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Novel analytical applications of porphyrin to HPLC post-column flow injection system for determination of the lanthanides

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Abstract

A novel indirect substitution spectrophotometric methodology using porphyrin is developed for the determination of some metals that do not react with porphyrin directly. The methodology is concerned with a multistep reaction system, which consists of 3 complexation reactions that occur in a sequence of EDTA with metal, EDTA with Cu(II) and porphyrin with Cu(II). The complexation reaction of Cu(II) with a cationic porphyrin, *meso*-tetrakis (4-*N*-trimethylaminophenyl)-porphine (ttmapp), which has a high molar absorptivity about $5 \times 10^5\,\mathrm{l\,mol^{-1}\,cm^{-1}}$, serves for spectrophotometric detection. This methodology was verified through individual determinations of 14 species of lanthanides following a batchwise procedure, which was further developed to an automatic procedure on a HPLC post-column flow injection system for simultaneous analysis of the lanthanides. Compared with the conventional post-column derivatization method using 4-(2-pyridylazo) resorcinol (PAR), the analytical sensitivity was greatly improved in this method and the results also showed good linearity.

Keywords: Porphyrin; Post-column flow injection analysis; Indirect substitution method; Lanthanides

1. Introduction

The quantitative analysis of the lanthanides has received much attention since 1960s for their important roles in industrial and geochemical fields [1,2]. Separation techniques, like high performance liquid chromatography (HPLC) and capillary electrophoresis, have proved to be necessary for the lanthanides analysis in many cases due to their extremely similar chemical properties. HPLC for the lanthanides analysis has been studied most intensely and in recent years, some sensitive detection devices, such as inductively coupled plasma atomic emission spectrometry (ICP-AES) [3] and inductively coupled plasma mass spectrometry (ICP-MS) [4,5], have been combined directly with HPLC as post-column detection systems in order to develop a method with both sensitivity and efficiency, but the interfaces between these instruments and column were generally complicated to deal with. Post-column derivatization for UV–vis spectrophotometry appears to be a more attractive approach to obtain sensitivity for its simplicity and low expenses, and some methods have been reported and also frequently utilized for the lanthanides analysis [6–13]. The reagents commonly used in post-column derivatization of the lanthanides include Arsenazo(I), Arsenazo(III), PAR and, etc., among which PAR has the highest molar absorptivities about $6 \times 10^4\,\mathrm{l\,mol^{-1}\,cm^{-1}}$ [14].

Porphyrin is a group of chemical analogues possessing a macrocycle, which consists of four pyrrole rings linked by methine bridges and gives rise to their characteristic spectrum (Soret band) with intense absorption in visible region (400–450 nm). The molar absorptivities of Soret band range over $2 \times 10^5 - 5 \times 10^5\,\mathrm{l\,mol^{-1}\,cm^{-1}}$, much higher than most colorimetric reagents, and this has led to extensive researches to explore the analytical applications of porphyrins as ultra sensitive spectrophotometric reagents [15]. Various derivatives of porphyrin, such as anionic porphyrin of *meso*-tetraphenylporphine trisulfonate (TPPS), cationic porphyrin of *meso*-tetrakis(4-*N*-methylpyridinyl)porphyrin (TMPyP) and some amphoteric porphyrins, have been synthesized to obtain a porphyrin with improved chemical and physical properties, but up to now, only a limited species of metal ions, such as Cd(II), Zn(II), Pd(II), and, etc., have been determined with porphyrins [16–23]. Furthermore, metals that can be determined by each porphyrin derivatives have never exceeded 4 or 5 species

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and there is no remarkable development in this field in recent years. The applications of porphyrin to HPLC for simultaneous analysis of these metals were also reported by pre-column derivatization [24–27], but the methodology appeared rather limited and inconvenient due to the inertity of porphyrin as metallic ligand, the complexation reactions of which are not only selective but also require rigid reaction conditions.

We have made extensive studies on the syntheses of some porphyrin derivatives and their complexation reactions with various metal elements [28,29]. Compared with other porphyrins, ttmapp has high molar absorptivity and also shows physical and chemical properties more suitable for spectrophotometry [30], but like all the other derivatives, ttmapp only react with a limited species of metals of Zn(II), Pb(II), Cu(II) and Pd(II). In order to find out more applications in the analytical field for porphyrin, a novel concept described as the indirect substitution method is suggested herein for the determination of some metals that do not react with ttmapp directly. The principle is illustrated in Fig. 1. EDTA is employed as a substitution agent between metals and Cu(II), and the absorption change owing to the complexation reaction of Cu(II) with ttmapp is detected out as the signal of sample. Given that all the reactions occur stoichiomitrically in sequence and the same concentrations of EDTA and Cu(II) are used, the quantity of metals complexed with EDTA in step 1 should be equal to the remaining quantity of Cu(II) after step 2, and this remaining quantity of Cu(II) could be detected out as the complex of ttmapp–Cu(II) in step 3.

It should be noticed that the coexistence of EDTA–metal complexes with Cu(II) in steps 2 and 3 may lead to electrophilic substitution between them resulting in deviation from the stoichiometry. Therefore, metals potentially applicable for this methodology are required capable of forming highly stable or inert complexes with EDTA. In this paper, lanthanides were selected to verify the effectiveness of this methodology. Following a batch wise procedure, 14 species of lanthanides were individually determined with good linearity and high sensitivity. The procedure was applied onto a FIA system of a four-channeled peristaltic pump, which was connected after a cationic exchange column and made the procedure simple and precisely controlled. Lanthanides were divided into 3 groups (Nd–La designated as light group, Tb–Sm as medium group and Lu–Dy as heavy group) according to the stabilities of their EDTA complexes, and each group was isocratically eluted and determined on the system.

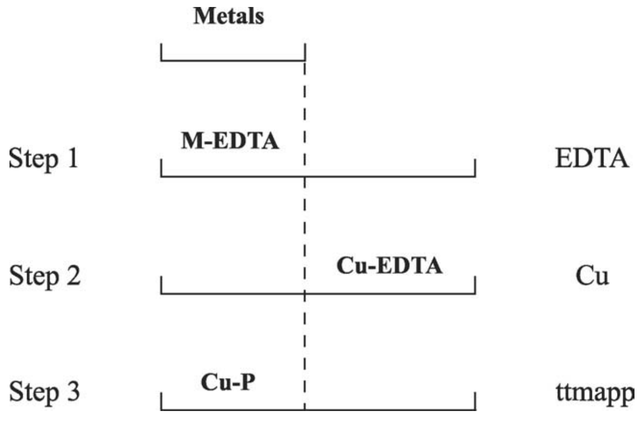

Fig. 1. Schematic illustration for principle of the indirect substitution method. The length of the solid lines represents the reagent concentration. M–EDTA, Cu–EDTA and Cu–P represent the complexes of EDTA with metals, EDTA with Cu and ttmapp with Cu, respectively.

2. Experimental

2.1. Instrumentation

Elutions were performed at a flow rate of 0.5 ml min^{-1} with a pump (CCPM, Toyosoda Industries, Japan), which was equipped with a 100 μl sample loop and a Rheodyne 7125 injection valve for sample injection. Column used for cation exchange chromatography was Partisil-10 SCX (4.0 mm × 250 mm, GL Science Inc., Japan), and for ion-pair chromatography ODS symmetry C_{18} (5 μm, 4.6 mm × 150 mm, Waters) and TSK gel ODS-120T (4.6 mm × 150 mm, Tosoh, Japan) column were used. Post-column FIA system consisted of a Gilson miniplus3 peristaltic pump (M&S Instruments Trading Inc.) and reaction coils (PTFE, 0.5 mm i.d.) of various lengths, with post-column reagents pumped at a flow rate about 0.06 ml min^{-1}. Signals were detected with an UV8020 photometer (Tosoh Co., Japan) and recorded with a type 3066 recorder (Yokokawa Elec., Japan). An UV8200 photometer (Shimadzu Co., Japan) was used for the photometric titration of ttmapp and other batch wise studies.

2.2. Reagents

All reagents, except for ttmapp, were purchased from Wako Pure Chemical Industries (Japan) and are of analytical grade. Tetratoluensulfonate salt of ttmapp was synthesized and purified in our laboratory following the procedure below: (1) *p*-dimethylaminobenzaldehyde (9 g) was dissolved in propionic acid (60 ml). The solution was refluxed for 30 min, into which pyrrole (2 g) prepared in propionic acid (10 ml) was added drop wise during the reflux. This solution was further refluxed for 30 min then was cooled down in ice bath, when a purple precipitate of *meso*-tetrakis (4-*N*-dimethylaminophenyl)-porphine (tdmapp) was obtained. This precipitate was separated by filtration and was washed with deionized water and acetone until the filtrate became colorless. (2) In methyl *p*-toluenesulfonate (10 ml), the obtained tdmapp was mixed and heated at 140 °C for 3 h, then this mixture was cooled down to room temperature. Pyridine was added drop wise into this mixture and a purple precipitate of ttmapp was obtained, which was filtrated and dried in vacuum. (3) This solid product was recrystallized from methyl alcohol.

The stock solution of ttmapp was prepared in 0.1 M nitric acid by dissolving 0.04 g of the synthesized powder into a 250 ml flask, the concentration of which was photometrically titrated with standard Cu(II) nitrate solution (for AAS, 1000 ppm) as 1.1×10^{-4} M following the absorption change at 433 nm (molar absorptivity: 4.8×10^{5} l mol^{-1} cm^{-1} for ttmapp) or at 411 nm (molar absorptivity: 4.6×10^{5} l mol^{-1} cm^{-1} for Cu(II)–ttmapp complex). The stock solution of EDTA (1.0×10^{-4} M) was prepared as aqueous solution of its disodium salt.

Eluent for cation exchange chromatography were prepared as aqueous solutions of 40% methanol (v), pH of which was adjusted to 4.6 with lithium hydroxide monohydrate. For isocratic elutions of the light, medium or heavy lanthanides, HIBA of 0.026, 0.04 or 0.08 M was added into the eluent and was degassed before use. Reagents used on post-column FIA system were prepared as follows: (1) EDTA (6.0×10^{-6} M) was prepared by diluting its stock solution in a buffer, which involves acetic acid (0.1 M), sodium acetate (0.2 M) and sodium hydroxide (0.12 M) making the main stream solution near pH 6.0 after the confluence; (2) Cu(II) (6.0×10^{-6} M) and ttmapp (4.4×10^{-6} M) were prepared as a mixture by diluting their standard or stock solution in 0.1 M nitric acid; (3) ascorbic acid (0.5%) was prepared by dissolving its sodium salt in a buffer, which involves acetic acid (0.025 M) and sodium acetate (0.8 M) making the mainstream solution near pH 5.0 after the confluence; (4) nitric acid was prepared as 1.9 M making the mainstream solution near pH 1.0 after the confluence. Solutions of the lanthanides were prepared by proper dilution from their respective standard solutions (for AAS). Deionized water was used throughout.

2.3. Procedures

2.3.1. Batch wise analysis of individual lanthanides

The applicability of this indirect substitution method for the analysis of individual lanthanides was verified through batch wise studies following a procedure below: (1) In a 50 ml flask, a sample solution (involving no more than 12.5 μg lanthanide) was prepared with ammonia buffer (pH 9) to give a total volume about 40 ml. (2) EDTA stock solution (0.5 ml, 1.0×10^{-4} M) was added to this solution, shaking about 20 s for complete reaction. (3) Cu(II) stock solution (0.75 ml, 7.9×10^{-4} M) was added next, shaking about 20 s for the complexation of Cu(II) with the remaining EDTA. (4) ttmapp (1.0 ml, 1.1×10^{-4} M), ascorbic acid (1.0 ml, 1%) and acetate buffer (pH 5.0) were added in sequence and mixed completely, holding the final solution for 5 m. (5) Nitric acid (2 ml) was added finally making pH value about 1.0. 6) The flask was filled up with water and the absorbance of this solution was measured at 433 nm.

Ascorbic acid was added to catalyze the complexation reaction of Cu(II) with ttmapp, the optimum pH for which ranges over pH 4.1–5.9 [29]. In this paper, pH 5.0 was used as the optimum. The Soret band of ttmapp appears around 411 nm at pH 5 and overlaps the spectrum of the formed Cu(II)–ttmapp completely, thus nitric acid was added to shift the Soret band to 433 nm ($\text{pH} \leq 1.2$) and the reaction was also quenched at the same time.

2.3.2. HPLC post-column FIA of lanthanides

HPLC post-column FIA system is schematically shown in Fig. 2. Reagents (R1–R4) were flowed into the mainstream of eluent at the same flow rate of 0.06 ml min^{-1}. Equal quantities of EDTA (R1, 6.0×10^{-6} M) and Cu(II) (R2, 6.0×10^{-6} M) were mixed in RC2 to form complexes of EDTA–Cu(II), thus no Cu(II) was available downstream for ttmapp (R2) to form complexes in RC3. Last step in this FIA procedure was the acidification of the mainstream in RC4 by nitric acid (R4), shifting the spectrum of ttmapp from 411 nm to 433 nm. Lanthanides ($\leq 1.4 \times 10^{-6}$ M) were injected (S) and separated on the column (C). The eluted lanthanides were mixed with EDTA in RC1, forming the complexes of EDTA–Lanthanide. Excess quantity of EDTA in the sample zone reacted with Cu(II) in RC2, where Cu(II) became excess and this excess quantity of Cu(II) should be equal to the quantity of the lanthanides injected. Under the catalysis of ascorbic acid (R3), this excess quantity of Cu(II) reacted with ttmapp in RC3 producing Cu(II)–ttmapp, the spectrum of which was separated from that of the remaining ttmapp by nitric acid in RC4. Lanthanides were detected out as the signals from the absorbance decrease at 433 nm.

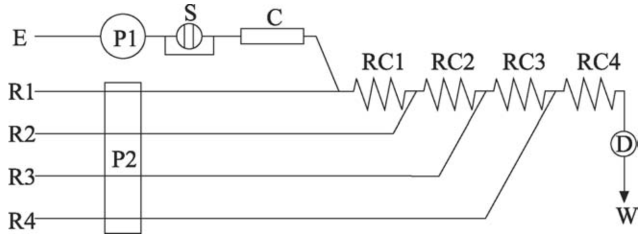

Fig. 2. Schematic diagram of HPLC post-column FIA system. E, eluent, aqueous mixture of methanol and HIBA; R1, EDTA; R2, mixture of Cu and ttmapp solution; R3, mixture of acetate buffer and ascorbic acid; R4, nitric acid; P1, HPLC pump; P2, peristaltic pump; S, sample injector; C, column; RC1–4, reaction coils; D, detector; W, waste.

3. Results and discussion

3.1. Batch wise analysis of individual lanthanides

The principle of this indirect substitution method is based on the stoichiometry of the three complexation reactions occurring in a sequence of EDTA with lanthanides, EDTA with Cu(II) and ttmapp with Cu(II). In most cases, this EDTA–Cu(II)–ttmapp reaction system is nonselective and all metals that can form highly stable or inert complexes with EDTA may respond to it. Therefore, the batch wise procedure was only performed as verification for the principle rather than practical use. The procedure proved to be convenient to evaluate the applicability of this indirect substitution method for the analysis of individual lanthanides, and also efficient for the pH optimization of the post-column FIA system, which was rather complicated to be dealt with in such kind of studies. Following the batch wise procedure, the responses of 14 lanthanides to this EDTA–Cu(II)–ttmapp reaction system were investigated at the optimum pH for the color reaction of ttmapp with Cu(II) (pH 5), and Fig. 3 shows the results of such indirect spetrophotometric titration for 3 species of Lu, Tb and La representing the heavy, medium and light group, respectively. Lu responded to the reaction system with a most sensitive and linear titration curve ($A = -5.078 \times 10^{5}\, C + 0.8026$, $C \leq 8.875 \times 10^{-7}$ M and $r^2 = 0.9986$, where A is the absorbance, C is the concentration of lanthanide), from which the responses of other lanthanides deviated gradually as atomic number decreases. The lightest La nearly showed no response to this reaction system. Such devi-

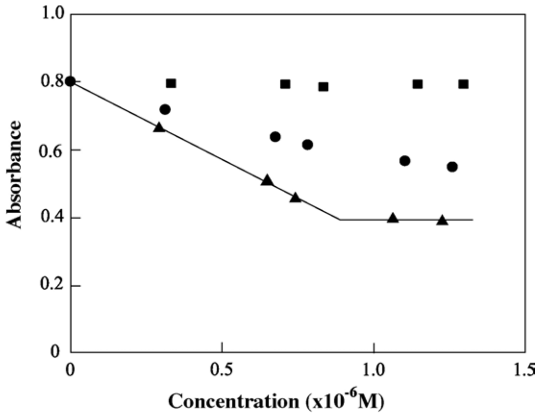

Fig. 3. Batch wise analysis of the individual lanthanides. [Cu] = 1.2 × 10^{-6} M, [ttmapp] = 2.2 × 10^{-6} M, [EDTA] = 1.0 × 10^{-6} M; acetate buffer (sodium acetate: 5.0 × 10^{-2} M, acetic acid: 2.5 × 10^{-2} M), pH 5.0; (■) La, (●) Tb, (▲) Lu.

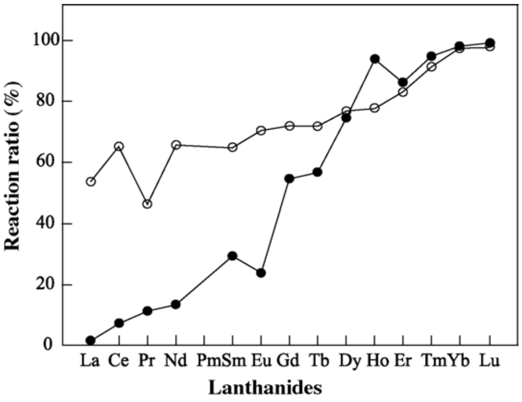

Fig. 4. Effects of pH on the reaction ratios of the lanthanides. (○) Ammonia buffer (1.0 × 10^{-3} M), pH 9; (●) acetate buffer, pH 5. Other conditions identical to Fig. 3.

ation is considered to occur in steps 2 and 3 (Fig. 1), where the formed EDTA–lanthanide complexes coexisted with excess quantity of Cu(II). This coexistence gives rise to an opportunity of electrophilic substitution between the excess Cu(II) and the EDTA–lanthanide complexes, leading to the sensitivity losses shown in Fig. 3. The EDTA–Lu complex is the most stable species among the EDTA–lanthanides [log $K_{\mathrm{La-EDTA}}$ = 19.80 (Lu)–15.64 (La)] and also more stable than EDTA–Cu(II) (log $K_{\mathrm{Cu-EDTA}}$ = 18.70) [31], thus it is considered that no electrophilic substitution occurred between Cu(II) and EDTA–Lu in the duration of their coexistence. This means the theoretical stoichiometry of the reaction system was obeyed in the case of Lu. Therefore, the titration curve of Lu was used as a standard to evaluate the reaction ratios of other lanthanides, which was calculated as a proportion of the slope of the response curve for each lanthanide ($C \leq 8.875 \times 10^{-7}$ M: the observed equivalent concentration) to that of Lu (defined as 100% reaction ratio).

The pH conditions in steps 1 and 2, where the complexation reactions of EDTA with the lanthanides and Cu(II) occurred consecutively, were investigated over a range of pH 3.0–10.0. The heavy group of lanthanides showed reaction ratios about 100% over wider pH ranges, among which Lu showed the maximum and stable reaction ratio (100%) over a range of pH 5.0–8.5. Such pH ranges became narrower with the decrease of atomic number, shifting slightly toward high pH side and the maximum reaction ratios also decrease. For Tb, the maximum reaction ratio about 73% was obtained over a range of pH 7.5–9.3. For La, the maximum reaction ratio of 55% was obtained only at pH 9.0, and decreased remarkably at higher or lower pH values. Based on these results, pH 9.0 was considered as the universal optimum pH for the complexation reactions of EDTA with all the lanthanides and Cu(II) on post-column FIA system. Fig. 4 shows the comparison of the reaction ratios obtained at pH 5.0 and pH 9.0 for each lanthanide. The deviations of the light and medium group occurred at pH 5.0 (Fig. 3) were suppressed at pH 9.0 to the maximum extent, but the determinations in rigid stoichiometry still could not be achieved. The effort to use some ligands, such as ammonia, to reduce the conditional stability constant of EDTA–Cu(II) also has been done but showed little effect.

3.2. HPLC post-column FIA of lanthanides

3.2.1. HPLC conditions

To investigate the optimum HPLC conditions, conventional post-column derivatization method using PAR was employed and the post-column FIA section of the apparatus shown in Fig. 2 was simplified to one channel for this purpose. Isocratic elutions of the lanthanides with water-based eluent proved to be successful by both cation exchange chromatography and ion pair chromatography, but it was found that such aqueous eluent was not fitful for the post-column FIA system because the color reagent of ttmapp was ready to adsorb to inner walls of PTFE flow tube. At least 30% (v) methanol should be involved in eluent to prevent the adsorption of ttmapp, while the addition of this amount of methanol made the separation of the lanthanides by ion-pair chromatography rather difficult. Therefore, cation exchange chromatography of the lanthanides was proposed for this indirect substitution method. Other organic solutions, such as ethanol, chloroform/methanol and acetonitrile were also investigated as eluent ingredients to solve the adsorption of ttmapp, but all these additives interfered the post-column complexation reaction of ttmapp with Cu(II) to some extent. A maximum of 70% (v) methanol in eluent was tolerable for the post-column FIA system and a minimum of 30% (v) was found necessary for solving the adsorption problem. Besides this, the addition of methanol also showed certain effect on suppressing band spreading of the eluted lanthanides. This was particularly preferable for this post-column FIA system, because the involvement of the multistep reactions after the column made the analysis a little time-consuming than conventional PAR method thus ready to suffer losses of resolution and sensitivity. Although higher composition of methanol in eluent could compensate such loss to a greater extent, eluent involving 40% (v) methanol was adopted on a consideration of analysis cost. Retention times for the lanthanides were also reduced slightly for the addition of methanol and no negative influence was observed on the signal resolutions of the eluted lanthanides.

The effect of HIBA concentration on the elution of the lanthanides was also investigated. The result showed that lanthanides were eluted in an order of Lu–La and retention times reduced with the increase of HIBA concentration. For the convenience of post-column FIA study and evaluation of the method, gradient elution was not performed and lanthanides were eluted isocratically with 0.026, 0.04, and 0.08 M HIBA for the respective lanthanide group to obtain complete elutions about 60 min. Further increase of HIBA concentration would reduce the color reaction rate of Cu(II) with ttmapp, which also depends on the catalyst concentration to be used.

3.2.2. Post-column FIA system

A four-channeled peristaltic pump was employed to construct the post-column FIA system to achieve the automation of the batch wise procedure for the analysis of lanthanides eluted from the column. The sequence of the multistep reactions and the precise control for the reaction time of each step were easily fulfilled on such continuous FIA system. The optimization of this post-column FIA system was carried out in 3 steps: (1) the optimization of the ascorbic acid-catalyzed complexation reaction of Cu(II) with ttmapp; (2) the optimization of the complexation reaction of Cu(II) with EDTA; (3) the optimization of the complexation reaction of EDTA with the eluted lanthanides.

Color reaction of ttmapp with Cu(II) catalyzed by ascorbic acid forms the basis of this post-column reaction system, serving to detect the signals corresponding to the eluted lanthanides. The principle of this method requires that all the complexation reactions in the system occur stoichiomitrically and on the other hand, the continuous FIA system imposes restriction on the complexation reaction rate. Therefore, concentration of ascorbic acid should be adopted as high as possible to catalyze the color reaction to be completed within a shortest period. By flowing water instead of EDTA, the optimum concentration of ascorbic acid and reaction coil length of RC3, which corresponds to the reaction time on FIA system, were investigated through monitoring the absorbance change at 433 nm owing to ttmapp. The complexation reaction ratio of ttmapp with Cu(II) could be estimated when the baseline, or the absorbance of ttmapp decreased to reach a stable state under the catalysis of ascorbic acid with certain concentration. Although ascorbic acid of higher concentration could catalyze the color reaction to be completed with shorter coil length, a blank absorption increased gradually at 433 nm when the concentration of ascorbic acid was raised above 0.5%. Such absorption may influence the analysis sensitivity depending on practical analysis conditions. For the convenience of discussion, a 0.5% ascorbic acid was used in this FIA system. Under the catalysis of 0.5% ascorbic acid, the reaction ratio of the complexation of ttmapp with Cu(II) reached to a maximum value by using a 5-m coil at RC3.

The chemicals used as substitution agent in this method are required to be able to form stable complexes with the lanthanides and Cu(II) within a minimum period of time. Among the complexation reagents investigated, e.g., EDTA, nitrilotriacetic acid (NTA), 1,2-cyclohexylenedinitrilotetraacetic acid (CyDTA) and diethylenetriaminepentaacetic acid (DTPA), EDTA proved to be appropriate for this purpose. By flowing EDTA from R1, the optimum conditions for the complexation reaction of EDTA with Cu(II) at RC2 was investigated. The maximum reaction ratio could be estimated from the baseline shifting, that is, the absorbance increase at 433 nm due to the complexation of EDTA with Cu(II). The signal reached near a maximum and stable state with 1-m coil of RC2, which was sufficient for the system because no obvious absorbance increase was observed with longer coils. The studies also showed that the reaction ratio did not vary noticeably in a wide pH range of 2.5–9.0, thus no special buffer needs to be used at R2.

The final step of the optimization for the post-column FIA system was to adjust the coil length and pH of RC1 to obtain maximum signals of the lanthanides eluted from column. A 1-m coil was proved sufficient for the complete reaction of EDTA with the eluted lanthanides, the signals of which reached near the maximum state and showed little change with longer coils. Batch wise studies have showed that pH 9.0 was the universal optimum for this step on a consideration of total lanthanides. Such high pH failed to be employed into this post-column FIA

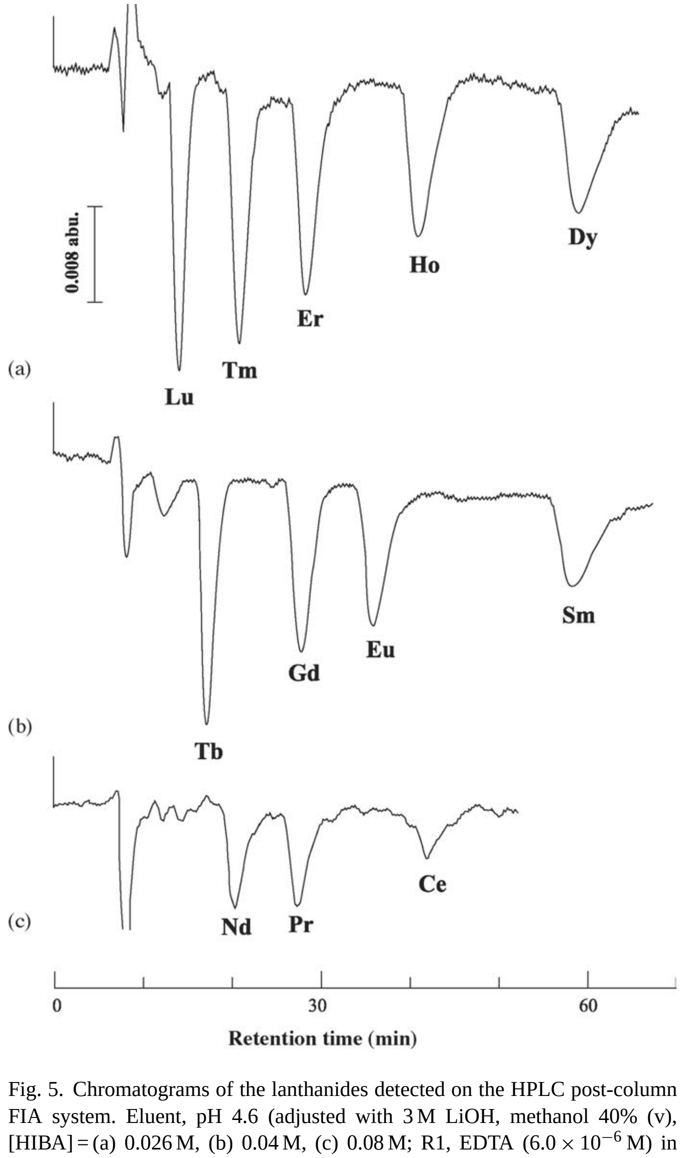

Fig. 5. Chromatograms of the lanthanides detected on the HPLC post-column FIA system. Eluent, pH 4.6 (adjusted with 3 M LiOH, methanol 40% (v), [HIBA] = (a) 0.026 M, (b) 0.04 M, (c) 0.08 M; R1, EDTA (6.0×10^{-6} M) in acetic acid (0.1 M), sodium acetate (0.2 M) and sodium hydroxide (0.12 M); R2, mixture of Cu (6.0×10^{-6} M) and ttmapp (4.4×10^{-6} M) in nitric acid (0.1 M); R3, ascorbic acid (0.5%) in acetic acid (0.025 M) and sodium acetate (0.8 M); R4, nitric acid (1.9 M); Sample concentration, 0.2 μg/ml for each lanthanides.

system due to the difficulty to choose a proper buffer solution with no interference on the following color reaction of ttmapp with Cu(II). The highest pH that the system could tolerate was found around pH 6.0, which was not a favorite value for the analysis of the light and medium group.

Based on the studies above, 13 species of lanthanides were eluted in 3 groups isocratically and were detected on the post-column FIA system followed. Chromatograms obtained are shown in Fig. 5. Similar to the result of the batch wise analysis, the detection sensitivity of these lanthanides were in sequence of the heavy, medium and light group. Yb also could be detected out with this system but the HIBA composition of eluent needs to be optimized for a better resolution to Lu. When the atomic number decreases, sensitivity loss occurred and La was not detected out under the conditions. The detection sensitivity of Tm, Tb and Nd were the highest within each lanthanide group, the calibration graphs of which are shown in Fig. 6 by comparison with the analysis result of Lu using conventional PAR method. Lu was used as a representative for the lanthanides in PAR method, which offers same sensitivity for all the lanthanides. The graph slopes for other species deviate from that of these 3 elements but not to a great extent. Compared with the result of PAR method, most of the lanthanides could be determined with greatly improved sensitivity by this indirect substitution method. Good linearity could be obtained up to 1.2–1.4 × 10^{-6} M. On the other hand, reasonable detection limits could not be obtained because the baseline noise caused by the postcolumn 4-channel peristaltic pump was difficult to control and needed some special techniques. In the case of Fig. 5 detection limits obtained was about 10 ng/ml (signal/noise = 2). To reduce noise level, detection wavelength could be changed to 411 nm by following the absorbance increase of Cu(II)–ttmapp complex, although the molar absorptivity at 411 nm is a little lower than at 433 nm.

The principle of this method is designed on the basis of stoichiometry of the three complexation reactions, but due to the electrophilic substitution, which occurred between Cu(II) and the complexes of EDTA–lanthanides with lower stability constants in the duration of their coexistence, made the analysis deviate from the stoichiometry. In the case of analysis only concerned with the light lanthanides, the extent of such deviation could be minimized through the adjustment of post-column FIA variables to suppress the electrophilic substitution extent. Other studies showed that the determination sensitivity for the light group could be raised about 3 times when the following post-column FIA variables were used: coil lengths of RC2 and RC3 were 0.8 and 3.0 m, respectively; the concentration of ascorbic acid was 5%. Lanthanum that could not be detected in Fig. 5 was also detected out apparently in this case. The reaction ratios of EDTA with Cu(II) and ttmapp with Cu(II) were reduced to some extent, whereas the analysis sensitivity was compensated due to the reduction of the coexistence duration of the EDTA–lanthanides and excess Cu(II).

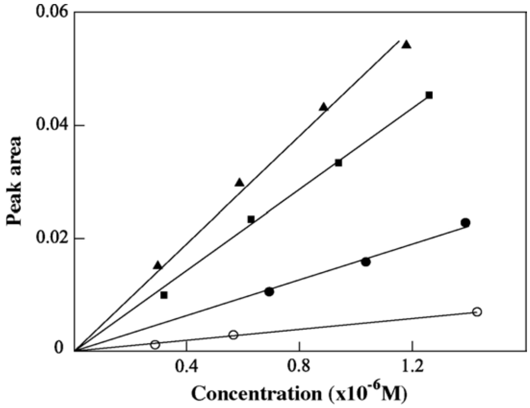

Fig. 6. Calibration graphs of the lanthanides and comparison of the sensitivity with the conventional PAR derivatization method. Reagent conditions used for the indirect substitution method are identical to Fig. 5., (▲) Tm, (■) Tb, (●) Nd. For conventional PAR method: eluent, [HIBA] = 0.026 M, pH 4.6; [PAR] = 0.2 mM in acetic acid (1 M) and ammonia (3 M), detected at 520 nm, (○) Lu; flow rate, P_1 = 0.5 ml/min, P_2 = 0.1 ml/min.

4. Conclusion

The analytical applications of the highly sensitive colorimetric reagent of porphyrin, molar absorptivity of which is about 5×10^5 l mol^{-1} cm^{-1}, were extended to a new field by the development of the novel indirect substitution methodology. The effectiveness of the method was verified through the determination of the lanthanides with a batchwise procedure, which was further applied to a HPLC post-column FIA system. The employment of flow injection technique simplified the procedure owing to the automation of the analysis. The system can be further optimized depending on the practical analysis requirement. Metals that can form highly stable or inert complexes with EDTA rapidly may respond to this EDTA–Cu(II)–ttmapp system, thus a more extensive application field of porphyrin may be expected.

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