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Determination of Cadmium, Copper, Lead and Zinc by Flow Voltammetric Analysis

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Flow analysis with voltammetric system for determination of cadmium, copper, lead and zinc has been developed. Mercury film working electrode (MFE) was prepared by on-line mercury deposition on a glassy carbon electrode (GCE). Cadmium, copper, lead and zinc were monitored simultaneously by anodic stripping voltammetry (ASV) after a sample was flowed through the electrochemical cell for deposition of the metals on mercury film. Detection limits for cadmium, copper, lead and zinc were 4, 7, 1 and 15 ppb, respectively, for deposition time of 20 s. Linear range of calibration graph for all metals was up to 100 ppb. The precisions (%RSD, n=11) for 25 ppb of the metals were 2-6%. The system has been applied to determine the trace metals in drinking water and wastewater samples.

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Trace heavy metals are very important in the environment due to their serious toxicity although presenting at very low concentrations. They may accumulate in food chain. The development of new methods for quantifying trace metals is required and challenged. Most of the sensitive and selective methods recently available such as ICP-MS, ICP-AES and GF-AAS are too expensive and are not practically applied in a developing country, i.e. Thailand. Alternative cost-effective methods based on combination of flow techniques for sample pretreatmeet e.g. preconcentration/separation with some spectrometric detection systems have been proposed.[1-3]

Voltammetry, especially anodic stripping voltammetry is a promising technique for the determination of trace elements.[4] It is relatively low-cost but provides high sensitivity and can be simultaneous determination of multi-elements. However, batch voltammetry consists of a time consuming and a tedious analysis procedure. Flow techniques have been applied to improve the performance of voltammetry.[5,6] For example, lead in blood was determined by hydrodynamic voltammetry in flow system which could be automated and easy to use.[7] Trace copper in water has been determined in a flow system after on-line extraction as its diethyldithiocarbamate into toluene.[8]

In this work, attempts have been made to develop a flow anodic stripping voltammetry for the simultaneous determination of cadmium, copper, lead and zinc. Conditions of the system were investigated. Several advantages were gained such as better sensitivity and peak resolution, lesser sample and reagent consumption, and faster than a batch procedure. Mercury film electrode can be repeatedly employed for more than 80 times. The proposed method has been applied to determine the trace metals in drinking water and wastewater samples.

Experimental

Chemicals

All chemicals were of analytical reagent grade and Milli Q water (Millipore) was used throughout for preparing of solutions. Acetate buffer (1 M, pH 4.6) was prepared by dissolving 68.04 g of sodium acetate trihydrate (Merck, Darmstadt, Germany) in water and glacial acetic acid (Merck) was added until pH of solution was 4.6, then diluting to 500 ml with water. The solution was then made up to 1000 ml with water. Other concentrations of buffer solutions were prepared by diluting 1 M buffer with water. Mercuric nitrate (Merck) was used to prepare a plating solution. Oxygen free nitrogen gas (99.9995 % N_2) was used for purging dissolved oxygen from a solution. A mixed standard stock solution of 100 ppm of cadmium, copper, lead and zinc were prepared from a 1000 ppm standard solutions for AAS of each ion (Merck). Other concentrations of mixed standard solutions were prepared by appropriate diluting the stock solution. Bottled drinking water samples were purchased from locally department stores.

Flow manifold

Two lines manifold was designed as shown in Fig. 1. Sample and acetate buffer solution were pumped with the same flow rate to merge together and flowed further to a mixing coil and then to a flow through electrochemical cell. Three electrodes cross flow cell (BAS, Indiana, USA) was employed. A voltammograph (BAS CV-50W, BAS) was used for square wave anodic stripping voltammetric analysis. All potentials were measured with respect to a Ag/AgCl reference electrode. A potential of working electrode, mercury thin film coated on a glassy carbon electrode,

was set at −1100 mV for a specified (deposition) time while the sample was flowing through the electrochemical cell. Then the flow was stopped and a square wave potential waveform (−1100 mV to +280 mV) was applied to the working electrode with a scan rate of 75 mV min^{-1} while current was measured, resulting a voltammogram. The flow was started again to clean the electrode and the line, and to prepare for the next sample. For electrochemical cleaning, the potential of the working was set to 0 mV for 20 s.

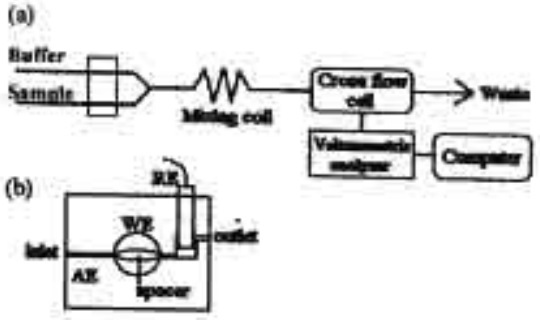

Fig. 1 Flow diagram of flow voltammetric system: (a) flow manifold and (b) details of flow cell; AE = auxiliary electrode, WE = working electrode and RE = reference electrode.

Analysis of samples

The drinking water samples were degassed by purging with oxygen free nitrogen gas before subjecting to analyze by the proposed system. The samples were also analyzed by graphite furnace atomic absorption spectrometry (GF-AAS) as a standard method for comparison. The recommended conditions for GF-AAS were employed.[9] Wastewater samples were digested before taking to analyze by the above methods. UV digestion was carried out by adding hydrogen peroxide (30%w/v/) to the sample and irradiating by UV in a home-made UV digestion unit for 6 h. The digested solution was put in a 50 ml volumetric flask and diluted to the mark with Milli Q water.

Results and discussion

Preparation of mercury film electrode

Mercury film coated on a glassy carbon electrode (GCE) was used as a working electrode (WE). Coating of Hg on a GCE was flowing a plating solution (500 ppm Hg^{2+} in a 0.1 M HNO_3) carried out by applying potential of -800 mV to GCE while through the flow cell. The resulted WE was tested for the determination of 100 ppb Cd standard solution. The WE could be repeatedly used for at least 80 analysis cycles, without the deviation of the peak height obtained more than 2% as shown in Fig. 2. The electrochemical cleaning of WE by applying a potential of 0 mV for 20 s after each analysis run was necessary for preventing of cross contamination of the metals.

Optimization

Flow rate. Flow rates of buffer and sample solutions were set to be equal. Total flow rate of the system was optimized in order to get higher peak current and good reproducibility. It was found that the higher flow rate provided the higher sensitivity and sample through put, but for a flow rate of more than 0.5 ml min^{-1}, the deterioration of mercury film was observed and leading to low reproducibility of the results. A total flow rate of 0.5 ml min^{-1} was chosen for further studies.

Concentration of buffer solution. Acetate buffer concentrations (0.2-1.0 M) were studied. The effect of buffer concentration on peak current of 70 ppm Cd and Pb was shown in Fig. 3. 0.5 M acetate buffer was chosen because the higher sensitivity and reproducibility of peak heights were obtained.

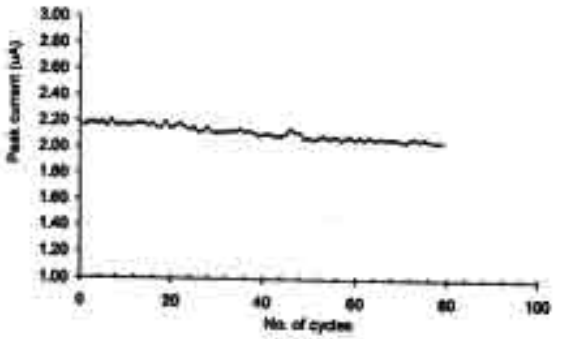

Fig. 2 Peak current of 100 ppb Cd for several analysis runs using the same mercury film.

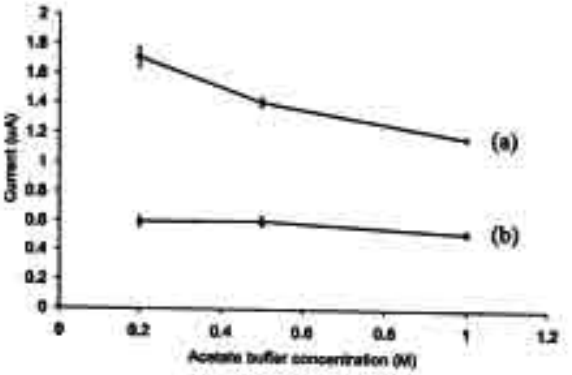

Fig. 3 Effect of buffer concentration on peak current of: (a) Cd and (b) Pb.

Mixing coil length. Mixing coils of 0, 50, 100 and 150 cm long were tried. The length of mixing coil did not much affect the peak height, but did affect the precision of the analyses. The mixing coil of 100 cm long was chosen due to the lowest %RSD of peak heights for all metals and rapid analysis were obtained.

The optimum conditions for flow anodic stripping voltammetry were summarized in Table 1.

Table 1 Optimum conditions for flow voltammetric system for the determination of Cd, Cu, Pb, and Zn.

Mercury plating	500 ppm Hg(II); -800 mV;10 min
Electrolyte	0.5 M acetate buffer pH 4.6
Flow rate (total)	0.5 ml min^{-1}
Mixing coil length	100 cm
Electrolysis potential	-1100 mV vs Ag/AgCl
Stripping (sweep) potential	-1100 to 280 mV
Sweep mode	Square wave
Sweep rate	75 mV s^{-1}

Calibration graph and precision

Using the conditions in table 1, a series of mixed standard solutions of cadmium, copper, lead and zinc (5, 20, 50, 70 and 100 ppb) was performed. The voltammograms obtained are depicted in Fig. 4. A calibration graph of each metal was constructed by

plotting peak currents obtained (μA) versus concentrations (ppb). The linear ranges, calibration equations, R^2 and detection limits (3σ) are presented in Table 2.

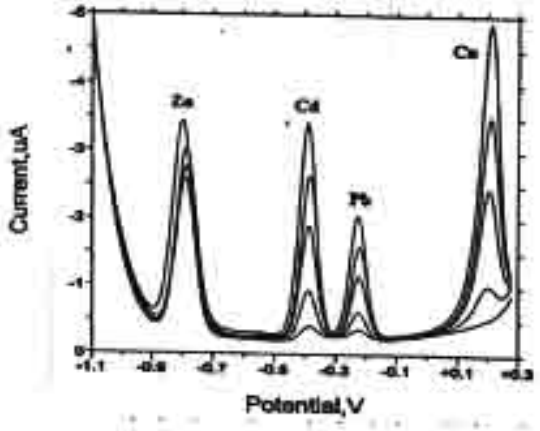

Fig. 4 voltammograms obtained from flow-ASV system; 5, 20, 50, 70 and 100 ppb each element.

Table 2 Calibration data for various elements.

Element	Linear range	Equation	R^2	Detection limit (ppb)
Cd	5-100	y=0.032x + 0.05	0.9956	4
Cu	10-100	y=0.043x + 0.50	0.9982	7
Pb	5-100	y=0.017x + 0.02	0.9986	1
Zn	20-100	y=0.007x + 1.20	0.9440	15

Precision of the method was evaluated by 11 replicate determinations of 25 ppb each element in a mixed metals standard solution. The relative standard deviations of Cd, Cu, Pb and Zn were found to be 3.8, 6.2, 3.6, and 3.0, respectively.

Analysis of samples

The results of the analyses of drinking water samples are presented in Table 3. Concentrations of the metals in bottled drinking water samples were less than detection limits, except Zn in Mont Fleur drinking water.

Table 3 Concentrations of metals in drinking water samples.

Sample	Concentration found (ppb)			
	Cd	Cu	Pb	Zn
Cooly fresh	-	-	-	-
Aura	-	-	-	-
Polaris	-	-	-	-
Mont Fleur	-	-	-	112
Hawiian	-	-	-	-
Minere	-	-	-	-
Vittel	-	-	-	-
Perrier	-	-	-	-
S. Pellegrino	-	-	-	-

- = below detection limits.

Concentrations of Cd and Pb in digested wastewater samples are summarized in Table 4. The results obtained agreed with those obtained from GF-AAS method.

Table 4 Concentrations of Cd and Pb in digested wastewater samples.

Sample	Concentration found (ppb) by method		
	GF-AAS	Flow-ASV	%different
Cadmium			
S1	25	26	4
S2	50	54	8
S3	7	4	-42
Lead			
S1	100	92	-8
S2	200	196	-2
S3	20	28	40

A flow voltammetric system was developed. Under suitable conditions, the system could be applied for the simultaneous determination of trace cadmium, copper, lead and zinc using square wave anodic stripping voltammetric mode. The sensitivity and selectivity (peak resolution) were improved from the conventional batch voltammetric system because a large area and thin mercury film electrode was employed, respectively. The same mercury film could be repeatedly used for at least 80 analysis runs. The proposed procedure consumed less sample and reagent, and was faster than a batch method. The system provided opportunity for automation and incorporation of on-line sample pretreatments.

Acknowledgements

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References

1. K. Grudpan, J. Jakmunee, and P. Sooksamiti, *Lab. Robotics and Autom.*, **1998**, *10*, 25.
2. P. Sooksamiti, H. Gekies, and K. Grudpan, *Analyst*, **1996**, *121*, 1413.
3. K. Grudpan, J. Jakmunee, and P. Sooksamiti, *J. Radioanal. Nucl. Chem.*, **1998**, *229*, 179.
4. A.W. Bott, *Curr. Sep.*, **1995**, *14*, 24.
5. C. Colombo, C.M.G. van den Berg, and A. Daniel, *Anal. Chim. Acta*, **1997**, *346*, 101.
6. J. Alpizar, A. Cladera, V. Cerda, E. lastres, L. Garcia, and M. Catasus, *Anal. Chim. Acta*, **1997**, *340*, 149.
7. S. Jacnicke, R.M. Sabarathinam, B. Fleet, and H. Gunasingham, *Talanta*, **1998**, *45*, 703.
8. T.J. Cardwell, I.H. Santos, and A.M. Bond, *Anal. Chim. Acta*, **1997**, *340*, 169.
9. *Analytical Methods for Furnace AAS B332*, Perkin-Elmer, Norwalk, CT.

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A Cost-Effective Gravitational Field-Flow Fractionation System

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Various possibilities for cost-effective field-flow fractionation (FFF) systems are proposed. Different components of the Gravitational FFF (GrFFF) systems are discussed. A simple GrFFF channel could be made of a cut plastic sheet of transparency and sandwiched by two lucite pieces. A gas pressure system from a gas cylinder (e.g. N_2) can be an alternative means for pumping. HPLC UV/Vis detector can be used for monitoring the particles. The read-out can be either a chart recorder or a simple computer-interfacing device. The proposed cost-effective system can be applied to separate Silica Gel 60G.

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Various separation techniques have been applied to different situation such as environment, pharmaceuticals, biotechnology and industry. Those include liquid chromatography, gas chromatography, electrophoresis, size-exclusion chromatography and field-flow fractionation.[1] The latter is relatively new. Field-flow fractionation (FFF) is commonly used for separation and characterization of various particles and macromolecules.[2,3] FFF instrumentation may be similar to HPLC. Although in some cases, FFF systems may be more complicated. There are now FFF instruments commercially available,[4,5] but rather expensive, especially in Thailand, therefore we are interested in making use of existing components, not only for basic research but also in term of instrumental development. We have experienced in development of low cost instrumentation.[6-8] In this paper, we outline the possibilities of cost-effective set-ups for gravitational FFF (GrFFF).

Experimental

A GrFFF system usually consists of components, represented schematically in Fig. 1.[9-12]

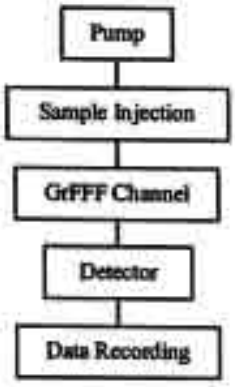

Fig. 1 Schematic diagram of a simple GrFFF system.

Possibilities of Components of a GrFFF system

Pumping system. A propelling device may be one of the following:

A gas pressure device: gas pressure based propulsion may utilize a cylinder of an inert gas e.g. N_2 to propel a carrier solution.[6,13]

A peristaltic pump: a peristaltic pump may be used.

A HPLC pump: a HPLC pumping system, a reciprocating pump, a syringe pump or a displacement pump, may be employed.[14]

Sample injection system.

Homemade injection device: an injection port can be homemade. A sample is injected into carrier stream via silicone rubber septum by a syringe with a hypodermic needle, through the port (Fig. 2) made of a plastic block.

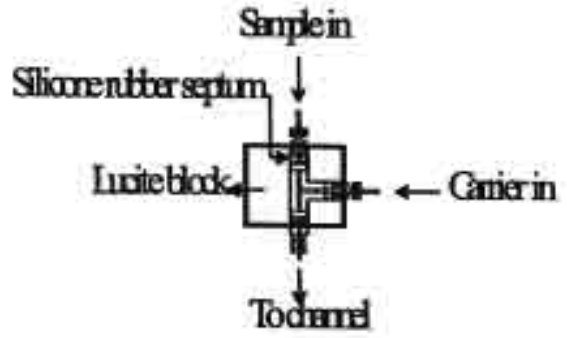

Fig. 2 A homemade injection port.

6-port injection valve: this type of valve is commercially available from various suppliers.

FFF separation unit

GrFFF channel: A simple channel can be assembled by using an overhead transparency sheet and two pieces of lucite blocks. The transparency sheet, usually 0.01 cm thick, can be cut to be a spacer and the lucite pieces (approximately 1 cm thick each)

are served as two walls. The spacer is sandwiched in between the two lucite walls and tightened with the bolts. The channel is shown in Fig. 3.

A detector. The following detectors may be employed: an UV/Vis detector, a fluorescence detector or a light scattering detector.

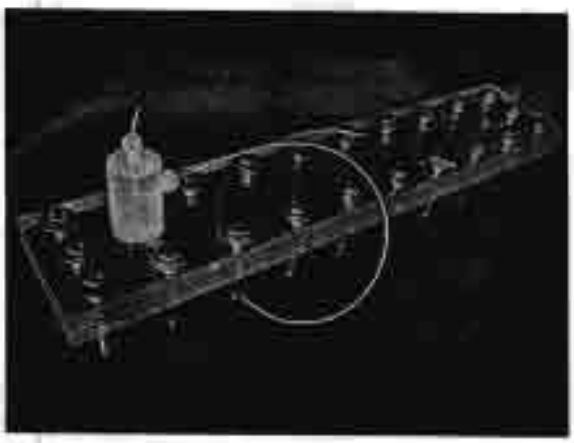

Fig. 3 GrFFF channel.

Data recording. Acquisition/read-out unit could be: a chart recorder, low-cost interfacing device (simple computer-interfacing device such as Pocket Sampler, which is commercially available (Dickamith, Australia)) and the LabVIEW (National Instruments™, USA).

Results and Discussion

A simple GrFFF system may compose of five components (Fig. 1). We set up a system by using alternative devices for each part.

As an alternative, using a cylinder gas, which can be generally available in any laboratory, N_2 gas-pressured propulsion to deliver the carrier was tried. The gas pressure produced pulse-free flow. Other alternatives, a peristaltic pump or a HPLC pump is rather relatively more expensive, though they are efficient and most widely employed in FIA and HPLC works.

A simple introducing device for sample was a homemade injection port (Fig. 2). The low concentration loaded sample is usually considered to avoid overloading effect. A small syringe with a needle (25µl) could be used. Also using 6-port valve is common for sample introduction and provides better reproducibility.

An UV/Vis detector, which was a commonly available in the analytical laboratory, is employed in this work.

A chart recorder was used. The low-cost interfacing device (Pocket Sampler) may be employed if digitized conversion was needed. A Pocket Sampler with software supplied is handy and easy for operating. The more powerful LabVIEW is also an option, however it is rather more expensive.

The GrFFF system and its application as a teaching tool.

A simple GrFFF consisting of N_2-gas pressurized pump, a homemade injection system, a GrFFF channel, an UV/Vis detector and the chart recorder was tested by using Silica Gel 60G (Merck, Germany). Results are represented in Fig. 4. A sample of thin layer chromatographic Silica Gel 60G (5-40 µm) was employed for the GrFFF's demonstration. The broad silica size ranges of 5-10 µm and 15-25 µm was prepared from Silica Gel 60G (5-40 µm) by repeated settling.[15] From the results obtained (Figs. 4A, 4B), the longer retention time, the smaller sizes of silica (5-10 µm) eluted and the flow rate effect of water carrier: the higher flow rate, the lower retention time; effect of relaxation time (stopped flow of carrier after the suspension was injected): the longer stop time the higher retention time obtained (Fig. 4C).[16]

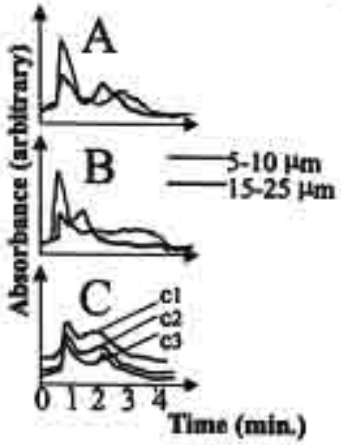

Fig. 4 The fractograms of Silica Gel samples. (A) Flow rate of 1.5 ml/min, stopped flow for 60 sec. (B) Flow rate of 4 ml/min, stopped flow for 60 sec. (C) Flow rate of 2 ml/min, stopped flow for (c1) 5, (c2) 10 and (c3) 30 sec.

In conclusion, various possibilities of simple gravitational field-flow fractionation set-ups are described. The components are cost-effective and easily available in a laboratory. Separation can be demonstrated for Silica Gel 60G of different sizes.

Acknowledgements

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References

1. F. Rouessac and A. Rouessac "*Chemical Analysis: Modern Instrumental Methods and Techniques*", 1998, John Wiley & Sons, Chichester.
2. J. C. Giddings, *Science*, 1993, *260*, 1456.
3. M. E. Schimpf, K. Caldwell and J. C. Giddings "*Field-Flow Fractionation Handbook*", 2000, John Wiley & Sons, New York.
4. http://www.fffract.com/public_html/products.htm
5. http://www.postnova.com/content/english/index_english.htm
6. K. Grudpan, C. Taylor, H. Sitter and C. Keller, *Fresenius J. Anal. Chem.*, 1993, *346*, 882.
7. K. Grudpan, P. Sritharathikun and J. Jakmunee, *LRA*, 2000, *12*, 129.
8. K. Grudpan, *LRA*, 2000, *12*, 127.
9. P. Reschiglian, D. Melucci, A. Zattoni and G. Torsi, *Annali di Chimica*, 1997, *87*, 677.

10. J. C. Giddings, M. N. Myers, K. D. Caldwell and J. W. Pav, *J. Chromatogr.*, **1979**, *185*, 261.
11. J. Plockova and J. Chmelik, *J. Chromatogr. A*, **2000**, *868*, 217.
12. J. Pazourek and J. Chmelik, *J. Chromatogr.*, **1995**, *715*, 259.
13. J. M. Calatayud "*Flow Injection Analysis of Pharmaceuticals Automation in the Laboratory*", **1996**, Taylor & Francis, London.
14. D. A. Skoog and J. J. Leary "*Principles of Instrumental Analysis*", **1992**, Harcourt Brace College Publishers, Florida.
15. R. J. Hunter "*Introduction to Modern Colloid Science*", **1993**, Oxford University Press, Oxford.
16. J. Pazourek , E. Urbankova and J. Chmelik, *J. Chromatogr.*, **1994**, *660*, 113.

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Combination of Field-Flow Fractionation and Flow Injection Analysis

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This paper presents a combination of field-flow fractionation (FFF) with flow injection analysis (FIA). FFF is a novel powerful separation technique for particles, while FIA having various advantages. Instrumentation of the set of the combination of gravitational FFF and FIA with chemiluminescence detection is proposed. Its possible application to silica sorbed iron of different particle sizes as a model is demonstrated.

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Field-flow fractionation (FFF) is a technique for size separation and characterization of particles and macromolecules. Separation by FFF takes place in a thin channel by applying an external driving force perpendicular to laminar flow of the carrier liquid.[1,2] Various driving forces have been employed in FFF such as centrifugal, flow, thermal gradients, electrical and gravitational. Gravitational field-flow fractionation (GrFFF) could be the most cost-effective and simple set up.[3-4]

Combination of FFF with one of other analytical tools such as GFAAS[3], ICP-AES or ICP-MS[4-6] results in size-based element distributions.

Flow injection analysis (FIA) has been demonstrated to provide several advantages, including simple instrumentation and economic aspects.[10-14] FIA has been combined with other analytical techniques but not yet with FFF. We have then attempted to hyphenate FFF with FIA for size-based element distribution.

In this paper, we demonstrate the combining gravitational field-flow fractionation (GrFFF) with reverse flow injection analysis with chemiluminescence (FIA-CL). The FFF is for separation of particle sizes of silica coated with goethite.[15] The FIA-CL is for iron determination which is based on catalytic reaction of alkali luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and hydrogen peroxide.[16-21]

Experimental

Instrumentation for Gravitational Field-Flow Fractionation-Reserve Flow Injection with Chemiluminescence

The GrFFF instrument equipped with the reverse FIA with chemiluminescence detection is depicted in Fig. 1. An isocratic pump (SpectraSERIES, USA) was used to propel a carrier in GrFFF system. The particles suspension samples were injected into the FFF channel (10 µl) and monitored for mass distribution by an UV/Vis detector (Linear Instruments Model 200 detector, USA) at a wavelength of 254 nm. The eluting particles from GrFFF flowed directly into the FIA-CL system for monitoring of iron in each fraction.

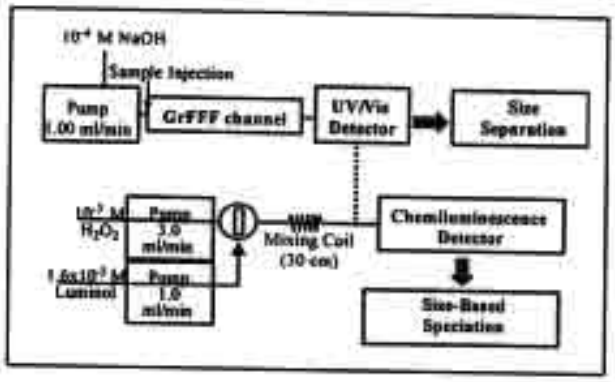

Fig. 1 Schematic diagram of the GrFFF equipped with FIA chemiluminescence detection.

For reverse FIA, luminol solution was injected, via a six-port injection valve (Upchurch, USA), into a stream of the mixture containing luminol and hydrogen peroxide which was delivered by a peristaltic pump (Eyela SPM 23, Japan) before merging with the line from GrFFF and finally entering into the CL. The chemiluminescence was monitored by a modified commercial liquid scintillation counter, with a spiral flow cell (Packard Radiometric Flo-one®\Beta Series A-100 Model A140K)(Canberra, USA).

Performance test was made by using model samples consisting of 5 µm and 10 µm silica particles coated with goethite (FeOOH).

Results and Discussion

Conditions used in the experiments are summarized in Table 1.

Table1 Experimental conditions used.

GrFFF set-up	
Concentration of sodium hydroxide carrier/ M	10^{-4}
Flow rate/ ml/min	1.00
Channel volume/ ml	0.57
Sample concentration/ mg/ml	2.0
Sample injection/ µl	10
FIA-CL set-up	
Luminol concentration/ M	1.6×10^{-3}
Hydrogen peroxide concentration/ M	10^{-1}
Flow rate of luminol/ ml/min	1.0
Flow rate of hydrogen peroxide/ ml/min	3.0
Injection volume of luminol/ µl	80
Mixing coil length/ cm	30

Fig. 2 shows the GrFFF fractogram (UV detector response for mass versus elution time) for the mixture of 5 µm and 10 µm silica coated goethite. Good resolution was obtained with a run of about 8 min. This would be a steric/hyperlayer GrFFF mode: the larger particles, the quicker elution observed.[4,26]

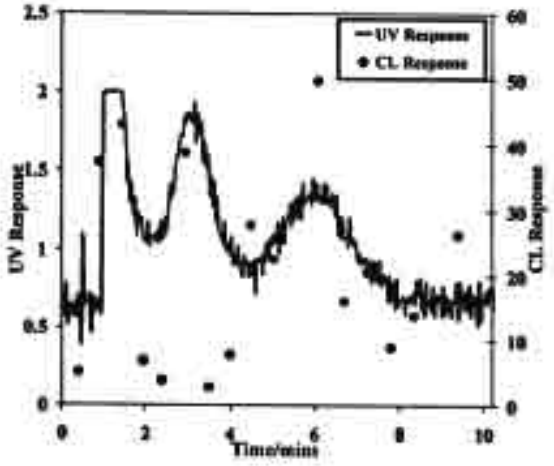

Fig. 2 UV absorbance and FIA-CL response based fractograms from 5 µm and 10 µm silica particles coated with goethite (FeOOH) obtained using GrFFF with FIA-CL for iron speciation.

Also shown in Fig. 2, the chemiluminescence responses (points) obtained from a series of injections of luminol/H_2O_2 into the GrFFF eluent prior to flowing into the FIA-CL system. It can be observed that the response due to the 5 µm fraction was higher than that of 10 µm fraction. The response corresponds to iron coated on silica. Since there was no digestion step in this experiment, it is likely that the CL responses should relate to the iron sorbed on surface area of the particles and not the total iron content in the goethite coatings. The nature of the CL responses and the possibility of including on-line digestion have been under investigation.

The combination of field-flow fractionation (FFF) with flow injection analysis (FIA) has been demonstrated for size based speciation.

Acknowledgements

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References

1. J. C. Giddings, *Science*, **1993**, *260*, 1456.
2. M. E. Schimpf, K. Caldwell and J. C. Giddings *"Field-Flow Fractionation Handbook"*, **2000**, John Wiley & Sons, New York.
3. P. Reschinglian, D. Melucci, A. Zattoni and G. Torsi, *J. Microcolumn Separations*, **1997**, *9(7)*, 545.
4. J. Pazourek (Licentiate) Thesis, Lund University, **1996**.
5. D. Melcci, G. Gianni, G. Torsi, A. Zattoni and P. Reschiglian, *J. Liq. Chrom. & Rel. Technol.*, **1997**, *20(16&17)*, 2615.
6. A. Bernard, B. Paulet, V. Colin and P. J.P. Cardot, *Trends in Analytical Chemistry*, **1995**, *14(6)*, 266.
7. C. Contaldo, G. Glo, F. Fagiolo, F. Dondi and R. Beckett, *Colloids Surf. A: Physicochem. Eng. Aspects*, **1997**, *120*, 27.
8. J.F. Ranville, D.J. Chittleborough, F. Shanks, R. Morrison, T. Harris, F. Doss and R. Beckett, *Anal. Chim. Acta*, **1999**, *381*, 315.
9. H.E. Taylor, J.R. Garbarino, D.M. Murphy and R. Beckett, *Anal. Chem.*, **1992**, *64*, 2036.
10. J. Ruzicka and E.H. Hansen *"Flow Injection Analysis"*, **1988**, John Wiley&Sons, New York.
11. K. Grudpan, P. Srithanathikun and J. Jakmunee, *LRA*, **2000**, *12*, 129.
12. Z. Fang *"Flow Injection Separtion and Preconcentration"*, **1993**, VCH, Weinheim.
13. K. Grudpan, C. Taylor, H. Sitter and C. Keller, *Fresenius J. Anal. Chem.*, **1993**, *346*, 882.
14. K. Grudpan, *LRA*, **2000**, *12*, 127.
15. G. M. Day (Honors) Thesis, Monash University, **1990**.
16. J. M. Calatayud *"Flow Injection Analysis of Pharmaceuticals Automation in the Laboratory"*, **1996**, Taylor & Francis, London.
17. Y. Zhou and G. Zhu, *Talanta*, **1997**, *44*, 2041.
18. P.J. Worsfold, R.F.C. Mantoura, E.P. Achterberg and A.R. Bowie, *Anal. Chim. Acta*, **1998**, *361*, 189.
19. W. Qin, Z. J. Zhang, and F.C. Wang., *Fresenius J. Anal. Chem.*, **1998**, *360*, 130.
20. E.C. Sarantonis and A. Townshend, *Anal. Chim. Acta*. **1986**, *184*, 311.
21. K. Okamura, T. Gamo, H. Obata, E. Nakayama, H. Karatani and Y. Nozaki, *Anal. Chim. Acta*, **1998**, *377*, 125.
22. K. Saitoh, T. Hasebe, N. Teshima, M. Kurihara and T. Kawasima, *Anal. Chim. Acta*, **1998**, *376*, 247.
23. H. Obata, H. Karatani and E. Nakayama, *Anal. Chem.* **1993**, *65*, 1524.
24. J. M. Lin, H. Arakawa and M. Yamada, *Anal. Chim. Acta*, **1998**, *371*, 171.
25. J. M. Lin and T. Hobo, *Anal. Chim. Acta*, **1996**, *323*, 69.
26. J. Pazourek and J. Chmelik, *J. Chromatogr.*, **1995**, *715*, 259.

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ANALYTICAL SCIENCES 2001, VOL.17 SUPPLEMENT

Flow Injection Sample Pretreatment for Determination of Lead by Flame Atomic Absorption Spectrophotometry

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Flow injection sample pretreatment for determination of lead by flame atomic absorption spectrophotometry has been developed. Utilizing an in-valve mini-column, packed with a solid phase extraction resin (Sr.spec™), lead was preconncentrated and separated from the matrix. The conditions for sorption and desorption of lead were investigated. A 1 M nitric acid solution and 0.005 M EDTA solution of pH 7 were suitable for soeption and desorption step respectively. A concentration efficiency of 11 min^{-1} was achieved. A linear calibration was obtained for 0.1-5.0 μg Pb and a single standard calibration can be applied. The method has been applied to the determination of lead in certified reference materials, leachates and digests of tin tailing samples. The system allows rapid analysis of very small sample volumes.

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Lead has toxicity effects to human health. Accordingly, the development of methods for its determination in variuos biological and environmental samples is both necessary and challenging. Several sensitive methods exist for lead quantitation such as GFAAS, ICP-MS, but a high instrumentation and operating cost are limitted the applications. Stripping voltammetric techniques have been widely used, but they suffer from interferences from metals presented in large amounts in mineral samples.[1] Other methods, for instance, FAAS, spectrophotometry are not sensitive or selective enough, so the preconcentration and separation step are required. Various techniques such as solvent extraction,[2] ion exchange,[3,4] extraction chromatography,[5-7] and precipitation[8] in combination with flow injection analysis have been developed for determination of lead in various samples, gaining several advantages over the conventional preconcentration/separation methods: rapid measurement, high sample throughput, fewer reagents and glass ware, avoidance of tedious work and prevention of contamination. However, conventional ion exchangers are not selective enough for application with a mineral sample which contains high concentrations of interfering ions and are ineffective for highly acidic sample solution. Recently, a novel extraction chromatographic resin (Sr.spec™) that exhibits an extremely strong retention of lead under acidic condition was developed.[9] This resin has been used in in-valve column for determination of lead in soil[10] samples by FAAS.

In this work, the conditions for separation/preconcentration of lead by flow injection in-valve mini-column packed with Sr. spec™ resin prior to the determination by AAS were investigated. EDTA solution was found to be the most effective eluent for eluting of sorbed lead from the resin. Relatively low concentration of EDTA (0.005 M) could be used, thus avoiding of burner clogging and high background signals. A single standard calibration of up to 5.0 μg Pb with a detection limit of 0.09 μg Pb was achieved. The method was applied for the determination of lead in some certified reference materials and in leachates and digests of tin tailing samples.

Experimental

Chemicals

All chemicals used were of analytical-reagent grade, except nitric acid and hydrofluoric acid (Merck, Damstadt, Germany) were of suprapure grade and sulfuric acid (Merck) was ultrapure grade. Deionized water was used for preparing of all solutions. Citric acid monohydrate, oxalic acid, tartaric acid, sodium citrate, ammonium oxalate monohydrate and ethylenediaminetetraacetic acid, disodium salt (EDTA) were obtained from Merck. Humic acid, sodium salt was obtained from Aldrich. Lead nitrate standard solution for ICP-AES (1000 ppm) was also from Merck. Sr.spec™ resin (80-100 μm grain diameter) was from Eichrome, Darien, USA. Certified reference materials (CRMs) of sediment and soil were obtained from Community Bureau of Reference (BCR) and International Atomic Energy Agency (IAEA), respectively.

Preparation of solutions

Lead standard solution. 100 ppm lead was prepared by diluting 1000 ppm lead standard with 0.1 M HNO_3. A series of

lead standard solutions were prepared by diluting the 100 ppm lead standard solution into a solution of 1 M HNO_3.

Eluent solutions. To obtain 0.5 M concentration of various eluents, the following amounts of chemicals were dissolved and made up to 1 l volume: EDTA: 1.88 g, ammonium oxalate: 7.141 g, sodium citrate: 17.85 g, tartaric acid: 7.542 g, citric acid: 10.559 g, and oxalic acid: 6.335 g.

FI manifold

Flow injection system (FIAS-400, Perkin Elmer) and atomic absorption spectrophotometer (AA 3300, Perkin Elmer) were used. A flow diagram of the system was designed as shown in Fig. 1.

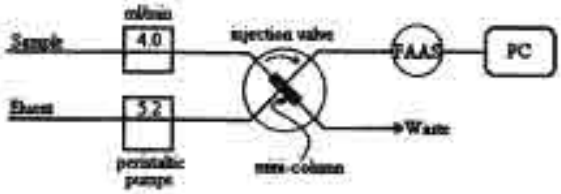

Fig. 1 Manifold for FI-in-valve mini-column determination of lead: FAAS = flame atomic absorption spectrophotometer and PC = personal computer.

Sample and eluent were propelled via peristaltic pump 1 and 2 respectively. An in-valve mini-column packed with Sr.Spec™ resin was incorporated within the injection valve, replacing one of the sample loops. While the valve was in the INJECT position, the eluent solution passed through the column to admit the sample into the detector. When the injection valve was turned to the LOAD position, a sample solution in 1 M HNO_3 passed through the column in an opposite direction, thus avoiding tighten packing of the column. After a specified loading time, the valve was automatically turned back to INJECT position and accumulated lead was eluted downstream for continuous monitoring by FAAS.

The detector conditions used were: wavelength set to 217.0 nm, fuel (C_2H_2) flow rate 3.7 l min^{-1}, air flow rate 10 l min^{-1}. A fuel flow rate, a hollow cathode lamp and burner position were adjusted until the maximum sensitivity of 10 ppm Pb aspirated was obtained. An aspiration rate was 5.0 ml min^{-1}.

Samples preparation

Digested tin tailing samples. Tin tailings were dissolved by microwave digestion system (MLS 1200 mega, Milestone, USA). 100 mg of sample was accurately weighed and put in a Teflon container. 2 ml conc. HNO_3 and 5 ml conc. HF were added. The sample containers were closed and assembled on a container block and then were digested in a microwave oven at about 100 °C for 30 min and at about 120 °C for 30 min. The digested solution and the remaining residue were transferred to another Teflon container. A 5 ml conc. H_2SO_4 was added and treated further open digestion on a hotplate in a fume cupboard for a few hour to evaporate HF. After cooling, the solution was transferred to a 50 ml volumetric flask and the volume is made up to the mark with 0.1 M HNO_3.

CRMs. The procedure used for digestion of CRMs was already reported elsewhere.[10]

Leached tin tailings. Ammonium acetate was used for leaching of exchangeable ions from the particles.[11] 1 g of tin tailing was weighed and put in a 25 ml polyethylene vial. 20 ml of 1 M ammonium acetate were added and the vial lid was tightly closed. The vials were shaken using an end-over-end shaker with rotating at 30 rpm for 6 h. After the particles were settled, the solution was transfered into a centrifuge tube. The solution was separated by centrifuging at 8000 rpm for 20 min. 15 ml of upper clear solution was taken into a 25 ml vial which contained 2 ml of conc. HNO_3 and 3 ml of deionized water.

Analysis of Samples

The above sample solutions were diluted with a 1 M HNO_3 before commencing to the determination of lead by the proposed system. For comparison purpose, inductively coupled plasma mass spectrometry (ICP-MS) (Elan 6000, Perkin Elmer, USA) was also used to analyze the samples. A sample was prepared by transferring 1 ml of sample solution into a sample tube, adding 4 ml of 1% HNO_3 solution and 50 ul of an internal standard solution (Rh-103). The standard solution were prepared by diluting of a multi-element standard solution to suitable concentrations. The conditions of the instrument: plasma power 1050 kW, radio frequency 40 MHz, argon gas for plasma, auxiliary and nebulizer of 15.0, 0.5 and 1.0 l min^{-1}, respectively, autolensing of ion lens and sample aspiration rate of 1.0 ml min^{-1} were employed.

Results and Discussion

Concentration of nitric acid as sample medium

It was found that a peak height of 0.8 μg Pb^{2+} did not significantly increase for increasing a concentration of nitric acid to higher than 1 M. This agreed with the previous report which Pb^{2+} can be sorbed efficiently on the Sr.Spec™ resin from a medium of 0.8-2.0 M HNO_3.[10] The 1 M HNO_3 was utilized in further studies.

Flow rate of eluent

Since the aspiration rate of a nebulizer was 5.0 ml min^{-1}, the lower flow rate of the eluent caused air bubble in the flow channel leading to low reproducibility of the results. The higher flow rate caused low sensitivity that could be due to low nebulization efficiency. The eluent flow rate of 5.2 ml min^{-1} was chosen.

Investigation for eluent

The peak heights for elution of 0.8 μg sorbed Pb by 0.05 M of the various eluents of different pH values are displayed in Fig. 2. It was found that EDTA was the best one as determined by peak height and RSD of triplicate injections. However, citrate and oxalate were also quite effective. The elution mechanism would be the strong formation of a complex with Pb^{2+} in the eluent solution. The EDTA solution of pH 7 was used in subsequent analyses because it provided the better precision, narrow and high peaks, and moderate residence time compared to the higher pH solution.

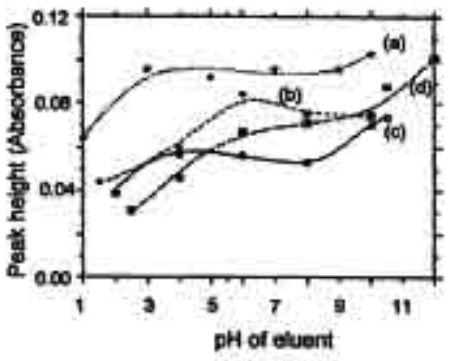

Fig. 2 Effect of various eluents of different pH : (a) EDTA, (b) oxalic acid, (c) tartaric acid and (d) citric acid.

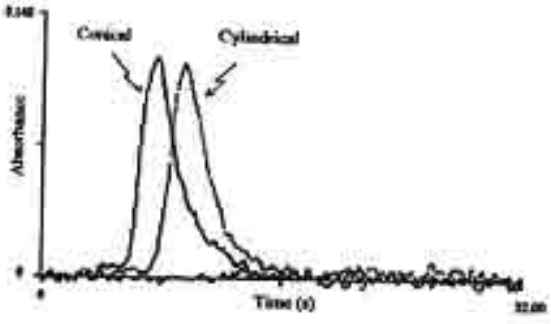

Fig. 3 Effect of column designs.

Concentration of EDTA

EDTA solution (pH 7) of different concentrations (0.00-0.01 M) were used as eluent for elution of 2 μg sorbed Pb^{2+} on the Sr.Spec™ resin. It was found that an EDTA concentration of 0.001 M (30 fold higher than Pb^{2+} concentration by mole) or more can elute the adsorbed Pb^{2+} effectively. The 0.005 M EDTA was chosen in order to ensure the completeness of elution and also to save the reagent.

Column designs

Two column designs: a cylinder (2 cm long, 3 mm i.d.) and a truncated cone (1 cm long, 2 and 4 mm i.d. at ends) were tried. The results are depicted in Fig. 3. The column dimension did not significantly affect the peak height (0.114±0.001 and 0.119±0.004 for cylinder and cone shaped column, respectively), but did affect the residence time in case of loading and elution with the same flow direction. This implied that the adsorption of lead on the column occurred in a small region at the beginning of the column. The distribution coefficient for lead extraction from 1 M HNO_3 solution into the resin was high enough to retain lead in a small area of the column. The eluent was effective enough to desorb accumulated lead from the column and had only small effect on the dispersion of the elution profiles.

Interferences

Effect of some possible interfering substances in environmental and mineral samples are shown in Table 1. The tolerance limit was defined as the concentration of the interfering ions that cause 5% deviation in peak height of 0.2 ppm Pb^{2+}. EDTA and humic acid were selected as representative of the complexing agents in the environment. Humic acid up to 20 ppm had no effect on lead sorption, but the concentration of higher than 10 ppm humic acid did cause problems with column blocking. Other interfering ions have been studied using a similar system.[10] It has been reported that Fe^{3+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Sr^{2+}, Cu^{2+} and Cd^{2+} do not interfere at concentrations up to 50 ppm.

Calibration graph and precision

Both normal calibration (plots of the ppm Pb versus the corresponding peak height) and single standard calibration (plot of μg Pb versus peak height obtained) can be used. The μg Pb can be varied by using only one standard solution of Pb^{2+} and varying the preconcentration time, so the μg Pb were calculated from the loading flow rate (q, ml min^{-1}), the preconcentration time (t, min) and the concentration of standard solution (C, μg ml^{-1}) : μg Pb equivalent = q (t) (C). The single standard calibration graph is shown in Fig. 4. The calibration equation (up to 5 μg Pb): μg Pb = 0.070 (peak height, A) + 0.007; r = 0.9995 was obtained. The detection limit (3σ) was 0.09 μg Pb and the relative standard deviations of 12 replicate injections of

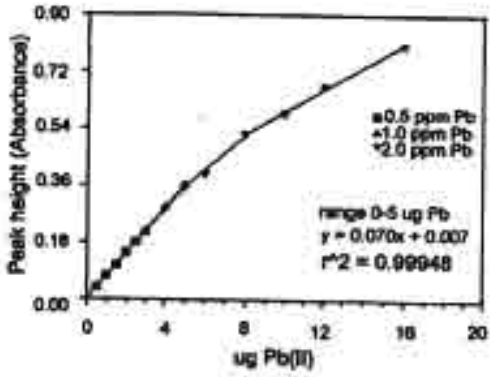

Fig. 4 Single standard calibration graph; three concentrations of lead standard solutions (0.5, 1.0 and 2.0 ppm Pb) were loaded.

Table 1 Tolerance limits of some interfering substances.

Interfering substances	Tolerance limit
EDTA	0.01 M
Humic acid	10 ppm
NaCl	0.25 M
$MgCl_2$	1.0 M
PO_4^{3-}	5000 ppm
Fe(III)	750 ppm

0.8 μg Pb and 1.0 μg Pb were 5.2 and 2.3 %, respectively.

Analysis of samples

Certified reference materials of soil and sediment, and tin tailing samples were analyzed by the developed method. The single standard calibration was applied for determining the concentrations of the samples. The results obtained are shown in Tables 2 and 3. The t-test was used to compare the results obtained from the proposed method and those obtained from the ICP-MS method or the certified values. It indicated that no significant difference was found at a 95% confidence level.

Table 2 Contents of lead in certified reference materials found by the FI-FAAS method.

Samples	Certified value (μg g^{-1})	Concentration found (μg g^{-1})	% difference
IAEA soil-7	60 (55-71)	58.4±3.2	-2.3
CRM-320 sediment	42.3±1.6	43.4±2.0	2.6

Table 3 Lead contents in tin tailing samples.

Samples*	Lead contents found (ppb) by		
	ICP-MS	FI-FAAS	% difference
1	128.7	124.0	-3.7
2	77.9	79.2	1.6
3	91.8	79.1	-13.8
4	97.7	98.3	0.6
5	255.8	257.6	0.7
6	239.0	254.0	6.3
7	194.8	212.0	9.2
8	188.6	186.2	-1.3
9	10.5	11.6	10.3
10	3.5	3.6	3.1

* 1-8 : digested tin tailing samples (1 min preconcentration)
9-10 : tin tailing leachates (2 min preconcentration).

It can be concluded that flow injection sample pretreatment system utilizing in-valve mini-column packing with Sr.Spec™ resin for the determination of lead in a complex matrix samples by flame atomic absorption spectrophotometry has been developed. Lead was sorbed on the resin from a 1 M HNO_3 medium and then eluted by a 0.005 M EDTA solution. The single standard calibration method can be applied for determining of lead in the amounts up to 5 μg, with a detection limit of 0.09 μg and precision of 5.2% (0.8 μg Pb, n=12). Concentration efficiency for the preconcentration was 11 min^{-1}. The proposed system has been applied for analysis of digest and leachate of tin tailing samples, and certified reference materials. The results obtained agreed well with those by ICP-MS method and the certified values, but the developed method is relatively economic.

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References

1. K. Yokoi, A. Yamoguchi and T. Koide, *Anal. Chim. Acta*, **1995**, *316*, 363.
2. M.N. Bale, D.P. Dave and A.D. Sawant, *Talanta*, **1995**, *42*, 1291.
3. Z. Fang and B. Welz, *J. Anal. Atom. Spect.*, **1989**, *4*, 543.
4. S.D. Hartenstein, J. Ruzicka and G.D. Christian, *Anal. Chem.*, **1985**, *57*, 21.
5. D. Rodriguez, P. Fernandez, C. Perez-Conde, A. Gutierrez and C. Camara, *Fresenius J. Anal. Chem.*, **1994**, *349*, 442.
6. R. Ma, W. van Mol and F. Adams, *Anal. Chim. Acta*, **1994**, *285*, 33.
7. Y.P. Pena, M. Gallego and M. Valcarcel, *Talanta*, **1995**, *42*, 211.
8. Z. Zhuang, X. Wang, P. Yang, C. Yang and Y. Benli, *Can. J. Appl. Spect.*, **1994**, *39*, 100.
9. E.P. Horwitz, M.L. Dietz, S. Rhoads, C. Felinto, N.H. Gake and J. Hooghton, *Anal. Chim. Acta*, **1994**, *292*, 263.
10. P. Sooksamiti, H. Gekies and K. Grudpan, *Analyst*, **1996**, *121*, 1413.
11. A. Tessier, P.G.C. Campbell and M. Bisson, *Anal. Chem.*, **1979**, *51*, 844.

ผลงานวิจัย ก5

ANALYTICAL SCIENCES 2001, VOL.17 SUPPLEMENT

Kinetic Determination of Iodine in Urine Using Stopped-Flow Injection

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A flow injection system in the stopped mode (stopped-FI) will be presented for determination of urinary iodine based on the catalytic effect of iodide on the redox reaction between Ce(IV) and As(III). Since the reaction is a first order process, kinetic determination of the catalyst using the stopped-FI can be carried out in two ways. The iodine content could be measured based on a linear plot between the logarithm of the absorbance of Ce(IV) taken at a fixed time against iodine concentration. For this type of calibration, the use of an automatically controlled stopped-FI should diminish the errors in the conventional batch method, which often arise from the imprecision of timing. With this procedure, the detection limit (3σ) of 3 μg l^{-1} and a sample throughput of 35 samples h^{-1} were achieved. The calibration can also be made using the relationship between the rate constant and the catalyst concentration. In this case the flow was stopped for, say 25 minutes depending on the iodine concentration, to record the kinetic profile of the disappearance of Ce(IV). The stopped-FI system is an alternative tool in the status assessment of iodine deficiency disorder.

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Iodine is an essential element for human. Contents of iodine in urine are normally used as a marker for status assessment of iodine deficiency disorder (IDD). The following values are guide for IDD status: <20 μg l^{-1} (severe); 20-49 μg l^{-1} (moderate); 50-100 μg l^{-1} (mild) and >100 μg l^{-1} (normal).[1] Since iodine concentration in urine samples is considerably low, it is necessary to use sensitive methods for this application.

There have been some publications that described methods for determination of urinary iodine. The methods concern techniques such as high performance liquid chromatography (HPLC),[2-5] ion-selective method[6] and ICP-MS.[7]

Amongst other methods, kinetic methods are also suitable for general trace analysis[8] and usually give a high sensitivity with sometimes cost-effective. The most common method for determination of urinary iodine is a kinetic spectrometric method with catalytic effect of iodide on a redox reaction between Ce(IV) and As(III). Sandell and Kolthoff first described analytical application of this reaction to determine iodide catalyst in 1934.[9] Possible mechanisms of the reaction were proposed in 1969.[10] Some applications of the Sandell and Kolthoff method were later described.[11-15] for determination of iodine using its kinetic effect. Nevertheless the classical method for urine was provided by Dunn et al.,[1,15] The method is carried out batchwise and requires sample digestion since urine contains varieties of species, which interfere the kinetics. The Sandell and Kolthoff method applied in flow based techniques,[16-20] including flow injection (FI) technique[21] have been described for this application as alternative procedure to the batch method. The main purpose of these automation techniques is for convenience and a better precision in taking reading signal at a fixed-time.

Recently our laboratories have proposed a method based on flow injection.[22] The method is based on the batch procedure of Dunn et al.'s[1,15] which requires choloric digestion of samples prior kinetic determination. After the digestion, iodine species are oxidised to iodate ion,[23] which is not the catalyst for Sandell and Kolthoff method. However non-published experimental results[24] have demonstrated that under the condition used, iodate ion is reduced to iodide ion in the presence of arsenious acid and chloride ion. Our results have shown in a batch system that the kinetics is a first-order type.

For first-order kinetics, in this case, disappearance of Ce(IV) is exponential, that is

$$[Ce^{IV}]_t = [Ce^{IV}]_0 e^{-kt} \quad (1)$$

where $k = k_{catalysed}[I^-] + k_{uncatalysed}$

Therefore,

$$-\ln[Ce^{IV}]_t = -\ln[Ce^{IV}]_0 + tk_{uncatalysed} + tk_{catalysed}[I^-] \quad (2)$$

$[Ce^{IV}]_0$ and $[Ce^{IV}]_t$ are the concentrations of Ce(IV) at the original and at time t respectively. $k_{uncatalysed}$ is the rate constant in the absence of iodide ion. $k_{catalysed}$ is the rate constant due to the concentration of the iodide catalyst, $[I^-]$. According to Beer's law, equation (2) can be written as

$$-\ln A_t = -\ln A_0 + tk_{uncatalysed} + tk_{catalysed}[I^-] \quad (3)$$

where A_0 and A_t are the absorbance of $[Ce^{IV}]_0$ and $[Ce^{IV}]_t$ respectively. Thus a linear calibration plot can be obtained as written in equation (4)

$$-\ln A_t = \text{slope}[I^-] + \text{intercept} \quad (4)$$

In this work, two kinds of kinetic method which are fixed-time and rate constant methods, were employed with stopped-flow injection (stopped-FI) for iodine. With the use of stopped-FI, kinetics profile of the disappearance of Ce(IV) can be studied. Under the condition in the flow injection technique that also provides a type of pseudo first-order kinetics, calibration for iodine can be made based on equation (4). This method is a fixed-time method. Another kinetic method, which utilized measuring of the first-order rate constant, was studied in the stopped-FI system.

Experimental

Flow injection manifold

Figure 1 schematically illustrates the set up of the flow injection system used. Optimization was described in the previous work.[22] An Ismatec peristaltic pump, model IS7610 was used for propelling reagents and sample plug. A Rheodyne injection valve, model 7125, fitted with Teflon loop (1.0 mm i.d.) was employed for injections of standards and samples. Cole Parmer Tygon tubings with i.d. of 0.75 mm, 2.25 mm o.d. and 0.75 mm wall, were used for the FI assembly and for making the 100 cm reaction coil. A Shimadzu spectrophotometer, model UV-2-01, Japan, with a tungsten lamp and a Philips flow cell of 0.01 ml volume was used for monitoring the absorbance of Ce (IV) at 420 nm. An Alltech chart recorder, model LR 93025, USA, was used for recording the signal from the spectrophotometer. The recorder was set at 50 mV f.s.d.

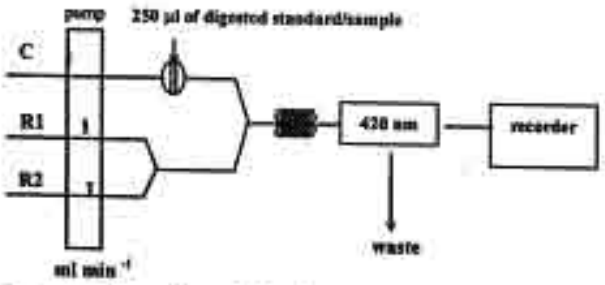

Fig. 1 The flow injection manifold for kinetic determination of iodine in urine. [C: water carrier, R1: 8.0×10^{-3} M Ce(IV) in 1.75 M H_2SO_4 , R2: 1.0×10^{-1} M As(III) + 0.8 M NaCl in 0.5 M H_2SO_4].

Reagents

All chemicals used in this work were AR grade, supplied by Merck (Germany). Deionized-distilled water was obtained from a Milli-Q system, Millipore, USA, and was used throughout.

Iodine standards

A stock of iodine standard (1,000 mg l^{-1}) was prepared by dissolving 0.1685 g of potassium iodate in water and making up to mark of a volumetric flask (100 ml). Further dilutions were made for working solutions of appropriate concentrations.

Ceric ammonium sulfate 0.008 M (R1)

Ceric ammonium sulfate 0.008 M was used as a reagent stream in the FI methods. This solution was prepared by dissolving 5 g of $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$ in 1 l of 1.75 M H_2SO_4.

Arsenious acid 0.1 M (R2)

As_2O_3 (10 g) and NaCl (47 g) were dissolved in 500 ml of water with heating on a hot plate. After cooling to the room temperature, 27.8 ml of conc. H_2SO_4 was added to this solution followed by dilution with water to one litre. This 0.1 M arsenious acid solution was used as reagent stream (Fig. 1).

Sample, standard and digestion

Casual urine samples were collected from students and were selected to cover to range of iodine concentration studied. The samples were frozen and thaw before digestion. The employed digestion method of samples and standards was a modified Dunn et al.'s.[22]

Procedure

After digestion, samples or standards were injected into the FI system depicted in Fig. 1 for determination of iodine.

Rate constant method

At the time of 41-second after sample injection into the manifold in Fig. 1, when the peak bottom rose in the usual continuous mode, the flow was stopped. Absorbance of Ce(IV) at 420 nm was continuously recorded until constant reading was reached (complete reduction to Ce(III)).

Fixed-time method

Operating procedure for this method is similar to that described for the rate constant method. At 41 seconds after injection, the flow was stopped for a period (1 min). The signal was then continually recorded during the stopped-flow period. The flow was restarted again at time t' (101 seconds after injection) to push away the sample zone resulted in the rise of absorbance back to the baseline. In the calibration, the stopped interval must be fixed for all working solutions and the samples.

Results and Discussion

Rate constant method

Fig. 2 shows kinetics of the reduction of Ce(IV) to Ce(III) which were monitored using the stopped-FI manifold in Fig. 1.

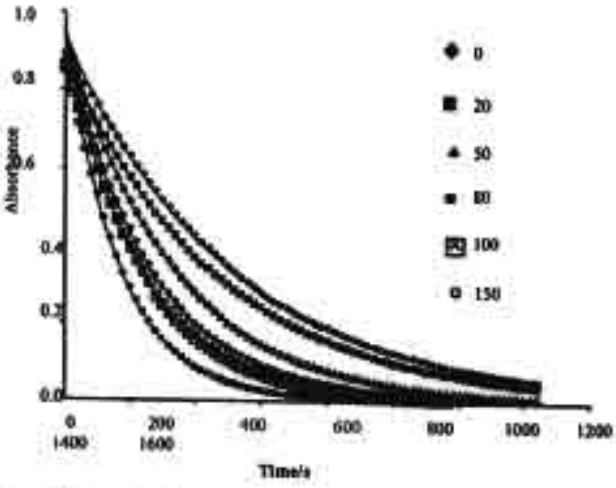

Fig. 2 Kinetics obtained from the stopped-FI system, showing reduction of Ce(IV) at different concentrations of iodine in sample plug from 0 to 150 µg l^{-1} (top to bottom). Full lines represent the exponential fittings.

Kinetic analysis was carried out using commercial software, ENZFITTER. The results in Fig. 2 show that each set of data agrees well with the theoretical exponential fit (with standard error < 10%). This indicates that in the monitoring plug, the process of decreasing in Ce(IV) concentration is a pseudo first-order type for the range of iodine concentration used. The plot between rate constant (RC), given by the software, and iodine concentration was linear ($RC = 2.1 \times 10^{-3}$ [I] + 1.43, $r^2 = 0.998$). This plot was used as the calibration for measuring iodine content in urine samples.

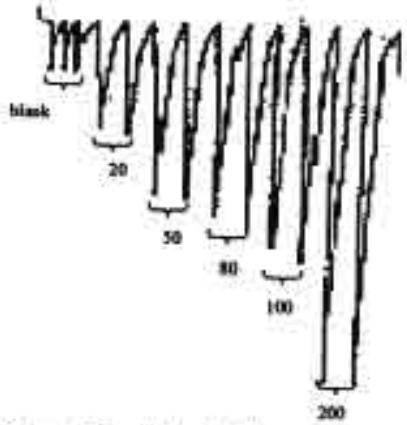

Fig. 3 Stopped-FI profiles of iodine standards (μg I l^{-1}) for the fixed-time kinetic method.

Fixed-time method

Results in Fig. 2 have demonstrated that the kinetics of disappearance of Ce(IV) are of first-order type over the concentration range of iodine catalyst of 0 to150 μg I l^{-1}. Thus, we should be able to use the relationship in equation (4) for the fixed-time method under the same condition in the stopped-FI manifold.

Figure 3 represents some of the signal profiles obtained from the stopped-FI manifold for iodine standards ranging from 0 to 200 μg l^{-1}. Linear calibration plot, made between the logarithm of Ce(IV) absorbance and iodine concentration, was satisfactorily achieved ($\ln A_t = 1.7 \times 10^{-3}$[I] + 2.41×10^{-1}, $r^2 = 0.994$). This calibration was used in determination of iodine in urine samples.

Method validation

Firstly, application of both the kinetic methods were tested using the stopped-FI. Analysis of twelve urine samples was carried out. According to linear regression test, there was no significant difference between the results given by the two methods at 95% confidence (slope = 0.880 ± 0.26 and intercept = 4.92 ± 23.3).

Although the rate constant method can be used in this application however the experiment takes a much longer time than the fixed-time method for a sample. The time taken to achieve a complete kinetics can be up to 25 min for the rate constant method whereas only 4 min is required to get a complete signal profile, in the fixed-time method. Hence, the latter method is more practical for real use and therefore selected for further investigation.

The stopped-FI manifold was used with fixed-time method to analyse another set of eleven samples. The results were compared with an inductively-coupled plasma mass spectrometric method[22] (ICP-MS). A regression line of y = 0.754±0.25x + 0.533±15.9 (x is ICP-MS and y is the stopped-FI) was obtained. This indicates that results of the two methods are not significantly different at 95% confidence. However it is observed that values of iodine contents given by the kinetic method is often lower than the ICP-MS method. This could possibly due to loss of iodine during acid digestion for the kinetic method whereas this is not likely to occur for the ICP-MS method since only dilution of urine was required.

General feature of the stopped-FI with fixed-time method

Some characteristic of the fixed-time method with the flow injection approach is summarized in Table 1.

Table 1 Feature of the stopped-FI with fixed-time method

FI parameter	Value
Detection limit (3σ)	3 μg l^{-1}
Sample throughput	35 samples h^{-1}
Reproducibility (100 μg l^{-1})	0.25%

Conclusion

This work demonstrated that kinetic methods, both the rate constant and the fixed-time methods, could be operated using flow injection technique. The calibrations are satisfactorily linear according to the experimental condition giving first-order kinetics. However for real application, the fixed-time method is more preferable. The procedure is suitable for large number of samples and can be alternative to the conventional batch method. The stopped-FI method is readily applicable for automation.

Acknowledgements

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References

1. J. T. Dunn, IDD newsletter, **1993**, *9*, 5. Accessed 26th January1999, from http://avery.ined.virgima.edu/~jtd/iccidd/idddocs/idd196bt m.
2. W. Buchberger and K. Winsauer, *Anal.Chim. Acta.*, **1985**, *3*, 347.
3. J. Odink, J. J. P. Bogaards, and H. Sandman, *J. Chromatogr.*, **1988**, *431*, 309.
4. J. Rendl, S. Seybold, and W. Borner, *Clin. Chem.*, **1944**, *40*, 908.
5. F. Moussa, M. C. R. Demy, F. Veinberg, F. Depasse, R. Gharbi, J. Y. Hautem and P. Aymard, *J. Chromatogr. B*, **1995**, *667*, 69.
6. Y. Yabu, K. Miyai, S. Hayashizaki, Y. Endo, N. Hata, Y. Iijima, and R. Fushimi, *Endocrinol. Japon.*, **1986**, *33*, 905.
7. M. Haldimann, B. Zimmerli, C. Als, and H. Gerber, *Clin. Chem.*, **1998**, *44*, 817.
8. T. Kawashima, S. Nakano, M. Tabata., and M. Tanaka, *Trends in Anal. Chem.*, **1997**, *16*, 132.
9. E. B. Sandell and I. M. Kolthoff, *J.A.C.S.*, **1934**, *56*,1426.
10. P. A. Rodriguez and H. L. Pardue, *Anal. Chem.*, **1969**, *41*, 1369.
11. T. Deguchi, A. Tanaka, I. Sanemasa, and H. Nagai, H. Bunseki Kagaku, **1983**, *32*, 23.
12. S. P. Mushtakova, L. F. Kozhina, L. M. Ivanova, A. K. Myshkina and O. A. Rytova, *J. Anal. Chem.*, **1998**, *53*, 214.
13. G. Aumont and J. C. Tressol, *Analyst*, **1986**, *111*, 841.
14. W. Chantore, S. Muangkaew, J. Shiowatana and D. Nacapricha, *Lab. Robotics and Automation*, **1999**, *11*,37.
15. J. T. Dunn, H. E. Crutchfield, R. Gutekunst and A. D. Dunn, *Thyroid*, 1993, **3**, 119.
16. P. J. Garry, D. W. Lashley and G. M. Owen, *Clin. Chem.*, **1973**, *19*, 950.

17. W. May, D. Wu, C. Eastman, P. Bourdoux and G. Maberly, *Clin. Chem.*, **1990**, *36*, 865.
18. K. Tsuda, H. Namda, T. Nomura, N. Yokoyama, S. Yamashita, M. Izumi and S. Nagataki, *Clin. Chem.*, **1995**, *41*, 581.
19. . S. Pino, S. L. Fang and L. E. Braverman, *Clin. Chem.*, **1996**, *42*, 239.
20. Z. Yaping, Y. Dongxing, C. Jixiang, L. Tianshiu and C. Huiqin, *Clin. Chem.*, **1996**, *42*, 2021.
21. J. Ruzicka and E. M. Hansen, *Flow Injection Analysis*, Wiley, New York, 2nd edn., 1988.
22. D. Nacapricha, S. Muangkaew, N. Ratanawimarnwong, J. Shiowatana, and K. Grudpan, *Analyst*, **2001**, *126*, 121.
23. N. Cheungchan and D. Nacapricha, unpublished work.
24. S. Suwannachoat, P. Wilairat and P. Tongkaew, unpublished work.

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A simple flow injection system with bead injection for trace iron determination

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Abstract

A simple and low cost flow injection (FI) system with bead injection (BI) was developed for determination of low concentration (μmol l^{-1}) of iron in water samples. Chelex-100 chelating resin beads, trapped in a jet ring cell, were employed. The intensity of red complex of 1,10-phenanthroline with Fe^{2+} was monitored using colorimetric detector with a LED green light source. Amount of total Fe (Fe^{2+} and Fe^{3+}) and Fe^{2+} can be evaluated by with and without reduction of Fe^{3+} using ascorbic acid. Lowest detectable levels of Fe^{2+} were 0.90 and 0.45 μmol l^{-1} for sample loading time of 3 and 5 min, respectively. Working range was up to 3.90 μmol l^{-1} using 0.3% w/v 1, 10-phenanthroline. Percent recoveries of spiked water samples (0.90–2.33 μmol l^{-1} of Fe^{2+}) were 100–110%.

Keywords: Flow injection; Bead injection; Trace iron determination; 1,10-Phenanthroline

1. Introduction

The concept of bead injection (BI), where functionalized beads are used as a solid surface to accommodate chemical reaction and to accumulate and retain the analyte, was first introduced to use with sequential injection technique [1–5]. Use of bead as a replaceable stationary phase helps to improve sensitivity, minimizes dilution and reduces cross contamination between analysis runs [5,6]. Sequential injection analysis (SIA) offers many advantages such as precise automatic control of reaction conditions and microfluidic manipulation of samples and reagents [1–5,7]. It also operates at microlitre scale, saving reagents while generating very small volumes of waste. On the other hand, while classical FIA uses much larger volumes of solutions, it can be fabricated from less expensive components and operated manually as demonstrated in this work. Flow injection (FI)

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is a well established technique that allows solutions flow for online reaction and detection [8–13]. Many tedious steps in manual batch wise operation such as mixing reagents in beakers can be eliminated. Even though FIA is not as sophisticated as SIA but its simplicity and low cost become the main benefits [14–18].

Since low-cost instrumentation is attractive to many laboratories, we have decided to develop a novel combination of FIA with BI technique. Such a home made FI–BI system has the advantage of using beads as replaceable solid phase. Iron and Chelex-100 are chosen as a model analyte and sorption beads, respectively, to demonstrate the system. Sorption of iron ions on Chelex-100 chelating resin beads reaches a maximum above pH 4, but the elution of sorbed iron is difficult. However, the transparent of Chelex-100 resin allows the transmittance detection on beads bed to be possible. The chemistry used in the present work is a simple detection of color intensity of the red complex $[(C_{12}H_8N_2)_3Fe]^{2+}$ at 524 nm [19,20]. The complex is developed when 1,10-phenanthroline reacts with Fe^{2+} that has been trapped on the resin beads. Attempts have been made to develop a procedure to determine low concentration of iron with a simple FI–BI system.

2. Experimental

2.1. Apparatus

The diagram of the system used in this work is represented in Fig. 1(a). Two peristaltic pumps (Ismatec) were employed, one for beads loading (16 ml min^{-1}) and another for buffer and sample pumping (3 ml min^{-1}). Tygon pump tubings and three way valves (Upchurch) were used for setting up the manifold. To save color reagent solution, 1,10-phenanthroline was introduced using a six port injection valve (FIAlab) instead of pumping it with buffer solution

The reaction cell (jet ring cell) was laboratory made and the detail of the structure is as shown in the diagram Fig. 1(a). One side of the cell was connected to LED green light source of 524 nm with a 47 nm spectrum half width. While another side having a photo transister (PT). This detector unit (Fig. 1(b)) gives a peak that reaches the highest point when there is the highest light absorption. Therefore, after the signal reaches the steady state for a few seconds, it is automatically adjusted back to the base line, and a peak is resulted. The detector was connected to a chart recorder (Perkin–Elmer model 056-1002) that was set for a 10 V fsd.

2.2. Reagents

Beads of chelating resin (iminodiacetic acid, Chelex-100) was from Sigma, Germany (Bio-Rad Laboratories). Their sizes were varied in the range of 50–100 dry mesh but only-35 wet mesh beads (wet bead size smaller than 35 mesh) were collected by sieving and used.

All other following reagents were purchased from Merck, Germany. Acetate buffer solution (0.1 M pH 4.5) was prepared from sodium acetate trihydrate and glacial acetic acid in de-ionized water. Standard Fe^{2+} solutions (0.45–9.00 μmol l^{-1}) were prepared from ammonium ferrous sulfate $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$. Standard Fe^{3+} solutions (0.45–3.60 μmol l^{-1}) were prepared from ferric nitrate. Color reagent used for Fe^{2+} was 1,10-phenanthroline-monohydrate prepared to a desired concentration in the acetate buffer solution. Ascorbic acid solution was a reducing agent to convert Fe^{3+} to Fe^{2+}.

2.3. Operation steps

Buffer solution was pumped through three way connector T and valves V2 and V4 into the jet ring cell at all time. Iron solution was also pumped all the time because it was connected to the same pump, see Fig. 1(a). However, the valve V1 and the tubing were connected in the way that a standard; sample solution containing Fe^{2+}, was sent back to the sample container when it was not a standard/sample loading step. This way, a standard/sample solution and number of pumps used can be reduced.

First, beads were loaded into the cell by pumping of bead suspension through valves V3 and V4

for 20 s. Then, the pump for bead suspension was stopped and V3 and V4 were switched off. Second, a standard/sample solution was loaded via valves V1 and V2 to the cell for the desired period of time and then the valves were switched off. Only the buffer solution was run for 1 min to wash all the iron in the flow line to the cell. Finally, 0.3% w/v 1,10-phenanthroline solution was introduced using a six port valve (V2) with 210 μl injection loop in order to consume only necessary amount of reagent. This color reagent reacts with Fe^{2+} captured on the beads and shows red color that can be monitored using a green light LED colorimeter. After each analysis, used beads can be discarded by flushing them out of the cell with the buffer solution.

3. Results and discussion

3.1. Characteristics of signal

As previously mentioned, the detection unit was built to automatically adjust the signal back to the base line after it reaches the maximum steady state for a few seconds. Therefore, there were two peaks obtained from each analysis run, as in Fig. 2. The first peak corresponded to bead loading.

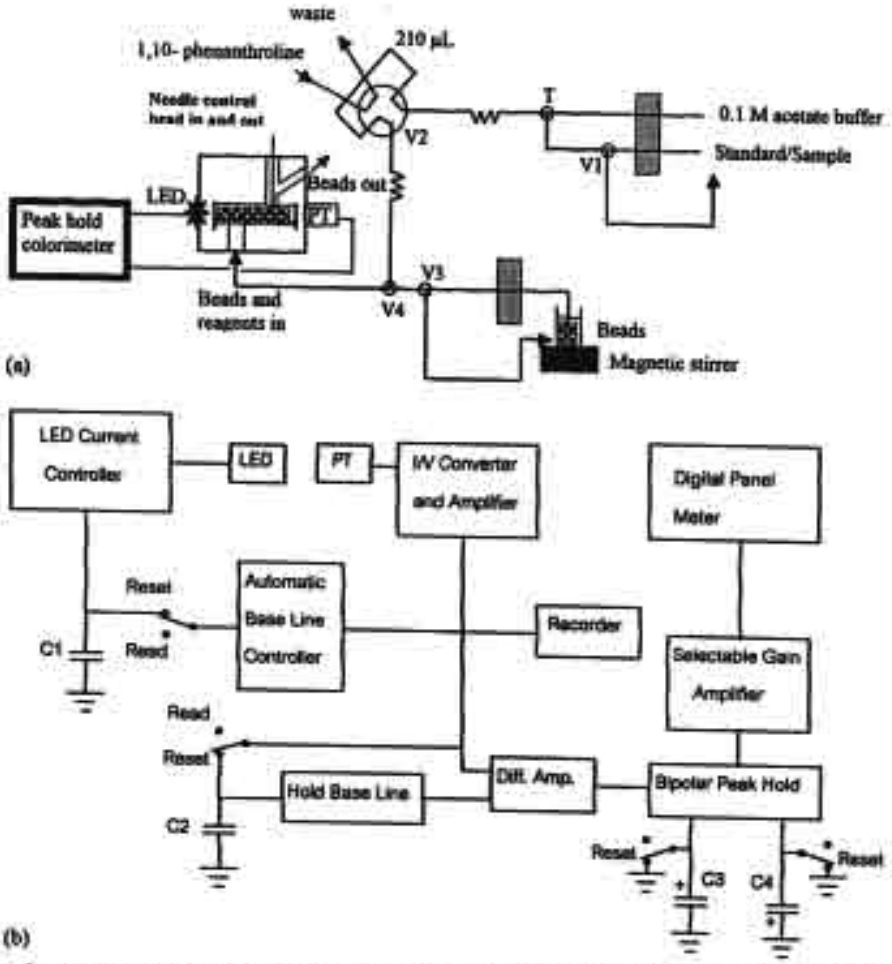

Fig. 1. (a) Flow injection set-up with bead injection for determination of iron (V1, V3 and V4 are three way valves; V2 is a six port injection valve and T is a three way connector). (b) Block diagram of the peak hold detector (LED, Light emitting diode; PT, Phototransistor).

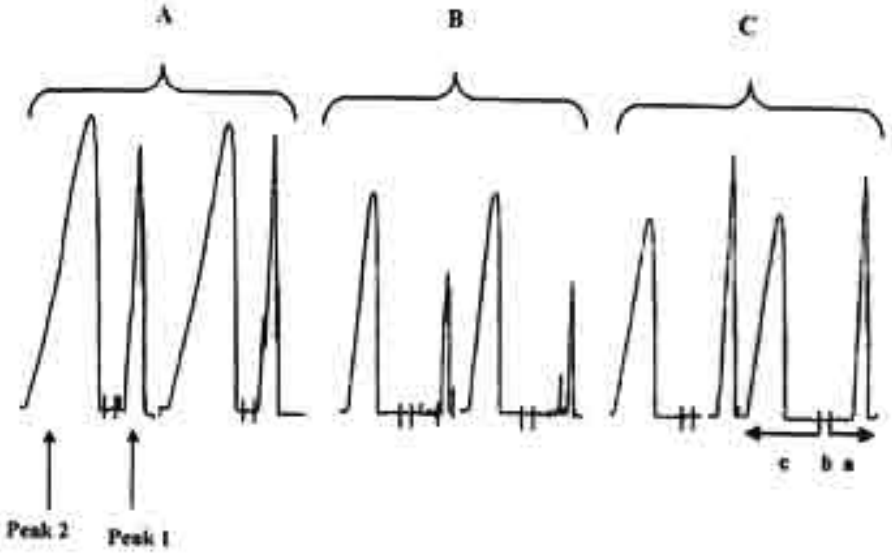

Fig. 2. Effect of bead loading time. Signals of sets A, B and C are of 9.00 μmol l^{-1} Fe^{2+} with 0.5% w/v 1,10-phenanthroline using a fixed sample loading time of 1 min and varied bead loading time of 20, 10 and 30 s, respectively. Signals from each analysis run consist of peak 1 and peak 2 that correspond to bead loading peak and analytical Fe peak, respectively, (see text). a, bead loading starts until the peak goes back to the base line; b, chart recorder is stopped; c, chart recorder starts before analytical peak appears until the peak goes back to the base line.

When beads entered the cell, they blocked the light source. As the signal reached the maximum (the cell was filled with beads), the signal went back down to the base line. The second peak was formed because of the maximum color intensity of the iron complex obtained when the color reagent solution reacted with iron trapped on the beads. This peak height directly relates to iron (Fe^{2+}) concentration on the beads and in the solution. At very high concentration of Fe^{2+}, the peak decay rate to the base line is slow. Thus, we observed the wide base peak at high concentration of iron.

3.2. Packing of beads in cell

It is important to control the homogeneity of resin bead suspension during different analysis run. Here bead suspension was stirred using magnetic stir bar at the fix rate so that at certain pump flow rate and fixed loading time, approximate same numbers of beads were introduced to the cell. Flow rate for bead loading step needs to be quite high (16 ml min^{-1}) to be able to transport these beads at the constant rate. This flow rate could be varied depending on size of beads used.

The uniformity of beads is also another important parameter. If they are too much different in size, beads may be packed in the cell differently each time. Amount of iron trapped may be different and would result in low precision. In addition, suitable and constant bead loading time is essential for good precision and for obtaining optimum analytical signals. The results shown in Fig. 2 were obtained from the fix loading time of 1 min of 9.00 μmol l^{-1}standard solution while the bead loading times were varied at 10, 20 and 30 s. This home made jet ring cell has approximated volume of about 50 μl. It was experimentally found that at 16 ml min^{-1} flow rate, the suitable bead loading time was 20 s. At shorter bead loading time (10 s) analytical signals (the second peaks) decreased due to less number of beads to trap iron. At longer bead loading time (30 s), analytical signals also decreased. There were excess beads outside the cell that could trap iron but

they were not detected because they were out of detection window. The reproducibility of the bead loading peak height was 4% R.S.D. from ten replicates.

3.3. Optimization of color reagent concentration

The experimental results (varying 1,10-phenanthroline concentrations of 0.05, 0.1, 0.3 and 0.6% w/v) showed that slightly excess concentration of 0.3% w/v of 1,10-phenanthroline offers optimum results for the determination of 9.00 μmol l^{-1} Fe^{2+} standard solution. At a higher concentration of 1,10-phenanthroline (up to at least 0.6% w/v), signals were not increased. At lower concentrations (0.1 and 0.05% w/v), signals were low because of insufficient amount of color reagent presented. We are interested in natural presented level iron (μmol l^{-1} or lower). Therefore, 0.3% w/v 1,10-phenanthroline was chosen for further experiments to save the reagent.

3.4. Calibration graph and effect of sample loading time

Sensitivity of the determination can be improved by using longer sample loading time. However, it is limited by the amounts of beads that cell can accommodate. Calibrations were obtained: $Y_1 = 0.0069X_1 - 0.542$, $(R^2 = 0.9999)$; $Y_2 = 0.0132X_2 - 0.3638$, $(R^2 = 0.9974)$; $Y_3 = 0.0144X_3 - 0.0831$, $(R^2 = 0.9452)$, where X and Y being μmol l^{-1} Fe^{2+} and peak height (V), for sample loading time of 1, 3 and 5 min, respectively. They show the significant changes in slopes between 1 and 3 min for sample loading time but only slight improve for 5 min loading time comparing with the 3 min one. The system allows 0.90 and 0.45 μmol l^{-1} Fe^{2+} to be determined for sample loading time periods of 3 and 5 min, respectively. Working range was about two order of magnitudes (up to 3.90 μmol l^{-1}).

3.5. Total iron determination

To determine Fe^{3+} and total iron using 1,10-phenanthroline reagent with the detection at 524 nm, Fe^{3+} needs to be prior reduced to Fe^{2+}. Although reducing agents, hydroxylammonium chloride, quinol or ascorbic acid can be used [13], ascorbic acid was chosen in this work because of its readily availability and its less toxicity. To test if ascorbic acid can be used effectively, peak heights obtained from analysis of 3.60 μmol l^{-1} Fe^{3+} with an addition of 5% w/v ascorbic acid were compared with those of Fe^{2+} of the same concentration. A mixture of Fe^{2+} and Fe^{3+} (1.80 μmol l^{-1} each) in the present of 5% w/v ascorbic acid was also analyzed and compared. It was confirmed that Fe^{3+} and total Fe could be determined utilizing ascorbic acid as a reducing agent. Optimum ascorbic acid concentration, from the concentrations investigated (0.5–5% w/v) for the particular concentration range of iron used in this work, was found to be 1% w/v. Too low ascorbic acid concentrations yielded low signals due to inefficient reduction of Fe^{3+} to Fe^{2+}.

3.6. Determination of iron in water samples

Table 1 summarizes the results of total iron determination and percent recoveries of spiked

Table 1
Percent recoveries of spiked iron in tap, pond and drinking water samples

Sample	[Fe] originally found (μmol l^{-1})	Spiked iron (μmol l^{-1})	Total [Fe] found (μmol l^{-1})	% Recovery of total Fe
Tap water	Fe^{3+} 0.82 ± 0.00	Fe^{2+} 1.80	2.78 ± 0.04	109
Tap water	Fe^{3+} 0.82 ± 0.00	Fe^{2+} 2.33	3.38 ± 0.04	110
Pond water	Fe^{3+} 0.64 ± 0.00 and total Fe 0.70 ± 0.00	Fe^{2+} 0.90	1.62 ± 0.19	100
Drinking water	Total Fe 0	Fe^{3+} 0.90 and Fe^{3+} 0.90	1.90 ± 0.10	106

iron in tap water, pond water and drinking water samples. The tap water sample did not contain Fe^{2+} in the level that can be detected. Iron in the form of Fe^{3+} is predominant. There are both Fe^{2+} and Fe^{3+} in the pond water sample. Fe^{3+} was estimated by subtraction of originally presented Fe^{2+} from total Fe. The drinking water sample did not show either Fe^{2+} or Fe^{3+}. Accuracy of the method was studied by spiking the samples, percent recoveries were found to be 100–110%. The positive recovery may be due to the interference from other elements in the samples.

4. Conclusion

A simple FI–BI system is proposed. A determination of trace iron in water samples using 1,10-phenanthroline with Chelex-100 resins was demonstrated. This inexpensive and manually easy to operate system offers online preconcentration for determination of low concentration ion. It improves analysis time because replaceable beads make elution steps unnecessary as compared with other preconcentration columns. This also reduces the possibility of cross contamination between runs. However, its drawbacks are that FI–BI uses large volumes of reagents and requires operators skill to carry out the assays in reproducible manner. It is hoped that this work on a model system will encourage others to exploit advantages of BI technique in combination with FI. Further investigation on online speciation of Fe^{2+} and Fe^{3+} or of other ions is in progress.

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References

[1] D.A. Holman, G.D. Christian, J. Ruzicka, Anal. Chem. 69 (1997) 1763.
[2] J. Ruzicka, A. Ivaska, Anal. Chem. 69 (1997) 5024.
[3] E.S.M. Lutz, H. Irth, U.R. Tjaden, J. van der Greef, Anal. Chem. 69 (1997) 4878.
[4] C.C. Oliveira, E.A.G. Zagatto, J. Ruzicka, G.D. Christian, Anal. Lett. 33 (2000) 929.
[5] J. Ruzicka, L. Scampavia, Anal. Chem. 4 (1999) 257A.
[6] S. Sole, A. Merkoci, S. Alegret, Trends Anal. Chem. 20 (2001) 102.
[7] J.F. van Staden, A. Botha, Anal. Chim. Acta 403 (2000) 279.
[8] J. Ruzicka, E.H. Hansen, Anal. Chem. 72 (2000) 212A.
[9] Z. Fang, Flow Injection Separation and Preconcentration, VCH, Weinheim, 1993.
[10] J.A. Sweileh, Microchem. J. 65 (2000) 87.
[11] H. Bagheri, A. Gholami, A. Najafi, Anal. Chim. Acta 424 (2000) 233.
[12] J.F. Liu, G.B. Jiang, Y.D. Feng, J. AOAC Int. 83 (2000) 1293.
[13] M.J. Pullin, S.E. Cabaniss, Water Res. 35 (2001) 363.
[14] K. Grudpan, C. Taylor, H. Sitter, C. Keller, Fresenius J. Anal. Chem. 346 (1993) 882.
[15] K. Grudpan, J. Jakmunee, Y. Vaneesorn, S. Watanesk, U. Aye Muang, P. Sooksamiti, Talanta 46 (1998) 1245.
[16] K. Grudpan, P. Sritharathikhun, J. Jakmunee, Anal. Chim. Acta 363 (1998) 99.
[17] K. Grudpan, P. Sritharathikhun, J. Jakmunee, Lab. Robotics Autom. 2 (2000) 129.
[18] K. Grudpan, K. Kamfoo, J. Jakmunee, Talanta 49 (1999) 1023.
[19] Standard Methods for the Examination of Water and Waste Water, American Public Health Association, American Water Works Association and Water Pollution Control Federation, 7th edition, Washington DC, 1989, pp. 3–102.
[20] H.M. Kingston, R.R. Greenberg, Anal. Chem. 55 (1983) 1160.

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Gravitational field-flow fractionation in combination with flow injection analysis or electrothermal AAS for size based iron speciation of particles

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Abstract

A simple gravitational field-flow fractionation (GrFFF) system was used for size separation of micron sized silica particles coated with hydrous iron oxide (geothite). The amount of iron on the particles was monitored either on-line by reverse-flow injection analysis (r-FIA) with chemiluminescence detection using luminol or off-line by electrothermal atomic absorption spectrophotometry (ETAAS). The combination of GrFFF with reverse FIA or with ETAAS has been demonstrated to be a cost-effective tool for size based iron speciation of particles.

Keywords: Gravitational field-flow fractionation; Flow injection analysis; Field-flow fractionation; Chemiluminescence; Iron speciation; Size based speciation

1. Introduction

There is currently much activity on the development of analytical methods for elemental speciation [1]. This is being applied in diverse fields such as medicine, agriculture, industry and the environment. While most work concentrates on determining the chemical forms of elements in samples there is also considerable interest in size based speciation. This involves determining the distribution of elements across the size range of the sample. In the field of environmental science this is important as particles size greatly influences the transport and fate of the elements associated with particles in air, soil and aquatic environments.

Size based speciation can be performed by combining separation and analytical methods. Recent examples include the off-line determination of metals (Cu, Fe, Mn) in beer by electrothermal atomic absorption spectrophotometry (ETAAS) after separation of metal complexes into different molecular weight fractions by size-exclusion chromatography (SEC) [2]. Inductively coupled plasma-mass spectrophotometry (ICP–MS) was employed for selenium speciation after capillary

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zone electrophoresis (SEC–CZE) of selenized yeast [3]. Sedimentation field-flow fractionation has been used on-line with ICP–MS for characterization of environmental colloids [4,5]. Flow FFF–ICP–MS was also used for determination of the size distribution and trace metal speciation of aquatic colloidal material [6,7]. Determination of size based elemental distributions of proteins [8] and trace metal analysis of humic acid from different origins in municipal waste were also performed by flow FFF–ICP–MS [9].

Natural aquatic and soil particles are thought to possess a coating of hydrous iron oxide and natural organic matter [10]. These coatings influence the uptake of contaminants such as trace metals, nutrients and biocides [11–13]. Thus the size distribution of readily released iron is of some importance in assessing the potential fate of some pollutants.

Field-flow fractionation (FFF) is a technique for size separation and characterization of particles and macromolecules [14]. FFF instrumentation is similar to that used in liquid chromatography. FFF takes place in a thin rectangular open channel and the separation is carried out by applying an external driving force perpendicular to the laminar flow of the carrier liquid [14]. Various driving forces can be employed in FFF (e.g. centrifugal, flow, thermal gradients, electrical and gravitational). This work used gravitational FFF (GrFFF) due to the simple experimental set up and low cost [15]. GrFFF can be set up by replacing the column in a standard HPLC system with the GrFFF channel. GrFFF is capable of separating particles in the diameter range of 2–50 μm and has been employed for biological samples such as red blood cells, parasites, wine yeast, and wheat starch [16–19]. The application of GrFFF for industrial particles has involved coal, silica and clay minerals [20–24].

GrFFF channel and separation mechanisms are illustrated in Fig. 1 [25]. A liquid carrier is pumped through the channel and a suspension of sample is injected (Fig. 1a). For micron size particles, larger particles elute earlier than the smaller ones and the elution mechanism involved is often called the 'steric elution mode'. In GrFFF, the hydrodynamic lift force can significantly affect the motion of particles [26]. Therefore, the GrFFF separation mechanism can also be referred to as the 'steric/hyperlayer elution mode'.

Combination of FFF with various analytical techniques has gained considerable interest recently, [14]. This has led to size based element distributions [5] and adsorption distributions [27,28]. In this paper we report work on the hyphenated method involving gravitational steric/hyperlayer FFF and flow injection (FI) with chemiluminescence (CL) detection (GrFFF–FIA–CL), which was first reported by Chantiwas et al. [29]. A schematic diagram of the GrFFF–FIA–CL instrument is given in Fig. 2a. The CL detection method used is based on the catalytic reaction of alkali luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and hydrogen peroxide. Trace amounts of iron catalyse the luminol oxidation, which emits light. Determination of iron based on this reaction has been reported previously, employing either normal or reverse FI [30–38]. In addition electrothermal AAS (ETAAS) analysis for iron was performed on size fractions collected from GrFFF as represented in Fig. 2b.

The objective of this paper is to demonstrate the effectiveness of these two new and relatively simple hyphenated analytical techniques (GrFFF–FIA–CL and GrFFF–ETAAS) for generating size based speciation data of iron. ETAAS was used for determination of the 'total' iron content of each fraction collected and the chemiluminescence reverse FI method was used to detect 'easily released' iron on the particles. The size separation and iron speciation was tested using model samples consisting of 5 and 10 μm HPLC silica particles coated with a thin layer of goethite (FeOOH).

2. Experimental

2.1. Gravitational field-flow fractionation

The components of the GrFFF instrument are represented in Fig. 2. The GrFFF system was low cost and easy to assemble. An overhead transparency sheet was cut to form the spacer. The channel dimensions were $0.095 \times 300 \times 20$ mm^3 for the

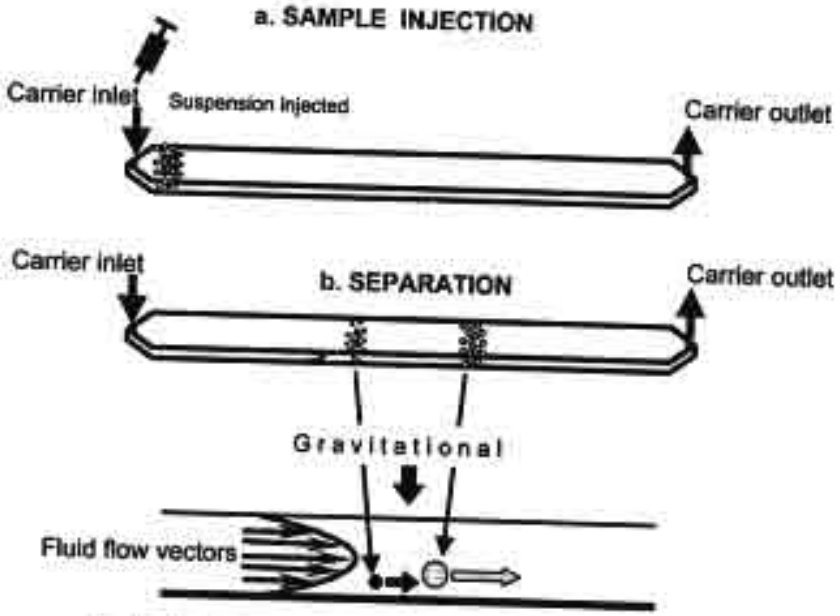

Fig. 1. GrFFF channel and steric hyperlayer separation mechanism.

channel thickness, length and breadth, respectively. The spacer was clamped between two of pieces of lucite plastic. An isocratic pump (SpectraSERIES, USA) was used to deliver the carrier solution. Particle suspensions were injected into the carrier stream with a hypodermic syringe (40 μl) through a home made injection port [39]. The concentration of particles in the FFF eluent was monitored by a UV detector (Linear Instruments Model 200 detector, USA) at an operating wavelength of 254 nm.

2.2. The electrothermal atomic absorption spectrophotometry

The ETAAS instrument was a Perkin–Elmer Model 5100 (Norwalk, CT) equipped with Zeeman correction, an HGA-600 graphite furnace and AS-60 autosampler. A pyrolytically coated graphite tube with L'vov platform was used. The ETAAS technique was used for trace iron determination of the eluted particles from the GrFFF system that were collected by a fraction collector (ISCO, Inc. Model RETRIEVER 500, Lincoln). Particle fractions were directly introduced as a slurry off-line into the ETAAS system for iron determination. ETAAS analysis was performed using the temperature program in Table 1 and with a wavelength of 248.3 nm, operating lamp current of 30 mA and sample volume of 10 μl was used. All fractions were homogenised by a vortex mixer before introducing into the atomiser. The modifier used for Fe determination was 1% of HNO_3 (5 μl). The calibration graph obtained using Fe standard solutions (10, 20, 40, 60 $\mu g\,l^{-1}$) was $Y = 0.00806X$, $r^2 = 0.997$.

2.3. Reverse-flow injection analysis with chemiluminescence detection (r-FIA–CL)

A luminol solution (8×10^{-6} M, 80 μl) was injected via a six-port injection valve (Upchurch, USA) into a stream hydrogen peroxide of (1×10^{-3} M) which was delivered by a peristaltic pump (Eyela SPM 23, Japan). After merging with an iron solution (standard/sample), the merged solutions were passed through a 30 cm mixing coil before finally entering the CL detector. The chemiluminescence intensity was monitored by a modified commercial liquid scintillation

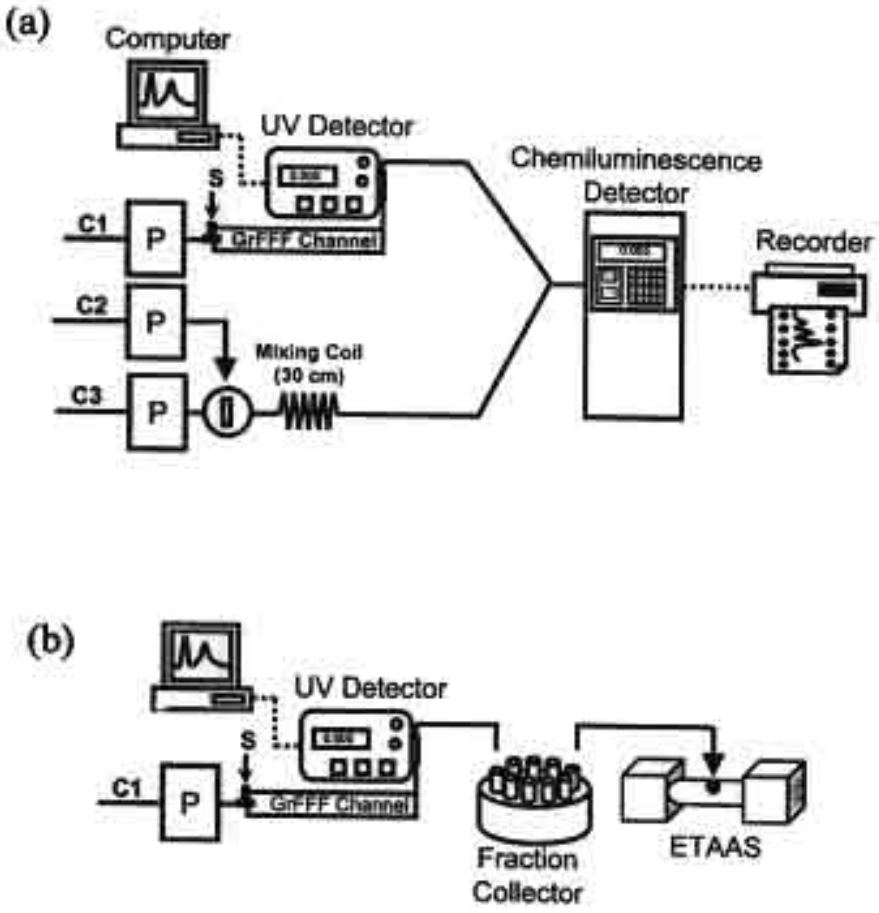

Fig. 2. Schematic diagram of the GrFFF coupled with (a) rFIA with chemiluminescence detection for iron determination. The reagents used were C1: 10^{-4} M NaOH (Flow rate 1.00 ml min^{-1}), C2: 8×10^{-6} M Luminol (Flow rate 1.0 ml min^{-1}), C3: 10^{-3} M H_2O_2 (Flow rate 3.0 ml min^{-1}) and (b) ETAAS.

counter, with a spiral flow cell (Packard Radiometric Flo-one®\Beta Series A-100 Model A140K, Canberra). The flow cell, Quick Change Flow Cell (serial number 7144) had a spiral shape and was

Table 1
Graphite furnace temperature program used for Fe analysis

Step	Furnace temperature (°C)	Time (s)		Internal Ar gas flow (ml min^{-1})
		Ramp	Hold	
1	110	1	15	300
2	130	7	5	300
3	20	7	10	300
4	1000	1	10	300
5	2400	2	15	0
6	2600	1	5	300

sandwiched between the two photomultiplier tubes. The volume of the flow cell was 200 μl. The calibration line for iron standards (10–80 $\mu g\,l^{-1}$) was $Y = 29.68X + 1373$, $r^2 = 0.996$.

2.4. Chemicals

All chemicals were analytical grade and were used without further purification. Water was obtained from a Milli-Q system (Milford, Massachusetts, MA). A 0.01 M solution of luminol was prepared by dissolving 5-Amino-2,3-dihydro-1,4-phthalazinedione (Sigma-Aldrich, Germany) (0.1772 g) in 100 ml of phosphate buffer. The buffer (pH 11) was prepared by dissolving Na_2HPO_4 (E. Merck, Germany) (0.7477 g) in 1000 ml of water and the pH was adjusted to be 11 with 2 M NaOH. Luminol solutions of appropriate concentrations were further diluted with the phosphate buffer. Hydrogen peroxide solution (0.001 M) was obtained by diluting H_2O_2 (30% volume) (Carlo Erba, Italy). An AAS iron (III) standard solution (1000 $mg\,l^{-1}$) (Merck, Germany) was diluted to appropriate concentrations. All reagents were freshly prepared and degassed for 12–15 min before use by using a sonication bath.

2.5. Silica particles

Chromatographic silica (5 and 10 μm) was obtained from used HPLC columns (CN packing from PARTISPHERE RTF Columns, WHATMAN, UK). The 5 μm particles were spherical and the 10 μm particles had an irregular shape.

2.6. Preparation of the goethite coated silica particles

The goethite coated silica was synthesized by a method developed based on the results reported by Stumm and O'Melia. It was used previously for the production of goethite coated kaolinite [40,41]. A solution of iron, (3.5 mM $Fe(NO_3)_3$) was gradually added to 10 ml of a suspension of silica particles (2 $mg\,ml^{-1}$). The pH was continually adjusted to 3.3 with 2 M HCl. The Fe solution addition rate was 1 $ml\,h^{-1}$ for the first 2 ml and 6 $ml\,h^{-1}$ for the remaining 12 ml. The mixture was continuously stirred. The suspension was then centrifuged and the silica residue was washed 4 times with Milli-Q water. The final suspension was dispersed in 10^{-4} M NaOH solution and heated in an oven at 60 °C for 24 h.

3. Results and discussion

3.1. Fe content of the Fe coated silica particles

The amount of iron on the coating silica was measured by ETAAS after being digested with *aqua regia* (HCl:HNO_3, v/v, 3:1). The concentration of iron was found to be 12.1 ± 0.2 $mg\,g^{-1}$ and 13.7 ± 0.7 $mg\,g^{-1}$ for the 5 and 10 μm particles, respectively. This indicates that the iron content of the two samples should be very similar.

Analysis of the Fe in the coated silica particles after acid dissolution (HCl:HNO_3, v/v, 3:1) was compared with slurry sampling (suspension of particles in Milli-Q water with the same dilution). It was found that the Fe content obtained from the slurry sampling method was lower than that measured after acid digestion. The recoveries were found to be $64 \pm 6\%$ and $59 \pm 10\%$ for the 5 and 10 μm particles, respectively.

Chen and Beckett reported almost complete recovery of Fe in a colloid sized (<1 μm) soil sample analysed by sedimentation FFF–ETAAS [41]. This suggests that the release of Fe coatings from these larger 5 and 10 μm sized particles is less efficient.

3.2. Thickness of the FeOOH layer on the silica particles

The thickness of the FeOOH coating on the particles (t) can be estimated if we assume the particle shape is spherical and coating layer is uniform. It is given by

$$t = \frac{\rho_{SiO_2} m_{FeOOH} d}{24\, \rho_{FeOOH} m_{SiO_2}} \quad (1)$$

where m_{SiO_2} is the known amount of sample mass injected, d is the diameter of the particles (5 μm),

ρ_{SiO_2} is the density of the silica (2.3 g ml^{-1}). We assume the iron coating on the particle is goethite (FeOOH) which has a density, ρ_{FeOOH}, of 3.8 g ml^{-1}. m_{FeOOH} is the mass of goethite on the coatings. The value of m_{FeOOH} was obtained from Fe based fractogram using the Fe content found in peak eluted between 3.75 and 7.75 min.

The calculated thickness of the goethite coating on the spherical 5 μm silica particles is found to be 5.1 nm, which is quite thin compared to the diameter of the silica particles of 5000 nm. The Fe content in the 10 μm sample peak was very similar, suggesting that the FeOOH coating thickness on the 10 μm particles could be approximately twice that of the 5 μm. However, the 10 μm particles are not spherical which could increase the specific surface area to some extent thus decreasing the actual coating thickness.

3.3. Separation of the Fe coated silica particles by GrFFF

Fig. 3 shows the GrFFF fractogram of a mixture of the 5 and 10 μm silica particles. The first peak is the void, which contains unretained particles. The 10 μm silica eluted before the 5 μm particles thus separation occurred by the steric/hyperlayer elution mechanism. Optical microscope pictures of the fractions collected at the maximum of the eluting peaks (Fig. 3) confirm the good separation obtained. The diameter scale was obtained using the empirical calibration expression:

$$\log t_r = -S_d \log d \quad (2)$$

and measuring the elution time (t_r) at the peak maximum of the 5 and 10μm particles. In Eq. (2) d is the diameter of the particles and S_d is the size based selectivity [42].

It was found by optical micrographs that there was a high proportion of small particles (< 1 μm) present in the 10 μm sample. These submicron particles would not be relaxed under the run condition performed here and would thus be eluted in the unretained void peak. This was confirmed by optical micrographs of the void peak fraction.

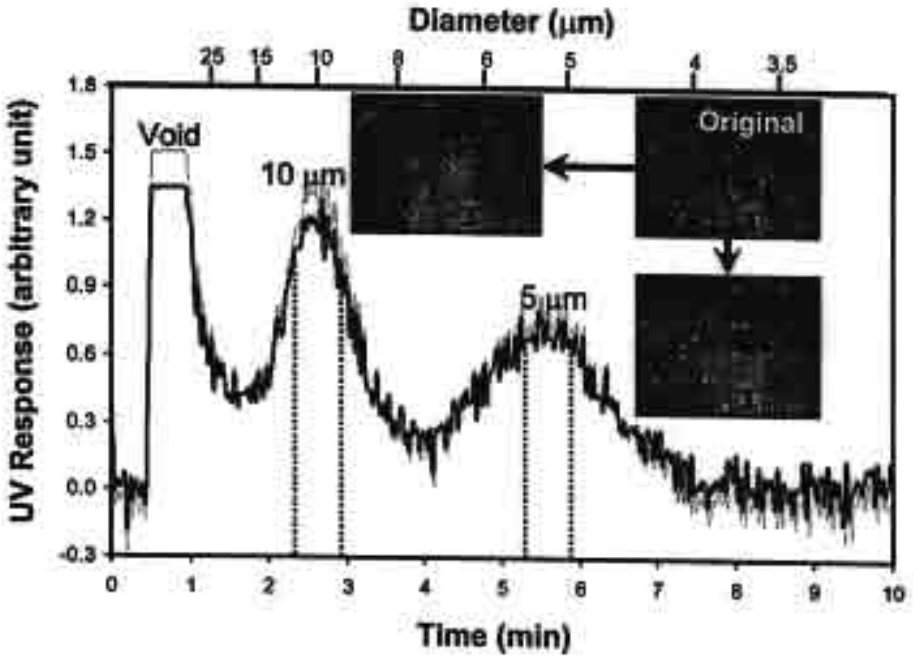

Fig. 3. The fractogram of a mixture of 5 and 10 μm goethite coated silica particles and the optical microscope pictures of the original mixture and fractions collected at the two peak maxima.

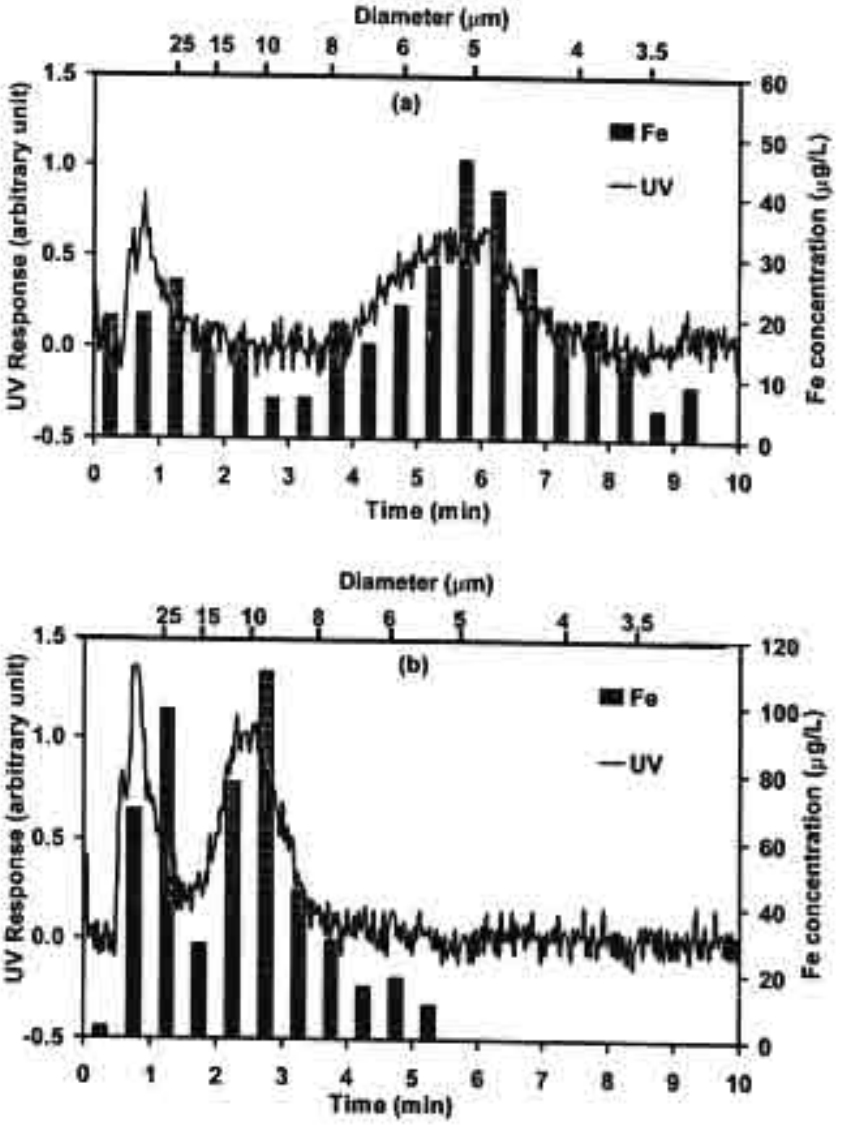

Fig. 4. UV detector fractograms and iron content of the fractions collected from GrFFF and determination by off-line ETAAS, (a) 10 μm, (b) 5 μm.

3.4. GrFFF–ETAAS of the Fe coated silica particles

Fig. 4 shows the fractograms of individual GrFFF runs of the 10 (Fig. 4a) and 5 μm (Fig. 4b) silica samples. The bar graphs represent the iron content in the collected fractions, which were determined off-line by ETAAS with slurry injection. It can be seen that the Fe concentration of each fraction follows quite closely the UV fractograms.

Computing the area under the Fe based fractograms ([Fe] vs elution time) including the void peak and comparing this with the Fe content of the whole sample digested with *aqua regia*, showed that 71 and 88% of the Fe on the particles was detected by slurry ETAAS, for the 5 and 10 μm samples, respectively. As noted above the recovery

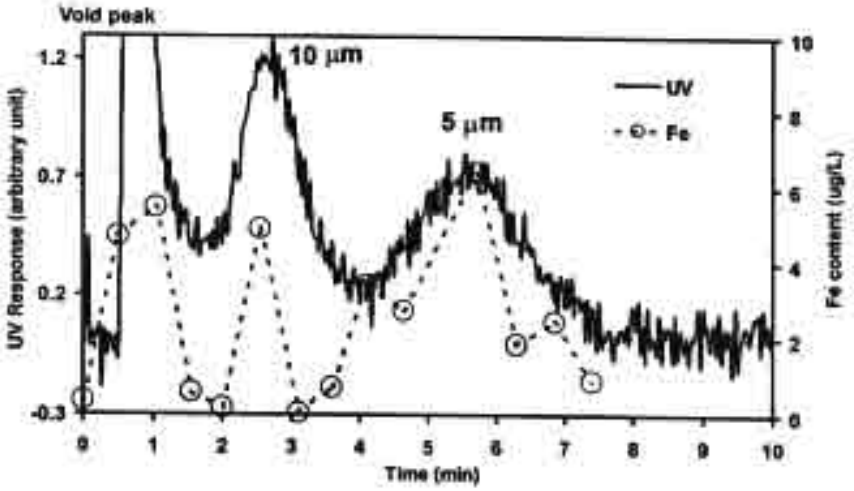

Fig. 5. UV (solid line) and Fe concentration (open circles with dashed line) fractogram of the mixture of 10 and 5 µm particles. The Fe concentration was determined on-line from the GrFFF combined with FIA–CL detection.

efficiency appears for these larger particles seems to be less than that found by Chen and Beckett for submicron particles (close to 100%) [41]. For the 10 µm particles a significant proportion of the Fe is associated with the fine particles found in the void peak which may be responsible for the higher proportion recorded by ETAAS (88%).

3.5. GrFFF–FIA–CL of the mixture of 5 and 10 µm of Fe coated silica particles

Fig. 5 shows the GrFFF fractogram for the mixture of 5 and 10 µm silica as well as the results of the online Fe analysis by r-FIA–CL. The eluant from the GrFFF was continuously flowed into the r-FIA–CL for Fe analysis. Each data point of the iron content corresponds to a signal obtained from an injection of the mixture of luminol and H_2O_2 into a flowing steam of the GrFFF eluent before entering into the CL detector.

The area of under the Fe profile ([Fe] vs elution time) was used to calculate the iron content of each individual peak. From the calculation, the Fe content in the 5 and 10 µm particles were found to be 0.57 and 0.14 mg g^{-1}, respectively. The iron content present in the 5 µm particles is higher than that in the 10 µm particles. This could suggest that the Fe content is related to the particle surface area. This is different from the trend in the Fe content obtained by GrFFF–ETAAS and after *aqua regia* digestion of the whole sample, where the Fe content in the two samples is approximately the same. It is also apparent that the r-FIA–CL method detects only a small proportion of the total Fe in the sample particles. In the case of the 10 µm particles, this is only about 1% of the total Fe obtained after *aqua regia* digestion.

4. Conclusion

In this work, we have attempted to apply simple GrFFF combined with FIA–CL or ETAAS as an approach to size based element speciation. We have tested this approach by employing two model silica particles which were coated with hydrous iron oxide (geothite). We have found that the simple GrFFF can be employed to separate micron sized particles, and when combined with another analytical device, such as FIA–CL or ETAAS, is a promising and cost-effective tool for size based element speciation of particles.

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References

[1] Special issue papers, J. Environ. Monit. 3 (2001) 1–101.
[2] R. Svendsen, W. Lund, Analyst 125 (2000) 1933.
[3] S. Mounicou, S. McSheehy, J. Szpunar, M. Potin-Gautier, R. Lobinski, J. Anal. At. Spectrom. 17 (2002) 15.
[4] D.M. Murphy, J.R. Garbarino, H.E. Taylor, B.T. Hart, R. Beckett, J. Chromatogr. 642 (1993) 459.
[5] J.F. Ranville, D.J. Chittleborough, F. Shanks, R.J.S. Morrison, T. Harris, F. Doss, R. Beckett, Anal. Chim. Acta 381 (1999) 315.
[6] M. Hassellov, B. Lyven, R. Beckett, Environ. Sci. Technol. 33 (1999) 4528.
[7] M. Hassellov, B. Lyven, C. Haraldsson, W. Sirinawin, Anal. Chem. 71 (1999) 3497.
[8] A. Siripinyanond, R.M. Barnes, J. Anal. At. Spectrom. 14 (1999) 1527.
[9] D. Amarasiriwardena, A. Siripinyanond, R.M. Barnes, J. Anal. At. Spectrom. 16 (2001) 978.
[10] R. Beckett, Surface and colloid chemistry in natural waters and water treatment, in: R. Beckett (Ed.), Surface Chemistry of Humic Substances in Aquatic Systems, Plenum, London, 1990, pp. 3–20.
[11] B. Chen, J. Hulston, R. Beckett, Sci. Total Environ. 263 (2000) 23.
[12] G.M. Day, B.T. Hart, I.D. McKelvie, R. Beckett, J. Reprod. Fertility Environ. Technol. 18 (1997) 769.
[13] G.M. Day, B.T. Hart, I.D. McKelvie, R. Beckett, Environ. Technol. 18 (1997) 781.
[14] M.E. Schimpf, J.C. Giddings, K. Caldwell (Eds.), Field-Flow Fractionation Handbook, Wiley, New York, Chichester, 2000, p. XVIII.
[15] P. Reschiglian, D. Melucci, A. Zattoni, G. Torsi, Annali di Chimica 87 (1997) 677.
[16] A. Bernard, C. Bories, P.M. Loiseau, P.J.P. Cardot, J. Chromatogr. B. 664 (1995) 444.
[17] A. Bernard, B. Paulet, V. Colin, P.J.P. Cardot, Trends Anal. Chem. 14 (1995) 266.
[18] R. Sanz, L. Puigno, P. Reschiglian, M.T. Galceran, J. Chromatogr. A. 919 (2001) 339.
[19] C. Contado, P. Reschiglian, S. Paccini, A. Zattoni, F. Dondi, J. Chromatogr. A. 871 (2000) 449.
[20] H. Meng, K.D. Caldwell, J.C. Giddings, Fuel Proc. Technol. 8 (1984) 313.
[21] K.A. Graff, K.D. Caldwell, M.N. Myers, J.C. Giddings, Fuel 63 (1984) 621.
[22] J. Pazourek, J. Chmelik, J. Chromatogr. A. 715 (1995) 259.
[23] J. Pazourek, E. Urbankova, J. Chmelik, J. Chromatogr. A. 660 (1994) 113.
[24] J. Pazourek, K.-G. Wahlund, J. Chmelik, J. Microcolumn Sep. 8 (1996) 331.
[25] P. Reschiglian, G. Toris, Chromotographia 40 (1995) 467.
[26] P.S. Williams, T. Koch, J.C. Giddings, Chem. Eng. Commun. 111 (1992) 121.
[27] R. Beckett, D.M. Hotchin, B.T. Hart, J. Chromatogr. 517 (1990) 435.
[28] J. Vanberkel, R. Beckett, J. Chromatogr. 733 (1996) 105.
[29] R. Chantiwas, J. Jakmunee, R. Beckett, I.D. McKelvie, K. Grudpan, Anal. Sci. 17 (2001) i423.
[30] A.R. Bowie, E.P. Achterberg, R.F.C. Mantoura, P.J. Worsfold, Anal. Chim. Acta 361 (1998) 189.
[31] V. Cannizzaro, A.R. Bowie, A. Sax, E.P. Achterberg, P.J. Worsfold, Analyst 125 (1999) 51.
[32] E.G. Sarantonis, A. Townshend, Anal. Chim. Acta 184 (1986) 311.
[33] P. Fletcher, K.N. Andrew, A.C. Calokerinos, S. Forbes, P.J. Worsfold, Luminescence 16 (2001) 1.
[34] S. Hirata, H. Yoshiharu, M. Aihara, Talanta 49 (1999) 1059.
[35] T. Kyuw, T. Fujiwara, H. Inoue, Y. Okamoto, T. Kumamaru, Anal. Sci. 14 (1998) 203.
[36] A.L. Rose, T.D. Waite, Anal. Chem. 73 (2001) 5909.
[37] K. Saitoh, T. Hasebe, N. Teshima, M. Kurihara, T. Kawashima, Anal. Chim. Acta 376 (1998) 247.
[38] W. Qin, Z.J. Zhang, F.C. Wang, Fresenius J. Anal. Chem. 360 (1998) 130.
[39] R. Chantiwas, J. Jakmunee, R. Beckett, K. Grudpan, Anal. Sci. 17 (2001) i1419.
[40] B. Chen, Investigation of pollutants associated with soil and sediment colloids, Ph.D. Thesis, Monash University, Melbourne, Australia, 2000, pp. 227.
[41] B. Chen, R. Beckett, Analyst 126 (2001) 1588.
[42] J.C. Giddings, M.H. Moon, P.S. Williams, M. Myers, Anal. Chem. 63 (1991) 1366.

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Simple flow injection system for colorimetric determination of iodate in iodized salt☆

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Abstract

This work presents a flow injection (FI) system that was developed for determination of iodate. The system utilizes the oxidation of iodide by the analyte to iodine, which subsequently forms tri-iodide. In the presence of starch, the blue I_3^-–starch complex is developed within the sample zone and can be colorimetrically detected at 590 nm. Optimization was carried out to make the system suitable for quantitating iodate added to table salts. To prevent accumulation of the blue complex residue on walls of tubing and the flow cell, a port was placed in the system for injection of 10^{-3} M thiosulfate plug (100 μl). An injection of this cleaning solution after each sample injection is recommended to avoid positive baseline shift. By means of the paired *t*-test, the amounts of iodine (mg I kg^{-1}) were statistically compared with the results determined by titration and by iodide ion selective electrode. No significant disagreement at 95% confidence was observed. The proposed system is very simple, uses common chemicals and provides rapid analysis (65 injections per h) with high precision (R.S.D. = 0.66%, $n = 10$). A detection limit of 2 mg I kg^{-1} salt can be achieved.

Keywords: Iodate; Iodized salt; Tri-iodide; Starch; Flow injection; ISE

1. Introduction

Addition of iodine to table salts is part of a preventive program for iodine deficiency disorder in many countries. Salts are commonly iodized with potassium iodide. However, for some tropical countries, including Thailand, salts are supplemented with potassium iodate for a longer shelf life. The recommended value for iodine supplement is 50 mg I kg^{-1}. Most, available methods for measuring iodine in iodized salt are for those supplemented with potassium iodide [1–4]. An ion chromatographic method is available for determination of iodate in iodized common salt [5], but sample throughput is considered low. Recently

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there was a report of an amperometric method for the iodate supplement [6], which provides a considerably high sample throughput at 35 injections per h. The method utilized the flow injection (FI) technique [7].

This work describes use of the well-known iodine chemistry, that is the Dushman reaction [8], for quantifying iodate content in iodized salt. Analysis is based on FI technique. Tri-iodide which is formed in the sample zone, after the reduction of iodate, intercalates inside starch molecules to produce a blue colored complex of tri-iodide starch (I_3^-–starch). Thus, instead of employing UV detection (I_3^-, 350 nm), the detection wavelength is shifted to the visible region (I_3^-–starch, 590 nm). This makes the system attractively economical. The method is new particularly for this application. Although some authors have used FI systems with detection of the starch complex, the systems were for other applications [9–11]. These works adapted detection of decolorization of the complex in a reagent stream, as determined by the concentration or activity of analytes. Examples of the works were the determination of amylase [9,10] and penicillin [11]. A group of authors [10] recommended pumping 0.2 M sodium hydroxide solution through the system to clean out the complex deposited on tubing wall. In this paper a more convenient way of cleaning is presented. The developed method was compared with the conventional titration method and with a potentiometric method.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent grade. Distilled water was used throughout. A stock of iodate standard was prepared by dissolving 2.1402 g of potassium iodate crystal (Univar, Australia) in water to 100.00 ml. Appropriate dilutions in 6% (w/v) sodium chloride were made in the preparation of working solutions.

The carrier stream was made by dissolving 2 g of potassium iodide(Univar, Australia) in a solution of 6% (w/v) sodium chloride and making up to 500 ml. The starch stream of 0.1% (w/v) starch solution containing 0.01 M sulfuric acid was daily prepared. Starch (0.5 g) (Merck, Germany) was mixed with a few milliliters of water to make slurry. The slurry was added to 500 ml of boiling water. After cooling, 5 ml of 1 M sulfuric acid (diluted from conc. H_2SO_4, Lab-scan, Ireland) was added to this solution.

The solution of 0.1 M thiosulfate was prepared by dissolving 2.5 g of solid sodium thiosulfate pentahydrate (Merck, Germany) in water and making up to 100 ml. This solution was diluted with water to obtain thiosulfate 1.0×10^{-3} M.

2.2. Sample

All samples were oven dried at 120 °C overnight and stored in desiccator until constant weight. A sample solution was prepared by dissolving an accurate weight of 30 g of salt in water and making up to 250.00 ml (note: filtration may be necessary for some sample solutions that contain particulate matter). By this preparation, a sample solution would contain approximately 12% (w/v) NaCl. The samples were analyzed directly using the titration method. For the developed FI and the potentiometric method, the liquid samples were diluted from 12 to 6% (w/v) salt before analysis.

2.3. Flow injection system

A FI manifold (Fig. 1) was set up and studied. An Ismatec peristaltic pump (model IS7610, Switzerland) was used for propelling reagents. Two Rheodyne injection valves (model 5020, USA) were used as V1 and V2 in the manifold. A Jenway spectrophotometer (model 6405, UK), fitted with a Philips flow cell of 10 μl volume, was used for monitoring the change of absorbance at 590 nm. PTFE tubing with i.d. of 0.5 mm was used throughout. An Alltech chart recorder (model LR 93025, USA) was used for recording signal.

2.4. Titration method

A 10.00 ml aliquot of 12% (w/v) salt solution was transferred into an iodine stoppered flask. To

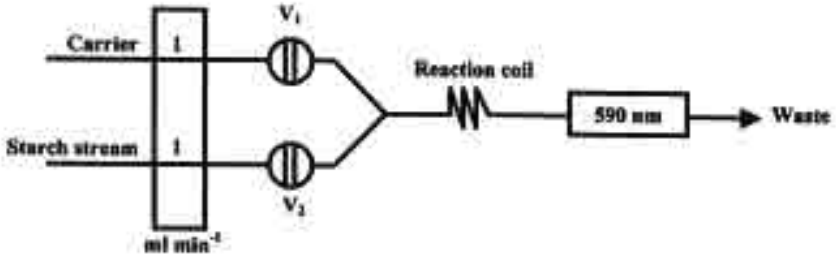

Fig. 1. The FI manifold for determination of iodate in iodized salt. Carrier: 2.5×10^{-2} M KI in 6% (w/v) NaCl, starch stream: 0.1% starch in 1.0×10^{-2} M H_2SO_4, V_1: 250 μl injection of sample solution or standard, V_2: 100 μl injection of 1.0×10^{-3} M $Na_2S_2O_3$.

the sample, 5 ml of 1 M H_2SO_4 and 10 ml of 10% (w/v) KI were added. The mixture was titrated against a standardized $Na_2S_2O_3$ solution (about 2.4×10^{-4} M), using 1% (w/v) starch as indicator.

2.5. Potentiometric method

Accurate 5.00 ml of a sample solution was directly transferred into a 50 ml volumetric flask which contained 12 ml of 2% (w/v) ascorbic acid. The volume was made up to the mark with water. After mixing, the solution was measured for the potential developed across the iodide-ISE and the saturated calomel electrode. Calibration was carried out with standard solution made from potassium iodate (1.0×10^{-6}–1.0×10^{-2} M) prepared to have the same liquid matrix as the sample (1.2% (w/v) NaCl and 0.5% (w/v) ascorbic acid).

3. Results and discussion

3.1. Manifold design

3.1.1. Background

Our present FI manifold (Fig. 1) was in fact modified form a previous manifold, which consisted of three channels. Recently, the three-channel manifold [14] was used in the validation of another FI system reported for determination of iodine in table salt using amperometric detection [6]. The system in Fig. 1 is made simpler than the previous system by combining together some of the reagents in the manifold and thus reduced to two-channel. Dispersion of an injected zone is less in the current manifold ($D = 2.6$) as compared with the former system ($D = 3.8$). The sensitivity (calibration slope) is increased by a factor of 1.5.

Levels of iodine found in iodized samples can be lower or higher than the recommended value. However, it is appropriate to prepare samples by dissolution to roughly 6% (w/v) salt before direct injection. Thus, the carrier stream of the system in Fig. 1 was designed to contain 6% (w/v) NaCl to prevent the Schileren effect [12]. Standard iodate solutions were also prepared in 6% (w/v) NaCl solution. The solid NaCl used in these preparations, can be of general grade or even sea salt (non-iodized).

3.1.2. Elimination of baseline shift

It was observed during analysis that there was accumulation of tri-iodide starch complex on tubing wall situated between the stream confluence and the flow cell. There may also be the deposition inside the flow cell. These resulted in positive shift of baseline. To diminish the problem, a valve (V2) was inserted in the system for injection of a plug of cleaning solution. A 100 μl injection of sodium thiosulfate, 1.0×10^{-3} M, was selected for washing off the starch complex. The complex is decolorized by thiosulfate to produce iodide, which does not form a colored complex with amylose molecule. An injection of the thiosulfate was carried out after every sample injection. Injection of sample was carried out approximately at 30 s intervals.

3.2. Optimization of the FI manifold

The objective of the experiment was to achieve a suitable signal reading (0.5–0.6 a.u.) for $2.5 \times$

10^{-5} M standard iodate solution. This concentration correspond to the recommended value at 50 mg I kg^{-1} of salt. Contents of iodine in real samples could be below or above this value.

Concentrations of reagents were adapted from the previous work of Jakmunee and Grudpan [6]. Three physical parameters, viz., injection volume at V1, flow rate and mixing coil length, were optimized one at a time. Repetitive injections of 2.5×10^{-5} M iodate solution were carried out in triplicate whereas concentrations of reagents were fixed at the values given in Fig. 1.

It was observed that increasing the volume to 250 μl raised up the signal to the desired absorbance value. This volume was chosen for optimization of the other parameters.

Flow rates were varied from 0.5 to 2.5 ml min^{-1}. The results showed that increasing the flow rate increased the sensitivity. This is probably due to the better mixing achieved at the confluence point in Fig. 1. In this work, the flow rate of 1 ml min^{-1} was selected for each flow channel in Fig. 1.

The system was most sensitive when no coil was used. The absorbance decreased from 0.615 to 0.485 a.u. when the coil length was increased from 0 to 1.5 m. In fact, any length could have been chosen but for this manifold a 120 cm coil was employed so that the absorbance for the highest concentration of iodate standard (4.0×10^{-5} M) was within the range of the detector.

3.3. Calibration

The calibration graph obtained for iodate concentration ranging from 5.0×10^{-6} to 4.0×10^{-5} M was linear: peak height (absorbance) = $25520[IO_3^-] - 0.052$, $r^2 = 0.999$ ($n = 4$). It was observed that the intercept was never at the origin. This may be due to the limitation of the reaction kinetics in the region of low concentrations or it could be the limitation of the dynamic range of detector.

3.4. Recovery

An experiment was carried out on four samples to evaluate the recovery of this method. Addition of potassium iodate standards (5.0×10^{-6}, 1.0×10^{-5} and 2.0×10^{-5} M) was made for a sample solution. The results given in Table 1 demonstrate that the method provides satisfactorily good recovery of the signal.

3.5. Effect of foreign ions

Normally our iodized salts are produced using sea salt. Five foreign ions that are present in seawater, viz. the remaining two halides and three major cations, were studied for possible affect on the analysis. As shown in Table 2, a standard solution, 2.0×10^{-5} M, was prepared containing, species of ions at concentration level normally found in a sample of sea salt (providing the salt was prepared from complete evaporation). The levels were in fact calculated based on the elemental abundance in seawater [13]. The results in Table 2 indicate that none of these ions gave rise to signal alteration of the pure standard outside ± 5%. Thus, it can be concluded that these foreign ions do not interfere with the proposed method.

3.6. Tolerance to pH of sample

Since iodized salts are produced using raw materials from various sources, a study was made to see if pH of sample solution has any effect on the FI signal. The experiment was carried out similarly to the optimization of parameters using injections of the same concentration of iodate standard. pH of the standard solution was adjusted (with HCl or NaOH) to 1.99, 5.15, 5.96, 7.05 and 9.09. The signal was constant for pH 5.15–9.09 (0.646–0.621 a.u.). The signal dropped to 0.502 a.u. for the lowest pH. This indicates that in acidic medium the yield of complex is less than at higher pHs. All of the salt samples used in this work did not have their solution pH outside the range of 6–8. Thus the method should be practical for most salts.

3.7. Application to real samples and validation

Analysis for iodine contents in nine samples was first carried out. The results were compared with those given by the titration method as shown in Table 3. According to paired t-test, no significant

Table 1
Recovery study of iodate added to samples

Sample	Sample concentration (M)	Added (M)*	Found (M)	Percentage recovery
S1	7.5×10^{-6}	5×10^{-6}	1.3×10^{-5}	110
		1×10^{-5}	1.9×10^{-5}	115
		2×10^{-5}	2.9×10^{-5}	107
S2	8.8×10^{-6}	5×10^{-6}	1.5×10^{-5}	124
		1×10^{-5}	1.9×10^{-5}	102
		2×10^{-5}	2.8×10^{-5}	96
S3	1.5×10^{-5}	5×10^{-6}	2.1×10^{-5}	120
		1×10^{-5}	2.6×10^{-5}	110
		2×10^{-5}	3.6×10^{-5}	105
S4	1.8×10^{-5}	5×10^{-6}	2.3×10^{-5}	100
		1×10^{-5}	2.9×10^{-5}	110
		2×10^{-5}	3.9×10^{-5}	105

* Concentration of standard iodate added to sample solutions.

Table 2
Effect of foreign ions on alteration of the FI signal of standard potassium iodate 2.0×10^{-5} M prepared in 6% (w/v) NaCl

Foreign species	Added as	Concentration added (M)*	Signal alteration (%)
F^-	NaF	1.4×10^{-4}	0
Br^-	NaBr	2.0×10^{-3}	0
K^+	KCl	2.0×10^{-2}	−1.2
Ca^{2+}	$CaCl_2 \cdot 2H_2O$	2.0×10^{-2}	−2.3
Mg^{2+}	$MgCl_2 \cdot 2H_2O$	1.0×10^{-1}	+3.2

* Based on normal levels present in sea salt.

Table 3
Iodine contents in table salts obtained from the colorimetric FI method and the titration method

Sample	Iodine content (mg I kg^{-1})±S.D.	
	Colorimetric FI ($n=3$)	Titration ($n=3$)
S5	16.5±0.1	18.5±0.2
S6	16.9±0.2	18.4±0.5
S7	24.3±0.2	24.2±0.2
S8	31.1±0.2	30.1±0.4
S9	31.5±0.1	33.5±0.4
S10	34.0±0.1	34.5±0.5
S11	42.2±0.2	40.6±0.6
S12	70.3±0.2	65.0±0.5
S13	71.9±0.1	65.8±0.8

different was found between the results of both methods at 95% confidence limit ($t_{observed} = 0.89$, $t_{critical} = 2.31$).

A further 12 samples of table salts were determined for iodine contents using the FI method and the method employing an iodide-ISE. The latter method requires addition of ascorbic acid into salt solution to reduce iodate ion to iodide ion before potentiometric detection. Iodine contents summarized in Table 4 demonstrate good agreement between the methods. The difference between both methods was not significant at 95% confidence ($t_{observed} = 0.77$, $t_{critical} = 2.20$).

3.8. Analytical features and advantage over other methods

The present method satisfactorily provides rapid analysis (65 injections per h) and thus is more rapid than the former FI method presented