-, Propionate, Acetate and Thiourea
≥1000	Mg^{2+}, Ca^{2+}, Ba^{2+}, AsO_3^-, Gluconate, HCO_3^-, NH_4^+, HPO_4^{2-}, SO_3^{2-}, BO_3^{3-} and $B_4O_7^{2-}$
≥100	SO_4^{2-}, $S_2O_3^{2-}$, Tartrate, Ascorbic Acid, PO_4^{3-} and CO_3^{2-}
≥10	Cr^{3+}
≥1	Cd^{2+}, Cu^{2+} (*), Co^{2+}, Hg^{2+}, Ni^{2+}, Mn^{2+}, Al^{3+}, Pb^{2+}, Fe^{2+} (**), Fe^{3+} (**), Sn^{2+} and $C_2O_4^{2-}$

* Interference is suppressed with thiourea.

** Interference is suppressed with fluoride in presence of oxygen ($Fe^{2+} \xrightarrow{O_2} Fe^{3+} \xrightarrow{F^-} [FeF_n]^{3-n}$).

Some cations and $C_2O_4^{2-}$ represent very serious interference in low concentration. Among these cations, Hg^{2+}, Pb^{2+} and Sn^{2+} are not expected to be present in pharmaceutical formulations or in several natural samples, such as waters and soils. The molar absorptivity (L mol^{-1} cm^{-1}) in 30% ethanol solutions for some important metal-DPKSH complexes[18] are as follows: Fe(II)/DPKSH, 2.2×10^4 (360 nm); Fe(III)/DPKSH, 2×10^4 (360 nm); Cu(II)/DPKSH, 3.6×10^4 (380 nm); Ni(II)/DPKSH, 4.9×10^4 (375 nm); Cd(II)/DPKSH, 1.2×10^4 (370 nm). These metals can, therefore, interfere with the zinc determination at 376 nm if they are present at concentrations equivalent to zinc in the system of interest. Such metals must be absent or masked with proper reagents such as fluoride for iron or thiourea for copper.

With respect to the gluconate anion, present in one of the studied sample, the interference is only −2%, in the 1000:1 ratio. This interference can be attributed to the competition between DPKSH and the gluconate for Zn(II). Formate, Na^+ and K^+ do not interfere in any concentration.

Determination of Zinc(II) in Pharmaceutical Samples

The accuracy of the recommended spectrophotometric procedure was evaluated by determining Zn(II) in the following pharmaceutical samples: "Zinc-Oligosol" (Labcatal) containing 0.470 mg zinc gluconate, 0.1 g glucose and 2 mL distilled water, indicated as a modifier in the course of cutaneous infections; "Zinc and Cuivre-Oligosol" (Labcatal) containing 0.470 mg zinc gluconate, 0.518 mg copper gluconate, 0.1 g glucose and 2 mL distilled water, indicated as a modifier for functional problems of the puberty, menopause and menstrual syndrome; "Gluconate of Zinc" (Nature's Bounty) containing 100 mg zinc gluconate (amounts present in 1 g sample), facilitates the carbohydrate digestion, prostate gland function, growth and maturity and development of new cells. "Colirio Moura Brasil" (Hoechst Marion Roussel S/A) containing 0.3 mg zinc sulfate, 15 mg boric acid, 0.7 mg borax and 1 ml naphazoline hydrocloridate (amounts present in 1 mL sample) an eyewash.

The results obtained from the DPKSH method, as the average of five determinations of each sample, are compared with those data from atomic absorption spectrometry (AAS) and all of them are in Table 2. Both of the methods gave very similar results and the highest difference is 10% for the zinc gluconate sample. The results from the DPKSH method are compared with those labelled in the samples and no significant difference was found at 95% confidence level, given better results than AAS.

Table 2. Determination of Zinc in Pharmaceutical Samples Using the DPKSH Method and the Atomic Absorption Spectrophotometry

Sample	DPKSH	AAS	Labelled Contents
Zinc-Oligosol[a]	0.0640 ± 0.0005	0.0668	0.0674
Zinc and Cuivre-Oligosol*,[a]	0.0651 ± 0.0002	0.0708	0.0674
Zinc Gluconate[b]	97.2 ± 0.2	109	100
Colírio Moura Brasil[a]	0.065 ± 0.002	0.064	0.068

* In presence of thiourea to mask copper.
[a] mg mL^{-1}.
[b] mg/tablet.

CONCLUSIONS

The proposed method using DPKSH is highly qualified for determination of zinc in pharmaceutical samples. It is a rapid, simple and very sensitive method. The complex Zn(II)/DPKSH is very stable allowing the preparation of many samples to be measured some hours later. The DPKSH method is an alternative method, with a very low number of steps, as liquid extraction, and with a very low cost in opposition to atomic absorption spectrometry, for example.

This method was successfully applied for zinc determination in pharmaceutical preparations and the results show a good agreement with the results obtained by AAS method, and a very good compliance with the labeled values.

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