

# Use of xylenol orange and cetylpyridinium chloride in rapid spectrophotometric determination of zinc in pharmaceutical products

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## Abstract

A simple, rapid, and sensitive spectrophotometric method for the determination of zinc(II) is performed, based on colour reaction between the metal ion and xylenol orange in the presence of surfactant cationic cetylpyridinium chloride. The important analytical parameters and their effects on the reported system are investigated. Zinc(II) reacts with the reagent and surfactant in the ratio 1:2:4 (metal:ligand:surfactant) in the pH range 5.0–6.0 to form a ternary complex with an absorption maximum at 580 nm. The reaction was extremely rapid at room temperature, and the absorbance value remains unchanged for at least 168 h. The apparent stability constant of the complex was found to be  $K = 1.05 \times 10^{10}$ , and the method adheres to Beer's law for 1–20  $\mu\text{g}$  zinc(II) per 25 ml with apparent molar absorptivity of  $1.1 \times 10^4 \text{ l mol cm}^{-1}$ . The effect of foreign ions was tested by taking a constant concentration of metal ion and determining its concentration in the presence of large number of foreign ions. The method was applied for determination of zinc(II) in dermal ointments where excellent agreement between reported and obtained results were achieved. The relative standard deviation was better than 2%. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Zinc determination spectrophotometry; Cetylpyridinium chloride; Pharmaceutical products

## 1. Introduction

A growing application of micelles in analytical chemistry involves the beneficial alteration of metal ion–ligand complex spectral properties via surfactant association [1–4]. Usually, the metal–chelate complexes formed in the micellar systems

are more stable than those formed in the absence of micelles. Surfactants and micellar systems are currently used in spectrophotometric determination of metals to solubilize reactants and products, and to improve the sensitivity and selectivity of these methods [5–9]. These effects show the advantage of such surfactant systems in the development of many new spectrophotometric methods for determining micro amounts of metals ions, anions, biological compounds, drugs and pesticides.

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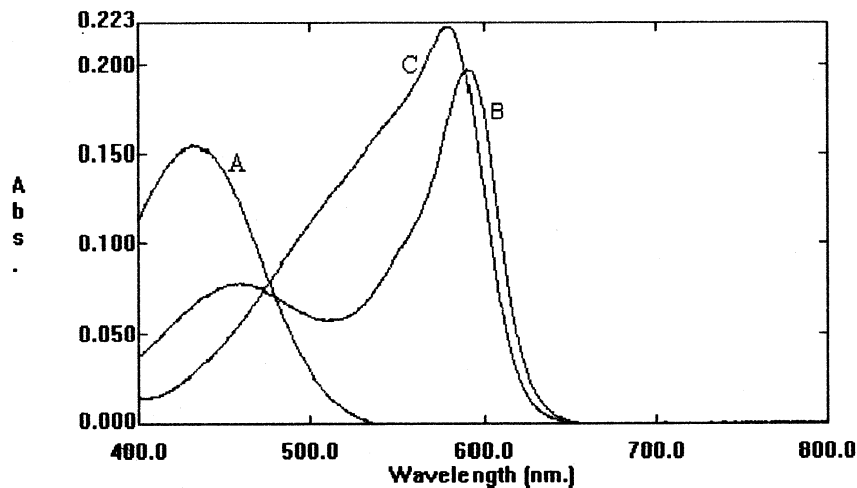


Fig. 1. Absorption spectra of zinc(II) ternary complexes and their reagent blank curves. (A) XO, (B) XO-CPC, (C) Zn(II)-XO-CPC. Conditions:  $[Zn(II)] = 2 \times 10^{-5} \text{ mol l}^{-1}$ ,  $[XO] = 10^{-4} \text{ mol l}^{-1}$ ,  $[CPC] = 10^{-3} \text{ mol l}^{-1}$ ; pH 5.5.

Table 1  
Comparison of reagents for the determination of zinc

Reagent	Surfactant	$\lambda_{\text{max}}$ (nm)	pH	$\epsilon \times 10^{-4}$ ( $\text{l mol}^{-1} \text{ cm}^{-1}$ )	Reference
Chrome azurol S	Tetradecyldimethyl-benzyl ammonium	510	–	9.5	[10]
Methylthymol blue	CPC	600	–	1.57	[11]
Stylbaso	CPC		8.0–10.0	5.6	[12]
Pyrocatechol violet	CPC	690	9.0	1.3	[13]
3,5-diBr-PADMAP		610	–	12.6	[14]
3,5-diBr-PADAP		570	–	13.0	[15]
Dithizone	Sodium dodecylsulfate	538	9.0	6.6	[16]
Xylenol orange	CPC	580	5.5	1.1	This work

Zinc is one of the very important metals that is widely used in the pharmaceutical industry, especially in manufacturing dermal ointment. Apart from this is the vital role zinc plays in biological systems. Many investigations have been made regarding the spectrophotometric determination of zinc and of these chrome azurol S [10], methylthymol blue [11], stylbaso [12], pyrocatechol violet [13], 2-[(3,5-dibromo-2-pyridyl)azo] dimethylaminophenol (3,5-diBr-PADMAP) [14], 2-[3,5-dibromo-2-pyridylazo] diethylaminophenol (3,5-diBr-PADAP) [15] methods have been commonly used. However, most of these methods lack

sensitivity ( $\epsilon < 1 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ ). Many reports describing the synthesis of new hydrazones and semicarbazones and their application to the

Table 2  
Regression analysis of the calibration graph and other quantitative data for Zn(II)

Concentration range ( $\mu\text{g/ml}$ )	0.04–0.8
Recovery (%)	99.7
Correlation coefficient	0.9998
Standard deviation	0.07
Relative standard deviation (%)	2.0

Table 3

ZnO determination standard by the proposed method, with the results compared with those obtained by atomic absorption spectrometry (AAS)

Standard	ZnO certified (%)	ZnO found (%)	
		AAS	Present method
Ointment, used in dermal treatment	27	26.75	26.80

spectrophotometric determination of zinc were published. They offer, however, no significant progress with respect to either sensitivity or selectivity in comparison with the dithizone method. Dithizone is still the most widely used reagent for zinc and the method is reasonably sensitive [16,17], but many problems are associated with their use. Dithizone is practically insoluble in water and combines with zinc ions to yield a non-polar coloured complex, generally extracted into solvents like chloroform and carbon tetrachloride. This is time consuming and tedious, and involves the use of chlorinated solvents. The complexing properties of xylenol orange have been used for the spectrophotometric determination of many ions. However, a little attention has been directed to determination of zinc in micellar media.

In this paper, we propose an improved spectrophotometric method that can be used to quantify zinc directly, based on the colour reaction between the metal ion and xylenol orange in the presence of surfactant cationic cetylpyridinium chloride. The method saves time and effort, as well as many chemicals. Different variables that affect the reaction of xylenol orange with zinc in micellar solutions as well as a detailed description of procedures are presented. The method proposed has been successfully applied to the determination of zinc contained in dermal ointment.

## 2. Experimental

### 2.1. Apparatus

A Shimadzu UV-2101PC double beam UV–visible spectrophotometer (Japanese model) with a

fixed slit width of 0.5 nm and its recorder were used. The curves of the visible spectra of reference and test solutions were recorded in 1-cm path-length cells over the wavelength range 360–800 nm. A 3420 Electrochemistry Analyser (Jenway) with a combined glass-saturated calomel electrode was used for pH measurements.

### 2.2. Reagents and solutions

Unless otherwise stated, all commercial reagents used were of analytical grade, without further purification, and their solutions were prepared by weighing with distilled water as solvent.

Zinc(II) stock standard solution ( $1 \times 10^{-2}$  mol  $l^{-1}$ ) was prepared by dissolving 0.7180 g  $ZnSO_4 \cdot 7H_2O$  (Labosi) in freshly distilled water and diluting to 250 ml in a calibrated flask.

Xylenol orange (XO) (Labosi) solution ( $1 \times 10^{-3}$  mol  $l^{-1}$ ) was prepared by dissolving 0.1900 g in 250 ml distilled water.

Cetylpyridinium chloride (CPC) (Labosi) solution ( $1 \times 10^{-2}$  mol  $l^{-1}$ ) was prepared by dissolving 0.8950 g in 250 ml distilled water.

Acetate buffers (0.5 mol  $l^{-1}$ ) of pH 4.0–6.0 were prepared by mixing appropriate volumes of 0.5 mol  $l^{-1}$  acetic acid (Riedel-de Haen) and 0.5 mol  $l^{-1}$  sodium hydroxide (Labosi) solutions.

### 2.3. Pharmaceutical preparation

A commercial pharmaceutical (Cod liver oil and zinc oxide ointment, 40g tube; SAIDAL -SPA pharm Ind, Algeria) agent was used with declared contents as follows.

- Cod liver oil, 20 g containing 600 UI vitamin A  $g^{-1}$  and 85 UI vitamin D  $g^{-1}$ .
- Zinc oxide, 27 g.

- Excipient, 100 g.

### 2.3.1. Properties

A topical agent that protects damaged tissues.

### 2.3.2. Indications

Skin diseases due to irritations, namely superficial burns over limited skin areas, sunburns, diaper rash, superficial non-oozing skin lesions, cracks of the breaks, chilblains.

### 2.4. General procedure

Transfer a sample solution containing no more than 500  $\mu\text{g}$  Zn(II) to a 25 ml volumetric flask, add 2 ml of  $1 \times 10^{-4}$  mol  $\text{l}^{-1}$  XO and 1 ml of  $1 \times 10^{-3}$  mol  $\text{l}^{-1}$  CPC, adjust the pH of the solution to 5.5, dilute to the mark with distilled water, mix well and allow to stand for about 30 min. Measure the absorbance of the coloured solution in a 1-cm cell at 580 nm against a reagent blank. All the experiments were carried out at room temperature. The zinc concentration was determined using a calibration graph prepared with standard solutions of zinc(II).

### 2.5. Analysis of zinc in dermal ointment

An accurately weighed 4.1 mg ointment was transferred into a 100 ml separatory funnel and treated with 10 ml diethyl ether and 10 ml nitric acid ( $0.5$  mol  $\text{l}^{-1}$ ) to dissolve the organic compounds and zinc oxide. Shake the contents for 10 min and allow the two phases to settle and separate. The aqueous phase containing 0.8894 mg zinc(II) was subjected to analysis by the recommended procedure.

## 3. Results and discussion

### 3.1. Characteristics of the reagent and the complex

The absorption spectra of XO and its zinc complex in the presence of surfactant CPC are shown in Fig. 1. In the presence of CPC, the

ternary complex (Zn(II)–XO–CPC), formed at pH 5.5, shows maximum absorbance 0.221 at 580 nm (curve C), the blank (XO) shows 0.154 at 432 nm (curve A) and XO–CPC shows 0.197 at 591.5 nm (curve B)

### 3.2. Conditions for complex formation

The influence of pH on the absorbance of ternary Zn(II) complexes were studied over the pH range 3.6–7.0 at 580 nm. The pH was adjusted to the desired value using acetate buffer. The relationship between pH and the absorbance of ternary complex is linear over the pH range 5–6. Therefore, the value pH 5.5 was selected in the recommended procedure for Zn(II) determination as the ternary complex. The effect of the acetate buffer concentration and volume on the complex was studied and the results demonstrate that it does not affect the absorbance signal of the system when the buffer is in the concentration and volume ranges of 0.5–1.0 mol  $\text{l}^{-1}$  and 0.5–3 ml acetate, respectively.

The effect of varying the concentration of XO ranging between  $10^{-3}$  and  $10^{-5}$  mol  $\text{l}^{-1}$  on the colour development at a constant concentration  $1 \times 10^{-3}$  mol  $\text{l}^{-1}$  zinc(II) and  $1 \times 10^{-3}$  mol  $\text{l}^{-1}$  surfactant was examined by measuring the absorbance at the optimum pH and at 580 nm. Full colour development was obtained in the presence of  $1 \times 10^{-4}$  mol  $\text{l}^{-1}$  reagent. Thus, a 2 ml solution of XO was selected as optimal for the general procedure.

The effect of the surfactant CPC concentration on the formation of Zn(II) ternary complex was studied over the CPC concentration range  $10^{-2}$  to  $10^{-5}$  mol  $\text{l}^{-1}$  in the final solution. The study revealed that maximum enhancement of the complex absorbance was obtained at a concentration of  $1 \times 10^{-3}$  mol  $\text{l}^{-1}$  CPC. The surfactant concentrations in these studies were above the critical micelle concentration of  $0.9 \times 10^{-3}$  mol  $\text{l}^{-1}$  CPC [2]. In the procedure, 1 ml surfactant is used.

The order of addition of the reagents was studied and the results demonstrated that the complex formation was not affected by it. Consequently, the order Zn(II) + XO + CPC + buffer was utilized in the proposed procedure.

At room temperature, the maximal colour development of ternary Zn(II) complex formation is completed immediately after all reagents were added, and the absorbance is stable for least 5 days. All the measurements were made 30 min after the preparation of the solution in all the experiments.

### 3.3. Complex stoichiometry

Quantitative information on complex formation is obtained by determining the composition and the stability constants of the complexes formed. The  $J_{ob}$  values of continuous variation and molar ratio methods indicated a 1:2:4 for the ternary Zn(II)–XO–CPC complex. The apparent stability constant of the ternary complex, determined by  $J_{ob}$  values of continuous variation and molar ratio methods [18], is  $1.05 \times 10^{10}$ .

### 3.4. Analytical characteristics of the method

A calibration graph for the determination of zinc was constructed under optimum experimental conditions as already described. Beer's law was obeyed over the concentration ranges 1–20  $\mu\text{g}$  zinc(II) per 25 ml with a molar absorptivity of  $1.1 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ .

### 3.5. Comparison with other methods

Table 1 compares the present method with some of the methods reported in the literature. It can be seen from Table 1 that the proposed method is superior to existing methods with respect to sensitivity.

### 3.6. Interferences

Under the optimal conditions, the selectivity of the proposed method was investigated by the analysis of samples containing 5  $\mu\text{g}$  zinc in the presence of various amounts of other ions. The alkaline earth metal ions, Ag(I), Cd(II), Hg(II) and Co(II) did not interfere. The interference from Fe(III) and Al(III) was eliminated by masking with sodium fluoride, and the interference of Ni(II), Cu(II) and Pb(II) was eliminated by masking with ethylenediamine tetraacetic acid.

### 3.7. Statistical analysis

The regression analysis of calibration graphs and other quantitative data for Zn(II) is presented in Table 2. Precision of the proposed method was obtained from five parallel determinations of 5  $\mu\text{g}$  Zn(II) per 25 ml.

### 3.8. Analytical application of the method

In order to confirm the usefulness of the proposed spectrophotometric method, it has been applied to the determination of zinc in an ointment used in dermal treatment (manufactured by SAIDAL-SPA pharm Ind., Algeria), where excellent agreement between reported and obtained results were achieved (Table 3). The recovery of zinc was 99.7% and the relative standard deviation was better than 2%.

## 4. Conclusion

First, the proposed method is simple and rapid. The maximal colour development of ternary Zn(II) complex formation is completed immediately after all reagents were added and the absorbance is stable for least 5 days. No heating or standing was needed. Zinc values obtained by the proposed method correlated closely with those obtained by atomic absorption spectrometry.

Second, in terms of sensibility, the result of the proposed method was as good as that obtained by other research (see Table 1).

Third, the proposed method can be used to quantitate zinc directly in some pharmaceutical products. The method saves time and effort as well as many chemicals.

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