A pH-dependent CT sensitive method for detection of trace Mn in milk by UV-spectrophotometry

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A R T I C L E   I N F O

A B S T R A C T

In this study, a new method was developed for the extractive pre-concentration/detection of trace Mn in milk prior to analysis by UV-spectrophotometry. The method is based on the pH-controlled intra-ligand charge transfer (ICT) complexation of modifier, 2-HBT in presence of trace Mn (II), and then the extraction of the complexes, where the hydrazine and hydrazone tautomers are predominant at pHs of 2.0/ 3.0, and pH 6.0 because of CT at pH 4.0. The free Mn (II) and total Mn levels of the samples were detected at 269 nm by UV-spectrophotometry. For total Mn, the method was validated by analysis of two certified milk samples. The results were statistically in good agreement with the certified values, and the precision was lower than 6.4%. After a pre-concentration of 62.5-fold, the limits of detection were 1.45/1.92 and 1.54/2.12 $\mu g\ L^{-1}$ in range of 5–165 and 5–150 $\mu g\ L^{-1}$ from the solvent-based and matrix-matched calibration curves, respectively. There is not a matrix effect from comparison of the calibration curves's slopes. The method was successfully applied to analysis of milk samples. In terms of speciation, the free Mn (II) and total Mn levels in milk were in the range of 7.65–30.4 $\mu g\ L^{-1}$, and 10.2–34.7 and 10.7–35.5 $\mu g\ L^{-1}$ after the microwave and ultrasound assisted extraction.

1. Introduction

Mn is an essential trace element that is present in all living cells. The human body in normal conditions contains about 10–20 mg of Mn, and it is present in numerous oxidoreductases and other enzymes exercising several biological functions (Oga, 2008; Goldhaber, 2003). A daily ingestion of at least 3 mg is considered as necessary (Goldhaber, 2003). Although Mn is considered an essential element, the data are insufficient to settle down a value for the daily ingestion (de Azevedo and Chasin, 2003). The fetal life and the first childhood are the most vulnerable periods to the Mn deficiency although rare (Jensen, 1995). This can cause dermatitis, problems in the glucose metabolism and of proteins, mitochondria abnormalities, infertilities, bad formation of the bones, decrease of the serum cholesterol, and others (ATSDR, 2000). Although the Mn gastrointestinal absorption is only 3–5%, foods constitute the largest source of Mn in general for the population, and they are also the primary source of the absorbed metal (de Azevedo and Chasin, 2003). The levels of daily alimentary ingestion range from 1.0 to 2.0, 2.0–5.0, 0.6–1.0, and 0.3–0.6 mg day^{-1} for age groups of 3–10-years old, > 10 years, 6 months, ≤ 6 months, respectively, (de Azevedo and Chasin, 2003). Excess of Mn can be toxic, and the nervous system seems to be the most vulnerable (Erikson et al., 2007). The Mn neurotoxicity in adults is

associated with symptoms of Parkinson disease while effects in children are not well characterized. Babies and children are quite sensitive to the Mn toxicity. Neonates accumulate more Mn than adults due to larger absorption of Mn (Garcia et al., 2007). The Mn concentration in milk and similar products vary from 0.02 to 0.49 $\mu g\ g^{-1}$ (de Azevedo and Chasin, 2003). Erikson et al. (2007) reported that Mn concentration in human milk ranged from 3 to 6 $\mu g\ L^{-1}$ while in bovine milk the Mn concentrations being about 21 $\mu g\ L^{-1}$. Human milk usually had a lower concentration while the infant formula content can vary drastically. In this case, the soy food deserves special attention. The plants absorb Mn in form of divalent ion (Cardoso et al., 2003), and the soy could concentrate it in its grains. A feeding bottle of soy drink can contain a Mn concentration many times higher than that found in the breast or cow milk. Therefore, the information on the Mn levels in this infant food is extremely important in assessing the risk to human health.

Due to its low concentrations in a variety of food samples, Mn determination requires either an accurate analytical method with sufficient sensitivity or a suitable pre-concentration tool for an improvement in sensitivity of the method and avoiding from the possible matrix effect. Graphite furnace-atomic absorption spectrometry (GF-AAS) and plasma techniques have been widely used for total Mn determination in several types of food samples. Most analytical techniques for metal

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determination need a sample preparation method which involves solubilization with complete or partial decomposition of the matrix, and most methods make use of acids with oxidizing properties. Mn and other metals were determined in breast milk by GF-AAS with Zeeman background correction after wet-ashing decomposition finding mean 43.2 μg L^{-1} and levels varying from 12.5 to 70.7 μg L^{-1} (Turan et al., 2001). Leotsinidis et al. (2005) determined Mn by GF-AAS in breast milk-finding levels ranging from 1.01 to 15.70 μg L^{-1}.

Several methods of slurry sampling have been frequently studied to minimize drawbacks of the digestion procedures, such as long analysis times, high cost, analyte loss or contamination and to get better limits of detection and sensitivity of the methods. Without any pre-concentration, direct analysis of milk and milk-based products were previously performed by sensitive, but expensive and time-consuming detection techniques such as inductive coupled plasma-optical emission spectrometry (ICP-OES), microwave induced plasma-optical emission spectrometry (MIP-OES) (McKinstry et al., 1999; Matusiewicz and Golik, 2004), and GFAAS using multivariate optimization (Cabrera et al., 1996; de Amorim et al., 2011). Also, combination of flame atomic absorption spectrometry (FAAS), flow injection-flame atomic absorption spectrometry (FI-FAAS) and ICP-OES with ultrasound assisted-cloud point extraction (UA-CPE) and an immobilized 8-hydroxyquinoline minicolumn as a pre-concentration tool (Sahin et al., 2010; Lemos et al., 2008; Lemos and David, 2010; Rod et al., 2006; Yalcin et al., 2012; Bezerra et al., 2006; Bruhn et al., 2002), including spectrophotometry (Gouda, 2014), was independently used in multi-element analysis of water, tea, milk, vegetable leaves, food and mussel and non-fat milk powder, including Mn.

The CPE is an attractive technique that reduces the consumption of the solvent, extraction time and the disposal costs. It has been used for the extractive pre-concentration after the formation of sparingly water-soluble complexes (Badera et al., 2014). When compared with other enrichment techniques, it has some advantages such as low cost, speed, good concentration efficiency, eco-friendly lower toxicity, and safety (Badera et al., 2014). It is a simple procedure with a high capacity to pre-concentrate (PF), since the metal can be collected in small volumes (0.2–0.5 mL) of the micellar phase. This allows PFs identical to those of other techniques without an additional re-extraction step.

In this study, a new UA-CPE method was developed for the pre-concentration/speciation analysis of free Mn (II) and total Mn from the matrix via two sample preparation procedures. For this purpose, 2-HBT is used as a modifier of poly (SMAnh) to obtain the stable metal–chelates at pH 2.0 and 3.0, respectively where hydrazinic tautomer is selectively prevalent because of ICT at pH 4.0. The formed metal–chelates is extracted into the micellar phase of Triton X-114 as extractant, diluted to a volume of 0.4 mL with THF, due to the high density of the micellar phase, and detected at 269 nm by spectrophotometry.

There are two special features at the heart of the study: (a) implementation of ultrasound energy shortens the extraction time and improves the extraction efficiency and (b) use of CTAB-SDS mixture (4:1, v/v) for amidic copolymer at pH 2.0 and only SDS for imidic copolymer at pH 3.0 in the extraction process provides an improvement in detection sensitivity/selectivity of the method by UV-spectrophotometry. To fully characterize the UA-CPE method, the variables affecting chelate formation and extraction efficiency were evaluated and optimized in detail.

2. Experimental

2.1. Instrumentation

The chelators were characterized by instrumental tools like FT-IR, ^{1}H NMR, and UV -Vis. The levels of free Mn (II) and total Mn were detected at 269 nm, including two shoulder peaks appearing at 240 and 292 nm, against a blank in THF by a double-beam spectrophotometer (Shimadzu 1800, Japan). An ultrasonic bath (40 kHz and 300 W) (UCS-10, Seoul,

Korea) was used for the extraction and pre-concentration of Mn from the matrix by UAE for sample preparation, and UA-CPE. A vortex (50 Hz and 12 W) (VM96-B, Korea) was used for the homogenization of the samples. A centrifuge (Universal 320 Hettich, England) was used to facilitate the phase separation. The pH measurements were performed via a pH meter (Selecta 2001 Sartorius, North America). A Labconco ultrapure water system (Kansas City, USA) was used to obtain water to be used in experiments, resulting water with a resistivity of 18.2 MΩ cm^{-1}.

2.2. Reagents, SRMs and solutions

In this study, all the chemicals used were of analytical purity; they were purchased from Sigma (USA) and Merck (Germany). At initial, all the containers were kept in 10% (v/v) HNO_3 for at least 24 h, and then washed five times with water. The stock chelator solutions (0.1% (w/v)) were prepared by dissolving 0.100 g of the solids in acetone–water (1:1, v/v), due to precipitation in alcohol. The Mn (II) working solutions (25 mg L^{-1}, $MnCl_2 \times 4\ H_2O$) were prepared by dilution of the solution of 1000 mg L^{-1} with water. The solutions of Oxalic acid, KCl, Triton X-114, CTAB-SDS (4:1, v/v), and only SDS in 100 mL of water (0.02 mol L^{-1}, 2.0% (w/v), 5.0% (v/v), and 4.0% (w/v)) respectively were independently prepared to improve the sensitivity of the method. Methanol, ethanol, acetonitrile, acetone, THF, acidic methanol and ethanol (1.0 mol L^{-1} HCl) were tested as the diluting agents. The McIlvaine buffer solutions (0.1 mol L^{-1}, pH 2.0–8.0) were prepared by mixing known volumes of and 0.1 mol L^{-1} Citric acid and 0.2 mol L^{-1} Na_2HPO_4, adjusting to the desired pH using dilute HCl or NaOH, and completing to 100 mL with water. Two certified milk samples (SRM 1549 Nonfat milk powder, SRM 8435 Whole milk powder), supplied from NIST (Gaithersburg MD, USA), were used to validate the method. Also, the Salicylic acid, H_2O_2-Citric acid mixture (3:2, v/v) (2.0% (w/v), 2.0 and 0.2 mol L^{-1}) sequentially for sonication of 20 min at 35 °C, and 20 plus10 min at 55 °C at pH 5.0 due to their reducing and chelating nature, and HNO_3-H_2O_2 mixture (4:1, v/v) for speciation of free Mn (II) and total Mn levels were used in the extraction of all the samples by UAE and MAE.

2.3. Sampling, sample preparation

For sampling, all glassware and plastic beaker/tubes were washed with a 0.5 mol L^{-1} HNO_3 solution for at least 24 h and thoroughly rinsed 6 times with ultrapure water before use. As Mn concentration in the samples is very low, even minor contamination at any stage of the sampling, sample storage, and handling or analysis has the potential to affect the accuracy of the results. The certified values are available for total Mn for the assessment of the method's accuracy. The milk/ milk-based products were randomly purchased from the local markets in Sivas, Turkey. Initially, all the samples were stored in a refrigerator until analysis. According to the procedures adopted from literature with slight modification (Khan et al., 2014; Abollino et al., 1998; Li et al., 2023; Topolski, 2011; Gürkan and Temel, 2020), all the samples with and without certification were pretreated and extracted as follows:

For microwave assisted extraction (MAE), aliquots of 0.2 and/or 0.3 g were accurately weighed directly into the digestion vessels. An acid mixture of conc. HNO_3-H_2O_2 (4:1, v/v) were added. The MAE was performed by 1000 W at 80 °C for 5 min, 1000 W at 50 °C for 5 min, and 1000 W at 190 °C for 20 min. After cooling for 30 min, the contents were transferred to 50 mL volumetric tubes and a dilution of 25 and 100-fold for certified samples and the milk-based samples, respectively, were made for analysis. The blank solutions were prepared by the procedure above without sample (Khan et al., 2014).

For UAE, aliquots of 0.3 g of the milk/milk-based products were separately weighed into a centrifuge tube of 50-mL, to which 5 mL of water was added to facilitate sample homogenization by vortexing for 2 min at 1200 rpm. After that, for analysis of free Mn (II) and total Mn, standard Mn (II) solutions (10 μg L^{-1}) was added to the homogenized sample solutions for reliability of the method and extraction of the Mn.

The centrifuge tubes were placed in a test tube rack and immersed in the ultrasonic bath (40 kHz, 300 W), keeping the water level equal to the extraction solvent level inside the tubes. In order to enhance the leaching of the analyte from the sample matrix to the solvent, sonication was carried out in the ultrasonic bath (300 W, 40 kHz) for free Mn(II) levels with 10 mL of 2.0%(w/v) Salicylic acid at 35 °C for 20 min, so as to obtain a pH value (herein, pH_{iep} 4.83) corresponding to the isoelectric point of casein, and, then, the tubes were centrifuged at 3000 rpm for 5 min. The resultant clear extraction solutions were diluted to a volume of 50 mL with water (Abollino et al., 1998; Li et al., 2023; Topolski, 2011; Gürkan and Temel, 2020). In fact, under these conditions casein precipitates, co-precipitating several macromolecular compounds (lipids); after filtration through a membrane filter, a homogenous and clear solution is obtained which cannot lead to any light-scattering in a cuvette of the μ-volume cell of 0.4 mL without risk of clogging.

For analysis of total Mn levels, the pre-sonicated and treated sample mixtures with 10 mL of 2.0% (w/v) Saliycylic acid for 20 min were sequentially exposed to further pretreatment 5.0 mL of 2.0 mol L^{-1} H_2O_2 and 0.2 mol L^{-1} Citric acid mixture (3:2, v/v) at 55 °C for 10 min (Abollino et al., 1998; Li et al., 2023; Topolski, 2011; Gürkan and Temel, 2020). The spiked samples at known concentration levels (10 μg L^{-1}) before extraction and pre-concentration were also prepared to calculate the recovery of the extraction procedure, including establishment of the matrix-matched calibration curves. To check if there is a matrix effect, a pooled sample for the matrix-matched calibration curve was prepared and contained equal amounts of the pre-treated samples. The linearity of the method was validated in the linearity range of 5–165 and/or 5–150 μg L^{-1} (n: 6) by spiking with known amounts of Mn (II) in a pooled matrix, including solvent-based calibration curve, because the extraction process is more sensitive to Mn (II). For all the pre-treatment processes, a blank control was carried out in the same way. Then, an aliquot (5.0 mL) of the final clear extract or pre-treated sample solutions with and without spiking (10 μg L^{-1}) for recovery studies was submitted to pre-concentration by UA-CPE prior to analysis by spectrophotometry at 269 nm. The Mn contents of the samples were determined by means of the matrix-matched calibration curves built using standards dissolved in the blank matrix extracts.

2.4. UA-CPE procedure

Aliquots of 5.0 mL of the pre-treated sample solutions for free Mn(II) and total Mn contents or a standard solution containing Mn(II) in the range of 5–165 μg L^{-1}, 25/200 μL of 0.1 mol L^{-1} of McIlvaine buffer solution at pH 2.0/3.0, respectively, 250/300 μL of 0.1%(w/v) of 2-HBT-modified copolymer solutions, 50/150 μL of 4.0% (w/v) CTAB-SDS (4:1, v/v) and only SDS, 100/200 μL of 2.0% (w/v) mol L^{-1} KCl, 250/300 μL of 0.02 mol L^{-1} oxalic acid and 800 μL of 5.0% (v/v) of Triton X-114 were sequentially added to a centrifuge tube of 50-mL, thoroughly mixed and diluted to mark with water. The contents of the flask were sonicated for phase separation in an ultrasonic bath (40 kHz, 300 W) for 5 min at 40 °C. Separation of the phases was accelerated by centrifuging at 3000 rpm for 7 min. Without cooling, the surfactant-rich phases became viscous. Then, the aqueous phase could be separated by using a syringe. Subsequently, the micellar phase was diluted to a volume of 0.4 mL with THF as diluent to decrease its viscosity and make the final volume feasible to transfer into the optical cell of 1.0 cm for the absorbance measurement at 269 nm against an analyte blank prepared under similar conditions. The free Mn (II) and total Mn levels of the samples, submitted to extractive pre-concentration by UA-CPE after pretreatment of samples with two UAE approaches, were determined by using the matrix-matched calibration to suppress the matrix effect. To establish the signal contributions resulting from the used reagents, a blank control solution subjected to the same UA-CPE procedure was measured in parallel with the samples or standard calibration solutions.
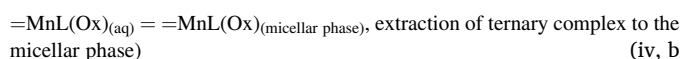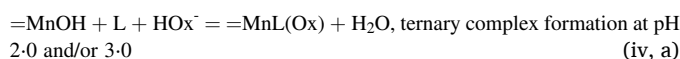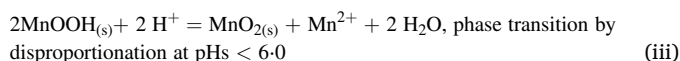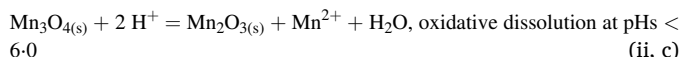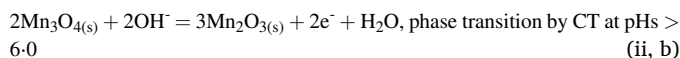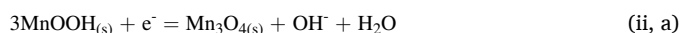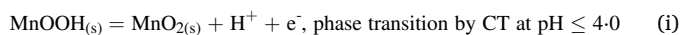
2.5. Statistical analysis

All data were subjected to one- and/or two-way analysis of variance (ANOVA) in SAS 9.1. The mean values were separated by LSD (Least Significant Difference) test at p- of 0.05. The p-values of less than 0.05 considered statistically significant.

3. Results and discussion

For extraction of trace Mn (II), poly (styrene-co-maleic anhydride) (poly(SMAnh) was modified with 2-HBT, to obtain chelators (called as poly(SMAm) and poly(SMIm)) by amidation and sequentially imidation reactions. The newly prepared chelators were thoroughly characterized by instrumental tools such as ATR-FTIR, ^{1}H NMR, TGA/DrTGA and DSC. The detailed spectral data and information's related to their characterization were extensively given in our previous studies (Gürkan and Zengin, 2023; Zengin and Gürkan, 2019).

From prior studies conducted with aim of method development by UA-CPE, it has been observed that a pH-dependent ICT complex is formed among all the other reagents and Mn (II). By a CT and tautomerization at a minimal pH of 4.0, the formed chelates in THF showed a characteristic band at 269 nm with shoulder peaks appearing at 240 and 292 nm for Mn (II) detection at 2.0 and 3.0, respectively. Their UV-Vis spectra in THF upon addition of Mn (II) in range of 2.5–40 μg L^{-1} are represented in Fig. 1(a, b) for 2-HBT modified chelators under optimal conditions. The findings have been also supported by observations appearing in two different studies, based on IR and UV spectral data for only 2-HBT and Cu chelation before and after its reaction with Curcumin with shift of 1580 or1561 cm^{-1} to 1633 cm^{-1} in the >N-C=N grouping, and 228/266–365 nm via ICT, in literature (Gvozdjakova and Ivanovičová, 1986; Calinescu et al., 2019) where the tautomers coexist in equilibrium. Considering all this information, because of pH-dependent/ICT sensitive complexation in micellar media, a wavelength of 269 nm in UV-region due to be more sensitive was adopted for absorbance measurements and used for further studies. It is thought that the complex formation mechanism by pH-induced tautomerization/ICT proceed with a series of steps in nanoscale as follows:

$$MnOOH_{(s)} = MnO_{2(s)} + H^+ + e^-, \text{ phase transition by CT at } pH \leq 4.0 \quad (i)$$

$$3MnOOH_{(s)} + e^- = Mn_3O_{4(s)} + OH^- + H_2O \quad (ii, a)$$

$$2Mn_3O_{4(s)} + 2OH^- = 3Mn_2O_{3(s)} + 2e^- + H_2O, \text{ phase transition by CT at pHs > 6.0} \quad (ii, b)$$

$$Mn_3O_{4(s)} + 2 H^+ = Mn_2O_{3(s)} + Mn^{2+} + H_2O, \text{ oxidative dissolution at pHs < 6.0} \quad (ii, c)$$

$$2MnOOH_{(s)} + 2 H^+ = MnO_{2(s)} + Mn^{2+} + 2 H_2O, \text{ phase transition by disproportionation at pHs < 6.0} \quad (iii)$$

$$=MnOH + L + HOx^- = =MnL(Ox) + H_2O, \text{ ternary complex formation at pH 2.0 and/or 3.0} \quad (iv, a)$$

$$=MnL(Ox)_{(aq)} = =MnL(Ox)_{(micellar phase)}, \text{ extraction of ternary complex to the micellar phase} \quad (iv, b)$$

where L and Ox are 2-HBT modified chelators and oxalic acid as tridentate (N, N, O donor atoms) and bidentate (O, O donor atoms) ligands for chelation and stabilization of at least one of Mn oxides or hydr (oxides) through their surface-active site groups at equal and/or lower pHs (especially where instable, highly reactive MnOOH and MnO_2 coexist in equilibrium at pH_{pzc} of 4.8). By a similar mechanism, it is also implied in literature (Chen et al., 2013; Li et al., 2020) that Mn (III), generated from the reaction of MnO_2 (with pH_{pzc} of 2.3–2.9) and oxalic acid by CT, gives a ternary complex with a quinoxaline-di-N-oxide antibiotics (carbadox), containing hydrazine moiety in its sidechain.
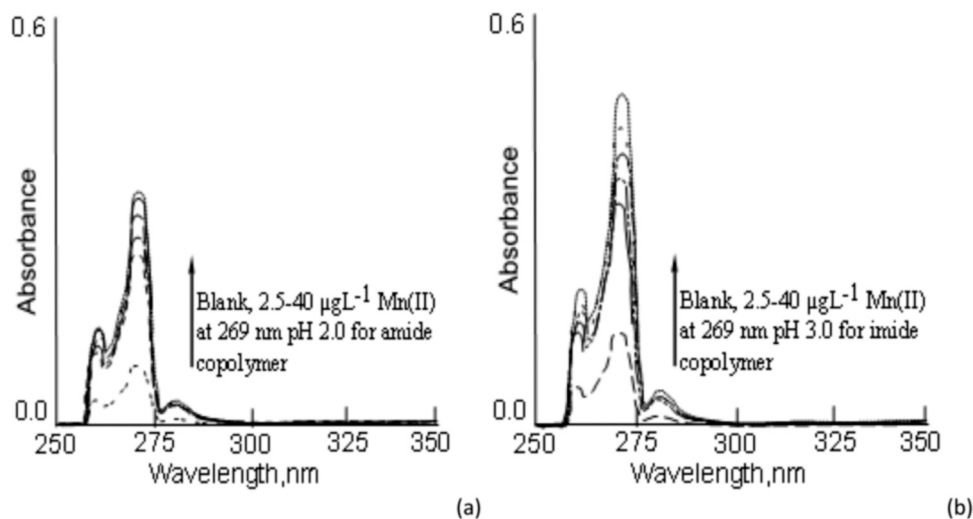
Fig. 1. (a, b) UV-Visible absorption spectra of 2-HBT modified copolymers in THF upon sequential addition of Mn (II) in range of 2.5–40 µg L^{-1} for amide and imide copolymers at pH 2.0 and 3.0, respectively under optimized reagent conditions.

3.1. Optimization of the UA-CPE system variables

Due to its simplicity and ease to use, the variables affecting the UA-CPE efficiency was optimized by univariate optimization, based on one-factor-at-a-time. In this approach, we observe the effect of one factor at a time on the analytical signal. Shortly, the main variables affecting the complexation and extraction efficiency for analysis of 100 µg L^{-1} Mn (II) were optimized step by step.

3.1.1. Effect of pH and buffer concentration

In the UA-CPE process, it is generally aimed at forming a binary or ternary metal chelate complex, according to the pH-dependent CT chelation properties and charges of the reagents participating in the complex formation. The pH plays a major role to ensure this extractable chelate complex formation, because the working pH affects the chemical forms in which both analyte and the modified amide/imide copolymers can be present. For these reasons, the effect of pH on the extraction of binary or ternary complex in the presence of only CTAB and/or CTAB-

SDS mixture (4:1, v/v) as both counter-ion and sensitivity enhancer for measurements of Mn (II) at 100 µg L^{-1} each was studied using 0.1 mol L^{-1} McIlvaine buffer in the pH range of 2.0–8.0. The results were represented in Fig. 2(a). As can be seen from the results, the best signal was obtained at pH 2.0 and 3.0 with a slightly lower sensitivity than those of the hydrazone tautomer at pH 6.0 for while it is obtained at pH 6.0 for complexation of Mn. The signals were drastically increased at lower pHs than 4.0 where the sensitivity increases with decreasing pH to pass through a minimum at pH 4.0. It is thought that the increase in the signal between pH 4.0 and 6.0 is due to the complexation of the one electron oxidation species of Mn(II) formed in the solution environment by CT and the interaction of the hydrazonic tautomers with the surface active site groups of at least one of the oxide or oxyhydroxide species of Mn (like MnOOH, MnO$_2$, Mn$_2$O$_3$ and/or Mn$_3$O$_4$ formed by pH-dependent, CT-sensitive reductive/oxidative dissolution and phase transformation in nanoscale) while the signal gradually decreases with increasing pH between pH 6.0–8.0. The increase in the signal at low pHs can be due to the pH-dependent CT complexation of Mn (II) with the
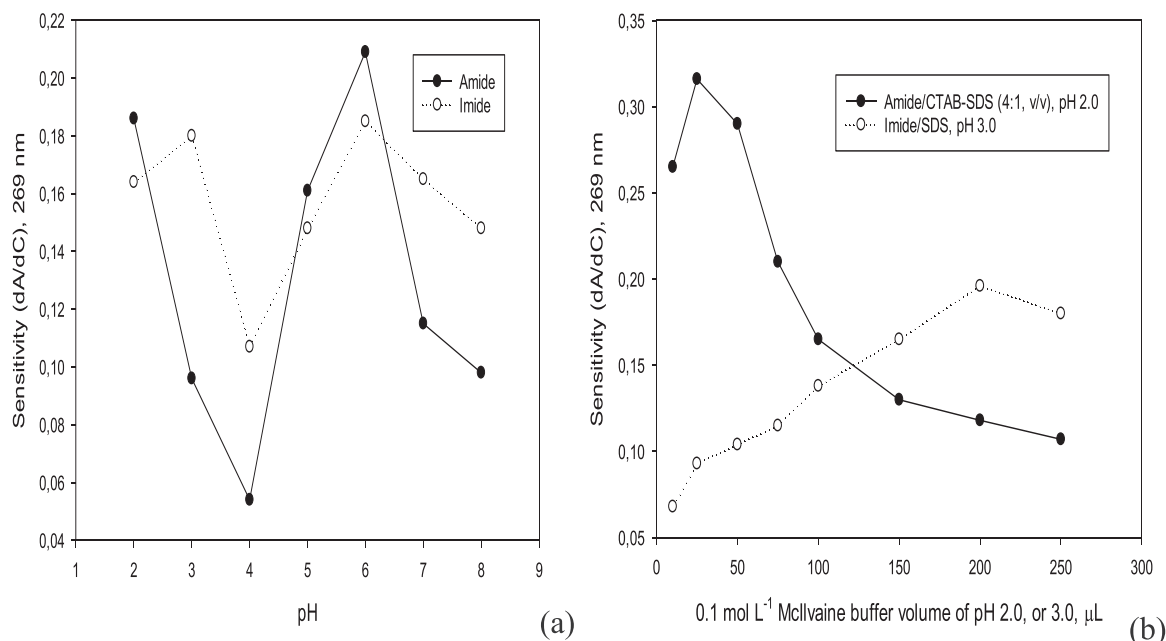

Fig. 2. The effect of pH (a) and (b) buffer (b) concentrations on sensitivity.

chelating copolymers where the hydrazinic tautomer predominates at equilibrium, with only SDS and CTAB- SDS mixture (4:1, v/v) present in the acidic environment, so as to cause a decrease in the blank signal/ improvement in signal reproducibility at low concentrations as ion-pair complex in the presence of CTAB as counter-ion for amidic copolymer and SDS as micellar enhancer for both amidic and imidic copolymer at pHs than 4.0. Shortly, at lower or higher pHs than 4.0, the increase or decrease in the signal can be due to the formation and phase trans-formation of the colloidal precipitates, $Mn(OH)_2$, MnOOH and soluble ionic complexes, $Mn(OH)Citr^-$, $Mn(OH)Ox$, and $Mn(OH)Ox_2^{2-}$ of Mn species with increasing hydroxyl concentration in presence of auxiliary ligands of oxalic/citric acids and stabilizers like pH-sensitive SDS and CTAB controlling particle growth in nanoscale (Rahman and Brown, 1983; Kagakani and Yokoyama, 1985; Mahmoud et al., 2022; Patel et al., 2014; Chen et al., 2013; Huangfu et al., 2015; Gray et al., 1978). Therefore, a pH of 2.0 and 3.0 for hydrazinic tautomer was chosen as optimal for quantitative extraction of Mn. Given that the pH value of 2.0 and 3.0 is more sensitive and selective than that for Mn^{2+} at iso-molar concentrations of 100 $\mu g\,L^{-1}$ in terms of feasibility of trace analysis, optimization of other analytical variables was performed at pH 2.0 and 3.0 where Mn (II) as analyte is used.

To improve the sensitivity and selectivity, the effect of the McIlvaine buffer concentration of 0.1 $mol\,L^{-1}$ in Fig. 2(b) was also studied in the range of 10–250 μL for 2-HBT modified copolymers, and the maximum absorbance was obtained at a buffer volume of 25 μL for amide copolymer while it is obtained at a buffer volume of 200 μL for imide copolymer. From the results, in lower and higher buffer volumes than 25 or 200 μL, it was observed that the signal either sharply or gradually decreased. Linear dependence of the sensitivity to the buffer volume is due to the fact that citrate selectively binds and stabilizes Mn species against the matrix components, via proton coupled electron transfer (PCET), in the presence of chelating copolymers at pH 4.0, given that a chelate complex has a stability constant ($logK_f$ 14.67) of $Mn(OH)Citr^-$ at pH 5.0–7.1 (Xyla et al., 1992; Wang and Stone, 2006; Li et al., 2020). Hence, it was decided to use the buffer volumes of 25 and 200 μL for further studies.

3.1.2. Effect of 2-HBT-modified copolymer concentrations

The affinity of Mn species toward the donor atoms of the chelating copolymers modified by 2-HBT is due to the hard and soft acid–base theory. It correlates the degree of metal hardness or softness to the observed strength of interaction with the N-, N-, and S-donor atoms on benzothiazole and hydrazine moieties by pH-dependent CT. Mn is considered as a redox sensitive metal essential for plants with its complex chemistry, where it generally exists in three oxidation states, Mn (II), Mn (III), and Mn (IV) in real sample matrices. Only the Mn (II) is available for plant uptake, as Mn (III) being a hard Lewis acid according to Pearson hard/soft acid-base rule (preferably coordinating through N, N,O-donor atoms), is unstable and Mn (IV) forms highly insoluble oxides and oxyhydroxides, and precipitates. In generally, the Mn oxides are among abundant, low-cost, stable, and eco-friendly compounds with their catalytic activities such as bulk, supported, nano-sized, colloidal forms and with high surface area in different reactions. Many redox active centers in the structure of these oxides could favor the occurrence of multi-electron and multi-step reactions (Chen et al., 2013; Huangfu et al., 2015; Gray et al., 1978; Xyla et al., 1992; Wang and Stone, 2006; Li et al., 2020). The –NH–NH–, =N–NH–, and C=O groups of the chelating two tautomeric forms of 2-HBT (hydrazine and hydrazono), including donor N- and S-atoms on the benzothiazole ring, used in this study are considered relatively soft bases leading to the observed high affinities between each other. Chelating ligands like polydentate hydrazone-based ligand, sulfonamide antimicrobial sulfamethazine, 2-mercaptobenzothiazole and β-cyclodextrin with Mn and nano-sized Mn oxides (Goyal and Srivastava, 1995; Mahmoudi et al., 2017; Gao et al., 2012; Najafpour et al., 2015; Dong et al., 2010), including inter-action of 2-HBT with As(III) and Hg species (Gürkan and Zengin, 2023;

Zengin and Gürkan, 2019), are chelated probably by specific interactions among chelators and extractant, and thus, the formed chelates are extracted to the core of the micellar aggregates.

In this sense, effect of each copolymer volume on the sensitivity was studied in the range of 50–400 μL of 0.1% (w/v). In this study, 2-HBT-modified copolymers act as soft bases, which tend to be large and very polarizable in the presence of Mn as a hard or soft metal ion depending on oxidation state because of pH-dependent CT, according to Pearson's hard–soft Lewis's acid–base principle. The maximum sensitivity was observed in a volume of 250 μL with a significant sensitivity difference for amidic copolymer while it is obtained in a volume of 300 μL for imidic copolymer as shown in Fig. 3. It is believed that this sensitivity difference is raised from specific interactions between the CTAB, and the free carboxylate group of the amidic copolymer not participating in the chelate formation with surface active end groups of Mn oxide or oxy (hydroxide) where CTAB acts as both a counter-ion and sensitivity-enhancer at pH 2.0. At higher volumes than 250 μL, the sensitivity was gradually decreased due to increase in the blank signal because of ion-pair formation among reagents without analyte. To ensure the complete complexation for the replicate measurements of 100 $\mu g\,L^{-1}$ Mn (II), it has been concluded that a volume of 250 and 300 μL^1 for each copolymer, respectively, is enough for further studies.

3.1.3. Effect of CTAB-SDS mixture (4:1, v/v), only SDS and Triton X-114 concentrations

The interaction of an analyte and surfactant can take places differently based on the nature of the analyte and the surfactant. A polar molecule can bind with surfactant forming micelles by electrostatic interactions, but non-polar molecule is partially solubilized or partitioning into hydrophobic micelle medium. Given that the copolymers outside the coordination center in the working pHs of 2.0 and 3.0, respectively, have ionizable carboxyl, –COOH, and non-ionizable carbonyl, C=O, groups; ionic surfactants, CTAB-SDS mixture (4:1, v/v) and only SDS, were obtained to improve their sensitivity and selectivity, and the signal stability was studied to facilitate the extraction of the Mn(II) to the micellar phase. The effect of 4.0% (w/v) CTAB-SDS mixture (4:1, v/v) amount on the sensitivity was studied in the range of 10–350 μL for amidic copolymer at pH 2.0, and the results were shown in Fig. 4(a). The best signal was obtained at a volume of 50 μL while it was obtained at a
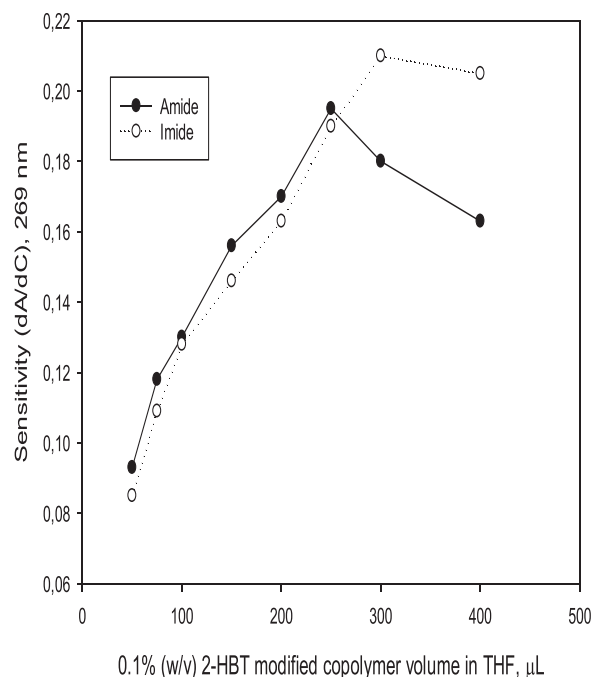
Fig. 3. The effect of 2-HBT-modified copolymer concentrations on sensitivity.
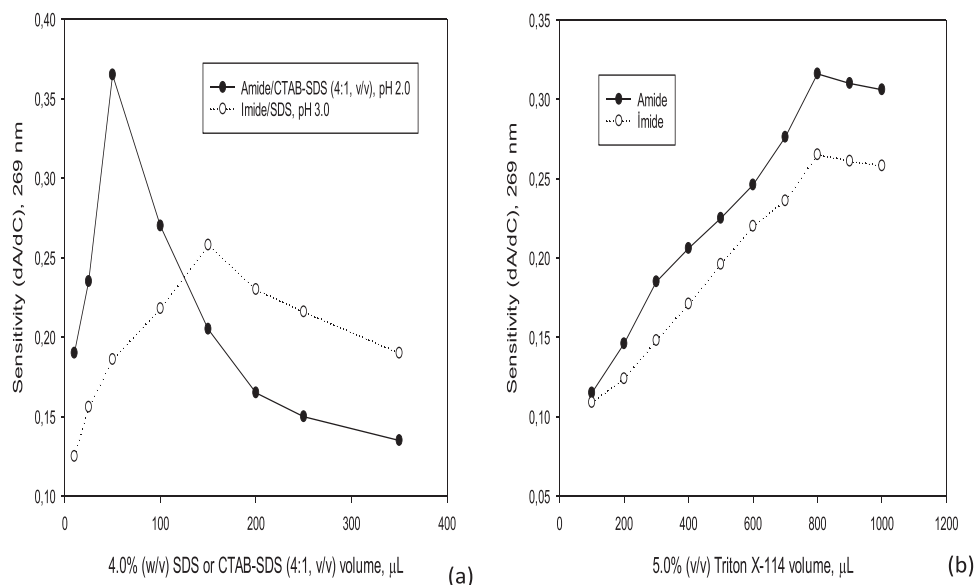
Fig. 4. The effect of CTAB-SDS mixture (4:1, V/v), only SDS (a) and Triton X-114 (b) concentrations on sensitivity.

much higher volume of 150 μL for the imidic copolymer at pH 3.0. The signal for the amidic copolymer is linearly increased in a narrow range of 10–50 μL and reached to a saturation point at lower volumes, to meet the charge of ionizable carboxyl group for electro-neutrality. Perhaps, this case can be due to possible synergistic effect of CTAB and SDS as a result of pH-dependent sharp change in critical micellar concentration of SDS below pH 4.0 and in size and shape of CTAB micelles with solubilized active polar additives like –COOH, -OH and –NH$_2$ (Rahman and Brown, 1983; Kagakani and Yokoyama, 1985; Patel et al., 2014). In the case of the imidic copolymer, the signal was decreased gradually at higher volume than 150 μL. This decrease can be due to an increase in the blank signal without analyte. Therefore, for further experiments, 50 and 150 μL for both copolymers were selected as optimal values.

Where the concentration of surfactant is one of a critical factor, Triton X-114 is non-ionic surfactant widely used in CPE due to achieve a good CPE that means increase the extraction efficiency by reducing the phase volume ratio, results in improvement of concentration factor. The effect of Triton X-114 concentration on the sensitivity was studied in the range of 100–1000 μL of 0.5% (v/v) in Fig. 4(b). The results showed that

the best signal was obtained with a volume of 800 μL for both the amidic and imidic copolymer. In fact, an apparent concentration of approx. 8.0 mmol L^{-1} for effective phase separation is 40-fold higher than the critical micelle concentration (CMC) of 0.2 mmol L^{-1}. At higher surfactant volumes than 800 μL, the signal was gradually decreased. This decrease in the signal might be related to the presence of the high amount of surfactant, resulting in an increase in the volume of the surfactant-rich phase, and, thus, leading to decrease in the pre-concentration factor. Also, the increase in viscosity of the surfactant-rich phase leads to poor sensitivity.

3.1.4. Effect of oxalic acid concentration

A 50 mL of a solution containing 100 μg L^{-1} of Mn (II) and known concentrations of other reagents, 25–500 μL of 0.02 mol L^{-1} oxalic acid in a pH medium of 2.0 and 3.0 was subjected to the CPE process. The best sensitivity as a function of the concentration of the oxalic acid is shown in Fig. 5(a). As can be seen from Fig. 4(a), the sensitivity linearly increases up to a volume of 250 and 300 μL for copolymers and reaches near quantitative extraction. However, the sensitivity gradually
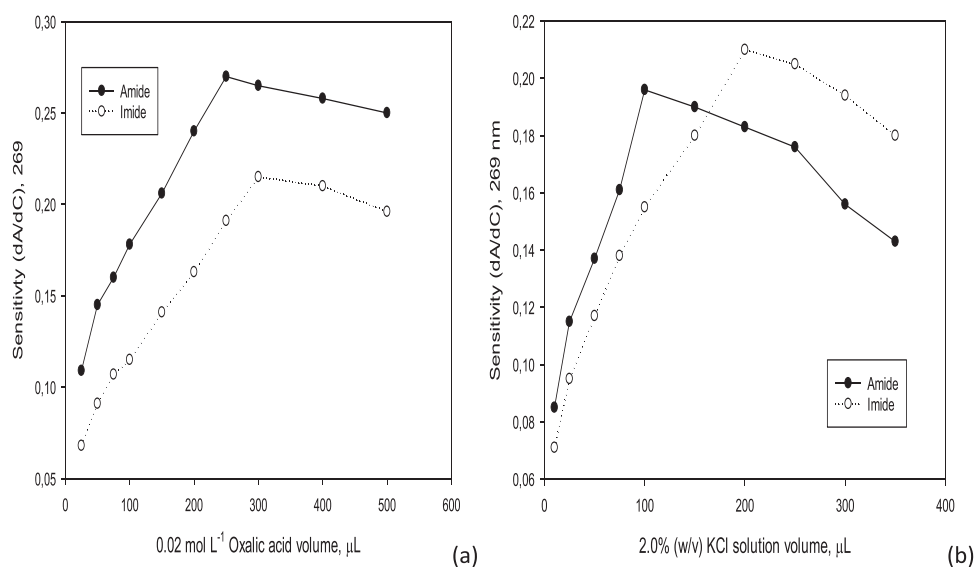

Fig. 5. The effect of Oxalic acid (a) and KCl (b) concentrations on sensitivity.

decreased at higher volumes than 250 and/or 300 µL. So, a volume of 250 and 300 µL oxalic acid chosen as an auxiliary ligand for especially effective chelation of Mn (III) formed because of CT among reagents in micellar phase was chosen to account for other extractable species that might potentially interference with the analysis of Mn (II). Moreover, it has been expressed in literature (Chen et al., 2013; Flynn and Catalano, 2019) that oxalic acid selectively caused to the greatest reductive dissolution of MnO_2 at pH 4.0.

3.1.5. The salting-out effect

The salt addition assists the solubilization or partitioning of solute into organic phase. The presence of electrolytes has been reported to reduce the cloud point temperature, and cause more efficient extraction. The lower clouding point is attributed to the electrolytes which promote dehydration of the poly(oxyethylene) chains. The salting-out phenomenon is in linear relation with desorption of ions from the hydrophilic polar parts of the micelles, increasing the interaction between micelles and consequently causing the precipitation of the surfactant molecules (Badera et al., 2014). For this purpose, the salting-out effect was investigated in the range of 10–35 µL of 2.0% (w/v) KCl in Fig. 5(b) and the highest sensitivity was obtained at a concentration of 100 and 200 µL for amide and imide copolymer, respectively. The signal gradually decreased with increasing KCl volume at higher volumes. This effect might be explained by the additional surface charge when the electrolyte volume is very high, thus changing the molecular architecture of the surfactant and, consequently, the micelle formation process. It is necessary to imply that different blank solutions without Mn (II) were also evaluated and no significant signal was obtained. Therefore, a KCl volume of 100 and 200 µL was concluded to be optimal for further studies.

3.1.6. Effect of UA-CPE parameters

To achieve an easy phase separation and efficient pre-concentration in the CPE processes, it is imperative to optimize the incubation time and temperature for the extractable complex formation. It was desirable to employ the shortest incubation time and the lowest possible equilibrium temperature, as a compromise between completion of extraction and efficient separation of phases.

The influence of equilibrium time and temperature was investigated in the range 2–20 and from 25° to 50°C, respectively. The results demonstrate that the equilibrium time of 5 min and a temperature of 40 °C for both copolymers were chosen for further experiments. The extraction efficiency for the chelate complexes at this temperature for both 2-HBT modified copolymers was quantitatively maximum and constant. Therefore, an equilibrium temperature of 40 °C was chosen to fulfill efficient separation conditions. Higher temperatures gradually lead to a decrease in the sensitivity. This decrease can be because dehydration between the hydrogen bonds occurs when increasing temperature, to cause a decrease in phase volume ratio.

To achieve a good separation, the effect of centrifugation rate and time on the extraction efficiency was also studied. The centrifugation rate was ranged from 1000 to 4000 rpm. It was observed that over 3000 rpm, surfactant phase completely settled for a quantitative recovery. Therefore, a centrifugation rate of 3000 rpm was concluded to be optimal. At the optimal centrifugation rate, recovery was also examined as a function of centrifugation time in the range of 1–15 min. Over 7 min, analytical signal was constant, stable, and reproducible because a complete separation occurred within this time and no appreciable improvements were observed for longer periods, indicating complete transfer of the surfactant phase to the bottom of the centrifuge tube. Therefore, a centrifugation time of 7 min was concluded to be enough for a quantitative recovery.

3.1.7. Effect of diluents

To facilitate the detectability of the sample solution by spectrophotometry, it was necessary to decrease the viscosity of the surfactant-rich phase. Different solvents, such as THF, acetone, acetonitrile, ethanol, methanol, and alcoholic solutions acidified with 0.2 mol L^{-1} HCl, were tried to select the one producing the best results regarding sensitivity, reproducibility, and stability of the signal. The best result was obtained with THF. A suitable aliquot of THF (approx. 0.2 mL) was added to the surfactant-rich phase after separation, in which the micellar phase is diluted to a volume of 0.4 mL for a pre-concentration factor of 62.5 from pre-concentration of optimal 25-mL sample (in the range of 5–35 mL). This amount of THF was chosen to ensure a sufficient volume of the sample for maximum sensitivity. For smaller volumes, the reproducibility of the signals was very poor, whereas for higher volumes, there was a decrease in the signal due to dilution.

3.2. Analytical figures of merit

The analytical features were achieved by means of calibration curves built using standards in solvent (herein, THF) and calibration curves built using standards dissolved in blank matrix extracts using known amounts of the Mn(II) (in the range of 5–165 µg L^{-1}), in the presence of chelating copolymers, CTAB-SDS (4:1, v/v), or only SDS plus Triton X-114, and KCl as salting-out agent in a citrate medium buffered to pH 2.0 and/or 3.0 for pre-concentration by UA-CPE under the optimal conditions. Linear relationships between the absorbance and Mn(II) concentration by spectrophotometry at 269 nm were obtained by (a) the aqueous standard calibration solutions prepared in THF, using the Mn (II) working solutions to reach concentration levels ranging from 5 to 165 µg L^{-1} and (b) the matrix-matched calibration solutions prepared by spiking into the sample blank extracts in THF before analysis by spectrophotometry, using the Mn(II) working solutions to reach concentrations ranging from 5 to 150 µg L^{-1}.

In terms of regression parameters, such as mean slopes and intercepts plus their standard deviations for each calibration curve, the results obtained with both calibration solutions can be compared to see whether there is any significant interference. In this sense, the matrix effect was studied by comparison of the slopes of the calibration curves in solvent and in matrix. The similarity of both slopes was checked by a hypothesis test, based on two-paired ANOVA analysis for 10 degrees of freedom at 95% confidence limit. The experimental values, t_{exp} of 1.78 and 1.21, are lower than the tabulated value, t_{tab} of 2.23, for six replicate measurements of each calibration (degree of freedom (DF) of 10, 95% CL). This result indicates that there is no significant difference between two mean slopes in terms of matrix effect, and as a result, the matrix-matched calibration will also be able to be used, instead of the standard addition method in the analysis of real samples.

Table 1 summarizes the analytical figures of merit. The limits of detection, defined as LOD= $3 \times S_{blank}/m$ (where LOD, S_{blank}, and m are limits of detection, standard deviation of the blank, and slope of the calibration curves, respectively) were 1.45/1.92 µg L^{-1} and 1.54/2.12 µg L^{-1} for the solvent based and matrix-matched calibration curves, respectively. After pre-concentration by UA-CPE, the pre-concentration factor (PF) calculated as ratio of the bulk aqueous sample solution volume to that of the diluted micellar phase with THF was 62.5 from 25 mL/0.4 mL. The sensitivity enhancement factor calculated as the ratio of the slope of the calibration curves with and without pre-concentration by UA-CPE was also approximately 43 and 40, respectively. The intra-day/inter-day precision (as RSDs% in same day and three consecutive days) for the measurements of the three concentration levels of Mn (II), (n: 5, 5, 25 and 100 µg L^{-1}) were found to be in the range of 3.1–5.2 and 3.8–6.3%, respectively. Analytical figures of merit of the method are extensively represented in Table 1.

3.3. The study of interferences

In view of the selectivity provided by spectrophotometry, the only interference may be attributed to the pre-concentration step. In fact, some metal ions may react with chelators to give more stable chelates

Table 1
Analytical figures of merit of the method for trace Mn detection.

Analyte	Calibration type	From regression analysis at 269 nm in THF				[a] LOD, $\mu g\ L^{-1}$	[a] LOQ, $\mu g\ L^{-1}$	Precision		Accuracy
		Linear range, $\mu g\ L^{-1}$	Slope $\pm$ SD	Intercept $\pm$ SD	Determination coefficient, r^2			Repeatability (RSD % for same day, 5, 25 and 100 $\mu g\ L^{-1}$, n: 5)	Intermediate precision (RSD% for three consecutive days, 5, 25 and 100 $\mu g\ L^{-1}$, n: 5)	Recovery %
By poly (SMAm) at pH 2.0	By the linear calibration curve in solvent	5–165	$(1.86 \pm 0.15) \times 10^{-3}$	$(1.22 \pm 0.09) \times 10^{-2}$	0.9935	1.45	4.84	3.1–5.2	3.8–6.0	93.0–96.5
	*By the matrix-matched calibration curve prepared from blank sample extracts	5–150	$(1.75 \pm 0.12) \times 10^{-3}$	$(1.28 \pm 0.09) \times 10^{-2}$	0.9950	1.54	5.14	3.5–5.5	4.1–6.3	91.0–94.0
By poly (SMIm) at pH 3.0	By the linear calibration curve in solvent	5–165	$(1.80 \pm 0.13) \times 10^{-3}$	$(1.25 \pm 0.11) \times 10^{-2}$	0.9941	1.92	6.39	3.2–5.1	4.3–6.2	92.5–96.1
	*By the matrix-matched calibration curve prepared from blank sample extracts	5–150	$(1.70 \pm 0.11) \times 10^{-3}$	$(1.30 \pm 0.12) \times 10^{-2}$	0.9955	2.12	7.06	3.7–5.8	5.0–7.1	92.0–95.3
[b] Matrix effect, %		+ 5.91%, + 5.56%								
[c] Sensitivity enhancement factor (EF)		43, 40								
[d] Pre-concentration factor (PF)		62.5								

*Based on externally spiking into the blank sample extracts at concentration levels of 5, 10, 25, 50, 75, 100 and 150 $\mu g\ L^{-1}$ of Mn (II) before analysis

[a] Limits of detection and quantification ((LODs, LOQs) for Mn (II) were determined according to the expressions at $3.0 \times s_{blank}/m$ and $10 \times s_{blank}/m$, respectively (where s_{blank} is the standard deviation of twelve consecutive blanks (n: 12), and m is the slope of the calibration curves prepared in solvent and from sample extracts).

[b] Based on comparison of slopes of two calibration curves established in solvent and sample extracts by using formula, $ME\% = (m_{solvent} - m_{matrix-matched} / m_{solvent}) \times 100$

[c] Ratio of slopes of calibration curves established with and without pre-concentration

[d] Ratio of the bulk aqueous sample solution volume (25 mL) to that of the micellar phase (0.4 mL)

than Mn, to cause a decrease in the extraction efficiency. As shown in Table 2, when a $\pm$ 5.0% variation in the absorbance is considered, most of the interferents in a tolerance ratio of 35–1250 had no obvious influence on the recovery of 100 $\mu g\ L^{-1}$ Mn (II) (n: 3) under optimal conditions. The results show that the method is highly selective in the

Table 2
The interference study for the selective extraction of 100 $\mu g\ L^{-1}$ Mn (II) (n: 3).

Co-existing ions	Tolerance ratio, [Interferent]/[analyte ion]	Recoveries %	RSDs %
NH_4^+, K^+, Na^+	1250:1	96.0–98.5	2.3–3.7
Ca^{2+}, Mg^{2+}, Sr^{2+}, NO_3^-, F^-	1000:1	95.5–98.5	2.5–3.5
Ba^{2+}, Cl^-, SO_4^{2-}, HPO_4^{2-}, $C_2O_4^{2-}$	750:1	95.0–99.5	2.5–4.0
Al^{3+}, Zn^{2+}, Fe^{2+}, Ni^{2+}	500:1	96.0–101.0	2.0–3.5
HCO_3^-, Br^-, Tartaric acid, Citric acid	350:1	95.0–97.0	2.5–3.7
As^{5+}, Sb^{5+}, Zn^{2+}, Ag^+	250:1	92.0–95.5	3.0–4.1
Cd^{2+}, MoO_2^{2+}, VO^{2+}, VO_2^+, Urea	200:1	93.0–96.0	2.5–3.7
Pb^{2+}, Bi^{3+}, NO_2^-, I^-	175:1	94.0–96.5	3.0–4.0
Fe^{3+}, [a]	150:1 (1500)	100.6–104.1	3.5–5.0
(As^{3+}, Sb^{3+})[b]	125:1 (1000)	92.0–95.5	2.5–4.0
(Formaldehyde)[c]	100:1 (750)	92.0–95.0	2.7–3.5
Sn^{2+}, [d]	75:1 (500)	91.0–94.5	3.5–4.5
(Co^{2+}, Cu^{2+})[e]	50, 35:1 (1000, 500)	102.0–105.5	4.0–5.5

[a] By using 1.0 mL of 0.02 mol L^{-1} NH_4F solution in medium buffered to pH 5.0

[b] After pre-oxidation of As^{3+}/Sb^{3+} to As^{5+}/Sb^{5+} with 0.01 mol L^{-1} H_2O_2 solution in alkaline medium

[c] After pre-treatment of 0.25 mL of 0.02 mol L^{-1} Na-metabisulfite, $Na_2S_2O_5$ around pH 5.0

[d] By using 0.2 mL of 0.025 mol L^{-1} $Na_2H_2P_2O_7$ solution in a medium buffered to pH 7.0

[e] By using selectively 2.0 mL of 10 mmol L^{-1} ethylendiamine/ 1.0 mmoL L^{-1} oxalic acid mixture (3:1, v/v) in a medium buffered to pH 5.0

presence of different interfering species. This selectivity may be due to the formation of more stable complexes of Mn species with functional site groups of the chelators when compared to other metal or metalloid ions studied. However, the interference of the Co^{2+}, Cu^{2+}, Sn^{2+}, As^{3+}/Sb^{3+} and Fe^{3+}, including formaldehyde in tolerance ratio of 35–150 on the absorbance can greatly be suppressed by use of suitable masking agent in a media buffered to pH of 5.0 or 7.0 before UA-CPE.

3.4. The method validation

The accuracy of the method was confirmed by analysis of two certified and two quality control samples, in terms of free Mn (II) and total Mn contents, after extraction by MAE and UAE, respectively. The results are given in Table 3(a, b). The results were in good agreement with the certified values for total Mn in the SRMs studied, according to one-way t-test at 95% confidence level. From measurements obtained by the matrix-matched calibration curve, the precision (as RSDs%) was lower than 7.4% and 8.3% for both the certified and the quality control samples, demonstrating the good reproducibility of the method. The recoveries were in range of 92.3–94.1% and 90–93% for the certified and the quality control samples, respectively where the recoveries for extraction of free Mn (II) with Salicylic acid with and without spiking (10 $\mu g\ L^{-1}$, n: 5) are in range of 76.9–79.3 and 91–93%, respectively. According to the one-way t-test and regression analysis, no significant difference between the certified values and the results found by the proposed method was statistically found where the calculated t-values of 1.49 and 2.06 are lower than the critical t-values of 2.78 (n: 5, 95% CL). All the results indicate that the applicability of the method, in speciation of Mn, is free of interference for the matrix effect in terms of accuracy and reliability.

Table 3(a)

The validation of the method by analysis of two certified milk samples (n: 5).

CRMs	*Certified, $\mu g\ g^{-1}$		Observed, $\mu g\ g^{-1}$		RSD %	Recovery %	* *Total Mn, $\mu g\ g^{-1}$	The one paired t-test[c], t_{exp}
	Free Mn (II)	Total Mn	[a]Free Mn (II)	[b]Total Mn				
SRM 1549 Nonfat milk powder	-	0.26 ± 0.06	0.200 ± 0.012	0.240 ± 0.012	6.0, 5.0	76.9, 92.3	0.248 ± 0.013	2.06
SRM 8435 Whole milk powder	-	0.17 ± 0.05	0.135 ± 0.010	0.160 ± 0.010	7.4, 6.2	79.4, 94.1	0.162 ± 0.012	1.49

*The total Mn levels of certified milk samples (as mean ± SD, 95% CL)

* * Total Mn values (as mean ± SD, n: 5) obtained by MAE and dilution of 25-fold

[a] The free Mn (II) levels (as mean ± SD, n: 5) obtained by UAE using Salicylic acid and dilution (25-fold)

[b] The total Mn levels (as mean ± SD, n: 5) obtained by UAE using Salicylic acid plus H_2O_2-Citric acid and dilution (25-fold)

[c] The experimental t -values calculated by using the one paired Student's t-test for free Mn (II) and total Mn levels (n: 5) without spiking where the critical t-value is 2.78 for 4 degrees of freedom at 95% CI

Table 3(b)

. The accuracy/precision studies.

Sample	*Total Mn, $\mu g\ L^{-1}$	Added, $\mu g\ L^{-1}$	Accuracy/precision for analysis of free Mn (II) /total Mn contents with and without spiking					
			On the same day (n: 5)			On three consecutive days (n: 3 ×5)		
			[a,b] Found, $\mu g\ L^{-1}$	Recovery %	RSD %	[a,b] Found, $\mu g\ L^{-1}$	Recovery %	RSD %
Infant formula	24.0 ± 1.0	-	19.3 ± 0.2, 23.5 ± 0.2	-	-, 5.7	18.8 ± 0.3, 23.1 ± 0.3	-	-, 8.3
		10, 10	28.3 ± 0.5, 32.5 ± 0.5	93, 92	5.4, 5.3	28.1 ± 0.6, 32.4 ± 0.6	91, 90	6.6, 6.4
Skimmed milk	15.5 ± 0.7	-	10.8 ± 0.2, 14.8 ± 0.3	-	-, 6.2	10.8 ± 0.2, 14.5 ± 0.03	-	-, 6.4
		10, 10	20.3 ± 0.5, 23.7 ± 0.5	93, 92.2	5.4, 5.2	20.1 ± 0.6, 23.3 ± 0.6	91, 90.3	6.6, 6.3

*Total Mn values (as mean ± SD, n: 5) obtained by MAE and dilution of 10-fold

[a] The free Mn (II) levels (as mean ± SD, n: 5) obtained by UAE using Salicylic acid and dilution (10-fold)

[b] The total Mn levels (as mean ± SD, n: 5) obtained by UAE using Salicylic acid plus H_2O_2-Citric acid, and dilution (10-fold)

3.5. The method applications

For applicability of the method to speciation of free Mn (II) and total Mn, the known aliquots of the milk-based samples with and without spiking at 10 $\mu g\ L^{-1}$ levels were in parallel taken into a centrifugation tube of 50-mL. The following steps were taken to determine free Mn (II)

Table 3(c)

The analysis results found by the developed UV spectrophotometric method with and without spiking (10 $\mu g\ L^{-1}$, n: 5).

Sample	*Total Mn, $\mu g\ L^{-1}$	Added, $\mu g\ L^{-1}$	By the matrix-matched calibration curve, 5, 15 and 30 $\mu g\ L^{-1}$						[c] The two-sample t-test, t_{exp}
			By UAE			By UAE			
			[a] Found, $\mu g\ L^{-1}$	Recovery %	RSD %	[b]Found, $\mu g\ L^{-1}$	Recovery %	RSD %	
Milk based drinks with fruit flavored, banana, strawberry, cherry, apricot, peach from same brands									
A1	12.5 ± 0.6	-	9.80 ± 0.5	-	5.1	11.9 ± 0.5	-	4.2	1.72
		10, 10	19.3 ± 0.8	95.0	4.1	21.5 ± 0.8	96	3.7	-
A2	10.7 ± 0.4	-	7.65 ± 0.4	-	5.2	10.2 ± 0.4	-	3.9	2.08
		10, 10	17.2 ± 0.7	95.5	4.1	19.5 ± 0.8	93	4.1	-
A3	14.2 ± 0.5	-	10.2 ± 0.5	-	4.9	13.7 ± 0.6	-	4.4	1.51
		10, 10	19.3 ± 0.8	91	4.1	23.1 ± 0.8	94	3.5	-
A4	13.1 ± 0.6	-	9.30 ± 0.4	-	4.3	12.5 ± 0.6	-	4.8	1.58
		10, 10	18.7 ± 0.7	94	3.7	21.7 ± 0.8	92	3.7	-
A5	16.7 ± 0.7	-	12.7 ± 0.6	-	4.7	16.3 ± 0.6	-	3.7	0.97
		10, 10	22.1 ± 0.8	94	3.6	25.5 ± 1.0	92	3.9	-
Milk based products: whole milk, skimmed milk, soy-based milk									
B1	12.8 ± 0.5	-	9.5 ± 0.5	-	5.3	12.3 ± 0.5	-	4.1	1.58
		10, 10	18.7 ± 0.8	92	4.3	21.8 ± 0.9	95	4.1	-
B2	15.5 ± 0.6	-	10.7 ± 0.5	-	4.7	15.0 ± 0.6	-	4.0	1.58
		10, 10	20.1 ± 0.8	94	4.0	24.5 ± 1.0	95	4.1	-
B3	19.6 ± 1.0	-	16.4 ± 0.7	-	4.3	19.0 ± 0.8	-	4.2	1.05
		10, 10	25.5 ± 1.0	91	3.9	28.5 ± 1.1	95	3.9	-
Milk: cow, sheep, goat									
C1	23.0 ± 1.2	-	16.5 ± 0.7	-	4.2	22.6 ± 1.0	-	4.4	1.13
		10, 10	25.5 ± 1.0	90	3.9	31.7 ± 1.2	91	3.8	-
C2	27.5 ± 1.3	-	21.5 ± 0.8	-	3.7	26.7 ± 1.1	-	4.1	0.58
		10, 10	30.8 ± 1.1	93	3.6	35.8 ± 1.3	91	3.6	-
C3	35.5 ± 1.5	-	30.4 ± 1.2	-	3.9	34.7 ± 1.3	-	3.7	0.90
		10, 10	39.5 ± 1.5	91	3.8	43.7 ± 1.6	90	3.7	-

*Total Mn values (as mean ± SD, n: 5) obtained by MAE and dilution of 100-fold

[a] The free Mn (II) levels (as mean ± SD, n: 5) obtained by UAE using Salicylic acid and dilution (100-fold)

[b] The total Mn levels (as mean ± SD, n: 5) obtained by UAE using Salicylic acid plus H_2O_2-Citric acid, and dilution (100-fold)

[c] The experimental t -values calculated by using the two-sample t-test for total Mn levels (n: 5) obtained by MAE and UAE where the critical t-value is 2.78 (DF: 8, 95% CI.

and total Mn levels. The free Mn (II) and total Mn levels of the samples were pre-treated by UAE and MAE for applicability and feasibility of the method. After the pre-treatment, the pre-treated sample solutions were submitted to UA-CPE procedure for analysis of free Mn (II) and total Mn. The results obtained are given in Table 3(c).

After the validation, the method, applied to milk-based samples, for free Mn (II) and total Mn levels, was conducted and submitted to UC-CPE process before analysis by UV-spectrophotometry at 269 nm (n: 5). The reliability of the method for determination of free Mn^{2+} and total Mn contents in the sample matrices, in terms of accuracy (as recovery%) and precision (as RSDs%), was evaluated by spiking with known concentrations of Mn(II) at 10 $\mu g\ L^{-1}$ (depending on Mn level of the matrix) to the samples after dilution (20–100 fold). The recoveries were reasonably satisfactory levels (in range of 91–95.5 and 90–96%) for both free Mn (II) and total Mn with RSD ranging from 3.6% to 5.3% and from 3.6% to 4.8% in Table 3(c), thus successfully verifying the reliability of the method and applicability to the selected milk products samples. Moreover, all the samples were in parallel analyzed by using MAE for determination of total Mn as well as UAE. After pre-concentration by UA-CPE, their free Mn (II) and total Mn contents were in the range of 7.65–30.4 and 10.2–34.7 $\mu g\ L^{-1}$, respectively, while their total Mn contents found by the present method after MAE are in range of 10.7–35.5 $\mu g\ L^{-1}$. In terms of total Mn levels, it has been observed that the results found by the present method after both MAE and UAE statistically are in good agreement with each other (for DF of 4, 95% CL), according to two-way ANOVA analysis.

Due to the lack of studies on Mn in milk and fruit flavored milk-based drinks, the results are compared with several pieces of literature. According to Knowles et al. (2006), the Mn concentration in the milk must be between 0.02 and 0.05 mg L^{-1}. All the selected milk samples were within this limit, with total Mn levels of 10.7–35.5 and 10.2–34.7 $\mu g\ L^{-1}$ found by the present method after MAE and UAE. In comparison to the permissible limits according to Flynn, Hurley (Flynn, 1992; Hurley, 1997) (0.03, 0.02 mg L^{-1}) and Malbe et al. (2010) (0.102 mg L^{-1}), the milk samples in this study did not exceed these limits, in such a way as not to present a threat to individuals consuming these products. The amount of Mn in high doses seriously affects human health. It leads to emotional and mental disturbances (lack of coordination and muscle stiffness) and causes impairment of neuromuscular and neurological control (Santamaria, 2008). The consumption of milk that contains high doses of Mn, that have exceeded permissible limits, leads to congenital disabilities, impaired bone development and causes impairment in male fertility. The brain is also very sensitive to an overabundance of Mn, and it is particularly vulnerable to this excess (Santamaria, 2008).

3.6. Comparison with other reported methods

The method was especially compared with spectrophotometry, ICP-OES and FAAS with and without FI in combination with CPE or UA-CPE, that had recently been reported in the literature for pre-concentration of trace Mn from various sample matrices. Considering the figures of merit achieved in Table 4, it is evident that the method has either a better or a comparable linear working range, including other key analytical features. Moreover, its detection limits (1.45/1.92 $\mu g\ L^{-1}$ and 1.54/

Table 4

Comparison of the method with the reported other methods.

Reagents	Surfactant type	Sample volume, mL	SRP diluting agent	Detection system	Linear range, $\mu g\ L^{-1}$	LODs[a], $\mu g\ L^{-1}$	RSD%, Recovery %	EF[b] (or PF[c])	Sample type	Ref.
Magneson I	Triton X-114	25	1.0 mL of 0.1 mol L^{-1} HNO_3 in ethanol	FAAS	10–400	2.9	1.1–1.3, 97.6–98.6	19	Water and food	Sahin et al., 2010
Br-TAO	Triton X-114	2.8	H_2SO_4	FAAS	Up to 150	0.5	5.4–6.3, 98–106	14	Food	Lemos et al., 2008
Me-BTABr	Triton X-114	3.0	-	FAAS	2.2–150	0.7	0.9–2.0, 99.6–101.5	16 or 17	Food	Lemos and David, 2010
PAN	Triton X-114	50	400 μL methanol in 0.1 mol L^{-1} HNO_3	FAAS	1–142	0.39	2.2, 95.9–99.3	49.1	Milk and water	Rod et al., 2006
8-HQ	Triton X-100	10	1.0 mL methanol in 0.1 mol L^{-1} HNO_3	FAAS	6.5–265	1.9	1.4–12, 98.4	10–20	Tea and water	Yalcin et al., 2012
Br-PADAP	Triton X-114	50	0.4 mL HNO_3 (1:1 v/v)	ICP-OES	5–30	0.83	2.6, 106	25	Vegetable leaves	Bezerra et al., 2006
Quinalizarin	Triton X-114	25	500 μL methanol	Spectrophotometry, 528 nm	5–200	0.80	1.35–2.60, 96–102.5	103/ 50	Water and food	Gouda, 2014
Azo-immobilized 8-HQ	-	4.2	-	Online pre-concentration/FI-FAAS	2.5–125	0.5	1.4–1.7,88.5–99.2	47	Mussel and non-fat milk powder	Bruhn et al., 2002
2-HBT modified poly (SMAm and SMIm) copolymers	Triton X-114	25	400 μL THF	Spectrophotometry, 269 nm	5–165	1.45, 1.92	3.1–6.2, 92.5–96.5	43, 40/ 62.5	Milk based products	The present study

SRP: Surfactant rich phase, Magneson I: p-nitrophenylazoresorcinol, PAN: 1-(2-pyridylazo)− 2-naphthol, Br-TAO: 4-(5′-bromo-2′-thiazolylazo)orcinol, Quinalizarin: 1,2,5,8-tetrahidroksiantrakinon, STZ: Sulfathiazole, Me-BTABr: 2-[2′-(6-methyl-benzothiazolylazo)]− 4-bromophenol, 8-HQ: 8-Hydroxyquinoline, Br-PADAP: 2-(bromo-2-pyridylazo)− 5-diethyl-amino-phenol, FAAS: flame atomic absorption spectroscopy, ICP OES: inductively coupled plasma optical emission spectrometry, S: spectrophotometry

[a] Limit of detection

[b] Enrichment factor

[c] Pre-concentration factor

2.12 μg L^{-1}) obtained by two calibration curves are either better than or comparable with that of other methods which use sensitive, but expensive, complicated and element selective detection such as FAAS, FI-FAAS and ICP-OES requiring expert user in his/her research area (Sahin et al., 2010; Lemos et al., 2008; Lemos and David, 2010; Rod et al., 2006; Yalcin et al., 2012; Bezerra et al., 2006; Gouda, 2014; Lemos et al., 2010), including spectrophotometry at 528 nm (Bruhn et al., 2002). Moreover, FAAS has limitations such as interference from flame, mono-elemental, limited application to small volumes, low sensitivity, poor precision, and possible memory effect. Also, ICP-OES has the limitations like continuum background emission, spectral overlaps, matrix effects, fluctuations in signal and background especially at low concentration levels. Therefore, for reliable analysis, it requires the use of an internal standard as well as skilled operator.

4. Conclusions

In the current work, new prepared amide/imide copolymers modified with 2-HBT for CT-sensitive complexation of Mn (II) in the presence of oxalic acid at pH 2.0/3.0, respectively, prior to analysis by combination of UA-CPE with UV-spectrophotometry have resulted in a significant improvement of figures of merit of the method besides the solvent-free extraction of toxic metals from complex matrices. The method provides the possibility of simple, cost-effective, accurate, and reliable determination of Mn (II) with comparable results of those of spectrophotometry in visible region at 528 nm, and element selective detection techniques like ICP-OES, and FAAS with and without FI, and it can be considered as an alternative tool to the sensitive, but complex and cost-effective spectrometric techniques such as ICP-OES and ICP-MS. Moreover, these techniques are not available in every analytical research laboratory and require skilled users of the field.

CRediT authorship contribution statement

H.B. Zengin: Supervision, Methodology, Validation, Formal analysis, Visualization, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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A pH-dependent CT sensitive method for detection of trace Mn in milk by UV-spectrophotometry

By   Zengin, HB (Zengin, H. B.) [1]

Abstract   In this study, a new method was developed for the extractive pre-concentration/detection of trace Mn in milk prior to analysis by UV-spectrophotometry. The method is based on the pH-controlled intra-ligand charge transfer (ICT) complexation of modifier, 2-HBT in presence of trace Mn (II), and then the extraction of the complexes, where the hydrazine and hydrazone tautomers are predominant at pHs of 2.0/ 3.0, and pH 6.0 because of CT at pH 4.0. The free Mn (II) and total Mn levels of the samples were detected at 269 nm by UVspectrophotometry. For total Mn, the method was validated by analysis of two certified milk samples. The results were statistically in good agreement with the certified values, and the precision was lower than 6.4%. After a pre-concentration of 62.5-fold, the limits of detection were 1.45/1.92 and 1.54/2.12 mu g L-1 in range of 5-165 and 5-150 mu g L-1 from the solvent-based and matrix-matched calibration curves, respectively. There is not a matrix effect from comparison of the calibration curves's slopes. The method was successfully applied to analysis of milk samples. In terms of speciation, the free Mn (II) and total Mn levels in milk were in the range of 7.65-30.4 mu g L-1, and 10.2-34.7 and 10.7-35.5 mu g L-1 after the microwave and ultrasound assisted extraction.

Keywords   Author Keywords: Mn (II); 2-Hydrazinobenzothiazole; Amide/Imide Copolymers; Milk products; UV-Spectrophotometry; Ultrasound-assisted cloud-point extraction
Keywords Plus: CLOUD POINT EXTRACTION; ATOMIC-ABSORPTION-SPECTROMETRY; OPTICAL-EMISSION SPECTROMETRY; MANGANESE; PRECONCENTRATION; ELEMENTS; SAMPLES; WATER; OPTIMIZATION; ESSENTIALITY

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2022	2021

JCI Category	Category Rank	Category Quartile
CHEMISTRY, APPLIED *in SCIE edition*	17/77	Q1
FOOD SCIENCE & TECHNOLOGY *in SCIE edition*	49/170	Q2

The Journal Citation Indicator is a measure of the average Category Normalized Citation Impact (CNCI) of citable items (articles and reviews) published by a journal over a recent three year period. It is used to help you evaluate journals based on other metrics besides the Journal Impact Factor (JIF).

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Journal information

JOURNAL OF FOOD COMPOSITION AND ANALYSIS

Publisher name: ACADEMIC PRESS INC ELSEVIER SCIENCE

Journal Impact Factor ™

4.3
2022

4.6
Five Year

JCR Category	Category Rank	Category Quartile
CHEMISTRY, APPLIED *in SCIE edition*	19/73	Q2
FOOD SCIENCE & TECHNOLOGY *in SCIE edition*	43/142	Q2

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