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Utility of formazans and cetylpyridinium chloride in rapid spectrophotometric determination of zinc in biological materials and pharmaceutical formulations

A.S. Amin [a,*], Y.M. Issa [b]

[a] *Chemistry Department, Faculty of Science, Benha University, Benha, Egypt*
[b] *Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt*

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Abstract

A facile, rapid and sensitive spectrophotometric method for the determination of zinc is performed, based on complexation reaction between the metal ion and 1,5-diphenyl-3-acetylformazan (**I**) 1-(*o*-carboxyphenyl)-3-acetyl-5-acetylformazan (**II**), 1-(*o*-carboxyphenyl)-3-acetyl-5-phenylformazan (**III**), and 1-(*o*-carboxyphenyl)-3-acetyl-5-m-tolylformazan (**IV**) in the presence of cationic surfactant cetylpyridinium chloride (CPC). The important analytical parameters and their effects on the reported system are investigated. Zinc reacts with the reagents (**I**–**IV**) and CPC in the ratio 1:1:2 (metal:reagent:CPC) in the pH range 8.5, 7.5, 5.5 and 6.5 to form a ternary complex with an absorption maximum 616, 656, 672 and 599 nm, respectively. The reaction was extremely rapid at room temperature, and the absorbance value remains unchanged for at least 1 week. The apparent stability constant of the complex were found to be 13.1 9.2, 11.4 and 12.3, and adheres to Beer's law for 0.05–3.50 μg per 10 ml of zinc. For more accurate analysis, Ringbom optimum concentration range was found from 0.08 to 3.20 μg per 10 ml of zinc. The apparent molar absorptivity, Sandell sensitivity, detection and quantification limits are also calculated. Taking a constant concentration of metal ion and determining its concentration in the presence of large number of foreign ions tested the effect of foreign ions. The method was applied for determination of zinc in serum, human hair and pharmaceutical formulations, where excellent agreements between reported and obtained results were achieved. The relative standard deviation was better than 1.67%.

Keywords: Formazans; Zinc determination; Spectrophotometry; Surfactant; Biological materials and pharmaceutical formulations

1. Introduction

Zinc is an essential element in the nutrition of animals and human. It acts as a cofactor in numerous enzymes and plays an important role in protein synthesis and cell division. It exerts a crucial influence on the maintenance of cell

* Corresponding author.
E-mail address: asamin2002@hotmail.com (A.S. Amin).

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membrane stability and in the function of immune system. It also constitutes an active ingredient in medical products intended for topical application. Zinc salts are used in ophthalmic solutions, in lotions intended for treatment of chronic skin diseases and incorporated as astringents in various solutions [1]. Zinc deficiency effects may be severe. They range from impaired neuropsychological functions and wound healing to growth retardation, immune disorders and dermatitis. On the other hand, zinc can be toxic when exposures exceed physiological needs. After single or short-term exposure to concentrations of zinc in water and beverages between 1.0 and 2.5 mg l^{-1}, poisoning incidents with symptoms of gastrointestinal distress, nausea and diarrhea were reported. Additionally, pharmacological intakes of zinc have been associated with effects such as leukopenia and/or hypochronic microcytic anemia and decreases in serum high-density lipoprotein concentration [2]. It is therefore, important to monitor the concentration of zinc and its salts in both biological materials and pharmaceutical formulations.

A growing application of micelles in analytical chemistry involves the beneficial alteration of metal ion-ligand complex spectral properties via surfactant association [3–6]. Usually, the metal-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 of these methods [7–12]. These effects show the advantages of such surfactant systems in the development of many spectrophotometric methods for determining micro amounts of metal ions.

In this work, we propose an improved spectrophotometric method that can be used to quantitative determination of zinc directly, based on the complexation reaction between zinc ion and 3-acetylformazans in the presence of the cationic surfactant cetylpyridinium chloride. The method saves time and effort, as well as many chemicals. Different variables that affect the reaction of formazans 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 in biological materials and pharmaceutical formulations.

2. Experimental

2.1. Apparatus

JASCO V530 UV–Vis double beam spectrophotometer (Japan) with a fixed slit width of 0.5 nm and X–Y recorder were used. The curves of the visible spectra of test and reference solutions were recorded in 10 mm pathlength quartz cells over the wavelength range 350–800 nm. An Orion research model 601A/digital ionalyzer fitted with a combined glass-calomel electrode was used for pH adjustment and checking the pH value of buffer solutions.

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 and dissolved in bidistilled water and/or ethanol as solvents.

Standard zinc solution, 10.0 μg ml^{-1} was prepared by dissolving 0.4398 g of zinc sulphate in distilled water, adding 5.0 ml of sulphuric acid (1 + 1) and diluted to 100 ml with bidistilled water in a calibrated flask. The stock solution was standardized by EDTA titration, it contains 1 mg ml^{-1} of Zn^{2+}. A 10.0 μg ml^{-1} solution was prepared by accurate dilution with bidistilled water.

3-Acetylformazans (**I**–**IV**) were prepared as recommended previously [13]. The 2×10^{-3} M reagent solution was prepared by dissolving an accurately weighed amount in absolute ethanol.

Thiel buffer solutions of various pH values ranging from 2.0 to 12.0 were prepared as recommended earlier [14]

Table 1
Characteristics of the complexes of Zn^{2+} with formazans (**I**–**IV**)

Parameter	I	II	III	IV
pH	8.5	7.5	5.5	6.5
λ_{max} (nm)	616	656	672	599
Stoichiometry	1:1:2	1:1:2	1:1:2	1:1:2
Logarithmic stability constant	13.1	9.2	11.4	12.3
Stability up to (h)	24	18	24	18
Beer's law limiting conc. (ng ml^{-1})	5.0–350	5.0–310	5.0–300	5.0–325
Ringbom optimum conc. (ng ml^{-1})	8.0–320	8.0–290	8.0–280	8.0–300
Detection limits (ng ml^{-1})	1.6	1.5	1.7	1.5
Quantification limits (ng ml^{-1})	5.0	4.8	5.1	4.9
Molar absorptivity (l mol^{-1} cm^{-1})	1.74×10^5	1.94×10^5	1.56×10^5	1.37×10^5
Sandell sensitivity (ng cm^{-2})	0.376	0.337	0.419	0.476
Regression equation[a]				
Intercept (a)	+0.007	+0.011	−0.006	−0.004
Slope (b)	0.266	0.297	0.239	0.210
Correlation coefficient (r)	0.9994	0.9990	0.9992	0.9998
Standard deviation (%)	0.80	0.83	0.76	0.87
Relative standard deviation (%)	1.53	1.66	1.45	1.60
Error (%)	±1.20	±0.96	±1.15	±0.75

[a] $A = a + bC$, where C is the concentration in μg ml^{-1}.

2.3. General procedure

A sample solution containing no more than 3.5 μg of Zn^{2+} was transferred to a 10 ml calibrated flask, and 2.0 ml of 5×10^{-4} M of formazan was added and mixed well. The pH was adjusted to the optimum pH value by adding 4.0 ml of the recommended buffer to the above mixture followed by 2.0 ml of 1×10^{-3} M CPC and diluted to the mark with distilled water. The solution was mixed well and the absorbance of the coloured complex was measured at the optimum wavelength recorded in Table 1 against a reagent blank prepared in the same manner. All the experiments were carried out at room temperature (25±2 °C). The zinc concentration was determined using a calibration graph prepared with standard solutions of zinc ions.

2.4. Procedure for blood serum

After collection, approximately 15 ml of the blood samples were left to clot in centrifuge tubes at room temperature; 15 min later, the blood clot was detached carefully from the glass wall with a rod and centrifuged immediately at 3000 rpm. A 5.0-ml portion of the clear supernatant serum was pipetted off and treated with 5.0 ml of 1.2 M trichloroacetic acid. The mixture was centrifuged at 3000 rpm, and the deproteinised supernatant was collected, the resulting solution was neutralized with 2.0 M NaOH until a pH value of ca. 3 was reached and analyzed by using the procedure described above.

2.5. Procedure for human hair

The hair samples were collected from healthy members of the laboratory by means of titanium oxide coated scissors. Each was cut in ca. 1.0 cm long pieces and treated with 20 ml acetone for 10 min. After several washings with deionized water to ensure complete removal of the organic solvent, the samples were treated three times with 1.0% v/v EXTRAN (MA-2, neutral, Merck), for 10 min. The samples were washed with bidistilled water until all the detergent was removed, and dried at 60 °C for 12 h. An accurately weighed amount of the sample (ca. 0.5 g) was then treated successively with 20 ml ca. 14 M HNO_3 and 2.0 ml 30% H_2O_2,

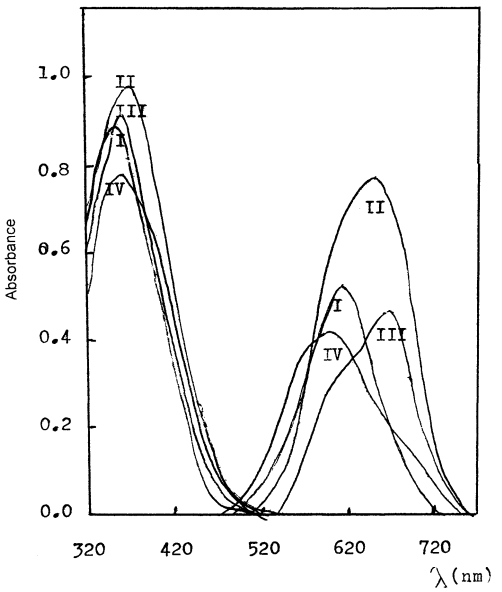

Fig. 1. Absorption spectra of (1×10^{-4} M) formazans (**I**–**IV**) and their (2.0 μg ml^{-1}) zinc complex in the presence of surfactant CPC.

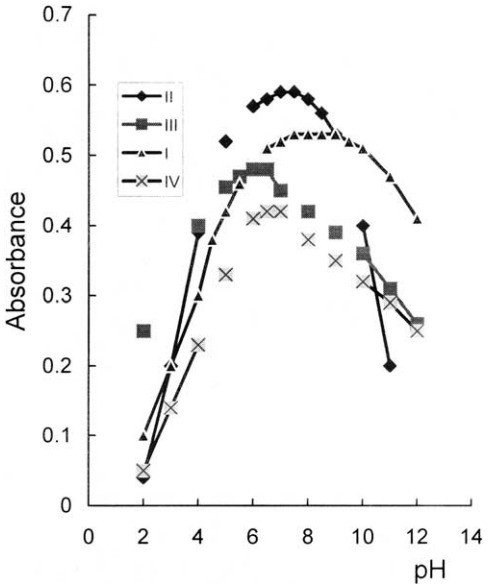

Fig. 2. Effect of buffer on the absorbance of 2.0 mg $ml^{-1}$of Zinc using fomazans (**I**–**IV**).

and after a period of 2.0 h, was heated almost to dryness. The resulting residue was treated with 1.0 ml ca. 14 M HNO_3 and then heated gently until complete decolorisation. Finally, the resulting solution was neutralized with 2.0 M NaOH, diluted to 100 ml with bidistilled water, and was finally analyzed, using the general procedure described above.

2.6. Procedure for pharmaceutical formulations

2.6.1. Hamoderme (talc powder)

The sample (25 mg) was treated with 3.0 ml of concentrated sulphuric acid, the solution was dried, washed with water and filtered to remove the white residue. The filtrate and washings were completed to 50 ml and analyzed for zinc as previously described.

2.6.2. Prozoline zinc (solution)

A 10 ml portion of the solution was evaporated to dryness and the soluble salts were dissolved with 2.0 ml concentrated sulphuric acid. The solution was filtered to remove the insoluble residue and washed three times with water. The filtrate was made up to 25 ml in a calibrated flask. An aliquot was analyzed for zinc per 10 ml as described above.

3. Results and discussion

3.1. Characteristics of the reagents and their complexes

The absorption spectra of formazans (**I**–**IV**) and their zinc complex in the presence of surfactant CPC are shown in Fig. 1. In the presence of CPC, the ternary complex (Zn^{2+}–R–CPC), formed showed λ_{max} at 616, 656, 672 and 599 nm, whereas the blank (R–CPC) showed λ_{max} at 358, 368, 362 and 360 nm for reagent **I**, **II**, **III** and **IV**, respectively.

3.2. Conditions for complex formation

The influence of pH on the absorbance of ternary Zn^{2+} complexes was studied over the pH

Table 2
Comparison of reagents for the spectrophotometric determination of zinc

Reagent	Surfactant	λ_{max} (nm)	pH	$\varepsilon \times 10^4$ (l mol^{-1}cm^{-1})	Reference
Chrome azural S	Zephiramine	510	–	9.5	[17]
Methylthymol blue	CPC	600	–	1.57	[18]
Stylbaso	CPC	576	8.0–10	5.6	[19]
Pyrocatechol violet	CPC	690	9.0	1.3	[20]
3,5-diBr-PADMAP[a]	–	610	–	12.6	[21]
3,5-diBr-PADAP[b]	–	570	–	13.0	[22]
Dithizone	SDS	538	9.0	6.6	[23]
Xylenol orange	CPC	580	5.1	1.1	[24]
Cadion 2B	Triton X-100	524	9.2	10.0	[25]
Hydrazidazol	Triton X-100	640	7.5	2.7	[26]
1-(2-Thiazolylazo)2-naphthol	Triton X-100	582	6.2–8.0	4.5	[27]
4-(2-Arsonophenylazo) salycilic acid	–	525	6.0	1.36	[28]
I	CPC	616	8.5	17.4	This work
II	CPC	656	7.5	19.4	This work
III	CPC	672	5.5	15.6	This work
IV	CPC	599	6.5	13.7	This work

[a] 3,5-diBr-PADMAP: 2-[(3,5-dibromo-2-pyridyl)azo]dimethylaminophenol.
[b] 3,5-diBr-PADAP: 2-[(3,5-dibromo-2-pyridyl)azo]diethylaminophenol.

range 2.00–12.50. Different buffer solutions (borate, universal, phosphate, acetate, and thiel buffers) were tested to choose the optimum buffer media. Thiel buffer solution is the optimum one and the best pH ranges were 7.0–9.5, 6.5–8.5, 4.5–6.5, and 6.0–7.0 for reagents **I**, **II**, **III** and **IV**, respectively (Fig. 2). The pH 8.5, 7.5, 5.5 and 6.5 were chosen for all further studies for Zn^{2+} complex of reagents **I**, **II**, **III** and **IV**, respectively. Moreover, the optimum volume of thiel buffer solution was found to be 4.0 ml for all further studies.

The effect of varying the reagent concentration ranging between 1×10^{-3} and 1×10^{-5} M on the colour development at a constant concentration 20 μg per 10 ml Zn^{2+} and 2×10^{-4} M surfactant was examined by measuring the absorbance at the optimum pH at λ_{max} for each system. Full colour development was obtained in the presence of 1×10^{-4} M reagent. Thus a 2.0-ml solution of 5×10^{-4} M reagent was selected as optimal for the general procedure.

In the presence of a surfactant, the deep red coloured complex was formed instantaneously. Of the eight surfactants tried, namely cetyltrimethyl ammonium bromide (CTMAB), cetylpyridinium bromide (CPB), cetylpyridinium chloride (CPC), benzyldimethyl tetradecylammonium chloride (Zephiramine), sodium lauryl sulphate, sodium dodecyldimethyl aminoacetate, emulsifier OP, Tween-80, and Triton X-100, cetylpyridinium chloride was the best because it gave the highest sensitivity and stability. Moreover, the effect of surfactant CPC concentration on the formation of Zn^{2+}- complex was studied over the range 10^{-3}–10^{-5} M in the final assay solution. The study revealed that maximum enhancement of the complex absorbance was obtained at a concentration of 2×10^{-4} M CPC.

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^{2+} + reagent + buffer + CPC was utilized in the proposed procedure.

At room temperature, the maximum colour development of the ternary complex is completed immediately after all reagents were added, and the absorbance is stable for at least one weak. Raising

Table 3
Comparison of the results obtained using the proposed and FAAS methods

Sample	Zn content found[a]												
	FAAS	I	*t*-test[b]	*F*-value[b]	II	*t*-test[b]	*F*-value[b]	III	*t*-test[b]	*F*-value[b]	IV	*t*-test[b]	*F*-value[b]
Serum I	1.16[c]	1.15	1.28	2.76	1.18	1.63	3.34	1.17	1.19	2.62	1.14	1.43	2.95
Serum II	0.93[c]	0.95	1.42	2.96	0.95	1.16	2.51	0.91	1.35	2.70	0.90	1.61	3.24
Serum III	1.10[c]	1.07	1.14	2.52	1.12	1.26	2.64	1.11	1.67	3.43	1.08	1.37	2.76
Hair I	188.[d]	190	1.36	2.80	186.8	1.09	2.41	1.85	1.26	2.69	190.4	1.09	2.33
Hair II	121.8[d]	120	1.57	3.19	122.5	1.43	2.92	121	1.48	3.04	123	1.22	2.58
Hair III	164.0[d]	160	1.67	2.42	165.4	1.11	2.37	162.5	1.57	3.24	161.8	0.99	2.13
Prozoline	2.48[e]	2.49	0.89	2.04	2.50	1.50	3.13	2.47	0.92	2.04	2.51	1.14	2.51
Hamoderme	4.92[f]	4.92	1.27	2.67	4.98	1.33	2.57	4.96	1.72	3.54	5.00	1.55	3.16

[a] Average of six determinations.
[b] Theoretical values for *t*- and *F*- at five degrees of freedom and 95% confidence limits are 2.57 and 5.05, respectively.
[c] Zinc concentration in μg ml^{-1}.
[d] Zinc concentration in μg g^{-1}.
[e] Zinc concentration in mg g^{-1} $ZnSO_4$, manufactured by the Nile Company for Pharm. and Chem. Ind. Cairo, Egypt.
[f] Zinc concentration in mg g^{-1} $ZnSO_4$, manufactured by Kahira Pharm. and Chem. Ind. Company, Cairo, Egypt.

the temperature up to 45 °C no change in the colour intensity was observed, whereas above which absorbance starts to decay with blue shift in λ_{max}.

3.3. Complex stoichiometry

Quantitative information on the complex formation is obtained by determining the composition and the stability constants of the complexes formed. Job's continuous variation and molar ratio methods indicated a 1:1:2 for the ternary complex Zn^{2+}:formazan:CPC. The apparent stability constant of the ternary complex, determined by Job value of continuous variation and molar ratio method are 1.26×10^{13}, 1.58×10^{9}, 2.51×10^{11} and 2.00×10^{12} for complexes of **I**, **II**, **III** and **IV**, respectively.

3.4. Quantification

The calibration graph was constructed following the general procedure. The linear regression equation was applied and the values of slope, intercept and correlation coefficient obtained are recorded in Table 1. Beer's law was obeyed in the range recorded in Table 1 for each system. The calculated molar absorptivity and Sandell sensitivity for each zinc complex with reagent (**I**–**IV**) are included in Table 1.

The standard deviation of the absorbance measurements for each system was calculated from a series of 13 blank solution. The limits of detection ($K = 3$) and of quantification ($K = 10$) of each method were established according to the IUPAC definitions ($C_l = KS_0/s$, where C_l is the limit of detection, S_0 is the standard error of blank determination, S is the slope of the standard curve and K is the constant related to the confidence interval [15]) and the calculated values were recorded in Table 1. The relative standard deviations at a concentration level of 2.0 μg per 10 ml of zinc (six repeating determinations) were calculated and recorded in Table 1. Comparison of the applicability of the reagents under consideration with those of other methods (Table 2) shows that they are among the most sensitive spectrophotometric reagents for zinc.

3.5. Interference

Under the optimum conditions, the selectivity of the proposed method was investigated by analysis of samples containing 2.0 μg ml^{-1} zinc in the presence of various amounts of other ions. The alkaline earth metal ions, Ag^{+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Al^{3+}, Ni^{2+}, Cu^{2+}, and Co^{2+} did not interfere. Masking with sodium fluoride (NaF) eliminated the interference from Fe^{3+}.

3.6. Analytical applications

In order to confirm the usefulness of the proposed spectrophotometric method, it has been applied to the determination of zinc in serum, human hair and pharmaceutical formulations. The selected pharmaceutical formulations were two commercially available ophthalmic solution (prozoline zinc) containing $ZnSO_4$, and an anti-mycosis powder (Hamoderme talc powder). The obtained recoveries for all samples were satisfactory and varied between 98.3 and 101.2%.

The accuracy of the developed method was checked by comparison of the results obtained by the proposed method to the values obtained by using FAAS as the reference method. The results are shown in Table 3.

The performance of the proposed procedure was assessed by calculation of the t- and F-values compared with FAAS method. Mean values were obtained in a Student's t- and F-tests and 95% confidence limits for five degrees of freedom [16], and the results showed that the calculated values did not exceed the theoretical ones.

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