NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

New Journal of Chemistry

ARTICLE

Efficient solid phase extraction of codeine from human urine samples using a novel magnetic molecularly imprinted nanoadsorbent and its spectrofluorometric determination

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Tayyebeh Madrakian*, Fariba Fazl, Mazaher Ahmadi and Abbas Afkhami

From a medical or clinical point of view, in order to assess toxicity, adverse effects, interactions and therapeutic efficiency, monitoring drugs levels in body fluids such as urine and plasma has become increasingly necessary. This paper, reports on a novel method for determination of trace concentration levels of codeine phosphate in human urine samples. In this regard, a solid phase extraction step, based on a novel magnetic molecularly imprinted polymer nanoadsorbent, has been developed prior to spectrofluorometric analysis of the drug. After optimization of various factors, such as pH, contact time, adsorbent dosage and desorbing solvent, the results showed that using the proposed method sensitive and accurate determination of the drug in the concentration range of 2.0-500.0 ng mL^{-1} with the detection limit of 0.67 ng mL^{-1} is achievable. The urine samples analyses results showed that a powerful method for codeine phosphate determination has been proposed.

Introduction

Codeine (methyl morphine) is one of the most important constituents of opium and can be prepared by prepared from morphine by methylation. Its phosphate form (COP) is usually used for the treatment of gently or moderate pain in clinic medication. Compared with morphine, the antitussive effect of codeine is approximately one-third of morphine, and its analgesic action is only one-twentieth of morphine's activity.[1] It is stronger than general antipyretic analgesics, and its function duration is similar to morphine. These properties lead to a wide prescription of codeine in cough syrups and tablets. It has moderate analgesic and weak cough suppressant effects. The analgesic effects are observed because only trace amounts of morphine are formed after the administration of codeine.[2] However, long-term use of codeine is addictive and excessive intake even cause death.[3, 4] Therefore, it is very necessary to analysis its content in urine or biological fluids.

Various approaches such as gas chromatography tandem mass spectrometry (GC-MS) [5-7], high performance liquid chromatography (HPLC) with ultraviolet detection [8, 9] and coupled with tandem mass spectrometric detection (HPLC-MS) [10], chemiluminescent [11, 12], capillary electrophoresis [13, 14], thin-layer chromatography (TLC) [15], fluorescence (FL) [16, 17], electrochemistry [1, 18] and chemometrics methods [19] have been reported for COP determination. Most of these methods always need a pretreatment step (such as liquid-liquid extraction and solid phase extraction) for enhancements some analytical parameters such as detection limit (sensitivity) and selectivity.

Todays, solid-phase extraction (SPE) is widely used for the extraction and preconcentration of analytes in various environmental, food and biological samples. It is the most popular clean-up technique due to factors such as convenience, cost, time saving and simplicity, and it is the most accepted sample pretreatment method todays.[20-25] At present, there are several types of sorbents for SPE, including normal-phase, reversed-phase, ionic, and other special sorbents. However, due to their unsatisfactory selectivity, these traditional sorbents usually cannot efficiently separate analytes in complex biological or environmental samples.[26]

A relatively new development in the area of SPE is the use of molecularly imprinted polymers (MIPs) for the sample clean-up and development of selective and sensitive analytical methods.[26-28] MIPs are synthetic polymers possessing specific cavities designed for a target molecule and are synthesized by the polymerization of different components. With the development of molecularly imprinted adsorbents, some disadvantages of MIPs have emerged and greatly restricted its application. It was excited for scientists to find that giving MIP magnetism was an effective approach to overcome the disadvantages of MIPs.[26, 29, 30] When used as a SPE sorbent, magnetic MIP bead can be dispersed into solution directly and separated via an external magnetic field, avoiding the operation of making packed columns as the traditional SPE adsorbents. Accordingly, an interest has been raised in preparation and application of magnetic MIPs in recent years.[26, 31-36] These studies greatly extended the preparation method of magnetic MIPs and made them more efficient as

Faculty of Chemistry, Bu-Ali Sina University, Hamedan, Iran
*Corresponding author. Tel. / Fax: +98-811-8257407
E-mail: madrakian@basu.ac.ir, madrakian@gmail.com (T. Madrakian)

SPE adsorbents. However, structure of the magnetic supporter (core), rather than a single Fe_3O_4 particle as reported in the previous studies, should be better designed to improve the magnetic performance and enhance the stability of MIPs.

At this wok, a highly selective magnetic COP-imprinted polymer nanoadsorbent has been synthesized using magnetite nanospheres (MNSs) as the magnetic core, a synthesized aminoimide monomer (AI) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker, ammonium persulfate as the initiator, and COP as the template. The prepared nanoadsorbent has been fully characterized and its affinity to the COP was experimentally evaluated. After optimization of various affecting factors (such as pH, adsorbent dosage and contact time), the adsorbent was successfully used for extraction of COP from infected human urine samples.

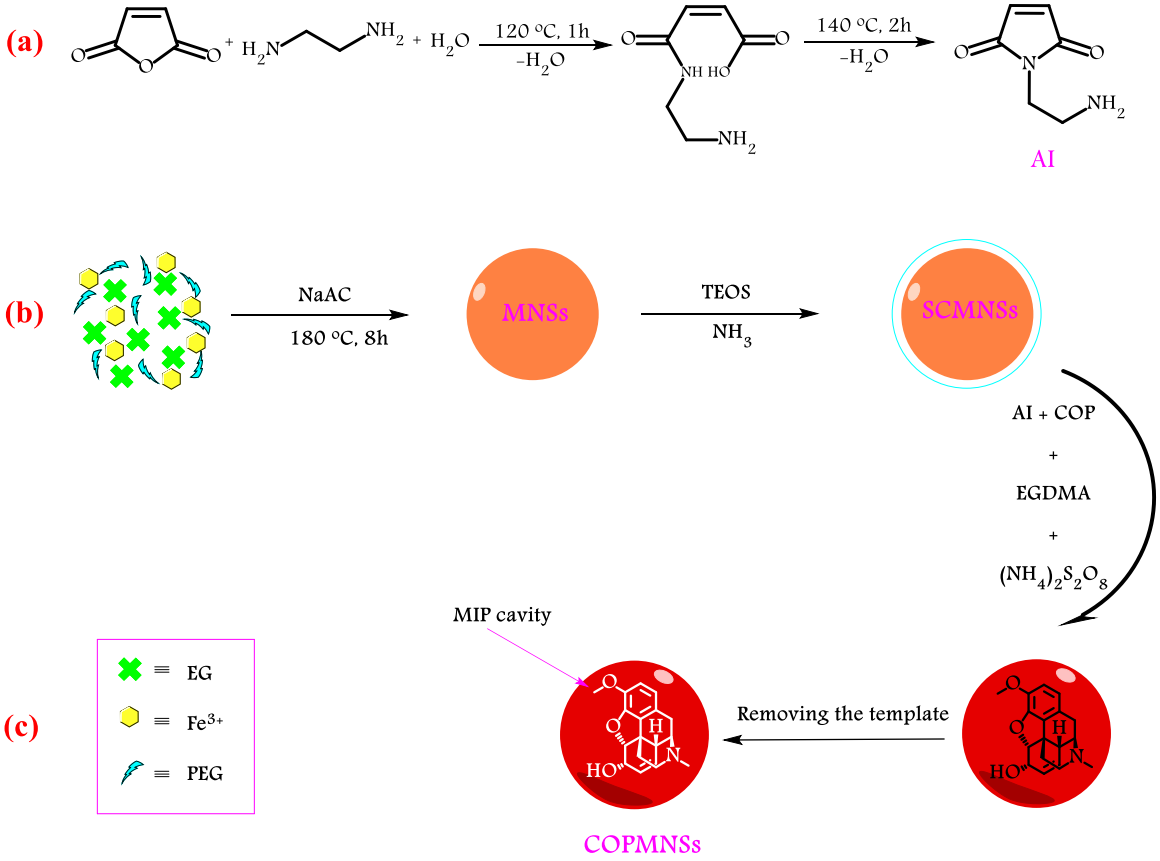
Experimental

Apparatus

Infrared spectra's of the polymeric adsorbent were collected using a FT-IR spectrometer (Perkin-Elmer model Spectrum GX) with the spectral range of 4000-400 cm^{-1}. A transmission electronic microscope (TEM, Philips-CM10) obtained the nanographs of the adsorbent with measurements operating at 100 KV. The crystal structure of the synthesized materials was determined by an X-ray diffractometer (XRD, 38066 Riva, d/G. via M. Misone, 11/D (TN) Italy) at ambient temperature. A Metrohm model 713 pH-meter was used for the pH adjustments. A 40 kHz universal ultrasonic cleaner water bath (RoHS, Korea) was used. A Knauer 1050 HPLC pump and a Knauer 2850 PDA detector with Macherey nagel column C18 (150 mm × 2.5 mm, particle size 4 µm) was used. For the instrumental control, data collection and processing, chromgate software was employed. A Perkin Elmer (LS50B) luminescence spectrometer was used for the determination of COP at λ_{em}= 348 nm (λ_{ex}= 214 nm).

Chemicals

Alborz Bulk Pharmaceutical Company (Kaveh Industrial zone, Saveh, Iran) provided COP. Standard solution of 200 mg L^{-1} COP was prepared with doubly distilled water (DDW) and stored at 4˚C darkly and working standard solutions of different COP concentrations were prepared daily by diluting the stock solution with DDW water. Ethylene glycol (EG) and polyethylene glycol (PEG, fw~8000 g mol^{-1}) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The AI functional monomer was synthesized according to our recently reported procedure. All of the other chemicals were purchased from Merck Company (Merck, Darmstadt, Germany). DDW water was used throughout this work. Britton-Robinson universal pH buffer was used for pH adjustment of the working solutions. It was consists of a mixture of 0.04 M H_3BO_3, 0.04 M H_3PO_4 and 0.04 M CH_3COOH that was titrated to the desired pH with NaOH and/or HCl solutions.



Scheme 1 Reactions involved in the synthesis of (a) the aminoimide monomer, (b) MNSs and COPMNSs adsorbents

New Journal of Chemistry

ARTICLE

Synthesis of the aminoimide monomer functional monomer

The aminoimide monomer used for preparation of the MIP layer was synthesized according to our recently reported procedure with some minor modification (Scheme 1a).[34] Briefly, the aminoimide monomer was synthesized by slow addition of maleic anhydride (1 g) to the solution of ethylenediamine (1 mL) in DDW (20 mL). The solution was heated to 120ºC for 1 h, until all the water was removed and ethylenediamine reacted with maleic anhydride through ring opening. Then, the unsaturated aminoimide monomer was prepared by heating the reaction product to 140ºC for 2 h.

Synthesis of the COP imprinted magnetic nanoadsorbent:

Preparation of silica coated magnetite nanospheres (SCMNSs) (Scheme 1b)

$FeCl_3.6H_2O$ (1.35 g) was dissolved in ethylene glycol (EG, 40 mL) to form a clear solution, followed by the addition of sodium acetate (NaAC, 3.6 g) and polyethylene glycol (PEG, 1.0 g). The mixture was vigorously ultrasonicated for 30.0 min, then refluxed at 180ºC for 8 h, and finally allowed to cool down to room temperature. The black products (MNSs) were washed several times with ethanol and dried at 60°C for 6 h.[37]

MNSs were coated with a silica layer to prevent iron oxide cores from leaching into the mother system under any acidic circumstances. Briefly, 0.10 g of MNSs nanospheres were dispersed in the mixture of ethanol (50 mL), DDW water (10 mL), and concentrated ammonia aqueous solution (3 mL), followed by the addition of tetraethylorthosilicate (TEOS, 2.0 mL). After stirring at room temperature for 1.5 h, the silica coated MNSs nanospheres (SCMNSs) were separated and washed with ethanol and DDW water.[38]

Preparation of COP-imprinted polymer coated SCMNSs (COPMNSs) and non-imprinted polymer coated SCMNSs (NIPMNSs) (Scheme 1c)

In order to prepare the COPMNSs, the amidoamine monomer was polymerized in the presence of SCMNSs (0.5 g), ammonium persulfate (0.1 g, as the initiator), EGDMA (0.2 mL, as the cross-linking monomer) and COP (0.01 g, as the template) in 30 mL DDW at 85ºC for 12 h according to Scheme 1c. The product was separated using a magnet and washed with methanol until no template molecule (COP) spectrofluorometricaly was detected in the washing solution and then washed overnight with methanol to complete remove the template. Finally, the product was dried under vacuum for 12 h. The NIPMNSs were also synthesized by the same procedure, only without the addition of the template.

SPE procedure for COP and its spectrofluorometric determination

To a 25.0 mL sample solution containing COP and 10.0 mL Britton-Robinson buffer solution of pH 6.5, 0.09 g of COPMNSs was added. The solution was shaken at room temperature for 35.0 min. Subsequently, the COP loaded COPMNSs were separated from the mixture with a permanent hand-held magnet within 60 s. The residual amount of the drug in the solution was determined spectrofluorometrically at λ_{em}= 348 nm (λ_{ex}= 214 nm). The adsorption percentage, i.e., the drug removal efficiency (%Re), was calculated using the following equation:

$$\%\mathrm{Re} = \left[\frac{(C_0 - C_t)}{C_0}\right] \times 100 \quad (1)$$

where C_o and C_t represent the initial and final (after adsorption) concentrations of COP in mg L^{-1}, respectively. Also, all the experiments were performed at room temperature.

Preconcentration studies for the determination of trace amounts of COP were performed by adding 110.0 mL of the solution containing 2.0-500.0 ng mL^{-1} COP and 60 mL of Britton-Robinson buffer of pH 6.5 to 0.09 g of COPMNSs; the solution was stirred for 35.0 min. The concentration of COP decreased with time due to adsorption by the adsorbent. The drug loaded nanospheres were separated by magnetic decantation, and desorption was performed with a 2.0 mL of methanol. The concentration of COP in the resulting solution was measured spectrofluorometrically at λ_{em}= 348 nm (λ_{ex}= 214 nm).

Adsorption isotherm studies

An adsorption isotherm describes the fraction of sorbate molecules that are partitioned between liquid and solid phase at equilibrium. Adsorption of COP by COPMNSs and NIPMNSs nanospheres was modeled using Langmuir and Freundlich adsorption isotherms.

Freundlich isotherm

The Freundlich isotherm is applicable to both monolayers (chemisorption) and multilayer adsorption (physisorption) and is based on the assumption that the adsorbate adsorbs onto the heterogeneous surface of an adsorbent.[39] The linear form of Freundlich equation is expressed as:

$$\ln q_e = \ln k_f + \frac{1}{n}\ln C_e \quad (2)$$

Where K_F and n are Freundlich isotherm constants related to adsorption capacity and adsorption intensity, respectively and C_e is the equilibrium concentration (mg L^{-1}).

Langmuir isotherm

The Langmuir isotherm assumes monolayer adsorption on a uniform surface with a finite number of adsorption sites [40]

Once a site is filled, no further sorption can take place at that site. As such, the surface will eventually reach a saturation point where the maximum adsorption of the surface will be achieved. The linear form of Langmuir equation may be written as:

$$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{1}{q_m} C_e \quad (3)$$

Where q_m is the maximum adsorption capacity of the substrate (mg of analyte/ g adsorbent) and K_L is a constant representing the strength with which the drug is bound to the adsorbent ($dm^3\ g^{-1}$).

Urine samples pretreatment

Post-dose urine samples were collected from healthy donors who received one 60 mg of codeine phosphate dose orally. All urine samples were stored at -20ºC. Then, 110 mL of each sample was adjusted to pH 6.5 using 0.01-0.1 mol L^{-1} HCl and/or NaOH and directly subjected to the SPE procedure. All experiments were performed in compliance with the relevant laws and institutional guidelines, and Behbood institute (Hamedan, Iran) has approved the experiments. Furthermore, in order to the method accuracy's evaluation, the urine samples were spiked with COP at ng mL^{-1} concentration levels.

Results and Discussion

In order to synthesize COPMNSs, MNSs were first synthesized by a solvothermal reduction method. Then, the synthesized nanospheres were coated with a layer of SiO_2 using TEOS silanization agent. Finally, the surface of the synthesized SCMNSs was imprinted with COP using the amidoamine monomer as the functional monomer, EGDMA as the cross-linker, COP as the template and ammonium persulfate as the polymerization reaction initiator.

Characterization of the synthesized adsorbents

The FT-IR spectra of the products were recorded to verify the formation of the expected products. The related spectra are shown in Fig. 1. The characteristic absorption band of Fe-O in magnetite (around 598 cm^{-1}) was observed in Fig. 1a. A strong peak at 1087 cm^{-1} in Fig. 1b is attributed to Si-O in SiO_2. The new absorption peaks at almost 1452 and 1725 cm^{-1} in Fig. 1c are assigned to C-N and C=O bands in the polymer-coated final product (COPMNSs), respectively. Moreover, new absorption peaks at 2956 and 3000 cm^{-1} are related to the stretching modes of the aliphatic C-H group [26]. Based on the above results, it can be concluded that the fabrication procedure has been successfully performed.

The XRD pattern of the COPMNSs in Fig. 2 shows diffraction peaks that are indexed to (3 1 1), (4 0 0), (5 1 1) and (4 4 0) reflection characteristics of the cubic spinel phase of magnetite (JCPDS powder diffraction data file no. 79-0418), revealing that the resultant nanospheres are mostly magnetite. The average crystallite size of the COPMNSs nanospheres was estimated to be 16 nm from the XRD data according to Scherer equation [23].

The TEM image of the COPMNSs in Fig. 3b, in comparison with SCMNSs image (Fig. 3a), indicates that the MNSs nanospheres are enwrapped rather homogeneously in SiO_2 shell, and further by the MIP layer. The average size of the synthesized nanospheres was estimated to be about 95 nm.

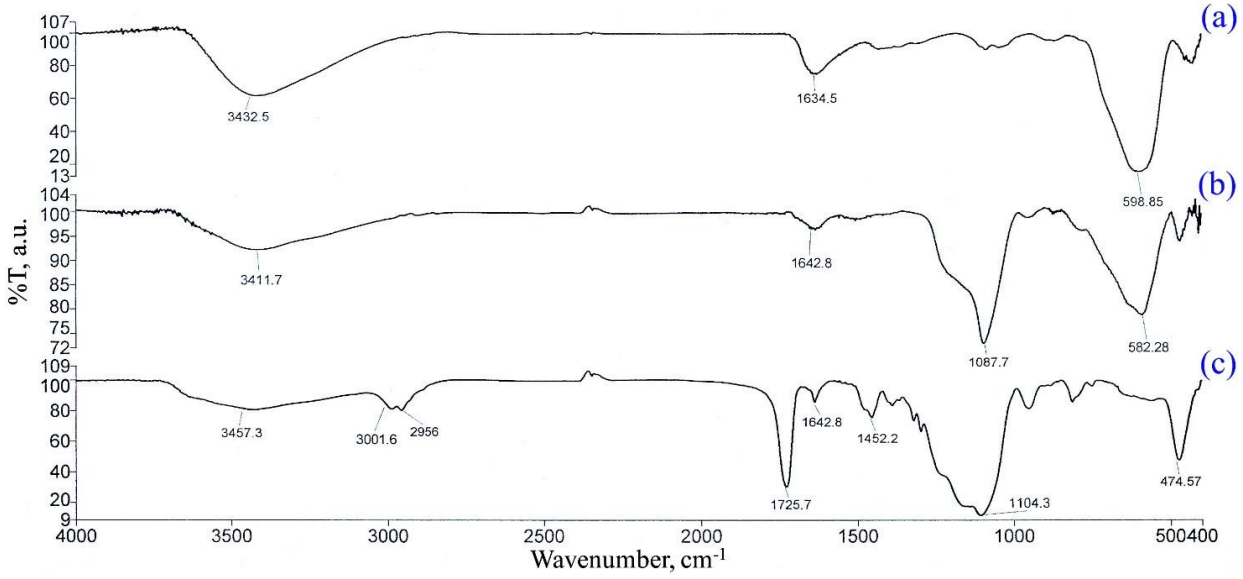


Fig. 1 FT-IR spectra of (a) MNSs, (b) SCMNSs and (c) COPMNSs

New Journal of Chemistry

ARTICLE

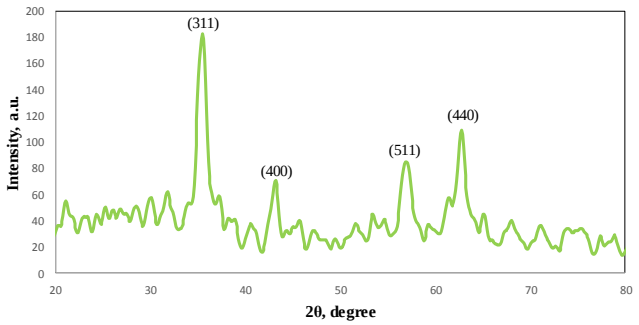


Fig. 2 XRD pattern for the COPMNSs nanospheres

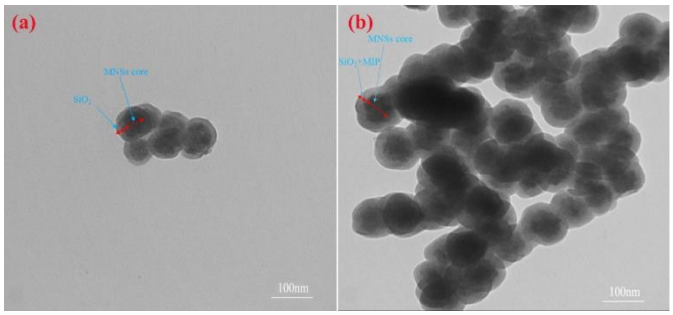


Fig. 3 TEM image of (a) SCMNSs and (b) COPMNSs nanospheres

Point of zero charge (pH_{PZC}) of the COPMNSs adsorbent

The pH_{PZC} of the COPMNSs was determined in degassed 0.01 mol L^{-1} $NaNO_3$ solution at room temperature. Aliquots of 20.0 mL 0.01 mol L^{-1} $NaNO_3$ were mixed with 0.03 g of the nanoparticles in several beakers. The initial pH of the solutions was adjusted at 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 using 0.01-0.1 mol L^{-1} of HNO_3 and/or NaOH solutions as appropriate. The initial pHs of the solutions were recorded, and the beakers were covered with parafilm and shaken for 24 h. The final pH values were recorded and the differences between the initial and final pH (ΔpH) of the solutions were plotted against their initial pH values. The pH_{PZC} corresponds to the pH where ΔpH=0.[26] The pH_{PZC} for the COPMNSs was determined using the above procedure and was obtained as 6.4. The results are shown at Fig. 4.

Effect of various factors affecting the COP removal efficiency

Various factors that could potentially affect the analyte removal efficiency (i.e. pH, contact time and nanospheres dosage) were optimized and here are the detailed results:

Effect of pH

Solution pH affects the adsorption process of the drug molecules by affecting both the aqueous chemistry and surface binding-sites of the adsorbent. The effect of pH on the COP removal efficiency was investigated in the range 3.0-10.0 using an initial COP concentration of 2.0 mg L^{-1} and a stirring time of 45 min, where the pH was adjusted using Britton-Robinson buffer. As elucidated in Fig. 5, the maximum value of COP removal appeared in pH=6.5 and at higher or lower pHs a significant decrease in COP removal efficiency can be seen. Due to the pK_a value of COP ($pK_{a(COP)}$= 8.32 [41]), At pH>8.32, the predominant form of COP is its neutral form and at the other range is its protonated form and at the other hand, the adsorbent surface charge at pH>6.4 is negative and at pH<6.4 is positive. So, electrostatic interactions aren't responsible for the related pH effect (Fig. 5). It can be suggested that hydrophobic interaction and hydrogen bonds between COP and the MIP cavities (mainly the amine functional group of AI) is responsible for the high observed removal efficiencies at pH of 6.5. For further optimizations the pH=6.5 was chosen.

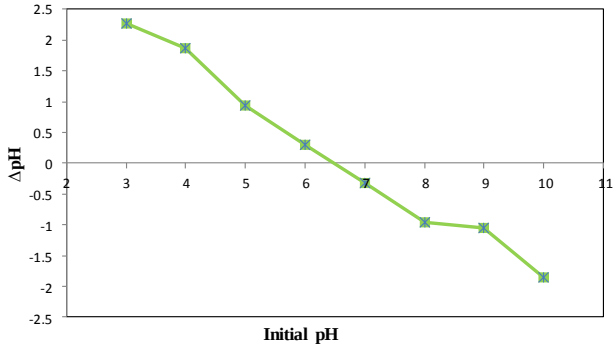


Fig. 4 Point of zero charge of the COPMNSs adsorbent

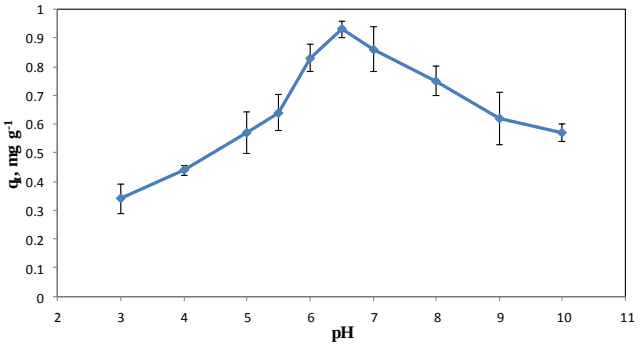


Fig. 5 Amount of COP uptake per gram of adsorbent at different pH values (Conditions: 0.04 g of COPMNSs, 25 mL of 2.0 mg L^{-1} of COP, agitation time of 45 min, N=3)

Effect of nanospheres dosage

The dependency of the adsorption of COP on the amount of modified nanospheres was studied at room temperature and at pH 6.5 by varying the adsorbent amount from 0.02 to 0.12 g in contact with 25.0 mL solution of 2.0 mg L^{-1} of COP with agitation time of 45 min. The results showed that the removal efficiency of COP increased by increasing amount of the COPMNSs due to the availability of higher adsorption sites. The adsorption reached a maximum with 0.09 g of adsorbent and maximum percentage removal was about 97%.

Effect of contact time

The effect of contact time on the adsorption of COP was studied to determine the time needed to complete remove of COP by COPMNSs from a 2.0 mg L^{-1} solution of the analyte at pH 6.5. A 0.09 g of the adsorbent was added into 25.0 mL of the COP solution. Fluorescence intensity of COP was monitored versus time to determine variation of the analyte concentration. It was observed that after a contact time of about 35 min, almost all the COP was adsorbed (%Re>98) and this time was much enough to reach semi-equilibrium condition.

Effect of various factors affecting the COP preconcentration efficiency

The aim of this step is providing the highest preconcentration factor and concentration of the adsorbed analyte into minimum possible volume of desorbing solvent. In this regard, various factor that can potentially affect the COP'S desorbing efficiency (i.e. type and volume of desorbing solvent, desorbing time and initial sample volume) have been optimized. The detailed results are given in bellow:

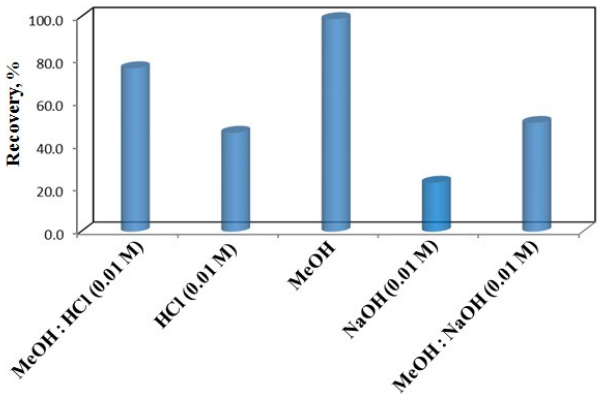


Fig.6 Effect of various eluents on COP recovery

Desorbing solvent

For desorption studies, COP loaded COPMNSs were first washed by DDW to remove the unadsorbed COP that loosely attached to the vial and adsorbent. In order to estimate the recovery of COP from COPMNSs, desorption experiments with different reagents (methanol (MeOH), 0.01 mol L^{-1} NaOH, 0.01 mol L^{-1} HCl, mixture of methanol: 0.01 mol L^{-1} HCl (1:1 v/v) and mixture of methanol: 0.01 mol L^{-1} NaOH (1:1 v/v)) were performed. After adsorption of COP, the adsorbent was magnetically separated and washed with DDW. Then 2.0 mL of the eluent was added to the COP loaded COPMNSs. Samples were collected after 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 and 45.0 min contact times to evaluate the COP recovery. The results (Fig. 6) showed that methanol is the most effective as a back-extracting solvent and can be used for the quantitative recovery of the analyte. Desorption rate was found to be rapid as almost 98% desorption completed at almost 20.0 min.

Initial sample volume

The effect of initial sample volume on the analyte adsorption was studied in the range 10.0-200.0 mL; 10.0 mL samples containing 2.0 mg L^{-1} of COP were diluted to 25.0, 50.0, 100.0, 110.0, 150.0 and 200.0 mL with DDW water. Then adsorption and desorption processes were performed under the optimum conditions (pH 6.5; contact time, 35.0 min; COPMNSs dosage, 0.09 g) as described in the experimental section. The results (results was not shown) showed that the analyte contents in the volumes up to 110.0 mL were completely and quantitatively adsorbed by the nanospheres, but there was a decrease in the amount adsorbed at higher volumes. Therefore, for the determination of trace quantities of the analyte, a sample volume of 110.0 mL was selected in order to having highest preconcentration factor.

Adsorption isotherms

For measuring the adsorption capacity of COPMNSs and NIPMNSs, the absorbents was added into COP solutions at various concentrations (under optimum condition), and the suspensions were stirred at room temperature, followed by magnetic removal of the absorbent. Adsorption of the COP by COPMNSs and NIPMNSs adsorbents were modelled using Freundlich and Langmuir adsorption isotherm models. The remained analyte in the supernatants was measured spectrofluorometrically, and the results were used to plot the isothermal adsorption curves as shown in Fig. 7. The equilibrium adsorption data were fitted to Langmuir and Freundlich isotherm models by linear regression. The resulting parameters are summarized in Table 1.

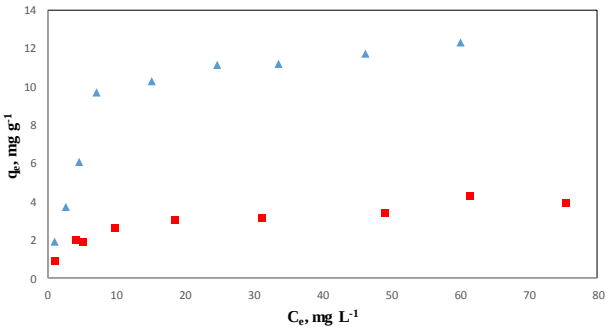


Fig. 7 Isothermal adsorption curves of COP on (▲) COPMNSs and (■) NIPMNSs adsorbents

The higher correlation coefficient obtained for the Langmuir model (> 0.99) indicates that the experimental data are better fitted into this model, and the adsorption of COP onto COPMNSs adsorbents is more compatible with Langmuir assumptions, i.e., adsorption takes place at specific homogeneous sites within the adsorbent. According to the results (Table 1), the maximum amount of COP that can be adsorbed by COPMNSs and NIPMNSs was found to be 13.44

and 4.06 mg g^{-1} at pH 6.5, respectively. The relatively high adsorption capacity of COPMNSs in comparison with NIPMNSs, shows that the adsorption of COP molecules takes place at a large number of specific homogeneous sites within the adsorbent (specific cavities of the MIP layer), beside of non-specific interactions which are approximately identical for both the COPMNSs and the NIPMNSs adsorbents.

Reusability and stability of the adsorbent

The reusability and stability of COPMNSs for the extraction of COP was assessed by performing thirteen consecutive separations/desorption cycles under the optimized conditions (Conditions: 0.09 g of COPMNSs, 110.0 mL of 0.2 mg L^{-1} of COP, agitation time of 35 min). Desorption of COP from the adsorbent was performed with methanol as described above. There was no significant change in the performance of the adsorbent during these thirteen cycles, indicating that the fabricated COPMNSs is a reusable and stable solid phase sorbent for the extraction of COP.

Analytical applications

The calibration graph was constructed from spectrofluorometric measurements of the desorbed COP after performing its adsorption/separation under the optimum conditions as described above. The calibration graph was linear in the range 2.0-500.0 ng mL^{-1} for a sample volume of 110.0 mL. The calibration equation is $I_F = 0.714C + 0.791$ with a determination coefficient of 0.995 (n = 10), where I_F is the fluorescence intensity of the eluate at λ_{em}= 348 nm (λ_{ex}= 214 nm) and C is the concentration of the analyte in ng mL^{-1}.

Table 1 Adsorption isotherm parameters of Langmuir and Freundlich models for the adsorption of the analyte onto COPMNSs and NIPMNSs adsorbents

Isotherm models	Langmuir			Freundlich		
	K_L (L mg^{-1})	q_{max} (mg g^{-1})	R^2	K_f ($mg^{1-1/n}$ $L^{1/n}$ g^{-1})	1/n (L g^{-1})	R^2
COPMNSs	2.55	13.44	0.997	1.01	0.42	0.859
NIPMNSs	0.75	4.06	0.992	0.08	0.32	0.952

Table 2 Assay of COP in infected human urine samples

Experiment No.	Spiked value (ng mL^{-1})	Found (ng mL^{-1})	Recovery percent	HPLC method
1	-	12.01±0.04*	-	11.71±0.07
2	10.00	21.80±0.03	98.1	23.41±0.06
3	20.00	31.43±0.01	97.3	32.04±0.02

*N=3

The limit of detection, defined as LOD =3S_b /m, where LOD, S_b and m are the limit of detection, standard deviation of the blank and the slope of the calibration graph, respectively, was found to be 0.67 ng mL^{-1} of COP . As the analyte in 110.0 mL of the sample solution was concentrated into 2.0 mL, a maximum preconcentration factor of 55.0 was achieved in this method. The relative standard deviations (RSD) for 100.0 and 10.0 ng mL^{-1} of the analyte were 0.91% and 1.73% (n = 5), respectively.

The analytical applicability of the proposed method was evaluated by determining the analyte in human urine samples. The results are given in Table 2. The results of the spiked samples show good recoveries of the proposed method and suggest that the method is a good accurate candidate for COP determination in the investigated samples. Furthermore, the results were compared using HPLC method[42], and are tabulated in Table 2. The results show that the two methods are in good agreement and the proposed method can be used for effective determination of COP in the urine samples.

Table 3 shows a comparison between the results obtained by the present method with those obtained by some other methods reported for the determination of COP. This comparison shows that the proposed method analytical performance is comparable with some sensitive instrumental method, such as HPLC, and in some cases, the method is superior on the analytical figure of merit bases. Other advantages of the proposed method are simplicity, less expensive, low LOD, wide linear range, easy adsorbent separation and high removal capacity of the adsorbent.

Table 3 Comparison of the proposed method with some previously reported methods for COP determination

Method	LOD (ng mL^{-1})	Sample	Ref.
HS-SPME [a]	1.0	Human hair	[43]
RPHPLC [b]	3.0	Human blood plasma	[42]
LC–MS	10.0	Human hair	[44]
LLME–HPLC [c]	0.6	Human urine	[9]
LC-MS/MS [d]	1.0	Human urine	[45]
MIP/SPE/FL	0.67	Human urine	This work

a Headspace Solid-Phase Microextraction
b Reverse-Phase High-Performance Liquid Chromatography
c Liquid-Liquid Microextraction followed by High-Performance Liquid Chromatography
d Liquid Chromatography tandem Mass Spectrometry

Conclusions

The novel molecularly imprinted nanoadsorbent, used for recognizing of codeine, was prepared using magnetite nanospheres as the magnetic core, a synthesized aminoimide monomer as the functional monomer, ethylene glycol dimethacrylate as the cross-linker, ammonium persulfate as the initiator and codeine as the template. The synthesized core-shell adsorbent has been fully characterized using XRD,

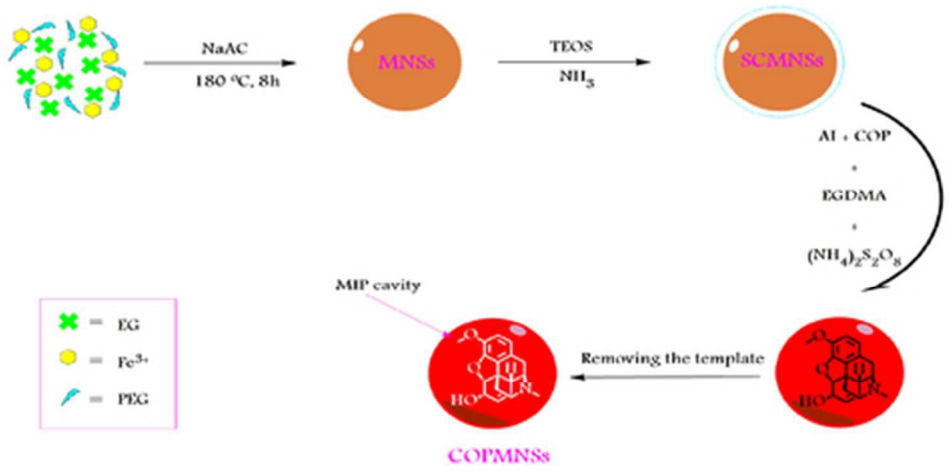
FTIR and TEM measurements. The recognition properties and applications based on the solid-phase extraction of the synthesized nanoadsorbent were evaluated. The resulting nanoadsorbent showed a relatively large adsorption capacity and fast binding kinetics for recognizing the drug, and the method showed high recovery, good accuracy and repeatability in determining of the drug in human urine samples.

Acknowledgements

This work was financially supported by the Bu-Ali Sina University Research Council and the Center of Excellence in Development of Environmentally Friendly Methods for Chemical Synthesis (CEDEFMCS), and the authors acknowledge the support provided for this work.

References

1. Y. Li, K. Li, G. Song, J. Liu, K. Zhang and B. Ye, *Sens. Actuat. B*, 2013, **182**, 401.
2. D. T. Gimenes, R. R. Cunha, M. M. A. d. C. Ribeiro, P. F. Pereira, R. A. A. Muñoz and E. M. Richter, *Talanta*, 2013, **116**, 1026.
3. B. A. Sproule, U. E. Busto, G. Somer, M. K. Romach and E. M. Sellers, *J. Clin. Psychopharmacol.*, 1999, **19**, 367.
4. M. Hakkinen, T. Launiainen, E. Vuori and I. Ojanpera, *Forensic Sci. Int.*, 2012, **222**, 327.
5. M. Krogh, A. S. Christophersen and K. E. Rasmussen, *J. Chromatogr.*, 1993, **621**, 41.
6. K. A. Rees, P. A. McLaughlin and M. D. Osselton, *J. Anal. Toxicol.*, 2012, **36**, 1.
7. U. Hofmann, S. Seefried, E. Schweizer, T. Ebner, G. Mikus and M. Eichelbaum, *J. Chromatogr. B,* 1999, **727**, 81.
8. H. He, S. D. Shay, Y. Caraco, M. Wood and A. J. Wood, *J. Chromatogr. B,* 1998, **708**, 185.
9. M. Shamsipur and N. Fattahi, *J. Chromatogr. B,* 2011, **879**, 2978.
10. Z. Hu, Q. Zou, J. Tian, L. Sun and Z. Zhang, *J. Chromatogr. B,* 2011, **879**, 3937.
11. G. M. Greenway, L. J. Nelstrop and S. N. Port, *Anal. Chim. Acta*, 2000, **405**, 43.
12. B. Qiu, X. Chen, H. L. Chen and G. N. Chen, *Luminescence*, 2007, **22**, 189.
13. M. E. Capella-Peiro, D. Bose, M. F. Rubert and J. Esteve-Romero, *J. Chromatogr. B,* 2006, **839**, 95.
14. T. Zhou, H. Yu, Q. Hu and Y. Fang, *J. Pharm. Biomed. Anal.*, 2002, **30**, 13.
15. D. S. Popa, R. Oprean, E. Curea and N. Preda, *J. Pharm. Biomed. Anal.*, 1998, **18**, 645.
16. B. Weingarten, H. Y. Wang and D. M. Roberts, *J. Chromatogr. A,* 1995, **696**, 83.
17. S. Gandhi, P. Sharma, N. Capalash, R. S. Verma and C. R. Suri, *Anal. Bioanal. Chem.*, 2008, **392**, 215.
18. A. Afkhami, H. Khoshsafar, H. Bagheri and T. Madrakian, *Sens. Actuat. B*, 2014, **203**, 909.
19. A. Afkhami, M. Abbasi-Tarighat, M. Bahram and H. Abdollahi, *Anal. Chim. Acta*, 2008, **613**, 144.
20. T. Madrakian, A. Afkhami, M. A. Zolfigol, M. Ahmadi and N. Koukabi, *Nano-Micro Lett.*, 2012, **4**, 57.
21. T. Madrakian, A. Afkhami, M. Rahimi, M. Ahmadi and M. Soleimani, *Talanta*, 2013, **115**, 468.
22. T. Madrakian, A. Afkhami, H. Mahmood-Kashani and M. Ahmadi, *J. Iran. Chem. Soc.*, 2013, **10**, 481.
23. T. Madrakian, A. Afkhami and M. Ahmadi, *Chemosphere*, 2013, **90**, 542.
24. A. Afkhami, R. Moosavi and T. Madrakian, *Talanta*, 2010, **82**, 785.
25. A. Afkhami, H. Bagheri and T. Madrakian, *Desalination*, 2011, **281**, 151.
26. T. Madrakian, A. Afkhami, H. Mahmood-Kashani and M. Ahmadi, *Talanta*, 2013, **105**, 255.
27. B. Sellergren, *Molecularly Imprinted Polymers: Man-Made Mimics of Antibodies and their Application in Analytical Chemistry*, Elsevier Science, 2000.
28. T. Madrakian, M. Ahmadi, A. Afkhami and M. Soleimani, *Analyst*, 2013, **138**, 4542.
29. Z. Lin, W. Cheng, Y. Li, Z. Liu, X. Chen and C. Huang, *Anal. Chim. Acta*, 2012, **720**, 71.
30. X. Mao, H. Sun, X. He, L. Chen and Y. Zhang, *Anal. Methods*, 2015, **7**, 4708.
31. Y. Ji, J. Yin, Z. Xu, C. Zhao, H. Huang, H. Zhang and C. Wang, *Anal. Bioanal. Chem.*, 2009, **395**, 1125.
32. W. Rao, R. Cai, Y. Yin, F. Long and Z. Zhang, *Talanta*, 2014, **128**, 170.
33. H. He, D. Xiao, J. He, H. Li, H. He, H. Dai and J. Peng, *Analyst*, 2014, **139**, 2459.
34. T. Madrakian, A. Afkhami, B. Zadpour and M. Ahmadi, *J. Ind. Eng. Chem.*, 2015, **21**, 1160.
35. X. Wang, L. Wang, X. He, Y. Zhang and L. Chen, *Talanta*, 2009, **78**, 327.
36. X. Kan, Q. Zhao, D. Shao, Z. Geng, Z. Wang and J.-J. Zhu, *J. Phys. Chem. B*, 2010, **114**, 3999.
37. M. Ahmadi, T. Madrakian and A. Afkhami, *Anal. Chim. Acta*, 2014, **852**, 250.
38. M. Ahmadi, T. Madrakian and A. Afkhami, *New J. Chem.,* 2015, **39** , 163.
39. H. Freundlich and W. Heller, *J. Am. Chem. Soc.*, 1939, **61**, 2228.
40. I. Langmuir, *J. Am. Chem. Soc.*, 1916, **38**, 2221.
41. E. Fuguet, M. Reta, C. Gibert, M. Rosés, E. Bosch and C. Ràfols, *Electrophoresis*, 2008, **29**, 2841.
42. V. Nitsche and H. Mascher, *J. Pharm. Sci.*, 1984, **73**, 1556.
43. F. Sporkert and F. Pragst, *Forensic Sci. Int.*, 2000, **107**, 129.
44. D. K. Huang, C. Liu, M. K. Huang and C. S. Chien, *Rapid Commun. Mass Spectrom.*, 2009, **23**, 957.
45. T. Nema, E. C. Chan and P. C. Ho, *J. Mass Spectrom.*, 2011, **46**, 891.



39x19mm (300 x 300 DPI)