Determination of Zinc and Magnesium in Food Samples via Cloud Point Extraction using Brilliant Green as a Reagent

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In this article, we present a sensitive and efficient method for the separation and determination of Zinc (II) and Magnesium (II) ions using a combination of liquid ion exchange and cloud point extraction (CPE). The process includes converting these ions into metal ion complexes by using EDTA at pH 10. These complexes are then bound electrostatically with Brilliant Green (BG) to form ion pair association complexes, which are subsequently transferred into a cloud point layer. The study involved optimizing the extraction conditions, including the EDTA concentration, temperature, heating duration, and surfactant volume. The concentration of EDTA for achieving higher extraction efficiency is 1×10^{-4} M in the presence of 100 µg of Zn(II) and Mg(II) per 10 mL of aqueous solution using 1×10^{-4} M of BG. The solutions should be heated at 85°C for Zn(II) and 80°C for Mg(II) for 20 minutes. The optimal volume of surfactants is 0.5 mL of Triton X-100 for Zn(II) and 0.6 mL for Mg(II). The study also includes an analysis of the impact of electrolytes and interferences and the spectrophotometric identification of Zn(II) and Mg(II) in various samples.

Keywords: cloud point extraction, separation, spectrophotometric determination, Zn(II), Mg(II)

Introduction

The use of green technologies is a central focus in the development of modern analytical chemistry [1,2]. In this field, separation, and preconcentration processes are critical steps that present a significant challenge to researchers seeking to enhance the methodology's selectivity and sensitivity. Several techniques have been developed to address these challenges, including liquid-liquid extraction, ion exchange, coprecipitation, and solid-phase extraction [3,4]. Among these, cloud point extraction (CPE) stands out as an environmentally friendly method for separation and preconcentration, offering numerous advantages over conventional liquid-liquid extraction. CPE is known for being accurate, inexpensive, fast, precise, and selective [5]. It is considered a green extraction procedure because it uses minimal amounts of toxic organic solvents [6,7]. The fundamental principle of traditional cloud point extraction involves the ability of a nonionic surfactant to form micelles in aqueous solutions when heated above a specific threshold temperature [8,9].

The determination of zinc is crucial for several reasons. Zinc is an essential trace element necessary for over 300 enzymes involved in protein and DNA synthesis, as well as cell division [10]. Its deficiency can lead to health issues like immune disorders, growth retardation, and inflammation. Industrially, zinc is used in alloys, coatings, batteries, and more, making accurate analysis vital for optimizing processes and reducing environmental impact. With natural zinc reserves depleting, recovering zinc from secondary materials like industrial waste is increasingly important. This efficient resource use also helps mitigate environmental pollution. Thus, precise zinc determination in various samples is vital for health, economic development, and environmental protection. In various analytical studies, zinc has been successfully extracted from real samples using a variety of organic reagents. compounds like 2-Hydroxy-1-naphthaldehyde thiosemicarbazone, 2-Hydroxy-1-naphthaldehyde Compounds such as isonicotinoyl hydrazone (HNINH), 2-Benzoylpyridine-4-phenyl-3-thiosemicarbazone (BPTSC), and 2-Hydroxy-1-naphthaldehyde benzoyl hydrazone (HNABH) have been employed for this purpose. These organic reagents form stable complexes with zinc ions, facilitating their extraction from complex sample matrices such as environmental water, soil, and biological fluids. Thus, in the study [11], zinc was extracted from real samples using the organic reagent 1-(4-(phenyldiazenyl)phenyl) azonaphthalene-2-ol. Another study [12] involved the extraction and separation of zinc, nickel, and cobalt using both Cyanex 272 (bis(2,4,4-trimethylpentyl) phosphinic acid) and Ionquest 801 (2-ethylhexyl phosphonic acid mono-2-ethylhexyl ester). This paper comprehensively analyzed the metal extractions of zinc, nickel, and cobalt using Ionquest 801 and

Cyanex 272 and their combinations. The findings revealed that these substances demonstrated nearly identical performance in terms of zinc selectivity over cobalt [13]. A simple method [14] was developed using the water-soluble polymer polyacrylic acid to separate and preconcentrate trace amounts of Zn, Cu, and Cd before atomic absorption analysis. This method was compared with traditional water-insoluble sorbents in all aspects and was found to be simple, precise, fast, and inexpensive. The recovery values were 98.0% for Zn, 99.7% for Cu, and 96.5% for Cd, with detection limits of 2.2 μg/L for Zn, 2.5 μg/L for Cu, and 1.8 μg/L for Cd. In another work [15], cloud point extraction (CPE) was utilized to extract Zn(II), Cu(II), and Cd(II) from tap water and river water samples. The analytes in the initial aqueous solution were complexed with 8-hydroxyquinoline, with the surfactant Triton X-114 added at a concentration of 0.06%. After optimizing the extraction conditions, good enrichment factors were achieved. The detection limits were 0.03 ng/mL for Zn(II), 0.72 ng/mL for Cu(II), and 0.15 ng/mL for Cd(II), with relative standard deviations lower than 3.1%.

Magnesium is a heavy metal that is frequently present in wastewater. There has been an increasing need for magnesium in the automobile, fertilizer, pulp, paper, and wastewater treatment sectors [16]. Furthermore, magnesium (Mg(II)) is a vital ion for the human body, acting as a cofactor for over 300 enzymatic reactions and playing a critical role in numerous metabolic pathways [17, 18]. Similarly, the extraction and determination of magnesium have also been explored using various organic reagents. Compounds like 8-Hydroxyquinoline, 1-(2-Pyridylazo)-2-naphthol (PAN), and Eriochrome Black T are commonly employed for the extraction of magnesium. In a study [19], di-(2-ethylhexyl) phosphoric acid (P204) was utilized for the extraction of magnesium to separate it from lithium.

The aim of this study is to evaluate the impact of contamination on Zn(II) and Mg(II) levels in fruits and vegetables using a simple, cost-effective, and safe technique [20-21]. Our approach involves the use of a combined method comprising liquid ion exchange and cloud point extraction (CPE) to achieve this goal. The process entails the conversion of these ions into metal ion complexes using EDTA at pH 10, followed by the electrostatic binding of these complexes with Brilliant Green (BG) to form ion pair association complexes, which are subsequently transferred into a cloud point layer. This study holds the potential to introduce a more efficient and environmentally conscious approach to the field.

Experimental part

Chemicals and reagents. Dithizone (98.0%), ethanol (95%), nitric acid (ACS reagent, 65%), and hydrochloric acid (ACS reagent, 37%) were procured from Merck. Eriochrome black T (99.96%), Brilliant green (99.98%), and magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) were acquired from Fluka. Zinc (PA), ethylenediaminetetraacetic acid disodium salt dihydrate (99%), and tetrachloromethane (Analar) were sourced from BDH Industries Ltd. Triton X-100 (99%, 1% solution) was purchased from MACLEAN Company, China. Deionized water Millipore was used for the preparation of all water solutions.

The samples of six different fruits and vegetables, namely bananas, oranges, Spinach, peaches, celery leaves, and lentils, were collected from a local market.

Preparation of solutions. The stock solution of Zn(II) with a concentration of 1 mg/mL was prepared by dissolving 1 gram of Zn metal in 15 mL of dilute (1:1) hydrochloric acid, with further dilution to 1L in a volumetric flask. For the preparation of the Mg(II) stock solution (1 mg/mL), 0.8360 grams of $MgCl2 \cdot 6H_2O$ were dissolved in 100 mL of distilled water. The stock solution of EDTA solution with a concentration of 1×10^{-2} M was prepared by dissolving 0.3802 grams of Na_2EDTA in 100 mL of distilled water. A solution of Eriochrome black T (0.02%) was prepared by dissolving 0.02 grams in 100 mL of distilled water. A Brilliant green solution (1×10^{-2} M) was prepared by dissolving 0.482 grams in 10 mL of distilled water. A Dithizone solution (1×10^{-2} M) was prepared by dissolving 0.0256 grams in 10 mL of CCl_4.

The working solutions were prepared from stock solutions by dilution with an appropriate solvent or buffer to achieve the desired concentration.

Preparation of samples. 1 gram of each fruit/vegetable sample was treated with 5 mL of 65% HNO_3, and the mixture was gently boiled over a water bath at 90°C for 1–2 hours or until a clear solution was achieved. Subsequently, an additional 2.5 mL of 65% HNO_3 was added, and the heating was continued until complete digestion occurred [22]. The resulting samples were then transferred to suitable storage flasks, protected from external influences, and processed according to the optimal conditions for each method, employing appropriate masking agents as necessary [23,24].

Instruments. A UV-Vis double beam spectrophotometer (Biochrom Libra S60, UK) was used for spectrophotometric studies and absorbance measurement. The thermostated water bath (Hamburg-90, England) maintained a constant temperature during the extraction experiments. A laboratory pH meter (E163694, CE, Germany) with a combined glass electrode was used to measure the pH of solutions.

Cloud point extraction (CPE) procedure. In a standard cloud point extraction (CPE) procedure, a 10 mL aqueous solution containing 100 μg of Zn(II) or Mg(II) ions, along with EDTA and Brilliant green, is prepared. A specific volume of the non-ionic surfactant TritonX-100 is added to this solution, and the pH is adjusted to 10. The mixture is then heated in a water bath to reach the appropriate temperature

and duration required for the formation of the cloud point layer (CPL). CPL was separated from the aqueous solution and dissolved in 5 mL ethanol. The absorbance of an ion pair association complex formed by Zn(II) and Mg(II) is determined by measuring at the respective wavelengths of maximum absorbance, which are λ_{max} = 630 nm for Zn(II) and λ_{max} = 624 nm for Mg(II). The concentrations of Zn(II) and Mg(II) remaining in the aqueous phase were quantified spectrophotometrically. Specifically, the Dithizone method was employed for the determination of Zn(II) ions, while the Eriochrome black T method for Mg(II) ions.

The distribution ratio (D) of the ion pair association complexes of Zn(II) or Mg(II) between the aqueous phase and the cloud point layer was calculated using Eq.1. The calibration curves constructed for ion determination in aqueous solutions using Dithizone for Zn(II), represented by the equation y = 0.077x + 0.0289 with an R^2 value of 0.9991, and Eriochrome black T for Mg(II), represented by the equation y = 0.0064x + 0.002 with an R^2 value of 0.9998.

$$D = [M(II)]_{CPL} / [M(II)]_{aq.} \quad \text{(Eq.1)}$$

where $[M(II)]_{CPL}$ and $[M(II)]_{aq.}$ – equilibrium concentrations of metal ions in the cloud point layer (CPL) and aqueous phase (aq).

Results and Discussion

The spectrometric experiments focused on the extraction of Zn(II) and Mg(II) ions, which involved the typical extraction procedure and evaluation of effective parameters. Figure 1 shows the UV-Vis absorption spectra of the Zn(II) and Mg(II) ion pair association complex with BG. The absorbance of a complex formed by Zn(II) and Mg(II) as an ion pair association is measured at the respective wavelengths of maximum absorbance, which are λ_{max} = 630 nm for Zn(II) and λ_{max} = 624 nm for Mg(II).

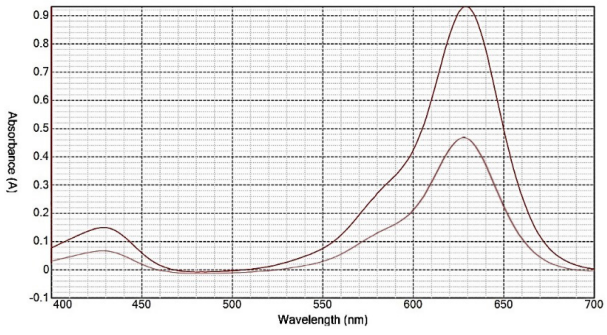

Figure 1. UV-Vis absorption spectrum of ion pair association complexes of Zn(II) with BG (A) and Mg(II) with BG (B).

Optimization of cloud point extraction procedure

Effect of EDTA concentration

The concentration of EDTA in cloud point extraction directly affects the formation of metal-EDTA complexes. Excessive concentrations can reduce efficiency and compromise selectivity, while insufficient levels may result in incomplete complexation and lower sensitivity. Determining the appropriate EDTA concentration is crucial for optimizing CPE performance. In this study, we investigated the concentration of EDTA in the range from 1×10^{-6} to 1×10^{-2} M. Figures 2a,b illustrates the impact of EDTA concentration on the formation, stability, and extraction efficiency of ion association complexes.

The results indicate that a concentration of 1×10^{-4} M EDTA was found to be the optimal concentration for achieving higher extraction efficiency for Zn(II) and Mg(II) ions. This concentration allows for the maximum formation rate of the metal anion complexes $ZnHY^-$ and $MgHY^-$, which are essential for forming the extracted ion pair association complex. Excessive levels of EDTA can reduce extraction efficiency by accelerating the reverse direction of the thermodynamic equilibrium, leading to increased dissociation and decreased extraction efficiency according to the mass action law.

Effect of Temperature

The temperature of the CPE process plays a significant role in controlling the behavior of systems containing nonionic surfactants. Increasing the temperature makes the surfactant more hydrophobic, forming water droplets that cause the separation phenomenon. However, the separation temperature depends on the quantity and structure of the surfactant used. The effect of temperature was studied in the range of 55°C to 95°C to determine the optimal temperature for the process. Figures 2c,d illustrates that the optimal temperature for excellent extraction of Zn(II) ions is 85°C, whereas for Mg(II) ions, it is 80°C.

The equilibrium constant of extraction K_{ex} at each temperature was calculated according to Eq.2. The enthalpy change (ΔH_{ex}) and entropy change (ΔS_{ex}) were determined from the slope and intercept of a plot of $\ln(K_{ex})$ against $1/T$, respectively. A change in Gibbs free energy (ΔG_{ex}) was calculated from Eq.3-4. The results are demonstrated below in Table 1.

$$K_{ex} = D/[M(II)]_{BG} \quad \text{(Eq. 2)}$$

$$\Delta G_{ex} = -R\,T\,Ln\,K_{ex} \quad \text{(Eq. 3)}$$

$$\Delta G_{ex} = \Delta H_{ex} - T\Delta S_{ex}, \quad \text{(Eq. 4)}$$

where $[M(II)]_{BG}$ – equilibrium concentrations of ion pair association complexes of Zn(II) and Mg(II) with BG; R – is the gas constant (8.314 J/(mol·K)), T – is the temperature in Kelvin.

Table 1. Thermodynamic parameters of CPE.

	ΔH_{ex}, kJ.mol^{-1}	ΔG_{ex}, kJ.mol^{-1}	ΔS_{ex}, J.mol^{-1}K^{-1}
Mg(II)	33.815	-57.996	68.50
Zn(II)	19.617	-59.763	112.139

Effect of Heating Time

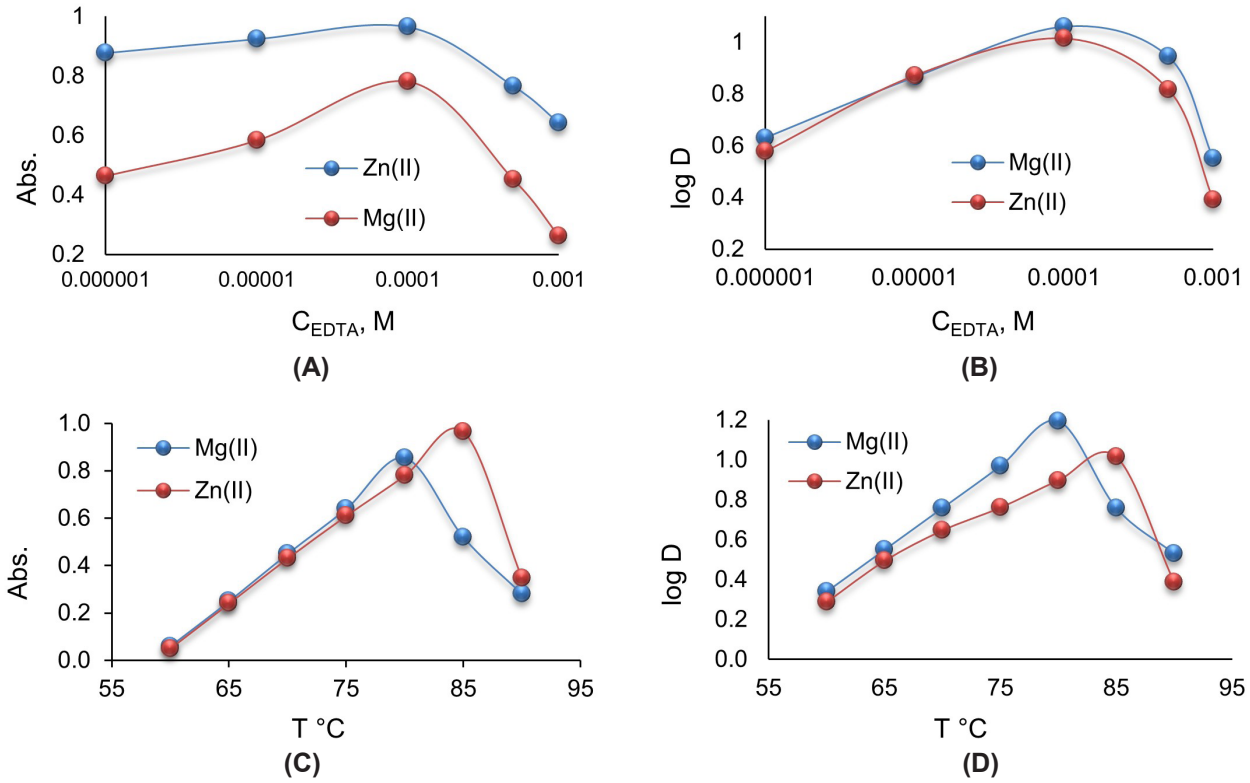

Figure 2. Effect of EDTA concentration (A, B) and temperature (C, D) on the formation and stability of the ion association complex (A, C) and on extraction efficiency (B, D).

The duration of heating in cloud point extraction should be adjusted to optimize the extraction process for maximum analyte recovery and selectivity. If the heating time is too short, incomplete phase separation may result in lower extraction efficiency. On the other hand, excessively long heating times can lead to degradation of the analyte or surfactant, which can compromise the accuracy of the results. The heating time varied from 5 to 30 minutes. Figures 3a,b show that heating the solution for 20 minutes was the best time for achieving excellent extraction efficiency for both metal ions. Heating the solution for longer than the optimal time decreased the extraction efficiency due to the effect of diffusion of micelles in the aqueous solution.

Effect of Surfactant Volume

Optimizing surfactant volume is crucial for maximizing CPE performance and achieving high analyte recovery. Increasing surfactant volume enhances micelle formation and analyte solubilization, improving extraction efficiency. However, excessive volumes can lead to emulsion formation, while insufficient volumes may result in incomplete solubilization. In the experimental setup, we evaluated volumes of TritonX-100 ranging from 0.1 to 1 mL. Results are presented in Figures 3c,d. The optimal volumes for higher extraction efficiency were 0.5 mL for Zn(II) and 0.6 mL for Mg(II) of TritonX-100.

Effect of electrolytes

Electrolytes in CPE can affect the process in two main ways. Firstly, they can influence the cloud point temperature by altering the solubility of surfactants. Secondly, they can impact the stability of the micelles formed during CPE. Low concentrations can enhance stability, while high concentrations may disrupt micelle formation. In this research, we investigated how the presence of strong electrolytes, including LiCl, NaCl, KCl, NH_4Cl, $MgCl_2$, and $CaCl_2$ at a concentration of 0.1M, impacts the efficiency of extraction processes. Our findings, which are detailed in Table 2, reveal that each type of electrolyte has a unique effect on extraction efficiency due to its specific behavior in aqueous solutions.

Table 2. Effect of different electrolytes on extraction efficiency.

Electrolyte	Zn(II)		Mg(II)	
	Abs.	D	Abs.	D
LiCl	1.722	22.45	1.542	25.43
NaCl	1.546	19.63	1.387	21.64
KCl	1.461	17.44	1.215	18.58
NH_4Cl	1.152	14.52	0.988	16.44
$MgCl_2$	1.637	21.26	1.463	22.87
$CaCl_2$	1.484	18.67	1.315	19.34

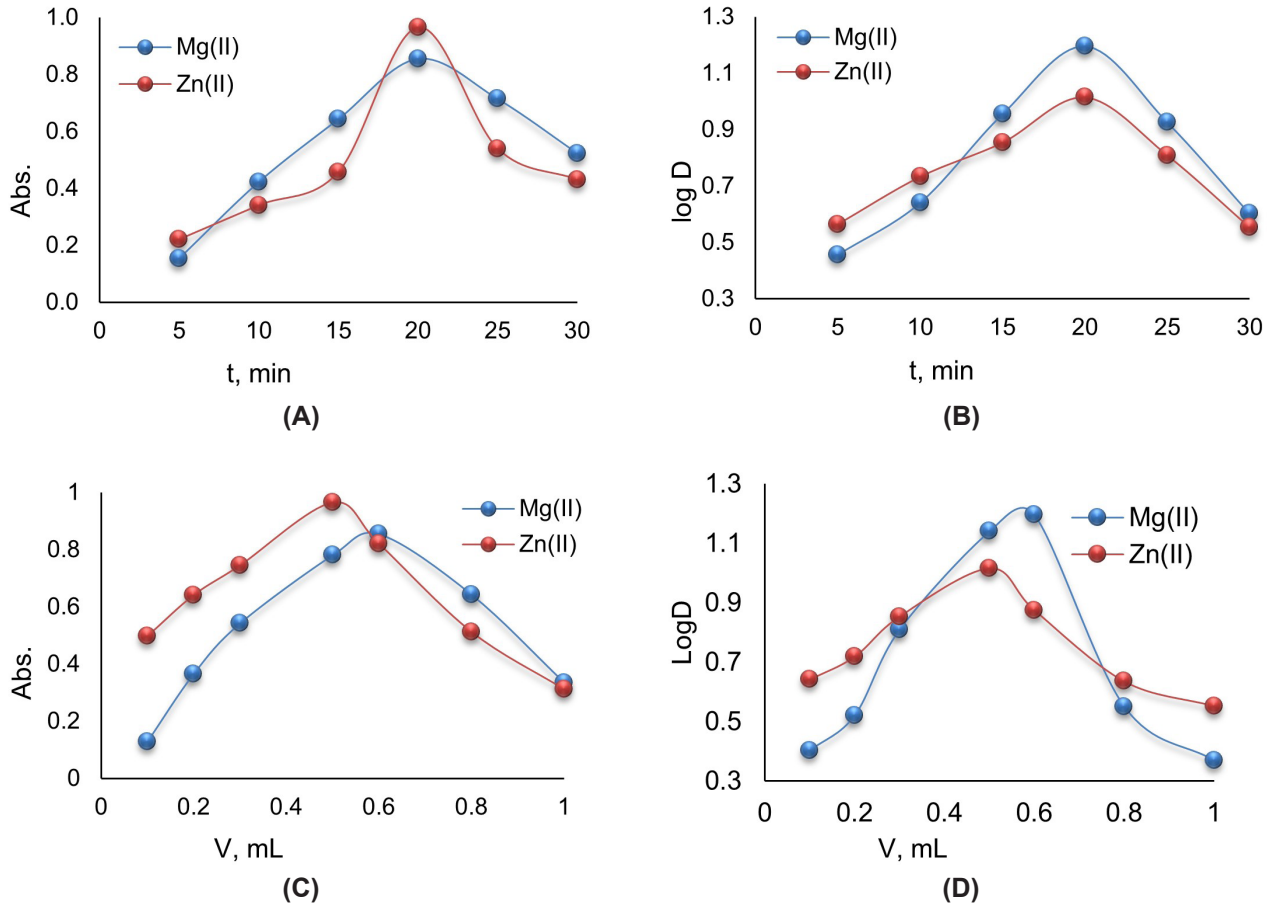

Figure 3. Effect of heating time (A, B) and TritonX-100 volume (C, D) on the formation and stability of the ion association complex (A, C) and on extraction efficiency (B, D).

Effect of interfering ions

Interfering ions can significantly affect the extraction process by competing with the target analytes for binding sites on the surfactant or chelating agent. This competition can lead to reduced extraction efficiency and selectivity. Also, interfering ions may alter the physicochemical properties of the system, such as the cloud point temperature or the stability of the micelles, further impacting the extraction process. Below the optimized requirements, the interfering effect of certain transition metal cations (Cd, Ni, and Co) on the recovery of Zn(II) and Mg(II) was evaluated to confirm the selectivity of the method. The results are presented in Table 3.

Table 3. Effect of interfering ions.

Metal ions	Zn(II)		Mg(II)	
	Abs.	D	Abs.	D
Cd(II)	0.721	7.54	0.483	7.46
Ni(II)	0.462	3.56	0.566	9.88
Co(II)	0.578	4.94	0.389	5.23

The results indicate a decrease in extraction efficiency when foreign ions of transition metals are present in an aqueous solution. This is because these foreign ions interact with Zn(II) and Mg(II) to form ion pair association complexes, which decreases the concentration of BG and EDTA in the aqueous solution. Consequently, the formation of ion-pair association complexes for the ions under study is also reduced.

Analytical characteristics of the method

At the optimized condition, the dynamic linear range for the determination of Zn(II) and Mg(II) via the liquid ion exchange method is from 0.1 to 10 mg L^{-1} (Figure 4). The calculated Limit of Detection (LOD) is 0.839 and 1.129 mgL^{-1} for Zn(II) and Mg(II), respectively, and the Limit of Quantification (LOQ) is 2.543 and 3.421 mgL^{-1} for Zn(II) and Mg(II), respectively, Table 4.

Table 4. Parameters for the determination of Zn(II) and Mg(II) via liquid ion exchange method.

Parameter	Zn(II)	Mg(II)
λ_{max} (nm)	630	624
Bear's law obeys (mgL^{-1})	0.1-10	0.1-10
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	5.422×10^3	2.327×10^3
LOD (mgL^{-1})	0.839	1.129
LOQ (mgL^{-1})	2.543	3.421

Table 5. Determination of Zn(II) and Mg(II) by the suggested method and comparison with FAAS.

No	Sample	The determined concentration of metal ions, mg L^{-1} by current method		by FAAS	
		Zn(II)	Mg(II)	Zn(II)	Mg(II)
1.	*Banana*	10.11±0.10	12.22±0.11	9.89±0.21	12.05±0.12
2.	*Orange*	12.51±0.14	4.38±0.12	12.02±0.11	4.51±0.01
3.	*Spinach*	11.30±0.20	8.61±0.14	10.88±0.12	8.57±0.04
4.	*Peaches*	10.31±0.21	9.85±0.21	10.34±0.01	9.80±0.05
5.	*Celery leaf*	10.35±0.01	5.24±0.11	10.28±0.12	5.25±0.22
6.	*Lentil*	11.28±0.21	6.28±0.22	11.18±0.23	6.25±0.12

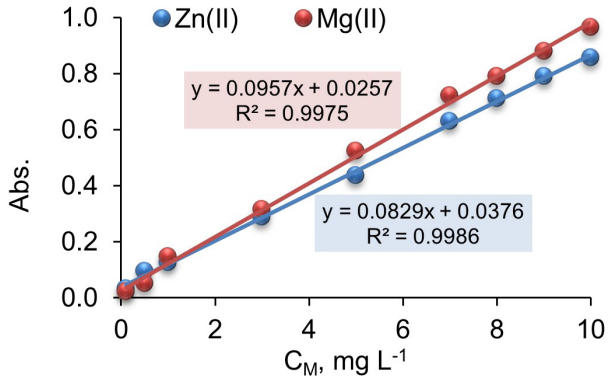

Figure 4. Calibration curves of Zn(II) and Mg(II) with BG.

Application

The procedure was finally utilized to determine Zn(II) and Mg(II) levels in six food samples obtained from a local market. Table 5 shows the results obtained using two methods: proposed CPE and flame atomic absorption spectroscopy (FAAS). The strong agreement between the procedure and direct analysis by FAAS indicates that this technique may be suitable for determining the Zn(II) and Mg(II) levels in various samples.

Conclusion

The study introduces a sensitive method for separating and determining Zn(II) and Mg(II) using ion pair association complex formation by liquid ion exchange. It involves the determination of the optimal concentration of EDTA and Brilliant green for higher extraction efficiency and studying the effects of different electrolytes and the presence of interfering metal cations in aqueous. This technique can be used to measure the levels of Zn(II) and Mg(II) in different samples. The results obtained through CPE and FAAS demonstrate the effectiveness of the proposed procedure, as shown by the recovery of metals applied to the samples.

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