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Determination of calcium in complex samples using functional magnetic beads combined with electrodeless/sheathless electrospray ionization mass spectrometry

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Conventionally, electrospray ionization ion trap mass spectrometry (ESI-ITMS) is not used for the analysis of metal ions because metal ions may be of too low mass to be trapped by the mass analyzer. Furthermore, metal ions can easily precipitate during ESI processes because of their poor volatilities. We present an approach for solving these two problems using milk as the sample to demonstrate the feasibility of using ESI-ITMS as the detection method for metal ions. Iron oxide magnetic beads with nitrilotriacetic acid (NTA) immobilized on their surface were used as affinity probes for calcium ions from dairy drinks. Ethylenediaminetetraacetic acid (EDTA) was used for the elution of the metal ions chelated by NTA on the beads. The EDTA eluent was analyzed by electrodeless/sheathless ESI-ITMS. The use of functional magnetic beads to trap metal ions from complex samples can effectively reduce the interference from sample matrix. Using the EDTA eluent as the sample avoids the problem of the low volatility of metal ions during ESI-ITMS analysis. This method gives measurements of calcium ions in dairy drinks quantitatively very close to the true values (<4% error). Copyright © 2006 John Wiley & Sons, Ltd.

Metal ions are usually determined by atomic absorption spectrometry.[1–4] Generally, sample pretreatment is required for complex samples to prevent distortion of the analyses by matrix interference and ion suppression effects. One of the effective methods developed for sample pretreatment is solid-phase microextraction (SPME) introduced by Pawliszyen and coworkers.[5–7] For example, fused-silica fibers coated with ion exchanger have been used as the extraction probes for trapping target metal ions.[7] Because of their high surface-to-volume ratio, nanoparticles are suitable adsorbents for solid-phase extraction.[8–12] Silica nanoparticles,[8] magnetic particles coated with gold nanoparticles,[9] diamond nanoparticles,[10] carbon nanotubes,[11] and titania-coated magnetic nanoparticles[12] have been used to extract and concentrate peptides and proteins from sample solutions. Magnetic materials have an advantage as affinity probes because they can be easily isolated from the sample solution after conjugating with their target species by the application of an external magnetic field.

Immobilized metal ion affinity chromatography (IMAC) beads modified with ligand-metal complex have been used to trap phosphorylated species from complex samples.[13–19] These IMAC substrates immobilized with ligands can be used as the adsorbents to trap metal ions from sample solutions. Thus, we proposed the use of iron oxide magnetic beads with immobilized nitrilotriacetic acid (NTA) as trapping adsorbents for metal ions. The magnetic beads were initially rinsed with ethylenediaminetetraacetic acid (EDTA) solution to remove metal ions from the surfaces since EDTA has superior chelating affinity for transition metal ions to NTA. The rinsed magnetic beads were employed to trap calcium ions from dairy drinks. After separation of the beads from the mixture, EDTA was used to elute the calcium from the surfaces of the magnetic bead-Ca(II) conjugates to generate EDTA-Ca(II) complex ions. The NTA-immobilized functional magnetic beads trap not only calcium ions, but also transition metal ions from the sample solution. Nevertheless, a calibration curve can be generated for the quantitative analysis of calcium ions using a given concentration of EDTA solution as the internal standard and eluent solution. Electrospray ionization ion trap mass spectrometry (ESI-ITMS) was used for the determination of EDTA-metal complex ions. Generally, ESI-ITMS is not suitable for the analysis of analyte ions with a mass number less than 50. Many metal ions have masses less than 50 but this value is, of course, increased to greater than 50 by formation of the EDTA adducts. An additional problem is the precipitation of metal ions, commonly observed in the direct analysis of metal ion samples by ESI-MS. EDTA-metal ion complexes have greater volatilities than the metal ions alone, thus alleviating the problem of the poor volatility of metal ions.

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We have demonstrated previously that a pulled bare fused-silica capillary can be used directly as the electrospray emitter[20] without coating with conductive material or connecting to an electrode on the outlet of the tip. Because a capillary is used as the emitter, a small volume of sample solution is sufficient to carry out ESI-MS analysis. We herein demonstrate the feasibility of electrodeless/sheathless ESI-MS using a pulled bare capillary as the emitter for the determination of calcium using the EDTA eluent as the sample.

EXPERIMENTAL

Reagents and materials

Methanol and hydrofluoric acid were purchased from Tedia (Fairfield, OH, USA). Acetonitrile, acetic acid, and ammonium hydrogen carbonate were obtained from Riedel-de Haën (Berlin, Germany). Sodium hydroxide, ammonium hydroxide, calcium chloride-2-hydrate, ammonium acetate, and trisodium citrate-2-hydrate were obtained from J.T. Baker (Phillipsburg, NJ, USA). EDTA, potassium phosphate, and magnesium chloride were purchased from Sigma (St. Louis, MO, USA). The fused-silica capillary (50-μm i.d. × 365-μm o.d.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). Non-fat milk [Ca(II): 100 mg/L] and yogurt [Ca(II): 1095 mg/L] were purchased from a local grocery store. Fe_3O_4@NTA-Ni magnetic beads (USPIO, 8–10 nm) were purchased from Taiwan Advanced Nanotech Inc. (Taoyuan, Taiwan).

Pretreatment for dairy drink samples

Dairy drinks (milk and yogurt, 100 μL) were mixed to homogeneity with deionized water (200 μL) and hydrochloric acid (20 μL, 1 N). The mixture was allowed to stand at ambient temperature for 10 min followed by the addition of glacial acetic acid (30 μL). After addition of a NaOH solution (120 μL, 1.0 N), a white precipitate appeared immediately. Ammonia solution (30 μL, 30%) was added to further neutralize the acidity. The mixture was then centrifuged at 2000 rpm for 10 min to separate precipitates from the solution and the supernatant was diluted 40-fold with methanol/0.05% ammonia solution (1:1, v/v). After these treatments, the solution had been diluted 200-fold from the original dairy drink. This solution was used as the sample for the follow-up trapping experiments using functional magnetic beads as affinity probes.

Using functional magnetic beads as affinity probes to trap metal ions

An EDTA (200 mg/L) solution (soln. A) was prepared in methanol/0.05% ammonia solution (1:1, v/v) for the elution of metal ions from the surfaces of the functional magnetic beads. Fe_3O_4@NTA-Ni magnetic beads (0.01 mL, 32 mg/mL) were rinsed with soln. A (1 mL) for 30 min to remove metal ions from the surfaces of magnetic beads. The beads were aggregated by a magnet adjacent to the sample vial and the solution was removed by pipette. The beads were then rinsed with the methanol/0.05% ammonia solution (1:1, v/v, 0.5 mL) and resuspended in the same solution (0.1 mL). The magnetic bead suspension (20 μL) was mixed with sample solution (100 μL) with vortex-mixing for 30 min. The solution was removed by pipette and the beads were rinsed with 100 μL methanol/0.05% ammonia solution (1:1, v/v) followed by the elution of metal ions with soln. A (0.05 mL) under vortex-mixing for 30 min. The EDTA-metal ion complex suspension was collected by applying an external magnetic field to aggregate the magnetic beads. The suspension was introduced into the ESI-ITMS system for mass spectrometric analysis using a pulled bare fused-silica capillary as the ESI emitter. Scheme 1 displays the procedures for this affinity experiment.

Fabrication of the pulled bare fused-silica capillary

A capillary (35 cm) was conditioned prior to fabrication, using a pump at 18 mmHg pressure, to rinse it successively with 1 N NaOH (for 30 min) and water (for 30 min). The inner surface of a capillary was modified by 3-aminopropyltriethoxysilane (APTES) to reverse the direction of electroosmotic flow. An APTES (10 mM) solution was flushed into the capillary for 10 min followed by successive flushes with deionized water (for 10 min) and air (for 10 min) using a pump (pressure: 18 mmHg). The modified capillary was placed in an oven at 110°C for 90 min to strengthen the cross-linking of APTES on the capillary wall. The capillary was allowed to stand at ambient temperature for 12 h. The pulled bare fused-silica capillary was fabricated by applying a small weight (50 g) on the lower end of a vertical capillary. The lower part of the capillary was heated and then quickly drawn to form a narrow capillary tip. After cooling to ambient temperature, the capillary tip was immersed in HF solution (25%) for 10 min. The i.d. of the fabricated capillary (30 cm) was 12 ± 3 mm, while the o.d. was 30 ± 3 mm. The pulled capillary was conditioned prior to the analysis by using a pump (pressure: 18 mmHg) to rinse it successively with water (for 10 min), EDTA solution (for 10 min), and the sample solution (for 10 min) before carrying out ESI-MS analysis.

ESI-ITMS configuration

The configuration of the capillary electrophoresis (CE)/ESI-MS system incorporating the pulled bare capillary as the ESI emitter has been described elsewhere.[20] Briefly, the pulled capillary tip, which was used as the spray emitter, was placed directly into an orthogonal ESI mass spectrometer. The capillary tip was ca. 7 mm from its metal cap orifice horizontally. No electrical contact was applied on the outlet of the capillary tip. The capillary was filled with the sample solution prior to ESI-MS analysis. The capillary inlet was placed in a vial containing the sample solution. A voltage of −1000 V was applied to the platinum electrode immersed in the sample reservoir during ESI-ITMS analysis.

Instrumentation

All the mass spectra were obtained using an Esquire 2000 ESI ion trap mass spectrometer (Bruker Daltonics, Leipzig, Germany) operated in the negative ion mode.

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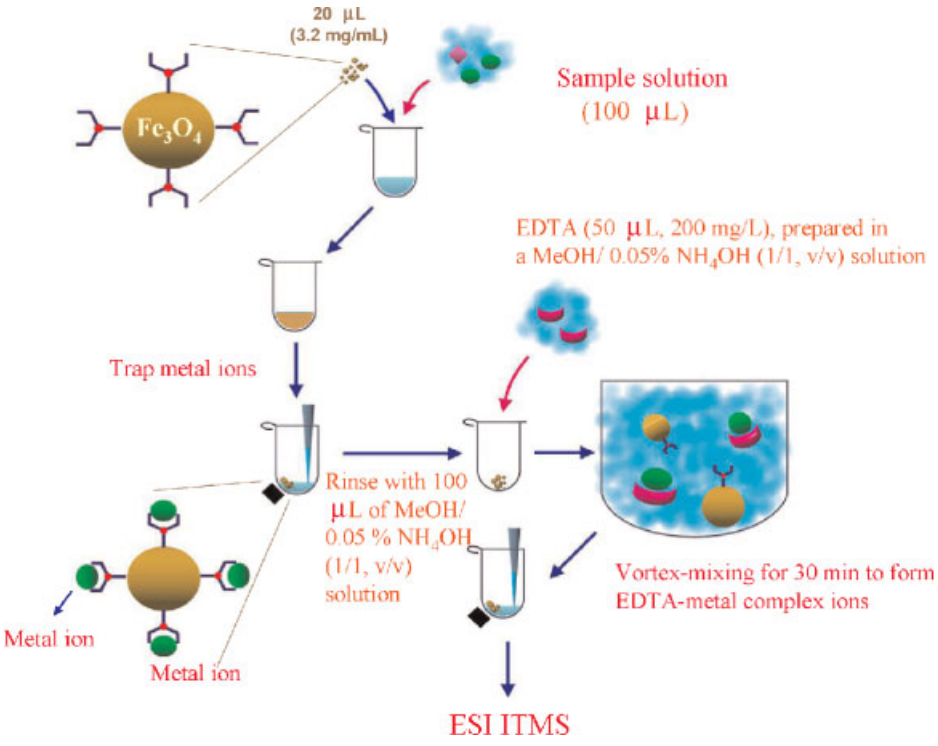

Scheme 1. Using the functional magnetic beads as affinity probes to trap metal ions from sample solutions.

RESULTS AND DISCUSSION

Figures 1(a)–1(f) present the ESI mass spectra of the eluted solutions obtained using functional magnetic beads to trap calcium ions from calcium standard solutions with various concentrations followed by the elution of EDTA. The $[EDTA+Ca^{2+}–3H^+]^-$ ion should appear at *m/z* 329 when calcium is present. However, even when no calcium is spiked in the sample (Fig. 1(a)), an ion with weak abundance appears at *m/z* 329. As the concentration of Ca(II) in the standard solution increases, the relative abundance of the anions at *m/z* 329 ($[EDTA+Ca^{2+}–3H^+]^-$) and 291 ($[EDTA–H^+]^-$) gradually increase in the spectra. Other ions at *m/z* 313, 344, and 351 correspond to $[EDTA+Na^+–2H^+]^-$, $[EDTA+Fe^{3+}–4H^+]^-$, and $[EDTA+Na^+ +Ca^{2+}–4H^+]^-$, respectively. The results demonstrate that the magnetic beads with immobilized NTA can trap metal ions from the sample solution. Furthermore, the trapped metal ions can be eluted by EDTA and determined by ESI-MS.

Figure 2 displays the plot of the ratio of the sum of the ion signal intensities of $[EDTA+Ca^{2+}–3H^+]^-$ at *m/z* 329 (I_{329}) and $[EDTA+Na^+ +Ca^{2+}–4H^+]^-$ at *m/z* 351 (I_{351}) to the sum of the signal intensities of the ions derived from EDTA, i.e. $[EDTA–H^+]^-$ at *m/z* 291 (I_{291}), $[EDTA+Na^+–2H^+]^-$ at *m/z* 313 (I_{313}), $[EDTA+Ca^{2+}–3H^+]^-$ at *m/z* 329 (I_{329}), $[EDTA+Fe^{3+}–4H^+]^-$ at *m/z* 344 (I_{344}), and $[EDTA+Na^+ +Ca^{2+}–4H^+]^-$ at *m/z* 351 (I_{351}), as a function of the concentration of Ca(II). The correlation coefficient of this plot of $(I_{329}+I_{351})/(I_{291}+I_{313}+I_{329}+I_{344}+I_{351})$ as a function of the concentration of calcium (0–9 mg/L) is 0.9992. On this basis, we can determine the concentration of calcium from unknown samples. Above a concentration of 9 mg/L calcium, the ratio of $(I_{329}+I_{351})/(I_{291}+I_{313}+I_{329}+I_{344}+I_{351})$ does not increase linearly with calcium concentration because the binding surfaces on the beads have achieved saturation. Even when the concentration of calcium ions in the sample is increased, the calcium eluted from the beads remains steady. It is possible to further extend the concentration range for

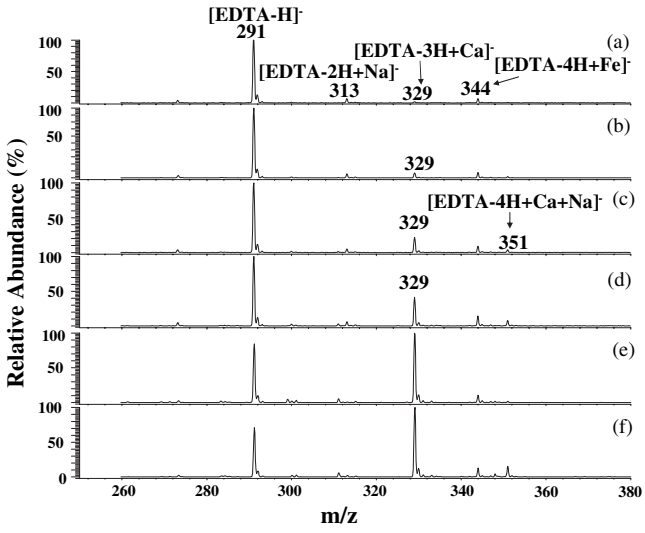

Figure 1. ESI mass spectra of the samples obtained using the functional magnetic beads to trap target ions from calcium standard solutions with various concentrations: (a) 0, (b) 1 mg/L, (c) 3 mg/L, (d) 5 mg/L, (e) 9 mg/L, and (f) 11 mg/L.

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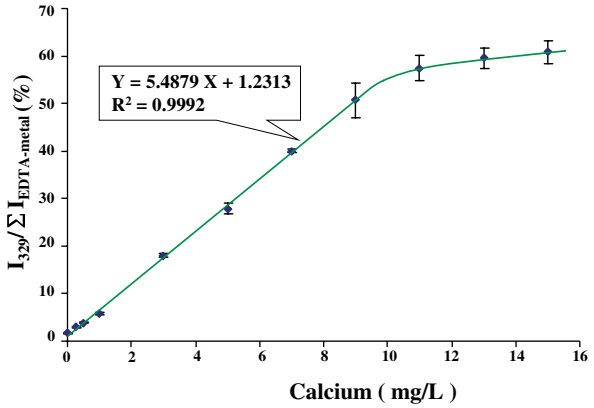

Figure 2. Plot of $I_{329}/\Sigma I_{EDTA\text{-}metal\ ions}$ as a function of the concentration of calcium.

quantitative analysis by increasing the number of magnetic beads used for extraction.

Alkali metal ions such as sodium and potassium are frequently present in real samples. Thus, we investigated the effects of alkali metal ions such as sodium and potassium ions on the determination of calcium by this current approach. The ESI mass spectrum obtained after using functional magnetic beads to trap target metal ions from the sample by spiking sodium citrate (10 mg/L) and potassium phosphate (10 mg/L) into a calcium solution (5 mg/L) is shown in Fig. 3. This experiment was repeated five times. On the basis of the experimental results and by using the plot in Fig. 2, the concentration of calcium in the sample solution is estimated as ca. 4.6 mg/mL (see Table 1). The presence of sodium and potassium ions in the sample solution causes about an 8% error from the true value (5 mg/mL). The result indicates that the presence of large amounts of alkali metal ions in the sample causes only small errors in the determination of calcium.

These calibrations were used to determine calcium in dairy drinks. The functional magnetic beads were used to trap calcium ions from diluted milk samples. This was followed by the elution of EDTA and analysis by ESI-MS. Figure 4(a)

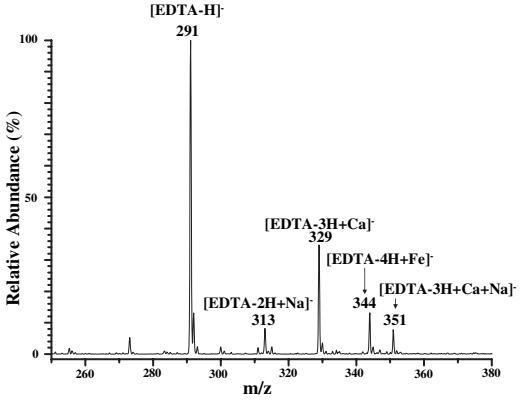

Figure 3. ESI mass spectrum of the calcium sample (5 mg/L) spiked with sodium citrate (10 mg/L) and ammonium phosphate (10 mg/L) obtained after using the functional magnetic beads to trap metal ions from the sample solution followed by elution of EDTA (0.05 mL, 200 mg/mL).

Table 1. Experimental results of the concentration of calcium in a sample containing calcium (5 mg/mL), sodium citrate (10 mg/L), and potassium phosphate (10 mg/L) obtained using the functional magnetic beads to trap metal ions followed by elution of EDTA and determination by ESI-MS

Sample	$I_{329}/\Sigma I_{EDTA\text{-}metal\ ions} \times 100\%$	Calcium (mg/L)
1	26.4	4.6
2	25.8	4.5
3	26.0	4.5
4	27.7	4.8
5	26.4	4.6
Average	26.5	4.6

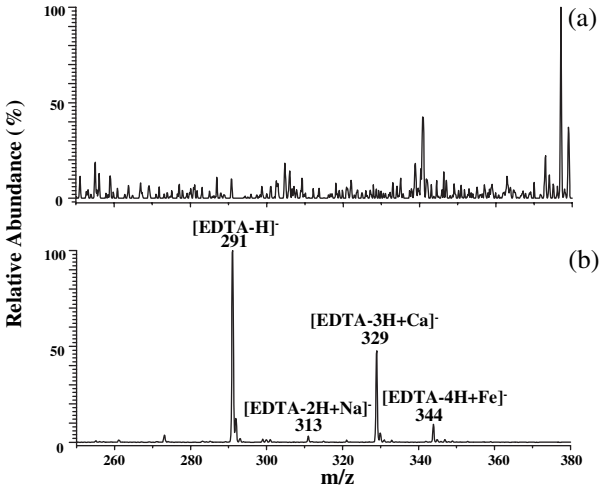

Figure 4. (a) ESI mass spectrum of the milk sample diluted 200-fold with methanol/0.05% ammonia solution (1:1, v/v) from the original sample solution. (b) ESI mass spectrum of the diluted milk sample obtained using the functional magnetic beads to trap metal ions from the sample solution followed by elution of EDTA (0.05 mL, 200 mg/L).

presents the direct ESI-MS analysis of a diluted milk sample, which has been diluted 200-fold with methanol/0.05% ammonia solution (1:1, v/v) without any pretreatment. The mass spectral quality is not good. However, Fig. 4(b) presents the ESI mass spectrum of the diluted milk sample obtained using the functional beads to trap target metal ions followed by the elution of EDTA dissolved in methanol/0.05% ammonia solution (1:1, v/v; 0.05 mL). A series of EDTA-metal complex ions is observed in the ESI mass spectrum. The ions at *m/z* 291, 313, 329, and 344 correspond to $[EDTA–H^+]^-$, $[EDTA+Na^+–2H^+]^-$, $[EDTA+Ca^+–3H^+]^-$, and $[EDTA+Fe^{3+}–4H^+]^-$, respectively. From the calibration curve in Fig. 2 and the result in Fig. 4(b), we estimated the concentration of Ca(II) in the sample as ca. 5.18 mg/L. Because the sample used in Fig. 4(b) has been diluted 200-fold with a methanol/0.05% ammonia solution (1:1, v/v) from the original milk sample, the original concentration of milk sample is ca. 1036 mg/L, which differs by only ~3.5% from the true value (1000 mg/L) provided by the milk producers.

The concentration of calcium in yogurt was determined using this approach. Figure 5 presents the ESI mass spectrum obtained after using functional magnetic beads to trap their

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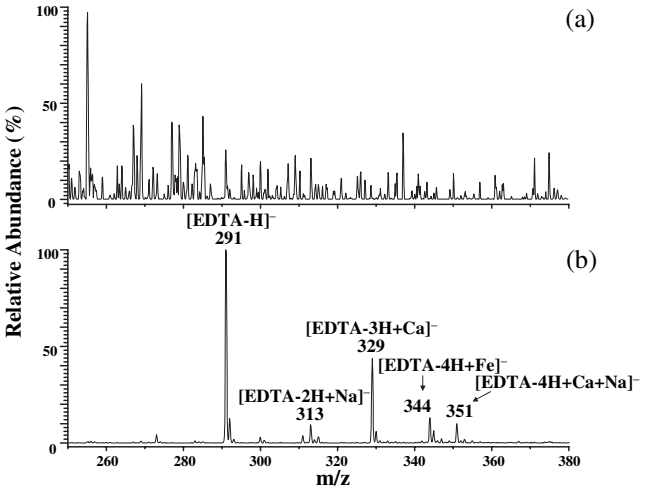

Figure 5. (a) ESI mass spectrum of the yogurt sample (100 μL) diluted 200-fold with methanol/0.05% ammonia solution (1:1, v/v) from the original sample. (b) ESI mass spectrum of the diluted yogurt (100 μL) obtained using the functional magnetic beads to trap metal ions from the sample solution followed by elution of EDTA (0.05 mL, 200 mg/L).

target metal ions from a yogurt sample, which had been diluted 200-fold with a methanol/0.05% ammonia solution (1:1, v/v) solution, followed by the elution of EDTA solution (0.05 mL), which was prepared in a solution of methanol/0.05% ammonia solution (1:1, v/v). Several EDTA-metal ion complexes are observed in the ESI mass spectrum. The ions at *m/z* 291, 313, 329, 344, and 351 correspond to $[EDTA–H^+]^-$, $[EDTA+Na^+–2H^+]^-$, $[EDTA+Ca^+–3H^+]^-$, $[EDTA+Fe^{3+}–4H^+]^-$, and $[EDTA+Ca^+–3H^+]^-$, respectively. From the calibration curve in Fig. 2 and the result in Fig. 5, we estimated the concentration of Ca(II) in the diluted yogurt sample as ca. 5.29 mg/L. The sample used to obtain Fig. 5 had been diluted 200-fold from the original yogurt, so the original concentration in the yogurt sample was ca. 1058 mg/L, also ~3.4% different from the value (1095 mg/L) provided by the dairy factory.

CONCLUSIONS

We have demonstrated that immobilized NTA magnetic beads can be used to trap calcium ions from dairy samples. After elution by EDTA, the EDTA-metal ion complex can be used for the determination of calcium in the sample solution. Dairy drinks generally contain a high concentration of calcium ions. Our functional magnetic beads are capable of trapping calcium ions from diluted dairy drinks and eliminating the interferences from the complex matrix. Many transition metal ions bind with EDTA, but we have demonstrated that we can use immobilized NTA magnetic beads to trap calcium from both milk and yogurt. The trapped calcium ions are then eluted by the EDTA solution for the quantitative analysis of calcium by ESI-ITMS. On the basis of our experiments, the results of the quantitative analysis of calcium using this approach for milk and yogurt are close to the values known to be present in dairy drinks. This approach should be suitable for the quantitative analysis of other metal ions. Although there are number of other analytical methods used for the determination of metal ions, this approach demonstrates the possibility of using ESI-ITMS for this application.

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