Article

JOURNAL OF THE CHINESE CHEMICAL SOCIETY

In-vitro Aluminum Determination and Preconcentration in Blood of Dialysis Patients Based on Ionic Liquid Dispersive Liquid-Liquid Biomicroextraction by 2-Amino-3-(1H-imidazol-4-yl)propanoic Acid

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(Received: Oct. 21, 2013; Accepted: Feb. 27, 2014; Published Online: May 6, 2014; DOI: 10.1002/jccs.201300531)

In this study, trace amounts of aluminum in serum of dialysis patients were chelated with 2-Amino-3-(1H-imidazol-4-yl)propanoic acid (Histidine) and determined by electro-thermal atomic absorption spectrometry (ETAAS). A fast and efficient method based on ionic liquid dispersive liquid-liquid bio-micro-extraction (IL-DLLBME) was developed for the determination of Al cation in human blood serum samples. In this work, a small amount of 1-Hexyl-3-methylimmidazolum hexafluorophosphate ([HMIM][PF_6]) as an extractant solvent was dissolved in acetone as a dispersant solvent and then the binary solution was rapidly injected by a syringe into the serum containing Al^{3+},Which have already in-vitro chelated by Histidine amino acid (Al-His) at pH = 6.5. After separation, the settled IL-phase was dissolved in ethanol up to 200 μL and 20 μL of samples injected into the ET-AAS by auto-sampler. Various parameters have been studied and optimized for 10 mL of sample. Under the optimum conditions, the enrichment factor (EF), limit of detection (LOD) and working range (peak area mode) were obtained 53, 15 ng L^{-1} and 0.05-4.1 μg L^{-1} respectively. In vitro Al chelation showed that His can significantly decrease aluminum concentration in serum of dialysis patients. Validation of methodology was confirmed by standard reference material (SRM).

Keywords: Aluminum; In vitro chelation; Dialysis patients; Histidine; Dispersive liquid-liquid bio-micro-extraction.

INTRODUCTION

Aluminum is a trivalent cation (Al^{3+}) found in humans, animals, and environment. Small quantities of dietary aluminum are not a significant source of concern in persons with normal elimination capacity.[1,2] Most heavy metals can cause disease in human body and essential metals such as; copper and zinc affected on human body in the case of deficiency or imbalance. Toxic effects of aluminum dependent on the amount of ingested, entry rate, tissue distribution, concentration achieved, and excretion rate.[2,3] High amount of aluminum is toxic for human body and is observed in all age groups with no predilection for any race or sex.[4] Aluminum also used for dialysis dementia.[5] Aluminum is used as a food additive, antacids, buffered aspirin an antiperspirants and first aid antibiotic an antiseptics.[6] In human body, approximately 95% of aluminum eliminated through renal but in renal dysfunction, that aluminum has the potential to accumulate in tissue.[7] Aluminum is absorbed from the gastrointestinal tract (GI) in the form of oral phosphate-binding agents (aluminum hydroxide), via dialysate on patients on dialysis and total parenteral nutrition (TPN) contamination. If a significant load exceeds the body's excretory capacity, the excess is deposited in various tissues, including bone, brain, liver, heart, spleen, and muscle.[2] Different pathways attributed to Al accumulation in the brain of Alzheimer's patients. Al bioavailability through food and environment is moderate. Alzheimer's patients are known to have altered permeability of the blood-brain barrier, permitting more Al entry into brain. Further, people suffering from renal dysfunction accumulate Al in their brain due to its insufficient removal from the body.[8] Aluminum brain concentrations should be lower than 2 μg/g and causes an oxidative stress within brain tissue.[9-11] Aluminum binds to various ligands in the blood and distributes to every organ, with highest concentrations ultimately found in bone and lung tissues. Efforts to remove Al from serum by direct hemodialysis have generally been unsuccessful, because 80% of aluminum ions are bound to serum proteins such as albumin and transferrin. Earlier agents such as dimercaptopropanol (DMP) and penicil-

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lamine were not successful for Al removal in serum[12,13] Patients in renal failure (RF) lose the ability to clear aluminum and are candidates for aluminum toxicity. Many factors increase the incidence of aluminum toxicity in RF patients. Aluminum reference values for human blood serum in dialysis patients are less than 60 μg L^{-1} and Describes reference intervals and additional information for interpretation of test results. May include intervals based on age and sex when appropriate. Intervals are Mayo-derived, unless otherwise designated. If an interpretive report is provided, the reference value field will state this. in healthy people is 1-3 μg L^{-1}. In normal tissue aluminum concentrations are greater in lung (60%) than bone and soft tissues. Higher concentrations are seen in uremia and higher still in dialysis encephalopathy.[14,15] All this findings cause alarming concern in public health, demanding accurate determination of aluminum ion at traces and sub-trace levels. Sensitive analysis techniques for determination of aluminum include; High performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICP-MS),[16] stripping voltammetry,[17] flame atomic absorption spectrometry (FAAS),[18] and electrothermal atomic absorption spectrometry (ETAAS)[19,20] that were frequently coupled with a prior pre-concentration and/or separation steps. However, the high instrumental and operational costs and the high detection limits are common disadvantages of many of these methods. Sample preparation procedures such as liquid-liquid extraction (LLE),[21] inductively coupled plasma optical emission spectrometry (ICP-OES),[22] solid phase extraction (SPE)[23] and Cloud point extraction (CPE)[24] are developed to simplify analytical approaches as it reduces costs. Dispersive liquid-liquid micro extraction (LLME) is a miniaturization of the traditional LLE technique, where the ionic liquids (ILs) is a drop of a few micro litres of a water-immiscible solvent that can be directly immersed in the sample and dispersed by organic solvent.[25,26] ILs has various advantages over traditional organic solvents but they depended on many parameters such as temperature, percentage of extraction.[27-29] In this work, ionic liquid dispersive liquid-liquid bio-micro-extraction (IL-DLLBME) based on histidine (His) amino acid were used for in-vitro chelation and pre-concentration aluminum in serum dialysis patients before determination by ETAAS. The effect of various parameters on aluminum bio-micro-extraction were investigated and discussed in detail. Also, aluminum can be mobilized and eliminated by dialysis when enough histidine ligand was used.

EXPERIMENTAL

Reagents and chemicals: All reagents were of trace analytical grade from Sigma Aldrich. High purity reagents were used for all preparations of the standard and sample solution. Aluminum stock solution was prepared from an appropriate amount of the nitrate salt of this analyte as 1000 mgL^{-1} solution in 0.01 mol L^{-1} HNO_3 (Merck). Standard solutions were prepared daily by dilution of the stock solution. 0.8 mol L^{-1} buffer acetate solution was used for adjusting pH at 6.5. Polyoxyethyleneoctyl phenyl ether (TX-100) as the anti-sticking agent and mixed of stereoisomers of histidine (DL-His) was also purchased from Sigma Aldrich. Ultrapure water was obtained from Millipore continental water system (Bedford, USA) and 1-Hexyl-3-methylimmidazolum hexafluorophosphate ([HMIM] [PF_6]) was prepared from Sigma Aldrich. All glass vessels used for the trace analysis were kept in 10% nitric acid solution for at least 24 h and subsequently washed with distilled water.

Apparatus: Determination of Aluminum was performed with a spectra GBC electro-thermal atomic absorption spectrometer (Model; Plus 932, Australia) using a graphite furnace module (GF3000, GBC). The operating parameters for the metal of interest were set as recommended by the manufacturer. A hollow cathode lamp operating at a current of 6 mA and a wavelength of 396.2 nm with a spectral bandwidth of 0.5 nm was used. All experiments were performed by using auto-sampler injector. The instrumental and extraction conditions and temperature programming for the graphite atomizer are listed in Table 1. The pH values of the solutions were measured by a digital pH meter (Metrohm 744). A Multiwave 3000 microwave sample preparation system (Anton Paar, Graz, Austria) was used in this study.

Sampling: The method of IL-DLLBME was developed by real samples includes; blood of dialysis patients, gastrointestinal patients (Aluminum Magnesium Anti acid syrup – Mgs Oral) and water samples. For sampling, all glass tubes were washed with a 0.5 mol L^{-1} HNO_3 solution for at least 24 h and thoroughly rinsed 6 times with ultrapure water before use. As aluminum concentra-

Table 1. Instrumental Conditions for aluminum determination by ET-AAS

Parameter	Al
Wavelength (nm)	396.2
Lamp current (mA)	5
Slit (nm)	0.5
LOD (μg L^{-1})	0.7
Range [a] (μg L^{-1})	2.5-85
Range [b] (μg L^{-1})	2.5-230
Method	ET-AAS

[a] Peak Height. [b] Peak Area

tions in whole blood and serum are very low, even minor contamination at any stage of sampling, sample storage and handling, or analysis has the potential to affect the accuracy of the results. For analysis in whole blood 5-10 µL, pure heparin (free Aluminum, 99.5%) is added to a 10 mL blood sample. The human blood sample was maintained at –20 °C in a cleaned glass tube. Serum and blood samples were collected from dialysis patients or gastrointestinal patients of Iranian petroleum industry hospital (Hospital Ward 7-IPIH, PIHO). The population of this study consisted of two groups: patients with aluminum exposed (n = 20, male, age 20-50) and healthy employees as control group (n = 20). Control group was selected from matched people of the same age and sex (only male) without diseases affecting. All samples were analyzed by IL-DLLBME and compared with microwave digestion method.

Procedure: A preconcentration procedure of IL-DLLBME was performed as follows: first, 0.5×10^{-5} mol L^{-1} of DL-histidine amino acid solution, 0.1 mL of triton X-100 1% (w/v), 0.4 mL of acetone as a dispersive solvent and a 1.2 mL buffer solution (pH = 6.5) were added to 10 mL of all standards and samples, then 0.1 g of [HMIM] [PF_6] was added for extraction of Al ions. Triton X-100, an emulsifier and anti-sticking agent, was added to the solution in order to raise the efficiency of the extraction procedure. A small amount of [HMIM] [PF_6] as an extractant solvent was dissolved in acetone as a dispersant solvent and then the binary solution was rapidly injected by a syringe into the serum containing Al^{3+}, which have already been in vitro chelated by histidine amino acid at pH = 6.5. For optimizing and recovery, 10 mL of Al (III) standard solution (0.5 µg L^{-1}) was used instead of the sample, and 0.08 g of [HMIM] [PF_6] was added to the Al-histidine complex. The resulting system was shaken for 3 min by ultrasonic shaking at 25 °C. In order to separate the phases, the turbid solution was centrifuged for 5 min at 3500 rpm and the aqueous phase

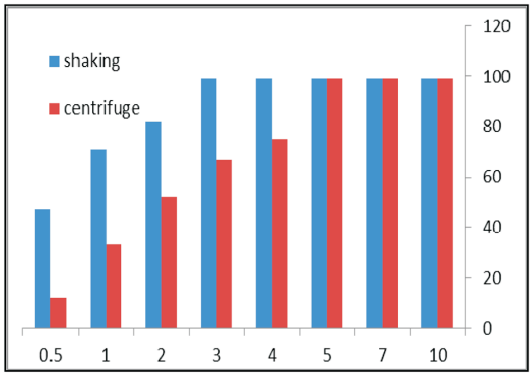

Fig. 1. The Effect of shaking and centrifuging on the aluminum extraction recovery.

Table 2. Extraction Conditions of aluminum by Proposed Method

Features	Value Al
Precision (RSD%, N = 10)	3.2
LOD of DILLBME (µgL^{-1})	0.015
Enrichment Factor	54
Working range of DLLBME (µgL^{-1})[a]	0.05-1.35
Working range of DLLBME (µgL^{-1})[b]	0.05-4.20
Correlation coefficient of DLLBME	0.9965

[a] Peak Height. [b] Peak Area

was removed with a transfer pipette (Figure 1). Finally, the settled IL-phase was dissolved in ethanol up to 200 µL and 20 µL of samples injected into the ET-AAS by its auto-sampler. In this research, other amino acid such as, arginine (Arg), and lysine (Lys) with positive charge and aspartic acid (Asp, negative charge) compared with histidine for aluminum micro-extraction by IL-DLLBME method. Extraction conditions were shown in Table 2.

RESULTS AND DISCUSSION

In-vitro aluminum chelation from serum is very hard because about 80% of aluminum ions are bound to serum proteins such as albumin and other compounds. However aluminum can be chelated by histidine bio-ligand at pH 6.5 as same as deferoxamine (DFO) in serum of dialysis patients and help us for Al removal from human body. In this study, we used IL-DLLBME method based on histidine bio-ligand for micro-extraction and determination of aluminum in water and human biological samples.

Effect of ETAAS conditions

In order to increase the accuracy, precision and repeatability, we used triton X-100 for blood samples. The influence of pyrolysis temperature on the absorbance was studied within a range of 900–1700 °C. The maximum absorbance was achieved within a range of 1300–1500 °C. Therefore, 1400 °C was selected as the working pyrolysis temperature. Once selected, a drying time of 30 s was chosen for water evaporation, and a long ramp time of 50 s was chosen as it allowed gradual elimination of organic matrix and avoided aluminum loss in pyrolysis temperature. The effect of atomization temperature on aluminum signal was studied within the range of 2000–3000 °C, and the maximum signal was obtained at approximately 2500 °C. Cleaning time and temperature were ordered at 4 s and 2600 °C respectively, and argon flow rate was 300 mL min^{-1} (Table 3 and Figure 2).

Table 3. Temperature program of ET-AAS for aluminum determination (Argon flow rate, mL min^{-1})

Step	Temperature (°C)	Ramp (s)	Hold (s)	Argon
Dry	130	20	10	300
Ash	1400	40	10	300
Atomize	2500	1	2	0.0
Clean	2600	1	3	300

Effect of biological ligand on aluminum micro-extraction

For aluminum extraction in human blood samples by IL-DLLBME, the effect of different biological ligands (BL) such as, arginine (Arg), lysine (Lys), aspartic acid (Asp) histidine (His), ornithine (Orn), thiophene (Thi) and Thi & Lys and Orn & Lys was investigated. The results showed us that His was better than others for aluminum extraction at pH = 6.5 (95%). The Orn & Lys had good recovery extraction (72%) but less than His amino acid (Figure 3). Increase of amino acid in serum could be helpful to aluminum extraction and its recovery depended on BL concentration in serum. The concentration of Histidine was one of the important parameters obtained from the optimized IL-DLLBME method. Through this investigation, the amount of His used was from 1×10^{-6} to 1×10^{-4} mol L^{-1}. The results obtained from this investigation showed that, by increasing His concentration up to 8.0×10^{-6} mol L^{-1}, the recoveries also increased. Figure 4 shows that 4.2×10^{-6} mol L^{-1} was the minimum His concentration necessary to achieve maximum extraction efficiency. Therefore, 5.0×10^{-6} mol L^{-1} ligand concentration was selected for further studies.

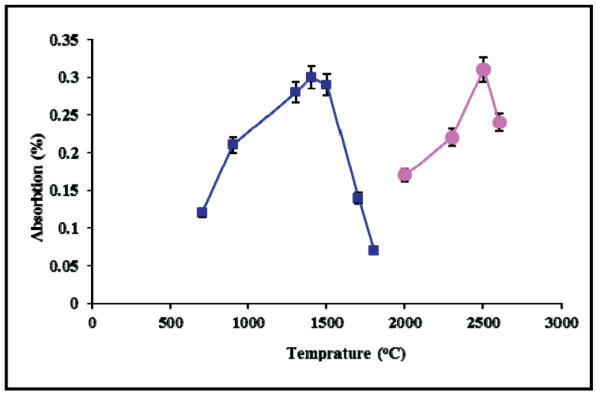

Fig. 2. The effect of atomization temperature on the aluminum signal.

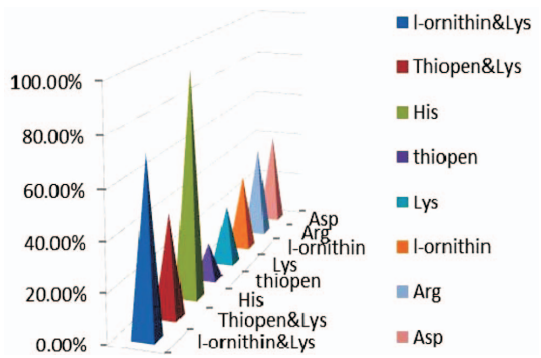

Fig. 3. Comparing of different amino acids on the aluminum DLLBME in serum samples.

Effect of pH range

The pH of the sample solution plays an important role in the pre-concentration and extraction procedure because the formation of soluble metal complexes and their stabilities in aqueous solutions are strongly related to the pH of the medium. To investigate the effect of pH, a set of solutions containing the aluminum metal ions at a concentration given in the general procedure were taken. The influence of sample pH on absorption of Al^{3+} was investigated using different pH values ranging from 2 to 12 for 0.5 μg L^{-1} Al^{3+}. The Histidine amino acid complexation was strongly conditioned by the pH of solutions and subsequently affected extraction efficiency of the complex. The results showed that the highest extraction efficiency for Al^{3+} was achieved at pH 6 to 7. Thus, we selected pH = 6.5 for further studies (Figure 5).

Effect of sample volume and amount of ionic liquid

In IL-DLLBME method, sample volume is one of the most important parameters to be studied. The effect of sam-

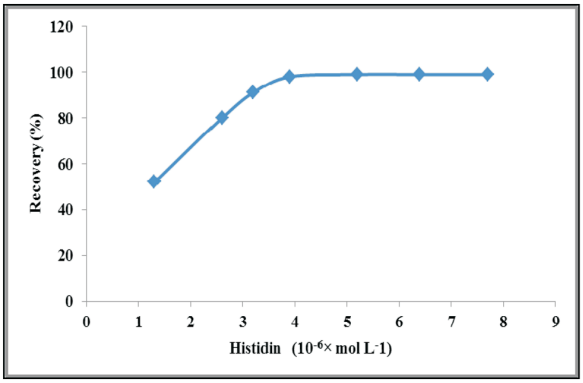

Fig. 4. The effect of His concentration on the aluminum DLLBME recovery (◆). Concentration of Al (III): 500 ng L^{-1}; amount of ionic liquid: 0.1 g; extraction time: 8 min; sample volume: 10 mL; N = 5; pH = 6.5.

ple volume was examined in a range of 1-30 mL for 0.5 μg L^{-1} Al (III). Quantitative extraction was observed between 1 mL and 15 mL. At higher volumes the recoveries decreased. It was also noticed that higher sample volumes partially solubilized the ionic liquid phase, leading to non-reproducible results. Therefore, a sample volume of 10 mL was selected for further experiments of IL-DLLBME (Figure 6). It was also observed that extraction efficiency of the system was remarkably affected by ionic liquid amount, so it was examined within the range of 0.02–0.2 g. Quantitative extraction was observed at higher than 0.08 g of [HMIM][PF_6]. Therefore, in order to achieve a suitable pre-concentration, 0.1 g of [HMIM][PF_6] was chosen as optimum leading to a final ILs (Figure 7).

Effect of various mineral acids and ethanol on recovery of extraction

Direct injection of ionic liquids into ETAAS was not possible, because ILs have high viscosity. The proposed method was evaluated based on back-extraction of aluminum from IL with a mineral acid and dilution by organic solution. Decreasing of the pH leads to dissociation and releasing of aluminum ions into the aqueous phase with 77% of extraction recovery (Figure 8). But in dilution of IL with ethanol, methanol, acetone and acetonitrile, 92%, 74%, 98% and 83% recoveries were obtained, respectively. The research showed that dilution of ionic liquid with ethanol solution has high efficiency extraction compared to acid back-extraction (Figure 9).

Effect of matrix

ETAAS is a very specific technique with low sensitivity to interference. Then, the potential interference effects occurring in this procedure are mainly related to the extraction during the pre-concentration step applied to the

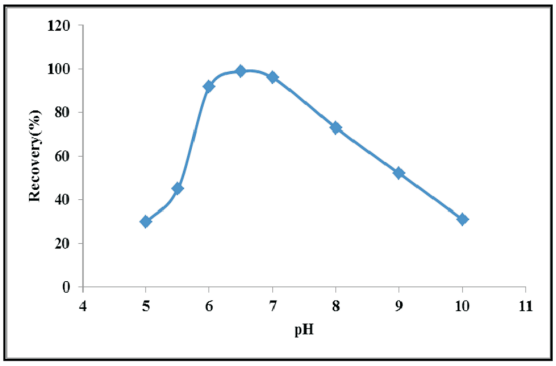

Fig. 5. The influence of samples pH on the aluminum DLLBME recovery (◆). Concentration of Al (III): 500 ng L^{-1}; amount of ionic liquid: 0.1 g; extraction time: 8 min; sample volume: 10 mL; N = 5; pH = 6.5.

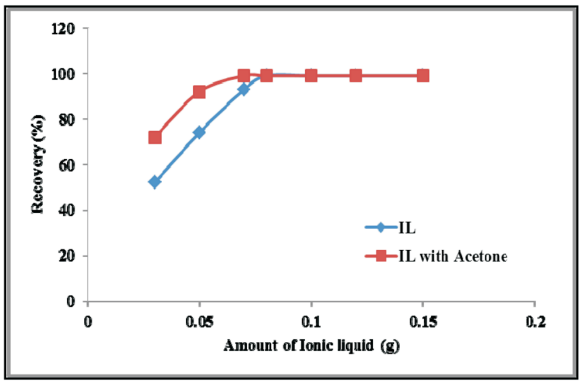

Fig. 7. The effect of [HMIM][PF_6] on the DLLBME recovery without acetone (♦). The effect of [HMIM][PF_6] on the DLLBME recovery with acetone (■). Concentration of Al (III): 500 ng L^{-1}; amount of ionic liquid: 0.1 g; pH = 6.5.

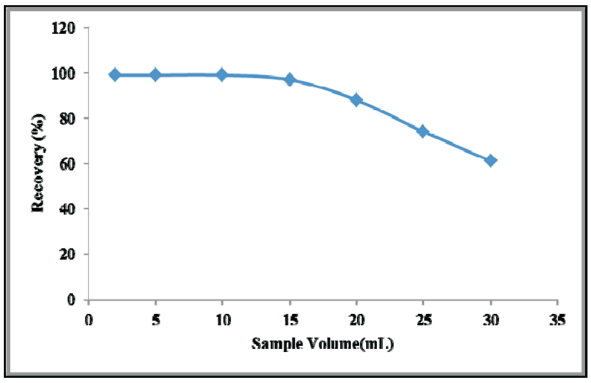

Fig. 6. The influence of sample volume on the aluminum DLLBME recovery (♦). Concentration of Al (III): 500 ng L^{-1}; amount of ionic liquid: 0.1 g; pH = 6.5.

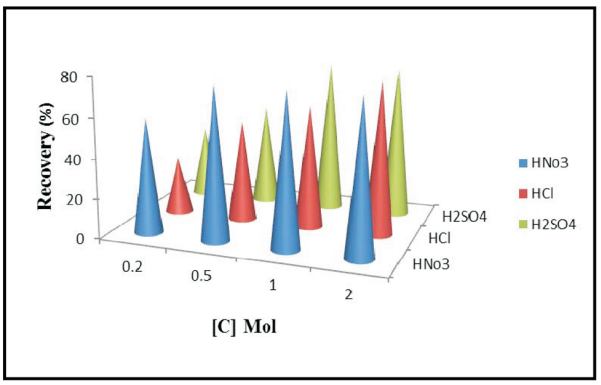

Fig. 8. The effect of mineral acids on the aluminum DLLBME recovery. Concentration of Al (III): 500 ng L^{-1}; amount of ionic liquid: 0.1 g; pH = 6.5.

target samples. Considering the samples of interest, the most probable metal ions' reported effect of potential interfering ions on the determination of aluminum were investigated. The procedure of IL-DLLBME was performed using a 10 ml sample containing 0.5 μg L^{-1} of analyte and 1–2 mg L^{-1} different concentration of matrix ions. The tolerate amounts of each ion were the concentration values tested that caused less than 5% of the absorbance alteration. The ions normally present in the sample do not interfere under the experimental conditions used. The results are shown in Table 4.

Method validation

The IL-DLLBME method was applied to determine Al (III) found as a base value in 10 mL of biological samples. The spiked serum, urine and water were prepared to demonstrate the reliability of the method for extraction and determination of aluminum (Table 5). The recovery of spiked samples was satisfactorily reasonable and was confirmed using addition method, which indicated the capability of the system in the determination of Al^{3+} in human blood and water samples. The method was validated by standard reference material (NIST SRM 2670a) (Table 6). The mean of aluminum concentration in blood samples in dialysis patients before and after dialysis were determined by IL-DLLBME. The results of dialysis patients (20-50 ages) by proposed method showed us that the concentration of aluminum in serum after dialysis was higher than before dialysis (98.24 ± 5.62 vs 13.17 ± 0.66, $P < 0.05$). Serum aluminum was significantly higher in dialysis patients and gastrointestinal patients than in normal control respectively (98.24 ± 5.62 vs 2.19 ± 0.13 and 27.83 ± 1.58 vs 2.19 ± 0.13, $P < 0.05$). The calibration curve of IL-DLLME method was linear between 0.05–4.20 μg L^{-1}. Precision and accuracy of IL-DLLBME help us for trace aluminum analysis in biological samples such as, hair and nail (Table 7).

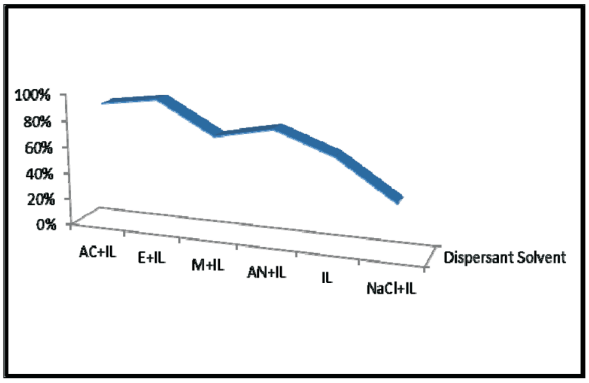

Fig. 9. The effect of ionic liquid dilution with organic solution on the aluminum DLLBME recovery. Concentration of Al (III): 500 ng L^{-1}; amount of ionic liquid: 0.1 g; pH = 6.5.

Table 4. The effect of matrix ions (ion conc./Al conc.)

Ions	Maximum tolerance ratio
K^+, Na^+, Li^+, Mn^{2+}, Co^{2+}, Ba^{2+}, Mg^{2+}, Ca^{2+}, Pb^{2+}, Ag^+, Ba^{+2}, CH_3COO^-, F^-, PO_4^{3-}, CO_3^{2-}	1200
NO_3^-, SO_4^{2-}, Cl^-, Zn^{2+}, Cu^{2+}, V^{3+}	600
Ni^{2+}, Fe^{3+}, Cr^{3+}	250

This work was performed using 10 mL of 0.5 (μgL^{-1}) Al standard solution (pH = 6.5).

Table 5. The results for tests of addition/recovery for trace aluminum determination in some real samples (μgL^{-1})

Sample	Added	Found[a]	Recovery (%)
Urine	---	1.10 ± 0.04	---
	0.2	1.29 ± 0.06	95
Plasma	---	0.93 ± 0.05	---
	0.5	1.41 ± 0.08	96
Blood	---	2.13 ± 0.07	---
	0.5	2.69 ±0.15	102
Serum	---	0.27 ± 0.02	---
	1	1.22 ± 0.06	96

[a] Mean of three determinations ± confidence interval ($P = 0.95$, $n = 3$), [b] Not Detected.

Table 6. Analytical results of aluminum determination in standard reference material (μg L^{-1})

SRM	Certified	Found[a]	Recovery (%)
Urine	4.02 ± 0.21	3.91 ± 0.18	97.26

Mean value ± standard deviation based on three replicate measurements, [a] NIST SRM 2670a, Aluminum in frozen dried urine, pH 6.5, -20 °C.

CONCLUSIONS

In this research, Ionic Liquid based dispersive liquid liquid bio micro-extraction (IL-DLLBME) combined with ETAAS was used for pre-concentration and ultra-trace determination of Al^{3+} in human serum of dialysis patient and water samples. Also, this method introduces histidine as a human ligand that can be used for in vitro aluminum removal from body with low toxic effect. Factors influencing in IL-DLLBME method was optimized. The proposed method has many advantages such as; ultra-trace analysis,

Table 7. The capability of different methods for determination of aluminum in human samples

Samples	ET-AAS	IL-DLLBME	ICP
Serum[a]	ND	0.56 ± 0.03	ND
Blood[a]	2.88 ± 0.16	2.74 ± 0.12	ND
BHT [a]	ND	1.76 ± 0.09	ND
Hair [b]	11.89 ± 0.63	12.11± 0.71	12.42 ± 0.65
Nail[b]	6.16 ± 0.34	6.28 ± 0.27	5.97 ± 0.33
Liver [b]	ND	1.28 ± 0.06	ND

(Mean value ± standard deviation based on three replicate measurements) (N = 3, P = 0.95), [a] μg L^{-1}, [b] 0.2 g human sample (μg/g) , Blood of hyper thyroidism.

Table 8. Comparison of results between the published and proposed method in this work

Method	Separation	PE[e]	%RSD[f]	LOD[d]	Ref.
SPF	SPE[a]-CPE[b]	25	>10	0.340	[30]
ICP MS	HPLC-UV	---	3.0	0.830	[31]
ETAAS	CPE[b]	37	4.7	0.090	[32]
ETAAS	CPE[b]	34.8	3.6	0.060	[33]
FAAS	CPE[b]	20	4.5	----	[34]
UV-VIS	CPE[b]	33	1.9	0.050	[35]
ETAAS	DLLME[c]	53	2.2	0.015	This work

SPE; Spectrofluorimetry, **ICP MS**; Inductively Coupled Plasma Mass Spectrometry, **ETAAS**; Electro-thermal Atomic Absorption Spectrometry, **FAAS**; Flame Atomic Absorption Spectrometry, **UV-VIS**; Ultraviolet–visible spectroscopy, **HPLC**; high performance liquid chromatography.
[a] Solid Phase Extraction, [b] Cloud Point Extraction
[c] Dispersive Liquid- Liquid Micro extraction
[d] Limit of Detection (μg L^{-1})
[e] Preconcentration Factor, [f] Relative Standard Deviation

simple, low cost, fast, reliable and Al removal in human serum samples. Validation of methodology was confirmed with SRM and spike samples. The results showed that the IL-DLLBME method is comparable to other powerful techniques as HPLC-UV-ICP-MS with high precision and accuracy (Table 8).

ACKNOWLEDGEMENTS

The authors are thankful to the Iranian occupational and environmental health laboratory of IPIHRI-OEHRC, PIHO, Dr. M. J. Kian and F. Eftekhar for their support for this work.

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