Selective ionic liquid ferrofluid based dispersive-solid phase extraction for simultaneous preconcentration/separation of lead and cadmium in milk and biological samples

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1. Introduction

Ferrofluids are stable magnetic nanoparticle suspensions in a carrier liquid. Basically, in ferrofluids, the single domain magnetic nanoparticles should be coated by a suitable material, to prevent particles from agglomerating [1]. The stability of the ferrofluid is due to the balance between repulsive (Brownian motions, steric and electrostatic forces) and attractive interactions (Van der Waals and dipolar attractive forces) [2]. Although the amount of magnetic nanoparticles in ferrofluids is about 3–10%, and many properties of ferrofluids, such as vapor pressure and density, are the same as the base fluid, these materials possess magnetic properties and fluidity simultaneously [3]. Therefore, the coating material and the carrier liquid have a crucial role in the field of ferrofluids. By choosing different carrier liquids and coating materials, wide applications of ferrofluids have been reported in different research areas such as biomedical [4], microelectronics [5], biological microelectromechanical systems [6] and also analytical chemistry [7–9]. As a result, research in this field has been focused on coating agent materials and carrier liquids.

For selecting an appropriate coating material, several criteria should be met. For instance, it should be non-toxic, low cost, chemically stable, and also compatible with carrier liquid. It has been proven that some chemical or physical surface modifications of coating material have improved its selectivity and/or affinity toward special species [10,11]. Due to its non-toxicity, low cost, long-term chemical stability, and easy surface modification, TiO2 has been proven to be an excellent choice of coating material [12]. Also, chelating agents are the most common modifiers which consist of donor atoms such as oxygen, nitrogen, and phosphorus. Due to the presence of these donor atoms, selective binding with certain species on the surface of coating material will occur [13]. In some publications, ferrofluids were used in field of analytical chemistry, but in all of them magnetic nanoparticles were coated by silica [7–9]. Also, the selectivity of the ferrofluid was not the purpose of these studies. In spite of the wonderful properties of TiO2 as an excellent coating agent, there is no report on using TiO2, and also modified TiO2, in the field of ferrofluids, although it can improve the selectivity of ferrofluids greatly. However, to provide a stable ferrofluid, in the next step, a unique structure and properties of carrier fluid are critical.

Ionic liquids (ILs) have been emerging in recent years as novel, stable, and environmentally friendly compounds with amazing properties such as negligible vapor pressure, low flammability, and...
liquid state in a broad temperature range [14]. Additionally, due to the presence of electrostatic, hydrogen bonding, and Van der Waals interactions, ILs possess unique structure, which provides a protective shell around magnetic nanoparticles and improves the ferrofluids’ stability greatly [15–17]. Therefore, by choosing ionic liquid as a carrier fluid, very stable ferrofluids can be prepared, which is suitable to use in sample preparation methods [18].

Determination of heavy metals in biological and in food samples has become a key interest because of their toxic effects on living beings [19,20]. Heavy metals such as Cd(II) and Pb(II) cause irreparable effects on the urinary tract, liver, blood chemistry and the cardiovascular system [21]. Several studies have been reported for preconcentration-separation of Cd(II) and Pb(II) such as solid phase extraction [22–25] liquid-liquid extraction [26] cloud point extraction [27,28] and dispersive liquid-liquid extraction [29,30]. In spite of some benefits, these methods suffer from many drawbacks. Therefore, improvements are necessary. In this study, we want to report a very simple, fast, efficient, and selective preconcentration-separation method which is assisted by ionic liquid ferrofluid for cadmium and lead.

The purpose of this work was to use TiO2 as an excellent coating material for magnetic nanoparticles and also improve its selectivity by loading 1-(2-pyridylazo)-2-naphthol on its surface. Therefore, to provide a stable ferrofluid, 1-Hexyl-3-methylimidazolium tetrafluoroborate was chosen as the carrier liquid. Therefore, selective ionic liquid ferrofluid based dispersive solid phase extraction (IL-FF-D-SPE) was used for preconcentration-separation of cadmium and lead in milk, human urine, and blood plasma samples combined with flame atomic absorption spectrometry (FAAS), which is a very fast, easy, cheap, and selective determination technique in comparison with other methods such as ICP-MS. Additionally, different parameters which affected the extraction recovery of Pb(II) and Cd(II) were investigated with the help of chemometrics method.

2. Experimental

2.1. Instruments

The determination of Pb(II) and Cd(II) was carried out using a Varian Spectra AA-400 atomic absorption spectrometer (Santa Clara, USA, www.varianinc.com), equipped with a deuterium background correction and an air-acetylene burner. The lamp currents were set at 5 and 4 mA for Pb(II) and Cd(II), respectively. All measurements were carried out in the peak height mode at 217.0 and 228.8 nm, using a spectral band width of 1.0 nm and 0.5 nm for Pb(II) and Cd(II), respectively. A pH meter Model 692 from Metrohm (Herisau, Switzerland, www.metrohm-ag.com) equipped with a glass combination electrode was used for the pH measurements. Additionally, for magnetic separations, a strong magnetic field (Nd 2Fe12B) magnet (1.31 T) was used. A Field emission scanning electron microscope (FESEM), model S-4160 (www.hitachi.com/procurement/network/japan) was used for preparation of SEM images. Fourier transform infrared spectra (FT-IR) were recorded from a KBr disk using an Equinox 55 Bruker (Billerica MA, USA, www.bruker.com) with the ATR method over the wavelength range of 400–4000 cm⁻¹. For separation of human blood plasma from a blood sample, a refrigerated centrifuge (Hettich, Universal 320R, www.hettichcentrifuge.co.uk, Buckinghamshire, England) equipped with an angle rotor (6 place, 9000 rpm, cat no 1620 A) was used.

2.2. Reagents

All chemicals used were of analytical reagent grade unless otherwise stated. All aqueous solutions were prepared using ultra-pure water. Water was purified using Milli-Q purification system. The stock standard solutions of Cd(II) and Pb(II) (1000 mgL⁻¹) were prepared from appropriate amounts of their nitrate salts (Merck, Darmstadt, Germany, www.merck.de) in ultra-pure water and working standard solutions were prepared by appropriate stepwise dilution of the stock standard solutions. 1-Hexyl-3-methylimidazolium tetrafluoroborate [Hmim][BF4] 98%, 1-Hexyl-3-methylimidazolium hexafluorophosphate [Hmim] PF6, 1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide [Hmim]TF2N, 1-(2-pyridylazo)-2-naphthol, tetra-n-butyl orthotitanate (TBOT), NH3, ethanol, and sodium dodecyl sulfate were purchased from Merck (Darmstadt, Germany). Nano-Fe3O4 was purchased from Sigma-Aldrich (St. Louis, MO, USA, www.sigmaaldrich.com, Fe3O4 spheres powder, <40 nm, purity > 98%). Buffer solution (pH=6.7, 1 molL⁻¹) was prepared by dissolving appropriate amounts of potassium dihydrogen phosphate (1 molL⁻¹) and sodium hydroxide (1 molL⁻¹). High purity HNO3, H2O2, HClO4 and HCl were purchased from Merck (Darmstadt, Germany), which were used for the digestion of milk, urine, and blood plasma samples throughout this project. The pipettes and vessels were cleaned before use by soaking in 10% nitric acid solution for at least 24 hours and then rinsed thoroughly with distilled water.

2.3. Synthesis of titana-coated magnetite nanoparticles (Fe3O4/TiO2)

First, 10 mL of tetra-n-butyl orthotitanate was dissolved in 70 mL ethanol to form a clear solution. Then, 0.2 g Fe3O4 nanoparticles were dispersed in this solution under sonication for approximately 5 min. While the suspension was stirred vigorously over a period of 15 min, a 1:5 (v/v) mixture of water and ethanol was added slowly with a dropper into this mixture. Then, the mixture was stirred further for 1 hour. Finally, after separating and washing the residue with ethanol, the obtained powder was oven-dried and calcinated at 200 °C for 6 h [31] (Fig. 1.).

2.4. Preparation of 1-(2-pyridylazo)-2-naphthol modified Fe3O4/TiO2

In the present work, 1-(2-pyridylazo)-2-naphthol has been immobilized on sodium dodecyl sulfate-coated Fe3O4/TiO2. The surface modification was done based on a previous report using some modifications [32]. In a 250 mL beaker, 1.0 g of Fe3O4/TiO2 nanoparticles were dispersed in 20 mL of 0.001 molL⁻¹ HCl, under sonication. During sonication of this mixture, to avoid any changes in ionic strength, 0.3 mL of 1 molL⁻¹ NaNO3 was added using a dropper. The pH of the solution was adjusted to 5. Then, 3 mL of 1 molL⁻¹ sodium dodecyl sulfate was added and solution was stirred for 1 h. After that 0.2 g 1-(2-pyridylazo)-2-naphthol was added and the solution was stirred for further 1 h at 60 °C in a water bath. Finally, the resulted suspension was filtered and dried under a vacuum.

2.5. Sample preparation

2.5.1. Human blood plasma sample

A fresh blood sample was obtained from a healthy volunteer a few minutes prior to this test. To obtain the blood plasma sample,
the blood sample was centrifuged for 10 min at 4500 rpm. Then, 3 mL of the plasma sample was transferred to a clean polyethylene tube and 3 mL of nitric acid and 2 mL hydrogen peroxide were added to the sample. The tube was tightly capped and centrifuged for 10 minutes at 4400 rpm. Then, the sample was placed in a hot water bath at 95 °C for 90 min. Finally, the digested sample was diluted with 30 mL of distilled water.

2.5.2. **Human urine sample**

A fresh urine sample was obtained from a healthy volunteer a few minutes prior to this test. First, 10 mL of the urine sample was transferred to a 50 mL beaker. Next, 10 mL of a mixture of 2:1 concentrated HNO3 and HClO4 was added to urine sample. The beaker was covered with a watch glass and heated on a hot plate (100 °C 15 min, 150 °C 10 min). Then, the watch glass was removed and the acid evaporated to dryness at 150 °C. During the next step, by adding 3 mL HClO4 to the resulting white residue, the mixture was heated at 160 °C until it was completely dried. Finally, 5 mL of 1 mol L⁻¹ HNO3 was added and the mixture was heated at 150 °C for 1 min. After cooling the mixture, the volume was made up to the mark in a 50 mL volumetric flask.

2.5.3. **Milk sample**

A milk sample was purchased from local supermarket in Tehran. One milliliter of milk was transferred to a PTFE digestion vessel. Afterwards, 5 mL of concentrated HNO3 and 2 mL H2O2 were added to the milk sample. The tube was tightly capped and centrifuged for 10 minutes at 4400 rpm. Then, the sample was placed in a hot water bath at 95 °C for 90 min. Finally, the digested sample was diluted with 30 mL of distilled water.

2.6. **Preparation of ferrofluid**

For the preparation of the ferrofluid, the adsorption of the stabilizing agent on sorbent is necessary; therefore, 10 mg of modified titana-coated magnetic nanoparticles with 1-(2-pyridyland 0.1 g [Hmim]BF4. After 30 min of sonication of this mixture, stable suspension of magnetic nanoparticles (ionic liquid-ferrofluid) was obtained. The resulting suspension had a sorbent fraction of 9.1% and ionic liquid fraction of 90.9%.

2.7. **Extraction procedure**

The sample or standard solution contains 40 μg L⁻¹ Pb(II) and 30 μg L⁻¹ Cd(II), NaNO3 (0% w/v), at pH 6.7, which were poured into a 50 mL tube. Then, by using a 1.0 mL syringe, the ionic liquid-ferrofluid, containing 0.03 g sorbent and 0.3 g ionic liquid, was injected into the sample solution to form a dark cloudy suspension (the volume of suspension was 0.28 mL). Due to the rapid injection of the ferrofluid into the aqueous sample, extraction was achieved within a few seconds. By using a strong magnet at the bottom of tube, the ferrofluid was settled and the solution became clear. After removing the supernatant by decanting, 1 mL nitric acid (1.59 mol L⁻¹) was added to the vial to desorb the Pb(II) and Cd(II) through 8 min sonication. Subsequently, a strong magnet was placed at the bottom of tube and immediately the solution became limpid. By using a syringe this clear solution was transferred to a vial for analyzing with FAAS.

3. **Result and discussion**

3.1. **Characterization of adsorbent**

Fig. 2 shows the SEM image of Fe3O4-coated nanoparticles with TiO2. As it can be concluded from SEM image, the Fe3O4 nanoparticles were coated successfully with TiO2 since the size of these nanoparticles has been changed from below 40 nm (before coating with TiO2) to the range of 60–70 nm after coating.

Fig. 3 gives the FT-IR spectra pattern of Fe3O4 nanoparticles (a), Fe3O4/TiO2 (b) and 1-(2-pyridylazol)-2-naphtol modified on Fe3O4/TiO2 surface (c) in the range of 4000–400 cm⁻¹. Compared with the peaks of Fe3O4 in Fig. 3(a), many new peaks appeared in Fig. 3(b and c). In Fig. 3(a–c), the presence of Fe-O band vibration at 534 cm⁻¹ is obviously observed. As seen in Fig. 3(b and c), the absorption peak at 550 cm⁻¹ assigned with stretching vibration of Ti-O band. Also, the absorption peak at 1041 cm⁻¹ is ascribed to Ti-O–H bond stretching. Peaks in region of 2864.4–2968.1 cm⁻¹ correspond to O–H stretch bond [33,12]. Due to presence of the band at 1640 cm⁻¹ which belong to pyridine ring of 1-(2-pyridylazo)-2-naphtol and 1436 cm⁻¹ which is attributed to N–N bond (Fig. 3(c)), the nanometer Fe3O4/TiO2 is successfully modified by 1-(2-pyridylazo)-2-naphtol. [34].

![Fig. 2. The SEM images of (a) Fe3O4 NPs and (b) TiO2-coated Fe3O4 NPs.](image-url)
3.2. Selection of ionic liquid for preparation of the ferrofluid

For selecting the best carrier fluid, apart from providing a stable ferrofluid, several criteria such as immiscibility with an aqueous solution, non-toxicity, having low vapor pressure to avoid any loss during extraction, and compatibility with coating material should be met. For this purpose, some ionic liquids such as 1-Hexyl-3-methylimidazolium tetrafluoroborate [Hmim]BF₄, 1-Hexyl-3-methylimidazolium hexafluorophosphate [Hmim]PF₆, and 1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [Hmim][Tf₂]N were selected and, by the qualitative observation of sedimentation of each ferrofluid, the stability of the suspensions was checked. Finally, 1-Hexyl-3-methylimidazolium tetrafluoroborate [Hmim]BF₄ was chosen.

3.3. Selection of eluent

Based on the desorption of Pb(II) and Cd(II) from modified nanoparticles in the acidic medium, this medium would be preferred in desorption step. To avoid any losses of sorbent, chloric acid was not chosen because this acid can dissolve Fe₃O₄ nanoparticles. In addition, based on the possibility of formation of precipitate of lead in presence of sulfate ions, sulfuric acid was not chosen too. Therefore, the possibility of desorption of lead and cadmium ions in acidic medium was examined by using nitric acid [35]. Also, some organic solvent such as ethanol, ethanol (1% HNO₃) and acetone were selected to examine the possibility of desorption of lead and cadmium ions. Therefore, nitric acid was chosen as the best eluent. The results are shown in Fig. 4.

3.4. Screening and optimization design

In this study, Plackett–Burman screening design was used to select the variables that mainly effect the extraction recovery of Pb(II) and Cd(II). Based on the preliminary experiments, the effects of seven factors, at two levels were investigated. Since pH would have significant effect on extraction recovery, to find the best pH value, both acidic and basic medium should be studied [36]. However in selecting of the two levels of pH, several criteria should be met. For instance, there was the possibility of protonation of 1-(2-pyridylazo)-2-naphthol at lower pH and also precipitation of metal ions at higher pH values. Therefore, pH 4 and 8 were selected. In addition, sorbent amount (0.01 and 0.03 g), ionic liquid amount (0.3 and 0.5 g), ionic strength (0 and 10% [w/v] NaNO₃), eluent volume (1 and 2 mL), eluent concentration (0.5 and 2 mol L⁻¹), and desorption time (5 and 10 min) were the other variables of interest in this experiment. The total design matrix showed 15 runs (12 + 3 center points) to be carried out randomly in order to eliminate the effects of extraneous or nuisance variables. The evaluation of the main effects was done based on ANOVA results and Pareto charts.

The normalized results of the experimental design were evaluated (F-95%), and the standard effect is estimated for computing a t-statistic for each effect. A parameter is considered as significant when its value is higher than t, all other variables are not significant factors in the studied range. As it can be concluded from Fig. 5(a and b), pH has significant positive effect on extraction recovery of Pb(II) and Cd(II). Also the results illustrated in Fig. 5(a and b) confirm that eluent concentration and desorption time have significant positive effects on extraction recovery of Pb(II) and Cd(II), respectively. And other parameters show no significant effect on the extraction recovery of both elements.

Therefore, the Box–Behnken design (BBD) was performed to evaluate the optimum condition of performance of IL-FF-D-SPE of Pb(II) and Cd(II) for the three significant parameters: pH, eluent concentration, and desorption time. The 3D response surface plots for the extraction recovery (%) of both elements were shown in Fig. 6. As seen in Fig. 6, an optimization showed that the best pH is 6.7, eluent concentration of 1.59 mol L⁻¹, and desorption time of 8 min.

The Box–Behnken design is suitable for exploration of the quadratic response surface and construction of a second order polynomial model which can be expressed as the following equations:

\[
Y_{(Cd)} = \alpha_0 + \alpha_1A + \alpha_2F + \alpha_3G - \alpha_4A^2 - \alpha_5AF \\
\;
+ \alpha_6AG - \alpha_7F^2 - \alpha_8FG - \alpha_9G^2
\]

\[
Y_{(Pb)} = \beta_0 + \beta_1A + \beta_2F + \beta_3G - \beta_4A^2 + \beta_5AF \\
\;
- \beta_6AG - \beta_7F^2 - \beta_8FG - \beta_9G^2
\]

where \(\alpha_0\) and \(\beta_0\) are intercepts, and \(\alpha_1-\alpha_9\) and also \(\beta_1-\beta_9\) are the regression coefficients, also \(Y_{(Cd)}\) and \(Y_{(Cd)}\) are response functions (extraction recovery) of lead and cadmium, respectively. In addition, A, F and G are pH, eluent concentration and desorption time, respectively. The quality of the fit of polynomial model equation was expressed by the coefficient of determination \(R^2\). However, to show more tangible how much obtained results are fitted to polynomial equations, the percentages of \(R^2\) (\(R^2\%\)) are reported here. These equations have \(R^2\%\) of 98.51% and 98.76% for lead and cadmium,
respectively. These results indicate that only 1.49% and 1.24% of the total variations were not explained by the model for lead and cadmium, respectively. At the optimum condition the highest extraction recovery of 99.3 and 99.8% were obtained for lead and cadmium, respectively.

3.5. Breakthrough volume

Due to the low concentration of trace metals in real samples, to achieve the higher preconcentration factor, the preconcentration step is inevitable. Therefore, after preconcentration step, samples with large volume should be taken into smaller volume. For this purpose, maximum applicable sample volume was determined by increasing the dilution of metal ions solution in range of 25–350 mL, while using the same amount of suspension (0.28 mL) for each sample and keeping the total amount of Pb(II) and Cd(II) fixed at 2.0 and 1.5 μg, respectively. The quantitative recoveries for Pb(II) and Cd(II) were found to be stable until 250 and 200 mL for Pb(II) and Cd(II), respectively, and the adsorbed Pb(II) and Cd(II) could be eluted with 1 mL of eluent. Therefore, the concentration factor (CF) of 250 and 200 can be achieved for lead and cadmium ions, respectively.

3.6. Effect of potentially interfering ions

In order to assess the applications of the recommended procedure, the effect of potentially interfering ion on the extraction recoveries of Pb(II) and Cd(II) were also examined. For this purpose, by spiking appropriate amounts of potentially interfering ions in the range of 50–15000 μg mL⁻¹ to 50.0 mL of solution containing 40 μg L⁻¹ of Pb(II) and 30 μg L⁻¹ of Cd(II) evaluation was done. The obtained results are summarized in Table 1.

3.7. Adsorption isotherms

The interactive behavior of solution and the adsorbent can be predicted by equilibrium adsorption isotherm model which is
important for evaluating the sorption capacity of sorbent and designing an adsorption system.

In the present study for achieving this purpose, the mg of Pb(II) and Cd(II) absorbed per gram of sorbent, $q_e$, versus equilibrium concentration ($C_e$) were investigated at a different initial concentration of Pb(II) ($0.25–20$ mg L$^{-1}$) and Cd(II) ($0.25–2.5$ mg L$^{-1}$). Then, by plotting $C_e/q_e$ versus $C_e$ the following equations (Eqs. (1) and (2)) were obtained for Pb(II) and Cd(II), respectively, which showed that the adsorption models were in agreement with Langmuir isotherm.

$$C_e/q_e = 0.0894C_e + 0.3402 \quad (R^2 = 0.9966) \quad (1)$$

$$C_e/q_e = 0.1071C_e + 0.0267 \quad (R^2 = 0.9997) \quad (2)$$

where $C_e$ is equilibrium concentration of Pb(II) and Cd(II) in the solution (mg L$^{-1}$), $q_e$ is amount of Pb(II) and Cd(II) adsorbed per unit mass of adsorbent (mg g$^{-1}$). By comparing the obtained results with Langmuir isotherm model, the maximum sorption capacity can easily be calculated. Therefore, the maximum sorption capacity of lead and cadmium on 1-(2-pyridylazo)-2-naphtol modified Fe$_3$O$_4$/TiO$_2$ was $11.18$ and $9.34$ mg g$^{-1}$, respectively.

### 3.8. Figures of merit

Under the optimal experimental conditions, calibration curves were obtained for each metal ion by simultaneous preconcentrating of $50$ mL of the sample solution. Table 2 features the analytical characteristics of the optimized method for each element, including linear range, correlation coefficient, limit of detection (LOD) and reproducibility expressed as a relative standard deviation (RSD). A concentration factor (CF) of about $250$ (for lead) and $200$ (for cadmium) were obtained as a ratio between the volume

<table>
<thead>
<tr>
<th>Ions</th>
<th>Concentration (μg mL$^{-1}$)</th>
<th>Extraction recovery (%)</th>
<th>Pb(II)</th>
<th>Cd(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>15000</td>
<td>$101.2 \pm 2.4$</td>
<td>$98.8 \pm 2.5$</td>
<td></td>
</tr>
<tr>
<td>K$^+$</td>
<td>10000</td>
<td>$97.8 \pm 2.2$</td>
<td>$99.1 \pm 2.5$</td>
<td></td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>1000</td>
<td>$96.9 \pm 2.5$</td>
<td>$100.5 \pm 2.6$</td>
<td></td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>50</td>
<td>$101.6 \pm 2.4$</td>
<td>$101.3 \pm 2.7$</td>
<td></td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>100</td>
<td>$98.4 \pm 2.6$</td>
<td>$97.6 \pm 2.4$</td>
<td></td>
</tr>
<tr>
<td>Ag$^+$</td>
<td>50</td>
<td>$98.9 \pm 2.5$</td>
<td>$100.7 \pm 2.6$</td>
<td></td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>50</td>
<td>$97.4 \pm 2.3$</td>
<td>$96.9 \pm 2.5$</td>
<td></td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>500</td>
<td>$99.2 \pm 2.2$</td>
<td>$96.5 \pm 2.4$</td>
<td></td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>10000</td>
<td>$96.5 \pm 2.3$</td>
<td>$97.2 \pm 2.6$</td>
<td></td>
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<tr>
<td>NO$_3^-$</td>
<td>15000</td>
<td>$100.7 \pm 2.4$</td>
<td>$98.4 \pm 2.6$</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6. Estimated response surface by plotting extraction recovery (%) versus pH and eluent concentration (a), pH and desorption time (b).

Table 1

Effect of coexisting ions on recovery of $40$ μgL$^{-1}$ of Pb(II) and $30$ μgL$^{-1}$ of Cd(II) ($n=3$).
of the sample solution and the final volume obtained after the elution step (1 mL).

### 3.9. Accuracy of the method

The validation of the presented procedure was performed by analyzing a certified reference material of water sample (NIST-1640A). It was found that the obtained results (12.23 ± 0.3 μg L⁻¹ Pb²⁺ and 3.7 ± 0.1 μg kg⁻¹ Cd²⁺) were in good agreement with the certified values (12.005 ± 0.040 μg kg⁻¹ Pb²⁺ and 3.961 ± 0.072 μg kg⁻¹ Cd²⁺), and no significant differences have been observed (t-test, *P*=0.05).

### 3.10. Analysis of real samples

The recommended methodology was applied for simultaneous extraction and FAAS determination of investigated toxic metal ions in milk, human urine, and blood plasma samples. Fresh urine and blood plasma samples were obtained from healthy volunteers a few minutes prior to testing. The results obtained are summarized in Table 3.

### 3.11. Comparison of analytical performance data with literatures

Comparison of this method with other SPE extraction methods used for preconcentration and FAAS determination of lead and cadmium ions from various real samples signifies that the presented method has some improvements compared with the earlier reported methods with significant merits (Table 4). In addition to selectivity of ionic liquid ferrofluid, the presented IL-FF-D-SPE method has lower limit of detection (1.21 μg L⁻¹ for lead and 0.21 μg L⁻¹ for cadmium), higher preconcentration factor (250 and 200 for lead and cadmium respectively), good repeatability (RSD%) and sorption capacity. All these results indicate that using 1-(2-pyridylazo)-2-naphtol modified Fe₃O₄/TiO₂ in IL-FF-D-SPE combined with FAAS is a very simple and sensitive and selective method for the preconcentration and determination of lead and cadmium ions.

### 4. Conclusion

In the present study, the determination of trace amounts of Pb(II) and Cd(II) in milk, human urine and blood plasma were investigated by a selective IL-FF-D-SPE. To improve chemical stability of magnetic nanoparticles, TiO₂ was used as coating...
ions were done simply by surface modification with 1-(2-pyridylazo)-2-naphtol. Also, to provide stable ferrofluid, 1-Hexyl-3-methylimidazolium tetrafluoroborate was used as carrier solvent. Therefore, for the first time a selective ionic liquid ferrofluid was used in dispersive solid phase extraction. Due to dispersion of sorbent in the aqueous phase, this method has a much faster in comparison with solid phase extraction which is a time-consuming column passing. Moreover, the magnetic separation greatly improved the separation rate. Therefore, no centrifugation is need for phase separation and sorbent is achieved from the aqueous bulk without using conical bottom glass tubes, which can be easily damaged and difficult to clean. Also in comparison with other literatures (Table 4), this sorbent and method have shown great advantages. In order to simplicity and low cost of this method, selective IL-FF-D-SPE is suitable for batch operations, and is capable for other samples with complicated matrices after a simple digestion step.

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