Determination of Uranium in Seawater Samples by Liquid Chromatography using Mandelic Acid as a Complexing Agent

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Abstract

The determination of uranium at different stages of the recovery process as well as in seawater is important in its recovery study. A previous study developed a high-performance liquid chromatography (HPLC) method for uranium determination in seawater using α-hydroxy isobutyric acid as a chelating agent. However, this method causes turbidity in process samples containing high amounts of iron, resulting in the clogging of the HPLC column. In the present work, use of mandelic acid as a chelating agent for uranium has been explored. Elution conditions were optimized for the separation of iron [Fe(III)] and uranium [U(VI)] by studying the effect of an ion interaction reagent, the concentration of mandelic acid, and methanol content in the mobile phase. Different parameters were optimized to develop off-line pre-concentration of uranyl-mandelate on the reversed stationary phase. The method offers quantitative recovery of uranium and linearity in the U(VI) concentration range of 0.5 ppb to 500 ppb and can be used for the determination of U(VI) in process samples with Fe/U amount ratios up to 3,000. The method has been successfully used for the determination of U(VI) in seawater samples and process samples. The developed methodology was validated by comparing the results with those of isotope dilution-thermal ionization mass spectrometry.

Introduction

The determination of uranium (U) in natural water systems is of interest in view of its role as nuclear fuel and its toxicity (1,2). Recovery of U from seawater (present at a few ppb levels) is proposed as an option for increasing its availability to meet the future energy requirements. Though concentration of U in seawater is low, the advantages of the dissolved state and almost inexhaustible quantities of U in seawater appear interesting for U recovery. Furthermore, U extracted from seawater qualifies as a green fuel, since the process leaves no mill tailings at the recovery site and U fission in nuclear reactors generates electrical energy without CO_2 emissions (3–5). Hence, recovery of U from seawater has gained importance in recent years and different processes are in the development stage for recovering U in an economic way (5,6). This requires methodologies to determine U at different stages of the recovery process as well as in the starting material.

Methods involving liquid chromatography (LC) are reported in the literature for the determination of U in seawater and other aqueous samples (7–10). α-Hydroxy isobutyric acid (α-HIBA) was used for the determination of U in groundwater samples with cation exchange as well as reversed-phase stationary phases (7,8). Hao et al. studied the different hydroxy carboxylic acid ligands for the pre-concentration of U, and a ligand-exchange system was devised wherein the analytes were concentrated as mandelic acid complexes, and then separated as HIBA complexes (9). Shaw et. al reported the use of chelation ion chromatography for the separation and determination of U from spiked seawater samples and simulated samples containing a large amount of iron [Fe(III)] (10).

An HPLC method was developed in a previous study for the determination of U in seawater using α-HIBA as a chelating agent (11). This method required the use of α-HIBA of pH 6–7 for the effective pre-concentration of U onto the reversed-phase (RP) column. However, when determining U in process samples containing high levels of Fe(III), the method was found to be unsuitable due to the clogging of the pre-concentration column by the precipitate formed in the feed solution. Process samples were collected from various stages of U recovery from seawater. The stripping of U from various organic-based adsorbents previously immersed in seawater was carried out in an elution reactor in a 1–2 M HCl medium. The elute contained vanadium (0.06–0.8 µg/mL) and U (0.09–0.9 µg/mL), along with Fe(III) (12). The source of Fe could be due to the leaching from the reaction vessels used for the acidic stripping of U in the recovery studies. It is also reported that RP-based methods lose the resolution capacity in the presence of a high concentration of Fe(III), and thus separation of U is affected (10). Previously reported methods were found unsuitable for the adoption of process samples, due to the presence of large amounts of Fe. Though a method has been reported showing good selectivity for U in the presence of Fe, the detection limit of U by this method was 20 ppb, which is not sufficient for seawater analysis (10). Furthermore, the selectivity of U over Fe was achieved at acidic conditions (≥ 0.5 M) which is not tolerable in RP–HPLC systems.

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The objectives of the present work are: (*i*) good separation between U(VI) and Fe(III) so that accurate determination of U based on its chromatographic peak area is achievable; (*ii*) the possibility to introduce the samples at pH ≤ 4.0 so that formation of turbidity is avoided; and (*iii*) quantitative recovery of U during the pre-concentration procedure.

In the present work, use of mandelic acid as a chelating agent for U present at ppb levels in seawater was explored employing a C_{18} RP column. Though both α-HIBA and mandelic acid are hydroxy carboxylic acids, the latter being more hydrophobic offers stronger retentions for the U complex on the RP stationary phase, and thus provides better recovery of U at a relatively lower pH, which is essential to minimize the hydrolysis of Fe(III). The elution conditions were optimized by studying the concentration of mandelic acid, MeOH content in the mobile phase, etc. to provide good separation of U(VI) and Fe(III). The method offers excellent recovery of U from seawater and process samples containing large amounts of Fe. The method was validated by comparing the values with those obtained from isotope dilution–thermal ionization mass spectrometry (ID–TIMS). The methodology is attractive for the determination of U in seawater and process samples from different stages of U recovery. The present approach eliminates the use of two different chelating agents viz. mandelic acid for pre-concentration and α-HIBA for elution, as reported previously (9).

Experimental

Instrumentation

Chromatographic studies were carried out using an HPLC system consisting of an L-7100 gradient pump (Merck Hitachi, Tokyo, Japan), and a Rheodyne injector (Model 7725i) with a 100 µL sample loop. C_{18} monolithic RP columns (50 mm × 4.6 mm and 100 mm × 4.6 mm, Chromolith, Merck, Darmstadt, Germany) and a C_{18} particulate RP column (150 mm × 4.6 mm, Purospher STAR, Merck) were used as stationary phases. The eluent from the column was monitored employing a variable wavelength photometric detector (L-7420) after reaction with a post-column reagent (PCR), which was added with a Hurst piston pump (Princeton, Indiana) into a low dead volume-mixing tee (Valco, Texas). An isocratic pump with all SS contact parts (Model 501, Waters Corporation, Massachusetts) was used as the concentrator pump for delivering the sample solution through the column. A Finnigan MAT-261 (Thermo Electron, Bremen, Germany) thermal ionization mass spectrometer equipped with a multi-Faraday cup detection system, using a double filament assembly was employed for the isotope dilution experiments.

Reagents

Freshly deionized water, purified with a Milli-Q system (Millipore, Bengaluru, India), was used for all the dissolutions and dilutions. α-HIBA (Lancaster, UK) and 2-Hydroxy-2-phenylacetic acid or mandelic acid (E-Merck, Darmstadt, Germany) were used as the chelating agents. Tetrabutylammonium bromide (Fluka, St. Gallen, Switzerland) was used as the ion interaction reagent. High purity reagents such as HNO_3, NH_4OH (Suprapure grade, E. Merck), etc. were used for the sample treatment. MeOH (Gradient grade, Merck) was used as the organic modifier for the mobile phase. Arsenazo(III) (Fluka, Switzerland) was used as the post-column reagent (PCR). NaCl, KCl, $MgCl_2$, $Ca(NO_3)_2$ and $SrCl_2$ (Thomas Baker, Mumbai, India) were used for preparing the simulated seawater. The concentration of U in a uranyl nitrate stock solution was determined, as mentioned previously, by employing a biamperometric method (13). ^{233}U was used as the spike for the isotope dilution method. Uranium and Tetravalent Actinides–UTEVA resin (Eichrom Technologies, Inc., Lisle, IL) was used for the purification of U for the mass spectrometric analysis. The seawater samples and the process samples were received from the Desalination Division of BARC.

Procedure

The procedure for the pre-concentration of U in seawater was almost similar to that which was reported in a previous work (11). The appropriate quantity of mandelic acid was dissolved in water to prepare a 0.5 M. solution. Different solutions were adjusted to the desired pH using NH_4OH and HNO_3, and were filtered through 0.45 µm Millipore membrane filters. Appropriate quantities of salts were dissolved in water to obtain simulated seawater, with concentrations of Na^+, Mg^{2+}, Ca^{2+}, K^+, and Sr^{2+} as 10500, 1350, 400, 380, and 133 ppm (w), respectively (6). The uranyl nitrate stock solution was standardized and the PCR solution was prepared as per the procedure mentioned previously (14). PCR solution was delivered at a flow rate of 0.3 mL/min. The seawater sample was acidified to pH 2–3 using HNO_3 and was heated to boiling for 15 min. The solution was cooled to room temperature and was filtered through 0.45 µm filters. The filtered solution was made-up to known volume and was divided into two portions; one for HPLC analysis, and the other for ID–TIMS analysis.

Table I. Sequence of Operations Involved in the HPLC Analysis of Seawater/Processed Samples

Step	Mobile phase	Stationary phase	Flow rate (mL/min)	Volume (mL)	Remark
1 (Conditioning)	0.075 M mandelic acid of pH 4.0	100 mm x 4.6 mm C_{18} monolith column	1	10	Column connected to HPLC pump
2 (Loading)	Sample prepared in 0.075 M mandelic acid (pH 4.0)	100 mm x 4.6 mm C_{18} monolith column	1–7	5–30	Column connected to S.S. pump
3 (Washing)	0.075 M mandelic acid of pH 4.0	100 mm x 4.6 mm C_{18} monolith column	1	10	Column connected to HPLC system
4 (Elution)	Gradient as given in Table II	100 mm x 4.6 mm C_{18} monolith column and 150 mm x 4.6 mm C_{18} particulate column connected in series, in the same direction	1	20	Column connected to HPLC system

In the portion used for HPLC experiments, 0.075 M mandelic acid was added and the pH of the solution was adjusted to 4.0. The different steps involved in the HPLC analysis are summarized in Table I. A blank was determined with 0.075 M mandelic acid of pH 4.0 before and after the sample analysis under identical conditions. The quantification of U was based on the area of the peak from the chromatogram.

The fraction for ID–TIMS analysis was mixed with a known amount of a pre-calibrated ^{233}U spike. The mixture was treated with 8 M HNO_3 and evaporated to near dryness. The treatment with 8 M HNO_3 was repeated three times to ensure a proper isotopic exchange between the sample and the spike isotopes. The U concentration and matrix separation was carried out using UTEVA resin as per the procedure reported in the literature (15). The spiked mixture was evaporated, dissolved in 3 M HNO_3, and loaded onto UTEVA resin taken in a glass column (4 mm, i.d.). The washing of the matrix elements was carried out with 3 M HNO_3. Finally, U was eluted by 0.05 M ammonium oxalate solution. The eluate was evaporated to dryness and the residue was dissolved in 1 M HNO_3 for loading onto the sample filament of a double rhenium filament assembly for TIMS analysis. The sample and the ionization filaments were heated to temperatures corresponding to heating currents of 2.2 A and 6 A, respectively. The mean value of the $^{233}U/^{238}U$ atom ratio was determined by taking a run summary from three blocks, each block consisting of 10–12 scans.

Results and Discussion

The possible interferences of matrix elements were from Fe and vanadium in the process samples. It has been shown that vanadium does not interfere under the RP conditions employing hydroxy carboxylic acids as eluent, and hence it was not considered in the present study (11). Matrix elements in seawater such as Na, K, Mg, Ca, and Sr do not retain under present RP-conditions. Though Arsenazo(III) is a chromogenic reagent specific for lanthanides and actinides, many other elements including Fe(III) are also known to form colored complexes with it, albeit with less molar absorbtivity (16). It was found that the molar absorbtivity of Fe(III)–Arsenazo(III) was 446 mol^{-1} dm^3 cm^{-1} at 650 nm, at which U(VI) elution was monitored.

Fe(III), U(VI) Separation study

Separation of Fe(III) and U(VI) on a modified RP column

It was reported recently from our laboratory that uranyl-mandelate is anionic in nature and its adsorption onto the RP stationary phase increases in presence of a cationic ion interaction reagent (IIR) (17). No information was available on the retention behavior of Fe(III) under similar conditions. Hence, the retention of Fe(III) and U(VI) were compared on an RP column dynamically modified with tetrabutlyammonium bromide as the IIR. Figure 1 shows the changes in the retention time (RT) of Fe(III) and U(VI) as a function of the concentration of IIR in the mobile phase. It is seen that the presence of IIR does not influence the retention of Fe(III) and a fairly long retention (~23 min) was observed. It can be seen from the figure that the separation between Fe(III) and U(VI) was sufficiently high at an IIR concentration of 0.02 M. However, from a practical point of view, it would not be attractive to use a cationically modified column for pre-concentration purposes, as separation takes a very long time and the peak shape is broad. Nevertheless, this study demonstrated that the kind of interaction of Fe(III) and U(VI) with the stationary phase is different, and thus it was decided to study the effect of the concentration of mandelic acid and MeOH content on the retention of Fe(III) and U(VI).

Effect of concentration of mandelic acid on the retention of Fe(III) and U(VI)

Figure 2 shows the effect of the concentration of mandelic acid in the mobile phase (pH 2.5) on the retention of Fe(III) and U(VI) using a 5 cm RP column. During this study, 20% MeOH (v/v) was included in the mobile phase. The retention of both Fe(III) and

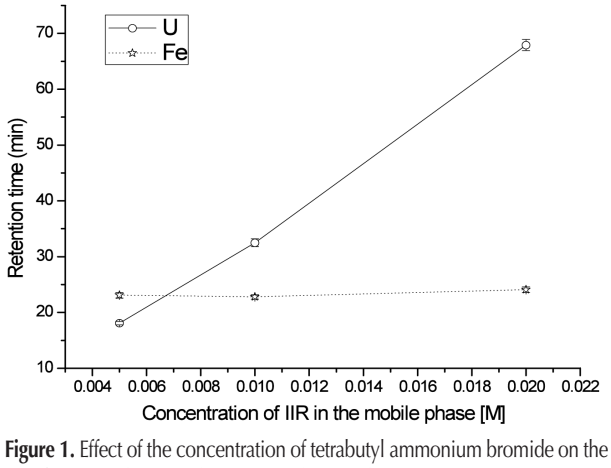

Figure 1. Effect of the concentration of tetrabutyl ammonium bromide on the RT of Fe(III) and U(VI). Chromatographic conditions: C_{18} (50 mm × 4.6 mm) chromolith column; 0.2 M mandelic acid (pH 2.5); flow rate: 1 mL/min; detection at 650 nm after post-column derivatisation with Arsenazo(III); 50 µg/mL each of Fe(III) and U(VI).

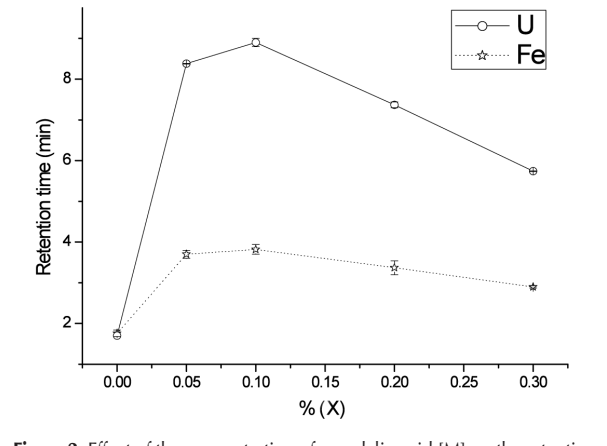

Figure 2. Effect of the concentration of mandelic acid [M] on the retention of Fe(III) and U(VI). Chromatographic conditions: C_{18} (50 mm × 4.6 mm) chromolith column; pH 2.5 mandelic acid, 20% (v/v) MeOH; 50 µg/g each of Fe(III) and U(VI); detection at 650 nm after post-column derivatisation with Arsenazo(III).

U(VI) shows a similar pattern with the change in the mandelic acid concentration in the mobile phase. In the absence of mandelic acid, both the ions elute out in the solvent front. As the concentration of mandelic acid increased from 0 to 0.1 M, the adsorption of both the ions increased due to the formation of a hydrophobic mandelate complex. However, as the concentration of mandelic acid increased further, the competition for the stationary phase from the undissociated mandelic acid molecules (due to pH 2.5) became significant, and the retention of both the ions decreased. Thus, 0.1 M mandelic acid was used in the mobile phase for further separation studies as it offers a good degree of resolution between Fe(III) and U(VI).

Effect of methanol content on the retention of Fe(III) and U(VI)

Figure 3 shows the effect of the retention of Fe(III) and U(VI) on an RP column as a function of MeOH percent (v/v) in the mobile phase. 0.1 M mandelic acid of pH 2.5 was used as the eluent. With the increase in MeOH content in the mobile phase, the retention of both Fe(III) and U(VI) decreased, with a drastic reduction in the RT of U(VI). These studies suggest that the use of a gradient system consisting of a gradually increasing mandelic acid (pH 2.5) concentration, and then a midway increase in MeOH content, will be appropriate for improving the peak shape of U(VI), and also for improving its separation from Fe(III). The gradient conditions were thus optimized for the separation of U, employing a C_{18} column as shown in Table II.

Preconcentration study

Since the present method employed mandelic acid, which forms complex with uranyl ion with greater hydrophobicity, operating parameters such as pH of mobile phase, concentration of chelating agent, sample-loading flow rate, volume of the sample solution and trace metal elution, etc., needed optimization. Due to previous experiences with α-HIBA, on-line coupling of the pre-concentration step with the HPLC system was not considered in view of the potential loss of uranyl-mandelate by adsorption onto the walls of PTFE and PEEK tubing (11). Since the SS pump used for the sample loading did not have a timer-control, the volume of the sample solution the passed through the pre-concentrator was determined by weighing the effluent. The selection of suitable elution conditions was required to evaluate the effect of all the parameters affecting the recovery of U by the C_{18} column.

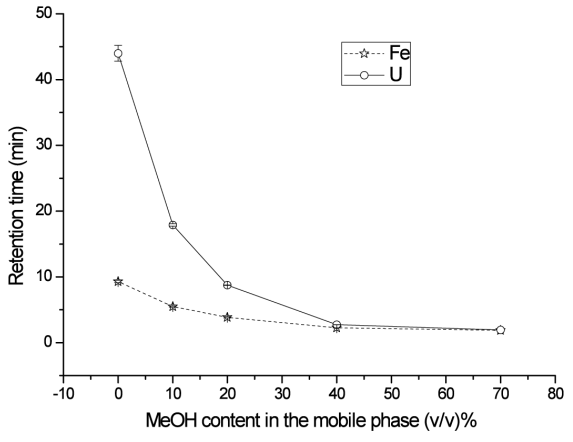

Figure 3. Effect of methanol content on the retention of Fe(III) and U(VI). Chromatographic conditions: C_{18} (50 mm × 4.6 mm) chromolith column; 0.1 M mandelic acid (pH 2.5); the rest of the conditions are the same as seen in Figure 2.

Suitability of eluent

It was reported by Hao et al. that mandelic acid is not a suitable ligand for the elution of U from a C_{18} column because of the very poor chromatographic efficiency (9). Therefore, a ligand exchange approach was done by incorporating α-HIBA as the eluting ligand for the separation step in their work. The RT of U on a 100 mm × 4.6 mm monolithic C_{18} column was determined employing different eluents such as 0.2 M α-HIBA (pH 2.5), 0.2 M mandelic acid (pH 2.5), MeOH (20%, v/v), and combinations of mandelic acid + MeOH and α-HIBA + MeOH. The RT of U(VI) was found to be 4.4 and 22.4 min using α-HIBA and mandelic acid (both 0.2 M and pH 2.5), respectively, while no elution was obtained using only 20% (v/v) MeOH until 35 min. Better elution efficiency of α-HIBA as compared to mandelic acid was attributed to the higher thermodynamic stabilities and the faster kinetics of the α-HIBA complexes. However, the presence of MeOH in the mandelic acid eluent drastically reduces the RT of U(VI) (also seen in Figure 3) and can become comparable to that obtained with α-HIBA. It was observed that the combinations of mandelic acid + MeOH and α-HIBA + MeOH resulted in the fast elution of U with RTs of 2.0 and 2.8 min, respectively. Hence, the combination of mandelic acid and MeOH is advantageous for efficient elution, apart from its ability to provide good separation between Fe(III) and U(VI). This implies that the use of ligand exchange was not very essential, and the use of α-HIBA was not explored for the separation of Fe(III) and U(VI). MeOH included in the mobile phase during the elution stage also improved the peak shape of U, and thus increased its sensitivity. Thus, the combination of mandelic acid (pH 2.5) and MeOH was used as the eluent for optimizing the parameters influencing the pre-concentration of U. These studies were carried out using a U(VI) solution prepared in simulated seawater.

Effect of concentration of mandelic acid on the recovery of U

The peak area per gm of a U(VI) solution, having passed through the concentrator column, was determined as a function of the mandelic acid concentration. Mandelic acid of pH 6.0 was used in this study, because α-HIBA showed the highest recovery

Table II. Optimized Gradient Condition for the Separation of U(VI)*

Time (min)	Mandelic acid (pH 4.0) [M]	Mandelic acid (pH 2.5) [M]	% MeOH (v/v)
0	0.075	0	0
3	0	0.18	15
19	0	0.18	35

* Chromatographic conditions: combination of 100 mm x 4.6 mm monolith C_{18} column and 150 mm x 4.6 mm C_{18} particulate column; mobile phase flow rate: 1 mL/min and post column reagent [0.15mM Arsenazo (III) and 0.01M urea in 0.1M HNO_3], flow rate: 0.3 mL/min.

of U at pH ≥ 6.0. Concentrator column conditioning, loading, and washing were carried out with mandelic acid of the given concentration. The elution was carried out by using the combination of mandelic acid (pH 2.5) and MeOH. The recovery was found to be satisfactory when the mandelic acid concentration was > 0.05 M. Thus, 0.075 M of mandelic acid was chosen for carrying out further pre-concentration studies.

Effect of pH of mandelic acid on the recovery of U

The effect of the pH of the conditioning and loading solutions on the recovery of U by the RP column is shown in Figure 4. The study was carried out by employing a 50 mm × 4.6 mm monolithic column. The column conditioning, U loading, and washing were carried out using 10 mL of 0.075 M mandelic acid of a given pH in the range of 2–6. When the pH of the solution was < 4, U was removed from the column during the washing stage itself. Good recovery was obtained with the mandelic acid solutions of pH 4–6. The increased dissociation of mandelic acid at higher pH levels helped in stronger complexation, and thus the retention increased. The strong hydrophobic nature of the mandelic acid complex enabled better retention and recovery at a relatively lower pH as compared to α-HIBA. Thus, pH 4.0 was chosen for carrying out further studies. A lower pH was preferred, as it would have minimized the hydrolysis of other metal ions present in the process seawater samples and prevented the formation of turbidity; however, α-HIBA required the pH to be in the range of 6.0–6.5 for an effective recovery of U. Though it is a practice to include a small percentage of methanol (1–5%) in the loading solution to wet the surface of the RP concentrator, it was not followed in the present study since the presence of MeOH adversely affected the breakthrough volume of the uranyl mandelate.

Effect of amount of the sample solution

This study was carried out to determine the maximum amount (volume) of the sample solution that can be passed through the column without affecting the quantitative recovery of U. This study was initially carried out employing a 50 mm × 4.6 mm column as the concentrator. The previously conditioned column was connected to the off-line pump and different volumes of simulated seawater containing U were passed through the column. The column was then subjected to washing, followed by elution as discussed previously. The shape and area of the U peak were examined over a range of 1 mL to 100 mL of simulated seawater containing 10 ppb of U(VI) at a flow rate of 1 mL/min. Though a linear relationship was observed between the U peak area and the loading volume up to 100 mL, it was found that the peak showed considerable broadening for volumes ≥ 50 mL. For example, the peak width was 1.6 min for 50 mL and was increased to 4.2 min for 100 mL. This observation indicated that though the concentrator column quantitatively retained U within the volume range studied, there was a definite spreading of the analyte taking place due to the self-elution by the loading/washing solution. This can have two deleterious effects: (*i*) restricting the detection limit of the method as a low-level concentration of the analyte would demand the passage of a large volume of the samples; and (*ii*) the probability for interference in view of the excessive broadening. Hence, it was decided to use a monolithic column of a larger dimension (100 mm × 4.6 mm) for pre-concentration and to introduce a particulate column for carrying out the separation. Table III shows the comparison of the U peak area as well as the peak width obtained for the passage of different amounts of U solution through the two different concentrator columns. It is shown that the peak area obtained using the combination of a 100 mm × 4.6 mm monolithic column for the concentration, and a 150 mm × 4.6 mm particulate column for the separation, yielded a better peak shape and a linear response for the sample solution amount up to 200 mL. This also indicates that in the case of the 100 mm × 4.6 mm monolithic column concentrator, the breakthrough volume for uranyl mandelate is greater than 200 mL.

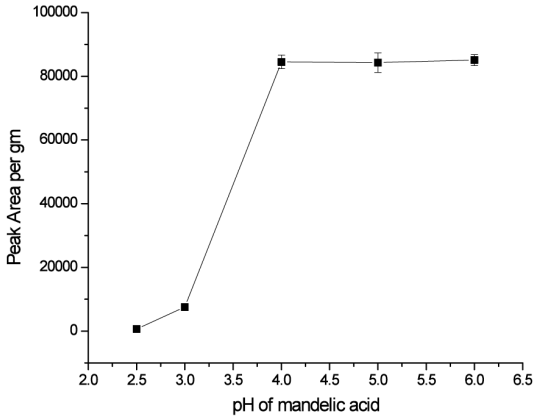

Figure 4. Effect of pH of mandelic acid on the recovery of U. Chromatographic conditions: 10 mL of 0.075 M mandelic acid of a given pH was used for conditioning, sample loading and washing of the concentrator column; 15 ppb U prepared in simulated seawater containing 0.075 M mandelic acid was passed through the concentrator column at a flow rate of 1 mL/min.

Table III. Comparison of the Data on the Response of U using Two Different Stationary Phases for Concentration and Separation*

50 mm × 4.6 mm monolith column as concentrator column and 100 mm × 4.6 mm monolith as separation column				100 mm × 4.6 mm monolith column as concentrator column and 150 mm × 4.6 mm particulate as separation column			
Amount of solution (gm)	Peak area	Area/gm	Peak width (min)	Amount of solution (gm)	Peak area	Area/gm	Peak width (min)
1.0045	68784	68476	1.3	0.9677	66792	69021	1.5
4.895	326409	66682	1.3	5.1083	330982	64793	1.6
25.0589	1676809	66915	1.5	25.098	1653834	65895	1.6
53.1483	3358753	63196	1.8	49.0583	3123911	63678	1.7
100.072	6189352	61849	4.3	94.911	6360941	67020	1.9
–	–	–	–	203.86	1.4E + 07	68033	2.3
Average area/gm		65424 ± 4%		Average area/gm		66407 ± 3%	

* Concentration of U: 10 ppb in simulated seawater solution. Elution conditions as per Table II.

In most of the reported methods, the elution of the analyte from the concentrator is done by back flushing (i.e., the sample loading is carried out in one direction and the elution is performed in the opposite direction) (8,9). It was decided to examine whether the back flushing of U-mandelate from the concentrator column helped in improving the peak shape. Chromatograms were recorded using a 50 mm × 4.6 mm monolithic column as the concentrator, and a 100 mm × 4.6 mm monolithic column as the analytical column. The pre-conditioned column was loaded with 50 gm of 5 ppb U(VI) prepared in simulated seawater containing 0.075 M mandelic acid with a pH of 4.0. In the first set of experiments, sample loading, column washing (10 mL), and elution were performed in the same direction of the flow. Subsequently, a chromatogram was obtained by carrying out the experiment in the same way, except the elution was carried out in the opposite direction of the sample loading. It was found that forward flushing yields sharper peaks as compared to back flushing, and hence this configuration of concentrator and analytical columns was used for further studies. The previously mentioned comparative study indicated that the diffusion of the analyte band must be occurring to a certain extent within the concentrator during the sample loading and washing stages for the entire range of injection volume studied.

Effect of the sample loading flow rate on the recovery of U

A 100 mm × 4.6 mm C_{18} monolithic column was used for carrying out pre-concentration whereas a combination of this monolithic column and a 150 mm × 4.6 mm C_{18} particulate column was used for the separation of the retained species. Due to its high porosity, the monolithic column offered the advantage of a low-pressure operation even at high sample loading flow rates (< 90 bar at 7 mL/min). Use of a particulate column with a greater capacity ensured good separation between U(VI) and Fe(III), even when the latter was present at a higher proportion. Thus, the use of the monolithic column in combination with the particulate column shortened the overall analysis time, ensured the complete transfer of analytes to the separating column, and offered better efficiency for separation. The conditioned monolithic column was connected to a pre-concentration pump. Approximately 10 mL of uranyl solution (5 ppb), prepared in simulated seawater containing 0.075 M of mandelic acid and pH adjusted to 4.0, was passed through the column at different flow rates in the range 0.5 mL/min to 7.7 mL/min. After the loading process, the washing and elution of the column was done as described previously. The uranyl peak area/gm of the sample solution passed was determined as a function of flow rate of the loading solution. It was observed that the sample loading the flow rate did not affect the recovery of uranyl-mandelate on the stationary phase up to 7.7 mL/min, which was the maximum flow rate possible with the concentration pump. The combination of the C_{18} monolithic column with a high permeability as a concentrator, and mandelic acid as a ligand forming a strong hydrophobic complex with U(VI), resulted in a pre-concentration system offering the flexibility of a sample loading rate depending upon the concentration and volume of the sample solution.

Table IV. Comparison of Peak Area/mL Obtained for U Under Different Injection/Loading Conditions*

Mode of sample introduction	Injected volume	Peak area	Area/mL
Direct injection	100 µL	64890	648900
using Rheodyne	2 x 100 µL	129038	645190
	5 x 100 µL	320517	641034
	15 x 100 µL	965698	643799
Off-line pre-concentration	3.8 mL	2453576	645678
pump	7.9 mL	5134080	649884
	16.0 mL	10532784	658299

* Concentration of U: 100 ppb in simulated seawater solution. Elution condition as per Table II.

Linearity and reproducibility of the method

The method optimized for the determination of U in seawater and process samples based on the above set of experiments is summarized in Table I. Linearity of the method with respect to the concentration of the uranyl solution was examined by loading 10–25 mL of simulated seawater samples containing a uranyl ion in the concentration range 0.5 ppb to 1000 ppb at a flow rate of 3 mL/min. A linear relationship between the peak area/gm of sample solution for U concentration was observed from 0.5 ppb to 500 ppb. The linearity plot of peak area versus concentration of U(VI) was generated over a narrow concentration range of 0.5 ppb to 50 ppb and the linear least squares regression analysis equation obtained was $Y = (2486 \pm 27) \times X$ and $R = 0.9998$. At concentrations above 500 ppb, the U peak area response showed saturation trends. The simulated seawater solution containing 5 ppb of U was repeatedly analyzed ($n = 12$) as per the previously mentioned method over a period of two days, and the reproducibility (% RSD) on the U peak area/gm of sample solution was found to be 3.6%. The detection limit of the method was found to be 0.2 ppb of U, using 30 mL of simulated seawater passed through the pre-concentrator, and considering the S/N of 3.

The quantitative recovery of U by the optimized method was also verified. 100 ppb of U solution prepared in a simulated seawater solution was injected directly through the 100 µL loop connected to the Rheodyne injector, and the chromatogram was recorded by running the elution gradient. Subsequently, multiple injections were given through the same 100 µL loop before carrying out the elution. Later on, the conditioned concentrator column was mounted onto the pre-concentration pump and known amounts of uranyl solutions were passed through the column, followed by washing, as mentioned previously. The loaded pre-concentrator column was then connected to the HPLC system and elution was performed. The area/mL obtained for the U peak under different injection/loading conditions are compared in Table IV. It is shown that the method offered a quantitative recovery for U from a simulated seawater solution.

Effect of Fe(III) on the recovery of U(VI)

One of the objectives of the study was to determine U in process samples containing larger proportions of Fe(III). Thus studies were carried out to find out the effect on recovery/quantification of U by including varying proportions of

Fe(III) and U(VI) in the simulated seawater containing 0.075 M mandelic acid and pH 4.0. Fe(III) to U(VI) amount ratio in the simulated sample was varied from 0 to 3000. The concentration of U was maintained at 10 ppb in all cases, and about 10 mL of the sample solution was passed through the column. The column conditioning, washing, and elution were performed as described previously. It was shown that the U peak area was not affected by the presence of Fe(III) in the sample up to a Fe/U amount ratio of 3000. In the chromatogram obtained for the simulated seawater sample containing Fe(III) and U(VI) in the proportion 3000:1, there was enough time difference (~1 min) between Fe(III) and U(VI) peaks to assure the unbiased determination of U. It was observed during these experiments that one of the extensively used columns exhibited a shorter RT (~14 min), compared to a relatively new concentrator column (~17 min, Figure 5B). However, there was no significant difference in the performance of the two columns during the pre-concentration of U(VI) from volumes as large as 200 mL of solution.

Analysis of seawater samples

The treated seawater samples were mixed with the required quantity of mandelic acid and pH of the solution was adjusted to 4.0. The HPLC analysis was performed as per the procedure discussed in Table I. Two seawater samples, and the two process samples were analyzed for the U concentration by the developed method. The process sample was 40 times diluted before passing it through the concentrator column. Figures 5A and 5B show the typical chromatograms obtained for the seawater sample and the process sample, respectively. As shown, the U peak was well separated from the Fe peak in the case of the process sample. The concentration of U in seawater was determined by a standard addition method employing HPLC. The results obtained by HPLC and ID–TIMS are given in Table V. It is shown that within the measurement of uncertainty, the results compare well between the two methods. A standard addition procedure was preferred, instead of internal calibration in HPLC, since the latter would depend on identifying another element with behavior nearly similar to that of U(VI).

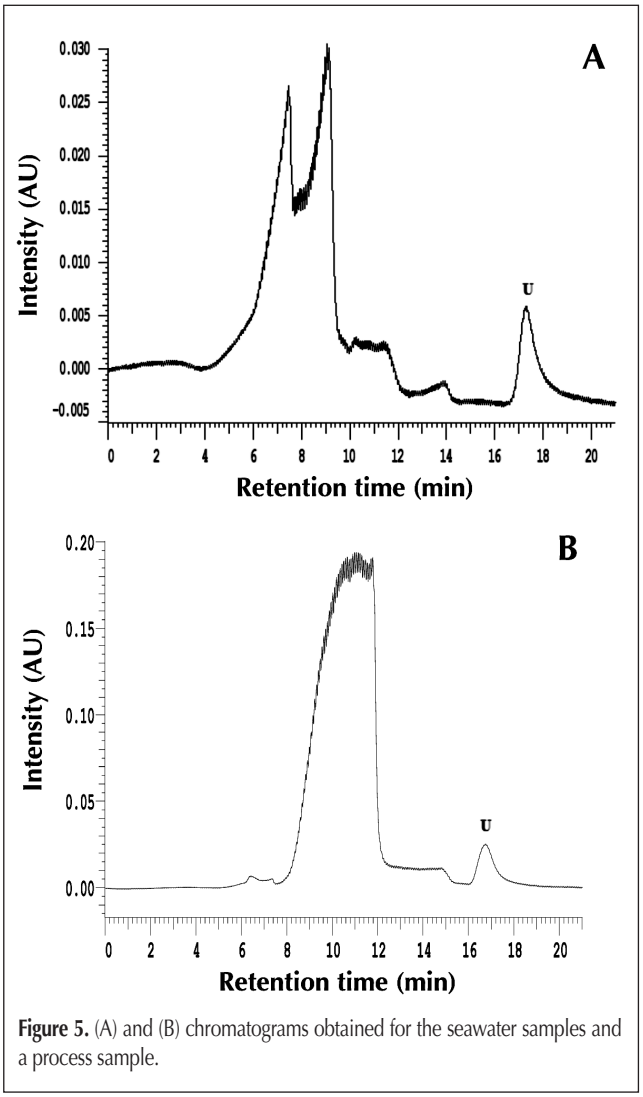

Figure 5. (A) and (B) chromatograms obtained for the seawater samples and a process sample.

Conclusion

Mandelic acid was used as a chelating agent for the U concentration and separation using a C_{18} RP column. The elution conditions were optimized by studying the effect of the concentration of mandelic acid, MeOH content in the mobile phase, etc., for the separation of U(VI) from Fe(III). The pre-concentration method for U is robust in terms of flow rate and volume of sample, and offers a tolerance of pH up to 4. The optimized LC separation offers the quantification of U in the presence of Fe up to an amount ratio of 3,000. The approach offers quantitative pre-concentration of U in the concentration range of 0.5 to 500 ppb. The method was validated by comparing the values with those obtained from ID–TIMS, and the methodology was applied for the determination of U in seawater and the process samples.

Matrix elements such as Na, K, Mg, and Ca in seawater often cause instrumental drift, isobaric polyatomic interferences, and signal suppression in the determination of trace levels of U by various analytical techniques such as ICP–MS, ICP–AES, etc. The present HPLC approach overcomes all these limitations in an economically viable way. The sample pre-concentrated by HPLC column can be introduced to AAS or ICP–MS, with the latter providing better selectivity.

Table V. Concentration of U in Seawater and Processed Samples Obtained by HPLC and ID-TIMS

Sample code	U Concentration (ppb)	
	HPLC*	ID-TIMS†
SW-1	3.1 ± 8%	3.2 ± 6%
SW-2	13.5 ± 7%	12.2 ± 5%
SWP-3	769 ± 3%	767 ± 2%
SWP-4	6.9 ± 6%	7.8 ± 5%

* Concentration determined by standard addition method.
† Mean of three determinations.

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