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Optimisation and Evaluation of Zinc in Food Samples by Cloud Point Extraction and Spectrophotometric Detection

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ABSTRACT

The cloud point extraction (CPE) method was developed to determine the zinc prior to Ultraviolet-visible (UV-Vis) spectrophotometry detection. Triton X-100was applied as extractant based on the complexation reaction of Zn(II) ions with ethylenediaminetetraacetic acid (EDTA). Under optimal conditions, the CPE was used to determine the concentration of zinc in canned food samples. The amounts of zinc found in the food samples were in the range of 0.005-0.007 mg/L with RSD of < 8 %. This confirmed that the proposed CPE method is suitable for the determination of zinc in food samples, indicating the concentration of zinc was within the permissible limit.

Keywords: *cloud point extraction, Triton X-100, zinc, UV-Vis spectrophotometry, food*

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INTRODUCTION

Zinc (Zn) is an element in Group 12, having an atomic number of 30 and a molecular weight of 65.38 g/mol. Zn can also come in the form of Zn^{2+} (ion) in its oxidation state, with its electron configuration written as [Ar] $3d^{10}4s^2$. Zn is a bluish-white, lustrous, moderately reactive metal, and also a strong reducing agent. It is hard and brittle at most temperatures but becomes malleable at 100-150°C. Owing to its properties, Zn is generally used as a coating (for metals) to prevent corrosion. In the food packaging industry, zinc is commonly used as a protective coating in canned food. Packaging, such as cans, containers, caps, and closures are usually coated on one/both sides. The internal coating protects the can from its contents, such as acidic soft drinks, and fishes, meats, and soups. Even though Zn has the ability to prevent metal damage, it is relatively soft and also reactive vis-à-vis inorganic and organic compounds [1-2].

Zinc oxide-modified epoxy phenolic lacquers are generally used as a coating for acidic food products to prevent sulphur-staining defects. However, the lacquer will be softened due to heat sterilisation, which allows the sulphur compounds to react with the zinc oxide, forming harmless zinc sulphide. It should be pointed out that direct contact between acidic food products with zinc sulphide salts would damage the internal protective coating of the can [3]. The can could also rust post-packaging due to the presence of oxygen in the cans' headspace being absorbed by the food products, especially oily fishes such as salmon, tuna, and sardines. Therefore, the consumption and exposure of zinc to food could result in long-term consequences on human health, especially on the nervous system and behaviour [4-6].

In the recent years, determining the concentration of zinc in canned food products is of considerable interest, especially in the context of ensuring that the zinc concentration in finished food products falls within the permissible range, as per the Food and Agriculture Organization (FAO)/ World Health Organization (WHO) [7] which was defined to be 150 mg/L. The Malaysian standard for Zn concentration is 100 mg/L. Techniques such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-OES), atomic absorption spectrometry (AAS), and UV-Vis spectrophotometer are widely used for detecting Zn(II) ions [8-10]. However, detecting metal ions at trace and very low concentrations in food samples using the aforementioned instrument are restricted due to insufficient sensitivity and matrix interferences.

A pre-concentration and enrichment step are necessary and valuable in order to improve detection of Zn in food samples. Cloud Point Extraction (CPE) method is regarded as a pre-concentration and enrichment step due to it being safe, environmentally friendly, simple, rapid, single-stage operation, and cost-effective [11-12]. CPE is comprised of three steps: (1) solubilisation of the analytes in the micellar aggregates; (2) clouding; and (3) phase separation for analysis. The solution will separate into two layers after being heated several times; the layers are aqueous-rich and surfactant-rich phases [13]. The hydrophobic compounds (analyte) in the solution will simultaneously bind and pull the micelles towards the surfactant-rich phase, which allows it to be extracted.

This separation process is suitable for polyoxyethylene surfactants due to the presence of two ethylene oxide parts in the micelle compound, which is responsible for the repellent effect at low temperatures (under hydrated condition). However, both will be attracted to each other at higher temperatures (under dehydrated condition); simply put, the attraction/repellent effect is temperature-dependent [14]. The basic principles of the CPE method are based on the clouding phenomenon of surfactants. It requires the modification of several parameters, such as the pH of the solution, the concentration of the chemicals being used, and also the temperature and incubation time in order to effect phase separation(s) [15].

Ultraviolet-visible spectrophotometer is still the most attractive and popular method being used in chemical analyses due to its simplicity, speed, and precision. Its combination of spectrophotometric detection with CPE was first proposed by [16] who studied CPE extraction for pre-concentration of Mn(II) and Zn(II) ions in water samples. Moreover, UV-Vis spectrophotometer guarantees a cost-effective solution for the analyses of heavy metals relative to other spectrophotometric methods [9].

Based on the aforementioned considerations, this study intends to detect the concentration of zinc in canned food samples that are common in Malaysian marketplaces using the pre-concentration CPE method prior to the UV-Vis spectrophotometric detection. In this approach, non-ionic surfactant Triton X-100 was used as an extractant to entrap Zn(II) ions based on the complexation reaction of Zn(II) ions with EDTA by the pH-dependent CPE method. EDTA is a well-known chelating agent reporting continuous high compatibility in the formation of metal-EDTA complexes. It is a suitable complexing agent for metals due to its highly stable molecule. The effects of the operating parameters, such as equilibration temperature, incubation time, concentrations of surfactant, and salt have been preliminarily explored in order to obtain the highest extraction efficiency of Zn(II) analyte in aqueous media prior to analysing food samples. As per literature, the investigation of the CPE method by utilisation of Triton X-100 and assisted by EDTA as its complexing agent to detect Zn in canned food samples has yet to be conducted.

METHODOLOGY

Reagents and chemicals

A viable Triton-X 100 (99 % with molecular weight: 80,000), nitric acid (HNO_3) (71 %), hydrochloric acid (HCl) (37 %), sodium hydroxide (NaOH), and 15 % (v/v) of trichloroacetic acid (TCA) were purchased from Merck (Darmstadt, Germany). Zinc sulfate ($ZnSO_4$) and ethylenediaminetetraacetic acid (EDTA) (99 %), a pH 10 buffer, and sodium carbonate (Na_2CO_3) were purchased from Sigma Aldrich (St. Louis, MO, USA). Three canned food samples, which are lychee in syrup, tomato puree, and pickled lettuce in brine were selected for this study. Fresh working standard solutions were prepared by diluting a stock solution, which was then kept stable during the day of experiments. Deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1} resistivity) was used throughout the experiment(s).

Instrumentation

The UV-Vis spectrophotometer (Model T60 UV-Vis Jenway spectrophotometer model 6715), with a 1 cm glass cell was used to obtain the absorption spectra and absorbance measurements. A centrifuge machine (Model Nuve NF 800 centrifuge, Behsa, Iran) was used to facilitate and accelerate the phase separation process. A pH meter (Model OH700, Switzerland) with a combined glass electrode, was utilised to determine the pH of the samples. The thermo-stated water bath (Model Memmert MNE 29L^{-1} Water Bath, Germany) was used to keep the temperature constant during the aqueous biphasic system (CPE) experiment.

Cloud point extraction (CPE) procedure

The CPE was designed to mimic the procedure outlined [17] with slight modifications to allow for the observation of the phase separation process; an aliquot of 4 mL standard solution of 0.01 mg L^{-1} Zn(II) was mixed with 4 mL of 0.01 mg L^{-1} EDTA in a centrifuge tube. The aliquot solution of Zn(II)-EDTA complex was adjusted to alkaline condition using 2 mL of a pH 10 buffer solution. Consequently, 40 % (v/v) of Triton X-100 was added, followed by a 4 mL of 1.5 mol L^{-1} Na_2CO_3 solution, which acted as the extractant and synergic reagents of the CPE method, respectively. Then, the mixture was left to stand in a thermostated bath at 60˚C for 15 minutes prior to the phase separation and later cooled to room temperature. The phase separation was completed as a resulting solution of the CPE via the increase in the viscosity quenched after the total separation time. The surfactant-rich phase (viscosity) was separated using a syringe needle from the corresponding aqueous-rich phase to minimise the possibility of cross-contamination from the analyte. The collected surfactant-rich phase volume was measured, and the surfactant-rich phase was isolated prior to it being analysed. The concentration of Zn(II) ions in the surfactant-rich phase were analysed using UV-Vis spectrophotometer at a wavelength of 250 nm. The reported data are in the form of an average of three measurements.

Optimisation of parameters study

The important variables affecting the stability and performance of the phase separation system include the pH, equilibration temperature, incubation time, surfactant concentration, and salt concentration, all of which were optimised and evaluated. The suitable pH condition for Zn(II)-EDTA complex was investigated by comparing the maximum absorbance signals between acidic (pH 4) and alkaline (pH 10) media. A solution complex of Zn(II)-EDTA was prepared by mixing the 1 mL of 0.01 M of Zn(II)-EDTA at a standard ratio 1:1. The effect of equilibration time was studied at 50˚C, 60˚C, 70˚C, 80˚C, 90˚C, and 100˚C. Also, the dependence of extraction efficiency of Zn(II) ions towards the CPE system upon incubation time was carried out within the range of 10, 15, 20, 25, and 30 mins. The effect of the concentration of surfactants on CPE system were conducted at 10 % (v/v), 20 % (v/v), 30 % (v/v), 40 % (v/v), 50 % (v/v), and 60 % (v/v), while the effect of the concentration(s) of salt required by the CPE system varied from 0.5 mol L^{-1}, 1.0 mol L^{-1}, 1.5 mol L^{-1}, 2 mol L^{-1}, and 2.5 mol L^{-1}. The extraction efficiency, EE (%), of Zn(II) ions based CPE system was calculated using Eq. (1):

$$\textit{Extraction efficiency (\%)} = \frac{(C_s V_s)}{(C_o V_o) \times 100} \qquad (1)$$

Where C_s represents the Zn(II) ions concentration in the surfactant-rich phase of volume (V_s), and C_o represents the Zn(II) ions concentration in the aqueous-surfactant mixture of volume (V_o). The experiment was performed in triplicates (*n=3*).

Preparation of food samples

Three samples of canned fruit and vegetable were used in this study. Canned lychee and tomato puree are classified as fruits, whilst pickled lettuce is classified as a vegetable. The samples were prepared with slight modifications [18]. Canned lychee and pickled lettuce were individually homogenised with sugar brine once opened. The sample solution was filtered from the fruit flesh using a muslin cloth, then double-filtered using a microfilter paper (0.45 µm x 47 mm). 2.5 mL of the 1 M HNO_3 solution was added into 10 mL of each of the sample. The sample was heated and evaporated near to dryness for an hour and cooled to ambient temperature. Then, the sample was diluted with 10 mL of deionised water, and the pH was adjusted to 4 using 0.01 M diluted NaOH. A similar procedure was used to prepare the canned tomato puree sample. It was heated and evaporated near to dryness for an hour, and afterward left to cool to ambient temperature. 20 mL of diluted tomato puree was centrifuged at 6000 rpm for 15 mins, then filtered using a microfilter paper (0.45 µm x 47 mm). The filtrate solution sample was adjusted to a pH of 4 using 0.01 M diluted NaOH. The (remaining) samples were used for other optimal CPE system studies.

RESULTS AND DISCUSSION

Optimisation of the CPE Method

Effect of pH

The pH in a CPE system plays a major role in the extraction of metal-chelate complexes since a suitable pH is required to realise sufficient hydrophobicity metal-chelate complexes reacting with a surfactant-rich phase volume. Thus, pH can be regarded as a primary parameter of CPE in determining the concentration of Zn(II) ions. In this study, the resulting absorption spectra of Zn(II)-EDTA complex at alkaline was selected at pH 10 (Supplementary data: Figure 1). It is confirmed that the actual absorption wavelength of Zn(II)-EDTA complex successfully took place at 250 nm. Contrarily, the acidic pH (pH 4) condition showed weak absorbance and low spectra. Therefore, further optimisation studies of CPE procedure was evaluated at a pH of 10.

EDTA is generally written as H4Y (tetraprotic acid). The hydrogens in H4Y represents four ionisable hydrogens. EDTA has four carboxyl groups and two amine groups, which act as electron pair donors (Lewis bases). Each of the four carboxyl groups and two amine groups contain a pair of unshared electrons that are capable of complexing with a metal ion. EDTA will generally react with metal ions to form a metal-EDTA complex [19-20] which can be represented using Eq. (2):

$$M^{2+} + H_2Y^{2-} \rightleftharpoons MY^{2-} + 2H^{+} \qquad (2)$$

Where M represents metal, and H2Y2- represents the chelating agent.

By carrying out the chelating reaction in an alkaline buffer solution, the H+ ion at EDTA structure can be removed, which gives the opportunity for the positively charged Zn(II) to bind or attach itself to the free carboxyl group of the EDTA molecule (Figure 1) [21]. It can be concluded that the Zn(II)-EDTA complex ions can completely be formed in an alkaline condition, possibly rendering the detection of Zn(II)-EDTA complex ions by UV-Vis spectrophotometer. This supposition is relatively similar to other works reporting that the pre-concentration of Zn(II) is most efficient in alkaline conditions at pH of 8-12 [22-24]. This occurs due to the increasing presence of cations, which then stop increasing in an alkaline environment, due to the solution reaching thermodynamic equilibrium for complex formation and extraction. It can be inferred that pH significantly influences the absorbance intensity for a complexing reaction. Therefore, a pH of 10 was selected as the optimum condition for studying the CPE system.

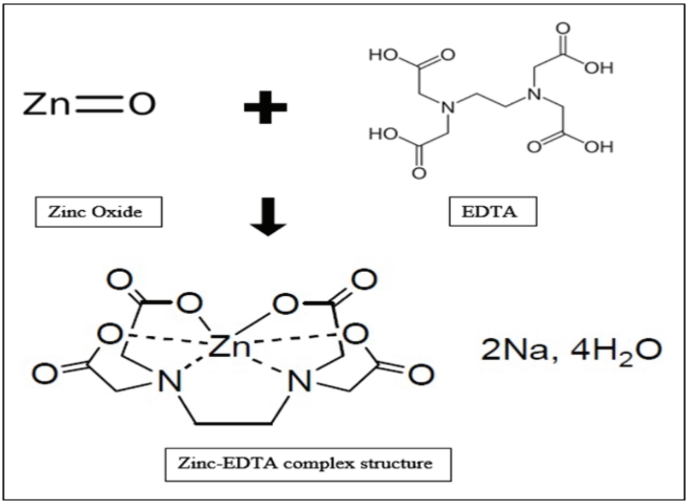

Figure 1: The General Formation of Zinc-EDTA Complex

Effect of equilibration temperature

The equilibration temperature is a crucial parameter in the CPE system due to the efficiency of the extraction of the analyte, depending on the cloud point temperature (CPT) of the surfactant. The temperature range used in this study was 20˚C to 100˚C. Figure 2 shows that at temperatures of 20˚C, 30˚C and 40˚C, there is no separation of phases, indicating that the CPE is ineffective at these temperatures. This could be due to the fact that at lower temperatures, the phase separation of the desired analytes becomes low, caused by the immobilisation of the analytes, which prevents the surfactants from reacting. Separation seems to slowly take place from 50˚C to 60˚C, which can be attributed to the transfer of Zn(II) ions into the extractant Triton X-100 surfactant-rich phase. Zn(II) ions recovery is optimal at 60˚C, where it reported an extraction efficiency of 48%. A small but significant decrease in the CPT of non-surfactant Triton X-100 (originally at 63-68˚C) to 60˚C takes place upon the addition of sodium carbonate, which induces the agent/synergic reagent in the CPE. This confirms that the equilibration temperature influences the isotropic phases of non-surfactant Triton X-100, which directly influence the extraction efficiency of Zn(II) ions in the CPE system. The non-surfactant Triton X-100 was obtained in a single isotropic solution phase region at temperatures far below the CPT, but the surfactant will separate into two co-existing isotropic phases at temperature ranges of the CPT and above. This influences the formation of the separation phase in CPE and the immobilisation of Zn(II) ions into the surfactant micelles. It should be pointed out that the presence of salt also decreases the CPT of the surfactant and increases the extraction efficiency of the Zn(II) ions.

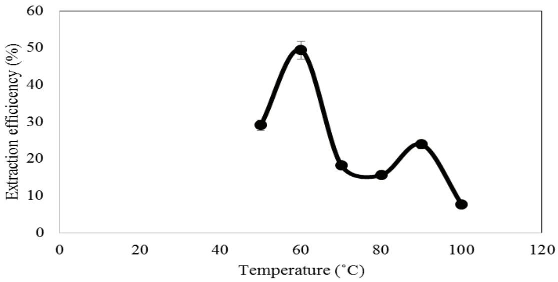

Figure 2: Effect of Equilibration Temperature. Condition: Ratio 1:1 of Zinc: EDTA Complex Solution, pH 10, 40% (v/v) of Triton X-100, 1.5 mol/L of Na_2CO_3 Solution, 15 Min

However, when the temperature exceeds 60˚C (significantly higher than CPT), the extraction efficiency of Zn(II) gradually decreased due to the migration (out) of the Zn(II) ions from the micelles' surfactant-rich phase. This is relevant to the surfactant phase volume, due to the fact that the increasing temperature will interfere with the interactions between surfactant-rich and aqueous-rich phases, which decreases the hydration of solutes. Also, the increasing temperature decreases the critical micelle concentration (CMC) of the non-surfactant Triton X-100, which will induce the surfactant to be more hydrophobic in order to effect an equilibrium shift, which favours the dehydration of ether oxygen. This results in an increase in the concentration of micelles [25]. The increasing temperature will result in the evaporation of the solution, affecting a decrease in the desired zinc analytes, which is far from economical. Therefore, the equilibration temperature at 60˚C was selected for further optimisation study.

Effect of incubation time

The parameter incubation time was studied for five to 30 mins. Incubation time is closely related to the equilibration temperature since it contributes to the efficiency of the Zn(II) ions' extraction efficiency. It can be seen in Figure 3 that the Zn(II) ions recovery remains nearly constant for five to 15 mins when heated. At 20 mins, the recovery of zinc increased dramatically, then decreased significantly after 20 mins. This is due to the fact that when CPE is above CPT, the solution will separate into two phases, which takes place when the CPE is kept at a constant temperature for an indefinite amount of time. When the CPE system reaches its optimal incubation time, the complex formation will reach its thermodynamic equilibrium, resulting in a high extraction of Zn(II) ions [23]. The optimum incubation time for Zn(II) ions extraction efficiency is 20 mins, with a 92 % zinc recovery rate. At five to 15 mins, the separation reaction is insufficient, because it did not reach its thermodynamic equilibrium, which means that the recoveries are inefficient and the extraction percentage low. At more than 20 mins, the extraction efficiency of Zn(II) ions rapidly decreases due to the instability of Zn(II) ions and surfactant complexes. A longer incubation time helps the CPE system achieve full phase separation between the surfactant-rich and aqueous-rich phases. However, when the incubation time exceeds that of the optimum time, the diffusion of micelles in the aqueous solution will increase, the density of the surfactant will decrease, and its volume will also increase. These changes will result in low extraction recoveries and decreased absorbances [24]. Incubation time for zinc extraction of 20 min was used for the next optimisation parameter.

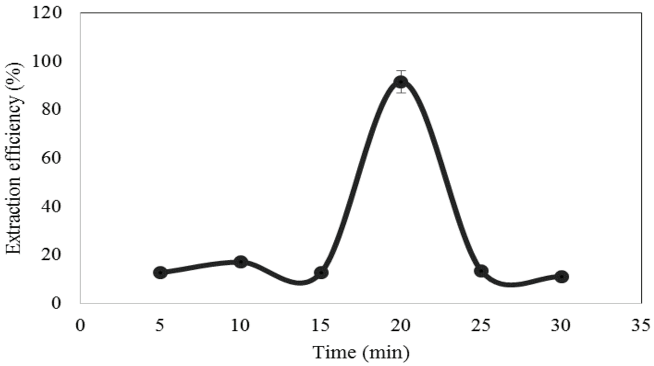

Figure 3: Effect of Incubation Time. Condition: Ratio 1:1 of Zinc: EDTA Complex Solution, pH 10, 40% (v/v) of Triton X-100, 1.5 mol/L of Na_2CO_3 Solution at 60˚C

Effect of concentration of surfactant

The performance of CPE for Zn(II) was observed under the presence of a non-ionic surfactant that influences the extraction efficiency and sensitivity of the extraction process. The utilisation of a surfactant is common for the extraction of metal-ligand complexes in a more efficient way without the use of organic solvents [26]. Triton X-100, also known as p-(1,1,3,3-tetramethyl butyl)phenoxy poly-(oxyethylene) glycol), was used as the CPE extractant due to its commercial availability, high purity, and low toxicity and cost. Concentrations of 10% (v/v) to 60% (v/v) Triton X-100 were used in the CPE method. Figure 4 shows that the Zn(II) ions recovery is directly proportional to the concentration of Triton X-100. Increasing the concentration of Triton X-100 will also increase the number of hydrophobic micelles, which results in a responsive increase of extractability of Zn(II) ions. This is in agreement with Yang *et al*., (2015) [12].

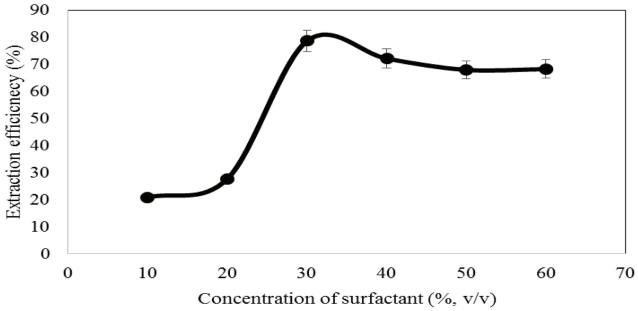

Figure 4: Effect of Triton X-100 Surfactant Concentration. Condition: Ratio 1:1 of Zinc: EDTA Complex Solution, pH 10, 1.5 mol/L of Na_2CO_3 Solution at 60˚C, 20 Min

However, at one point, the Zn(II) ions recovery will slightly decrease when the concentration of the surfactant exceeds 30% (v/v) of Triton X-100. This occurrence could be due to the presence of a high amount of surfactant, which increases the volume of the surfactant-rich phase. The increasing viscosity of the surfactant-rich phase leads to poor sensitivity. The results show that zinc extraction recoveries are highest at 30% (v/v) of Triton X-100, at a 78% zinc extraction efficiency. Low surfactant concentrations (10% (v/v) and 20% (v/v)), as shown in Figure 5, causes low recoveries of the desired Zn(II) ions due to the insufficient reactions between the Zn(II)-EDTA complex ions. The Triton X-100 quantitatively entrap the ternary (hydrophobic) complex [26]. The optimum concentration of the surfactant used for further optimisation in this study is 30% (v/v).

Effect of concentration of salt

Sodium carbonate (Na_2CO_3) salt was added as a synergic reagent (inducing agent) in the CPE system due to its ability to improve phase separation between surfactant-rich and aqueous-rich phases. The concentration of Na_2CO_3 used in this work was 0.5-2.5 mol/L. The Zn(II) recovery increased up to 94% (Figure 5) when 1.0 mol/L of Na_2CO_3 salt solution was added. It was observed that the presence of salt will decrease the cloud point (salting-out), which increases the efficiency of the extraction process. The lower cloud point is attributed to salts promoting the dehydration of the poly(oxyethylene) chains. Therefore, the optimum concentration of Na_2CO_3 is 1.0 mol/L. The salting-out phenomenon is directly related to the desorption of ions to the hydrophilic parts of the micelles, which increases the inter-attraction between micelles, consequently leading to the precipitation of the surfactant molecules [27].

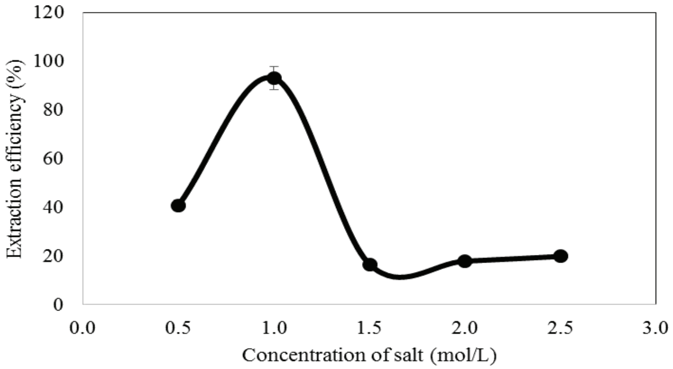

Figure 5: Effect of Na_2CO_3 Salt Concentration. Condition: Ratio of 1:1 of Zinc: EDTA Complex Solution, pH 10, 30 % (v/v) of Triton X-100, at 60˚C, 20 Min

However, the overly-high concentration of salt will reduce the ability of the surfactant to extract the analyte and to instantly decrease the recovery of the desired analyte. At a high concentration of salt, the surfactant tends to be more soluble in the sample solution, which consequently decreases the effectiveness of the CPE [28]. This is evident when 1.5-2.5 mol/L of Na_2CO_3 solution is added, where the extraction efficiency of Zn(II) ions gradually decreased, reporting a constant low zinc recovery performance (Figure 6). This effect can be attributed to the additional surface charge when the Na_2CO_3 concentration is very high, which changes the molecular architecture of the surfactant, and consequently, the micelle formation process. Therefore, 1.0 mol L^{-1} of Na_2CO_3 salt concentration was selected as the optimum parameter in the CPE study.

Validation and application of the CPE method to real samples

In order to confirm the applicability of the proposed method, the optimised CPE was used to determine the concentration of zinc in canned food samples under conditions of 1:1 ratio of Zn(II)-EDTA complex ion, a pH of 10, 30% (v/v) of Triton X-100, 1.0 mol L^{-1} of Na_2CO_3 concentration, temperature of 60˚C, and 20 mins of incubation time. The initial pH of the food samples inside the can be measured in order to understand the nature of the food samples itself. All of the canned food samples were maintained and tested under acidic conditions, at pH of 3.8 for canned pineapple, 4.12 for canned lychee, and 4.22 for the canned pickled lettuce.

The linear calibration graph was in the range of 0.001-0.020 mg/L with a correlation of determination (R2) was 0.9676. The limits of detection (LOD) and limits of quantification (LOQ) were calculated using S/N*3 and S/N*10 respectively, where S/N is the signal to noise ratio. The LOQ was 0.001 mg/L and the LOD was 0.0003 mg/L. The amounts of zinc found in the food samples were in the range of 0.001-0.005 mg/L with relative standard deviation (RSD) was < 10% as shown in Table 1. These trace amounts could be due to the can being immune to the food content, and remaining unaffected, with no internal corrosion. This also confirms that the trace amount of zinc in the canned food samples was below the permissible limit of 150 mg L^{-1}, and the Malaysian standard of Zn of 100 mg L^{-1}, which means that the canned food is safe for human consumption [7].

It should be pointed out that food additives such as sugar, syrups, salts, or other materials present in canned foods can significantly influence the corrosion rates inside the can. Thus, there is a possibility both of organic and inorganic substrates arresting the corrosion process. The trace amounts of zinc detected in the samples could be from the food contained within the can itself, since fish, fruits, and vegetables are by themselves rich in vitamins and minerals [29]. Thus, this CPE method is suitable for determining the concentration of Zn(II) ions in food samples.

Table 1: The Concentration of Zinc Traces Found In Food Samples

Concentration (mg L^{-1})				
Canned food samples	Added Zn(II) standard	Amount Zn(II) in Food Sample after added Zn(II) standard	Zn(II) found	RSD (%), (*n* =3)
Lychee	0.012	0.017	0.005	5.546
Canned pineapple	0.012	0.013	0.001	9.594
Pickled lettuce	0.012	0.017	0.005	2.773

CONCLUSION

A simple pre-concentration CPE method was successfully developed utilizing Triton X-100 and Na_2CO_3 as its extractant and synergic reagents, respectively. EDTA was used as a complexing agent for detecting the presence of zinc in food samples. The CPE was optimised by evaluating the concentrations of surfactant and salt, pH, temperature, and time. The zinc content in the canned food samples was found to be under permissible levels, which confirms the viability of the proposed CPE for the detection of trace amounts of metals in food samples.

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REFERENCES

[1] M. Jaishankar, T. Tseten, N. Anbalagan, B. B. Mathew and K. N. Beeregowda, 2014. Toxicity, mechanism and health effects of some heavy metals, *Interdisciplinary Toxicology, 7*(2), pp. 60-72. Doi: 10.2478/intox-2014-0009

[2] G. U. Chibuike and S. C. Obiora, 2014. Heavy metal polluted soils: effect on plants and bioremediation methods, *Applied and Environmental Soil Science, 2014*, p. 12. Doi: 10.1155/2014/752708

[3] P.K.T. Oldring and Nehring, 2007. *Packaging materials: 7. Metal packaging for foodstuffs, International Life Sciences Institute Europe Report Series*. ILSI Europe.

[4] J. Nordqvist, 2017. What are the health benefits of zinc?. *Medical News Today*. Retrieved from https://www.medicalnewstoday.com/articles/263176.php.

[5] R. Singh, N. Gautam, A. Mishra and R. Gupta, 2011. Heavy metals and living systems: An overview, *Indian Journal of Pharmacology, 43*(3), pp. 246-253. Doi: 10.4103/0253-7613.81505

[6] L. M. Plum, L. Rink and H. Haase, 2010. The essential toxin: impact of zinc on human health, *International Journal of Environmental Research and Public Health, 7*(4), pp. 1342-1365. Doi: 10.3390/ijerph7041342.

[7] Food and Agriculture Organization (FAO)/World Health Organization (WHO), 1984. List of maximum levels recommended for contaminants by the Joint FAO/ WHO Codex Alimentarius Commission. Second Series. CAC/FAL, Rome, 3, pp.1-8.

[8] R. Gürkan, Ü. Aksoy, H. İ. Ulusoy and M. Akçay, 2013. Determination of low levels of molybdenum (VI) in food samples and beverages by cloud point extraction coupled with flame atomic absorption spectrometry, *Journal of Food Composition and Analysis, 32*(1), pp. 74-82. Doi: https://doi.org/10.1016/j.jfca.2013.08.005.

[9] S.J. Kulkarni, S. Dhokpande, and D.J. Kaware, 2015. A review on spectrophotometric determination of heavy metals with emphasis on cadmium and nickel determination by U.V. Spectrophotometry, *International Journal of Advanced Engineering Research and Science* (IJAERS), *2*(9), pp. 35-38.

[10] G. Peng, Q. He, G. Zhou, Y. Li, X. Su, M. Liu and L. Fan, 2015. Determination of heavy metals in water samples using dual-cloud point extraction coupled with inductively coupled plasma mass spectrometry, *Analytical Methods, 7*(16), pp. 6732-6739. Doi: 10.1039/C5AY00801H.

[11] N. Jalbani and M. Soylak, 2015. Preconcentration/separation of lead at trace level from water samples by mixed micelle cloud point extraction, *Journal of Industrial and Engineering Chemistry, 29*, pp. 48-51. Doi: https://doi.org/10.1016/j.jiec.2015.02.025.

[12] X. Yang, G. Lu, R. Wang, Y. Xie, C. Guo, X. Yi, *et al.*, 2015. Competitive solubilisation of 4,4′ dibromodiphenyl ether, naphthalene, and pyrene mixtures in Triton X series surfactant micelles: The effect of hydrophilic chains, *Chemical Engineering Journal, 274*, pp. 84-93.

[13] M. Bilal, T. G. Kazi, H. I. Afridi, M. B. Arain, J. A. Baig, M. Khan, *et al.*, 2016. Application of conventional and modified cloud point extraction for simultaneous enrichment of cadmium, lead and copper in lake water and fish muscles, *Journal of Industrial and Engineering Chemistry, 40*, pp. 137-144. Doi: https://doi.org/10.1016/j.jiec.2016.06.015.

[14] A. Saha, S. B. Deb, A. Sarkar, M. K. Saxena and B. S. Tomar, 2016. Simultaneous preconcentration of uranium and thorium in aqueous samples using cloud point extraction, *RSC Advances, 6*, pp. 20109-20119. Doi: 10.1039/C5RA23734C

[15] X. Yang, Z. Jia, X. Yang, G. Li and X. Liao, 2017. Cloud point extraction-flame atomic absorption spectrometry for pre-concentration and determination of trace amounts of silver ions in water samples, *Saudi Journal of Biological Sciences, 24*(3), pp. 589-594. Doi: https://doi.org/10.1016/j.sjbs.2017.01.030.

[16] H. Watanabe and H. Tanaka, 1978. A non-ionic surfactant as a new solvent for liquid—liquid extraction of zinc(II) with 1-(2-pyridylazo)-2-naphthol, *Talanta, 25*(10), pp. 585-589. Doi: https://doi.org/10.1016/0039-9140(78)80151-9.

[17] D. Citak and M. Tuzen, 2010. A novel preconcentration procedure using cloud point extraction for determination of lead, cobalt and copper in water and food samples using flame atomic absorption spectrometry, *Food and Chemical Toxicology, 48*(5), pp. 1399-1404. Doi: https://doi.org/10.1016/j.fct.2010.03.008.

[18] R. Gürkan and N. Altunay, 2015. Determination of total Sn in some canned beverages by FAAS after separation and preconcentration, *Food Chemistry, 177*, pp. 102-110, 2015. Doi: https://doi.org/10.1016/j.foodchem.2015.01.015.

[19] Z. Ezzeddine, I. Batonneau-Gener, Y. Pouilloux, H. Hamad, Z. Saad and V. Kazpard, 2015. Divalent heavy metals adsorption onto different types of EDTA-modified mesoporous materials: Effectiveness and complexation rate, *Microporous and Mesoporous Materials, 212*, pp. 125-136. Doi: https://doi.org/10.1016/j.micromeso.2015.03.013.

[20] Y. Song, M.-T. Ammami, A. Benamar, S. Mezazigh and H. Wang, 2016. Effect of EDTA, EDDS, NTA and citric acid on electrokinetic remediation of As, Cd, Cr, Cu, Ni, Pb and Zn contaminated dredged marine sediment, *Environmental Science and Pollution Research, 23*(11), pp. 10577-10586. Doi: 10.1007/s11356-015-5966-5.

[21] C.P. Spencer, 1958. The Chemistry of ethylenediamine tetra-acetic acid in sea water, *Journal of the Marine Biological Association of the United Kingdom, 37*(1), pp. 127-144. Doi: 10.1017/S0025315400014880.

[22] E.B. Rehab and A-Z.E. Mohamed, 2018. Cloud point extraction using BBTSC reagent for pre-concentration and determination of zinc in some environmental water samples by flame atomic absorption spectrometry, *American Journal of Chemistry, 8*(2), pp. 51-56. Doi:10.5923/j.chemistry.20180802.05.

[23] X.P. Yang, Z.H. Jia, X.C. Yang, N. Luo and X.J. Liao, 2017. Determination of trace zinc in water, soil and rabbit blood samples using cloud point extraction coupled with ultraviolet-visible spectrophotometry, *Applied Ecology and Environmental Research, 15*(3), pp. 537-548. Doi: 10.15666/aeer/1503_537548.

[24] S. K. Jawad and F. H. Hayder, 2015. Determination and Extraction of Zinc (II) Via Cloud Point Methodology*, International Journal of Applied Chemical Sciences Research, 3*(1), pp. 1-12.

[25] A. Saliza, S. M. R. Nur and M. S. Noorashikin, 2018. Performance of sodium salts as an inducing phase separation agent on the polyethylene glycol/β-cyclodextrin cloud point extraction for the determination of propylparaben, *Desalination and Water Treatment, 105*, pp. 234-244. Doi: 10.5004/dwt.2018.22028

[26] R. Gürkan, U. Kır and N. Altunay, 2015. Development of a simple, sensitive and inexpensive ion-pairing cloud point extraction approach for the determination of trace inorganic arsenic species in spring water, beverage and rice samples by UV–Vis spectrophotometry, *Food Chemistry, 180*, pp. 32-41. Doi: https://doi.org/10.1016/j.foodchem.2015.01.142.

[27] S. Z. Mohammadi, T. Shamspur, D. Afzali, M. A. Taher and Y. M. Baghelani, 2016. Applicability of cloud point extraction for the separation trace amount of lead ion in environmental and biological samples prior to determination by flame atomic absorption spectrometry, *Arabian Journal of Chemistry, 9*, pp. S610-S615. Doi: https://doi.org/10.1016/j.arabjc.2011.07.003.

[28] J. Dong, K. B. Sharpless, L. Kwisnek, J. S. Oakdale and V. V. Fokin, 2014. SuFEx-Based Synthesis of Polysulfates, *Angewandte Chemie International Edition, 53*(36), pp. 9466-9470. Doi: 10.1002/anie.201403758.

[29] J. L. Slavin and B. Lloyd, 2012. Health Benefits of Fruits and Vegetables, *Advances in Nutrition, 3*(4), pp. 506-516. Doi:10.3945/an.112.002154.