IRON(II) ION IMPRINTED POLYMER FOR Fe(II)/Fe(III) SPECIATION IN WINE

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Abstract

In this paper, we proposed a procedure for the determination of Fe(III) and total Fe(II)+Fe(III) in wine samples employing newly synthesized Fe(II) ion imprinted polymer (Fe(II)-IIP) as a sorbent incorporated in dispersive solid phase extraction. Cross-linked precipitation copolymerization using 4-vinylpyridine (functional monomer), trimethylolpropane trimethacrylate (cross-linking agent) and 2,2′-bipyridyl as a specific for Fe(II) ligand was used for the synthesis of Fe(II)-IIP. The optimization experiments showed quantitative sorption of both Fe(II) and Fe(III) on the surface of Fe(II)-IIP at pH 7, however selective sorption of only Fe(II) could be achieved in the presence of fluoride ions as a masking agent for Fe(III). The Langmuir adsorption isotherm best described the adsorption of Fe(II) on the surface of the sorbent on the at least two kinds of binding sites with higher and lower affinities as defined by Scatchard plot. The analytical procedure developed allows the determination of iron(III) and total Fe(II)+Fe(III) in wine samples, with limits of quantification 0.1 mg L\(^{-1}\). The relative standard deviation varied in the range 3-7% for Fe content 0.1-10 mg L\(^{-1}\).

Keywords: Fe(II) ion imprinted polymer; Fe(II); Fe(III); speciation; wine

1. Introduction

Wine is a widely consumed alcoholic beverage and evidently important source of trace elements in human diet. Since the element bioavailability is highly variable between chemical forms, information on trace element speciation in wine samples is important analytical task from both viewpoints: human health and wine “health”. The content of Fe in wine is defined from its concentrations in grapes (Fe is part of certain enzymes) and depends on the maturity of the grape,
the soil type and the soil pH as well as on the contaminations during wine storage in metal containers. Iron is an essential element and necessary for the growth and activity of *Saccharomyces cerevisiae* during the alcoholic fermentation, Fe is also involved in oxidation processes and Fe(III) concentration is responsible for the so called white casse in wines (reactions with proteins in the presence of phosphates [1,2]. However total concentration of Fe is not a good predictor for the occurrence of these processes. Exact Fe speciation (according to the definition of IUPAC) is really difficult problem in wine samples due to relatively complicate wine matrix, however from practical point of view fractionation and determination of main Fe species in wine: organically bound Fe and kinetically labile complexes of Fe(II) and Fe(III) is enough for reliable conclusions during the wine making process and for wine stability during storage.

Various extraction procedures for Fe species separation have been proposed, based generally on ion exchange resins [3, 4], liquid–liquid and microextraction [5-7], cloud point extraction [8] and solid phase extraction (SPE) [9-14]. Highly selective ion imprinted polymers, incorporated as efficient sorbents in SPE procedures have been recently used in nonchromatographic speciation procedures. Ion imprinted polymers are new generation synthetic materials with artificially generated recognition sites able to specifically rebind a target ion. Their high selectivity is explained by the polymer memory effect toward the metal ion interaction with a specific ligand, coordination geometry, metal ion coordination number, ion charge, and size. For example our group reported on the application of IIPs for Hg and Cr speciation in waters [15, 16]. Numerous studies on IIPs and their application for selective preconcentration and separation of ferrous [17] and ferric [18-22] ions mostly from water samples have been also reported. According to authors’ knowledge, IIPs have never been used for Fe(II)/Fe(III) speciation in any samples. So far no information on the selectivity and application of IIPs for the selective separation of metal species with a different oxidation states, but almost equal coordination geometry and ion size.

The main purpose of this study is focused on the synthesis of new Fe(II) ion-imprinted polymer (Fe(II)-IIP) and its application for the determination and speciation of Fe in wine. Fe(II)–IIP is synthesized via cross-linked precipitation copolymerization using 2,2′-bipyridyl (BP) as a specific for Fe(II) ligand. Critical comparison between extraction efficiencies, adsorption capacities, and selectivity’s of the Fe(II)-IIP and non-imprinted (non-IIP) polymer gels are presented. Finally, analytical scheme for iron fractionation in wine samples is developed based on both the high selectivity of Fe(II)-IIP toward Fe(II) and Fe(III) in the presence fluoride anions as masking agent.
2. Materials and methods

2.1. Materials

All reagents were of analytical-reagent grade and all aqueous solutions were prepared in high-purity deionized water (DW) (Millipore Corp., USA). The stock standard solutions of Cu(II), Cd(II), Fe(III), Mn(II), Pb(II) and Zn(II) (1000 µg mL\(^{-1}\)) were Titrisol, (Merck, Germany), in 2% HNO\(_3\). For the preparation of aqueous Fe(II) solutions, (NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\).6H\(_2\)O (Sigma-Aldrich Co. LLC., USA) was used. A stock solution of 100 mg L\(^{-1}\) Fe(II) was daily prepared by dissolving the required amounts of Fe(II) salt in 100 mL of DW. Working solutions with desired concentration were prepared by appropriate dilution of the stock solution.

2,2’-Bipyridyl (Sigma-Aldrich Co. LLC., USA), TMPTMA, 2,2’-azo-bis-isobutynitrile (AIBN) (Merck, Germany) and acetonitrile (ACN) (Labscan, Ireland) were used without further purification, 4-VP (Merck, Germany) was used after distillation. The pH was adjusted with NH\(_4\)OH or HNO\(_3\). Deionized water was used in all experiments.

The commercial Amberlite XAD-8 resin for polar compounds (dipole moment 1.8, porosity 25, BDH, Poole, UK) a non-ionic macromolecular styrene divinilbenzene copolymer was used to separate hydrophobic Fe species.

2.2. Apparatus

The Flame AAS measurements were carried out with a Perkin Elmer Model AAnalyst 400 atomic absorption spectrometer in air/acetylene flame. Instrumental parameters were optimized according to the Instrument Manual.

The IR-spectra (4000–400 cm\(^{-1}\)) were recorded on an IRAffinity-1 Fourier Transform Infrared (FTIR) spectrophotometer with MIRacle Attenuated Total Reflectance Attachment (“Shimadzu”, Japan).

A scanning electron microscope (SEM, JEOL JSM-5500, Japan) was used for the determination of the microbead shape and size.

Specific surface area and pore size distribution were measured through nitrogen adsorption–desorption isotherms at 77 K using a Quantachrome NOVA 1200 apparatus (Quantachrome UK Ltd. England).

A microprocessor pH meter (Hanna Instruments, Portugal) was used for pH measurements.

The centrifuge EBA 20 (DJB Labcare Ltd, England) used to separate micro particles and extracted metal ion solution in batch experiments.
2.3. Synthesis of the Fe(II) ion-imprinted and non-imprinted sorbents

The Fe(II) ion-imprinted (Fe(II)-IIP) and non-imprinted (non-IIP) polymer gels were synthesized as described earlier with minor modification [23]. The Fe(II)-IIP was prepared via a cross-linking dispersion copolymerization using 4-VP (0.546 mmol) as a functional monomer, TMPTMA (0.894 mmol) as a cross-linking agent, AIBN (16.8 mg) as an initiator, Fe(II) as a template ion (0.114 mmol), BP as a non-polymerizable chelating agent (0.342 mmol) and acetonitrile (24 mL) as a solvent. The solution was saturated with dry nitrogen for 15 min and copolymerization was carried out at temperature 60 °C for 24 h. The polymer particles obtained were pink-red colored. They were recovered by centrifugation, washed with ACN to remove unreacted monomers and other ingredients. Iron was removed from the polymer network by several, sequential elution steps using 4 mol L$^{-1}$ HNO$_3$ as eluent. This procedure was repeated until the Fe concentration (template ions) in the eluate solution is below the LOQ as measured by ETAAS. Then Fe-IIP was dried in a vacuum oven at 60 °C. Non-IIP sorbent was synthesized in the same procedure as described above, in the absence of chelating agent and template ion. Reaction scheme for Fe(II)-IIP preparation is shown in Fig. 1.

2.4. Adsorption and desorption experiments

The adsorption studies of the Fe(II) ions by the prepared sorbents were carried out in batch experiments, as follows. A portion of standard solution containing 5 µg Fe(II) was added to a 10 mL test solution and adjusted to a desired pH value. Polymer gel particles of ca. 50 mg were added to this solution and stirred with an electric shaker for 30 min. The suspension was centrifuged at (5000 rpm), supernatant removed and the polymer gel washed twice with DW. The Fe(II) was eluted from the polymer particles with 5 mL 4 mol L$^{-1}$ HNO$_3$. Iron content in the effluante (supernatant after sorption) and eluate solutions was determined by FAAS. The degree of sorption of metal ions was defined by comparing the initial cation amount $A_i$ (µg) and equilibrium non-adsorbed cation amount $A_{\text{eff}}$ (µg) after sorption on the copolymer gel, using equation:

$$D_S \% = \left[ \frac{(A_i - A_{\text{eff}})}{A_i} \right] \times 100$$

The degree of elution of adsorbed Fe(II) ions was calculated using equation:

$$D_E \% = \left[ \frac{A_{\text{el}}}{(A_i - A_{\text{eff}})} \right] \times 100$$

where $(A_{\text{el}}, \mu g)$ is the amount of metal cations in solution after elution process.

The effect of the masking agents on the degree of sorption of Fe(II) and Fe(III) on Fe(II)-IIP and non-IIP, was studied by the described above procedure after addition of 1 ml 0.1 mol L$^{-1}$ NH$_4$F, CH$_3$COONH$_4$, tartaric acid or 0.05 mol L$^{-1}$ EDTA solutions to 50 mg of the sorbent at pH 7.
The effect of initial concentration of Fe(II) ion on the adsorption capacity of Fe(II)-IIPs and non-IIP was studied by adding 50 mg of the sorbents to Fe(II) solutions with increasing concentrations in the range 50–400 µmol L\(^{-1}\) at pH 7. Suspensions were shaken for 30 min at 250 rpm and 25.0 °C by using shaker. The adsorption capacity \(Q_e\), the amount of Fe(II) adsorbed by 1 g of sorbent at equilibrium, was calculated according to the equation:

\[
Q_e = \frac{(C_0 - C_e) \cdot V}{m},
\]

where: \(Q_e\) is the mass of Fe(II) ions adsorbed per unit mass of the sorbent, µmol g\(^{-1}\); \(V\) is volume of the solution, \(m\) is the mass of the sorbent, g; \(C_0\) is concentration of Fe(II) ions in the initial solution, µmolL\(^{-1}\); \(C_e\) is equilibrium concentration of Fe(II) in solution after adsorption, µmolL\(^{-1}\).

### 2.5. Selectivity experiments

Selectivity experiments were performed in a batch system by adding 50 mg Fe(II)-IIPs or non-IIP into 10 mL aqueous solution containing 0.5 µg mL\(^{-1}\) of Fe(II)/Cd(II), Fe(II)/Cu(II), Fe(II)/Fe(III), Fe(II)/Mn(II), Fe(II)/Pb(II) or Fe(II)/Zn(II). The mixture was shaken (250 rpm) at pH 7 for 30 min. The retained Cd(II), Cu(II), Fe(II), Fe(III), Mn(II), Pb(II) and Zn(II) were desorbed from the polymer gel particles with 5 mL 4 mol L\(^{-1}\) HNO\(_3\). The concentration of metal ions in the eluate and eluate was measured by FAAS.

### 2.6. Analytical procedure for Fe fractionation in wine

As a first step the organically bounded Fe was retained on an Amberlite XAD-8 resin and removed from wine sample – 10.0 mL wine was passed through a column filled with a XAD-8 resin, pre-treated as described [9]. The sample was divided into two aliquots of 5.0 mL each. Total content of kinetically labile Fe(II)+Fe(III) was measured in the first aliquot. The pH of the second aliquot was adjusted to 7 (NH\(_3\)), 50 mg sorbent and 1 mL of 1 mol L\(^{-1}\) NH\(_4\)F were added for selective sorption of Fe(II). The suspension was shaken (250 rpm) for 30 min and in the effluvate obtained Fe(III) was measured by FAAS. The content of Fe(II) is calculated as a difference between total kinetically labile Fe and Fe(III) measured after selective sorption of Fe(II).

### 3. Results and discussion

#### 3.1. Synthesis of Fe(II)–IIP and characterization studies

One of the most widely synthesis schemes for the preparation of effective ion-imprinted sorbents is trapping of non-polymerizable ligand in copolymeric matrices [24]. According to this method, binding sites are created by polymerization of the functional and crosslinking monomers.
in the presence of complex between target metal ion and suitable chelating agent (template). In the present work, preparation of Fe(II)–IIPs consists of several steps (Fig. 1). Firstly, the template–monomer (prepolymerization) complex was formed by non-covalent interactions between the functional monomer (4-VP) and the template molecule (Fe(II)–BP complex). Secondly, the copolymer network was formed by the precipitation copolymerization of the prepolymerization complex with TMPTMA as a cross-linker. Finally, Fe(II) ions were removed from copolymer network prepared leaving behind some specific binding sites with functional groups in a predetermined orientation and cavities with special size of templates.

Scanning electron micrographs of the obtained Fe(II)-IIP and non-IIP copolymer particles are shown in Fig. 1S (suppl.mat.). It is seen that the synthesized non-IIP particles are in the shape of spheres with mean diameters (determined from the micrographs) 170 nm (Fig. 1S(a)). The surface structure and morphology of the Fe(II)-imprinted sorbent is different - Fe(II)-IIP is in the form of bigger aggregates of irregular particles (Fig. 1S(b)). Rough surface of ion imprinted polymers as compared to the non imprinted polymer suggests that cavities were formed on the sorbent surface after template removal [25].

In the FTIR spectra of the both polymer gels the characteristic C=O stretching bands, which belongs to ester functional group in the structure of TMPTMA, appear at 1724 cm\(^{-1}\). The most important information in FTIR spectra is based on the C–C and C–N bands in the structure of pyridine ring (between 1600 and 1430 cm\(^{-1}\)). These bands are found at 1598 cm\(^{-1}\) (stretching vibration absorption of C—N bond), 1558 cm\(^{-1}\) and 1456 cm\(^{-1}\) (stretching vibration absorption of C=C bond) in the spectra of non-IIP. The vibration frequencies of C—N band are shifted to 1638 cm\(^{-1}\) for Fe(II)-IIP, which is an indication that a metal–ligand bond is formed between Fe(II) and chelating groups of BP in imprinted copolymer gels [26, 27].

The N\(_2\) adsorption-desorption isotherms and the specific surface area (\(S_{\text{BET}}\)), total pore volume (\(V_{\text{total}}\)) and average pore diameter (\(D_{\text{average}}\)) for the imprinted and non-imprinted polymer gels are presented in Fig. S2 (Suppl. mat.) and Table 1. The results for average pore diameters for Fe(II)-IIPs and non-IIP (9 nm and 28 nm, respectively) suggested a mesoporous structures for the synthesize polymer gels. In addition, the BET surface area and pore size of Fe(II)-IIP (2 m\(^2\) g\(^{-1}\) and 0.01 cm\(^3\) g\(^{-1}\), respectively) are lower than these of the non-IIP (Table 1). These typical results after imprinting are explained with the incorporation of Fe(II)-BP complex in polymer network which causes a certain filling of the pores and reduced adsorption of N\(_2\) [28].

3.2. Optimization of chemical parameters for sorption and desorption of Fe(II) onto Fe(II)-IIP
The acidity of the solution is the most critical parameter controlling the degree of sorption of metal ions because the pH value affects both the metal binding sites on the adsorbent surface and the metal chemistry in the solution. The synthesized polymer gels contained pyridine groups in both the polymer matrix and the immobilized chelating agent in Fe(II)-IIP which means that both specific and nonspecific interactions of Fe(II) with sorbents will depend on pH. The adsorption efficiency of the Fe(II)-IIP and non-IIP polymer gels toward Fe(II) was tested from an aqueous solutions at pH values in the range 3–8. As seen from Fig. 2., the degree of ferrous ions sorption was enhanced for both sorbents with an increasing pH to 7. At low pH, the pyridine groups in the polymer matrix and chelating agent BP are protonated and positively charged (pKₐ=5 for polyvinylpyridine [28] and pKₐ=4.33 for BP) and the adsorption of Fe(II) on the sorbent particles is prevented due to the electrostatic repulsion between them. When the pH increased, the sorbent surface became less positively charged, because of partial deprotonation which increases the ability of the pyridine for complex formation with the Fe(II) in aqueous solution. At any pH, the sorption affinity of the Fe(II)-IIP for Fe(II) ion was higher than those of the non-IIP. Quantitative sorption for Fe(II) (98±2%) was achieved at pH 7 with Fe(II)-IIP, while the degree of sorption for non-IIP was around 65% (Fig. 2). Obviously, the specific interaction between chelating ligand BP and Fe(II) does not take place in the case of non-IIP, explaining the observed lower degree of sorption. The degree of sorption for both studied sorbents decreased at pH>7, most probably related to the partial hydrolysis of the ferrous hydroxide. Finally the pH of 7 was selected as optimal for the SPE of Fe(II) by Fe(II)-IIP and non-IIP in the further investigations.

The elution of Fe(II) from the loaded Fe(II)-IIP particles was investigated by using various concentrations and volumes of HNO₃ and EDTA solutions as desorption agents following the general procedure (Section 2.4). It was found that 4 mol L⁻¹ HNO₃ is quite effective for quantitative elution (>95%) of Fe(II) from the sorbent (Table 1S Suppl. mat.). Thus, 5 mL of 4 mol L⁻¹ HNO₃ solution was used as eluent in the subsequent experiments.

The kinetics of both sorption and desorption processes of Fe(II) were investigated by batch procedure with 50 mg of Fe(II)-IIP for 5–60 min. Quantitative sorption was reached for 20 min while for quantitative desorption 40 min has to be used.

3.3. Adsorption isotherms and Scatchard plots

Adsorption isotherms are useful instruments to predict the mechanism of sorption and the sorption efficiency and capacity toward target ions. Experimental data for adsorption isotherms constructed through static adsorption experiments (Fig. 3S Suppl. mat.) are used for the calculations of results summarized in Table 2. As might be expected the adsorption capacity of
Fe(II)-IIP is significantly higher than this for non-IIP at the corresponding initial concentrations. This indicates that the cavities created after removal of the template ion in imprinted polymer gels are more complementary toward the Fe(II) imprinted ion and in coordination geometries, which benefits binding of Fe(II) to the recognition sites.

The linearized Langmuir and Freundlich isotherm equation (Fig. 4S and 5S) and the isotherm parameters calculated from their analysis are presented in Table 2. Higher $R^2$ values (> 0.95) achieved for the adsorption of Fe(II) on the imprinted and non-imprinted polymer gels show that the Langmuir equation gives better fit to the adsorption isotherm than the Freundlich isotherm model. The slope of the curves (Fig. 4S) was used for the calculation of theoretical (monolayer) capacities ($Q_{\text{max, teor}}$) of the Fe(II) on the Fe(II)-IIP and non-IIP which are 28.01 μmol g$^{-1}$ and 19.15 μmol g$^{-1}$ respectively and agreed very well with experimentally obtained values of 28.30 μmol g$^{-1}$ and 18.70 μmol g$^{-1}$.

In order to study the binding properties of the Fe(II)-IIP and non-IIP, Scatchard analysis was performed by using the saturation binding data (see explanation in Suppl. Mat.). The Scatchard plot for Fe(II)-IIP (Fig. 3 ) consists of two linear parts with different slopes, which undoubtedly indicated that at least two kinds of binding sites with higher and lower affinities could exists on the surface of the ion imprinted sorbent. The linear regression equations for the left (higher affinity binding sites) and right (lower affinity binding sites) parts of the curve in the figure were $Q_e/C_e = -2.863 Q_e + 59.384$ ($R^2 = 0.991$) and $Q_e/C_e = -0.222 Q_e + 6.463$ ($R^2 = 0.967$), respectively. Based on the values of the slope and the intercept of the biphasic curve (Fig. 3), the calculated values for $K_D$ were 0.349 and 4.50 μmol L$^{-1}$ and for $Q_{\text{max}}$ were 13.76 and 29.11 μmol g$^{-1}$, respectively. It might be concluded that the binding sites on the surface of the Fe(II)-imprinted polymer gel could be classified into two distinct groups with different specific binding properties - bipyridyl groups (specific interaction) and pyridine group (non-specific interaction) with Fe(II).

For non-IIP one straight line was observed which reveals only lower affinity binding sites on the surface of non-imprinted polymer particles (Fig. 3). The linear regression equation was $Q_e/C_e = -0.162 Q_e + 3.101$ ($R^2 = 0.994$). $K_D$ and $Q_{\text{max}}$ were 6.165 μmol L$^{-1}$ and 19.12 μmol g$^{-1}$, respectively. The adsorption capacity of non-IIP toward Fe(II) was lower than that of Fe(II)-IIP and suggests that non-IIP does not possess specific adsorption abilities toward Fe(II). These results of Schatchard analysis are typical for a pair of the imprinted and non-imprinted polymers obtained by the non-covalent approach [30, 31].

3.4. Selectivity experiments
The high selectivity toward templated ion is an important characteristic of the imprinted polymers due to specific recognition sites created in the obtained three-dimensional polymer network. The competitive ion recognition studies were performed in order to investigate the adsorption selectivity of the synthesized Fe(II)-IIP sorbents toward selected metals like Cd(II), Cu(II), Fe(III), Mn(II), Pb(II) and Zn(II), which often coexisted in wine samples. The experiments were carried out with Fe(II)-IIP and non-IIP in batch mode at pH 7. The distribution coefficients (D) for Fe(II) and competitive ions: Cd(II), Cu(II), Fe(III), Mn(II), Pb(II) and Zn(II) were calculated according to the equation:

\[ D = \frac{(A_i - A_{\text{eff}})}{A_{\text{eff}}} \]

The selectivity coefficient \( S_{\text{Fe(II)/Me}} \) for the binding of ferrous ions in the presence of competitor species was obtained according to the equation:

\[ S_{\text{Fe(II)/Me}} = \frac{D_{\text{Fe(II)}}}{D_{\text{Me}}} \]

where \( D_{\text{Fe(II)}} \) and \( D_{\text{Me}} \) are the distribution coefficients for Fe(II), and Cd(II), Cu(II), Fe(III), Mn(II), Pb(II) or Zn(II), respectively. The effect of imprinting on the selectivity could be evaluated through the comparison of selectivity coefficients \( S_{\text{Fe(II)/Me}} \) obtained for imprinted Fe(II)-IIP and non-IIP polymer gels.

A relative selectivity coefficient \( \rho \) which represents the adsorption selectivity and affinity of the Fe(II)-IIP towards the template ion related to non-IIP was calculated according to the equation:

\[ \rho(S_{\text{Fe(II)/Me}}) = \frac{S_{\text{Fe(II)-IIP}}}{S_{\text{non-IIP}}} \]

The degrees of sorption, \( D_S \) of studied metal ions can be seen in Figure 4, calculated distribution coefficients (D), selectivity coefficients \( S_{\text{Fe(II)/Me}} \) between Fe(II) and Cd(II), Cu(II), Fe(III), Mn(II), Pb(II) and Zn(II) and calculated relative selectivity coefficients \( \rho(S_{\text{Fe(II)/Me}}) \) between the Fe(II)-IIP and non-IIP are summarized in Table 3. Although some ions have similar ionic radii, the same charge, and high affinity toward the BP’ ligand, significant differences in their extraction characteristic \( D_S \) (Fig. 4), were observed. The results indicated higher selectivity of Fe(II)-IIP toward Fe(II) even in the presence of competitors Cd(II), Cu(II), Mn(II), Pb(II) and Zn(II) ions. The \( D_S \) values of Fe(II)-IIP sorbent toward Fe(II) were largest and decreased in order Fe(II)>Cu(II)>Zn(II)>Cd(II)>Pb(II)>Mn(II) due to the coordination-geometry selectivity of the sorbent which could provide the ligand groups arranged in a suitable way required for coordination of Fe(II) [32]. The high values of the relative selectivity coefficients \( \rho(S_{\text{Fe(II)/Me}}) \) (eq. 5) convincingly demonstrated superiority of Fe(II)-IIP sorbent over non-IIP (Table 3). However the results presented in Table 3 showed that Fe(II)-IIP exhibits the lowest selectivity towards Fe(III) ions \( (S_{\text{Fe(II)/Fe(III)}}) = 5.4 \). This result might be explained by the same stoichiometry of the complexes
of Fe(II) and Fe(III) with BP – [Fe(BP)₃]²⁺ and [Fe(BP)₃]³⁺ and the close values of the stability constants. Since the separation of Fe(II) and Fe(III) with Fe(II)-IIP was the main goal of this investigation, another way to increase the selectivity of the sorbent is to use masking agent which forms stable complexes with one of the studied species. The effect of various masking agents used predominantly for Fe(III) masking on the degree of sorption of Fe(II) and Fe(III) on the Fe(II)-IIP polymer gel is shown in Table 4. Two key conclusions could be drawn from results obtained. First, among all tested masking agents (tartaric acid, EDTA, NH₄F and CH₃COONH₄) only fluoride anions effectively mask Fe(III) ions most probably because of very high stability of complex FeF₄⁻. As far as Fe(II) do not form fluoride complex selective quantitative retention of Fe(II) in the presence of fluoride ions was observed. The degree of sorption of Fe(II) in the presents of fluoride anions was 97±2%, while this value for Fe(III) was <3% (Fig. 4). The selectivity of the Fe(II)-IIP towards Fe(III) ions increased remarkably – S_{Fe(II)/Fe(III)} = 1584.3 (Table 3). Second, the tartaric acid (found in wine samples) does not influence on the degree of sorption of Fe(II) and Fe(III) consequently wine matrix will not affect the extraction efficiency and selectivity of Fe(II)-IIP polymer gel toward Fe(II) and Fe(III) in real samples.

3.5 Application

Iron exists in wine as hydrophobic complexes with wine organic matter mostly polyphenols in complex with proteins and polysaccharides and labile Fe(II) and Fe(III) species. From practical point determination of labile species, especially labile Fe(III) is most important for reliable estimation of wine stability during long storage. In the present study analytical procedure developed for Fe fractionation consists of two main step: (i) separation of hydrophobic fraction of Fe on the Amberlite XAD-8 resin and (ii) selective determination of labile Fe(III) in the presence of fluoride ions after sorption of Fe(II) on the synthesized Fe(II)-IIP polymer gel (section 2.6; see Scheme 1 in Supplementary material). The developed procedure was applied to red and white wine after addition of different ratios Fe(II)/Fe(III). Iron additions were performed after removal of hydrophobic fraction as far as it is known that polyphenol/protein complex has very high adsorption ability toward Fe species. The accuracy of the method and the matrix effects of real samples on the selective separation of Fe(III) were assessed by the recovery experiments from the prepared spiked samples. Results obtained are presented in Table 4. The recovery values obtained for Fe(III)/Fe(II) were in the range 95-105% with standard deviations less than 3% independently of the Fe(II)/Fe(III) ratios. Moreover, the results demonstrated that the wine matrices had no considerable effect on the separation and determination of Fe(III). The repeatability of the synthesis of Fe(II)-IIP sorbent was investigated by using five different batches, prepared
independently under identical experimental conditions. The relative standard deviation for the degree of sorption of Fe(II)/Fe(III) in the presence and absence of fluoride ions for all prepared sorbents was less than 6% confirming good reproducibility of synthesis procedure. The reusability of the Fe(II)-IIP particles was studied for 50 sorption/desorption cycles in model aqueous and wine samples – relative standard deviation for aqueous samples varied in the range 3-5% for both Fe species for 50 cycles, while for wine samples after 20 sorption/desorption cycles RSD was degraded to more than 20% especially for the selective sorption of Fe(II). Evidently some organic wine components were strongly bounded on the surface of the sorbent and blocked sorbent functional groups.

The procedure was applied for the determination of Fe(III) in 30 monavarietal wine samples from Bulgaria. Total Fe content for all samples was below OIV permissible limit of 5 mg L\(^{-1}\) and the Fe(III) content varied between 0.2 mg L\(^{-1}\) to 0.7 mg L\(^{-1}\).

3.6 Analytical figures of merit

Analytical characteristics of developed procedure for Fe fractionation were defined by 5 parallel analyses of spiked red and white wine samples. Usually Fe content in wine is enough high and measured by FAAS, however developed procedure could be easily combined with Electrothermal AAS in order to reach lower detection limits (although this is less important for winemakers). The results obtained by using FAAS can be summarized as follow: LOD and LOQ calculated according to the 3σ and 10 σ criteria are respectively 0.03 mg L\(^{-1}\) and 0.1 mg L\(^{-1}\) for total Fe and Fe(III). The relative standard deviation varied in the range 3-7% for Fe content 0.1-10 mg L\(^{-1}\) for red and white wine sample.

4. Conclusions

New Fe(II)-IIP was synthesized by cross-linking dispersion copolymerization using 2,2’-Bipyridyl as selective ligand for Fe(II). The experiments performed showed the high selectivity of the sorbent toward Cd(II), Cu(II), Mn(II), Pb(II) and Zn(II), but low selectivity toward Fe(III). It might be concluded that ion imprinted polymers could not be used for selective separation of metal ions with only different oxidation states if template complexes have similar stoichiometry. Although separation of both Fe species was achieved in the presence of fluoride ions as a masking agent for Fe(III). Simple analytical procedure based on dispersive SPE was developed for Fe(II) and Fe(III) determination in wines. The procedure was easy and fast, suitable for routine application and meets the requirements of wine producers.
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Appendix Supplementary material

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Fig. 1. Scheme of the Fe(II)-IIP preparation.

Fig. 2. pH-dependence of the degree of sorption of Fe(II) ions with Fe(II)-IIP and non-IIP (three parallel experiments).
Fig. 3. Scatchard plots for the adsorption of Fe(II) ions onto Fe(II)-IIP and non-IIP.
Fig. 4. Degrees of sorption ($D_s$) of template and competitive ions with Fe(II)-IIP and non-IIP (three parallel experiments).

Table 1

Surface properties of Fe(II)-IIPs and non-IIP.

| Sample   | $S_{BET}$ | $V_{total}$ | $D_{average}$ |
|----------|-----------|-------------|---------------|
|          | m$^2$ g$^{-1}$ | cm$^3$ g$^{-1}$ | nm          |
| Non-IIP  | 55        | 0.39        | 28           |
| Fe(II)-IIP | 2        | 0.01        | 9            |
### Table 2
Experimental and fitting parameters for the Langmuir and Freundlich isotherm model for the adsorption of Fe(II) ions onto the Fe(II)-IIP and non-IIP at temperature 25°C.

| Constants          | Fe(II)-IIP | Non-IIP |
|--------------------|------------|---------|
| $Q_{\text{max, exp}}$ (µmol g$^{-1}$) | 28.30      | 17.80   |
| **Langmuir isotherm model** ($C_e/Q_e = C_e/Q_{\text{max}} + 1 / b.Q_{\text{max}}$) |
| $Q_{\text{max, teor}}$ (µmol g$^{-1}$) | 28.01      | 19.15   |
| $b$ (L µmol$^{-1}$) | 0.520      | 0.186   |
| $R^2$              | 0.998      | 0.995   |
| **Freundlich isotherm model** ($\ln Q_e = \ln k_F + n^{-1} \ln C_e$) |
| $k_F$ (µmol g$^{-1}$) | 335.81     | 62.70   |
| $n_F$              | 5.010      | 3.99    |
| $R^2$              | 0.916      | 0.825   |

In the Langmuir and Freundlich equations: $C_e$ (µmol L$^{-1}$) is the equilibrium concentration of Fe(II) in the solution, $Q_e$ (µmol g$^{-1}$) is the adsorption capacity of the adsorbed ferrous ions onto the sorbents at equilibrium, $Q_{\text{max}}$ (µmol g$^{-1}$) is the theoretical maximum adsorption capacity, $b$ (L µmol$^{-1}$) is the Langmuir constant that relates to the affinity of binding sites; $k_F$ and $n$ are Freundlich constants incorporating all factors that affect the adsorption process such as capacity and intensity.
Table 3
Distribution ($D$), selectivity coefficients ($S_{Fe(II)/Me}$) and relative selectivity coefficients $\rho(S_{Fe(II)/Me})$ of Fe(II)-IIP and non-IIP for Fe(II).

| Ion     | Fe(II)-IIP | Non-IIP | $\rho(S_{Fe(II)/Me})$ |
|---------|------------|---------|----------------------|
|         | $D$        | $S_{Fe(II)/Me}$ | $D$ | $S_{Fe(II)/Me}$ |                 |
| Fe(II)  | 49.0       | 2.1     |                     |
| Fe(III) | 24         | 5.4     | 1.5                 | 1.4             | 1.4             |
| Cd(II)  | 0.4        | 126.0   | 0.8                 | 2.7             | 46.6            |
| Cu(II)  | 0.9        | 57.5    | 2.2                 | 1.0             | 60.3            |
| Mn(II)  | 0.3        | 147.0   | 0.3                 | 7.5             | 19.5            |
| Pb(II)  | 0.4        | 132.5   | 1.1                 | 2.0             | 67.5            |
| Zn(II)  | 0.8        | 62.4    | 1.7                 | 1.2             | 50.0            |
| Fe(II)+F$^-$ | 24.0   | 2.0     | 1.9                 | 1.1             | 1.8             |
| Fe(III)+F$^-$ | 0.03  | 1584.3  | 0.08                | 28.2            | 56.1            |

Table 4
Influence of the masking agents on the degrees of sorption ($D_S$) of Fe(II) and Fe(III) ions at SPE with Fe(II)-IIP (50 mg sorbent; 10 mL samples with 0.1 mol L$^{-1}$ masking agent; pH 7; 5 mL 4 mol L$^{-1}$ HNO$_3$ as eluent; three parallel experiments).

| Ion     | $D_S$, % | Tartaric acid | EDTA | NH$_4$F | CH$_3$COONH$_4$ |
|---------|----------|---------------|------|---------|----------------|
| Fe(II)-IIP |          |               |      |         |                |
| Fe(II)   | 96±2     | <3            | 98±2 | 76±3    |                |
| Fe(III)  | 89±2     | <3            | <3   | 55±3    |                |
| Non-IIP  |          |               |      |         |                |
| Fe(II)   | 96±2     | <3            | 95±2 | 68±3    |                |
| Fe(III)  | 70±3     | <3            | 7±3  | 58±3    |                |
Table 5
Recovery studies for the determination of Fe(II) and Fe(III) in wine samples by dispersive SPE and FAAS measurements, using Fe(II)-IIP sorbent for selective sorption of Fe(II) (10 mL sample solution, 50 mg sorbent, pH 7, 30 min sorption time; three parallel determinations).

| Sample   | Added Fe, mg L\(^{-1}\) | Found Fe, mg L\(^{-1}\) | Recovery, % | RSD, % |
|----------|-------------------------|-------------------------|-------------|--------|
|          | [mean±sd]               |                         |             |        |
| Merlot   | 0                       | 1.6±0.04                |             |        |
| Merlot   | 2.0 Fe(III)             | 1.9±0.05 Fe(III)        | 95±3        | 2.6    |
| Merlot   | 4.0 Fe(II)              | 4.1±0.09 Fe(II)         | 102±2       | 2.1    |
| Chardonnay | 0                    | 0.9±0.02                |             |        |
| Chardonnay | 1.0 Fe(III)           | 1.01±0.02 Fe(III)       | 101±2       | 1.9    |
| Chardonnay | 2.0 Fe(II)             | 2.1±0.05 Fe(II)         | 105±3       | 2.3    |
Supplementary materials

Iron(II) ion imprinted polymer for Fe(II)/Fe(III) speciation in wine.

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Figures

Fig. 1S Scanning electron micrograph of non-IIP (A) and Fe(II)-IIP (B) particles at a ×10000 magnification.
Fig. 2S N₂ isotherms of samples (A) Fe(II)-IIP and non-IIP and (B).
Tables

Table 1S

Degree of elution $D_E$ (%) for Fe(II) from Fe(II)-IIP using different eluents (50 mg Fe(II)-IIP).

| Eluent Concentration of HNO$_3$, mol L$^{-1}$ | $D_E$, % |
|--------------------------------------------|----------|
| 0.5                                        | 55.5±4   |
| 1.0                                        | 67.8±4   |
| 2.0                                        | 88.9±2   |
| 4.0                                        | > 99     |

| Eluent volume of 4 mol L$^{-1}$ HNO$_3$, mL |
|---------------------------------------------|
| 2                                           | > 99     |
| 5                                           | > 99     |
| 10                                          | > 99     |

| Concentration of EDTA (5 mL), mol L$^{-1}$ | $D_E$, % |
|---------------------------------------------|----------|
| 0.1                                         | 75.4±3   |
| 0.5                                         | 87.2±2   |

Adsorption isotherms

Adsorption isotherms are defined as a graphical representation showing the distribution of absorbable analyte between the liquid and solid phases at various equilibrium concentrations. They are useful instruments to predict the mechanism of sorption and the sorption efficiency and capacity toward target ions. In order to study the adsorption capacity of Fe(II)-IIP and non-IIP, adsorption isotherm experiments were carried out by static adsorption experiments. The maximum experimental sorption capacity ($Q_{max,exp}$) of the imprinted and non-imprinted polymer gels were determined after saturation of the sorbents with Fe(II) ions under optimum conditions at room temperature.

The constructed adsorption isotherms (Fig. 3S) showed that the amount of Fe(II) adsorbed per unit mass of polymer gels increased with the initial concentration of Fe(II) and reached plateau values, determining the experimental adsorption capacity values.
In order to understand the interaction between adsorbate and adsorbent the equilibrium data were further analyzed using the Langmuir and Freundlich isotherm models, respectively. The Langmuir isotherm is based on the theoretical principle that only a single adsorption layer (monolayer) exist on the adsorbent surface [1]. It assumes additionally that all the active sites on the adsorbent surface are homogenous and there is no interaction between them. The Freundlich isotherm describes the extent of heterogeneity of the adsorbent surface including a multilayer adsorption [2]. Experimental data presented in Fig. 3S were used for fitting the curves for Langmuir (Fig. 4S) and Freundlich (Fig. 5S) isotherm models.

Fig. 3S  Experimental sorption isotherms for Fe(II) sorption onto the Fe(II)-IIP and non-IIP at temperature 25 °C (three parallel experiments).
Fig. 4S Langmuir isotherms for adsorption of Fe(II) on the Fe(II)-IIP and non-IIP.
Fig. 5S Freundlich isotherms for adsorption of Fe(II) on the Fe(II)-IIP and non-IIP.

**Scatchard analysis**

The Scatchard equation is:

\[
\frac{Q_e}{C_e} = \left(\frac{Q_{\text{max}} - Q_e}{K_D}\right)
\]

where \(Q_e\) is the amount of Fe(II) bound to sorbent at equilibrium, \(C_e\) is the free Fe(II) concentration at equilibrium, \(K_D\) is the equilibrium dissociation constant of binding sites and \(Q_{\text{max}}\) is the apparent maximum binding amount of Fe(II) [3]. The values of \(K_D\) and \(Q_{\text{max}}\) could be calculated from the slope and intercept of the linear curve plotted as \(Q_e/C_e\) versus \(Q_e\). The shape of Scatchard plot is related to the type of the interaction of absorbate with the adsorbent: straight line obtained by plotting \(Q_e/C_e\) versus \(Q_e\) suggest only one type of binding sites on the surface of the sorbent; if Scatchard plot shows a deviation from linearity, the adsorbent presents more than one type of binding sites.
**Scheme 1.** Analytical procedure for Fe fractionation in wine

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