Spectrophotometric determination of zinc in foods using N-ethyl-3-carbazolecarboxaldehyde-3-thiosemicarbazone: Evaluation of a new analytical reagent

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Abstract

N-ethyl-3-carbazolecarboxaldehyde-3-thiosemicarbazone (ECCT) is proposed as a new sensitive reagent for the extractive spectrophotometric determination of zinc(II). The ECCT forms yellow colored species of zinc(II) at pH range 3.0–5.5 and the complex was extracted into benzene. The Zn(II)–ECCT complex shows maximum absorbance at 420 nm with molar absorptivity and Sandell's sensitivity being 1.55×10^4 lit mol^{-1} cm^{-1} and 4.212×10^{-3} μg cm^{-2}, respectively. The system obeys Beer's law in the range of 0.4–6.0 mg/l, with an excellent linearity in terms of correlation coefficient value of 0.999. Most of the common metal ions generally found associated with zinc do not interfere. The repeatability of the method was checked by finding relative standard deviation (RSD). The developed method has been successfully employed for the determination of zinc(II) in foods. Various certified reference materials (NIST 1573, NBS 1572 and NIST SRM 8435) have been tested for the determination of zinc for the purpose of validation of the present method.
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Keywords: N-ethyl-3-carbazolecarboxaldehyde-3-thiosemicarbazone; Zinc(II); Extractive spectrophotometry; Foods

1. Introduction

Zinc occurs exclusively in +2 oxidation state. Zinc deposits are not only important commercially for the zinc they contain, but also because of their association with other valuable elements. About one-third of the present zinc production goes into the galvanizing of ferrous metals. Brass alloys consume another one-third of the world zinc production, while the remaining zinc is converted into a number of chemical products (Fisher, 1975; Giroux, Durieux, & Schechter, 1976; William, 1983).

Zinc is an essential element in the nutrition of animals including human beings (Brunborg, Julshamn, Nortvedt, & Frøyland, 2006; Saun, 2005). It acts as a cofactor innumerous of enzymes and plays an important role in protein synthesis and cell division. It exerts a crucial influence on maintenance of cell membrane stability and function of immune system. 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, poisoning incidents with symptoms of gastrointestinal distress, nausea and diarrhea are reported (Hernick & Fierke, 2005). Zinc is present in many foods, soil and is also found in a number of pharmaceutical samples, causing environmental pollution. Concentration of zinc greater than 5.0 mg/l affects the potable water nature in alkaline waters. It is clear that zinc is an essential element and has significant importance, both biologically and industrially. When the quantity is more than what is required, zinc produces toxic effects. Hence, separation and determination of zinc(II) from its associated metal ions is indispensable.

Thio- and phenyl thiosemicarbazones are important sulphur and nitrogen containing organic reagents find a wide

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range of applications in medicine (Hall et al., 2000) and agriculture, where zinc co-ordinates with these reagents to form stable complexes. The revived (Casas, Garcia-Tasende, & Sordo, 2000; Garg & Jain, 1988; Singh, Garg, & Singh, 1978) literature revealed that only a few thio- and phenyl thiosemicarbazones were employed for extractive determination of zinc(II). The review of literature indicates only a few thiosemicarbazones have been exploited for the spectrophotometric determination of zinc(II). Not much attention has been paid for the extractive spectrophotometric determination of zinc(II) with thiosemicarbazones or other organic chelating reagents (Benamor, Belhamel, & Draa, 2000; Herrador, Jimenez, & Asuero, 1987; Hoshi, Yotsuyanagi, & Aomura, 1977; Leyva, Pavon, & Pino, 1972; Nevado, Leyva, & Ceba, 1976; Sarma, Kumar, Reddy, Kumar, & Reddy, 2002) this has prompted the researchers to make a systematic investigation for utilizing N-ethyl-3-carbazolecarboxaldehyde-3-thiosemicarbazone (ECCT) first time for the extractive spectrophotometric determination of zinc(II) in microgram quantities. The title reagent ECCT was proved more stability when complexed with Zn(II) and found that the color of the complex is stable for more than 48 h. ECCT was extracted zinc selectively when associated with following metal ions Mn(II), Mg(II), Mo(VI), W(VI) Ca(II), Cr(III), Fe(II) and Zr(IV), it indicates that ECCT was stable reagent for extractive determination of zinc(II). ECCT is cheap, stable in high temperatures, easier to dispense and store. In our previous studies, we are developed the new analytical methods for determining the transition metals from various samples like environmental, biological and pharmaceutical samples using thio- and phenylthiocarbazones (Sarma et al., 2002, 2003, 2005; Reddy et al., 2004, 2003, 2002). In our ongoing research work, now we reporting the new analytical method for the determination of zinc(II) in foods using ECCT as a complexing reagent.

The analysis of zinc from various foods was recently studied by (Reddy, Kumar, Sarma, & Reddy, 2002) and (Sarma et al., 2006) using benzildithiosemicarbazone (BDTSC) and pyridioxal-4-phenyl-3-thiosemicarbazone (PPT) as chelating reagents by spectrophotometrically and other researchers reported on zinc recovery from food staffs by various analytical techniques (Martínez, Rincón, & Ibáñez, 2006; Ruz et al., 2006; Santelli, Bezerra, SantAna, Cassella, & Ferreira, 2005).

## 2. Materials and methods

2.1. Apparatus

Shimadzu 240 UV–Vis spectrophotometer with 1.0 cm quartz cell was used for absorbance studies. An Elico LI-120 digital pH meter was used for pH adjustment. A Perkin–Elmer 2380 atomic absorption spectrometer was used for the comparison of results.

2.2. Reagents

A 0.5 g N-ethylcarbazolecarboxaldehyde (ECC) was dissolved in 25.0 mL ethanol and mixed in a flask containing 1.5 g thiosemicarbazone dissolved in 25.0 mL of 1:1 ethanol–water mixture. The resulting reaction mixture was refluxed on water bath for 2 h. It was allowed to stand at room temperature until pale yellow crystals were formed (Cristofol, Rojas, & Pavon, 1991). These were separated and recrystallized from ethanol (Scheme 1).

Molecular formula and molecular weight of ECCT are $C_{16}H_{16}N_4S$, 296, respectively. ^{1}H NMR data (CDCl_3/DMSO): δ 1.43 (t, 3H, –CH_2–CH_3); 4.41 (q, 2H, –CH_2–CH_3); 7.18–8.48 (m, 9H, Ar–H and –NH_2); 9.16 (s, 1H, –CH=N), 11.62 (s, 1H, NH). IR Data (KBr): The ECC stretching frequency was observed at 1678 cm^{-1}. After reacting ECC with thiosemicarbazide corresponding Shieff base ECCT is formed and its IR spectrum indicated new stretching frequency at 3241 and 3142 cm^{-1}. These two absorptions are assigned to –NH_2 and –NH groups of thiosemicarbazone, respectively. Another new absorption at 1626 cm^{-1} is attributed to >C=N–. A new absorption at 1236 cm^{-1} was observed for the thiocarbonyl group. These spectra therefore suggest that the condensation between the carbonyl group of ECC and NH_2 group of the 3-thiosemicarbazide has taken place leading to the formation of ECCT.

Hence, the structural formula of the reagent ECCT may be given as follows:

N-Ethyl-3-carbazole carboxaldehyde-3-thiosemicarbazone (ECCT)
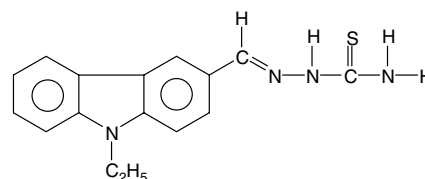
N-Ethylcarbazolecar -boxaldehyde   Thiosemicarbazone   ECCT
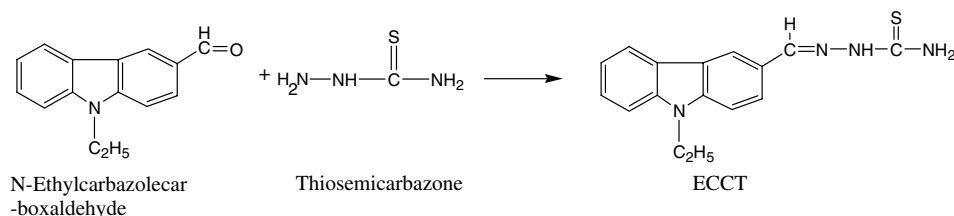
Scheme 1.

2.3. Preparation of standard solution of zinc(II)

A sample of 2.085 g of zinc chloride was taken in a litre standard flask. This was then dissolved and made up to 1 l with double distilled water. The exact content of zinc was determined, gravimetrically by 8-hydroxyquinoline (Vogel, 1961). The working solutions were obtained by diluting the stock solution to the requisite concentrations with double distilled water. All reagents used were of analytical reagent grade unless otherwise stated.

2.4. Buffer solutions

1.0 mol/l sodium acetate and 1.0 mol/l acetic acid solutions were prepared in double distilled water. Suitable portions of these solutions were mixed to get the desired pH.

2.5. Collection of food samples, preparation of solutions and analytical procedure

The food samples analyzed were brought from the local market during the month of June. The samples cleaned and dried in open air, preventing them from mineral contamination. The dried sample was pulverized in a mortar for the purpose of analysis, to a convenient size. . Ten grams of the powdered leafy/chilli or 10 mL of milk sample was taken in a silica crucible, heated to oxidise organic matter, and ashed at 550 °C, in a muffle furnace for 4–5 h. The ash was then dissolved by heating with 10 mL of 2 N hydrochloric acid, filtered through an acid, washed filter paper (Whatmann No. 41) and then washed with hot water. The filtrate and washings were collected in a 25 mL volumetric flask and finally, made up to the mark with double distilled water.

2.6. Analytical procedure for certified reference materials

About 0.1 g of each sample was dissolved in 10 mL of aqua-regia. They were heated to near dryness and the nitrate was expelled from the residue, using 5 mL of concentrated hydrochloric acid. Each residue was extracted into double distilled water separately and made upto 50 mL in volumetric flasks.

2.7. General procedure

To an aliquot of a working standard solution containing 12.5–150 μg zinc(II), were added pH 6.0 buffer (3 mL), 0.5% reagent solution (2 mL) and a salting-out agent, 0.1 mol/l magnesium sulphate (1 mL). The mixture was shaken two times with 10 mL portions of benzene each time for 1 min and allowed to stand for a few minutes. The two organic phases were collected into a 25 mL volumetric flask and made up to the mark with benzene. The absorbances of all the organic phases were measured at 420 nm against the reagent blank.

3. Results and discussion

3.1. Absorption spectra of the reagent and Zn(II)–ECCT complex

From the spectra it is clear, that the Zn(II)–ECCT complex and the reagent have maximum absorbances at 420 and 350 nm, respectively. The reagent has minimum absorbance at the maximum absorbance of the complex and does not interfere in the determination of zinc(II). Hence, 420 nm is chosen as the optimum wavelength for further studies.

3.2. Effect of pH on the extraction of Zn(II)–ECCT complex

The effect of pH on the formation of Zn(II)–ECCT complex is studied to find out the optimum pH for zinc(II) determination. pH studies are carried out using hydrochloric acid–potassium chloride (pH 1.0–2.6), sodium acetate–acetic acid (pH 3.4–6.5) and ammonium chloride–ammonium hydroxide (pH 7.0–11.0) buffers. The optical densities of the complex in the organic layers collected are measured at 420 nm, using their corresponding reagent banks. From the results, it is observed that the complex exhibits maximum absorbance at the pH 6.0. Hence, further studies have been carried out at pH, 6.0.

3.3. Effect of solvents on the extraction of Zn(II)–ECCT complex

The extraction of zinc(II) (1.0 mL of 1.67×10^{-3} mol/l) at pH 6.0 using ECCT (1.0 mL of 5.02×10^{-3} mol/l) in various solvents has been investigated. The results indicate that the extraction of zinc(II) varies with the nature of solvent follows the order (absorbance): benzene (0.869), xylene (0.843), chloroform (0.754), carbon tetrachloride, cyclohexane (0.723), chlorobenzene (0.712), n-butanol, amyl alcohol (0.634), methyl isobutyl ketone (0.545) and isoamyl alcohol (0.543). Among above solvents, benzene is found to extract the complex effectively, hence benzene is chosen for all further studies.

3.4. Effect of reagent concentration on the absorbance of Zn(II)–ECCT complex

The effect of reagent concentration has been studied at constant volumes: 1.0 mL of 1.67×10^{-3} mol/l zinc(II) solution, 3.0 mL of pH 6.0 buffer solution and 1.0 mL of ECCT solution containing different concentrations ranging from 1.67 to 16.73×10^{-3} mol/l to obtain the maximum color formation. The absorbances of these phases are measured at 420 nm, against their corresponding reagent blanks. It is absorbed from the absorbance values that, a maximum fivefold excess of the reagent is sufficient to get maximum color formation of the complex.

3.5. Effect of salting-out agents on the extraction of Zn(II)–ECCT complex

Various salting-out agents, such as lithium nitrate, lithium sulphate, magnesium sulphate and ammonium chloride are used to enhance the metal complex extraction in to organic phase, in a single stage. It is noticed that the presence of 1.0 mL of 0.1 mol/l magnesium sulphate facilitated a maximum absorbance. Hence, with 1.0 mL of 0.1 mol/l magnesium sulphate solution as a salting out agent and fivefold molar excess of the reagent used for zinc(II) is quantitatively extracted in to the organic phase.

3.6. Time stability of the color reaction

The absorbance values of Zn(II)–ECCT complex are monitored at different intervals of time, at 420 nm and found that the color of the complex is stable for more than 48 h.

3.7. Applicability of Beer's law, molar absorptivity, Sandell's sensitivity and correlation coefficient

The complex system confirms to Beer's law in the range 0.4–6.0 mg/l of zinc(II). The molar absorptivity and Sandell's sensitivity of the complex are 1.55×10^4 lit mol^{-1} cm^{-1} and 4.212×10^{-3} μg cm^{-2}, respectively. The correlation coefficient value of Zn(II)–ECCT system, with independent variable as concentration in μg/mL and dependent variable as absorbance, was found to be 0.999. This indicates excellent linearity between the two variables.

3.8. Ringbom plot for Zn(II)–ECCT complex system

The two outstanding advantages of Ringbom plot are: (a) at a glance it represents the concentration range, in which the error in the analysis is minimum (this corresponds to the nearly linear portion of the plot) and (b) in any concentration range, the accuracy of the analysis can easily be evaluated. The Ringbom plot is drawn (Fig. 1) between $\log C$ of zinc(II) and $(I–T)$ where, T is the transmittance. The plot has a sigmoid shape with linear at intermediate concentration values from 1.2 to 3.2 mg/l which indicates that zinc(II) is precisely determined in the range 1.2–3.2 mg/l. From above plot, the ratio between the relative error in concentration and photometric error is 2.875 for photometric error of one percent, $\Delta P = 0.01$. Hence, the relative error in concentration is 0.02875.

3.9. Precision and accuracy of the developed method

To asses the precision and accuracy of the method, determinations are carried out at different concentrations of zinc(II), under optimum conditions. The results show that the standard deviation of the method is found to be not more than 0.607 and the coefficient of variation does not exceed 0.969%. It is evident from these results that the method is precise besides being accurate.
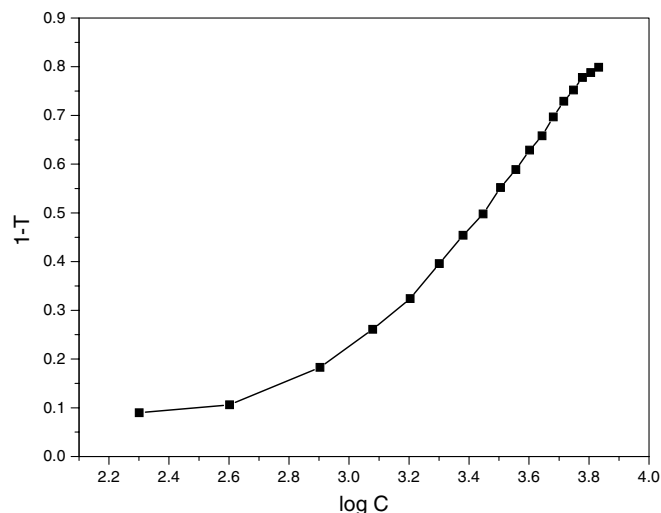

Fig. 1. Ringbom plot of Zn(II)–ECCT complex. Zn(II); 200–6800 μg/l; ECCT; 1.0 mL of 8.24×10^{-3} mol/l; pH, 6.0; λ_{max}; 420 nm.

3.10. Determination of the composition of Zn(II)–ECCT complex

Spectrophotometric investigation of the Zn(II)–ECCT complex made to obtain composition of the complex.

3.10.1. Job's method of continuous variation

The plot corresponding to its absorbance versus mole fraction of the metal ion is confirmed that one mole of zinc(II) reacts with one mole of the reagent showing the composition of the complex as 1:1 (M:L).

3.10.2. Mole ratio method

The absorbance and molar proportion of the metal ion is observed that one mole of the reagent and one mole of the metal ion participate in the complex formation.

3.10.3. Slope ratio method

Two series of mixtures, one with constant metal and varying concentrations of reagent and the other with constant reagent and varying concentration of metal were prepared using 3.345×10^{-3} mol/l solutions of the reagent and zinc(II). The absorbance values of these are noted at 420 nm, against their corresponding reagent blanks and graphically represented in Fig. 2. The above two plots indicate the formation of 1:1 complex, between metal and ligand, under experimental conditions.

From all the three methods mentioned above, it is evident that the composition of zinc(II) and ECCT in the complex is 1:1 (M:L).

3.11. Effect of foreign ions on the extraction of Zn(II)–ECCT complex

In order to assess the possible analytical applications of this color reaction, the effect of some foreign ions is examined, by carrying out determinations of 87.5 μg of zinc(II)
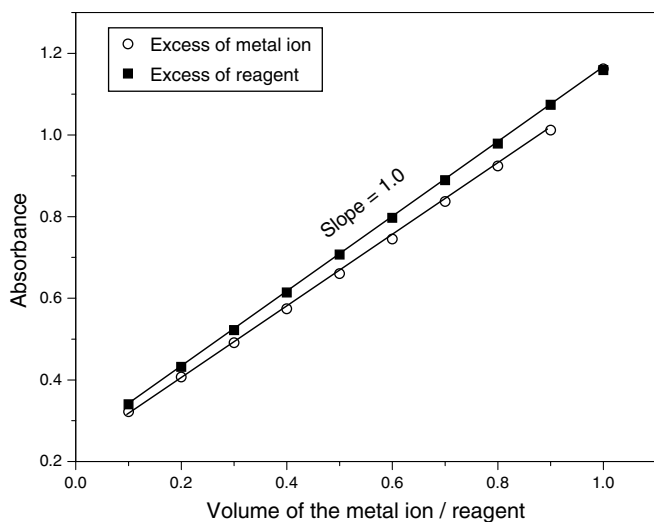
Fig. 2. Slope ratio method of Zn(II)–ECCT complex. Excess of metal ion: Zn(II); 1.0 mL of 3.35×10^{-3} mol/l; ECCT; 0.1–1.0 mL of 3.35×10^{-3} mol/l; pH, 6.0; λ_{max}; 420 nm; Excess of reagent: Zn(II): 0.1–1.0 mL of 3.35×10^{-3} mol/l; ECCT: 1.0 mL of 3.35×10^{-3} mol/l; pH, 6.0; λ_{max}: 420 nm.

with a known amount of foreign ion solution, using the recommended analytical procedure. An error of $\pm 2\%$ in absorbance reading is considered tolerable.

The results indicated that Mn(II), Mg(II), Mo(VI) and W(VI) do not interfere, even present up to five thousand folds excess. Ca(II), Cr(III), Fe(II) and Zr(IV) can be tolerated up to two thousand folds excess Pb(II), Ni(II), Pd(II), Cu(II), Se(IV), Cu(IV) and Cd(II) interfere severely even present in trace amounts.

Anions like fluoride, tartrate, thiocynate and oxalate do not interfere in the determination even when present upto five thousand folds excess. Chloride, bromide, sulphate nitrate and citrate do not have any effect on the extraction of zinc(II), even when present up to two thousand folds excess. Thiourea, thiosulphate, EDTA and phosphate interference of Cu(II) and Pd(II) can be suppressed by using 1.0 mL of 0.3% oxalate solution as masking agent. Pb(II), Ce(IV), Se(IV), Cd(II) and Ni(II) can be avoided by using 1.0 mL of 0.5% thiocynate solution.

Based on above results, the present method when compared with other reported spectrophotometric methods (Table 1) was found to be more sensitive and selective. The other existed reagents like benzildithiosemicarbazone and 1,2-cyclohexanedionedi-thiosemicarbazone are proved some of transition metal ions, such as copper, nickel, cobalt, lead, manganese, silver, mercury, cadmium, iron and vanadium are interfere and also less sensitive when compared with ECCT. On the other hand, remaining reagents which mentioned in Table 1, such as glyoxaldithiosemicarbazone, 1,3-cyclohexanedionedi-thiosemicarbazone, methylglyoxal bis(4-phenyl-3-thiosemicarbazone), xylenol orange and cetylpyridium chloride are reported as less sensitive. It also offers advantages like reliability and reproducibility in addition to its simplicity instant color development and less interference.

4. Applications of the developed method

The proposed method is applied for the determination of the zinc(II) in foods and certified reference materials.

4.1. Determination of zinc(II) in foods

Foods like chilli, leafy vegetable and milk samples were analyzed for zinc(II) using the proposed method. The con-

Table 1
Comparison of present method with other reported spectrophotometric methods

Reagent	λ_{max} (nm)	pH	Molar absorptivity (L /mol cm $\times 10^4$)	Applicability of Beer'slaw (ppm)	M:L	Remarks	References
Benzildithiosemicarbazone	395	9.5	0.42	1.0–18.0	1:1	Cu(II), Ni(II), Co(II), Pb(II), Mn(II), Ag(I) are interfere and less sensitive	Hoshi et al. (1977)
Glyoxaldithiosemicarbazone	433	9.0–11.0	1.30	NR	1:1	Less sensitive	Nevado et al. (1976)
1,3-Cyclohexanedionedithiosemi-carbazone	570	6.3	1.42	NR	NR	Less sensitive	Benamor et al. (2000)
Xylenol orange and cetylpyridium chloride	580	5.0–6.0	1.1	1.0–20.0	1:2:4	Less sensitive	Herrador et al. (1987)
Methylglyoxal bis(4-phenyl-3-thiosemicarbazone)	445	6.0–8.5	0.21	0.2–0.4	1:1	Less sensitive	Leyva et al. (1972)
1,2-Cyclohexanedionedithiosemi-carbazone	415	1.1–6.6	0.73	NR	1:2	Hg(II), Cu(II), Cd(II), Fe(II), Ni(II), Co(II), V(V) are interfere and less sensitive	Sarma et al. (2002)
N-Ethyl-3-carbazolecarboxaldehyde-3-thiosemicarbazone	420	3.0–5.5	1.55	0.4–6.0	1:1	Highly sensitive and selective	PM

M:L, metal:ligand; PM, present method; NR, not reported.

Table 2
Determination of zinc(II) in food samples

Food samples	Amount of zinc(II)[a] found		Present method	
	AAS method	Present method	SD	RSD (%)
Chilli samples[b]				
Sample location				
Rangampet	20.4	20.1	0.23	1.1
Ithepalli	19.5	19.2	0.24	1.2
Chandragiri	19.0	18.5	0.18	1.0
Muthyalareddypalli	30.0	29.5	0.28	0.9
Alipiri	17.5	16.9	0.21	1.2
Tiruchanoor	26.0	25.1	0.22	0.9
Pudi	27.0	26.3	0.30	1.1
Renigunta	22.0	21.1	0.23	1.0
Akkarampalli	20.0	19.9	0.23	1.1
Gajulamandyam	22.0	21.7	0.21	0.9
Leafy vegetables[b]				
Name of the sample				
Carrot (*Daucus carota*)	82.5	82.4	0.37	0.4
Mulla tota kura (*Amaranthus spinosus*)	26.0	25.6	0.19	0.7
Payalaaku (*Portulaca oleracea L.*)	21.0	20.7	0.14	0.7
Potato (*Ipomea batatas L.*)	51.0	50.6	0.19	0.4
Chinta (*Tamarindus indica*)	35.5	35.3	0.16	0.4
Pappu kura (*Portulaca oleracea*)	24.0	23.6	0.15	0.6
Thotakura (*Amaranthus gangeticus*)	56.0	55.7	0.19	0.3
Chukkaku (*Rumex vesicarius*)	92.5	92.4	0.24	0.3
Milk samples[c]				
Animal origin				
Buffalo	3.00	2.96	0.03	1.0
Cow	3.90	3.86	0.03	0.8
Goat	4.20	4.16	0.05	1.2
Sheep	2.40	2.38	0.02	0.8
Dairy	3.70	3.56	0.05	1.4

[a] Average of five determinations.
[b] Concentration in μg/g.
[c] Concentration in μg/mL.

Table 3
Determination of zinc in certified reference materials

Certified reference material	Zinc[a] in mg/kg		Present method	
	Certified value	Present method	SD	RSD (%)
Tomato leaves (NIST 1573)	62.0	61.7	0.20	0.3
Citrus leaves (NBS 1572)	29.0	28.5	0.18	0.6
Whole milk powder (NIST SRM 8435)	28.8	28.6	0.15	0.5

[a] Average of five determinations.

tent of the zinc(II) present in the organic solution was determined by using a calibrated plot and the results obtained were confirmed by direct atomic absorption spectrometer. These results are given in Table 2.

4.2. Determination of zinc(II) in certified reference materials

The present method is applied for the determination of zinc(II) in certified reference materials, such as Tomato leaves (NIST 1573), Citrus leaves (NBS 1572) and Whole milk powder (NIST SRM 8435). The concentration of zinc was determined by following the procedure described in general procedure and the results are presented in (Table 3).

5. Conclusions

First time we are attempted the new rapid and sensitive reagent for determination of zinc(II) from foods and certified reference materials. In the present investigation, the authors introduced ECCT to the field of extractive spectrophotometric determination of zinc(II). The reagent has been proved to be a highly sensitive reagent for zinc(II) compared with other existing extractive spectrophotometric determination methods. The selectivity of the reagent is improved by proper use of masking agents to suppress the interference of metal ions. The results of the proposed method are comparable with AAS. The good synchronization of experimental values with the certified reference materials clearly demonstrates the utility of this method for the determination of zinc(II) in foods.

Acknowledgements

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