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Nickel–aluminum layered double hydroxide as a nanosorbent for selective solid-phase extraction and spectrofluorometric determination of salicylic acid in pharmaceutical and biological samples

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ABSTRACT

The nickel–aluminum layered double hydroxide (Ni–Al LDH) was synthesized by a simple co-precipitation method and used as a solid-phase extraction (SPE) sorbent for separation and pre-concentration of trace levels of salicylic acid (SA) from aqueous solutions. Extraction of analyte is based on the adsorption of salicylate ions on the Ni–Al (NO_3^-) LDH and/or their exchanging with LDH interlayer NO_3^- ions. The retained analyte on the LDH was stripped by 3 mol L^{-1} NaOH solution and its concentration was subsequently determined spectrofluorometrically at λ_{em} = 400 nm with excitation at λ_{ex} = 270 nm. Various parameters affecting the extraction efficiency of SA on the Ni–Al (NO_3^-) LDH, such as pH, amount of nano-sorbent, sample loading flow rate, elution conditions, sample volume and matrix effects were investigated. In the optimum experimental conditions, the limit of detection (3 s) and enrichment factor were 0.12 μg L^{-1} and 40, respectively. The relative standard deviation (RSD) for six replicate determinations of 10 μg L^{-1} SA was 2.3%. The calibration graph using the pre-concentration system was linear in the range of 0.3–45 μg L^{-1} with a correlation coefficient of 0.9985. The optimized method was successfully applied to the determination of SA in blood serum, willow leaf and aspirin tablet.

1. Introduction

Salicylic acid (SA), 2-hydroxybenzoic acid, is one of the most important materials used in the pharmaceutical industry. Owing to its antiseptic properties, SA is used in the food industry, in the production of dyes and artificial scents, in cosmetics and medicine. Because of its effect on skin cells, SA is also used in several shampoos to treat dandruff and in sunscreen lotions as a topical therapeutic to protect the skin from sun damage. It is also a product of decomposition of acetylsalicylic acid (ASA) (aspirin), which is a common anti-inflammatory, analgesic and antipyretic drug used in topical preparations and tablets [1]. In the human blood (pH ≈ 7.4), SA is present as the deprotonated form (salicylate ion). Salicylate is highly toxic when taken in overdose and the amounts that produce toxicity may be only twice the daily dose taken for chronic pain [2]. Therefore, it is important to develop valid methods to quantify SA at concentrations naturally occurring in biological fluids, such as serum. However, this task may be difficult because salicylic acid has been reported to be present at very low concentrations in subjects not taking aspirin [3].

The most frequently used method for the clinical analysis of SA is "Trinder test" which is based on the formation of purple–violet complex of SA–Fe (III) ions that can be monitored spectrophotometrically [4]. However, this complex is strongly affected by interference from substances bearing enol and phenol groups. For this reason, in complex matrices, i.e., clinical samples, a preliminary separation step prior to SA determination could be a good choice.

Solid-phase extraction (SPE) is a well-established sample pretreatment technique in pharmaceutical, biomedical and environmental field, and plays a very important role in modern analytical science. SPE has become to the forefront compared to other pre-concentration and/or separation techniques, as it offers several advantages such as flexibility to choose the solid phase, high recovery, low cost, simplicity, higher enrichment factors, low consumption of organic solvents over liquid–liquid extraction, safety with respect to hazardous samples, and the ability of combination with different detection techniques in the form of on-line or off-line mode [5]. In order to control the analytical parameters such as selectivity, removal affinity and adsorption capacity, the choice of suitable sorbents for SPE is very important.

Layered double hydroxides (LDHs) are a class of synthetic two-dimensional nano-structured anionic clays and have received considerable attention in recent years due to their versatility and usefulness in a wide range of technological applications as anion exchangers, adsorbents, medicine stabilizers, catalysts,

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molecular sieves, polymer composites, and bioactive materials [6–17]. LDHs can be represented by the general formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}[A^{n-}_{x/n}\cdot mH_2O]^{x-}$, where M^{2+} is a divalent cation such as Mg^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+} or Cu^{2+}; M^{3+} is a trivalent cation such as Al^{3+}, Fe^{3+}, or Cr^{3+}; A^{n-} is an interlayer anion such as CO_3^{2-}, SO_4^{2-}, NO_3^-, Cl^- or OH^-; m and x are the numbers of moles of co-intercalated water and M^{3+} per formula weight of the compound, respectively [18]. The value of x is typically between 0.2 and 0.33 [19], even though there is no strict limitation to this value. LDHs are built up of brucite-like sheets and part of divalent cations (M^{2+}) originally coordinated octahedrally by hydroxyl groups, are isomorphously replaced by trivalent cations (M^{3+}), affording the positively charged layers in the presence of charge-balancing anions. The excesses of the positive charges are balanced by the anions (A^{n-}) in the interlayer. The anions in the interlayer can be exchanged by other anions [15]. Hydrogen bonded water molecules may also occupy the remaining free space between layers. Due to their large surface area and high anion exchange capacity [20,21], LDHs have the potential to be good adsorbents for solid-phase extraction of organic and/or inorganic anions from aqueous solutions.

In the present work, Ni–Al (NO_3^-) LDH was synthesized and structurally and morphologically characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM). Then, a simple method was developed for the separation and pre-concentration of trace amounts of SA from complex matrices using a column packed with Ni–Al (NO_3^-) LDH nano-particles as solid-phase extractor. The effects of various experimental parameters on the extraction efficiency were investigated and optimized. To evaluate the applicability of the proposed method, it was applied to the determination of SA in pharmaceutical and biological samples.

2. Experimental

2.1. Apparatus and instruments

Fluorescence spectra and intensity measurements were carried out using a FP-6200 spectrofluorometer (JASCO Corporation, Tokyo, Japan) with a wavelength range of 220–730 nm (with 1 nm intervals) for excitation and emission. The instrument equipped with a 150 W xenon lamp, 1.0 cm quartz cell, dual monochromaters (silicon photodiode for excitation and photomultiplier for emission), Peltier thermostatted single cell holder (model ETC-272), and supported with PC-based Windows® Spectra Manager™ software for JASCO Corporation version 1.02. The slit widths for both excitation and emission were set at 5 nm and the fluorescence spectra were recorded at a scan rate of 250 nm min^{-1}. Fluorescence intensities were measured at 400 nm with excitation at 270 nm at 25 °C. A 2 mL polypropylene cartridge (30 mm × 7 mm i.d., Shafa Co., Iran) packed with 200 mg of Ni–Al (NO_3^-) LDH and fitted with small cotton beads at both ends to prevent material losses was used to pre-concentrate the analytes in SPE procedures. The flow rate of solution through the column was controlled with an air-driven fluid pump model P34112 (Taiwan). A schematic diagram of the SPE system is given in Fig. 1.

In order to structural study of the LDH, XRD measurements were performed on a Brucker AXS (D8 Advance) X-ray powder diffractometer (Cu K_α radiation source, λ = 0.154056 nm) between 5° and 65° generated at 40 kV and 35 mA at room temperature. Prior to the XRD measurement, LDH cake was ground into powder and then pressed flat in the sample slot. In addition, FT-IR spectra (4000–400 cm^{-1}) were recorded on a Vector 22 (Bruker, Germany) Fourier transform infrared spectrometer using the KBr disk method with a ratio sample/KBr of 1:100 by mass. A scanning

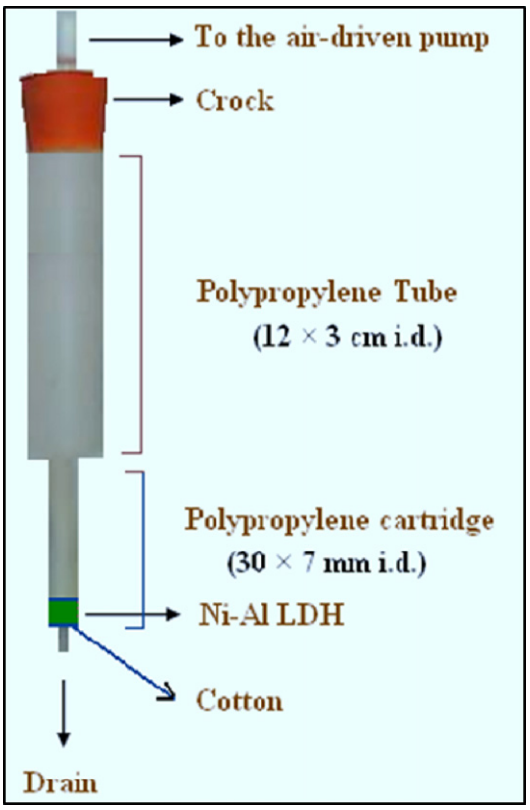

Fig. 1. Schematic diagram of the applied SPE system.

electron microscope (SEM), model P Scan Vega 2 (Czech Republic), was additionally used to examine the morphological characteristics of the sorbent. Ni and Al analysis of the LDH were carried out by flame atomic absorption spectrometry with a Varian SpectrAA 220 (Mulgrave, Victoria, Australia) instrument. A centrifuge (Beckman GS-6, USA) was used to accelerate the phase separation. The pH values were measured with a Metrohm pH-meter (model 827, Switzerland), supplied with a glass-combined electrode. An electronic analytical balance (Mettler Toledo, PB303, Switzerland) was used for weighting the solid materials.

2.2. Standard solutions and reagents

All chemicals used were of analytical-reagent grade and all solutions were prepared with doubly distilled deionized water (Shahid Ghazi Co., Tabriz, Iran). A 1000 mg L^{-1} stock solution of salicylic acid (Fluka) was prepared by dissolving appropriate amount of reagent in deionized water, and stored at 4 °C after preparation. Working standard solutions were obtained daily by suitable stepwise dilution of the stock solutions with deionized water and shaking them just prior to use. All salts used for the interference study, methanol, NaOH, HCl (37%) and LDH precursors, i.e., purified nickel nitrate hexahydrate ($Ni(NO_3)_2{\cdot}6H_2O$, 99%) and aluminum nitrate nonahydrate ($Al(NO_3)_3{\cdot}9H_2O$, 99%) were purchased from Merck (Darmstadt, Germany). pH adjustments were performed with HNO_3 and NaOH (0.01–2.0 mol L^{-1}) solutions. The pipettes and vessels used for the trace analysis were kept in 15% (v/v) nitric acid at least overnight and subsequently washed three times with deionized water prior to use.

2.3. Preparation of nickel–aluminum layered double hydroxide

Ni–Al (NO_3^-) LDH was prepared by co-precipitation method with controlled pH, and followed by hydrothermal treatment as

described elsewhere [22,23]. The synthesis was carried out under a N_2 atmosphere, and all of the solutions were prepared using deionized water to avoid contamination. In the present work, the Ni^{2+}:Al^{3+} molar ratio chosen for the synthesis of the LDH precursors was 2:1 to obtain stable layered compounds. For this purpose, 0.581 g Ni $(NO_3)_2{\cdot}6H_2O$ and 0.375 g Al $(NO_3)_3{\cdot}9H_2O$ were added into 30 mL deionized water under vigorous stirring at room temperature. The reaction mixture pH was adjusted to 9.6 by the addition of 2 mol L^{-1} NaOH solution. Then, the obtained slurry was subjected to hydrothermal treatment at a constant temperature of 100 °C for about 24 h. Afterward, the resulting precipitate was separated by centrifugation at 4000 rpm for 10 min and washed three times with deionized water, and dried at 60 °C for 6 h.

2.4. Column preparation

Ni–Al (NO_3^-) LDH was employed to create the solid-phase extraction column as follows: the column was prepared by introducing 200 mg of Ni–Al (NO_3^-) LDH into an empty 2 mL polypropylene cartridge using the dry packing method. Small portion of cotton was placed on both ends of the column. Before loading the sample, 3 mL of 3 mol L^{-1} NaOH solution was passed through the column to clean it. Then, the column was conditioned by passing only 2 mL of deionized water through the column prior to each use. After loading, the retained sample in the column was stripped with a 2.5 mL of 3 mol L^{-1} NaOH as a desorbing and/or ion-exchanger solution.

2.5. Sample preparation

2.5.1. Preparation of plant samples

A 100 mg portion of freshly weighted willow leaves was transferred into a mortar and complete crushing was carried out in the presence of 3 mL methanol. Afterward, the resulting mixture was transferred into centrifuge tube and a 3 mL portion of methanol was then added. After centrifugation at 5000 rpm for 15 min, a 200 μL portion of the supernatant solution was transferred into 100.0 mL volumetric flask and after dilution with deionized water, the concentration of SA was determined as described in Section 2.6.

2.5.2. Preparation of pharmaceutical samples

Five tablets of aspirin (ASA, 80 mg per tablet) were weighted and crushed to a fine powder in a mortar. An accurately measured amount (10 mg) of powdered drug was transferred into a glass beaker and dissolved in the deionized water. The solution was filtered through a Rund filter paper (blue band, no. 300210), transferring into a 250.0 mL volumetric flask, and then diluted to the mark with deionised water. Alkaline hydrolysis of the solution was employed in order to obtain complete transformation of ASA to SA [24]. For this purpose, a 100 μL portion of the obtained solution was transferred into a 100.0 mL volumetric flask and a 2 mL portion of 3 mol L^{-1} NaOH solution was then added. After dilution with deionized water, the pH was adjusted to 5 by adding diluted HNO_3 solution. Afterward, the volume was made up to the mark with the deionized water and the concentration of SA was determined as described in Section 2.6.

2.5.3. Preparation of biological samples

Human blood sample was obtained from healthy subjects who were not taking any drugs containing salicylates. Whole blood was allowed to coagulate before separating the serum by centrifugation at 4000 rpm for 15 min. Then, a 300 μL portion of serum was directly transferred into a 100.0 mL volumetric flask and after dilution to the mark with the deionized water, the concentration of SA was then determined as described in Section 2.6.

2.6. General procedure

For solid-phase extraction and pre-concentration of salicylate ions, a 100.0 mL portion of aqueous standard or sample solution containing salicylic acid in the range of 0.3 – 45 μg L^{-1} (pH 5) was passed through the Ni–Al (NO_3^-) LDH nano-sorbent in a micro-column at a flow rate of 2.0 mL min^{-1}. The pH of the solution was adjusted using minimum volume of 0.01 mol L^{-1} HNO_3 and/or NaOH. The total volume added for pH adjustment never exceeded 1% of the total volume. The use of buffer was knowingly avoided to restrict the addition of foreign anions, which may adversely affect the SA retention. After loading, the retained analyte on the micro-column was stripped by 2.5 mL of 3 mol L^{-1} NaOH solution at an elution rate of 1.0 mL min^{-1} and salicylate concentration in the eluted solution was subsequently determined spectrofluorometrically at λ_{em} = 400 nm with excitation at λ_{ex} = 270 nm. A blank solution was also run under the same conditions without adding the analyte. The column could be used repeatedly after regeneration with 3 mol L^{-1} NaOH solution and deionized water, respectively.

3. Results and discussion

3.1. Characterization of nano-sorbent (Ni–Al (NO_3^-) LDH)

The powder X-ray diffraction (XRD) is a very powerful technique for characterizing the structure of materials. Fig. 2 shows XRD pattern of Ni–Al (NO_3^-) LDH. It can be observed the characteristic reflections of (0 0 3), (0 0 6), (0 1 2), (0 1 5) and (0 1 8) planes of a crystalline LDH. In the zone close to 2θ = 60–62° the typical doublet of (1 1 0)–(1 1 3) planes of LDH was also observed. It can be seen that Ni–Al (NO_3^-) LDH exhibits the characteristic reflections of hydrotalcite-like LDH and no other crystalline phases were present, which are in agreement with the results reported by other researchers [25–29].

The infrared absorption spectroscopy is another useful tool for the characterization of LDHs, involving the vibrations in the octahedral lattice, the hydroxyl groups and the interlayer anions. The absorption band around 3423 cm^{-1} shown in the FT-IR spectrum of the Ni–Al (NO_3^-) LDH precursor (Fig. 3) can be assigned to the stretching vibration of the hydroxyl groups of LDH layers and interlayer water molecules. The bending mode of water molecules is responsible for the weak band at 1634 cm^{-1}. The band with maximum peak at 1384 cm^{-1} belongs to stretching vibration of NO_3^- ions intercalated in the interlayer gallery. Finally, the bands at 805 cm^{-1}, 563 cm^{-1} and 432 cm^{-1} can be ascribed to M–O stretching modes and M–O–H bending vibrations.

Scanning electron microscopy was employed to explore the morphology of the nano-sorbent. SEM image of Ni–Al (NO_3^-) LDH (Fig. 4) shows an aggregate that consists of crystallites were collected as small pseudo-spherical platelets after hydrothermally treated at 100 °C for about 24 h. The approximate sizes of the particles fall in the 25–200 nm range. Microphotography clearly evidences the nano-structural strains, which leads to non-rigid platelets shape.

The flame atomic absorption spectrometry (FAAS) was used here to assess the atomic composition of the LDH. For this purpose, 50 mg of the LDH was dissolved with a few drops of concentrated HNO_3 and diluted to 50 mL with deionized water. Ni and Al analysis were then performed by FAAS after appropriate dilution with deionized water. According to the obtained results, the Ni^{2+}:Al^{3+} ratio (1.96:1) is in agreement with the expectations considering the proportion of metal salt precursors used in the LDH synthesis (2:1).

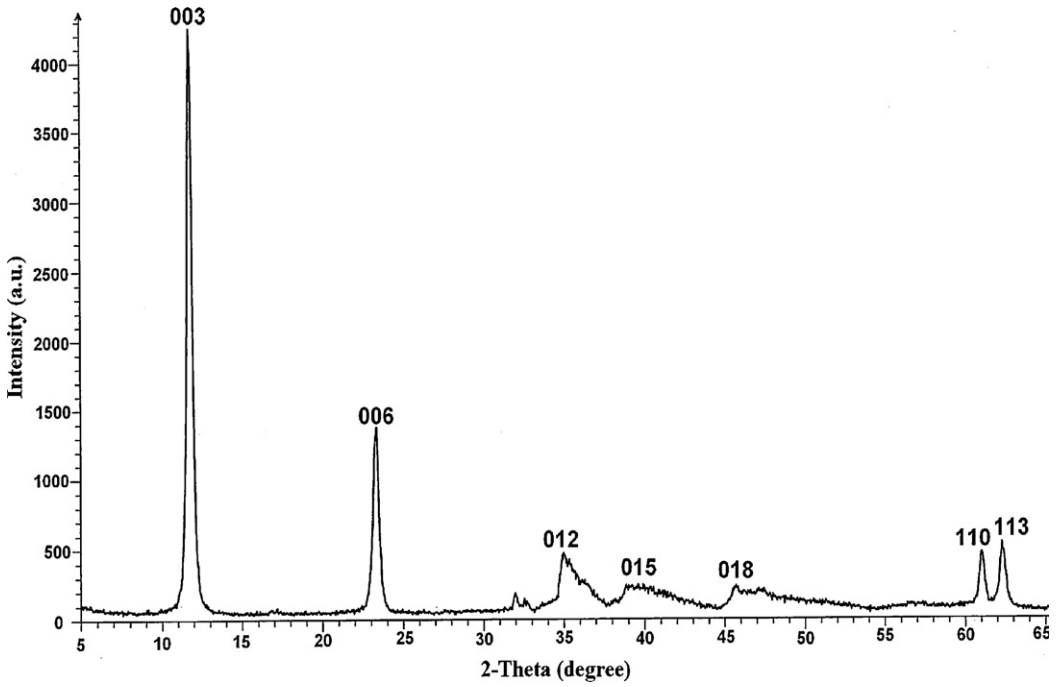

Fig. 2. XRD pattern of Ni–Al (NO_3^-) LDH.

3.2. Optimization of SPE conditions

3.2.1. Effect of pH

The effect of pH on the retention of SA was investigated by applying the proposed extraction and elution procedure to the sample solutions. The pH of each solution was adjusted to values ranging from 2 to 12 with minimum volume of 0.01 mol L^{-1} HNO_3 and/or NaOH. It was found that the recovery increased as the pH was increased from 2 to 4.5, and above pH 4.5, the recovery remained constant (data not shown). The pK_a value of SA in aqueous solution is 2.98 [30] and at pH > 4.5, SA mainly exists as deprotonated form (salicylate ion). Nevertheless, at pH < 4.5, not only the amount of salicylate ion was decreased due to its protonation, but also the amount of LDH was decreased because of high solubility of LDH nano-particles in strong acidic media. An increase in the concentration of the competing OH^- anions at pH above 9.0 might also be responsible for the observed decrease in the recovery at higher pH. Therefore, pH 5 was selected as the working pH. We did not use any buffer solution for pH adjusting, because this may affects the retention of SA due to the competition of the anionic species for the active sites of the nano-sorbent.

3.2.2. Effect of flow rate of sample loading

The influence of the sample loading flow rate on the recovery was investigated between 0.5 and 5.0 mL min^{-1}. Based on the obtained results, the flow rate in the range of 0.5–2.0 mL min^{-1} had no significant effect on the recovery of SA according to the procedure of column experiments. However, higher flow rates led

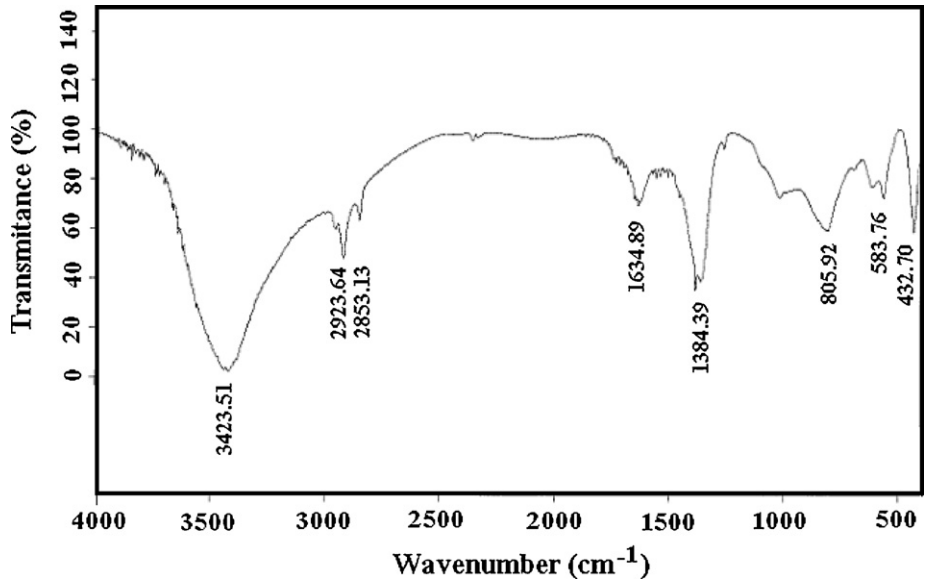

Fig. 3. FT-IR spectrum of Ni–Al (NO_3^-) LDH.

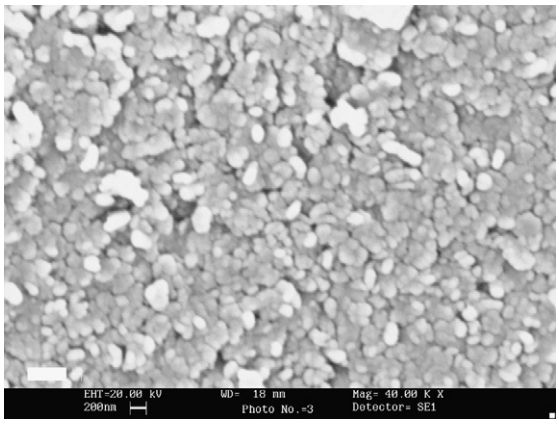

Fig. 4. SEM image of Ni–Al (NO_3^-) LDH.

to a continuous decrease in the recovery values as the interaction (adsorption and/or ion exchanging) time between the salicylate ions and the sorbent was decreased. Thus, the loading flow rate of 2.0 mL min^{-1} was selected for further experiments.

3.2.3. Optimization of elution conditions

The choice of elution reagent and its optimum concentration should be carefully taken into account. For this reason, various stripping reagents such as NaOH, NaCl and Na_2CO_3 were tested to find the best stripping solution for the retained SA. Among these, NaOH solution provided higher recovery. It was found that 3.0 mol L^{-1} NaOH was sufficient for complete elution of the retained salicylate ions from the column.

By keeping the eluent concentration of 3.0 mol L^{-1} NaOH, the effect of elution volume and flow rate on the recovery of salicylate was also investigated. The recovery was found to be quantitative when elution volume and flow rate were chosen between the ranges of 2.0–5.0 mL and 0.2–1.5 mL min^{-1}, respectively (data not shown). Thus, 2.5 mL of 3.0 mol L^{-1} NaOH with elution flow rate of 1.0 mL min^{-1} were selected as the optimum elution values.

3.2.4. Effect of the amount of Ni–Al (NO_3^-) LDH nano-sorbent

The influence of Ni–Al (NO_3^-) LDH amount on recovery was also studied. For this aim, a different amount of sorbents was added in the range of 50 and 300 mg into the micro-column. The test solution of 100 mL sample including 1.0 μg of SA was passed through the column at optimum conditions. The results showed that the amount of optimum sorbent was in the range of 150–300 mg of Ni–Al (NO_3^-) LDH for maximum extraction of SA. From these results, 200 mg of nano-sorbent was used in all further experiments as an optimum.

3.2.5. Sample volume and pre-concentration factor

The effect of sample solution volume on the salicylate adsorption was investigated by passing 10–250 mL sample solutions containing 1.0 μg of salicylate at a flow rate of 2.0 mL min^{-1} according to the recommended procedure. Recovery of salicylate was found to be quantitative when sample volume was chosen between the ranges of 10–100 mL. Above 100 mL, the recovery decreased for the analyte. So, by analyzing 2.5 mL of the final solution after the pre-concentration of 100 mL of sample solution, an enrichment factor was found as 40.

3.2.6. Sorption capacity

The sorption capacity of the sorbent was calculated by the batch technique. For this process, 100 mg of the sorbent was added to 50.0 mL of solution containing 50.0 mg L^{-1} of salicylate ions and stirred for 60 min with magnetic stirrer and filtered through a filter paper. Enriched salicylate ions onto LDH nano-particles were stripped with 5 mL of 3.0 mol L^{-1} NaOH and concentration of SA ions were determined spectrofluorometrically after dilution. As a result, capacity of the Ni–Al (NO_3^-) LDH for SA was found to be 4.8 mg g^{-1}.

Table 1
Tolerance limits of interfering species in the determination of 10 μg L^{-1} of SA.

Coexisting species	Interferent to analyte ratio
Na^+, K^+, Ca^{2+}, Mg^{2+}, NO_3^-	1000:1
CO_3^{2-}, HCO_3^-, I^-, BrO_3^-	500:1
Br^-, SO_4^{2-}, Cl^-, CH_3COO^-	250:1
IO_3^-, aspirin, ascorbic acid, epinephrine, mesalamine	100:1
F^-, citrate	50:1
$H_2PO_4^-$, HPO_4^{2-}	10:1

3.2.7. Reusability of the Ni–Al (NO_3^-) LDH nano-sorbent

In order to investigate the recycling of the Ni–Al (NO_3^-) LDH nano-sorbent, the column packed with 200 mg sorbent were rinsed with 2.5 mL of 3.0 mol L^{-1} NaOH and 2.0 mL deionized water, respectively, before application in the next one. After at least 300 times of recycling, there is no obvious decrease or increase for the recovery of analyte. The results indicate that the Ni–Al (NO_3^-) LDH nano-sorbent is stable as well as no carryover of analyte during SPE procedure, showing good reusability. In fact, high stability of the Ni–Al (NO_3^-) LDH nano-sorbent is one of the advantages of the proposed SPE system.

3.2.8. Study of interferences

For application of recommended solid-phase extraction to real samples, the effects of some coexisting ions and substances, with various pK_a values, on the recovery of SA were investigated. In these experiments, different amounts of interfering ions and compounds were added to the test solutions containing 10 μg L^{-1} of SA and then followed according to general procedure. The tolerance limit was considered if it resulted in a ±5% variation in recovery of SA. As can be seen in Table 1, most of examined cations and anions did not interfere with the extraction and determination. The results also showed that pharmaceuticals such as aspirin, ascorbic acid, epinephrine, mesalamine (4-amino salicylic acid) do not interfere in retention and elution of SA when their concentrations are 100-fold more than SA.

Table 2
Optimum conditions and analytical performance of the proposed method for SA determination.

Experimental conditions	
pH	5
Amount of sorbent (mg)	200
Eluent volume (mL)	2.5
Eluent concentration (mol L^{-1} NaOH)	3
Sample flow rate (mL min^{-1})	2
Maximum sample volume (mL)	100
Excitation wavelength (nm)	270
Emission wavelength (nm)	400
Analytical parameters	
Linear range (μg L^{-1})	0.3–45
Intercept	35.42
Slope	21.53
Detection limit (μg L^{-1})[a]	0.12
Correlation coefficient	0.9985
Relative standard deviation (RSD%) (n = 6)[b]	2.3 (10)
Enrichment factor[c]	40

[a] Calculated as three times the standard deviation of the blank signal.
[b] Value in parentheses is the SA concentration (μg L^{-1}) for which the RSD was obtained.
[c] Enrichment factor calculated as the ratio between the volume of the initial aqueous solution and the final elution volume.

Table 3
Determination of SA in different real samples (results of recoveries of spiked samples analysis).

Samples	Added SA ($\mu g\,mL^{-1}$)	Found SA[a] ($\mu g\,mL^{-1}$)	Recovery (%)
Blood serum[b]	–	6.5 ± 0.4	–
	10.0	16.1 ± 0.3	96.0
	20.0	26.2 ± 0.5	98.5
	Added SA ($mg\,g^{-1}$)	Found SA ($mg\,g^{-1}$)	
Willow leaf[c]	–	6.3 ± 0.2	–
	9.0	15.4 ± 0.3	101.1
	18.0	24.0 ± 0.3	98.3
Aspirin tablet[d]	–	374.4 ± 3.0[e]	–
	90.0	460.9 ± 3.4	96.1
	180.0	553.1 ± 2.9	99.3

[a] Mean of three experiments ± standard deviation.
[b] Obtained from the blood bank of the Shahid Madani Hospital, Azarshahr, Iran.
[c] Collected from local source, Azarshahr, Iran.
[d] Obtained from Pars Daru Co., Iran (each tablet with 0.165 g average weight contains 80 mg ASA).
[e] Equal to 80.7 ± 0.6 mg ASA per tablet.

3.2.9. Analytical performance

Optimized experimental parameters and analytical characteristics of the method were given in Table 2. Under these experimental conditions, analytical features of the proposed method such as, linear range of calibration curve, limit of detection (LOD), accuracy and precision were examined. A linear calibration graph was obtained in the range of between 0.3 and 45 $\mu g\,L^{-1}$ of SA in the initial solution, with a correlation coefficient of 0.9985. The linear regression equation was $F = 21.53\,C_{(SA)} + 35.42$, where F is the fluorescence intensity in arbitrary unit and $C_{(SA)}$ is SA concentration in $\mu g\,L^{-1}$, respectively. The LOD of this method, evaluated as the concentration corresponding to three times the standard deviation of six replicate measurements of blank solution using the pre-concentration method, was found to be 0.12 $\mu g\,L^{-1}$ for the pre-concentration of 100 mL of sample solution. The relative standard deviation (RSD) resulting from the analysis of six replicates of 100 mL solution containing 10 $\mu g\,L^{-1}$ SA was 2.3%. As the amount of SA in the sample solution was measured after a final volume of 2.5 mL, the solution was concentrated by a factor of 40.

3.2.10. Analysis of real samples

To test the reliability of the proposed procedure, the method was employed to determine the trace amounts of SA in different real samples, i.e., blood serum, willow leaf and aspirin tablet. In order to verify the accuracy of the established procedure, recovery experiments were also carried out by spiking the samples with different amounts of SA before any pretreatment. Table 3 shows the obtained results. As can be seen, recoveries between 96.0 and 101.1% were obtained, which confirm the accuracy of the proposed method. There was no available certified reference material (CRM) of SA to test the validity of the proposed method but the results of the method were compared with the results of a standard method [4] (Table 4). Applying the paired t-test, no significant difference at 95% confidence level was observed. It can be concluded that the proposed method is accurate and free from systematic errors.

Table 4
Validation results of SA analysis in the real samples by standard spectrophotometry method compared with the proposed method under the optimum conditions.

Samples	Concentration found[a]	
	Standard method [4]	Proposed method
Blood serum	7.4 ± 0.2 ($\mu g\,mL^{-1}$)	6.5 ± 0.4 ($\mu g\,mL^{-1}$)
Willow leaf	6.1 ± 0.3 ($mg\,g^{-1}$)	6.3 ± 0.2 ($mg\,g^{-1}$)
Aspirin tablet	82.6 ± 0.7 (mg ASA per tablet)	80.7 ± 0.6 (mg ASA per tablet)

[a] Mean of three experiments ± standard deviation.

4. Conclusions

The Ni–Al (NO_3^-) LDH nano-sorbent prepared in this study has shown excellent potential for use as a new SPE sorbent for salicylate extraction from aqueous solution. As far as we know, this is the first time that the Ni–Al (NO_3^-) LDH has been used as packing material for the selective solid-phase extraction of salicylate ions from aqueous solutions. Nanometer-sized Ni–Al (NO_3^-) LDH is a good choice for the separation and pre-concentration of organic ions in aqueous samples due to its low cost when compared to commercially available sorbents. It can be concluded that the coupling of novel Ni–Al (NO_3^-) LDH nano-sorbent for SPE procedure with spectrofluorometric detection exhibited excellent selectivity, repeatability, and ease of operation. The method was successfully applied to determine SA in blood serum, willow leaf and aspirin tablet.

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