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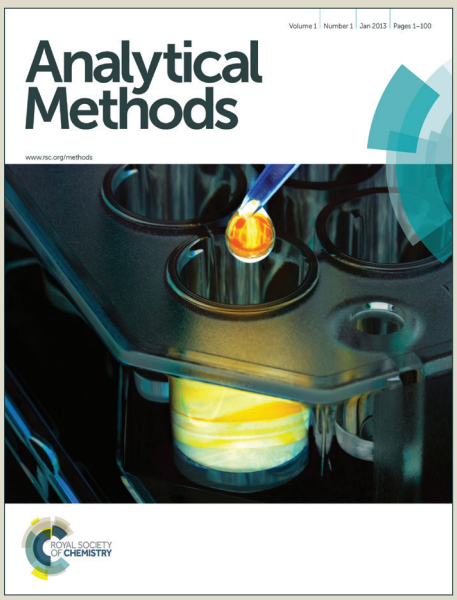

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ARTICLE

Spectrofluorometric determination of venlafaxine in biological samples after selective extraction on the superparamagnetic surface molecularly imprinted nanoparticles

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Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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This paper reports on a procedure for selective extraction of venlafaxine (VEN) drug from biological samples before its sensitive spectrofluorometric determination. In this regard, a layer of VEN-imprinted polymer was coated onto the surface of magnetite nanoparticles. The synthesized nanoparticles were characterized using FT-IR, VSM, XRD and TEM measurements. Various factors that could potentially affect the extraction efficiency of VEN (i.e. solution pH, nanoparticle dosage and contact time) have been optimized using one-at-a-time method. The prepared magnetic adsorbent could well dispersed in water medium and could be easily separated magnetically from the medium after loading with the adsorbate. The loaded VEN could be easily desorbed with a mixture of methanol and HCl and determined spectrofluorometrically at 598 nm. The results showed that using the proposed method, VEN can be determined spectrofluorometrically in the linear concentration range of 2.0-400.0 ng mL^{-1} with a limit of detection as low as 1.2 ng mL^{-1}. The results of human urine and blood serum analysis showed that the method is a good candidate for biological samples analysis purposes.

Introduction

Venlafaxine (VEN) is an antidepressant drug, which selectively inhibits reuptake of norepinephrine and serotonin, and slightly inhibits reuptake of dopamine, without significant affinity for muscarinic, histaminergic or adrenergic receptors.[1] VEN was introduced into treatment in 1990s. It was prepared as a racemic mixture, but the two enantiomeric forms have different impact on reuptake of neurotransmitters in the synaptic slit.[2, 3] VEN is mostly used in the treatment of major depressive disorder and against its recurrences. It is also administered in the therapy of fears, social phobias, sudden fears and agoraphobia [2]. However, an overdose of VEN results in the most common symptoms such as depression, serotonin toxicity, seizure, or cardiac conduction abnormalities [4].

From a medical or clinical point of view, monitoring drug levels in body fluids such as urine and plasma has become increasingly demanded in order to assess toxicity, adverse effects, interactions and therapeutic efficiency. Therefore, the development of sensitive and reliable analytical methods for the determination of VEN is a basic requirement for the study of this analyte in different types of samples with complex matrices. Literature reveals that, chromatography has been the dominant technique to detect trace levels of VEN.[5-7] However, these methods face the drawbacks of being expensive, laborious and require pre-treatment of the samples.

Sample preparation is crucial for obtaining meaningful results from the analysis of real samples, since it is the most tedious and time-consuming step and a possible source of imprecision and inaccuracy of the overall analysis. 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 pre-treatment method today.[8-13] At present, there are several types of sorbents for SPE, including normal-phase, reversed-phase, ionic, and other special sorbents [14]. However, due to their unsatisfactory selectivity, these traditional sorbents usually cannot separate analytes efficiently in complex biological or environmental samples.

A relatively new development in the area of SPE is the usage of molecularly imprinted polymers (MIPs) for the sample clean up and the development of selective and sensitive analytical methods.[14-19] MIPs are synthetic polymers possessing specific cavities designed for a target molecule and are synthesized by the polymerization of different components. In the most common preparation process, monomers form a

complex with the desired template through covalent or non-covalent interactions and then joined by using a cross-linking agent. After removing the template by chemical reaction or extraction, binding sites are exposed which are complementary to the template in size, shape, and position of the functional groups, and consequently allow its selective uptake.[20] The use of MIPs for SPE involves conventional SPE where the MIP is packed into columns or cartridges [21, 22] and batch mode SPE in which the MIP is incubated with the sample.[23] A major advantage of MIP-based SPE, related to the high selectivity of the sorbent, is achievement of an efficient sample clean up.

This work reports on the synthesis of a VEN-imprinted polymer coated magnetic nanoadsorbent for selective solid phase extraction of VEN from human blood serum and urine samples. The concentration of VEN in the extract was determined spectrofluorometrically. This procedure is simple, rapid, selective and sensitive.

Experimental

Reagents and materials

Venlafaxine hydrochloride, (1-[2-(dimethylamino)-1-(4-methoxy-phenyl) ethyl] cyclohexanol hydrochloride, Fig. 1) was purchased from Sigma-Aldrich Company. High purity reagents from Merck, Darmstadt were used. Double Distilled water (DDW) was used in all experiments. The VEN stock solution was prepared in DDW and working standard solutions of different VEN concentrations were prepared daily by diluting the stock solution with DDW. In the present study, Britton-Robinson universal buffer was used for pH adjusting of the working solutions.

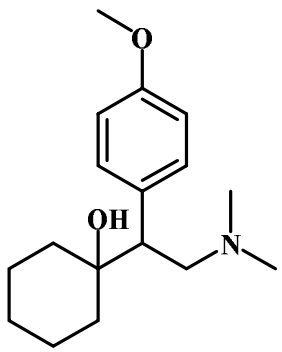

Fig. 1 The chemical structure of venlafaxine

Apparatus

The magnetic properties of the synthesized nanoparticles were measured with a vibrating sample magnetometer (VSM, 4 in. Daghigh Meghnatis Kashan Co., Kashan, Iran). The size, morphology and structure of the nanoparticles were characterized by transmission electronic microscopy (TEM, Philips, CM120, 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. Metrohm 713 pH-meter equipped with a combined glass calomel electrode was used for the pH adjustments at 25±1 °C temperature.

VEN concentration was determined by a Perkin-Elmer luminescence spectrometer model LS-50B. A Perkin-Elmer model Spectrum GX FT-IR spectrometer using KBr pellets recorded the mid-infrared spectra of the investigated nanoparticles in the region 4000-400 cm^{-1}. A 40 ± 5% kHz (power: 100 W) ultrasonic water bath (DSA100-SK2, Korea) was used in this work.

Preparation of silica coated magnetite nanoparticles (SCMNPs)

The magnetite nanoparticles (MNPs) were prepared by the conventional co-precipitation method, with minor modifications.[8] An 11.68 g of $FeCl_3.6H_2O$ and 4.30 g of $FeCl_2.4H_2O$ were dissolved in 200 mL DDW with vigorous stirring at 85 ^oC under nitrogen gas atmosphere. Then, 20 mL of 30% (w/v) aqueous NH_3 solution was added to the solution. The color of the bulk solution changed from orange to black immediately. The magnetite precipitates were washed twice with DDW and once with 0.02 mol L^{-1} sodium chloride by magnetic decantation. Then, to the magnetite nanoparticles prepared above (0.8 g) was added an aqueous solution of tetraethoxy silane (TEOS, 10% (v/v), 80 mL), followed by glycerol (60 mL). The pH of the suspension was adjusted at 3.6 using glacial acetic acid, and the mixture was then stirred and heated at 90 ^oC for 2 h under the nitrogen atmosphere. After cooling to room temperature, the suspension was washed sequentially with DDW and methanol.

Preparation of VEN-imprinted polymer coated SCMNPs (VENMNPs)

At the first step of imprited polymer synthesis, the functional aminoimide monomer, was synthesized according to the previously reported method.[24] This monomer was prepared by the addition of 1 g maleic anhydride slowly to the solution of 1 mL of ethylene diamine in 20 mL of DDW. Then, the solution was heated to 120 ^oC for 1 h, until water was removed and ethylene diamine joined to maleic anhydride by opening the anhydride ring. In the next step, the unsaturated monomer of aminoimide was prepared by heating the product to 140 ^oC for 2 h (Scheme 1a) in order to prepare VENMNPs (Scheme 1b), unsaturated aminoimide monomer was polymerized in the presence of SCMNPs (0.5 g), ammonium persulfate (0.1 g) as the initiator, ethylene glycol dimethacrylate (0.2 ml) as the crosslinking monomer and VEN (0.01g) as the template in 30 mL DDW at 85 ^oC for 12 h. The products were separated by a magnet and washed overnight with a mixture of methanol: 0.01 mol L^{-1} HCl (2:1 v/v) until no template molecule (VEN) was detected spectrofluorometrically in the washing solution. Then, the resulting nanoparticles were dried under the vacuum for 12 h. As a blank, non-imprinted polymer coated SCMNPs (NIPMNPs), which did not contain the template, was also prepared in parallel with the VENMNPs by using the same synthetic protocol in the absence of the template.

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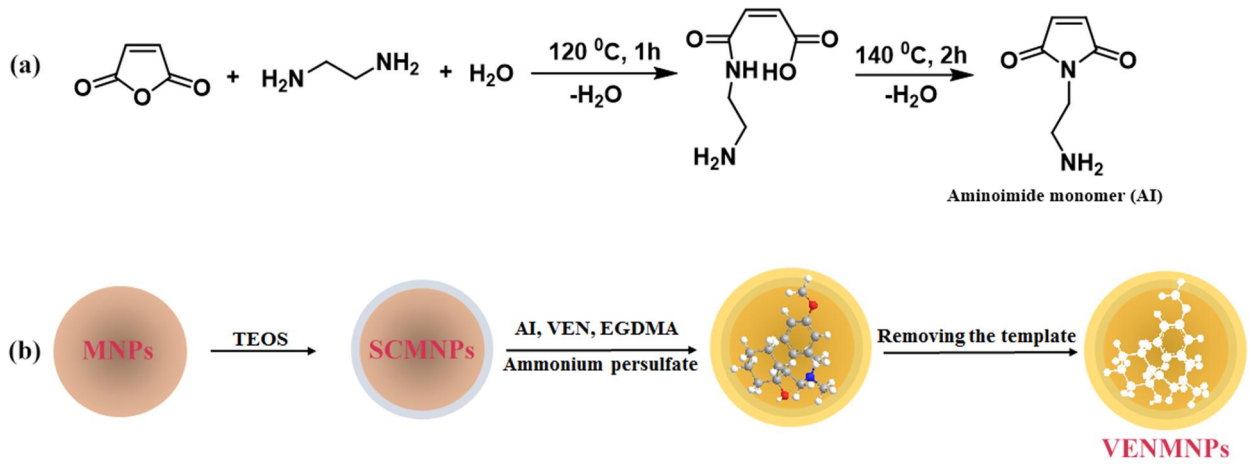

Scheme 1 Reaction involved in the synthesis of (a) aminoimide monomer and (b) VENMNPs.

VEN removal, preconcentration and spectrofluorometric determination

To a 25.0 mL sample solution containing VEN and 10.0 mL Britton-Robinson buffer solution of pH=7.0, a 0.04 g of VENMNPs was added. The solution was shaken at room temperature for 30.0 min. Subsequently, the VEN loaded VENMNPs were separated from the mixture with a permanent hand-held magnet within 60s. The residual amount of the drug in the solution was determined spectrofluorometrically at λ_{em}= 598 nm and λ_{ex}= 274 nm (Emission and excitation spectra of VEN are shown in Fig. 2). The adsorption percentage, i.e., the drug removal efficiency (%Re), was determined using the following equation:

$$\% \text{ Re } = [\frac{(C_0 - C_e)}{C_0}] \times 100 \quad (1)$$

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

Preconcentration studies for the determination of trace amounts of VEN were performed by adding 90.0 mL of the solution containing 2.0- 400.0 ng mL-1 of VEN and 50.0 mL of Britton-Robinson buffer of pH 7.0 to 0.04 g of VENMNPs and the solution was stirred for 30 min. The concentration of VEN decreased with time due to the adsorption by VENMNPs. The VEN loaded nanoparticles were separated with magnetic decantation and desorption was performed with a 2.0 mL of the mixture of methanol: 0.01 mol L-1 HCl (2:1 v/v). Then, the concentration of VEN in the resulting solution was measured spectrofluorometrically at λ_{em}= 598 nm (λ_{ex}= 274 nm).

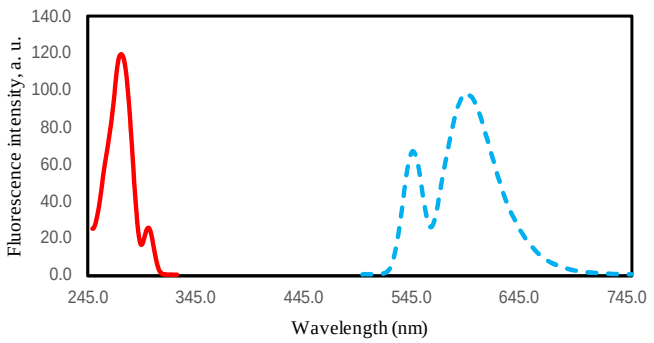

Fig. 2 Fluorescence excitation (——) at λ_{em}=598 nm and emission spectra (- - - -) at λ_{ex}=274 for VEN in water at pH 7.0 at room temperature.

Real samples treatment

Drug-free human urine samples were collected from healthy donors. All urine samples were stored at -20 °C. Then, 40.0 mL of each sample was diluted with 50.0 mL of a Britton-Robinson buffer (pH=7.0) solution and directly subjected to SPE procedure. The urine samples were spiked with VEN at ng mL^{-1} concentration levels.

The human blood serum sample was stored in a refrigerator after collection. A 2.0 mL of the sample was spiked with 0.5 mL of VEN solution at different concentrations of VEN. Then;

1.0 mL acetonitrile was added to deproteinize the serum. The precipitated proteins were separated by centrifuging at 4000 rpm and the clear supernatant was transferred into a volumetric flask and diluted with the Britton-Robinson buffer of pH 7.0 up to 100.0 mL total volume.

Results and Discussion

Characterization of the synthesized adsorbents

The resulting nanosized imprinted polymer was characterized by FTIR spectra, XRD, TEM and VSM analyses.

The FTIR spectra for the products in each step of the VENMNPs synthesis were recorded to verify the formation of the expected products. The related spectra are shown in Fig. 3. The characteristic absorption band of Fe-O in Fe_3O_4 (around 635 cm^{-1}) is observed in Fig. 3a. A strong peak at about 1054 cm^{-1} in Fig. 3b is attributed to Si-O band in SiO_2. Two new absorption peaks at 1722 cm^{-1} and 1155 cm^{-1} in Fig. 3c are assigned to C=O and C-N bands in the polymer-coated final product (VENMNPs), respectively [8]. Based on the above results, it can be concluded that the fabrication procedure has been successfully performed.

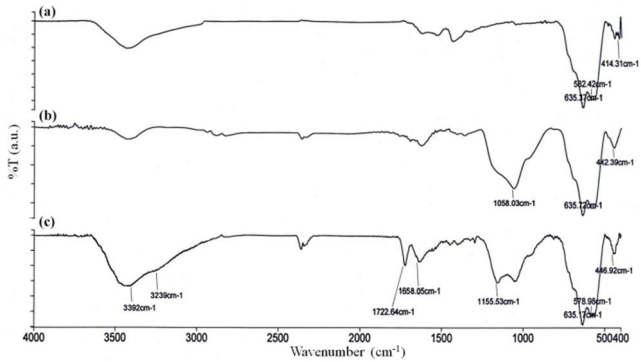

Fig. 3 FT-IR spectra of (a) MNPs, (b) SCMNPs and (c) VENMNPs

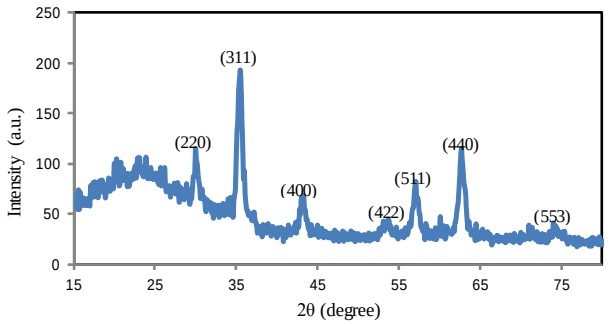

Fig. 4 XRD pattern for VENMNPs nanoparticles.

The XRD pattern of VENMNPs (Fig. 4) shows diffraction peaks that are indexed to (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), (4 4 0) and (5 5 3) reflection characteristics of the cubic spinel phase of Fe_3O_4 (JCPDS powder diffraction data file no. 79- 0418), revealing that the resultant nanoparticles are mostly Fe_3O_4. The average crystallite size of the VENMNPs nanoparticles was estimated to be 12 nm from the XRD data according to Scherrer equation.[11]

The TEM image of the VENMNPs in Fig. 5 indicates that the MNPs nanoparticles are enwrapped rather homogeneously in SiO_2 shells, and further by an MIP layer. The average size of the synthesized nanoparticles is estimated to be about 24.5 nm.

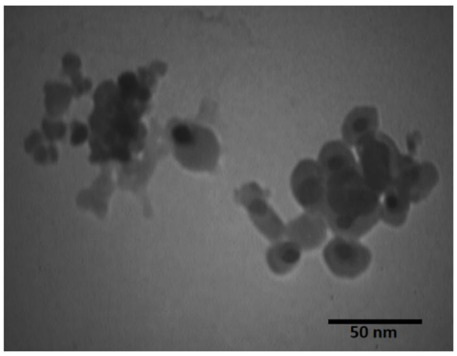

Fig. 5 TEM image of VENMNPs nanoparticles.

The magnetization curves of the bare MNPs, SCMNPs and VENMNPs recorded with VSM are illustrated in Fig. 6. As shown in Fig. 6, the magnetization of the samples would approach the saturation values when the applied magnetic field increases to 10,000 Oe. The saturation magnetization of the MNPs was 69.82 emu/g. For SCMNPs and VENMNPs, the saturation magnetization were 41.40 and 35.89 emu/g, respectively. These results show that magnetic properties are hardly affected by the surface modification. A magnetization reduction of about 40.70% was observed between the bare and SiO_2-coated Fe_3O_4 nanoparticles (SCMNPs), and about 13.31% between SCMNPs and VENMNPs. This may be related to the nanoparticles size effect, the increased surface disorder, and the diamagnetic contribution of SiO_2 and imprinted polymer layers.

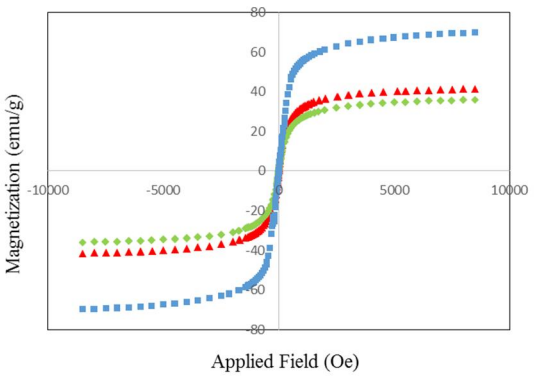

Fig. 6 Magnetization curves obtained by vibrating sample magnetometer (VSM) at room temperature: (■) bare MNPs; (▲) SCMNPs; and (♦) VENMNPs nanoparticles.

Point of zero charge (pH_{PZC}) of VENMNPs nanoparticles

The pH_{PZC} for the VENMNPs was determined in degassed 0.01 mol L^{-1} NaCl solution at room temperature. Aliquots of 30.0 mL 0.01 mol L^{-1} NaCl 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 and 9.0 using 0.01 mol L^{-1} of HCl and/or NaOH solutions as appropriate. The initial pH of the solutions was 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 [14]. The pH_{PZC} for VENMNPs was determined using the above procedure and was obtained as 6.2. The results are shown in Fig. 7.

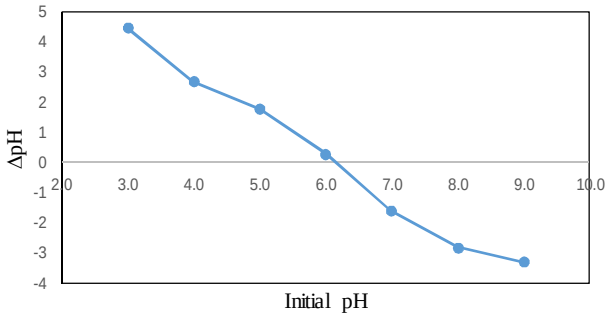

Fig. 7 Point of zero charge (pH_{pzc}) for VENMNPs nanoparticles.

Effect of various factors affecting the VEN removal efficiency

Various factors that can potentially affect the drug removal efficiency (i.e. pH, contact time and nanoparticles dosage) were optimized using "one-at-a-time" method. The detailed results are given below:

EFFECT OF PH

One of the important factors affecting the removal of the drug from aqueous solutions is the pH of the solution. The dependence of the drug sorption on pH is related to both the drug chemistry in the solution and the ionization state of the functional groups of the sorbent that affects the availability of binding sites. Drug chemistry usually depends on its pK_a (or pK_b) and one can predict ionization state of acidic or basic functional groups of the molecule at each pH. In the case of the adsorbent, responsible parameter is point of zero charge (pH_{PZC}). The point of zero charge is a characteristic of the metal oxides (hydroxides) and of fundamental importance in surface science. It is a concept relating to the phenomenon of adsorption and describes the condition when the electrical charge density on the surface is zero. The surface charge of VENMNPs with primary amine groups (belongs to amine group of the functional monomer) and hydroxyl groups (belongs to SiO_2 core-shell layer that has been partially covered by MIP layer) is largely dependent on the pH of the solution. The pH_{PZC} causes by the amphoteric behavior of hydroxyl and amino surface groups, and the interaction between surface sites and the electrolyte species. When brought into contact with aqueous solutions, hydroxyl groups of surface sites can undergo protonation or deprotonation, depending on the solution pH, to form charged surface species.

The effect of pH on the VEN removal efficiency was investigated in the range 3.0–11.0 using an initial VEN concentration of 2.0 mg L^{-1} and a stirring time of 45 min, where the pH was adjusted with Britton-Robinson buffer. Figure 8 indicates that the adsorbent provided highest affinity to VEN at pH 7.0. This is reasonable, because VEN significantly present in its protonated form at the pH< 9.4 (pK_a=9.4 [25]). On the other hand, the adsorbent surface charge at the pH<6.2 is positive and negative at pH >6.2. Therefore, in pH range of about 6.2-9.4, electrostatic attraction is responsible for high VEN removal efficiencies. At pH>9.4 VEN is in its neutral form and electrostatic attraction forces lost is responsible for low VEN removal efficiencies.

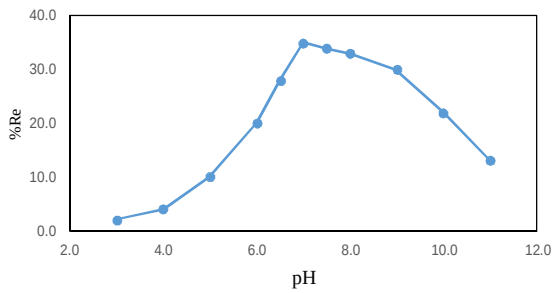

Fig. 8 Removal efficiency of VEN at different pHs (Conditions: 0.01 g of VENMNPs, 25 mL of 2.0 mg L^{-1} of VEN, agitation time of 45 min).

EFFECT OF NANOPARTICLES DOSAGE

The dependence of the adsorption of VEN on the amount of modified nanoparticles was studied at room temperature and at pH=7.0 by varying the adsorbent amount from 0.01 to 0.05 g in contact with 25.0 mL solution of 2.0 mg L^{-1} of VEN with agitation time of 45 min. The results showed that the removal efficiency of VEN increased by the increasing the amount of VENMNPs due to the availability of higher adsorption sites. The adsorption reached a maximum with 0.04 g of the adsorbent and maximum percentage removal was about 98%.

EFFECT OF CONTACT TIME

The effect of the contact time on the adsorption of VEN was studied to determine the time needed to remove VEN by VENMNPs from a 2.0 mg L^{-1} solution of the drug at pH 7.0. A 0.04 g of the adsorbent was added into a 25.0 mL (containing 10.0 mL of buffer solution of pH 7.0) of the drug solution. Fluorescence intensity of VEN was monitored versus time to determine variation of the drug concentration. It was observed that after a contact time of about 30.0 min, almost all of the drug was adsorbed (%Re>98) and this time was much enough to reach semi-equilibrium condition.

Effect of various factors affecting the VEN preconcentration efficiency

The aim of this step was providing the highest preconcentration factor and concentration of the adsorbed drug into minimum possible volume of the desorbing solvent. In this regard, various factor that can potentially affect the drug desorbing efficiency (i.e. type and volume of desorbing solvent, desorbing time and initial sample volume) were optimized using "one-at-a-time" method. The detailed results are given in below:

Desorption processes

For desorption studies, VEN loaded on the VENMNPs were first washed by DDW to remove the unadsorbed VEN that loosely attached to the vial and adsorbent. In order to estimate the recovery of VEN from VENMNPs, desorption experiments with different solvents (methanol, 0.05 mol L^{-1} HCl, mixture of methanol: HCl 0.01 mol L^{-1} (2:1 v/v), mixture of methanol: 0.01 mol L^{-1} NaOH (2:1 v/v)) were performed. After adsorption of VEN, the adsorbent was magnetically separated and washed with DDW. Then 2.0 mL of the eluent was added to the VEN loaded VENMNP. Samples were collected after 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 and 45.0 min contact times to evaluate VEN recovery. The results showed that the mixture of methanol: 0.01 mol L^{-1} HCl (2:1 v/v) is the most effective as a back-extracting solvent and can be used for the quantitative recovery of the drug. Desorption rate was found to be rapid as almost 98% desorption completed at almost 15.0 min.

Initial sample volume

The effect of initial sample volume on the drug adsorption was studied in the range 10.0–170.0 mL; 10.0 mL samples containing 0.02 mg L^{-1} of VEN were diluted to 25.0, 50.0, 100.0, 125.0, 150.0 and 170.0 mL with DDW. Then adsorption and desorption processes were performed under the optimum conditions (pH 7.0; contact time, 30.0 min; VENMNPs dosage, 0.04 g) as described in the experimental section. The results showed that the drug content in the volumes up to 100.0 mL was completely and quantitatively adsorbed by the nanoparticles, but there was a decrease in the amount became adsorbed at higher volumes. Therefore, for the determination of trace quantities of the drug, a sample volume of 100.0 mL was selected in order to having highest preconcentration factor.

Adsorption isotherms

The capacity of the adsorbent is an important factor that determines how much sorbent is required to quantitatively remove a specific amount of the drug from the solution. For measuring the adsorption capacity of VENMNPs and NIPMNPs, the absorbents was added into VEN solutions at various concentrations (under the optimum condition), and the suspensions were stirred at room temperature, followed by magnetic removal of the absorbent. An adsorption isotherm describes the fraction of the sorbate molecules that are partitioned between the liquid and the solid phase at equilibrium. Adsorption of the VEN by VENMNPs and NIPMNPs adsorbents were modelled using Freundlich [26] and Langmuir [27] adsorption isotherm models. The remained drug in the supernatants was measured spectrofluorometrically at λ_{em}= 598 nm (λ_{ex}= 274 nm), and the results were used to plot the isothermal adsorption curves as shown in Fig. 9. The equilibrium adsorption data were fitted to Langmuir and Freundlich isotherm models by linear regression. The resulting parameters are summarized in Table 1.

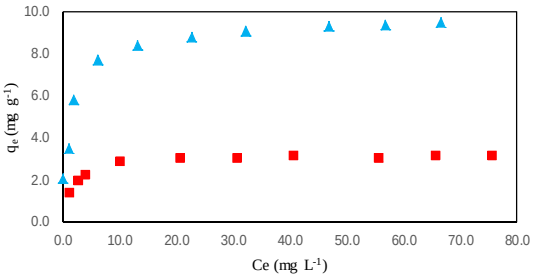

Fig. 9 Isothermal adsorption curves of VEN on (▲) VENMNPs and (■) NIPMNPs adsorbents.

Table 1 Adsorption isotherm parameters of Langmuir and Freundlich models for the adsorption of the drug on VENMNPs and NIPMNPs adsorbents.

Isotherm models	Langmuir			Freundlich		
	K_L (L mg^{-1})	q_{max} (mg g^{-1})	R^2	K_f	1/n	R^2
VENMNPs	0.65	8.44	0.999	3.47	0.24	0.919
NIPMNPs	0.39	3.26	0.998	1.52	0.18	0.859

The regression coefficients, r, for fitting the experimental data points to the linear forms of the Langmuir equations can be considered as a measure of goodness of fit. It is seen that Langmuir model is almost better successful in representing experimental isotherm data versus Freundlich model, and adsorption of VEN on VENMNPs adsorbents is more compatible with Langmuir assumptions, i.e., adsorption takes place at specific homogeneous sites within the adsorbent. The Langmuir model is based on the physical hypothesis that the maximum adsorption capacity consists of a monolayer adsorption, that there are no interactions between adsorbed molecules, and that the adsorption energy is distributed homogeneously over the entire coverage surface. This sorption model serves to estimate the maximum uptake values where they cannot be reached in the experiments.

According to the results (Table 1), the maximum amount of VEN that can be adsorbed by VENMNPs and NIPMNPs was found to be 8.44 and 3.26 mg g^{-1} at pH 7.0, respectively. The relatively high adsorption capacity of VENMNPs in comparison with NIPMNPs, shows that the adsorption of VEN molecules takes place at a large number of specific homogeneous sites within the adsorbent (specific cavities of the MIP), besides non-specific interactions which are approximately identical for both VENMNPs and NIPMNPs adsorbents.

Reusability and stability of the adsorbent

The reusability and stability of VENMNPs for the extraction of VEN was assessed by performing ten consecutive separations/desorption cycles under the optimized conditions (Conditions: 0.04 g of VENMNPs, 100.0 mL of 0.02 mg L^{-1} of VEN, agitation time of 30 min). Desorption of VEN from the adsorbent was performed with a mixture of methanol: 0.01 mol L^{-1} HCl (2:1 v/v) as described in section *3.4.1*. There was no significant change in the performance of the adsorbent during these cycles, indicating that the fabricated VENMNPs are a reusable and stable solid phase sorbent for the extraction of VEN.

Analytical applications

Calibration graph was constructed from spectrofluorometric measurements of the desorbed VEN after performing its adsorption/separation under the optimum conditions described above. The calibration graph was linear in the range 5.0 - 400.0 ng mL^{-1} for a sample volume of 100.0 mL. The calibration equation was $I_F = 1.93C + 1.36$ with a determination coefficient of 0.996 (n = 7), where I_F is the fluorescence intensity of the eluate at λ_{em}= 598 nm (λ_{ex}= 274 nm) and C is the concentration of the drug in ng mL^{-1}. 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 1.2 ng mL^{-1} of VEN. As the drug in 100.0 mL of the sample solution was concentrated into 2.0 mL, a maximum preconcentration factor of 45.0 was achieved in this method. The relative standard deviations (RSD) for 50.0 and 20.0 ng mL^{-1} of the drug were 1.09% and 1.86% (n = 5), respectively.

The analytical applicability of the proposed method was evaluated by determining the VEN content of different amounts of VEN spiked healthy human urine and human blood serum samples using VENMNPs adsorbent. The results are given in Table 2. The results show good recoveries of the proposed method for the VEN added to the biological samples and the method is a good candidate for VEN determination in the investigated samples.

Table 2 Assay of VEN in human urine samples by means of the proposed method.

Sample	Spiked value (ng mL^{-1})	Found (ng mL^{-1})	Recovery percent
Human urine			
	-	ND*	-
	10.0	9.7	97.0
	50.0	48.9	97.8
Human blood serum			
	-	ND*	-
	10.0	9.8	98.0
	50.0	51.2	102.4

*Not detected

Table 3 Comparison of the proposed method with the reported methods for VEN determination.

Method	LOD (ng mL^{-1})	Linear range (ng mL^{-1})	Sample	Ref.
SWV- HMDE[a]	124	-	Pharmaceutical formulations	28
NAF-CNT-GCE[b]	3.44[f]	10.57-17254.28[f]	Pharmaceutical formulations, blood serum and urine	4
HS-SPME-GC-NPD[c]	3	10-40000	post-mortem whole blood	29
CPE-HPLC[d]	2	10-800	Human plasma	30
HPLC-FL[e]	1.0	-	Human plasma	31
MIP/SPE/FL	1.2	2.0-400.0	Human blood serum and urine	This work

[a] Anodic stripping square wave voltammetry using a hanging mercury drop electrode.
[b] Adsorptive stripping differential pulse voltammetric determination employing Nafion–carbon nanotube composite glassy carbon electrode.
[c] Headspace-solid phase microextraction with GC detection.
[d] High-performance liquid chromatography using cloud-point extraction and fluorimetric detection.
[e] High-performance liquid chromatography with fluorimetric detection.
[f] Original data have been reported as mol L^{-1} concentration unit.

This comparison shows that the analytical performance of the proposed method is comparable with some sensitive instrumental methods, such as HPLC, and in some cases, the proposed method is much better. Other advantages of the proposed method are simplicity, less expensive procedure, low

LOD, wide linear range, easy adsorbent separation and high removal capacity of the adsorbent.

Conclusions

In order to prepare a magnetic nanoadsorbent, a venlafaxine-imprinted polymer was coated on the magnetite nanoparticles surfaces. The experimental results indicated that the sorption of venlafaxine onto the imprinted polymer was much better than non-imprinted polymer. The imprinted polymer also presents an advantage of high adsorption capacity and high chemical stability. Furthermore, the adsorbent is easily attracted by a magnet and can be separated from the liquid medium that enables an easy separation process. Based on the Langmuir isotherm analysis, the adsorption capacity was found to be about 8.44 mg g^{-1}. The method was successfully applied to the analysis of venlafaxine in the biological samples. The comparison analytical performance of the method with other previously reported methods indicated that a powerful method for venlafaxine determination in biological samples has been proposed.

Acknowledgements

This work support by the Bu-Ali Sina University Research Council and Center of Excellence in Development of Environmentally Friendly Methods for Chemical Synthesis (CEDEFMCS) and the authors acknowledge to that for providing to this work.

Notes

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