CoFe$_2$O$_4$ nano-particles functionalized with 8-hydroxyquinoline for dispersive solid-phase micro-extraction and direct fluorometric monitoring of aluminum in human serum and water samples

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**HIGHLIGHTS**

- CoFe$_2$O$_4$ nano-particles (NPs) was functionalized with 8-hydroxyquinoline (8-HQ).
- An ultrasound-assisted method was applied for functionalization of CoFe$_2$O$_4$ NPs.
- The obtained sol solution was applied as a dispersed solid-phase extractor.
- The developed method was used for separation and in situ determination of Al(III) ion.
- The method was applied to determine Al(III) ion in human serum and water samples.

**GRAPHICAL ABSTRACT**

**ABSTRACT**

A simple dispersive solid-phase micro-extraction method based on CoFe$_2$O$_4$ nano-particles (NPs) functionalized with 8-hydroxyquinoline (8-HQ) with the aid of sodium dodecyl sulfate (SDS) was developed for separation of Al(III) ions from aqueous solutions. Al(III) ions are separated at pH 7 via complex formation with 8-HQ using the functionalized CoFe$_2$O$_4$ nano-particles sol solution as a dispersed solid-phase extractor. The separated analyte is directly quantified by a spectrofluorimetric method at 370 nm excitation and 506 nm emission wavelengths. A comparison of the fluorescence of Al(III)–8-HQ complex in bulk solution and that of Al(III) ion interacted with 8-HQ/SDS/CoFe$_2$O$_4$ NPs revealed a nearly 5-fold improvement in intensity. The experimental factors influencing the separation and in situ monitoring of the analyte were optimized. Under these conditions, the calibration graph was linear in the range of 0.1–300 ng mL$^{-1}$ with a correlation coefficient of 0.9986. The limit of detection and limit of quantification were 0.03 ng mL$^{-1}$ and 0.10 ng mL$^{-1}$, respectively. The inter-day and intra-day relative standard deviations for six replicate determinations of 150 ng mL$^{-1}$ Al(III) ion were 2.8% and 1.7%, respectively. The method was successfully applied to direct determine Al(III) ion in various human serum and water samples.

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1. Introduction

Aluminum (Al) is the third most abundant metal in the earth's crust. It is widespread throughout nature, air, water, plants and consequently in all the food [1]. It is not considered to be an essential element in humans, but its toxicity is known. Al may enter the human body by mouth, intravenous infusion and by environment, drinking water, food and pharmaceutical products. However, the studies have revealed that when it reached a certain concentration in the human body, it caused and catalyzed many diseases. Al(III) ion has been considered as a possible cause of renal osteodystrophy, Shaver's disease, Parkinson’s disease and Alzheimer’s disease particularly in patients with chronic renal failure [2]. In addition, accumulation of Al in bone has been shown to result in bone loss [3]. Normally, Al is found at low levels in most drinking waters because it is still used as a flocculating and coagulating agent in potable water treatment units. The World Health Organization has established the maximum permissible content of Al in drinking water at 0.2 mg L\(^{-1}\) [4]. Therefore, there is a strong need for Al monitoring in environmental and clinical chemistry due to its negative roles in the human life.

Several methods have been reported for the determination of Al (III) ion in different matrices. These include high performance liquid chromatography [5,6], atomic absorption spectrometry [7,8], inductively coupled plasma optical emission spectrometry [9,10], inductively coupled plasma mass spectrometry [11,12], electrochemical techniques [13,14], spectrofluorimetry [15,16] and spectrophotometry [17,18]. However, most of these methods have important limitations and problems that are related to need a sample preparation system due to the presence of interfering species or the presence of Al(III) ion below the detection limit, time consuming, expensive and/or complicated analysis systems and hard operation. So, it is necessary to develop a simple, selective and highly sensitive method for in situ detection of Al(III) at trace levels in complex matrices such as serum samples.

In 2003, a rapid and simple clean-up technique named dispersive solid phase extraction (DSPE) was developed by Anastassiades et al. [19]. DSPE is based on the SPE methodology, but the sorbent is directly added into the sample solution without conditioning. This technique is a surface dependent approach that enables the isolation of target analytes from matrix solution by sorption on a solid sorbent. It is thus of interest to use this method for in situ measurements. In this case, solid sorbent particles were dispersed into a sample solution and their interaction with analyte can be generated the detectable signal (e.g., fluorescence). This method is a quick, easy, cheap, effective, rugged and safe (QuEChERS) approach for the analysis. In general, it does not need to time-consuming and laborious separation/desorption step, and measurement was occurred directly.

Nanoscience is one of the most important researches in modern science. The use of nano-sized materials offers major advantages due to their unique size and physicochemical properties [20,21]. Because of the widespread applications of nanoparticles (NPs) in the fields of physics, chemistry, biology, and medicine, much attention has been paid to the synthesis of different kinds of NPs. Furthermore, the surface properties of NPs allow to functionalize them by various capping ligands to make them enable for a range of applications. To the best of our knowledge, there are only a few reports involving the detection of Al(III) ion using NPs-based assays [22-24]. 8-Hydroxyquinoline (8-HQ) has been known as one of the most sensitive ligands used for the determination of Al(III) ion by fluorometric detection [25]. It forms a highly fluorescent complex with Al(III) ion which is relatively free from interfering species commonly present in water and biological samples. However, the direct monitoring of Al(III) ions in the trace level is difficult. Therefore, there is a crucial need for preconcentration steps before the analysis. Another solution for this problem is the signal amplification to achieve the high sensitivity in the low concentration of analyte.

Magnetic NPs are advantageous for analytical methods because they have a large surface-to-volume ratio, are comparable in size to many analytes of interest, are readily dispersible in solution, and have physical properties that are useful for enhancing signal detection [26]. Ferrofluids i.e., CoFe₂O₄ NPs also have the advantage of multiple synthetic routes for chemical functionalization. Thus, 8-HQ functionalized CoFe₂O₄ NPs seems promising to be adopted as a basis for the development of a fluorometric method for direct determination of Al(III) ion. In this work, an ultrasound-assisted method was used for functionalization of CoFe₂O₄ NPs with 8-HQ in the presence of SDS (Scheme 1). The functionalized NPs were employed for dispersive solid-phase microextraction and in situ monitoring of Al(III) ion in aqueous solutions. Several experimental variables affecting the method sensitivity were investigated in details. The method was applied to determine trace amounts of Al(III) ion in water and human serum samples with satisfactory results.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical-reagent grade and ultrapure deionized water obtained from Ghazi Serum Co. (Tabriz, Iran) was used throughout the work. CoFe₂O₄ NPs precursors i.e., ferric chloride (FeCl₃·6H₂O) and cobalt nitrate (Co(NO₃)₂·6H₂O), sodium hydroxide, aluminum nitrate (Al(NO₃)₃·9H₂O), 8-hydroxyquinoline (8-HQ), sodium dodecyl sulfate (SDS), sodium dihydrogen phosphate (NaH₂PO₄·2H₂O), ascorbic acid, 1,10-phenanthroline, hydrogen peroxide and all salts used for the interference study were purchased from Merck (Darmstadt, Germany). Stock solution of Al(III) at a concentration of 1000 mg L\(^{-1}\) was prepared by dissolving appropriate amount of its salt in deionized water. Working standard solutions were obtained by appropriate dilution of the stock solutions before analysis. A stock solution of 8-hydroxyquinoline was prepared by dissolving appropriate amount of this reagent in 5 mL ethanol (Merck) and diluting to 50 mL with deionized water. A stock phosphate buffer solution (0.2 mol L\(^{-1}\)) was prepared by dissolving appropriate amounts of NaH₂PO₄·2H₂O in deionized water and adjusting to pH 7.0 by adding 0.1 mol L\(^{-1}\) NaOH solution. All the plastic and glassware used for the trace analysis were immersed in 15% nitric acid for 12 h and rinsed with deionized water before use.

2.2. Apparatus and instruments

Fluorescence spectra and intensity measurements were carried out using a FP-6200 spectrofluorometer (JASCO Corp., Japan) with a wavelength range of 220–730 nm for excitation and emission. The instrument is equipped with a 150 W xenon lamp and 1.0 cm quartz cell, and supported with PC-based Windows® Spectra Manager TM software for JASCO Corporation version 1.02. The slit widths for excitation and emission were set at 5 nm and 10 nm, respectively, and the fluorescence spectra were recorded at a scan rate of 250 nm min\(^{-1}\).

To structure study of the as-prepared NPs, powder X-ray diffraction (XRD) measurements were performed by employing a D8 Advance (Bruker AXS, Germany) instrument with Cu Kα radiation source (1.54 Å) between 8 and 80° generated at 40 kV and 35 mA at room temperature. In addition, FT-IR spectra (4000–400 cm\(^{-1}\)) were acquired with a Vector 22 (Bruker, Germany) Fourier transform infrared spectrometer using the KBr pellet technique with a ratio sample/KBr of 1:100 by mass. The size
and shape of CoFe₂O₄ NPs were characterized by scanning electron microscope (SEM) model LEO1430vp (Carl Zeiss, Germany) and transmission electron microscopy (TEM) model 906 E (Zeiss, Germany).

The pH adjustments of the solutions were monitored using a digital pH-meter model 827 (Metrohm Ltd., Switzerland) supplied with a glass-combined electrode. Ultrasonic treatment was provided by an ultrasonic bath (SONICA, Italy). An electronic analytical balance model PB303 (Mettler Toledo, Switzerland) was used to weight the solid materials.

2.3. Synthesis of CoFe₂O₄ nano-particles

The CoFe₂O₄ NPs were prepared according to Massart method as described elsewhere [27]. Briefly, a solution containing ferric chloride (40 mL, 1 mol L⁻¹) and cobalt nitrate (10 mL, 2 mol L⁻¹ in 2 mol L⁻¹ HCl) was quickly added to sodium hydroxide (500 mL, 0.7 mol L⁻¹) under vigorous stirring. The obtained solution was heated at 95 °C for 30 min and the black precipitate was separated with a permanent handheld magnet after cooling down to room temperature. To get free particles from sodium and chloride ions, the product was washed with dilute nitric acid, and then washed with deionized water until the pH value of supernatant was about 7–8 so that the color turned to black-brown. The as-prepared product was treated with 0.5 mol L⁻¹ Fe(NO₃)₃ for 30 min, then centrifuged and washed through several cycles with deionized water. Afterward the supernatant was discarded and the precipitation was further washed with acetone for removal of the residual water, and kept in air-dry at room temperature for 2 h. In order to apply them to fluorescence measurements, it is necessary to bring initially the solid nano-particles into a water-soluble form. So, a stock solution of CoFe₂O₄ NPs was obtained by adding 5.0 mg of dry CoFe₂O₄ NPs to 50 mL of 0.025 mol L⁻¹ nitric acid. These non-agglomerated particles are stable in water over a wide range of pH values and in polar protic solvents such as ethanol [28].

2.4. Preparation of 8-HQ functionalized CoFe₂O₄ nano-particles

An ultrasound-assisted method was used for functionalization of the as-prepared CoFe₂O₄ NPs. For this purpose, an aliquot of 20 mL of 5 mmol L⁻¹ 8-HQ solution and 10 mL of 18 mmol L⁻¹ SDS solution were individually poured into a 100-mL beaker. Then, 750 μL of CoFe₂O₄ NPs stock solution was added into the mixture. Finally, to impregnate 8-HQ/SDS onto CoFe₂O₄ NPs, the mixture was sonicated for 20 min at room temperature.

2.5. Sample preparation

2.5.1. Water samples

Water samples including tap water, spring water, river water and bottled mineral water were chosen for the analysis. The bottled mineral water was purchased from local market and the others were collected from local sources. After sampling, water samples (except mineral water) were filtered through a round filter paper (blue band, no. 300210) to remove suspended particulate matter and then stored in a dark place at 4 °C. Finally, proper aliquots of each sample solution were analyzed by following the procedure described in Section 2.6.

2.5.2. Human serum

Human blood samples were obtained from healthy volunteer and patients’ ones at Al-Zahra hospital (Tabriz, Iran). To prepare serum samples, they were drawn into the test tube and treated with ethylenediaminetetraacetic acid. These samples were centrifuged at 3000 rpm for 10 min and then allowed to stand at 4 °C until the phase separation was done. The serum samples were kept in a freezer (−80 °C) until analysis. A 500 μL of each serum sample was placed in a centrifuge tube and 2.0 mL of acetonitrile was added to precipitate proteins. After vortex-mixing, the sample was centrifuged at 3500 rpm for 15 min, and the supernatant was transferred into a 5 mL volumetric flask and diluted to the mark.

Scheme 1. Schematic illustration of synthesis of SDS and 8-HQ coated CoFe₂O₄ NPs and their Al(III) ions binding to produce the Al(III)–8-HQ/SDS/CoFe₂O₄ NPs resulting fluorometric responses.
with deionized water. An appropriate aliquot of this solution was taken for analysis using the procedure described in the following section.

2.6. General procedure

An aliquot of standard or sample solution containing Al(III) in the range of 0.1–300 ng mL⁻¹ was transferred into a 5.0-mL volumetric flask, and 1.5 mL of 8-HQ functionalized CoFe₂O₄ NPs sol solution was added into the solution. Then, pH of the solution was adjusted to nearly 7 by adding 0.5 mL of 0.2 mol L⁻¹ phosphate buffer solution and diluted to the mark with deionized water. After shaking, the solution was allowed to stand for 15 min. Finally, the resultant solution was transferred into a 1 cm quartz cell, and the fluorescence intensity was measured at 506 nm with excitation at 370 nm.

3. Results and discussion

3.1. Characterization of 8-HQ functionalized CoFe₂O₄ nano-particles

Identification of the crystalline phases of NPs was performed by XRD analysis and the patterns of the as-prepared materials are shown in Fig. 1a. As can be seen, both of the XRD patterns of

Fig. 1. (a) XRD patterns of CoFe₂O₄ NPs and 8-HQ/SDS/CoFe₂O₄ NPs; (b) FT-IR spectra of CoFe₂O₄ NPs, 8-HQ and 8-HQ/SDS/CoFe₂O₄ NPs; (c) SEM image of CoFe₂O₄ NPs; and (d) TEM image of CoFe₂O₄ NPs.
CoFe₂O₄ NPs and 8-HQ/SDS/CoFe₂O₄ NPs exhibit a spinel structure with the diffraction peaks for (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), (4 4 0), (6 2 0) and (5 3 3) planes, which are in accordance to that of standard CoFe₂O₄ XRD pattern (JCPDS No. 22–1086). This shows that the crystal structure of CoFe₂O₄ NPs is not destroyed after functionalization with 8-HQ/SDS.

To characterize the surface nature of the NPs, the infrared absorption spectroscopy was used. Fig. 1b shows the FT-IR spectra of CoFe₂O₄, 8-HQ and 8-HQ/SDS/CoFe₂O₄ NPs in the region from 400 to 4000 cm⁻¹. The presence of the —OH absorption band between 3000 and 3500 cm⁻¹ is clear for 8-HQ, CoFe₂O₄ and the 8-HQ modified CoFe₂O₄. An obvious peak is observed at 1577 cm⁻¹ which could be attributed to C—O stretching vibrations. The absorption bands of C=C and C=N stretching vibrations are found at 1507 cm⁻¹ and 1637 cm⁻¹, respectively. The peak found at 1384 cm⁻¹ could be assigned to the ring stretching vibration of 8-HQ. The C—H out-of-plane wagging vibrations of the quinoline groups of 8-HQ are observed at 736, 802 and 824 cm⁻¹ [29]. The above-mentioned peaks were pronounced for both of 8-HQ and 8-HQ/SDS/CoFe₂O₄, while they were not found in the case of CoFe₂O₄ NPs. Also, the FT-IR spectrum of 8-HQ/SDS/CoFe₂O₄ NPs exhibits three peaks at 2957, 2921 and 2850 cm⁻¹, which are attributed to the asymmetric and symmetric C—H stretching vibrations of the CH₃ and CH₂ units in SDS structure [30].

These results indicate that the 8-HQ and SDS molecules have successfully attached on the surface of CoFe₂O₄ NPs.

In addition, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to obtain information on the particle size and morphology of as-prepared NPs. The size of the NPs should be sufficiently small to prepare stable magnetic colloids. SEM image of CoFe₂O₄ NPs is shown in Fig. 1c. It can be seen that the CoFe₂O₄ NPs had nearly uniform distribution of particle size and the average diameter of CoFe₂O₄ NPs was less than 50 nm. Moreover, TEM analysis of the colloidal solution reveals that both spherical and semi-spherical particle morphologies are present (Fig. 1d).

3.2. The ultrasonic irradiation role in synthesis of 8-HQ modified CoFe₂O₄ nano-particles

The ultrasonic irradiation was used for the synthesis of 8-HQ modified CoFe₂O₄ NPs. The sound waves create small, highly energetic gas/vapor filled microbubbles, which, upon implosive collapse, generate intense microjets, shock waves, and locally high pressures and high temperatures. The energetic bubbles scavenge surfactants from the solution and transport these reactive materials to the surface of NPs. On the other hand, the hydrodynamic interaction of bubbles in the bubble cloud and

![Fig. 2. Effect of (a) pH, (b) 8-hydroxyquinoline concentration, (c) SDS concentration and (d) CoFe₂O₄ NPs concentration on ΔIₕ (ΔIₕ is the difference between the fluorescence intensity of Al(III)—8-HQ/SDS/CoFe₂O₄ NPs system and Al(III)—8-HQ complex in bulk solution.) Conditions: Al(III) ion concentration; 150 ng mL⁻¹ and pH 7.0.](image-url)
the subsequent mixing of fluid enhance the dispersion of surfactant and NPs dissolved in the bulk fluid and drive them together at velocities of hundreds of meters per second for inelastic impact at the point of meeting, resulting in the high reacting speed [31,32]. Upon reaching the NPs/liquid interface, the electrostatic interaction between positively charged surfaces of CoFe$_2$O$_4$ NPs (pH < 8) and negatively charged surfactant (SDS), resulting in a dense coverage of the surfactant material on the NPs. In this research, 8-HQ was incorporated into the inner hydrophobic part of produced ad-micelles or hemi-micelles of SDS to produce an suitable and selective assembly (denoted as 8-HQ/SDS/CoFe$_2$O$_4$ NPs) for separation and in situ determination of Al(III) ion.

3.3. Optimization of the synthesis conditions of 8-HQ/SDS/CoFe$_2$O$_4$ nano-particles

To accomplish the optimum conditions for impregnation of 8-HQ on the CoFe$_2$O$_4$ NPs, the effect of several parameters such as the pH value, the concentrations of 8-HQ, SDS and CoFe$_2$O$_4$ NPs, and sonication time on the analytical signal was investigated by one-at-a-time method. Analytical signal ($\Delta I_F$) was the difference between the fluorescence intensity of Al(III)–8-HQ/SDS/CoFe$_2$O$_4$ NPs system and Al(III)–8-HQ complex in bulk solution. A 150 ng mL$^{-1}$ solution of Al(III) ion was used for all measurements and each experiment was repeated three times.

3.3.1. Effect of pH

The influence of pH on the analytical signal was studied. For this purpose, the pH of each solution was adjusted to between 3 and 12 using minimal volumes of diluted nitric acid or sodium hydroxide solution, or both. The results, shown in Fig. 2a, revealed that the maximum analytical signal was obtained at pH < 7. For CoFe$_2$O$_4$ NPs, the point of zero charge is nearby pH 8 [33,34]. So, the surface charge of the NPs is positive at pH < 8. The CoFe$_2$O$_4$ NPs with positively charged surfaces (pH < 8) can strongly adsorb negatively charged surfactants such as SDS. Hence, the SDS surfactant is able to form hemi-micelles or ad-micelles on the surface of CoFe$_2$O$_4$ NPs, and 8-HQ molecules could be trapped by the micelles. At pH > 8, a deterioration of the signal was observed. It can be ascribed to negatively charged surface of the NPs. Consequently, the synthesis was performed in the initial pH of solution (pH = 5) without adding any acidic or basic solution.

3.3.2. Effect of 8-hydroxyquinoline concentration

The effect of chelating agent concentration on the analytical signal was examined and the results are shown in Fig. 2b. The CoFe$_2$O$_4$ NPs were treated with 8-HQ solutions in the range of 0.34–8.5 mmol L$^{-1}$. Major improvement in the analytical signal was obtained as the chelating agent concentration increased up to 3.4 mmol L$^{-1}$, which is sufficient for total complexation. The concentrations above 3.4 mmol L$^{-1}$ had no significant effect on the analytical signal. Accordingly, 3.4 mmol L$^{-1}$ 8-HQ solution was chosen as optimum chelating agent concentration.

3.3.3. Effect of SDS concentration

The effect of SDS concentration on the analytical signal was investigated in the range between 1.0 and 11.0 mmol L$^{-1}$ SDS. The results (Fig. 2c) indicated that the analytical signal increases with

![Fig. 3. Effect of pH (a), amount of dispersed solid-phase extractor (b), incubation time (c) and temperature (d) on the fluorescence intensity of 8-HQ/SDS/CoFe$_2$O$_4$ NPs in the presence of 150 ng mL$^{-1}$ Al(III) ion.](image-url)
the increase in SDS concentration up to 3.0 mmol L\(^{-1}\) and then remains constant until 7.0 mmol L\(^{-1}\). Micelle formation in the aqueous solution might be responsible for the observed slightly decrease in the analytical signal at higher concentrations of SDS. So, 6.0 mmol L\(^{-1}\) of SDS solution (lower than its critical micellar concentration [35]) was recommended as an optimum concentration.

3.3.4. Effect of CoFe\(_2\)O\(_4\) nano-particles concentration

The influence of concentration of CoFe\(_2\)O\(_4\) NPs sol solution on the analytical signal was investigated in the range from 1.0 to 10.0 mg L\(^{-1}\). As can be seen from Fig. 2, the analytical signal increases with the increase in CoFe\(_2\)O\(_4\) NPs concentration up to 2.0 mg L\(^{-1}\). In the case of concentrations higher than 3.0 mg L\(^{-1}\), the analytical signal is decreased probably due to the self-absorption of the fluorescence emission by the excess of CoFe\(_2\)O\(_4\) NPs in the spectrophotometer cell. Hence, 2.5 mg L\(^{-1}\) of CoFe\(_2\)O\(_4\) NPs colloidal solution was selected for subsequent experiments.

3.3.5. Effect of ultrasound irradiation time

To investigate the effect of sonication time on the analytical signal, the mixture from precursors were subjected to ultrasound irradiation in an ultrasonic water bath in the range between 5 and 60 min. According to the obtained results (results are not shown), the analytical signal increased by increasing the sonication time up to 20 min and remained constant afterward. Therefore, 20 min was selected as the optimum sonication time.

3.4. Optimization of dispersive solid-phase micro-extraction conditions

To obtain the highest fluorescence intensity of the analyte, the effect of parameters influencing the complex formation between Al(III) and 8-HQ immobilized on CoFe\(_2\)O\(_4\) NPs including pH, amount of dispersed solid-phase extractor (8-HQ/SDS/CoFe\(_2\)O\(_4\) NPs concentration in a sol solution), incubation time, temperature and dissolved oxygen were investigated. A 150 ng mL\(^{-1}\) Al(III) ion solution (5.0 mL) was used for all measurements and each experiment was repeated three times.

As the complex formation between Al(III) and 8-HQ strongly depends on the pH value of the medium, preliminary studies were carried out to determine the pH corresponding to the maximum fluorescence intensity. For this purpose, the pH values of sample solution were adjusted to a range of 4–12 using minimal volumes of diluted HNO\(_3\) or NaOH, or both. As shown in Fig. 3a, the maximum fluorescence intensity was achieved between pH 6.5 and 8.0. As the CoFe\(_2\)O\(_4\) NPs surfaces are negatively charged at pH > 8, weak interactions leading to decrease in the stability of 8-HQ/SDS/CoFe\(_2\)O\(_4\) NPs might be responsible for the observed decrease in the fluorescence intensity at higher values of pH. On the other hand, the coordination ability between Al(III) ion and 8-HQ is decreased at pH > 8 owing to existence of the analyte as AlO\(_2^+\) in basic media. Consequently, pH 7 was selected as an optimum value, and 0.5 mL of 0.2 mol L\(^{-1}\) phosphate buffer solution (pH 7) was used for this purpose.

The influence of the amount of dispersed solid-phase extractor on the analytical signal was studied by varying the volume of the prepared 8-HQ/SDS/CoFe\(_2\)O\(_4\) NPs sol solution (Section 2.4) from 0.5 to 3.0 mL. As can be seen from Fig. 3b, the maximum analytical signal was achieved with 1.5 mL of the sol solution. However, higher volumes led to a decrease in fluorescence intensity values probably due to self-absorption of the fluorescence emission by the excess of 8-HQ/SDS/CoFe\(_2\)O\(_4\) NPs in the spectrophotometer cell, as we mentioned in Section 3.3.4.

The effect of incubation time on the fluorescence intensity was investigated. For this purpose, the solutions with different reaction times in the range of 5–50 min were prepared. As shown in Fig. 3c, the fluorescence intensity increases with the increase in the reaction time from 5 to 15 min and then remains constant. Consequently, 15 min was selected as an optimum incubation time.

The effect of temperature on the fluorescence intensity was also studied. The fluorescence intensity decreased by about 67.6% when the temperature increased from 25 °C to 70 °C (Fig. 3d). However, in this range, the shapes of the emission spectra remained unaltered. This fact is probable that higher temperatures lead to weaker electrostatic interactions that can lead to the heterogeneity of the binding sites and decrease the fluorescence intensity. Moreover, thermal quenching is another factor that decreases the fluorescence intensity as the temperature increases. The deactivating states are thermally activated at high temperature and non-radiative relaxation mechanisms, therefore, become dominant. Then, the excited state of complex will have highly energetic electrons at higher temperature increases, which subsequently return to the ground state via non-radiative process that lowers the fluorescence intensity [36]. Therefore, a room temperature (25 °C) was chosen as an operational temperature for further experiments.

In addition, one of the best-known collisional quenchers is molecular oxygen. The process involves dynamic collision between molecular (triplet) oxygen and the excited electronic state of the fluorophore and leads to a reduction of its intensity. In order to obtain reliable measurement result, the effect of dissolved oxygen on the fluorescence intensity was investigated. The dissolved oxygen was removed by filled N\(_2\) into cuvette for about 20 min. Based on the obtained results, the fluorescence intensity of the solution is almost the same before and after removing dissolved oxygen. Therefore, in the present work, the effect of dissolved oxygen in the solution can be neglected.

3.5. Spectral studies

Fig. 4 shows the fluorescence spectra of (a) Al(III)–8-HQ/SDS/CoFe\(_2\)O\(_4\); (b) Al(III)–8-HQ; (c) 8-HQ/SDS/CoFe\(_2\)O\(_4\) and (d) 8-HQ solution. As can be seen from Fig. 4d, 8-HQ barely has very weak fluorescence intensity at 506 nm wavelength in aqueous solution due to photo-induced electron-transfer (PET) process from lone pair electron of quinoline nitrogen atom. The fluorescence intensity of 8-HQ can be enhanced by adding of Al(III) ion as a result of PET inhibition [24]. However, other non-radiative transition pathways compete always with the fluorescence relaxation and cause usually dramatically lower or, in some cases, e.g., low concentration of fluorophore, completely eliminate
emission. A common example of quenching is observed with the collision of an excited state fluorophore and another non-fluorescent/fluorescent molecules in solution, resulting in deactivation of the fluorophore and return to the ground state. In this case, the excited state lifetime and the quantum yield of the affected fluorophore are reduced [37,38]. This could be a problem, particularly in the low concentration of fluorophore, leading to decrease in sensitivity of the method.

We found that the immobilization of 8-HQ on the surface of CoFe$_2$O$_4$ NPs with the aid of SDS ionic surfactant caused an obvious enhancement in the fluorescence intensity of 8-HQ compared with the free 8-HQ in bulk solution (Figs. 4c and d). Moreover, a comparison of the fluorescence of Al(III)–8-HQ complex in bulk solution and that of Al(III) ion interacted with 8-HQ/SDS/CoFe$_2$O$_4$ NPs revealed a nearly 5-fold improvement in intensity (Figs. 4a and b). The observed enhancement in the fluorescence intensity reflects the suppression of the nonradiative decay process due to increasing the rigidity of the system and elimination of collisional quenching. As shown in Fig. 5, the enhancement factor, calculated as the ratio of calibration curve slope of Al(III)–8-HQ/SDS/CoFe$_2$O$_4$ to that obtained for Al(III)–8-HQ complex in bulk solution, was found as 5. This provided a nearly 5-fold improvement in sensitivity of the method.

The resonance light scattering (RLS) technique is available to provide some insight into the process responsible for the formation of the complex, which is shown in Fig. 6. RLS spectra were obtained by simultaneously scanning the excitation and emission monochromators (namely, $\Delta \lambda = 0 \text{ nm}$) of spectrofluorometer from 350 to 600 nm. It can be seen that RLS intensity of 8-HQ is greatly enhanced when it is immobilized on the surface of CoFe$_2$O$_4$ NPs in the presence of SDS using an ultrasound-assisted procedure (Figs. 6b and d). In addition, the RLS intensities of 8-HQ and 8-HQ/SDS/CoFe$_2$O$_4$ NPs are obviously enhanced in the presence of Al(III) ion, indicating the complex formation between Al(III) ion and free 8-HQ in bulk solution (Fig. 6c) and also 8-HQ immobilized on the surface of CoFe$_2$O$_4$ NPs in a sol solution (Fig. 6a).

### 3.6. Study of Interferences

The tolerance limits of some potentially interfering substances on the fluorescence intensity of Al(III)–8-HQ/SDS/CoFe$_2$O$_4$ NPs system were evaluated. In these experiments, different amounts of interfering ions or compounds were added to a test solution containing 150 ng mL$^{-1}$ Al(III) prior to the ‘General procedure’ being carried out. The tolerance level was set at the highest amount of interfering ions or compounds causing less than ±5% change in the analytical signal of the analyte (the results are presented in Table 1). With the exception of Cr(III), Fe(II), Fe(III), Zn(II) and fluoride ion, all other ions or compounds examined did not interfere with the determination of 150 ng mL$^{-1}$ Al(III) ion.

For the elimination of these interferences, ascorbic acid, 1,10-phenanthroline (Phen) and hydrogen peroxide were used [17,24,39]. Cr(III) can be oxidized by hydrogen peroxide, Fe(II) and Zn(II) can be camouflaged with Phen and form a stable complex (Fe(III) must be reduced to Fe(II) by ascorbic acid before masking with Phen). In the absence of masking agents Cr(III), Zn(II), Fe(II) and Fe(III) interfered with Al(III) determination when they were present in 10 times the weight ratios of interfering ions to Al(III), but in the presence of masking agents the tolerable weight ratios of the interfering ions to Al(III) became 300 for Fe(II), Fe(III) and Cr(III) and 500 for Zn(II). As regards, the amounts of these interfering species, in water samples and biological samples, are below their tolerable levels, so there would be no interferences from these species in Al(III) ion determination.

The most interfering ion during the determination of Al(III) in water samples was fluoride. The interference of fluoride arises because Al(III) ion can form a series of stable compounds with F$^-$. According to the thermodynamic constants of stability, Zr(IV) reacts rapidly to combine with F$^-$ in preference to the Al(III) ion and the zirconium fluoride molecules formed effectively release the Al(III) ions that it can be determined spectrofluorometrically [40]. A concentration of 0.8 mg L$^{-1}$ Zr(IV) was therefore used as a precautionary measure to guard against fluoride interference in water samples.

<table>
<thead>
<tr>
<th>Interfering substances</th>
<th>Tolerance limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine, ascorbic acid</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Cysteine, K$^+$, Na$^+$, ClO$_4^-$, CH$_3$COO$^-$, Cl$^-$, Br$^-$, BrO$_3^-$</td>
<td>1000</td>
</tr>
<tr>
<td>Citrate, adenine, oxalic acid, B$_2$$^+$, Ba$^{2+}$</td>
<td>800</td>
</tr>
<tr>
<td>Zr$^{4+}$, Pb$^{2+}$, As(V), Mg$^{2+}$, EDTA</td>
<td>500</td>
</tr>
<tr>
<td>Fe$^{3+}$, Fe$^{2+}$, Co(III), Zn(II)</td>
<td>300</td>
</tr>
<tr>
<td>MnO$_4^-$, Cu$^{2+}$, VO$<em>4^{3-}$, Ag$^+$, Co$</em>{2+}$, Cd$^{2+}$, F$^{-}$</td>
<td>100</td>
</tr>
</tbody>
</table>

* In the presence of 0.005 mol L$^{-1}$ 1,10-phenanthroline.
* In the presence of 0.001 mol L$^{-1}$ ascorbic acid + 0.005 mol L$^{-1}$ 1,10-phenanthroline.
* In the presence of 0.006 mol L$^{-1}$ H$_2$O$_2$.
* In the presence of 0.8 mg L$^{-1}$ Zr(IV).
Table 2
Comparison of analytical characteristics of the presented method with other reported techniques for determination of Al(III).

<table>
<thead>
<tr>
<th>Method</th>
<th>Linear range (ng mL⁻¹)</th>
<th>LOD (ng mL⁻¹)</th>
<th>RSD (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>2.7–2160</td>
<td>1.35</td>
<td>1.0</td>
<td>[6]</td>
</tr>
<tr>
<td>ETAAS</td>
<td>0.35–20</td>
<td>0.11</td>
<td>&lt;5</td>
<td>[8]</td>
</tr>
<tr>
<td>ICP-DES</td>
<td>Up to 200</td>
<td>0.25</td>
<td>3.1</td>
<td>[10]</td>
</tr>
<tr>
<td>Voltammetry</td>
<td>3.24–32.4</td>
<td>2.43</td>
<td>3.2</td>
<td>[14]</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>10–2000</td>
<td>1.0</td>
<td>&lt;2</td>
<td>[17]</td>
</tr>
<tr>
<td>Spectrofluorometry</td>
<td>1.35–108</td>
<td>0.405</td>
<td>-</td>
<td>[23]</td>
</tr>
<tr>
<td>DISPME – spectrophotometry</td>
<td>0.1–300</td>
<td>0.03</td>
<td>1.7</td>
<td>This study</td>
</tr>
</tbody>
</table>

a High-performance liquid chromatography.
b Electrothermal atomic absorption spectrometry.
c Inductively coupled plasma optical emission spectrometry.
d Dispersive solid-phase micro-extraction.

3.7. Analytical figures of merit

Under the optimum conditions described, the calibration graph using the developed system for Al(III) ion was linear in the range of 0.1–300 ng mL⁻¹ with a correlation coefficient of 0.9986. The regression equation was \( \Delta F = 2.5615 \frac{C_{\text{Al(III)}}}{C_0} + 10.782 \), where \( \Delta F \) is the fluorescence intensity difference between sample solution (8-HQ/SDS/CoFe₂O₄ NPs in the presence of Al(III) ions) and blank (8-HQ/SDS/CoFe₂O₄ NPs in the absence of Al(III) ions) in arbitrary unit, and \( C_{\text{Al(III)}} \) is the concentration of Al(III) ions in ng mL⁻¹. The detection limit of the present work was calculated after application of the procedure to blank solutions. The limit of detection and limit of quantification, defined as \( 3 \frac{S_b}{m} \) and \( 10 \frac{S_b}{m} \) (where \( S_b \) is the standard deviation of the blank and \( m \) is the slope of the calibration curve) were 0.03 ng mL⁻¹ and 0.10 ng mL⁻¹, respectively. The precision of the method was evaluated by repeated analysis of Al(III) ion during the course of experimentation on the same day and on different days under the optimized experimental conditions. The inter-day and intra-day relative standard deviations for six replicate determinations of 150 ng mL⁻¹ Al(III) ion were 2.8% and 1.7%, respectively. A comparison of our method with some other published methods for the determination of Al(III) ion is shown in Table 2. As can be seen, the LOD of the method are superior to those of the other reported methods. Also, the method is relatively rapid and simple as compared with previously reported procedures.

Table 3
Determination of Al(III) in real samples (results of recoveries of spiked samples with different amounts of Al(III) ion).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Added Al(III) (ng mL⁻¹)</th>
<th>Found Al(III) (ng mL⁻¹)</th>
<th>Recovery (%)</th>
<th>t-Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td>–</td>
<td>3.94 ± 0.16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>44.30 ± 1.22</td>
<td>100.9</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>80.0</td>
<td>85.5 ± 1.04</td>
<td>101.9</td>
<td>2.59</td>
</tr>
<tr>
<td>Spring water</td>
<td>–</td>
<td>10.89 ± 0.84</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>52.43 ± 1.06</td>
<td>103.8</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>80.0</td>
<td>91.00 ± 1.66</td>
<td>100.1</td>
<td>0.11</td>
</tr>
<tr>
<td>River water</td>
<td>–</td>
<td>33.18 ± 0.63</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>72.83 ± 2.60</td>
<td>99.1</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>80.0</td>
<td>114.30 ± 0.76</td>
<td>101.4</td>
<td>2.54</td>
</tr>
<tr>
<td>Bottled mineral water</td>
<td>–</td>
<td>8.60 ± 0.35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>47.55 ± 0.58</td>
<td>97.4</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td>80.0</td>
<td>87.32 ± 0.88</td>
<td>98.4</td>
<td>2.52</td>
</tr>
<tr>
<td>Serum samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>–</td>
<td>97.15 ± 1.61</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>136.75 ± 0.58</td>
<td>99.0</td>
<td>1.19</td>
</tr>
<tr>
<td>Sample 2</td>
<td>–</td>
<td>87.06 ± 1.23</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>126.75 ± 0.62</td>
<td>99.2</td>
<td>0.86</td>
</tr>
<tr>
<td>Diabetic patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>–</td>
<td>158.6 ± 2.62</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>198.2 ± 1.28</td>
<td>99.0</td>
<td>0.54</td>
</tr>
<tr>
<td>Sample 2</td>
<td>–</td>
<td>137.2 ± 1.30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>176.5 ± 1.01</td>
<td>98.2</td>
<td>1.19</td>
</tr>
<tr>
<td>Ovarian cancer patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>–</td>
<td>280.90 ± 1.43</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>321.40 ± 1.28</td>
<td>101.2</td>
<td>0.67</td>
</tr>
<tr>
<td>Sample 2</td>
<td>–</td>
<td>300.50 ± 1.96</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>339.80 ± 0.90</td>
<td>98.2</td>
<td>1.34</td>
</tr>
</tbody>
</table>

a Mean of three experiments ± standard deviation.
b Recovery (%) = [100 × (Found – Base)/Added] × 100. "Base" and “Found” refer to the amount of Al(III) in samples before and after spiking, respectively.
c t-critical = 4.3 for n = 2 and P = 0.05.
d From drinking water system of Azarshahr, Iran.
e Obtained from local source, Azarshahr, Iran.
f Obtained from local source, Azarshahr, Iran.
g Obtained from local market, Azarshahr, Iran.
h Obtained from Al-Zahra hospital, Tabriz, Iran.
To evaluate the accuracy of the developed method, it was first applied to the determination of Al(III) ion in a standard reference material (NIST SRM 1643e, trace elements in water). The certified amount of Al(III) ion in the SRM is 141 ± 8.6 ng mL⁻¹. The obtained value for Al(III) ion was 138.95 ± 5.41 ng mL⁻¹ (mean of three determinations ± standard deviation), which is in good agreement with the certified value according to the Student t-test. The method was then applied for the determination of Al(III) ion in several water samples i.e., bottled mineral water, tap water, river water and spring water. Table 3 shows the obtained results. It should be noted that a preliminary step for remove the interfering effect of fluoride ions is necessary. The recovery experiments were carried out by spiking the samples with different amounts of Al(III) before any pretreatment prior to preparation and analysis according to the general procedure. As can be seen, the relative recovery values between 97.4 and 103.8% were obtained, which again confirm the accuracy of the method and its independence from the matrix effects.

The method was also used to determine Al(III) ion in human serum samples obtained from diabetic patients, cancer patients and healthy volunteers. The results are given in Table 3. According to the obtained results, the recoveries are close to 100%, indicating that there is no serious interference in such samples and the presented method could be applied for determination of Al(III) ion in complex matrices.

4. Conclusions

In this research, a simple dispersive solid-phase micro-extraction method based on the 8-HQ functionalized CoFe₂O₄ NPs was developed for the separation and in situ fluorometric detection of trace levels of Al(III) ion in aqueous solutions. An ultrasound-assisted procedure was used to prepare the 8-HQ functionalized CoFe₂O₄ NPs with the aid of SDS ionic surfactant. The experimental results indicate that the immobilization of 8-HQ on the CoFe₂O₄ NPs can be caused a greatly enhancement in the resonance light scattering intensity compared with the free 8-HQ in bulk solution. Moreover, a comparison of the fluorescence of Al(III)–8-HQ complex in bulk solution and that of Al(III) ion interacted with 8-HQ/SDS/CoFe₂O₄ NPs revealed a nearly 5-fold improvement in intensity. This provided a nearly 5-fold improvement in sensitivity of the presented method. The method was successfully used for the direct determination of Al(III) ion in various human serum and water samples. In comparison with most of other methods, this method is quick, simple and has high sensitivity. No additional clean-up steps are required, thus the method saves time, labor, money and solvent use compared with the tedious traditional solid-phase extraction method.

Acknowledgment

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References