

## The Use of Phenanthraquinone Monophenyl Thiosemicarbazone for Preconcentration, Ion Flotation and Spectrometric Determination of Zinc(II) in Human Biofluids and Pharmaceutical Samples

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A rapid flotation methodology for zinc(II) separation and enrichment from human biofluids is established. At pH 6.0 and ambient temperature, using oleic acid (HOL) as a foaming reagent, zinc(II) was separated with phenanthraquinone monophenyl thiosemicarbazone (PPT) as a new flotation collector for Zn(II). The floated red colored 1 : 2 Zn(II)-PPT complex was measured spectrophotometrically at 526 nm with a molar absorptivity of  $1.83 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ . Beer's law was obeyed over a concentration range 0.05-1.0 mg L<sup>-1</sup> in the aqueous as well as in the scum layers. The proposed preconcentration flotation methodology was applied to determine Zn(II) in human biofluids. Application was, also, extended to determine Zn(II) in pharmaceutical samples and natural water samples spiked with known amounts of Zn(II) with a preconcentration factor of 100 and a detection limit of 10 ng mL<sup>-1</sup>. The method was verified by comparison of the spectrophotometric results with flame atomic absorption spectrometric (AAS) measurements. Moreover a postulation for the mechanism of flotation is proposed.

**Key Words :** Ion flotation, Preconcentration, Zn(II), Biofluids, Drugs

### Introduction

Zinc is an essential microelement for growth, reproduction and development of living mammals. In addition to its role in catalysis and gene expression, zinc stabilizes the structure of proteins and nucleic acids, preserves the integrity of subcellular organelles, participates in transport and has an important role in viral and immune phenomena.<sup>1</sup> Zinc deficiency in human is clearly associated with certain clinical syndromes and possibly implicated in others. Growth retardation, anorexia, lethargy, hypogonadism, alopecia, confusion, apathy and delayed wound healing are variable manifestations of zinc deficiency in human.<sup>1</sup>

Recently, the interests for health-related and environmental sciences are widely and rapidly increased and the information about trace heavy metals is also becoming more important. Therefore, development of accurate determinations methods for them is necessary to fit such requirements. As a result, various types of powerful and elegant instruments have been developed up to present time for a qualitative and quantitative analysis of materials in view of composition as well as structure. Besides, various instrumental techniques have been extensively developed to improve the sensitivity, precision, selectivity and efficiency of analytical methods; but the combination of an appropriate preconcentration technique with these methods remains unsettled in the field of trace inorganic analysis to achieve successful analyses in many kinds of samples.<sup>2</sup>

The solvent extraction,<sup>3-5</sup> adsorption, ion exchange,<sup>6,7</sup> and coprecipitation<sup>8</sup> are commonly used as preconcentration techniques for the spectrometric determination of trace

heavy elements. However, these methods need relatively long time and troublesome operation for a large volume of sample.

A flotation technique,<sup>9-30</sup> on the other hand, is rapid and convenient compared to such preconcentration techniques and can be performed with an unskillful technique. This method is also able to concentrate many elements from a large sample volume of several liters to less than 10 mL, simultaneously.

The flotation technique is defined as a separation procedure by which various substances in a solution are selectively floated to the surface with the aid of tiny gas bubbles. The flotation technique can be classified into precipitate flotation and ion flotation.<sup>30</sup> In ion flotation technique, the desired trace ions in an aqueous solution are converted into hydrophobic species by adding complexing agents and/or surfactants, floated with the aid of numerous bubbles and concentrated in a scum or copious foam layer on the solution surface.<sup>30</sup>

The aim of the present study is to introduce phenanthraquinone monophenyl thiosemicarbazone (PPT) as a new reagent for the flotation of Zn(II) in media of diverse origin. In this study, trace amounts of Zn(II) were converted into Zn-PPT complex by adding PPT and then the complex in the aqueous sample solution was rendered hydrophobic by the addition of oleic acid (HOL) surfactant and floated to the scum layer by vigorous shaking. The floated substance was collected and the Zn(II) concentration was determined spectrophotometrically and verified by flame atomic absorption spectrometry (AAS). The various experimental variables such as pH, temperature, amount of PPT, type and amount of

surfactant, and so on, were optimized in the flotation procedure. Finally, this procedure was applied for the determination of Zn(II) in human biofluids, pharmaceutical samples and in natural water samples spiked with known amounts of Zn(II).

## Experimental Section

### Chemicals and solutions.

**Synthesis of phenanthraquinone monophenyl thiosemicarbazone (PPT),<sup>31</sup> (Scheme 1):** A hot solution of 4-phenylthiosemicarbazide (0.334 g, 2 mmol in ethanol) was added to (0.416 g, 2 mmol) 9,10-phenanthraquinone dissolved in the least amount of glacial acetic acid. The resulting solution was boiled under reflux for 1 h. The PPT crystals were filtered off, washed with ethanol, recrystallized from absolute ethanol and finally dried in a vacuum desiccators over anhydrous CaCl<sub>2</sub>.

The foaming reagents tested for the procedure were sodium palmitate (NaPL), sodium stearate (NaST), oleic acid (HOL), cetyltrimethylammonium bromide (CTAB) and Tween 80. Tween 80 was made as 0.5% aqueous, while NaPL, NaST as 99.7% propanolic solution. CTAB was used as 95% ethanolic solution. An oleic acid (HOL) stock solution ( $6.36 \times 10^{-2}$  mol L<sup>-1</sup>) was prepared by dispersing 20 mL of HOL food grade (*d* 0.895), in 1 L kerosene.

Zinc stock solution (1000 mg L<sup>-1</sup>) was prepared by dissolving 1.000 g of zinc metal in 15 mL of 1 : 1 HCl and dilute to 1 L with doubly distilled water (DDW). All other reagents used were of analytical reagent grade. Doubly distilled water (DDW) was used throughout this work.

To check the accuracy and precision of the proposed flotation procedure a certified reference material was analyzed viz.: stream sediment SARM 52 prepared by MINTEX (Ranburg, Republic of South Africa).

Pharmaceutical samples like Mineravit (E.I.P.I. Co.), Megavit Zinc (Amriya Pharm. Ind.) and Vitaplus Zinc (CID) were selected for the analysis of Zn(II).

**Instrumentation:** Two types of flotation cells were used throughout this study as it has already been described.<sup>20-28</sup>

The concentration of Zn(II) was determined using a Unicam UV 2100 UV/vis spectrometer and was confirmed by flame AAS measurements at 214 nm with a Perkin-Elmer 2380 atomic absorption spectrometer (USA) with a Pye Unicam (England) hollow cathode lamp and a conventional 10 cm slit burner head for air acetylene flame.

Adjustment of pH of the sample solutions was carried out in the range of 1-10 using 0.1 mol L<sup>-1</sup> HCl and/or 0.5

mol L<sup>-1</sup> NaOH. To monitor the pH of the media, a Hanna Instruments 8515 digital pH meter was used.

### Analytical procedures.

**Flotation-separation of Zn(II):** In an Erlenmeyer flask, 2.0 mL of  $1 \times 10^{-3}$  mol L<sup>-1</sup> PPT solution was added to an aliquot containing  $2 \times 10^{-5}$  mol L<sup>-1</sup> Zn(II) and 1 mL of 0.05 mol L<sup>-1</sup> Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>; the pH was adjusted to 6.0. The mixture was shaken well for few seconds to allow complete complexation of PPT with Zn(II) ions. A red color of Zn-PPT complex developed instantaneously. All contents were quantitatively transferred into flotation cell and its volume was adjusted to 10 mL. Then, 3 mL of  $2 \times 10^{-4}$  mol L<sup>-1</sup> HOL was added. The flotation cell was shaken upside down for 2 min by hand. Vigorous shaking of the flotation cell in the presence of a surfactant (HOL) creates bubbles in the solution which enhance the floatability of Zn-PPT complex. At equilibrium, a foamy layer was obtained and the aqueous solution in the cell became completely cleared of the colored complex. The processed aqueous phase was run off through the bottom of the cell. The scum layer, in which Zn(II) was concentrated, was taken into a small vial to determine Zn(II) by spectrophotometric and AAS measurements.

**Spectral detection:** A suitable volume of the scum layer was transferred to a 2-cm quartz cell and the absorbance was measured at 526 nm against a reagent blank to determine the concentration of Zn(II).

To determine the concentration of Zn(II) by flame AAS, the scum layer was stripped with 2 mL of (1 : 1) HNO<sub>3</sub> and the resultant clear solution was aspirated directly to the flame to measure Zn(II) concentration at 214.0 nm.

The flotation efficiency (F, %) was calculated from the relation

$$F = (C_s/C_i) \times 100$$

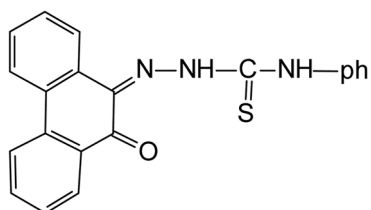
Here C<sub>i</sub> and C<sub>s</sub> are the concentration of Zn(II) in the initial aqueous and scum layers, respectively.

### Sample analysis.

**Analysis of water samples (Recovery test):** Tap water, Nile River water and sea water samples were collected from the city of Mansoura (Mansoura is an agro-industrial area in the Nile Delta, Egypt) and its neighborhood. Samples were filtered using a 0.45 μm pore size membrane filter to remove any suspended particulate matter and immediately treated with few milliliters of conc. HNO<sub>3</sub> to prevent possible hydrolytic precipitation of some mineral salts.

Different concentrations of Zn(II) viz.: 0.5 μg mL<sup>-1</sup> and 1 μg mL<sup>-1</sup>, were introduced to 20 mL aliquots of water samples, and 2 mL of 2.5X 10<sup>-3</sup> mol L<sup>-1</sup> PPT were added. The same previous steps of flotation, metal stripping and spectrophotometry were carried out and the recovery percentages were calculated.

**Analysis of human biofluids:** Blood serum and urine samples were collected from 10 healthy children and from 10 cases of Nephrotic Syndrome (NS). Serum and urine samples (5 mL each) were acidified using 2 mL of HClO<sub>4</sub> : HNO<sub>3</sub> (1 : 3) mixture, boiled to near dryness, dissolved in DDW and brought to 50 mL in a calibrated flask. Into



Scheme 1

flotation cell (a), 2 mL of the cold solution of the pretreated sample was introduced and the flotation process was completed as before.

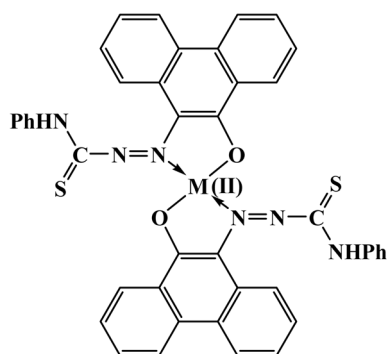
**Analysis of pharmaceutical samples:** The selected zinc containing drug samples were brought into solution by adopting the following procedure. Each sample was treated separately with concentrated nitric acid on a hot-plate, at a low temperature, to avoid violent spurting. The residue of each sample was cooled and again 1 : 1 nitric acid is added. The temperature of the hot-plate was gradually increased to 300 °C. The residue obtained was dissolved in nitric acid (1 : 1) and was slowly heated for 2 h to procure a dry mass. Finally, the residue was dissolved in a minimum amount of DDW. The sample solution was quantitatively transferred into a 100 mL volumetric flask and then made to the mark with DDW and analyzed for Zn(II), by using the recommended general procedure.

## Results and Discussion

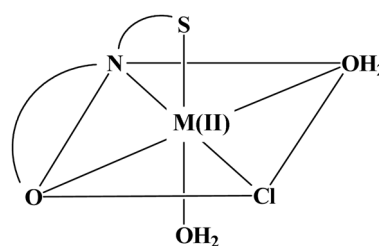
**PPT and its metal complexes.** If we consider the structure of the chromogen PPT, Scheme 1, and its metal complexes, Scheme 2 and 3, it is apparent that the chromogen molecule contains a number of reactive sites involving -N-NH, C=S and C-NH in association with the condensed benzenoid rings, which are known to chelate strongly with a whole raft of elements in Groups 1b, 2b, 3b, 4b, 5b and 6b of Periods 4, 5 and 6 of the Periodic Classification, with chelation through the N, S and/or O atoms.

PPT can react with several ions as a bidentate ligand (Scheme 2), in the thione form coordinating via N=N and the enolic carbenyl oxygen with the displacement of hydrogen atom from the latter group to form hydrophobic complexes.<sup>22</sup> PPT can also act as a tridentate ligand (Scheme 3) in the enolthione form coordinating via the C=S, N=N and the enolic carbonyl OH with the displacement of hydrogen atom from the latter group.<sup>23</sup>

In general, PPT has the ability to form a stable complex of 1 : 2 molar ratio with most divalent metal ions. However, Khalifa *et al.*,<sup>23</sup> showed that two kinds of complexes could be formed with a copper ion: Cu: PPT in the aqueous solution of pH 3.0-3.5 and the Cu(PPT)<sub>2</sub> at pH ≥ 6.5.



Scheme 2



Scheme 3. PPT as a tridentate ligand.

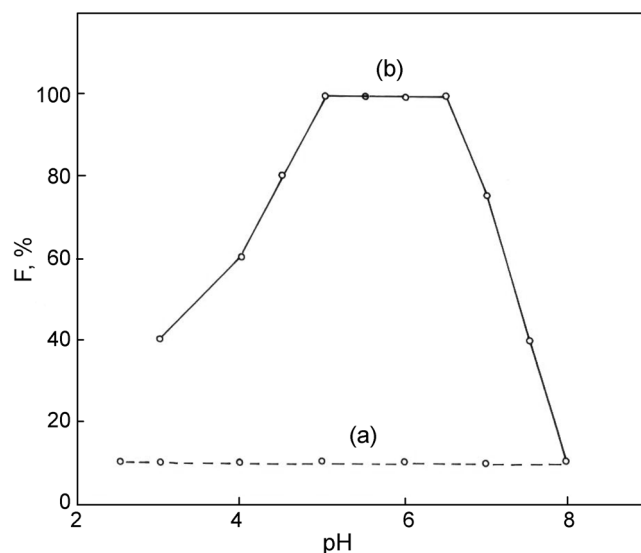


Figure 1. Influence of pH values on the flotation efficiency (F, %) of Zn(II): (a) in the absence of PPT and (b)  $2.5 \times 10^{-3}$  mol L<sup>-1</sup> PPT and  $2 \times 10^{-4}$  mol L<sup>-1</sup> HOL.

### Influence of experimental variables.

**Influence of pH:** The pH of solution is a very important factor for metal chelate formation and for the flotation process. Therefore, the effect of the pH on the flotation of Zn-PPT chelate was studied in the pH values ranging from 2.0 to 10. The results are shown in Figure 1. In the absence of PPT (graph a) the flotation efficiency of Zn(II) is very low over the pH range tested. Maximum flotation efficiency (> 95%) was obtained for Zn with PPT over pH values ranging from 4.5 to 7.5. The role of the reagent PPT is quite evident from comparison of graphs (a) and (b). The PPT reagent forms Zn-PPT complex with zinc ions rendering them more hydrophobic and easily separated from the solution bulk. At higher pH values the decrease in the flotation efficiency may be attributed to the formation of a white emulsion and due to the formation of excessive foams of sodium oleate. This will hinder the reaction to complete. Additionally, zinc can form hydroxide precipitates in a strongly basic solution.

**Selection of a suitable surfactant:** The addition of foaming agents is very advantageous because they transform the hydrophilic surface of the species floating in hydrophobic and so they facilitate the separation of the formed metal chelate from the aqueous phase to the scum layer.

To select properly an appropriate surfactant for the pro-

**Table 1.** Applicability of different foaming reagents for ion flotation of zinc using PPT as a complexing agent ( $Zn(II) = 10 \mu\text{g}$ ,  $PPT = 2 \times 10^{-3} \text{ mol L}^{-1}$ ,  $I_c = 0.01 \text{ mol L}^{-1}$ ,  $pH = 6.0$  at  $25^\circ\text{C}$ )

Surfactant	Recovery, R %
1. Non ionic	
- Tween 80	Foam, no flotation
2. Cationic	
- CTAB	Foam, no flotation
3. Anionic	
i. Single	
- NaST	80
- HOL	100
- NaPL	90
ii. In pairs	
- NaST + NaPL	85
- HOL + NaPL	80

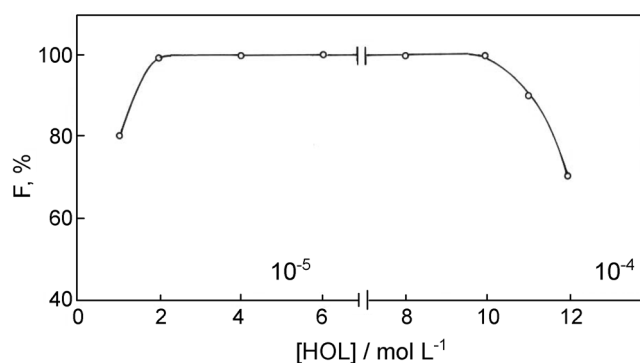
cedure, several foaming reagents were tested at pH 6.0 using  $2.5 \times 10^{-3} \text{ mol L}^{-1}$  PPT and 5 mL of surfactant solution (with specified concentration for each one).

It was found that the cationic surfactant (CTAB) and the non ionic tenside (Tween 80) were inadequate. They frothed well at pH 6.0, but they didn't help to separate the Zn-PPT complex from the processed solution. The surface of the formed Zn-PPT complex particles might be of the same charge as that of the CTAB cationic surfactant causing their ineffectiveness compared to the anionic surfactants used.

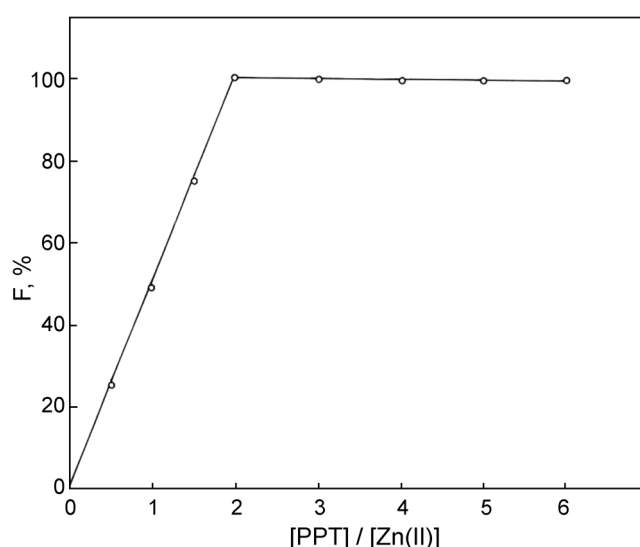
Among the anionic surfactants singly tested (NaPL, NaST and HOL) and in pairs (NaST/NaPL and HOL/NaPL), HOL was the most helpful (Table 1), achieving the highest recovery value of  $\sim 100\%$ . The results obtained by the pairs NaST/NaPL and HOL/NaPL were also satisfactory, but this combination of agents formed too much scum, which was very difficult to decompose by acid. When HOL was used as a single agent the stripping of Zn from the scum layer was easier and faster. Therefore, HOL was selected for the procedure.

**Influence of oleic acid (HOL) concentration:** The concentration of HOL is an important parameter; up to a certain limit the flotation efficiency (F,%) increases as the concentration of HOL increases. Figure 2, shows that the flotation efficiency of Zn(II) gradually increases as the concentration of HOL increases reaching  $\sim 100\%$  over a wide range of HOL concentration ( $2 \times 10^{-5}$ - $8 \times 10^{-4} \text{ mol L}^{-1}$ ). At a higher HOL concentration the surfactant molecules will gather together to form a microball called a micelle.<sup>32</sup> Micelles compete with the colligend molecule (Zn-PPT complex) and since they stay in solution, they reduce the effectiveness of separation. Also, the concentration of surfactant changes the bubble size with the size getting smaller as the surfactant increases. This makes creamier foam.<sup>32</sup> A suitable concentration of HOL ( $2 \times 10^{-4} \text{ mol L}^{-1}$ ) was used throughout this work.

**Influence of PPT concentration:** A sufficient amount of the ligand is needed for the complex formation of the trace metal ions under other optimum conditions. The flotation



**Figure 2.** Influence of oleic acid (HOL) concentration on the flotation efficiency (F,%) of Zn(II).

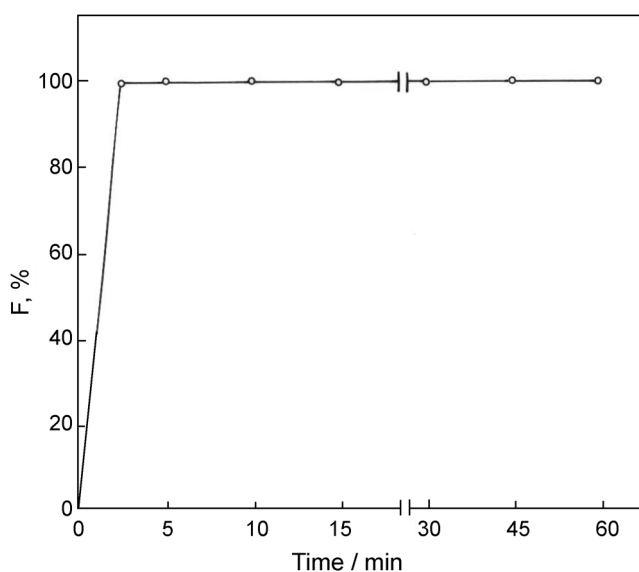


**Figure 3.** Influence of the phenanthraquinone monophenyl thio-semi-carbazone reagent (PPT) concentration.

efficiency (F, %) was measured in aqueous solution of various PPT ( $n = 5$ ) concentrations; the results are shown in Figure 3 as a function of  $[PPT]_{(aq,i)}/[Zn(II)]_{(aq,i)}$ . The figure clearly shows that (1) the flotation of Zn(II) becomes possible in the presence of PPT; (2) the value for (F, %) increases with an increase in the PPT concentration and reaches  $\sim 100\%$  at 2.0 showing clear abrupt points. Based on these findings, it was estimated that Zn(II) was floated as  $Zn : PPT = 1 : 2$  ( $n = 5$ ) complex. Figure 3, also shows that increasing the concentration of PPT doesn't affect the flotation process. A concentration of  $2.5 \times 10^{-3} \text{ mol L}^{-1}$  PPT was chosen to account for other floatable species in real samples.

**Influence of temperature:** Maximum flotation efficiency was achieved at temperature values ranging from 20 to  $45^\circ\text{C}$ . The proposed flotation procedure was performed at room temperature *i.e.*  $\sim 25^\circ\text{C}$ .

**Influence of shaking time:** Figure 4, shows the influence of shaking time of the original solutions (*i.e.* before flotation) on the flotation efficiency of Zn(II) at pH 6.0 in the presence of  $2.5 \times 10^{-3} \text{ mol L}^{-1}$  PPT and then adding  $2 \times 10^{-4} \text{ mol L}^{-1}$  HOL at room temperature. As it can be noticed, the



**Figure 4.** Influence of shaking time on the flotation efficiency (F, %) of Zn(II) in the presence of  $2.5 \times 10^{-3}$  mol L<sup>-1</sup> PPT and  $2 \times 10^{-4}$  mol L<sup>-1</sup> HOL.

flotation efficiency reaches its maximum (~100%) after about 2 min and remains constant. Proper shaking allowed the successful complexation of PPT and Zn(II) to form hydrophobic Zn-PPT complex that is easily floated by HOL. A shaking time of 3 min was used in the subsequent experiments.

**Effect of ionic strength:** The effect of the ionic strength ( $I_c$ ) on Zn(II) floatability was studied by using different ionic strength adjusters. The results of this investigation are given in Table 2. The data proved that the flotation recoveries of zinc show only a slight variation over a wide concentration range of the ionic strength.

**Influence of volume:** A series of experiments was conducted to float different concentrations of Zn(II) from different aqueous volumes using suitable large flotation cells under the recommended conditions. The results obtained revealed that, up to 10  $\mu$ g of Zn(II) could be quantitatively separated from one liter into 10 mL of HOL with a pre-concentration factor of 100. Accordingly, the detection limit

**Table 2.** Dependence of the zinc flotation recoveries on the ionic strength ( $I_c$ ) of the solution

Ionic strength ( $I_c$ ), mol L <sup>-1</sup>	Adjuster	Recovery, %
0.01	NaCl	100.0
	Na <sub>2</sub> SO <sub>4</sub>	99.5
	MgCl <sub>2</sub>	99.0
0.05	NaCl	99.5
	Na <sub>2</sub> SO <sub>4</sub>	99.0
	MgCl <sub>2</sub>	98.0
0.50	NaCl	99.6
	Na <sub>2</sub> SO <sub>4</sub>	98.5
	MgCl <sub>2</sub>	98.2

**Table 3.** Influence of foreign ions on the pre-concentration and determination of Zn(II), 0.2  $\mu$ g. Tolerance error (5%)

Foreign ions	Concentration, mg L <sup>-1</sup>	Recovery, R %
K <sup>+</sup>	$1 \times 10^4$	104
Na <sup>+</sup>	$1 \times 10^4$	103
Ca <sup>2+</sup>	$1 \times 10^3$	101
Mg <sup>2+</sup>	$1 \times 10^3$	96
Al <sup>3+</sup>	10	99
Fe <sup>3+</sup>	0.5 <sup>b</sup>	99
Cd <sup>2+</sup>	0.5 <sup>a</sup>	99
Co <sup>2+</sup>	0.5	98
Pd <sup>2+</sup>	0.5	97
Hg <sup>2+</sup>	0.5	99
Cu <sup>2+</sup>	0.5	96
Pb <sup>2+</sup>	0.5	100
Ni <sup>2+</sup>	0.5	99
Mn <sup>2+</sup>	0.5	100

<sup>a</sup>After addition of 1 mL of 0.05 mol L<sup>-1</sup> Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, <sup>b</sup>After addition of 1 mL 0.05 mol L<sup>-1</sup> ascorbic acid.

of such a separation procedure is 0.01  $\mu$ g mL<sup>-1</sup>.

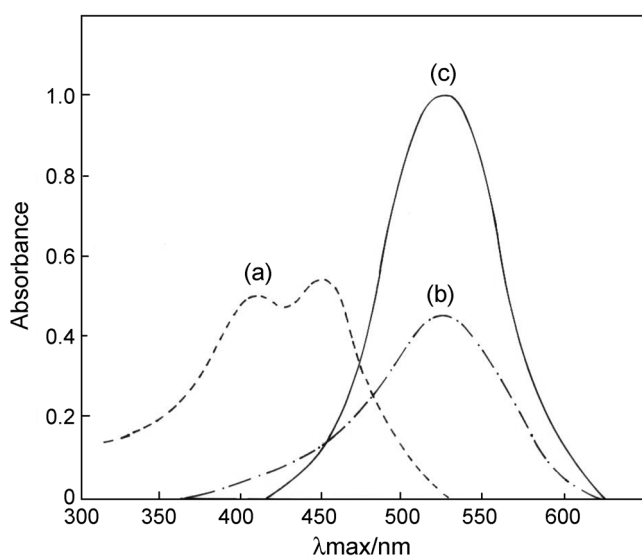
**Interfering effects of various foreign ions:** The effect of foreign metal ions on the flotation of Zn(II) with PPT has been studied in detail. Solutions containing 0.2  $\mu$ g of zinc(II) in 25 mL solution and various excesses of several cations and anions were prepared and the recommended flotation procedure was followed. The criterion for interference was fixed at a 5% variation of the average recovery percentage (R, %), calculated for the established level of zinc. The obtained results are shown in Table 3. The experimental data showed that Na(I), K(I), Ca(II), Mg(II), and Al(III) don't interfere in the recovery of Zn(II) whereas Cd(II), Cu(II), Hg(II), Fe(III) and Pd(II) have little interfering effects (~2%). All of these interferences were completely controlled by adding excess PPT ( $2 \times 10^{-3}$  mol L<sup>-1</sup>) and/or masking agents. In this regard, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> was used to suppress the interferences of Cd(II), Pd(II), Hg(II) and Cu(II). Fe(III) was reduced to Fe(II) by ascorbic acid before flotation of Zn(II). Cadmium forms analogous compound of the same color of Zn-PPT complex, but it was found that upon using the method of simultaneous equation,<sup>33</sup> 0.2  $\mu$ g of Zn(II) was successfully determined in the presence of 0.5 mg L<sup>-1</sup> of Cd(II). Anions such as acetate, Cl<sup>-</sup>, PO<sub>3</sub><sup>-</sup>, ClO<sub>4</sub><sup>-</sup>, I<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, oxalate, citrate and tartarate do not interfere.

Moreover, the effects of the same studied foreign ions on the flame AAS determination of Zn(II), were investigated. No single anion or cation caused a variation in the response by more than  $\pm 5\%$ . It is of great interest to note that the selected investigated elements (usually present as main element and traces in water and/or blood) have no deleterious effect on the analyte separation and determination under the recommended conditions.

#### Characterization of Zn-PPT complex.

##### Absorption spectra of PPT and Zn-PPT complexes:

The absorption spectra of the PPT, Zn-PPT complex in the aqueous and in the scum layer, are shown in Figure 5. PPT has two characteristic maxima at 410 nm and 450 nm (graph



**Figure 5.** Absorption spectra of (a) PPT in aqueous acetone solution, (b) Zn-PPT complex in aqueous solution and (c) Zn-PPT complex in oleic acid (HOL) layer.

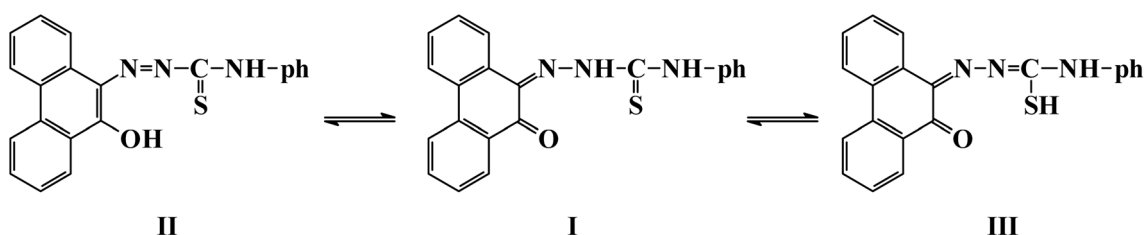
a). The presence of these two maxima is attributed to the keto enol and thioenol equilibrium (Scheme 4).

The absorption spectrum of Zn(II)-PPT complex is completely different from that of PPT. It is observed that (i) the  $\lambda_{\max}$  of Zn-PPT exhibits a red shift of 76 nm from that of PPT (curve b); (ii) only a single Zn-PPT chelate is formed in the aqueous and in the scum layer; having a maximum absorbance at 526 nm and (iii) the absorbance of Zn-PPT in the HOL layer (curve c) is much more than that of Zn-PPT in the aqueous solution (curve b). This proves that the species are highly concentrated in the HOL layer.

PPT is yellowish in color. Chelated with Zn(II), PPT changes its color drastically to red color in accordance with the assumption that zinc ions are bonded to PPT by complex formation.

**Stability of Zn-PPT chromogenic system:** The absorbance of the Zn-PPT complex reaches a maximum value instantly at room temperature and remains constant for 48 h at least. The stability constant was calculated to be  $1.26 \times 10^{11}$ .

**Composition of the floated Zn-PPT complex.** The stoichiometry of the Zn-PPT complex was studied under the established experimental conditions by the continuous variation ratio. The results obtained indicated that the composition of the complex was 1 : 2 (Zn<sup>2+</sup> : PPT), Scheme 2.



**Scheme 4.** Reaction equilibrium.

**Analytical figures of merit.** The effective molar absorption was calculated from the data obtained by the measurements of the organic phase absorbance as the condition of flotation was completed. The calibration graph obtained was a straight line passing through the origin over the concentration range mentioned above. The effective molar absorption coefficient ( $\epsilon$ ) at  $\lambda_{\max}$  526 nm was  $1.83 \times 10^5$  L mol<sup>-1</sup> cm<sup>-1</sup>. The Zn-PPT complex obeys Beer's law from 0.05 to 1.0 mg L<sup>-1</sup> with an optimum range.

In order to validate the Zn(II) flotation procedure, the proposed method was applied to a certified reference material *viz.* stream sediment SARM 52 prepared by MINTEK (Ranburg, Republic of South Africa); certified zinc concentration was 1.32 ppm. Analytical recoveries were also determined. The average content found ( $n = 5$ ) in the reference material was 1.28 ppm with a precision as relative standard deviation, RSD, up to 5.0%. The average analytical recovery ( $n = 5$ ) obtained was about 98.5 %.

**Comparison between PPT and other principal reagents for zinc determination.** The proposed PPT reagent can be compared to some common previously reported reagents: dithizone, pyridyl azonaphthol (PAN) pyridylazoresorcinol (PAR)<sup>34-36</sup> and TAN<sup>37</sup> reagents. In the dithizonemethod, the molar absorptivity of zinc dithizonate in CCl<sub>4</sub> solution (at  $\lambda_{\max} = 538$  nm) is  $9.26 \times 10^4$  L mol<sup>-1</sup> cm<sup>-1</sup>, zinc is extracted in a double batch step and the extraction process is relatively slow. In the PAN method, the molar absorptivity of the chloroform solution of the zinc-PAN complex is  $5.2 \times 10^4$  L mol<sup>-1</sup> cm<sup>-1</sup> at  $\lambda_{\max} = 560$  nm), the reagent absorbs little at the  $\lambda_{\max}$  of the zinc complex. On the other hand, PAR allowed zinc to be determined with, a sensitivity similar to that for the PAN method. TAN was also used to extract Zn(II) into chloroform with a molar absorptivity of  $5.0 \times 10^4$  L mol<sup>-1</sup> cm<sup>-1</sup> at  $\lambda_{\max} = 581$  nm. In the present flotation methodology, the molar absorptivity of zinc(II)-PPT complex in the scum layer is  $1.83 \times 10^5$  L mol<sup>-1</sup> cm<sup>-1</sup> (at  $\lambda_{\max} = 526$  nm), zinc is separated in a single batch, the reagent PPT does not absorb at the  $\lambda_{\max}$  of zinc-PPT complex, the flotation process is rapid (< 10 min) and the scum layer can easily be measured spectrophotometrically or aspirated directly into the flame for AAS determination after elution. The hazardous effects of chloroform are completely avoided.

On the other hand, there are different reports for Zn(II) separation by colloid precipitate flotation<sup>9-13</sup> or by ion flotation.<sup>14-17</sup> In these reports Zn(II) was separated using different reagents such as aluminium hydroxide,<sup>9</sup> hydrated iron hydroxide (Fe<sub>2</sub>O<sub>2</sub>·xH<sub>2</sub>O),<sup>10</sup> iron(III)tetramethylene-dithio-

carbamate [Fe(TMDTC)<sub>3</sub>],<sup>11</sup> ferric hydroxide and aluminum hydroxide, cobalt(hexamethylenedithiocarbamate) [Co-(HMDTC)<sub>3</sub>],<sup>12</sup> iron(hexamethylenedithiocarbamate) [Fe-(HMDTC)<sub>3</sub>],<sup>13</sup> 1-Naphthoic acid<sup>15</sup> and 1,10-Phenanthroline.<sup>16</sup> The figures of merits (detection limits, precision and recovery percentage) of the proposed ion flotation procedure are in a good comparison with those of previously reported reagents. Moreover, the color of Zn-PPT complex formed remains stable for long time which facilitates the simple measurements by the very simple spectrophotometry.

**Mechanism of flotation.** In studies concerning separation via flotation, the role of surfactant is very important. The nature of the interaction between oleic acid surfactant and the complex formed (Zn-PPT) must be studied to approach the actual mechanism of flotation. The proposed mechanism may proceed through: i) a physical interaction; ii) by forming a hydrogen bond between the hydrophilic part of HOL and the active sites in the ligand complex or iii) by an interaction between oleic acid and the complex, formed in solution through a coordinate bond forming a self-floatable (Zn-PPT-HOL) species. In all cases, the hydrophobic part of the surfactant attaches to air bubbles and floats separating the analyte-containing species.

In the present investigation, the mechanism of flotation is proposed to be physical in nature. This suggestion has been confirmed based on the following observations and experimental data: 1) The floated species has the same colour (red) as that obtained in the aqueous solution (especially in high reactants concentrations). 2) Raising the temperature above 45 °C causes a marked decrease in the flotation efficiency of Zn(II). This may be attributed to the weak bonding between the oleic acid and the Zn(II)-PPT complex that is easily destroyed upon heating.

### Analytical application

**Determination of zinc in human biofluids.** The determination of the total zinc level in human serum and urine samples was carried out by spectrophotometry and AAS after the flotation preconcentration was carried out. The serum and urine samples were collected from a healthy child (among healthy control group). The analytical results are summarized in Table 4, together with the relative standard deviations (RSDs) of the observed values estimated from three replicate analyses. In Table 4, the analytical results for

**Table 4.** Analytical results of total zinc in human blood serum and urine samples

Sample	Intra-day assay <sup>a</sup>		Inter-day assay <sup>b</sup>	
	Observed value <sup>c</sup>	RSD <sup>d</sup> (%)	Observed value	RSD (%)
Blood serum µg/dl	120	2.5	125	5.0
Urine, µg/dl	21	2.1	24	3.8

<sup>a</sup>Three replicate analyses on a single day. <sup>b</sup>Three replicate analyses on three different days. <sup>c</sup>Mean (n = 3). <sup>d</sup>Relative standard deviation was evaluated from three replicate analyses.

intra-day assay (three replicate measurements on a single day) and the inter-day assay (three replicate measurements on each of three days) measurements are shown for comparison. As is seen in Table 4, the RSDs of each observed value in the intra-assay measurements was below 2.5% while that in the inter-assay measurements was below 5%. Thus, the present flotation preconcentration method is considered to be sufficiently precise for the quantification of zinc in human biofluids.

The analytical results for blood serum and urine samples of children with nephrotic syndrome are shown in Table 5. The mean value of total content of zinc in the serum of children with nephrotic syndrome (NS) children was lower than the mean value for healthy controls. Hypozincaemia in NS may attributed to the protein loss especially albumin (since 60-70% of zinc is carried over albumin.<sup>38</sup> On the other hand, the mean value of the total zinc concentration in urine of NS is higher than the mean value for healthy control.

**Recovery yields of zinc in natural water samples.** The developed procedure for the determination of Zn(II) using a flotation methodology and spectrophotometry was examined

**Table 6.** Recovery (R, %) of known Zn(II) concentrations added to different water samples

Water sample	Zn(II) / µg mL <sup>-1</sup>		Recovery (%)	RSD, %
	Added	Found		
Tap water	0.5	0.51	102	2.9
(Mansoura City)	1.0	1.03	103	3.2
Nile River	0.5	0.48	96	4.3
(Mansoura City)	1.0	0.98	98	2.4
Sea Water	0.5	0.52	104	3.2
(Ras El-Bar)	1.0	1.02	102	1.3

Mean values were obtained using seven known samples

**Table 5.** Analytical results for total Zn(II) in human blood serum and urine samples collected from 10 healthy children and 10 cases of primary nephrotic syndrome (PNS)

Sample	Spectrophotometry		AAS		Sample Source <sup>c</sup>
	Observed value <sup>a</sup>	RSD <sup>b</sup> (%)	Observed value	RSD (%)	
Blood serum, µg/dl	90 [83-119.5]	2.5	89	4.0	PNS <sup>d</sup> (M,F, 1-16Y)
Urine, µg/dl	42.5 [30.5-62.5]	3.0	44	1.5	PNS (M,F, 1-16Y)
Blood serum, µg/dl	124.5 [95-150]	4.0	125	3.0	Healthy controls (M,F, 1-16Y)
Urine, µg/dl	23.4 [17.5-28.0]	2.7	22.5	2.5	Healthy controls (M,F, 1-16Y)

<sup>a</sup>Mean (n = 10). The values in square parentheses indicate the concentration range of blood serum and urine samples. <sup>b</sup>Relative standard deviation. <sup>c</sup>Samples were collected from Mansoura University Hospital of Children (Mansoura, Egypt). <sup>d</sup>Primary Nephrotic Syndrome: a clinical state characterized by heavy proteinuria and hypoalbuminaemia; often associated with odema, hypercholesterolaemia and hyperlipidaemia.

**Table 7.** Determination of Zn(II) in some pharmaceutical samples by the proposed flotation technique

Drug (Source)	Spectrophotometry		AAS	
	Observed value <sup>b</sup>	RSD <sup>c</sup> (%)	Observed value	RSD (%)
Mineravit (E.I.P.I.Co.)	15.2 (15.0) <sup>d</sup>	3.0	14.9	2.9
Megavit Zinc (Amriya Pharm. Ind)	24.2 (23.9)	2.7	23.7	3.2
Vitaplus Zinc (CID)	18.3 (18.2)	3.6	18.0	2.9

<sup>d</sup>Calculated values (mg/capsule), <sup>b</sup>Mean (n = 3), <sup>c</sup>Relative Standard Deviation.

for several natural-water samples, Table 6. The recoveries of spiked known additions to different water samples lay within the range 96-104%.

**Determination of Zn(II) in some pharmaceutical samples.** The proposed flotation procedure was successfully applied to the determination of Zn(II) in some zinc-containing drugs, Table 7, with satisfactory results.

### Conclusion

In the present study zinc(II) has been enriched and separated successfully from media of diverse origin by ion flotation using PPT, as a complexing agent, prior to its spectrophotometric and AAS determination. The recommended preconcentration procedure is fast (10 min). The necessary equipment for flotation is simple and inexpensive. The use of a small amount of surfactant and tiny gas bubbles (vigorous shaking) necessary to perform a proper flotation cannot permit any serious contamination risks, which could be manifested by high blank volumes.

In the present investigation, the author is introducing PPT as a new reagent to the field of flotation-separation and spectrometric determination of zinc(II) in media of diverse origin. The molar absorptivity value of the complex Zn-PPT ( $1.83 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ ) reveals that the reagent PPT is comparable to or more sensitive for zinc(II) than some other principal reagents for zinc determination.<sup>9-17</sup>

One of the most important advantages of the recommended method is the ability to analyze a larger volume of sample solution and to obtain a great preconcentration factor (100), as well as excellent recoveries of the trace element investigated. The effective flotation using only HOL indicates that the addition of PPT makes the sublimate sufficiently hydrophobic and doesn't need two surfactants. The use of only one foaming agent needs a less amount of conc. HNO<sub>3</sub> (2 mL) and makes the matrix of the final solutions tested by flame AAS less complex. A number of associated elements don't interfere with zinc(II) determination. The selectivity of the reagent is also improved by use of excess amounts of PPT and/or masking agents like thiosulfate to suppress the interference of some metal ions such as Hg(II), Cd(II) and Cu(II). This method has great potentiality for the successful determination of Zn(II) in pharmaceutical, biological and environmental samples.

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