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Study on speciation of aluminum in human serum using zwitterionic bile acid derivative dynamically coated C18 column HPLC separation with UV and on-line ICP-MS detection

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ABSTRACT

A C18 column dynamically coated with zwitterionic bile acid derivative, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), was used for direct injection high performance liquid phase chromatography (HPLC)–ultraviolet visible (UV)/inductively coupled plasma mass spectrometry (ICP-MS) speciation of aluminum in non-spiked human serum. Small-molecule Al-complex compounds of Al-citrate (Al-Cit) and large-molecule Al-protein compounds of Al-transferrin (Al-Tf) were chosen as the model species and their retention behaviors on CHAPS modified C18 column were studied with UV and on-line ICP-MS detection in detail. Under the optimal conditions, large-molecule Al-protein compounds and small-molecule Al-complex compounds could be separated in 4 min, and their concentrations were on-line determined by ICP-MS. The detection limits of the method were 0.74 and 0.83 ng mL^{-1} with the RSD of 2.8% and 3.0% ($n = 7$) for Al-Tf and Al-Cit, respectively. The developed method was applied to the speciation of Al in healthy human serum and chronic hemodialysis patient serum. To the best of our knowledge, this is the first report on the simultaneous quantification of both Al-protein compounds (e.g. Al-Tf) and small-molecule Al-complex compounds (e.g. Al-Cit) in healthy human serum at low concentration levels. Compared with the reported methods in the literature, this method has several attractive features such as simplicity, rapidness, no sample preparation required, and it provides a new strategy for the speciation of trace elements in human body fluids.

1. Introduction

More and more researches indicate that aluminum (Al) in body fluids is toxic to humans; excess of Al may cause serious neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [1–4]. However, toxic mechanism of Al is not clear until now because the toxicity and biological effects of Al are dependent not only on its total concentration but also on its existing species [5,6]. Therefore, speciation of Al in human body fluids is of great significance in analytical chemistry and bio-medical fields.

Hyphenated techniques by combining various chromatographic techniques (including capillary electrophoresis (CE)) with different atomic spectrometry/mass spectrometry are the most efficient techniques for the speciation of Al in human body fluids [7]. Al was initially proposed to exist as alumino-organic complexes instead of free Al (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_4^{-}$) in human serum by size-exclusion chromatography (SEC) and electrothermal atomic absorption spectrometry (ETAAS) [8]. Then studies, performed by ultrafiltration and ultramicrofiltration with ETAAS, suggested that ~90% Al species existed as high molecule weight (HMW) Al-protein compounds, while ~10% Al species as low molecule weight (LMW) Al-complex compounds in human serum [9]. Further studies on Al speciation in human serum by combining ion-exchange high performance liquid chromatography (HPLC)/fast protein liquid chromatography (FPLC)/CE with ETAAS [10–13]/inductively coupled plasma mass spectrometry (ICP-MS) [14–17] have proved that the main existing species of HMW-Al and LMW-Al were Al-transferrin (Tf) and Al-citrate (Cit), respectively. As confirmed by these researches, both proteins and small molecules bound with Al play very important roles in human serum, for example, Al-Tf has potential neurotoxicity, and Al-Cit can transmit through the cell membrane, which is important in the process of aluminum poisoning.

However, there are still some problems associated with the speciation of Al in human serum by hyphenated techniques. Firstly, due to the low concentration of Al in healthy human serum and high extraneous Al levels in the environment, the sensitivity of the hyphenated techniques is not enough for Al speciation especially for LMW-Al analysis in non-spiked healthy human serum.

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Secondly, because of the instability of Al species, sample preconcentration techniques cannot be an effective complement to hyphenated techniques. Therefore, the overwhelming majority of research work on the speciation of Al in human serum are focused on the Al spiked human serum. Up to now, only one report based on convective-interaction media (CIM) chromatography–ICP-MS with efficient cleaning procedure for eluents and chromatographic supports was published recently for quantitative analysis of Al-Tf in non-spiked human serum [19]. However, this work did not provide the content information of LMW-Al in non-spiked healthy human serum. Therefore, to develop an easy, rapid, sensitive and reliable method for the simultaneous analysis of both LMW-Al and HMW-Al species in human serum becomes one of the most challenging tasks to analytical chemists [18].

It is well established that surfactant-mediated HPLC by using a micellar mobile phase is one of the most efficient techniques for rapid separation of large and small molecules. Because proteins are soluble in micellar mobile phase, surfactant-mediated HPLC allows direct injection of untreated biological fluids into conventional reversed-phase column. By using the "restricted access media" with a hydrophobic interior and a hydrophilic exterior, proteins were sterically excluded from the column owing to the external hydrophilic layer, whereas small molecule accessible to the internal hydrophobic region was retained. With the use of 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), a bile acid derivative as the stationary phase modifier, HPLC separation of inorganic ions, theophylline and caffeine was successfully realized by Haraguchi et al. [20]. In subsequent work, the same group proposed a method for the speciation of trace elements in salmon egg cytoplasm by surfactant-mediated HPLC–ICP-MS [21]. These results encouraged us to use CHAPS as a stationary phase modifier for HPLC separation of large-molecule Al (Al-Tf) and small-molecule Al (Al-Cit) in human serum.

The aim of this work is to develop a new method based on CHAPS modified C18 column HPLC combined with UV and on-line ICP-MS detection for the speciation of Al in human serum, especially in non-spiked healthy human serum. With Al-Cit and Al-Tf as model Al species, the retention behaviors of both small-molecular Al-complex compounds and large-molecular Al-protein compounds were studied, and the optimal chromatographic separation conditions were established. The developed method was applied to the speciation of Al in human serum to evaluate its potentiality for elemental speciation in human body fluids.

Table 1
Operating conditions for the analytical instrument.

HPLC	
Stationary phase	CHAPS-coated C18 column
Mobile phase	0.2 mmol L^{-1} Tris–HNO_3 (pH 7.4) + 0.2 mmol L^{-1} CHAPS
Flow rate	0.7 mL min^{-1}
Injected value	10 μL
Column temperature	25 °C
UV	
Light source	Deuterium lamp
Detection wavelength	220 nm for small-molecule Al-complex compounds; 280 nm for large-molecule Al-protein compounds
ICP-MS plasma	
Rf power	1150 W
Rf matching	1.5 V
Sampling depth	6.8 mm
Carrier gas	1.1 L min^{-1}
Time-resolved data acquisition	
Scanning mode	Peak-hopping
Dwell time	100 ms
Integration mode	Peak area
Determined isotope	^{27}Al

2. Experimental

2.1. Apparatus

The HPLC system consisted of a LC-10AD high-pressure pump, CTO-10A column oven, SPD-10AV UV spectrometry detector, C-R6A chromatopac (Shimadzu, Japan), C18 column (Kromasil filler, 5 μm, 250 mm × 4.6 mm i.d., supplied by Prof. Feng's group, Department of Chemistry, Wuhan University, China). ICP-MS (HP 7500a, Agilent, USA) was used as an on-line detector for HPLC. Optimization of the ICP-MS instrument (i.e., lens settings, sampling depth, carrier gas flow rate) was performed with conventional Babington nebulization method (PN-ICP-MS) prior to being connected with HPLC. The instrumental operation conditions are summarized in Table 1. A Mettler Toledo 320-s pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China) with a combined electrode was used to adjust the pH.

2.2. Standard solution and reagents

All reagents used were at least of analytical reagent grade. High purity deionized water obtained by a Milli-Q system (18.2 MΩ cm, Millipore, Molsheim, France) was used throughout this work.

A stock solution (1.0 mg mL^{-1}) of Al was prepared by dissolving corresponding $Al(NO_3)_3{\cdot}9H_2O$ (Shanghai Reagent Factory, Shanghai, China) in high purity deionized water. A stock Na_3Cit solution (1.48×10^{-2} mol L^{-1}) was prepared by dissolving corresponding $Na_3Cit{\cdot}2H_2O$ (Chengdu Chemical Reagent Factory, Chengdu, China) in high purity deionized water.

A stock Al-Cit solution (16 μg mL^{-1} as Al) was prepared weekly by mixing 1 mL stock $Na_3Cit{\cdot}2H_2O$ solution and 4 mL of 0.100 mg mL^{-1} Al solution and diluting them to 25 mL by 10 mmol L^{-1} Tris–HNO_3 buffer solutions (pH 7.4). Tris base was purchased from Sigma, USA; HNO_3 (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) was purified by a sub-boiling system before use.

Al-Tf solution (10 μg mL^{-1} as Al) was prepared as described below. 30 mg transferrin (Sigma, USA) and 0.1 mL of 0.100 mg mL^{-1} Al solution were added into a 1 mL vial, and it was diluted to the calibrate by 0.05 mol L^{-1} Tris–HNO_3 buffer (pH 7.4) containing 0.024 mol L^{-1} $NaHCO_3$ (Beijing Reagent Factory, Beijing, China). The Al-Tf solution was stored at 4 °C in a refrigerator.

The working standards solutions were freshly prepared by stepwise dilution of the stock solution with high purity deionized water every day.

A stock CHAPS (Amresco, USA) solution of 30 mmol L^{-1} was prepared. And a human serum protein standard solution was prepared by dissolving albumin (40 g L^{-1}) in 0.05 mol L^{-1} Tris–HNO_3 buffer (pH 7.4) containing 0.024 mol L^{-1} $NaHCO_3$, and stored at 4 °C in a refrigerator.

2.3. Experimental procedures

2.3.1. Modified C18 column

The procedure for the preparation of CHAPS-coated C18 column was similar to Refs. [20,21]. Briefly, the CHAPS-coated C18 column was prepared by pumping 30 mmol L^{-1} CHAPS aqueous solution through the C18 column at a flow rate of 0.7 mL min^{-1} until the absorbance of the flowing solution observed on UV detector reached the maximum, which indicated that the maximum adsorption capacity of C18 column for CHAPS was obtained. Afterwards, 2 mmol L^{-1} CHAPS aqueous solution was flowed through the

Table 2
The effect of pH on recovery of Al-Cit and Al-Tf (mean ± S.D., $n = 3$).

Al species	Recovery (%)							
	pH 3	pH 4	pH 5	pH 6	pH 7	pH 7.4	pH 8	pH 9
Al-Cit	75 ± 6.2	78 ± 5.4	84 ± 3.7	88 ± 2.8	98 ± 4.3	102 ± 1.6	88 ± 6.1	82 ± 1.6
Al-Tf	73 ± 4.3	65 ± 7.2	10 ± 2.2	0	81 ± 6.3	103 ± 1.0	116 ± 1.1	116 ± 1.0

modified column for 2 h, and then rinsed with high purity deionized water for ca. 1 h. After conditioning the column with mobile phase, the CHAPS-coated C18 column was ready for the separation of target Al species.

2.3.2. HPLC–ICP-MS system

To combine HPLC with ICP-MS, the HPLC outlet (0.7 mL min^{-1}) was connected via a minimum length piece of Teflon tubing (i.d. 0.5 mm, length 30 cm) to a Babington nebulizer situated in a spray chamber.

The mobile phase of 0.2 mmol L^{-1} Tris–HNO_3 buffer solution (pH 7.4) was spiked with 0.2 mmol L^{-1} CHAPS to prevent the degradation of the separation column, and the flow rate was fixed as 0.7 mL min^{-1}. The sample was directly injected into the HPLC after pre-equilibrium of the separation column (15 min) and the elution behaviors of various Al species were examined with the UV absorption and on-line ICP-MS detection.

2.3.3. Controlling blank Al level

To avoid contamination by extraneous Al, all laboratory ware was made of polyethylene or Teflon material and thoroughly cleaned by soaking in 10% high purity nitric acid for at least 48 h. Immediately prior to use, all acid-washed ware was rinsed well with high purity deionized water. In order to decrease the blank Al level, the Tris–HNO_3 buffer solution was purified by flowing through a 724# acid cation exchange resin column (≤200 mesh, 2.54 × 10 cm, Hangzhou Zhengguang Resin Co. Ltd., Hangzhou, China) prior to use. Before use, the 724# acid cation exchange resin was washed with high purity deionized water to eliminate the impurities, then soaked in 8% (m/v) NaOH for 30 min, and rinsed with high purity deionized water to neutral pH. Next 7% (m/v) HCl was used to soak the resin 30 min for three times, then washed with high purity deionized water and dried.

2.3.4. Sample preparation

Synthetic human serum samples were prepared by adding corresponding Al-Cit and Al-Tf at a molar ratio of 1:1 in human serum protein standard solution and mixing them for 30 min. Subsequently, they were subjected to the chromatographic separation.

Healthy human serum samples were provided by the Hospital of Wuhan University (Wuhan, China), and chronic hemodialysis patient serum samples were obtained from Wuhan Union Hospital (Wuhan, China). The serum samples were mixed with the same volume of 0.05 mol L^{-1} Tris–HNO_3 buffer (pH 7.4) and stored at 4 °C before separation. Analyses were proceeded within 3 days.

For total aluminum determination, 0.5 mL serum samples were transferred into PTFE vessel, and 1.5 mL sub-boiled concentrated HNO_3 was added. Then the microwave digestion (WX-3000 microwave accelerated system, EU Chemical Instruments Co. Ltd., Shanghai, China) was performed according to the following heating programs: 3 atm for 1 min, 8 atm for 2 min and then 10 atm for 3 min. After digestion, the PTFE vessel was put on the ECH-1 temperature control heating panel (Sineo microwave chemistry technology Co. Ltd., Shanghai, China) and the sample solution was heated to very small volume to remove the superfluous acid. Then the digest was diluted to 2 mL with high purity deionized water prior to PN-ICP-MS detection. The blank sample was prepared with the same procedure without the addition of serum samples.

3. Results and discussion

3.1. Optimization of chromatographic separation conditions for various Al species

3.1.1. Effect of mobile phase pH

Variation of mobile phase pH will influence the existence of Al species, thus the effect of mobile phase pH on the recovery of small-molecule Al-complex compounds and large-molecule Al-protein compounds from the column was examined in the pH range 3–9 by using Al-Cit and Al-Tf as the model Al species, and the results are listed in Table 2. As could be seen, the variation of mobile phase pH has a more obvious effect on Al-Tf. In the studied pH range of 3–9, the recovery of Al-Cit was gradually increased with the pH increasing from 3 to 7.4, and then was slightly decreased with further increasing pH from 7.4 to 9, while the recovery of Al-Tf was decreased remarkably with increasing pH from 3 to 6, and then increased with the further increasing pH from 6 to 9. The recovery of 102% and 103% was obtained for Al-Cit and Al-Tf at physiological pH of 7.4, respectively. A very poor recovery of Al-Tf at the pH near the isoelectric point of Tf (5.6–6.6) was found due to the precipitation of Tf at its isoelectric point. And a lower recovery for Al-Cit was found at a lower pH, the possible reason is the protonation of citric acid/citrate at various pHs. However, it should be mentioned that the variation of mobile phase pH did not affect the retention time for both Al species studied. Therefore, a physiological pH of 7.4 was employed for the subsequent experiments.

3.1.2. Effect of buffer concentration

By keeping the pH of mobile phase as 7.4 and the concentration of CHAPS as 0.2 mmol L^{-1} in mobile phase to prevent the degradation of the separation column, the effect of buffer concentration on the recovery of Al-Cit and Al-Tf on the CHAPS modified C18 column was examined with the concentration of Tris–HNO_3 buffer solution changing from 0 to 1.5 mmol L^{-1}, and the results were given in Table 3. It can be seen that the buffer concentration variation has more influence on the recovery of Al-Tf than that of Al-Cit. Al-Cit and Al-Tf could not be quantitatively recovered (81.5% and 57.0% for Al-Cit and Al-Tf, respectively) from the CHAPS-coated C18 column by elution with only pure water but they could be eluted

Table 3
The effect of buffer concentration on recovery of Al-Cit and Al-Tf (mean ± S.D., $n = 3$).

Al species	Recovery (%)				
	0 mmol L^{-1}	0.2 mmol L^{-1}	0.5 mmol L^{-1}	1 mmol L^{-1}	1.5 mmol L^{-1}
Al-Cit	82 ± 8.5	102 ± 1.4	96 ± 4.4	93 ± 10	90 ± 4.7
Al-Tf	57 ± 10	103 ± 5.4	92 ± 6.7	85 ± 9.0	81 ± 4.6

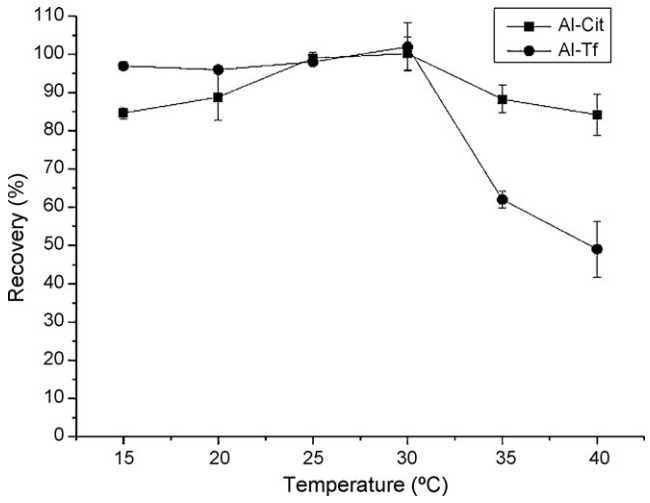

Fig. 1. Effect of temperature on recovery of Al-Cit and Al-Tf from the CHAPS-coated C18 column ($C_{Al\text{-}Cit}$ = 0.5 μg mL^{-1} and $C_{Al\text{-}Tf}$ = 0.5 μg mL^{-1}; for other experimental conditions, see Table 1).

and well recovered from the column by using 0.2–0.5 mmol L^{-1} of Tris–HNO_3 buffer solution. The recoveries will decrease slightly with the increase of buffer concentration. These characteristics may be associated with the varied ionic strength which affected the solubility characteristics of proteins.

3.1.3. The effect of column temperature and mobile phase flow rate

The fluctuation of surrounding temperature might lead to the changes of target Al species, therefore, the effect of column temperature on the recoveries of both Al-Cit and Al-Tf was studied. The experimental results in Fig. 1 show that the quantitative recoveries of two Al species on CHAPS-coated column were obtained with the column temperature varying in the range of 15–30 °C. However, when the temperature is higher than 30 °C, the recoveries of two studied species will decrease along with the increment of temperature, especially for Al-Tf. The Al-Tf would be deactivated in relatively high temperature, resulting in a non-quantitative recovery.

The effect of the flow rate of the mobile phase on the resolution of Al-Cit and Al-Tf was also investigated. The results indicated that when the flow rate was varied from 0.7 to 0.3 mL min^{-1}, the separation time was extended without obtaining better resolution of Al-Cit and Al-Tf chromatographic peaks. When the flow rate was higher than 0.7 mL min^{-1}, the resolution of the two chromatographic peaks became worse. Therefore, the mobile phase flow rate of 0.7 mL min^{-1} was selected for subsequent experiments.

Based on the above experimental results, the following optimized chromatographic conditions were used for the separation of different aluminum species: column temperature: 25 °C, mobile phase: 0.2 mmol L^{-1} Tris–HNO_3 buffer solution + 0.2 mmol L^{-1} CHAPS and pH 7.4, flow rate: 0.7 mL min^{-1}.

3.2. The retention behaviors of various Al species on modified C18 column and on-line HPLC–ICP-MS detection system

The retention behaviors of small-molecule Al-complex compounds (Al-Cit, Al-oxalic acid, Al-EDTA, Al-F) and large-molecule Al-protein compounds (Al-Alb, Al-Tf) on CHAPS modified C18 column were examined by UV detection, and the experimental results were given in Table 4. It could be seen that the retention time was around 2.4 min for the large-molecule Al-protein compounds; and >2.7 min for small-molecule Al-complex compounds. Obviously,

Table 4
The retention time of Al species.

Al species	Average retention time (min)	RSD% (n = 5)
Al^{3+} ($Al(NO_3)_3$)	2.94	4.5
Al-Cit	2.72	5.4
Al-Oxalic acid	2.73	3.6
Al-EDTA	2.92	4.1
Al-F	2.85	3.4
Al-Alb	2.43	5.3
Al-Tf	2.41	3.3

Detection of small-molecule Al-complex compounds at 220 nm and large-molecule Al-protein compounds at 280 nm.

quantitative fraction separation between large-molecule Al-protein compounds and small-molecule Al-complex compounds was realized, but the separation among different small-molecule Al-complex compounds or large-molecule Al-protein compounds was impossible in the proposed system. It could be concluded that the main retention mechanism of CHAPS modified C18 column was size-exclusion mode. As shown in Fig. 2, with CHAPS modification, the stationary phase has a hydrophobic interior and a hydrophilic exterior, large-molecule Al-protein compounds were sterically excluded from the stationary phase due to the external hydrophilic layer, thus eluted first, and no denaturalization and irreversible adsorption was observed. The small Al-complex compounds were allowed to interact with the internal hydrophobic region. In this region, the minor control force was the electrostatic or hydrophobic interactions, which would lead to the separation of small Al-complex compounds. However, this interaction was too weak to separate the small Al-complex compounds well.

Taking Al-Cit and Al-Tf as model species for small-molecule and large-molecule Al species, respectively, the retention behaviors of different Al species on CHAPS modified C18 column were further investigated by on-line ICP-MS detection, the chromatograms were shown in Fig. 3. As could be seen, the retention times on the modified C18 column were about 2.72 min for Al-Cit and 2.36 min for Al-Tf, which was consistent with the results obtained by UV detection. Further studies indicated that the recoveries for small-molecule Al-complex compounds and large-molecule

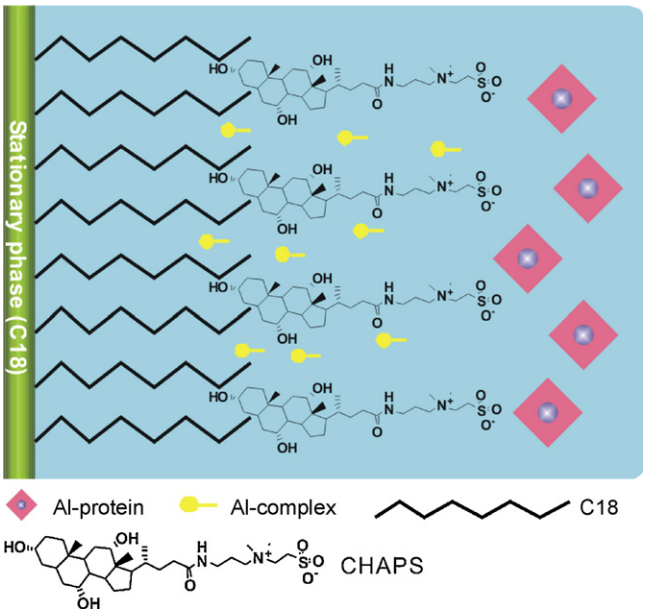

Fig. 2. A schematic diagram of the retention mechanism of Al species on CHAPS modified C18 stationary phase.

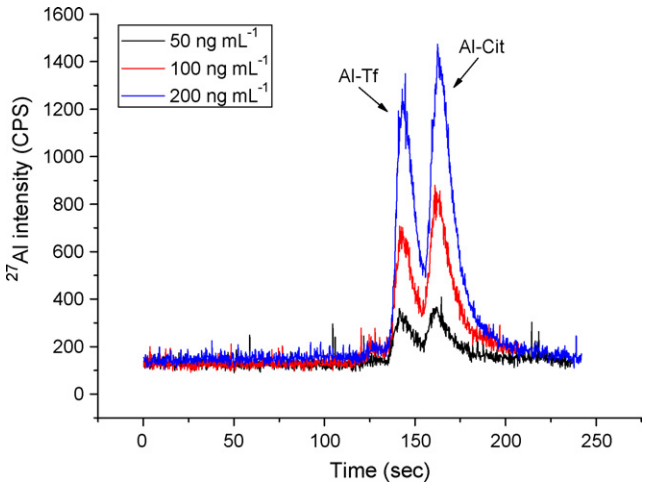

Fig. 3. Chromatogram of Al-Cit and Al-Tf by HPLC using the CHAPS-coated C18 column (for experimental conditions, see Table 1).

Al-protein compounds were in the range of 88.4–95.6% and 85.8–106%, respectively. All these results indicated that a quantitative separation of small-molecule Al-Cit and large-molecule Al-Tf could be achieved under the proposed chromatographic conditions.

3.3. Analytical performance

Under the optimal chromatographic conditions, the retention times of Al-Tf and Al-Cit obtained by on-line HPLC-ICP-MS were about 2.37 ± 0.02 min and 2.71 ± 0.02 min, respectively. In accordance with IUPAC recommendations, the limits of detection (LODs, 3σ) for Al-Tf and Al-Cit were 0.74 and 0.83 ng mL^{-1}, and the relative standard deviations (RSDs) were 2.8% and 3.0% ($c = 50$ ng mL^{-1}, $n = 7$), respectively. The linear range was 5–10,000 ng mL^{-1} ($R > 0.999$) for both Al-Tf and Al-Cit.

A comparison of this work with several other approaches [22–25] for Al speciation in human serum was given in Table 5. As could be seen, the separation time in this work was the shortest, which could improve the analytical efficiency, and prevent the transformation of Al species during separation. Also, the sample preparation (1:1 diluted with 0.05 mol L^{-1} Tris–HNO_3 (pH 7.4)) was simpler comparing with other methods in which the spiking procedure and derivative pretreatment were involved, which also could effectively avoid the transformation of Al species. The

Table 6
The concentration of Al-Cit and Al-Tf in synthetic human serum by HPLC-ICP-MS (mean ± S.D., $n = 3$).

	Added (ng)	Determined (ng)	Recovery (%)
Al-Cit	0.5	0.53 ± 0.03	107
	1	1.04 ± 0.06	104
	2	1.89 ± 0.11	94.6
	5	5.03 ± 0.03	101
Al-Tf	0.5	0.48 ± 0.01	96.4
	1	1.01 ± 0.01	101
	2	1.84 ± 0.23	92.2
	5	5.05 ± 0.08	101

LODs for Al obtained in this work were lower than that obtained by DHAB-derivative HPLC–UV, 8-HQ HPLC-fluorimetry, and off-line anion-exchange FPLC–ETAAS methods. Although the LODs for Al obtained in this work were a little higher than that obtained by CIM chromatography–ICP-MS and anion-exchange HPLC-HR–ICP-MS methods, as could be seen in Table 5, the injected volume (10 μL) of this work was much smaller than that of 1000 μL for CIM chromatography–ICP-MS and 100 μL for anion-exchange HPLC–HR-ICP-MS, and HR-ICP-MS which is not available in most laboratories is more expensive than quadrupoles ICP-MS used in this work.

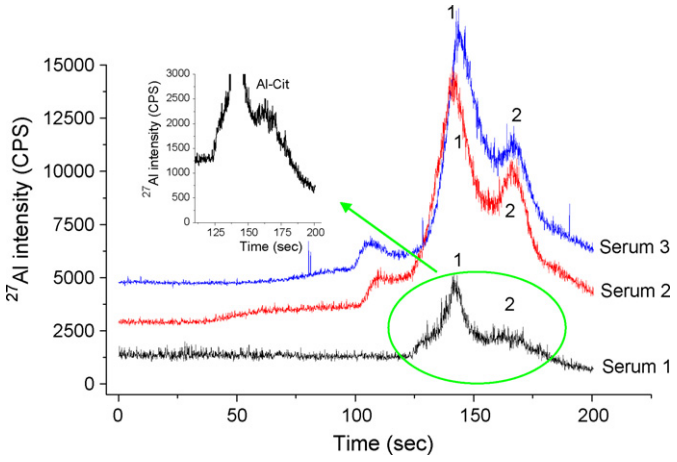

Fig. 4. Chromatogram of different Al species in human blood serum by CHAPS-coated C18 column HPLC–ICP-MS (1: Al-Tf, 2: Al-Cit; Serum 1 stands for healthy human serum sample, Serums 2 and 3 stand for chronic hemodialysis patient serum samples; for experimental conditions, see Table 1).

Table 5
Comparison of different analytical approaches for Al analysis in human serum.

Analytical technique[a]	Sample preparation	Injected volume (μL)	LOD (ng mL^{-1})	Separation time (min)	Reference
CHAPS-coated C18 column HPLC–ICP-MS	1:1 dilute with 0.05 mol L^{-1} Tris–HNO_3 (pH 7.4)	10	0.74-0.83	4	This work
SEC & CIM chromatography–ICP-MS	5-fold dilute with 50 mmol L^{-1} Tris–HCl + 30 mmol L^{-1} $NaHCO_3$	1000	0.15	45	[19]
DHAB-derivative HPLC–UV	Add 2 mol L^{-1} HCl and stand for 5 h, then add methanol and centrifuge, then add DHAB, buffer solution (pH 8) and heat at 70 °C for 20 min, and dilute with water	100	6	12	[22]
8-HQ (KD) HPLC-Fluorimetry	Threefold dilute with water, and react with 8-HQ with the ratio of 1:3	10	1	10	[23]
Off-line SEC and anion-exchange FPLC–ETAAS	Spike with certain amount of $AlNO_3$ and stand for 5 h	500	<1	20	[24]
Anion-exchange HPLC–HR-ICP-MS	Spike with Al (no clear description)	100	0.1	50	[25]

DHAB: 2,2′-dihydroxyazobenzene; 8-HQ: 8-hydroxyquinoline; KD: kinetic-differentiation mode; HR: high resolution.

Table 7
Analytical results (mean ± S.D., $n = 3$) for Al species in human serum.

Sample	Added (ng mL^{-1})	Determined by HPLC–ICP-MS (ng mL^{-1})		Sum of Al-Cit and Al-Tf (ng mL^{-1})	Percentage of total Al (%)		Total Al by PN-ICP-MS (ng mL^{-1})
	Al^{3+}	Al-Cit	Al-Tf		Al-Cit	Al-Tf	
Serum 1[a]	–	2.2 ± 0.2	27.9 ± 2.6	30.1	7.1[b]	90.6[b]	30.8 ± 2.9
	20	2.5 ± 0.8	44.9 ± 5.3	47.4	5.3[c]	94.7[c]	–
	50	11.1 ± 2.4	73.9 ± 7.3	85.0	13.1[c]	86.9[c]	–
Serum 2[d]	–	43.7 ± 5.7	288.7 ± 7.0	332.4	13.8[b]	91.1[b]	317.0 ± 34.9
Serum 3[d]	–	50.8 ± 5.0	291.6 ± 15.8	342.4	15.2[b]	87.4[b]	333.5 ± 33.3

[a] Healthy human serum sample.
[b] Determined value by HPLC–ICP-MS/total Al by PN-ICP-MS × 100%.
[c] Determined value by HPLC–ICP-MS/sum of Al-Cit and Al-Tf × 100%.
[d] Chronic hemodialysis patient serum sample.

3.4. Sample analysis

3.4.1. Synthetic human serum samples

To validate the accuracy and precision of the proposed method, synthetic samples were analyzed by CHAPS modified C18 column HPLC–ICP-MS. The retention times of Al-Tf and Al-Cit were about 2.41 and 2.77 min, respectively, which was in accordance with that obtained by standard solution. The analytical results for various Al species in synthetic human serum by CHAPS modified C18 column HPLC–ICP-MS were shown in Table 6. As could be seen, the concentrations of Al-Tf and Al-Cit obtained by the proposed method were in good agreement with the expected values. The recoveries for Al-Cit and Al-Tf were 94.6–107% and 92.2–101%, respectively.

3.4.2. Human serum samples

The proposed method was successfully applied for the speciation of Al in healthy human serum (Serum 1) and chronic hemodialysis patient serum (Serums 2 and 3) samples. Fig. 4 was the chromatograms for the speciation of Al in human serum samples, and two chromatographic peaks were observed for all three analyzed human serum samples. The retention times of these two peaks were 2.38 and 2.75 min, which referred to Al-Tf and Al-Cit, respectively. As could be seen from the enlarged figure for healthy human serum in Fig. 4, the low level Al-Cit was well separated from Al-Tf. And the HPLC–ICP-MS analytical results for Al species in human serum were shown in Table 7. The concentrations of Al-Cit and Al-Tf in healthy human serum were 2.2 and 27.9 ng mL^{-1}, respectively. For chronic hemodialysis patient serum, the determined value for Al-Cit was ranged in 43.7–50.8 ng mL^{-1}, while the concentration of Al-Tf was in the range of 288.7–291.6 ng mL^{-1}. As could be seen, the sum of the concentration of Al-Cit and Al-Tf obtained by HPLC–ICP-MS were about equal to the total Al concentration determined by PN-ICP-MS after digestion for both healthy human and chronic hemodialysis patient serum samples, which indicated that citrate and transferrin were the main carriers of Al in human serum. From Table 7, it was also found that 87.4–91.1% Al was large-molecule Al-transferrin in human serum, while small-molecule Al-complex compounds was only 7.1–15.2% of total Al in human serum. This study confirms that the distribution of Al (Al-Tf and Al-Cit) in non-spiked healthy human serum is the same as that found for chronic hemodialysis patient serum with high Al levels.

To further investigate the distribution of Al in human serum, the spiked healthy human serum with different Al^{3+} concentration levels was also analyzed by HPLC–ICP-MS. Certain amount of $Al(NO_3)_3$ was added in the healthy human serum sample (Serum 1), and the samples were held for 30 min to achieve the distribution equilibrium of aluminum prior to analysis. The results in Table 7 demonstrated that most Al^{3+} added in human serum was combined with transferrin, indicating that transferrin was the main carrier of Al in healthy human serum. To explain this phenomenon, a transformation equilibrium of Al in human serum [26,27] was shown in Fig. 5, the affinity of Cit for Al $\beta_{AlCit} = [AlCit]/[Al^{3+}][Cit^{3-}] = 1 \times 10^8\ M^{-1}$ [28], and the affinity of the C-site of Tf for Al $\beta_{AlTfC} = [Tf_C HCO_3{}^- Al^{3+}]/[Al^{3+}][Tf_C HCO_3{}^-] = 1 \times 10^{13}\ M^{-1}$ [29]. And the physiological concentration of Tf and Cit were 40 μmol L^{-1} [30] and 100 μmol L^{-1} [31], respectively. Under these conditions, it suggested that about 93% of μmol L^{-1} level of Al would be bound to Tf [26]. In this work, the results for Al speciation in human serum showed that 87.4–91.1% Al was existed as Al-Tf, and with standard addition of 20–100 ng mL^{-1} Al, most Al was bound to Tf, which was consistent with the results reported in the literature [17,26].

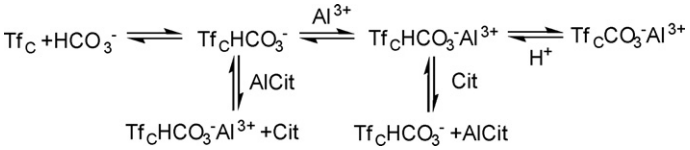

Fig. 5. A scheme of transformation equilibrium of Al in human serum.

4. Conclusion

A new approach which enabled reliable determination of different Al species in non-spiked human serum by CHAPS modified C18 column HPLC separation combined with UV and on-line ICP-MS detection was developed in this paper. Quantitative fraction separation between large-molecule Al-protein compounds and small-molecule Al-complex compounds in healthy human serum and chronic hemodialysis patient serum samples was realized under the optimal conditions. To the best of our knowledge, this is the first time to realize the simultaneous quantification of both HMW-Al and LMW-Al in healthy human serum at low concentration levels. And the analytical results show that the amount of Al-Tf accounts for 84.7–91.1% of the total Al, however, the content of Al-Cit is relatively low, which takes only 7.1–15.2% of the total Al; the distribution of Al in healthy human serum with low Al levels is the same as that found for chronic hemodialysis patient serum and spiked human serum with high Al concentrations. Compared with the reported literature, this method has several attractive features such as simple, rapid (separated in 4 min), and no sample preparation required, which can effectively improve the analytical efficiency and prevent the transformation of Al species. It can be used as a primary separation technique when applying 2D chromatography techniques for element speciation, thus it provides a new strategy for the speciation of trace elements in human body fluids.

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