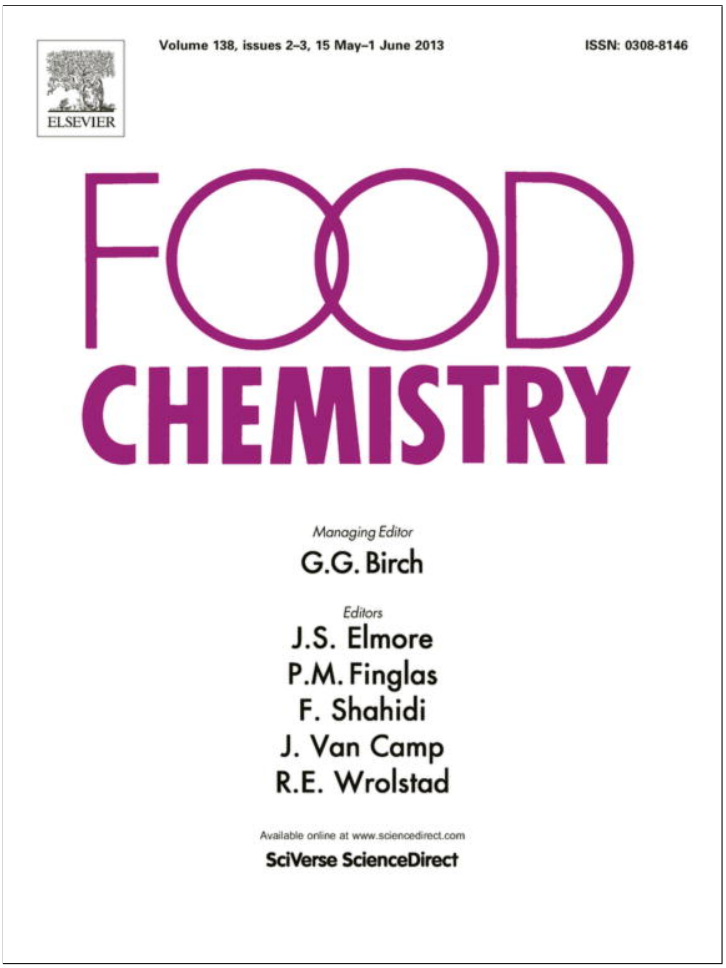

Food Chemistry 138 (2013) 1641–1647

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of total iron in food samples after flow injection preconcentration on polyurethane foam functionalized with *N,N*-bis(salicylidene)-1,3-propanediamine

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ARTICLE INFO

Article history:
Received 17 November 2011
Received in revised form 8 July 2012
Accepted 9 November 2012
Available online 20 November 2012

Keywords:
Polyurethane foam
N,N-bis(Salicylidene)-1,3-propanediamine
Preconcentration
Iron

ABSTRACT

A highly selective flow injection sorption system was developed for the fast determination of total iron in food samples. Iron (III) was reduced to iron (II) by ascorbic acid and preconcentrated on a mini-column packed with polyurethane foam (PUF) functionalized with *N,N*-bis(salicylidene)-1,3-propanediamine (SPDA). The retained Fe (II) was eluted with hydrochloric acid and subsequently reacted to 2,4,6-tri(2′-pyridyl)-1,3,5-triazine (TPTZ) then measured at 593 nm. The procedure has resulted preconcentration factor 36, sample frequency 20 h^{-1} and detection limit 18 $\mu g\ L^{-1}$. The precision (RSD) was found to be 5.7% and 3.1% at concentration levels 0.1 and 5.0 $\mu g\ mL^{-1}$ iron (II), respectively. Finally, the method was successfully applied to determination of total iron in reference material and food samples.

1. Introduction

Owing to its biological importance, iron is one of the most frequently analysed elements. The level of iron is important to the health of mammals, and iron accumulated in iron deposits of the body as iron-ferritin, where levels below 12 $\mu g\ L^{-1}$ indicate loss of iron causing anaemia (Aggett et al., 2002). It is important to determine trace amounts of iron for environmental protection, hydrogeology, chemical processes and public health studies (Pourreza & Mousavi, 2004). Natural waters contain various chemical forms of iron depending on the geological area and other chemical components. Iron (II) is normally less present in river water (Sangi, Jayatissa, Kim, & Hunter, 2004), and iron (III) can precipitate rapidly by the formation of hydrous iron oxide and hydroxides, which can absorb other trace metals. Thus, iron ion controls the mobility, bioavailability and toxicity of other trace metals in the natural water system (Forence & Batley, 1980). The circulation of iron in the environment is not fully understood, owing to relatively low contents in complex matrices (Andersen, 2005). Toxic trace metals can be incorporated in the prevailing iron precipitates which are controlled by the presence of complexing or precipitating agents as well as by the redox state of iron depending on the conditions of water, chemical and biological conditions of the environment (Ugo, Moretto, Rudello, Birriel, & Chevalet, 2001).

Various methods for quantitative analysis of iron have been developed; they are inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), electrochemistry, and ion chromatography (IC). Though all of these methods are highly sensitive, main disadvantages are the necessity of expensive and sophisticated instrumentation (Lunvongsa, Oshima, & Motomizu, 2006).

Spectrophotometric methods are less expensive and easy instrument operation but they suffer from the high limit of detection. The 4-(2-pyridylazo)-resorcinol (PAR) as spectrophotomteric reagent was used for the determination of iron in ground water (Klamtet, 2004).

Flow injection analysis (FIA) system equipped with a simple detector such as a UV–Vis spectrophotometer is one of the most effective and suitable approach for routine analysis, mainly owing to its simplicity, low instrumentation cost, high sample throughput and robustness (Lunvongsa et al., 2006).

Preconcentration procedures are introduced to increase analyte concentration to a measurable level. Among those, liquid–liquid extraction (Agrawal, Menon, & Pancholi, 2003), solid phase extraction (SPE) (Mahmoud, Hafez, Osman, Yakouta, & Alrefaay, 2010), bio-sorption (Baytak & Turker, 2005), cloud point extraction (Wu

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http://dx.doi.org/10.1016/j.foodchem.2012.11.054

et al., 2008), membrane filtration (Moghimi, 2008), coprecipitation (Durana et al., 2009) and ion exchange extraction (El-Shahat, Moawed, & Zaid, 2003). Solid sorbents are now routinely used in various research and application areas for their advantages over the classical solvent extraction or other preconcentration techniques (Mohamed & Al Saadi, 2001). Silica gel, activated carbon, cellulose, polyurethane foam, alumina, poly(acrylonitrile), styrene-divinylbenzene matrix, and clay have been used (Safavi, Iranpoor, & Saghir, 2004).

Preconcentration of iron have been done on several chelating reagents loaded on many solid sorbents such as methylthymol blue loaded on naphthalene (Pourreza & Mousavi 2004), 1,10-phenanthroline loaded on cation exchange resin (Ozyurek et al., 2007), *N,N′*-ethylene-bis-(ethane sulfonamide) loaded on activated carbon (Karacan & Aslantas 2008), 2-aminotiazole modified silica gel (Roldana, Alcantaraa, Padilhab, & Padilha, 2005), and 1-nitroso-2-naphthol immobilised on alumina (Mahmoud et al., 2010).

Coupling between FIA and SPE have revealed good approach to achieve both high productivity and sensitivity (Cassella et al., 2001). Polyurethane foam (PUF) has been utilised in preconcentration of iron both when unloaded (Cassella, 2002), after immobilised with 1,10-phenanthrolin (Bhattacharya, Roy, & Chakraborty, 1990) and by visual colorimetry (Michio, Hiroaki, Shinseki, & Takashi, 2003).

N,N-bis(Salicylidene)-1,3-propanediamine (SPDA) compound belongs to the class of Salens which are capable to form stable complexes with Fe (II) (Salem, El-Sheikh, & Zaki, 1994). A polystyrenedivinylbenzene-based macroreticular resin was functionalised with bis-(*N,N′*-salicylidene)1,3-propanediamine) and used for the determination of Cu(II), Ni(II), Co(II), Zn(II), Fe(II), Mn(II), Pb(II), Cd(II) and Cr(III) (Dev & Rao, 1996). Also, this reagent was used in membrane electrodes and in optical sensors (Shabany, Shabani, Dadfarnia, Gorji, & Ahmadi, 2008).

The present work describes the use of SPDA–PUF as a new sorbent for the preconcentration of iron (II). A full automated experimental design was made by coupling the SPE system on-line to the UV–Vis spectrometer and used for optimization of the chemical and hydrodynamic variables that affect the efficiency. The model system has been used for the rapid determination of total iron in food samples.

2. Experimental

2.1. Instrumentation

UV–Vis spectrophotometer model UV 1650PC (Shimadzu, Japan), equipped with Helma flow cell and UV2.10 probe software for kinetic data acquisition was used for recording the absorbance. The pH adjustment was made by pH metre model 780 Metrohm, (Herisau, Switzerland). Ultra pure water obtained from Elix Ultra pure UV water purification instrument (Massachusetts, USA) was used in all preparations and deionised water for washing purposes. All solutions were propelled by FIAS – 400 (Perkin Elmer, USA) peristaltic flow injection pump provided with Tygon tubes (1.14 mm internal diameter) and connexions made of Teflon. Five-port rotary valves were used to select preconcentration or elution steps. The flame atomic absorption spectrometer (FAAS), MKI-Solaar 32 Unicom 969 (England), was used a standardized method to analyse the total iron in the real samples as a comparative to the method developed. An aliquot from the prepared samples from liver, vegetables, fruits or supplements were aspirated into the FAAS under the recommended operational conditions. The lamp current, wavelength and fuel flow were set at 15 mA, 248.3 nm, and 0.6 $L\,min^{-1}$, respectively.

2.2. Reagents and solutions

N,N-bis(Salicylidene)-1,3-propanediamine (SPDA) was purchased from Aldrich (Miluakee, USA). Open – cell polyether – type PUF with density 55 $kg\,m^{-3}$ was supplied from the ContiTech Foampolster GmbH Company (Löhne, Germany). Iron (II) standard solution was prepared by diluting atomic absorption standard solution 1000 $mg\,L^{-1}$ of iron (II) sulphate hexahydrate (Redl de Haen, Germany). Working solution with concentration of 4 $\mu g\,mL^{-1}$ iron (II) was prepared daily by suitable dilution from the standard solution.

A solution from TPTZ reagent (0.0128 mol L^{-1}), used as a spectrophotometric reagent for iron (II), was prepared by dissolving 0.4 g from 2,4,6-tri(2′-pyridyl)-1,3,5-triazine (TLC grade,>99% purity, Fluka, Switzerland) in 100 mL 0.1% (V/V) nitric acid. Then, the resulted solution was diluted with 0.1 mol L^{-1} acetic/sodium acetate buffer (pH 5.8) to final concentration 4×10^{-4} mol L^{-1}.

Acetate buffer series were prepared by mixing appropriate volumes of acetic acid (0.1 mol L^{-1}) to sodium acetate solution (0.1 mol L^{-1}) in the pH range 3–6. Phosphate buffer (pH 6.0) was prepared by mixing appropriate volume of both of sodium hydrogen phosphate and sodium dihydrogen phosphate. Unless otherwise stated, the chemicals utilised in this work were of analytical grade. The glassware was cleaned before use by soaking in nitric acid 10% (v/v) and rinsed with deionised water.

2.3. Sample collection

Thirteen food samples were analysed. One certified reference material (bovine muscles BCR-CRM 184), six vegetables (eggplant, potatoes, tomato, green pepper, parsley and spinach), three fruits (apples, strawberry and banana) and three supplements (enriched rice, wheat bread and bovine liver) samples were collected from a nearby shop (Giza, Egypt). Vegetable and fruit samples were cleaned very well by washing many times with doubly distilled water to remove any dust. When necessary, the outer shell was removed from vegetables and fruits and cut into small pieces. Each specimen was dried at 100 °C for 24 h till constant weight then grounded into fine powder in porcelain mortar.

2.4. Sample preparation

2.4.1. Dissolving the bovine muscles and liver

Muscle and liver samples were decomposed according to the reported method (Burham, Abdel-Azeem, & El-Shahat, 2009). The sample was put in a clean and dry beaker and dried at 105 °C for 24 h. After this, it was transferred into a clean and dry crucible then ignited in a muffle furnace at 200 °C, then at 400 °C and finally at 600 °C, for 6.0 h at each temperature level. An accurately weighed 0.50 g from the final sample was mixed to 5.0 mL 65% (w/v) HNO_3 and 2.0 mL from H_2O_2 30% (v/v) and heated in closed digestion system till nearly dryness. The residue was neutralised by 10% (w/v) NaOH and diluted to 20.0 mL with ultra pure water then solid ascorbic acid (0.20 g) was dissolved in the sample to reduce ferric to ferrous iron and adjusted to pH 5.5 by acetate buffer.

2.4.2. Dissolving the vegetables, fruits and supplements

Digestion of the dry sample was effected according the method reported by Lemos et al. (2007). An accurately weighed 0.50 g from the powdered sample was mixed to 20 mL of 14 mol L^{-1} nitric acid solution into a clean glass beaker. The acid digestion was carried out by heating the mixture on hotplate within fume cupboard till nearly dryness. After cooling, ultra pure water was added to the final digest then it was neutralised to pH 7 by 2.5 mol L^{-1} sodium hydroxide solution. Ascorbic acid was added and pH was adjusted to 5.5 and made up to 50 mL final volume.

2.5. Preparation of the mini-column

A cylindrical polyethylene tube (3.5 cm length and 3 mm i.d.) was dry-packed with 300 mg plugs from SPDA–PUF material under vacuum suction to minimize channels. Both ends of the mini-column were sealed with plastic septa and fitted to the outer tubing. To clean and condition the sorbent, the packed mini-column column was washed with 0.5 mol L^{-1} hydrochloric acid; ultra pure water till the effluent solution has become neutral, and finally pre-conditioned by 2 mL acetate buffer pH 5.5. Good signal reproducibility and reasonably stable performance of the FIA system have been achieved.

2.6. FIA manifold and on-line preconcentration

The flow system, operating in time-based mode, was manifested using peristaltic pump fitted with Tygon tubes of 1.14 interior diameters. A schematic diagram of the flow injection manifold in both of the preconcentration and elution modes is shown in Fig. 1. In case of preconcentration mode, the sample (S) containing 4 μg mL^{-1} Fe (II) ions adjusted to pH 5.5 with acetic/acetate buffer is pumped through the SPDA–PUF mini-column at a flow rate 2.25 mL min^{-1}. The analyte was retained onto the mini-column and the effluent containing unadsorbed iron (II) and matrix in the sample was discharged into the waste route (W). The preconcentration time was typically 90 s. In the elution mode, a 200 μL eluent loop filled with 0.1 mol L^{-1} hydrochloric acid (E) was switched into the injection position (for 60 s) where the loop contents were displaced into the carrier stream (CR) of ultra pure water flowing at 2.0 mL min^{-1} and displaces the analyte from the sorbent. The eluate was derived to the mixing coil and reacted with TPTZ reagent. A blue colour is developed which was passed to the flow cell of the spectrophotometer. The absorbance signal was recorded at 593 nm where narrow peaks with stable baselines were obtained. The peak height was used to quantify iron (II) in the solution. After complete recording of the signal, the injection valve was returned to the preconcentration mode for the next cycle.

3. Results and discussions

3.1. Detection method

Based on the previously reported method for spectrophotometric determination of iron (II) by reaction to TPTZ (Dahlén & Karlsson, 1999), and the flow-injection determination of copper(II) by reaction to cysteine in presence of iron(III) where iron(II) was produced and iron(II)–TPTZ complex was measured (Wei, Teshima, Ohno, & Sakai, 2003). In this work, modifications to the previous methods have been done to enable pH control as strictly as possible by mixing the TPTZ reagent with acetate buffer stream at pH 5.5. This would enhance the stability of iron (II)–TPTZ complex and thereby maximise the absorbance signal thus improving the precision. Herein, the analyte ions in the carrier stream, passing at 2.25 mL min^{-1}, was reacted in a mixing coil to the TPTZ reagent passing at 1.5 ml min^{-1} where a blue coloured solution was developed and reached the flow cell of the spectrophotometer and measured at 593 nm. Finally, the analytical signals were recorded as peak height absorbance and taken as function of Fe (II) concentration.

3.2. Synthesis of SPDA–PUF sorbent

A half gram of the dried PUF plugs (1 cm length and 3 mm diameter) was soaked in 50 mL from 5 mol L^{-1} hydrochloric solution while stirring for 2 h then washed with water till acid free. After this, the plugs were suspended in 100 mL from 0.1 mol L^{-1} hydrochloric acid – ice mixture and 50 mL of 0.5 mol L^{-1} sodium nitrite solution was added while stirring. Then, the plugs were separated from the mixture and added to SPDA solution (0.5 g reagent in 100 mL 10% (w/v) sodium hydroxide solution) and kept at temperature below 4 °C for 24 h. A canary yellow material was obtained which was washed several times with ethanol and ultra pure water then left to dry in ambient temperature. The FT-IR spectrum of SPDA–PUF sorbent showed two additional bands that do not appear in the spectrum of untreated PUF. The first characteristic peak appeared at 1638 cm^{-1} may be assigned to the C=N group

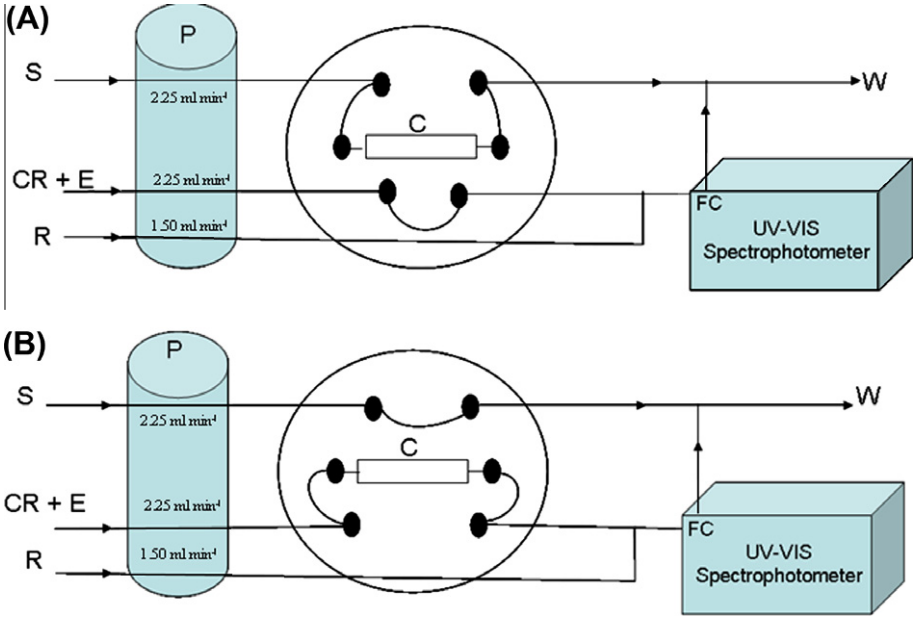

Fig. 1. Schematic diagram of the flow system for preconcentration and determination of iron (II); S: sample; E: eluent; CR: water carrier; R: TPTZ solution; P: peristaltic pump; C: SPDA–PUF mini-column; FC: flow cell; W: waste; (A): preconcentration mode; (B): elution mode.

stretching in SPDA reagent indicating its anchoring to PUF. The second peak was observed at 1416 cm^{-1} which might be contributed by N=N stretching indicating the conversion of toluidine groups in PUF to the diazotized foam. These findings confirm that SPDA molecule was covalently bonded to the PUF successfully via azo coupling.

3.3. Optimization of on-line preconcentration method

Experimental procedure was optimised to establish the best chemical and flow conditions for the retention and elution of the iron (II). Standard solutions containing 4.0 $\mu g\,mL^{-1}$ of Fe (II) were employed in this study.

3.3.1. Chemical optimization

The influence of sample pH on the retention of iron (II) onto the functionalized SPDA–PUF was investigated in the range 3.0–7.0. The flow system was optimised by loading iron (II) solution (4.0 $\mu g\,mL^{-1}$) onto the SPDA–PUF mini-column for 90 s followed by direct elution of the metal ion into the spectrophotometer. For this purpose, the pH values of solutions were adjusted to 3.0–6.0 by using acetate buffer solutions and at pH 7 with phosphate buffer. The maximum absorbance occurred at pH 5.5 as shown in Fig. 2. Acetate buffers pH 5.5 was chosen as the optimum in subsequent experiments. Beyond or less than pH 5.5, the peak height absorbance was found to be strongly decreased which might be due to the possible precipitation of the iron (II) hydroxide at pH > 5.5 or due to the less ionisation of the chelating groups in SPDA ligand at pH < 5.0.

The sorption of iron (II) ions onto SPDA–PUF is negligible under acidic conditions ($pH \leqslant 3.0$). Therefore, an elution experiment is expected to be performed with mineral acids such as nitric or hydrochloric acid solution because they can rapidly alter the pH of the medium which would facilitate fast desorption of iron (II). Results indicated no significant difference between the obtained signals by using either one of these two acids. However, nitric acid was avoided since it could shorten the life time of the mini-column by destroying the PUF matrix due to its oxidative action. Elution by common organic solvents such as acetone, methanol and ethanol was excluded because they caused plugging of the mini-column, thus increasing the back-pressure in the mini-column and resistance to the flow of solution through it. Hydrochloric acid was recommended as eluent because it did not influence the baseline signal of the TPTZ reagent and it has no oxidative action on the foam matrix unlike nitric acid. Further advantage obtained form the use of hydrochloric as eluent was to prevent oxidation of Fe (II). Therefore, hydrochloric solutions at various concentrations varying from 0.05 to 0.30 mol L^{-1} were examined as eluent. Best signal height was achieved at acid concentration 0.2 mol L^{-1} which revealed complete recovery of Fe (II). Hence, the 0.2 mol L^{-1} acid concentration was chosen as adequate eluent in the subsequent experiments.

The volume of the eluent was optimised carefully since the use of excess eluent volume may lead to peak broadening and decreases the concentration factor. On the other hand, less volume than the optimum value would not completely desorb iron (II) from the minicolumn. In order to optimise the eluent volume, individual samples containing 4.0 $\mu g\,mL^{-1}$ iron (II) were passed through the minicolumn under the optimised conditions. Then, the foam sorbent was injected with various volumes from the 0.2 mol L^{-1} hydrochloric acid solution in the elution step. Eluent loops with volumes 100, 200, 300, 400 and 500 μL were successively fixed into the injection valve in the peristaltic pump. Each loop was filled with the eluent and examined separately and the corresponding signal height was recorded. The results showed maximum and constant peak height with eluent volumes from 100 and 200 μL. Larger volumes than 200 μL have led to gradual decrease in the signal height and appearance of peak tailing which might be due to the distribution of the Fe (II) ions in eluent stream and the possibility of diffusion through the carrier solution.

3.3.2. Flow optimization

Sample flow rate has significant effect in the on-line preconcentration systems because it controls the amount of analyte flow through the mini-column. Thus, careful investigation of this parameter is very important to set an optimum sample flow rate that allows maximum mass transfer from liquid to solid-phase without loss of analytical throughput. For this purpose, sample solution was passed through the modified column at various flow rates between 0.75 and 4.5 mL min^{-1}. The analytical signals were maximum and constant in the range from 0.75 to 2.5 mL min^{-1}. Although low flow rates provides quantitative retention of the

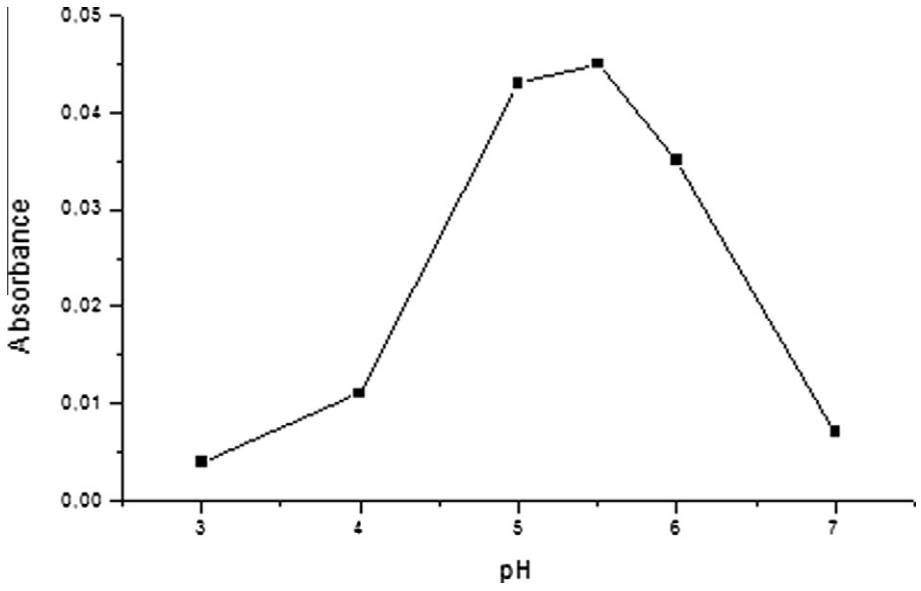

Fig. 2. Effect of pH on the signal of iron (II) from SPDA–PUF column and TPZ reagent at 593 nm and 2.25 mL min^{-1} and elution with 200 μl from 0.2 mol L^{-1} HCl solution.

metal ion but it decreases the sample throughput, resulting in long analysis time. At flow rates higher than 2.5 mL min^{-1}, the absorbance signal gradually decreases which might be due to the insufficient contact time between the iron (II) ions in the mobile phase and the chelating sites in the solid phase. Therefore, the sample flow rate 2.25 mL min^{-1} was selected as the optimum value in subsequent experiments as a compromise between efficiency and sensitivity.

The influence of the eluent flow rate was examined by changing the carrier flow rate while 200 μL form the eluent was injected into the carrier. Flow rates of the carrier solution in the range of 1–4 mL min^{-1} were studied. Quantitative desorption values has been obtained in the range 1.5–4.0 mL min^{-1}. Less eluent flow rate than 1.5 mL min^{-1} has resulted in a decrease in the peak height absorbance and instability in the shape of the signal due to the production of broad peaks. The lower the eluent flow rate the broader was the signal. Therefore, the 2.25 mL min^{-1} was selected flow rate for both of sample and eluent solutions to keep the pump at constant speed throughout the cycle.

The preconcentration time has significant effect on the sensitivity of time-based systems due to higher amount from the metal ion can be retained by increasing the elapsed time. However, preconcentration time cannot be increased indefinitely because washing of the mini-column by the sample itself might lead to dimensioning in the concentration efficiency. Investigation of this parameter was carried out by varying the preconcentration time from 10 to 120 s. The obtained results have indicated a linear increase in the analytical signal up to 100 s, confirming that quantitative sorption of the analyte and practically either no or negligible leaching out of the retained iron (II) ions takes place within this time period of sample flow. This suggestion could be used to improve the limit of quantification by employing higher preconcentration times. At prolonged preconcentration times than 100 s, the signal height is not directly proportional to time and the peak height increases nonlinearly. Noteworthy, longer preconcentration periods could be employed in case of the need to obtain higher enrichment factors but this, of course, on the expense of decreasing the sample frequency. Finally, a 90 s preconcentration time was recommended during this work as balance in order to achieve a reasonably high throughput and relevant sample consumption.

The capacity of the SPDA–PUF sorbent for sorption of iron (II) was carefully investigated because it govern both of the concentration range in which the method could be utilised and the type of natural samples the method can be applied. For this purpose, a series of model solutions containing 50.0–150.0 μg mL^{-1} iron (II) were individually shaken with containing 0.1 g sorbent. The recovered signals increased proportionally with increasing the concentration till reaching levelling off at 90 μg mL^{-1} concentration level which implied saturation of the sorbent. At this point, the maximum capacity was found to be 0.30 mg g^{-1}.

Lifetime of the mini-column was evaluated by measuring the height of the analytical signal of 4 μg mL^{-1} iron (II) solution at the end of each working day to investigate whether the retention efficiency still practically working effectively or adversely affected by the multiple use of the mini-column. Furthermore, the total number of cycles was counted daily. Results have shown good performance and reproducible signal height for at least 100 cycles a day. Plus, under the applied conditions of precocentration and elution, the mini-column could be employed for unlimited use which conveys high chemical stability of the sorbent.

3.4. Matrix effect

The potential interference due to common co-existing ions encountered in natural samples was tested by the on-line preconcentration method. Interference in the determination of 4.0 μg mL^{-1} Fe (II) was studied systematically in order to evaluate the selectivity of the proposed procedure. The tolerance limit was recognised at ± 5% relative error in the analytical signal. The cations Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Mn^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+} and the anions Cl^{-}, PO_4^{3-}, SO_4^{2-}, $C_2O_4^{2-}$ were examined. It is obvious that the effect of the most tested foreign ions is negligible. The strongest adverse effect was found with those metal ions which could be retained into the mini-column by forming complex with the SPDA ligand such as copper (30 mg L^{-1}) and cobalt (50 mg L^{-1}). Also, chromium and zinc are found to interfere but less than Cu(II) and Co(II). Alkali, alkaline earth elements and chloride did not show interference effect up to the studied ratio. Finally, the obtained data revealed adequate selectivity of the developed procedure for the determination of Fe (II) ions in most real samples because the existence of those strongly interfering ions is lower than the tolerance concentration.

3.5. Analytical performance

The analytical performance data for the developed procedure under the optimised conditions for preconcentration and elution are presented in Table 1. The flow system showed linearity within the concentration range from 0.05 to 5.0 μg mL^{-1} for 90 s preconcentration time. The linear working range of the proposed procedure is 0.05–5.0 μg mL^{-1}. Without preconcentration steps, the analysis range for iron ion by FAAS is 0.05–8.0 μg mL^{-1}. While the analysis can be done directly by FAAS, however, the attractive side of the proposed study is using UV–Vis spectrophotometer as a detector which is easily available almost in all laboratories and automated on-line analysis. The sample frequency was found to be 20 h^{-1}. For determination of the enrichment factor, two identical series of model solutions were prepared. The metal ion in the first set was determined by passing the samples directly into the flow system in absence of the mini-column and metal ion in the other set was determined after preconcentration on the SPDA–PUF mini-column. The calibration graph under the optimum chemical and flow conditions of preconcentration in the linear range between 0.2 and 3.0 μg mL^{-1} by the manifold shown in Fig. 1 could be represented by the equation $A = 0.007814$ (Fe(II), μg mL^{-1}) + 0.00037 ($r = 0.9972$). By using of direct determination in the flow system without preconcentration in the same concentration range, the calibration equation was $A = 0.000215$ (Fe(II), μg mL^{-1})−0.0005 ($R = 0.99919$).

The experimental enrichment factor calculated as the ratio of the slopes of the calibration graphs obtained with and without preconcentration was found to be 36 for 90 s preconcentration time. The concentration efficiency (Lemos, Bezerra, & Amorim 2008), defined as the product of the enrichment factor and the sampling frequency per number of samples analysed per minute was calculated and was also found to be 12 min^{-1}.

The limit of detection (LOD), defined as the iron (II) concentration that gives absorbance signal equivalent to three times the standard deviation of the blank ($n = 5$), was found to be

Table 1
Analytical performance data of the on-line preconcentration/separation method for total iron determination.

Enrichment factor	36
Preconcentration time (s)	90
Concentration efficiency (min^{-1})	12
Sample frequency (h^{-1})	20
Linear range (μg mL^{-1})	0.05–0.5
Regression equation	$A = 0.00037 + 0.00781$ [Fe(II), μg mL^{-1}]
Correlation coefficient	$r = 0.9972$
Limit of detection (μg L^{-1})	18.0
Precision (RSD, $n = 5$) (%)	5.7 (0.1 μg mL^{-1})
Sample volume (mL)	3.37

0.018 μg mL^{-1} in 3.0 mL sample solution. The limit of quantification (LOQ), calculated as the concentration that gives a absorbance signal equivalent to ten times the standard deviation of the blank (n = 5), was found to be 0.06 μg mL^{-1}. The precision of the procedure, calculated as the relative standard deviation in sample solutions containing 0.1 and 5.0 μg mL^{-1} iron (II), was found to be 5.7% and 3.1%, respectively by five measurements. Further improvement in the enrichment factor and LOD could be achieved by increasing preconcentration time without lowering in the analytical efficiency by applying the proposed system to lower concentration samples for prolonged preconcentration time than 90 s. This enabled quantitative sorption of iron (II) without significant loss in the peak height absorbance even after pumping of large sample volume.

3.6. Accuracy

To study the accuracy of the proposed method, the content of iron (II) in bovine muscles BCR-CRM 184 certified reference material was determined. Both of the certified and found values are compiled in Table 2. According to the procedure described by Linsinger (2005), the expanded uncertainty was calculated from the equation $U\Delta = 2(U^2_m + U^2_{crm})^{1/2}$ at a coverage factor of two (k = 2), corresponding to a level of confidence of approximately 95%. The results showed the value of $U\Delta$ is higher than the absolute diffrenec between the measured and certified values (Δ_m = 3.8 μg g^{-1}) which confirm there is no significant difference between the measured and certified values. Also, the obtained recovery was found to be satisfactory (95.2%) showing that the proposed approach is suitable for analysis of biological samples.

3.7. Analysis of food samples

Results for analysis of food samples by the developed SPDA–PUF method are depicted in Table 3. The iron content was in the range 17.6–257, 14.2–30.5 and 17.9–200 μg g^{-1} for vegetables, fruits and supplement samples, respectively. In the studied vegetables, the lowest concentration or iron was found in eggplant and the highest concentration was noticed in spinach. In the selected fruits, the maximum iron level was found in banana, followed by strawberry and finally apples. For supplements, the content of iron in liver was too high compared to enriched rice or bread.

The results achieved for the analysed samples were compared to those obtained by standard FAAS method. As presented in Table 3, it is clear that, the results obtained by the developed procedure are in good agreement with those obtained by FAAS method which confirms the reliability of the SPDA–PUF method. Also, high precision was obtained for the SPDA–PUF method since the corresponding RSD values were in the range 1.1–6.3% which were comparable to those obtained by the FAAS method with RSDs varied between 0.7% and 3.8%.

Moreover, satisfactory agreement for the analysis data obtained by the present method and reference methods especially those reported for Turkey samples (Tokalıoglu & Gürbüz, 2010) which prove relevant accuracy. However, the content of iron in most analysed samples is greater than those in the reference values which indicate the high contamination found in Egyptian vegetables, fruits and supplements due to the pollution in farm soil and irrigation water. Finally, the analysis results proved that the proposed preconcentration procedure is not affected by matrix interferences and can be satisfactory applied for on-line iron (II) preconcentration in food samples.

Table 2
Results for determination of iron (II) in certified reference material (BCR-CRM 184 bovine muscle) after preconcentration with SPDA–PUF sorbent.

Iron (II) amount (μg g^{-1})		Recovery (%)	Error (%)	$U\Delta$[a]
Certified[b]	Found[c]			
79.0 ± 2.0	75.2 ± 1.6	95.2	−3.8	5.1

[a] Expanded uncertainty of difference between result and certified values.
[b] Certified value ± uncertainty as reported in certificate.
[c] Mean value ± standard deviation (n = 3) and 95% confidence limit.

Table 3
Determination of iron content in food samples by the proposed on-line SPDA–PUF and standard FAAS methods (n = 4).

Sample	Found (mean ± SD) μg g^{-1}		
	SPDA–PUF method	FAAS method	Reference method
Vegetables			
Eggplant	17.6 ± 0.8	19.0 ± 0.2	14.5 ± 0.7[a]
Potato	19.1 ± 0.3	20.8 ± 0.6	15.5 ± 0.1[a]
Tomato	46.2 ± 0.5	44.9 ± 1.3	39.8 ± 1.3[b]
Green pepper	32.1 ± 1.6	33.7 ± 0.5	28.2 ± 0.2[a]
Parsley	95 ± 3.0	93.2 ± 2.9	89.4 ± 2.3[a]
Spinach	257 ± 5	259.0 ± 3.7	260 ± 3[a]
Fruits			
Apples	14.2 ± 0.9	13.8 ± 0.1	15.6 ± 0.2[a]
Strawberry	21.9 ± 0.7	19.6 ± 0.5	19.5 ± 0.2[a]
Banana	30.5 ± 1.2	28.4 ± 1.1	27.5 ± 0.7[a]
Supplements			
Rice (enriched)	27.0 ± 0.5	28.1 ± 1.0	22.3 ± 0.95 [c]
Wheat bread	26.9 ± 1.1	27.5 ± 0.8	19.4 ± 0.7[d]
Bovine liver	200.0 ± 12	198.2 ± 6.5	194 ± 20[e]

[a] Tokalıoglu and Gürbüz (2010).
[b] Durukan, Şahin, Şatıroğlu, and Bektaş (2011).
[c] Kosse, Yeung, Gil, and Miller (2001).
[d] Yıldız, Citak, Tuzen, and Soylak (2011).
[e] Ichinoki, Miyanaga, Hattori, and Fujii (2005).

4. Conclusions

The use of PUF chemically modified with SPDA reagent as new sorbent for packing a minicolumn has been successfully applied to the on-line preconcentration and spectrophotometric determination of iron by TPTZ. The method presented high selectivity and adequate sensitivity. The cellular structure of PUF–SPDA packed in the developed manifold improves significantly the analytical performance of the proposed procedure because it permits the employment of high flow rates resulting to better enhancement factors. The developed procedure is very simple, rapid, inexpensive, and high tolerance to interference ions. Analytical features such as detection limit, enrichment factor and precision the method has been demonstrated to be satisfactory for trace analysis of iron. Also, the precision and recovery obtained in the analysis of either certified biological material or food samples showed adequate accuracy of the results. Finally, the proposed method proved to be feasible for iron determination in various food samples.

References

Aggett, P. J., Agostini, C., Axelsson, I., Bresson, J. L., Goulot, O., Hernell, O., et al. (2002). Iron metabolism and requirements in early childhood: do we know enough?: a commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition, 34*, 337–345.

Agrawal, Y. K., Menon, S. K., & Pancholi, Y. (2003). Liquid–liquid extraction, preconcentration and trace determination of selenium with rotaxane. *Indian Journal of Chemistry, 42A*, 3000–3005.

Andersen, J. E. T. (2005). A novel method for the filterless preconcentration of iron. *Analyst, 130*, 385–390.

Baytak, S., & Turker, A. R. (2005). The use of Agrobacterium tumefacients immobilized on Amberlite XAD-4 as a new biosorbent for the column preconcentration of iron(III), cobalt(II), manganese(II) and chromium(III). *Talanta, 65*(4), 938–945.

Bhattacharya, S., Roy, S. K., & Chakraborty, A. K. (1990). Spectrophotometric determination of traces of iron after extraction of Fe(II)-phenanthroline complex on polyurethane foam. *Talanta, 37*(11), 1101–1104.

Burham, N., Abdel-Azeem, S. M., & El-Shahat, M. F. (2009). Removal of Pb and Cd from aqueous media and fish liver using novel polyurethane foam functionalized with pyrazolone as a new metal ion collector. *Central European Journal of Chemistry, 7*(3), 576–585.

Cassella, R. J. (2002). On-line solid phase extraction with polyurethane foam: Trace level spectrophotometric determination of iron in natural waters and biological materials. *Journal of Environmental Monitoring, 4*(4), 522–527.

Cassella, R. J., Salim, V. A., Jesuıno, L. S., Santelli, R. E., Ferreira, S. L. C., & Carvalho, M. S. (2001). Flow injection determination of cobalt after its sorption onto polyurethane foam loaded with 2-(2-thiazolylazo)-*p*-cresol (TAC). *Talanta, 54*(1), 61–67.

Dahlén, J., & Karlsson, S. (1999). Determination of iron(II) in natural waters by capillary zone electrophoresis using on-capillary complexation with 2,4,6-tri(29-pyridyl)-1,3,5-triazine. *Journal of Chromatography A, 848*(1–2), 491–502.

Dev, K., & Rao, G. N. (1996). Synthesis and analytical properties of a chelating resin functionalised with bis-(*N*,*N*′-salicylidene)1,3-propanediamine ligands. *Talanta, 43*(3), 451–457.

Durana, C., Buluta, V. N., Gundogdua, A., Ozdesa, D., Yildirima, N., Soylak, M., et al. (2009). Carrier element-free coprecipitation with 3-phenly-4-o-hydroxybenzylidenamino-4,5-dihydro-1,2,4-triazole-5-one for separation/preconcentration of Cr(III), Fe(III), Pb(II) and Zn(II) from aqueous solutions. *Journal of Hazardous Materials, 167*(1–3), 294–299.

Durukan, I., Şahin, C. A., Şatıroğlu, N., & Bektaş, S. (2011). Determination of iron and copper in food samples by flow injection cloud point extraction flame atomic absorption spectrometry. *Microchemical Journal, 99*, 159–163.

El-Shahat, M. F., Moawed, E. A., & Zaid, M. A. A. (2003). Preconcentration and separation of iron, zinc, cadmium and mercury, from waste water using Nile blue A grafted polyurethane foam. *Talanta, 59*(5), 851–866.

Forence, T. M., & Batley, C. E. (1980). Chemical speciation in natural waters. *CRC Critical Reviews in Analytical Chemistry, 9*, 219–296.

Ichinoki, S., Miyanaga, S., Hattori, M., & Fujii, Y. (2005). Selective determination of iron in river water and standard bovine liver by solvent extraction with *N*-benzoyl-*N*-phenylhydroxylamine followed by reversed-phase HPLC. *Journal of Liquid Chromatography & Related Technologies, 28*, 1417–1429.

Karacan, M. S., & Aslantas, N. (2008). Simultaneous preconcentration and removal of iron, chromium, nickel with *N*,*N*′-etylenebis-(ethane sulfonamide) ligand on activated carbon in aqueous solution and determination by ICP-OES. *Journal of Hazardous Materials, 155*(3), 551–557.

Klamtet, J. (2004). Development of flow injection analysis system for determination of iron (II) and total iron in ground. *NU Science Journal, 1*, 5–13.

Kosse, J. S., Yeung, A. C., Gil, A. I., & Miller, D. D. (2001). A rapid method for iron determination in fortified foods. *Food Chemistry, 75*, 371–376.

Lemos, V. A., Bezerra, M. A., & Amorim, F. A. C. (2008). On-line preconcentration using a resin functionalized with 3,4-dihydroxybenzoic acid for the determination of trace elements in biological samples by thermospray flame furnace atomic absorption spectrometry. *Journal of Hazardous Materials, 157*(2–3), 613–619.

Lemos, V. A., Passos, A. S., Novaes, G. S., Santana, D. A., Carvalho, A. L., & Silva, D. G. (2007). Determination of cobalt, copper and nickel in food samples after pre-concentration on a new pyrocatechol-functionalized polyurethane foam sorbent. *Reactive and Functional Polymers, 67*(6), 573–581.

Linsinger, T. P. J. (2005). Comparison of measurement results with the certified value, Application Note 1. Available from: <http://www.erm-crm.org>.

Lunvongsa, S., Oshima, M., & Motomizu, S. (2006). Determination of total and dissolved amount of iron in water samples using catalytic spectrophotometric flow injection analysis. *Talanta, 68*(3), 969–973.

Mahmoud, M. E., Hafez, O. F., Osman, M. M., Yakouta, A. A., & Alrefaay, A. (2010). Hybrid inorganic/organic alumina adsorbents-functionalized-purpurogallin for removal and preconcentration of Cr(III), Fe(III), Cu(II), Cd(II) and Pb(II) from underground water. *Journal of Hazardous Materials, 176*(1–3), 906–912.

Michio, Z., Hiroaki, I., Shinseki, H., & Takashi, Y. (2003). Visual colorimetry of iron(II)-1,10-phenanthroline after preconcentration onto polyurethane foam. *Bunseki Kagaku, 52*(3), 201–203.

Moghimi, A. (2008). Preconcentration and determination of chromium species using octadecyl silica membrane disks and ammonium pyrrolidine dithiocarbamate. *Asian Journal of Chemistry, 20*, 427–440.

Mohamed, E. M., & Al Saadi, M. S. M. (2001). Selective solid phase extraction and preconcentration of iron(III) based on silica gel-chemically immobilized purpurogallin. *Analytica Chimica Acta, 450*(1–2), 239–246.

Ozyurek, M., Celik, S. E., Berker, K. I., Guclu, K., Tor, I., & Apak, R. (2007). Sensitivity enhancement of CUPRAC and iron(III)-phenanthroline antioxidant assays by preconcentration of colored reaction products on a weakly acidic cation exchanger. *Reactive and Functional Polymers, 67*(12), 1478–1486.

Pourreza, N., & Mousavi, H. Z. (2004). Solid phase preconcentration of iron as methylthymol blue complex on naphthalene tetraoctylammonium bromide adsorbent with subsequent flame atomic absorption determination. *Talanta, 64*(1), 264–267.

Roldana, P. S., Alcantaraa, I. L., Padilhab, C. C. F., & Padilha, P. M. (2005). Determination of copper, iron, nickel and zinc in gasoline by FAAS after sorption and preconcentration on silica modified with 2-aminotiazole groups. *Fuel, 84*(2–3), 305–309.

Safavi, A., Iranpoor, N., & Saghir, N. (2004). Directly silica bonded analytical reagents: synthesis of 2-mercaptobenzothiazole–silica gel and its application as a new sorbent for preconcentration and determination of silver ion using solid-phase extraction method. *Separation and Purification Technology, 40*(3), 303–308.

Salem, I. A., El-Sheikh, M. Y., Zaki, A. B., & Nickel, U. (1994). Iron(II)-bis (salicyladimine) Schiff-base complexes catalyzed decomposition of hydrogen peroxide. *International Journal of Chemical Kinetics, 26*(9), 955–962.

Sangi, M. R., Jayatissa, D., Kim, J. P., & Hunter, K. A. (2004). Determination of labile Cu^{2+} in fresh waters by chemiluminescence. Interference by iron and other cations. *Talanta, 62*(5), 924–930.

Shabany, M., Shabani, A. M. H., Dadfarnia, S., Gorji, A., & Ahmadi, S. H. (2008). Solid phase extraction of zinc with octadecyl silica membrane disks modified by *N*,*N*′-disalicylidene-1,2-phenylendiamine and determination by flame atomic absorption spectrometry. *Ecleticn Química São Paulo, 33*(2), 61–66.

Tokalıoglu, S., & Gürbüz, F. (2010). Selective determination of copper and iron in various food samples by the solid phase extraction. *Food Chemistry, 123*, 183–187.

Ugo, P., Moretto, L. M., Rudello, D., Birriel, E., & Chevalet, J. (2001). Trace iron determination by cyclic and multiple square-wave voltammetry at Na®on coated electrodes. application to pore-water analysis.. *Electroanalysis, 13*, 661–668.

Wei, J., Teshima, N., Ohno, S., & Sakai, T. (2003). Catalytic flow-injection determination of sub-ppb copper(II) using the redox reaction of cysteine with iron(III) in the presence of 2,4,6-tris(2-pyridyl)-1,3,5-triazine. *Analytical Sciences, 19*(5), 731–735.

Wu, P., Gao, Y., Cheng, G., Yang, W., Lv, Y., & Hou, X. (2008). Selective determination of trace amounts of silver in complicated matrices by displacement-cloud point extraction coupled with thermospray flame furnace atomic absorption spectrometry. *Journal of Analytical Atomic Spectrometry, 23*, 752–757.

Yıldız, O., Citak, D., Tuzen, M., & Soylak, M. (2011). Determination of copper, lead and iron in water and food samples after column solid phase extraction using 1-phenylthiosemicarbazide on Dowex Optipore L-493 resin. *Food and Chemical Toxicology, 49*, 458–463.