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Cloud point extraction with/without chelating agent on-line coupled with inductively coupled plasma optical emission spectrometry for the determination of trace rare earth elements in biological samples

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ABSTRACT

The on-line incorporation of cloud point extraction (CPE) with/without 8-hydroxyquinoline (8-Ox) as chelating agent into flow injection analysis associated with inductively coupled plasma optical emission spectrometry (ICP-OES) for determining trace rare earth elements (REEs) is presented and evaluated. The significant parameters affecting on-line cloud point extraction of REEs such as sample pH, flow rate, 8-Ox concentration, Triton X-114 concentration were systematically studied. Under the optimized conditions, with the consumption of 3.0 mL sample solution, the limits of detection (3σ) were ranged from 41.4 pg mL^{-1} (Yb) to 448 pg mL^{-1} (Gd) with relative standard deviations (RSDs) of 1.0% (Eu)–5.9% (Sm) for on-line CPE–ICP-OES with 8-Ox as chelating agent, and 69.0 pg mL^{-1} (Sc) to 509.5 pg mL^{-1} (Sm) with RSDs of 2.9% (Yb)–7.5% (Ho) for on-line CPE–ICP-OES without 8-Ox as chelating agent, respectively. The sample throughput of 17 samples h^{-1} was obtained for both systems. The developed methods of on-line CPE–ICP-OES were validated by the analysis of certified reference material (GBW07605, tea leaves) and real biological samples of pig liver, *Auricularia auricula* and mushroom.

1. Introduction

Rare earth elements (REEs) have been widely used in functional materials, catalysts and some fertilizers in agriculture, especially in China. Consequently, more and more REEs are getting into the environment and finally enter the human body through food chain [1]. Several deleterious effects due to occupational and environmental exposure to REEs have been reported [2,3], and some studies have demonstrated that trace of REEs has inhibitory as well as stimulatory effects on the crystallization of urinary stones [4]. Therefore, the topic of the safety of REEs intake has been the subject of continual attention in analytical chemistry, and the determination of REEs in biological, food and environmental samples is becoming more and more important.

For determination of REEs in the practical samples, many analytical techniques have been used. These include: X-ray fluorescence spectrometry (XRF) [5], neutron activate analysis (NAA) [6], inductively coupled plasma mass spectrometry (ICP-MS) [7,8] and inductively coupled plasma optical emission spectrometry (ICP-OES) [9,10]. However, the sensitivity of XRF is lower than that of ICP-OES, while NAA requires very expensive nuclear reactor or particle accelerator. ICP-MS has been acknowledged as one of the most powerful techniques for REEs determinations, but the high cost limits its extensive application. ICP-OES is the commonly used technique for the determination of trace REEs due to its capability of rapid multi-element detection over a wide concentration range with relatively low detection limits. However, direct ICP-OES determination of trace REEs in biological samples sometimes is difficult due to the deficient detection limits and the matrix effects resulting from the major constituents such as organic compounds and inorganic salts. In order to achieve accurate and reliable analytical results, an efficient separation and preconcentration step prior to their determinations is required [11].

The separation/preconcentration methods reported in the literature are usually based on coprecipitation [12], liquid–liquid extraction [13], solid-phase extraction [7,10], ion-exchange [14], high performance liquid chromatography (HPLC) [15], etc. However, most of the above-mentioned methods are complicated and time-consuming. Thus, the development of a simple and rapid separation/preconcentration technique for determination of REEs is essential.

Cloud point extraction (CPE) technique is based on the fact that most non-ionic surfactants can form micelles in aqueous solutions and become turbid when they are heated beyond a temperature called the cloud point temperature (CPT). Above the CPT the micellar solution separates into a small volume of surfactant-rich phase and a diluted aqueous phase, in which the surfactant concentration is close to the critical micellar concentration (CMC). Any analyte sol-

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ubilized in the hydrophobic core of the micelles, will separate and become concentrated in the small volume of the surfactant-rich phase [16]. CPE offers several advantages including low cost, safety, and a high capacity to concentrate a wide variety of analytes, and has been used for the separation/preconcentration of trace metals in various samples with complicated matrices [17–23]. However, in conventional CPE, some manually operated procedures, such as heating, centrifugation, cooling, removal of supernatant and dilution, are always involved. These processes are not only fussy but also tend to result in large reagent consumption. Moreno Cordero and co-workers [24] were the first to recognize the advantages of combining CPE with flow injection analysis (FIA); however, sample preconcentration using the cloud point methodology was performed off-line in their experiments. Fang et al. [25] proposed the on-line incorporation of CPE to FIA for the first time. The authors evaluated the analytical performance of the on-line CPE–FIA system by using hematoporphyrin as a model test compound. More recently, Ortega et al. realized on-line incorporation of CPE with FI for the determination of gadolinium [26] and dysprosium [27] in urine by ICP-OES. The method was based on the complexation of analytes with 2-(5-bromo-2-pyridylazo)-5-diethylamin-ophenol in the presence of non-ionic micelles of PONPE-7.5, resulting in the retention of the surfactant-rich phase in a microcolumn packed with cotton. Yan and co-workers [28] used ammonium pyrrolidinedithiocarbamate (APDC) as the chelating agent and Triton X-114 as the extractant and a microcolumn packed with silica gel to entrap the analyte-containing surfactant-rich phase, and developed a new flow injection on-line micelle-mediated preconcentration and separation procedure for electrothermal atomic absorption spectrometry (ETAAS) determination of trace lead in biological samples. Using dithizone as the chelating agent, Triton X-114 as the extractant and a microcolumn packed with cotton wool to entrap the analyte-containing surfactant-rich phase, a CPE coupled with on-line FI system was presented by Garrido et al. [29] for spectrophotometric measurement of low levels of Hg(II) spiked in natural water samples.

However, until to present, the majority applications of cloud point extraction to preconcentrate metals are based on the formation of hydrophobic chelates in the surfactant aggregate by choosing specific chelating agents. It should be noted that the less chelating agent used, the better for the facilitation of the CPE operation procedures. Based on this consideration, a new CPE operation mode of CPE without the addition of any chelating agent was proposed recently [30,31], but the extraction was off-line processed.

8-Hydroxyquinoline (8-Ox) can react with REEs ions to form hydrophobic chelates which could be extracted by organic solvents, and it was widely applied to the separation/preconcention of REEs [7,32]. The aim of this work was to explore the feasibility of on-line coupled CPE system with/without chelating agent to ICP-OES for the determination of trace REEs in biological samples. The significant parameters affecting on-line CPE system with/without chelating agent were studied. The procedures and analytical performance of the two on-line CPE systems have been critically compared through the evaluation of precision and limits of detection. The two on-line CPE systems were used to the determination of trace REEs in biological samples with satisfactory results.

2. Experimental

2.1. Apparatus

IRIS Intrepid II XSP ICP-OES (Thermo., USA) (radial ICP torch, concentric model nebulizer, cinnabar cyclonic spray chamber) was used for REEs determinations. The optimum operation conditions were summarized in Table 1. The pH values were controlled with a Mettler Toledo 320-S pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China) supplied with a combined electrode. An IFIS-C flow injection system (Ruimai Tech. Co. Ltd., Xi'an, China) and a self-made PTFE microcolumn (20 mm × 2.0 mm i.d.) packed with silica gel were used in the on-line separation/preconcentration process. The operation sequence of on-line CPE coupled with ICP-OES determination was identical with that reported previously [33].

Table 1
Operation parameters of Intrepid XSP Rasial ICP-OES.

RF generator power	1150 W
Frequency of RF generator	27.12 MHz
Coolant gas flow rate (L min^{-1})	14
Auxiliary gas flow rate (L min^{-1})	0.5
Carrier gas flow rate (L min^{-1})	0.6
Max integration times (s)	30
Analytical wavelength (nm)	Ce 413.380, Dy 353.170, Er 337.271 Eu 381.967, Gd 335.047, Ho 345.600 La 408.672, Lu 261.542, Nd 401.225 Pr 414.311, Sc 361.384, Sm 330.639 Tb 350.917, Tm 313.126, Y 324.228 Yb 328.937

2.2. Standard solutions and reagents

The stock standard solutions (1 mg mL^{-1}) of REEs were prepared by dissolving their SpecPure® oxides (Merck, Darmstadt, Germany) or nitrates using the conventional method, and diluting to a certain volume with high purity deionized water. Standard solutions and test solutions were obtained by diluting the stock solution with 2% (v/v) HNO_3. 8-Hydroxyquinoline (8-Ox) solution of 0.1 mol L^{-1} was prepared by dissolving 1.45 g 8-Ox (Shanghai Reagent Factory, Shanghai, China) in 100 mL of 0.1 mol L^{-1} HCl. Solution (1.0%, w/v) of the non-ionic surfactant Triton X-114 (Acros Organics, New Jersey, USA) was prepared in high purity deionized water and was used without further purification. The stock solutions of interfering elements (K^+, Na^+) were prepared from their analytical reagents by dissolving KCl and NaCl (A.R., Shanghai Reagent Factory, Shanghai, China) in high purity deionized water. The stock solutions of interfering elements (Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}, Pb^{2+} and Al^{3+}) were prepared by dissolving certain amount of their inorganic salts ($CaCO_3$, $Mg(NO_3)_2$, $Fe(NO_3)_3$, $ZnCl_2$, $Pb(NO_3)_3$ and $Al(NO_3)_3$) (A.R., Shanghai Reagent Factory, Shanghai, China) in 2% (w/v) HCl. The stock solutions of interfering anions ($H_2PO_4^-$, Cl^-, SiO_3^{2-}, SO_4^{2-}, HCO_3^-) were prepared from their analytical reagents by dissolving certain amount of $NH_4H_2PO_4$, NH_4Cl, Na_2SiO_3, NH_4SO_4, NH_4HCO_3 in high purity deionized water. Buffer solution of 0.1 mol L^{-1} NaAc–HAc (pH 6.0) and 0.1 mol L^{-1} NH_4Cl–$NH_3{\cdot}H_2O$ (pH 10.0) were prepared for pH adjustment. All reagents used were specpure or at least of analytical reagent grade. All stock standard solutions were stored in polyethylene bottles in a refrigerator held at 4 °C. All glassware was kept in 10% nitric acid for at least 24 h and washed three times with high purity deionized water (18.2 MΩ cm, Millipore, Molsheim, France) before use.

2.3. Microcolumn preparation

A total of 24 mg of silica gel was filled into a PTFE microcolumn plugged with a small portion of absorbent cotton at both ends. Before use, high purity deionized water and 0.1 mol L^{-1} buffer solution (pH 6.0 NaAc–HAc or pH 10.0 NH_4Cl–$NH_3{\cdot}H_2O$) were sequentially passed through the microcolumn in order to clean and condition it for the following use.

2.4. Procedures (on-line FI-CPE)

The operation sequence of on-line CPE–ICP-OES determination was identical with that reported previously [33]. Before loading the microcolumn, it was conditioned for the preconcentration at the desired pH with buffer-diluted solution.

Step 1(a): the injector valve was in the fill position and pump 1 was activated. The solution of REEs (standard solutions or samples) and 0.1% (w/v) Triton X-114 with (or without) 0.5 mmol L^{-1} 8-Ox were placed in a plastic tube, and the resultant solution was adjusted to pH 6.0 with 0.01 mol L^{-1} HCl (or pH 10.0 with 0.1 mol L^{-1} $NH_3{\cdot}H_2O$), then loaded into the FIA manifold at a flow rate of 1.6 mL min^{-1}, the solution passed through the collection microcolumn, and the surfactant-rich phase containing the analytes was adsorbed onto the microcolumn packed with silica gel (24 mg).

Step 2(a): the injector valve was still in the fill position, whereas pump 1 was stopped and pump 2 was activated, so that an air flow was introduced to remove the residual solution in the microcolumn. Meanwhile, a 0.3 mL of 0.5 mol L^{-1} HCl was introduced.

Step 3(b): the status of the two pumps was the same as step 2, but the injector valve was turned to the inject position. An air flow was introduced to drive the eluent into the microcolumn for eluting the collected analytes directly into the nebulizer of the plasma.

It should be pointed out, Ohashi and co-workers [34] have reported that the extractions of Ln(III) without adding chelating agent were caused by the impurities containing in the commercial non-ionic surfactants. Therefore, the special attention was paid to the procedure blank, and the blank test was proceeded throughout all the work. For preparation of the blank samples, the solution of 0.1% (w/v) Triton X-114 with (or without) 0.5 mmol L^{-1} 8-Ox were placed in a plastic tube, and the resultant solution was adjusted to pH 6.0 with 0.01 mol L^{-1} HCl (or pH 10.0 with 0.1 mol L^{-1} $NH_3{\cdot}H_2O$). The blank solution was subjected to on-line CPE and the blank values were determined. Fortunately, no remarkable blank signal could be detected. To ensure the accuracy of the experiment results, all experiments were carried out at ambient temperature and corrected against the reagent blank.

2.5. Sample preparation

(1) 0.5 g of the certified reference material (GBW07605, tea leaves) was weighed and heated to 100 °C in the presence of 3 mL HNO_3, the resulting sample solution was heated to near dryness, and the residue was dissolved in 1.5 mol L^{-1} HNO_3. Then, the digest was transferred into 10 mL of flask, adjusted to pH 6.0 and diluted to the calibration with high purity deionized water for analysis.

(2) Pig liver, *Auricularia auricula*, mushroom were obtained from local market (Wuhan, China) and frozen in refrigerator. After washed with high purity deionized water and airing dried at 80 °C for 3 h, the pig liver was cut into pieces, the *A. auricula* and mushroom were smashed. All these samples were dried again at 105 °C till constant weight. The percentage of dry weight/fresh weight was calculated for analysis.

1.0 g of the above samples (pig liver, *A. auricula*, mushroom) were weighed and dissolved in 3 mL HNO_3 under mild heating. After the sample solution became to transparence, 10 drops of $HClO_4$ were added, and the resultant solution was vaporized to near dryness. The residue was dissolved in 1.5 mol L^{-1} HNO_3, and then transferred into 25 mL of flask, adjusted to pH 6.0 with 0.01 mol L^{-1} HCl (or pH 10.0 with 0.1 mol L^{-1} $NH_3{\cdot}H_2O$) and diluted to the calibration with high purity deionized water for analysis.

3. Results and discussion

Two different systems, on-line CPE with and without 8-Ox as chelating agent were preliminarily investigated, the significant parameters affecting on-line cloud point extraction of REEs were studied and optimized.

3.1. The choice of collection microcolumn

A homemade microcolumn packed with suitable filtering material was employed to carry out phase separation. In a preliminary study, silica gel, silylanization silica gel and absorbent cotton were tested as the microcolumn packing materials to perform the on-line collection of the analyte-entrapped micelles. It was found that three kinds of tested filtering materials all can collect the analyte-entrapped micelles in the two on-line CPE systems, while absorbent cotton cannot be regenerated and has to be changed after each run, which makes the on-line process be very difficult. Both silica gel and silylanization silica gel can be reused when they were served as filtering materials, but silica gel is much cheaper. Therefore, silica gel was chosen as the filtering material in the following work.

An appropriate microcolumn size is also important for the present flow injection (FI)-CPE–ICP-OES system. A larger microcolumn would provide higher retention efficiency for the analytes, but a high resistance against the solution passage would be encountered, thus a poor precision would be obtained. By using a smaller microcolumn, better precision and very sharp peaks could be obtained but the filtering capacity of the microcolumn was reduced. Based on these considerations, the internal diameter of the microcolumn was chosen as 2 mm in this work. By fixing the internal diameter of the microcolumn of 2 mm, the effect of microcolumn length on the retention efficiency was studied with microcolumn length varying within the range 10–40 mm. The experimental results showed that the optimal retention was achieved with microcolumn length of 20 mm. Therefore, a microcolumn with an effective length of 20 mm and inner diameter of 2 mm was applied to obtain suitable sensitivity and precision.

3.2. Optimization of the on-line separation/preconcentration process

3.2.1. Effect of pH

The pH plays an important role in metal–chelates formation and subsequent extraction. The effects of pH on the signal intensity of REEs ions in both on-line CPE with/without 8-Ox were studied with pH varying from 4.0 to 11.0, and the experimental results were shown in Figs. 1 and 2, respectively. For on-line CPE with 8-Ox as chelating agent (Fig. 1), the chelating reaction between REEs ions with 8-Ox plays a dominant role when the medium pH changing from 4.0 to 9.0. When the medium pH was in the range of 4.0–5.5, only part of the REEs were extracted into the surfactant-rich phase due to the incomplete formation of REEs-8-Ox complexes in this pH range. When the medium pH was in the range of 5.5–9.0, REEs were completely retained on the collection microcolumn due to the facts that they can form the Triton X-114 extractable REEs-8-Ox complexes with 8-Ox and therefore was retained by the silica gel. When the medium pH was higher than 9.0, the hydrolysis reaction plays a dominant role, the hydrolysis of REEs would occur prior to their chelation with 8-Ox which lead to the low extraction efficiency [35]. However, for on-line CPE without 8-Ox as chelating agent (Fig. 2), REEs were quantitatively retained only at pH higher than 9.0. One of the possible reasons was that the formation of cationic complexes between REEs ions and Triton-114 through its polyoxyethylene groups [31,36], and the other possible reason was that the hydrolysis of REEs occurred with the increase of pH, and the hydrophobic hydrates can be extracted by Triton X-114 and

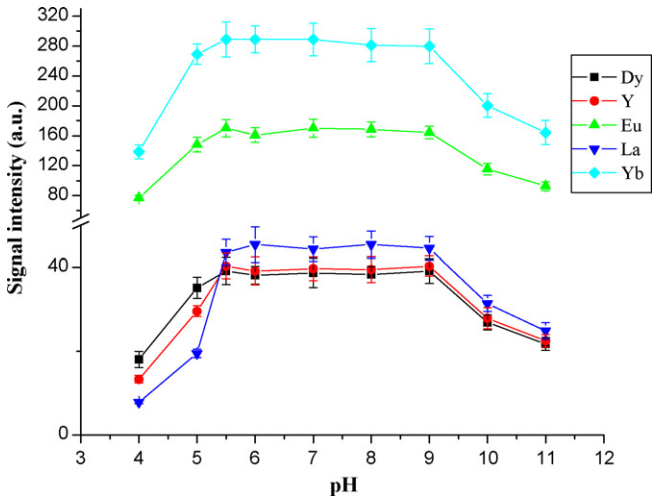

Fig. 1. Effect of pH on analytical signal intensity for on-line CPE–ICP-OES with 8-Ox as chelating agent. REEs: 0.1 μg mL^{-1}; 8-Ox: 0.5 mmol L^{-1}; Triton X-114: 0.1% (w/v); sample flow rate: 1.6 mL min^{-1}; elution: 0.3 mL of 0.5 mol L^{-1} HCl with flow rate of 1.6 mL min^{-1}.

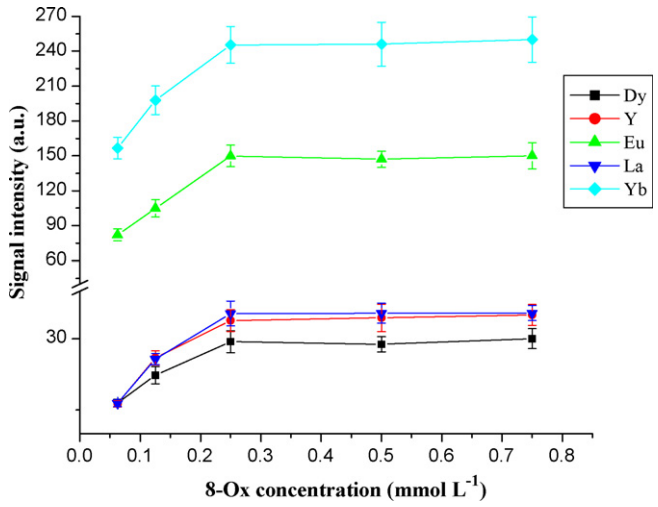

Fig. 3. Effect of 8-Ox concentration on analytical signal intensity for on-line CPE–ICP-OES with 8-Ox as chelating agent. REEs: 0.1 μg mL^{-1}; pH: 6.0; Triton X-114: 0.1% (w/v); sample flow rate: 1.6 mL min^{-1}; elution: 0.3 mL of 0.5 mol L^{-1} HCl with flow rate of 1.6 mL min^{-1}.

then was retained by the silica gel [37]. Consequently, in the following work, pH 6.0 and pH 10.0 were used for on-line CPE with and without 8-Ox as chelating agent, respectively.

3.2.2. Effect of 8-Ox concentration

The variation of the analytical signal as a function of the concentration of 8-Ox in the range of 0.0625–0.75 mmol L^{-1} was studied, and the experimental results in Fig. 3 demonstrated that the analytical signals of REEs were accentuated by 8-Ox at concentration up to about 0.25 mmol L^{-1}. The maximum analytical signals were achieved with this concentration and remained constant up to the highest amount studied. For further studies, an 8-Ox concentration of 0.5 mmol L^{-1} was selected.

3.2.3. Effect of Triton X-114 concentration

The amount of Triton X-114 concentration also plays an important role in the subsequent extraction. And the effect of Triton X-114 concentration on the extraction efficiency of REEs was studied in the concentration range of 0.005–0.5% (w/v). Fig. 4 was the effect of Triton X-114 concentration on the REEs signal intensity for on-line CPE with 8-Ox as chelating agent. As could be seen, the analytical signal intensity of REEs was enhanced with the increase of the concentration of Triton X-114 from 0.005% to 0.05%, and kept constant with the further increase of Triton X-114 concentration to 0.2% (w/v). For the on-line CPE without 8-Ox as chelating agent, a quantitative extraction of REEs was obtained with the Triton X-114 concentration less than 0.3% (Fig. 5). After Triton X-114 concentration was beyond 0.2% for on-line CPE with 8-Ox and 0.3% for on-line CPE without 8-Ox, the further increase in the concentration of Triton X-114 was led to a gradual decrease in the analytical signal of REEs. This could be attributed to that a larger volume of surfactant-rich phase would be obtained when higher amounts of surfactant are used, resulting in a decrease of concentration of the analytes. Accordingly, a Triton X-114 concentration of 0.1% (w/v) was employed for both on-line CPE systems.

3.2.4. Effects of equilibration temperature

The effect of the equilibration temperature was investigated in this work, and the results indicated that there was no significant influence of the extraction temperature on the extraction of REEs for the two CPE systems from 20 °C to 90 °C. So, ambient tempera-

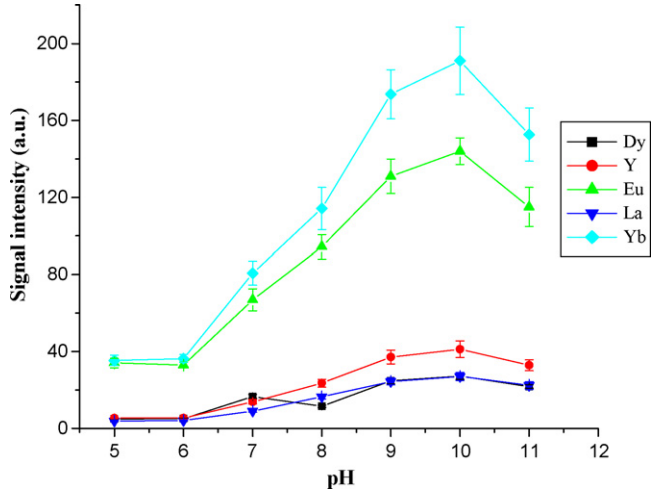

Fig. 2. Effect of pH on analytical signal intensity for on-line CPE–ICP-OES without 8-Ox as chelating agent. REEs: 0.1 μg mL^{-1}; Triton X-114: 0.1% (w/v); sample flow rate: 1.6 mL min^{-1}; elution: 0.3 mL of 0.5 mol L^{-1} HCl with flow rate of 1.6 mL min^{-1}.

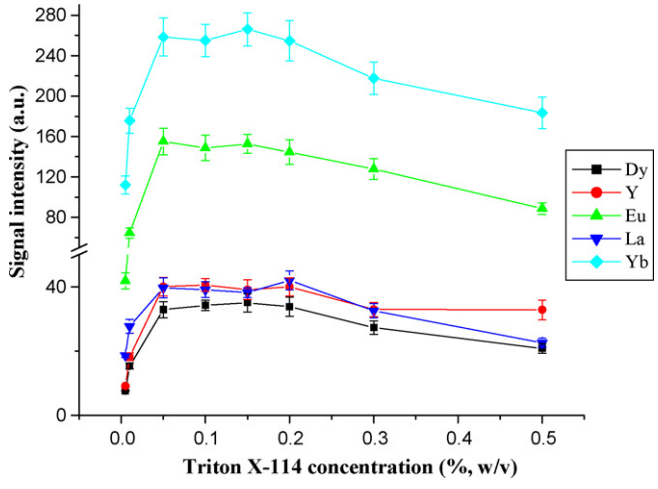

Fig. 4. Effect of Triton X-114 concentration on analytical signal intensity for on-line CPE–ICP-OES with 8-Ox as chelating agent. REEs: 0.1 μg mL^{-1}; pH: 6.0: 8-Ox: 0.5 mmol L^{-1}; sample flow rate: 1.6 mL min^{-1}; elution: 0.3 mL of 0.5 mol L^{-1} HCl with flow rate of 1.6 mL min^{-1}.

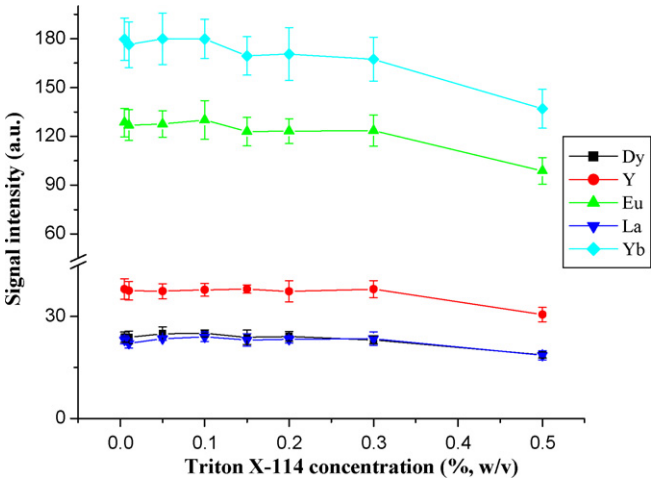

Fig. 5. Effect of Triton X-114 concentration on analytical signal intensity for on-line CPE–ICP-OES without 8-Ox as chelating agent. REEs: 0.1 μg mL^{-1}; pH: 10.0; sample flow rate: 1.6 mL min^{-1}; elution: 0.3 mL of 0.5 mol L^{-1} HCl with flow rate of 1.6 mL min^{-1}.

ture was chosen for equilibration temperature for both on-line CPE systems.

3.2.5. Loading rate of sample

The sample flow rate through the microcolumn is a key factor to analytical efficiency, since this is one of the steps that control the time of analysis. Fig. 6 was the effect of sample flow rate on the signal intensity of REEs for on-line CPE with 8-Ox as chelating agent. As could be seen, there was no obvious effect of sample flow rate on the signal intensity when the sample flow rate was varying from 0.5 mL min^{-1} to 1.6 mL min^{-1}. Similar results were obtained for the on-line CPE without 8-Ox. To improve analytical efficiency, 1.6 mL min^{-1} was chosen as the loading rate of sample for both on-line CPE systems.

3.2.6. Elution

In order to elute the collected analytes on the microcolumn effectively, 0.1–2.0 mol L^{-1} HCl and HNO_3 as eluent were examined for on-line CPE with 8-Ox as chelating agent. Both HCl and HNO_3 were found to be efficient to elute the collected analytes when their concentrations were beyond 0.1 mol L^{-1}. By fixing the eluent concentration of 0.5 mol L^{-1} HCl, the effect of the eluent volume on the analyte elution was also investigated and it was found that 0.3 mL of eluent could quantitatively elute the analyte from the microcolumn. As a result, 0.3 mL of 0.5 mol L^{-1} HCl was employed as eluent for on-line CPE with 8-Ox as chelating agent.

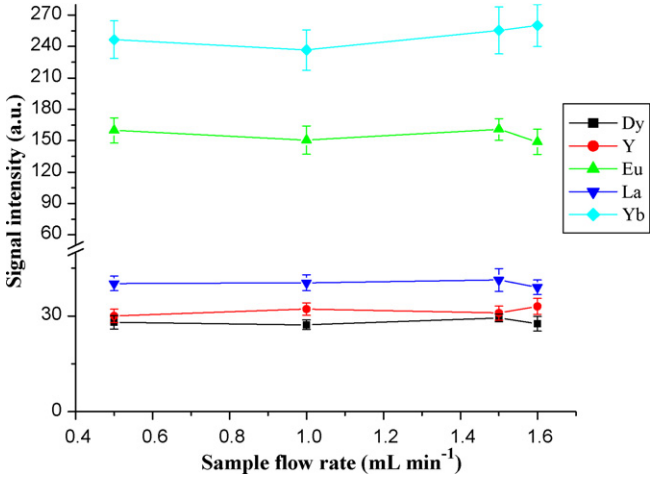

Fig. 6. Effect of loading rate of sample on analytical signal intensity for on-line CPE–ICP-OES with 8-Ox as chelating agent. REEs: 0.1 μg mL^{-1}; pH: 6.0; 8-Ox: 0.5 mmol L^{-1}; Triton X-114: 0.1% (w/v); elution: 0.3 mL of 0.5 mol L^{-1} HCl with flow rate of 1.6 mL min^{-1}.

Table 2
Effect of foreign ions on the determination of REEs for both on-line CPE–ICP-OES.

Coexisting ions	Tolerance limit of ions (μg mL^{-1})	
	With 8-Ox	Without 8-Ox
Na^+	10,000	5,000
K^+	5,000	5,000
Mg^{2+}	1,500	1,000
Ca^{2+}	1,000	1,000
Al^{3+}	100	100
Pb^{2+}	50	50
Zn^{2+}, Fe^{3+}	20	50
Cl^-	5,000	5,000
$H_2PO_4^-$	2,000	2,000
HCO_3^-	2,000	1,000
SiO_3^{2-}, SO_4^{2-}	1,000	1,000

The flow rate of the eluent was also examined, and it was found that there was no significant variation of the signal intensity when the eluent flow rate was in the range of 0.5–1.6 mL min^{-1}. In order to improve the analytical efficiency, 1.6 mL min^{-1} of eluent flow rate was selected for on-line cloud point extraction of REEs with 8-Ox as chelating agent.

The elution conditions for on-line CPE without 8-Ox as chelating agent were also investigated, and the similar results were obtained. Therefore, 0.3 mL of 0.5 mol L^{-1} HCl with flow rate of 1.6 mL min^{-1} was employed as elution conditions for both on-line CPE systems.

3.3. Effects of coexisting ions

The effects of common coexisting ions on REEs extraction and subsequent determination in the two on-line CPE–ICP-OES systems were investigated. For this purpose, solutions containing 50 ng mL^{-1} of REEs and the added coexisting ions were prepared and operated according to the recommended procedure. The tolerance limits of coexisting ions, which gave less than a 10% error for the determination of REEs, were evaluated, and results were listed in Table 2. As could be seen, the tolerance limit of Na^+ and Mg^{2+} for on-line CPE–ICP-OES with 8-Ox coupling as chelating agent was larger than that obtained for on-line CPE–ICP-OES without 8-Ox coupling as chelating agent, whereas the tolerance limit of Zn^{2+}

Table 3
Analytical performance of both on-line CPE–ICP-OES.

Element	Detection limit (pg mL^{-1})		Enrichment factor		RSD (%)	
	a	b	a	b	a	b
Ce	182	295	8.9	5.7	4.6	4.7
Dy	99.1	159	8.6	6.0	3.4	5.4
Er	247	245	8.0	8.1	1.8	4.3
Eu	66.4	80.1	8.6	7.3	1.0	5.4
Gd	448	500.5	8.1	7.2	5.8	4.0
Ho	114	236	9.2	5.2	1.9	7.5
La	88.1	151.1	8.7	5.5	5.8	5.0
Lu	78.2	101.4	8.1	6.5	3.3	3.8
Nd	264	295	8.6	7.8	2.1	3.8
Pr	367.7	407.7	8.0	7.2	5.5	4.6
Sc	43.9	69.0	8.5	5.4	3.0	4.7
Sm	409.5	509.5	7.9	6.5	5.9	4.9
Tb	286	395	8.7	6.3	3.1	7.2
Tm	255	361	8.7	6.2	3.2	4.4
Y	95.5	102.5	8.2	7.9	1.8	5.0
Yb	41.4	72.1	8.9	5.9	3.7	2.9

a: On-line CPE-ICP-OES with 8-Ox as chelating agent; b: on-line CPE-ICP-OES without 8-Ox as chelating agent.

Table 4
Determination of REEs in certified reference material (GBW07605, tea leaves) by on-line CPE-ICP-OES with and without 8-Ox as chelating agent (mean values and 95% confidence intervals, $n = 3$).

Element	Determined value ($\mu g\,g^{-1}$)		Certified value ($\mu g\,g^{-1}$)	t-Test[c]	
	a	b		a	b
Ce	0.92 ± 0.14	1.03 ± 0.04	1.0 ± 0.1	2.31	1.30
Dy	0.067 ± 0.005	0.077 ± 0.002	0.074	6.06	5.20
Er	N.D.	N.D.	–		
Eu	0.016 ± 0.002	0.020 ± 0.002	0.018 ± 0.002	3.46	3.46
Gd	0.09 ± 0.005	0.096 ± 0.007	0.093	2.59	1.73
Ho	0.018 ± 0.005	0.020 ± 0.002	0.019	0.87	1.73
La	0.57 ± 0.1	0.61 ± 0.1	0.60 ± 0.03	1.04	0.35
Lu	0.0062 ± 0.002	0.0068 ± 0.002	0.007	3.46	0.58
Nd	0.44 ± 0.07	0.41 ± 0.05	0.44	0	2.60
Pr	0.11 ± 0.01	0.124 ± 0.01	0.12		1.15
Sc	0.081 ± 0.01	0.087 ± 0.01	0.085 ± 0.009	0.99	0.43
Sm	0.086 ± 0.01	0.083 ± 0.01	0.085 ± 0.017	0.25	0.69
Tb	N.D.	N.D.	0.011		
Tm	N.D.	N.D.	–		
Y	0.37 ± 0.05	0.37 ± 0.04	0.36 ± 0.03	0.866	1.02
Yb	0.046 ± 0.007	0.041 ± 0.009	0.044 ± 0.004	1.15	1.30

a: On-line CPE-ICP-OES with 8-Ox; b: on-line CPE-ICP-OES without 8-Ox; N.D.: not detectable; c: $t_{0.05,2} = 4.30$.

Table 5
Analytical results of REEs in biological samples by both on-line CPE–ICP-OES (mean values and 95% confidence intervals, $n = 3$).

Element	Measured ($ng\,g^{-1}$) Pork liver		*Auricularia auricula*		Mushroom	
	a	b	a	b	a	b
Ce	173.2 ± 8.9	160.9 ± 14.3	23.5 ± 5.2	20.3 ± 4.2	18.1 ± 3.7	20.1 ± 4.7
Eu	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dy	48.0 ± 3.7	47.0 ± 5.4	4.6 ± 0.4	4.1 ± 0.9	1.9 ± 0.2	1.6 ± 0.2
Er	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Gd	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ho	N.D.	N.D.	N.D.	N.D.	5.2 ± 0.7	5.5 ± 1.2
La	69.8 ± 9.6	63.5 ± 10.6	105.4 ± 18.8	115.2 ± 14.6	39.1 ± 5.4	37.0 ± 8.6
Lu	5.8 ± 0.9	6.0 ± 1.2	11.5 ± 1.7	10.3 ± 1.2	N.D.	N.D.
Nd	N.D.	N.D.	5.1 ± 0.7	5.2 ± 0.7	N.D.	N.D.
Pr	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sc	11.6 ± 2.7	10.0 ± 2.2	2.6 ± 0.4	2.8 ± 0.4	1.6 ± 0.5	1.4 ± 0.2
Sm	42.3 ± 6.7	44.8 ± 8.9	7.9 ± 1.2	8.8 ± 1.7	3.9 ± 0.2	N.D.
Tm	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Y	15.5 ± 2.4	17.1 ± 3.2	2.6 ± 0.4	2.4 ± 0.4	1.8 ± 0.2	1.9 ± 0.2
Tb	N.D.	N.D.	N.D.	N.D.	7.6 ± 0.5	7.8 ± 1.2
Yb	7.5 ± 0.9	6.9 ± 0.7	1.7 ± 0.2	1.55 ± 0.2	N.D.	N.D.

a: On-line CPE-ICP-OES with 8-Ox; b: on-line CPE-ICP-OES without 8-Ox; N.D.: not detectable.

and Fe^{3+} for the former was less than that for the latter. However, all the tolerance limit values of the coexisting ions for both systems were higher than their concentrations typically found in biological samples, indicating that the developed methods could be applied for the determination of trace REEs in the practical samples.

3.4. Analytical performance

The calibration curves were obtained after the calibration standards were subjected to the on-line CPE with and without 8-Ox as chelating agent, respectively, and good linearity were obtained for both on-line CPE–ICP-OES in the concentration of 2–1000 $ng\,mL^{-1}$ for Dy, Eu, Ho, La, Lu, Sc, Y, Yb and 5–1000 $ng\,mL^{-1}$ for Ce, Er, Gd, Nd, Pr, Sm, Tb, Tm. Under the optimum conditions described above, the sample throughput was 17 h^{-1} for both on-line CPE–ICP-OES. The limits of detection (LODs, based on three times standard deviations of the blanks by eight replicates) and the relative standard deviations (RSDs, $C = 50\,ng\,mL^{-1}$, $n = 7$) together with the enrichment factor of the two on-line CPE–ICP-OES systems were summarized in Table 3. As could be seen, LODs for REEs obtained by on-line CPE–ICP-OES with 8-Ox as chelating agent were slightly better than that obtained by on-line CPE-ICP-OES without 8-Ox as chelating agent, and the RSDs for these two methods were comparable.

3.5. Validation and applications

For the validation of the proposed methods, both on-line CPE–ICP-OES systems were applied to the analysis of the certified reference material (GBW07605, tea leaves), and the analytical results were listed in Table 4. Applying the t-test, the $t0.05$, two values for the certified reference material of GBW07605 was listed in Table 4. As could be seen, there did not exist statistical differences between determined values and certified ones except Dy, which means that the determined values obtained by the proposed method coincided very well with the certified values.

Two developed methods were also applied to the determination of trace REEs in real biological samples (pig liver, *A. auricula*, mushroom), and the analytical results were given in Table 5. As could be seen, the analytical results obtained by both on-line CPE systems were in good agreement.

4. Conclusion

Cloud point extraction (CPE) with and without 8-Ox as chelating agent on-line coupled with ICP-OES for the measurement of trace REEs in biological samples were developed and critically compared. The experimental results indicated that: (1) the use of

on-line CPE greatly simplifies the extraction procedure; (2) LODs for REEs obtained by on-line CPE–ICP-OES with 8-Ox as chelating agent were slightly better than that obtained by on-line CPE–ICP-OES without 8-Ox as chelating agent, and the RSDs of the two methods were comparable; (3) both on-line CPE–ICP-OES systems had good selectivity and could be used for the biological sample analysis. The overall process of both on-line CPE–ICP-OES were successfully applied to the determination of trace REEs in biological samples (GBW07605 tea leaves, pig liver, *A. auricula* and mushroom), and they are characterized with automation, simplicity, selectivity, safety, low cost and are suitable for the determination of trace REEs in biological samples. Moreover, the on-line CPE–ICP-OES without any chelating agent could be a new strategy for the analysis of other trace elements in biological, environmental and food samples.

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