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ARTICLE TYPE

Enantioselective solid phase extraction prior to spectrofluorometric determination: a procedure for determination of naproxen enantiomers in the presence of each other

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A method for simultaneous spectrofluorometric determination of naproxen drug enantiomers has been proposed. The method based on three procedures. These procedures have their precision limitation that has been fully investigated and optimized condition has been proposed. In this regard, magnetite nanospheres have been chemically modified with L-(-)-Tryptophan (Try-MNSs). The synthesized nanospheres have been characterized by FT-IR, XRD, VSM and TEM measurements. The result showed that Try-MNSs have good enantioselectivity toward S-(+)-Naproxen and could efficiently remove that in the presence of its enantiomer. Using the proposed procedures, precise naproxen drug enantiomeric composition analysis in concentration range of 100.0-1800.0 ng mL^{-1} is achievable. Furthermore, S-(+)-Naproxen determination in 2.0-50.0 ng mL^{-1} concentration range with detection limit of 0.7 ng mL^{-1} is feasible.

Introduction

A wide range of chemical compounds used in pharmaceutical formulations feature one or more chiral centers. Each enantiomer of these chiral compounds exhibit different biological activities in living systems during biomedical and pharmacological processes. Nowadays, the significance of chirality in biological processes is well recognized and the strict regulations established by health authorities on the commercialization of chiral drugs, has led to the requirement for developing analytical and preparative methods for enantioseparation.[1, 2]

Naproxen (Nap) belongs to the class of non-steroidal anti-inflammatory drugs. After oral administration, naproxen is partially metabolized to its 6-O-desmethylated metabolite; then, both compounds are excreted in urine unchanged or conjugated with glucuronic acid or sulphate.[3-5] It has one chiral center which gives rise to two optical isomers and their pharmacological activity resides in S-(+)-enantiomer (S-Nap), while the R-(-)-enantiomer (R-Nap) causes some unwanted side effects.[6] Therefore, it is preferable to use pure S-Nap and marketing as a single enantiomer in drugs in order to decrease dosage and undesirable side effects.

Several methods have been proposed to analysis and separation of the Nap enantiomers such as inclusion crystallization[7], enzymatic kinetic method [8], chiral stationary phase HPLC [9], chiral mobile phase HPLC[10], molecular imprinting polymers chiral stationary phase [11], supercritical fluid chromatography [6] and capillary electrophoresis [12]. 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 today.[3, 13] 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 separate analytes efficiently in complex biological or environmental samples. [14]

In this work, a chiral magnetic nanoadsorbent have been synthesized (chiral surface modified nanospheres) by chemical attachment of L-(-)-Tryptophan onto magnetite nanospheres surface (Try-MNSs). Try-MNSs showed good enantioselectivity toward S-Nap comparing to R-Nap. The experimental result showed that, under optimum condition, selective S-Nap preconcentration in the presence of R-Nap can be achieved prior to its spectrofluorometric concentration analysis. The results of this work promise a methodology for Nap drug enantiomeric composition analysis.

Experimental

Reagents and materials

(S)-(+)-Naproxen (S-Nap), (R)-(-)-Naproxen (R-Nap) and L-(-)-Tryptophan were purchased from Sigma-Aldrich Company (USA). All of the other chemicals used were of analytical reagent grade and were purchased from Merck Company (Darmstadt, Germany). Double distilled water (DDW) was used throughout the work. All glassware were soaked in dilute nitric acid for 12 h and then thoroughly rinsed with DDW. The enantiomers stock solutions were prepared in methanol solvent and the working standard solutions of different enantiomers concentrations were prepared daily by diluting the stock solution with DDW. 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.

Apparatus

The size, morphology and structure of the nanospheres were characterized by transmission electronic microscopy (TEM, Philips-CMC-300 KV). The crystal structure of the synthesized nanospheres was determined by an X-ray diffractometer (XRD, 38066 Riva, d/G. via M. Misone, 11/D (TN) Italy) at ambient temperature. The magnetic properties of the synthesized nanospheres were measured with a vibrating sample magnetometer (VSM, 4 in. Daghigh Meghnatis Kashan Co., Kashan, Iran). The mid-infrared spectra of the synthesized nanospheres in the region 4000- 400 cm^{-1} were recorded by an FT-IR spectrometer (Perkin-Elmer model Spectrum GX) using KBr pellets. A Perkin Elmer (LS50B) luminescence spectrometer was used. A Metrohm model 713 pH-meter was used for pH measurements. A 40 kHz universal ultrasonic cleaner water bath (RoHS, Korea) was used.

Preparation of magnetite nanospheres (MNSs) and silica coated magnetite nanospheres (SCMNSs)

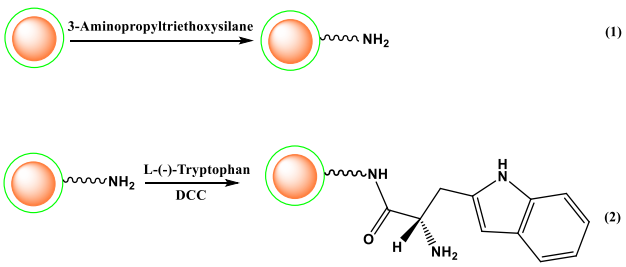
MNSs were synthesized by solvothermal reduction method with minor modifications. [15] Typically, $FeCl_3.6H_2O$ (1.35 g) was dissolved in ethylene glycol (40.0 mL) to form a clear solution, followed by the addition of sodium acetate (3.6 g) and polyethylene glycol (1.0 g). The mixture was ultrasonicated vigorously for 30.0 min and then refluxed at 180 ℃ for 8 h, and then allowed to cool down to room temperature. The black products were washed several times with ethanol and DDW water and then dried at 60 ℃ for 6 h. Also, SCMNSs were prepared according to previously reported method with minor modifications [16]. Typically, 0.5 g of MNSs was dispersed in 60.0 mL ethanol and 10 mL of DDW water by sonication for 15 min, followed by the addition of 1.0 mL ammonium hydroxide (25%) and 3.0 mL tetraethoxysilane sequentially. The mixture was reacted for 12 h at room temperature under continuous stirring. The resultant product was collected by a permanent hand-held magnet, and rinsed consecutively six times with ethanol and DDW water. Finally, the obtained SCMNSs were dried under vacuum at 60 ℃ for 3 h.

Preparation of L-(-)-Tryptophan modified SCMNSs (Try-MNSs)

SCMNSs was modified by L-(-)-Tryptophan by two steps that showed in Scheme 1.

In the first step, the surface of SCMNSs was modified with primary amine functional groups by the aid of 3-aminopropyltriethoxysilane reagent using below procedure: Typically, 0.8 g of SCMNSs was dispersed in 60.0 mL ethanol and 10 mL of DDW by sonication for 15 min, followed by the addition of 1.0 mL ammonium hydroxide (25%) and 2.0 mL 3-aminopropyltriethoxysilane sequentially. The mixture was reacted for 12 h at room temperature under continuous stirring. The resultant product (NH_2-SCMNSs) was collected by an external magnetic field, and rinsed six times with ethanol and DDW water. Finally, the NH_2-SCMNSs obtained were dried under vacuum at 60℃ for 3 h.

In the second step, the NH_2-SCMNSs were L-(-)-Tryptophan-functionalized by reacting with L-(-)-Tryptophan in methanolic suspension by the aid of dicyclohexylcarbodiimide (DCC) condensation agent [17]. In a typical procedure, 0.4 g of L-(-)-Tryptophan were dissolved in 50 mL methanol at 50 ℃ and sonicated for 10 min. Then, to this solution, 0.4 g of DCC was added and the solution was stirred for several minutes. Finally 1.0 g of NH_2-SCMNSs was added to the above solution and the solution was stirred again for 24 h at 50℃. The resultant product was collected by an external magnetic field, and washed to remove the unreacted molecules and the by-products with methanol and DDW water. Finally, the resultant nanospheres obtained were dried under vacuum at 60 ℃ for 12 h.



Scheme 1 Overall route for modification of the magnetite nanospheres with L-(-)-Tryptophan.

Removal and preconcentration experiments

To a 25.0 mL sample solution containing the enantiomers and 10.0 mL Britton-Robinson buffer solution of pH 6.0, a 0.02 g of Try-MNSs was added. The solution was shaken at room temperature for 30.0 min. Subsequently, the S-Nap loaded Try-MNSs were separated from the mixture with a permanent hand-held magnet within 60s. The residual amount of the enantiomers in solution was determined spectrofluorometrically at λ_{em}= 356 nm (λ_{ex}= 271 nm). The adsorption present, i.e., the drug removal efficiency (%R), was determined using the following equation:

$$\%R = \left[\frac{C_0 - C_t}{C_0}\right] \times 100 \qquad (1)$$

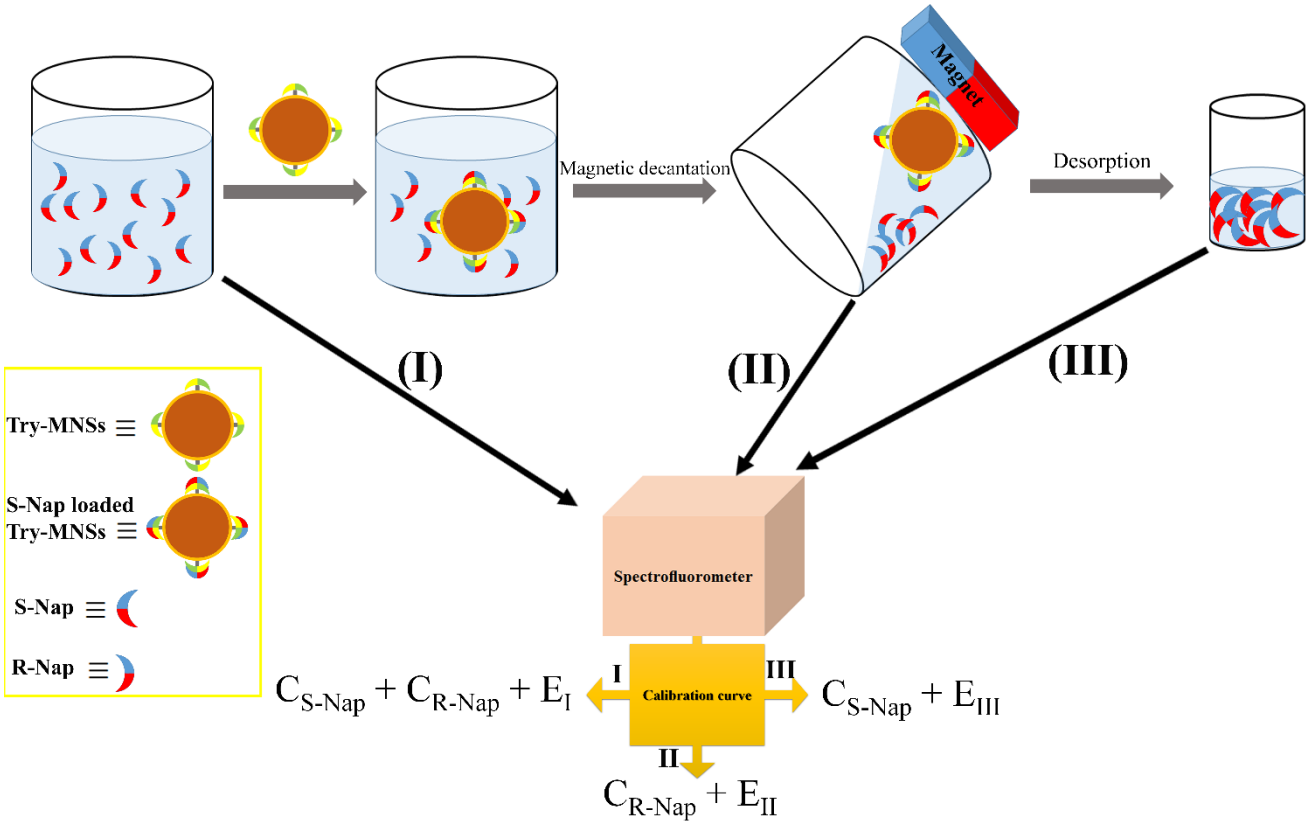
where C_o and C_t represent the initial and final (after adsorption) concentrations of the enantiomers in mg L^{-1}, respectively. Also all the experiments were performed at room temperature.

Preconcentration studies for determination of trace amounts of S-Nap were performed by adding 150.0 mL of solutions containing 2.0-50.0 ng mL^{-1} of S-Nap and 100.0 mL of Britton-Robinson buffer of pH 6.0 to 0.02 g of Try-MNSs and the solutions were stirred for 30 min. The concentration of S-Nap decreased with time due to the adsorption by Try-MNSs. The S-Nap loaded nanospheres were separated with magnetic decantation and desorption was performed with 2.0 mL of a 1:1 (v/v) mixture of methanol/sodium hydroxide aqueous solution (0.01 mol L^{-1}). The concentration of S-Nap in the resulting solution was measured spectrofluorometrically at λ_{em}= 353 nm (λ_{ex}= 271 nm).

Overall route for the enantiomeric composition analysis

In order to simultaneous determination of S-Nap and R-Nap and Nap enantiomeric composition analysis, three procedures were followed. Scheme 2 shows the summarized procedures. Each of this procedures are binary combination of three routes (i.e. routes I, II and III). Route I involve spectrofluorometrically determination of total the enantiomers concentrations at pH=6 (λ_{ex}= 271 nm, λ_{em}= 356 nm). Route II involve spectrofluorometrically determination R-Nap after S-Nap removal from enantiomers mixture via

adsorption by the adsorbent at pH=6 (λ_{ex}= 271 nm, λ_{em}= 356 nm). Route III involve spectrofluorometrically determination of S-Nap after its preconcentration in methanol/NaOH mixture solvent (λ_{ex}= 271 nm, λ_{em}= 353 nm). Fig. 1 shows excitation-emission spectra of Nap in pH=6 and 1:1 (v/v) methanol/NaOH (0.01 M) mixture solvent.



Scheme 2 Overall description of the proposed method.

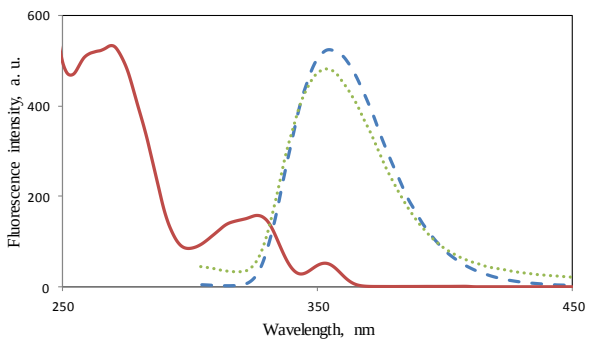


Fig. 1 Fluorescence excitation (——) and emission spectra at λ_{ex}=271 nm for Nap in water (pH 6.0) (- - - -) and in 1:1 (v/v) methanol/NaOH (0.01 M) (……) in room temperature.

Procedure (I-II):

This procedure involve spectrofluorometrically concentration determination of total Nap using route I and R-Nap using route II by calibration curve of IF = 0.5389 C + 3.266 (calibration curve 1) in concentration range of 100.0-1800.0 ng mL^{-1} . Inasmuch as R-Nap and S-Nap having no synergism effect on their corresponding florescence signal (that was experimentally checked out) and their signal is additive, one can determine total concentration of Nap (C_{Nap}=$C_{S\text{-}Nap}$+$C_{R\text{-}Nap}$) using route I (Scheme 2), R-Nap concentration using route II and S-Nap concentration using the difference of total concentration of Nap and R-Nap ($C_{S\text{-}Nap}$=C_{Nap}-$C_{R\text{-}Nap}$).

Procedure (I-III):

This procedure involve spectrofluorometrically concentration determination of total Nap using route I and calibration curve 1 and S-Nap after preconcentration using route III by calibration curve of IF = 18.522 C + 9.354 (calibration curve 2) in concentration range of 2.0-50.0 ng mL^{-1}. R-Nap concentration can be calculated using the difference of total concentration of Nap and S-Nap concentration ($C_{R\text{-}Nap}$=C_{Nap}-$C_{S\text{-}Nap}$).

Procedure (II-III):

This procedure involve spectrofluorometrically concentration determination of R-Nap using route II and calibration curve 1, S-Nap using route III and calibration curve 2 and total Nap concentration from summation of S-Nap and R-Nap concentration (C_{Nap}=$C_{S\text{-}Nap}$+$C_{R\text{-}Nap}$).

Each of the proposed procedure has its corresponding precision limitation (i.e. E_I, E_{II} and E_{III}) and optimized condition to minimize this limitation level that is discussed further in "Results and Discussion" section.

Results and Discussion

Characterization of the investigated nanospheres

The magnetization curves of the bare MNSs, SCMNSs and Try-MNSs recorded with VSM are illustrated in Fig. 2. As shown in

Fig. 2, 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 MNSs was 41.47 emu/g. For SCMNSs and Try-MNSs, the saturation magnetizations were 30.13 and 26.92 emu/g, respectively. These results showed that magnetic properties are hardly affected by the surface modification. A magnetization reduction of about 27.3% was observed between the bare and SiO_2-coated Fe_3O_4 nanospheres (SCMNSs), and about 10.7% between SCMNSs and Try-MNSs. This may be related to the nanospheres size effect, the increased surface disorder, and the diamagnetic contribution of the SiO_2 layers.

The FT-IR spectra of the products in each step of the Try-MNSs synthesis was 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 580 cm^{-1}) was observed in Fig. 3a. A strong peak at about 1100 cm^{-1} in Fig. 3b is attributed to Si-O in SiO_2. Two new absorption peaks at 1652 cm_{-1} and 1262 cm_{-1} in Fig. 3c are assigned to C=O and C-N bands in the Try-MNSs, respectively. Moreover, the new absorption peaks at 3227 and 3073 cm_{-1} are related to the stretching modes of the primary amino groups. [18] Based on the above results, it can be concluded that the fabrication procedure has been successfully performed.

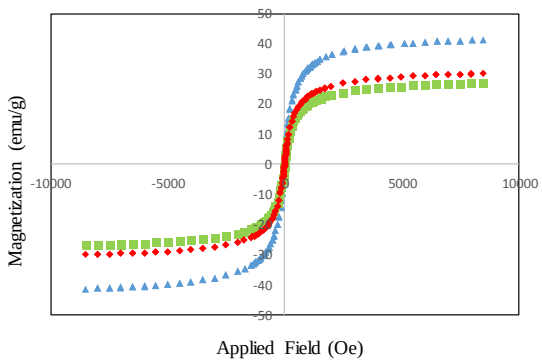


Fig. 2 Magnetization curves obtained by vibrating sample magnetometer at room temperature: (▲) bare MNSs; (♦) SCMNSs and (■) Try-MNSs.

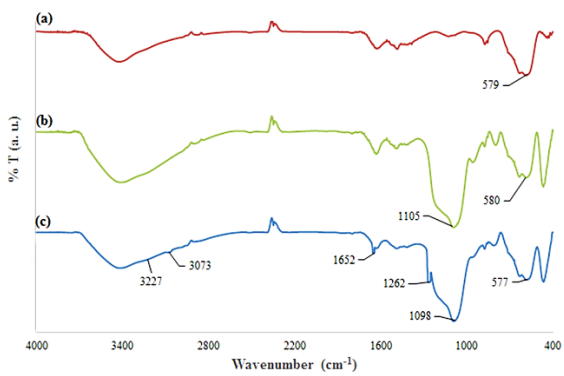


Fig. 3 FT-IR spectra of (a) MNSs, (b) SCMNSs and (c) Try-MNSs.

The XRD pattern of Try-MNSs (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 nanospheres are mostly Fe_3O_4. The average crystallite size of the Try-MNSs nanospheres was estimated to be 8.0 nm from the XRD data according to Scherrer equation. [19]

The TEM image of the MNSs in Fig. 5a indicates that spherical monodisperse nanoparticles with an average diameter of about 80 nm were synthesized. Figure 5b indicates that MNSs successfully coated with a core-shell layer of SiO_2 (with about 20 nm thickness). Figures 5c depict Try-MNSs. This Fig. shows that after L-(-)-Tryptophan functionalization process, core-shell layer thickness have been increased by about 5 nm, that is mainly due to coating effect of primary amine functionalizing agent and rarely due to L-(-)-Tryptophan coating process contribution (see Scheme 1).

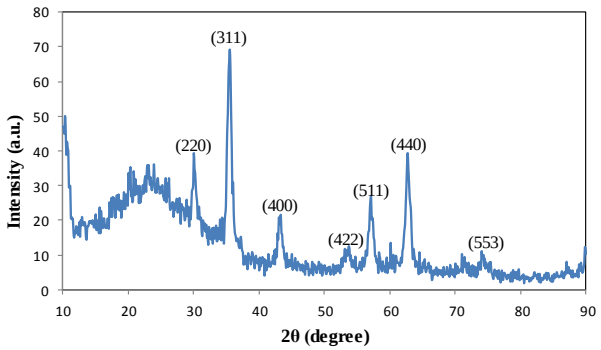


Fig. 4 XRD pattern of Try-MNSs.

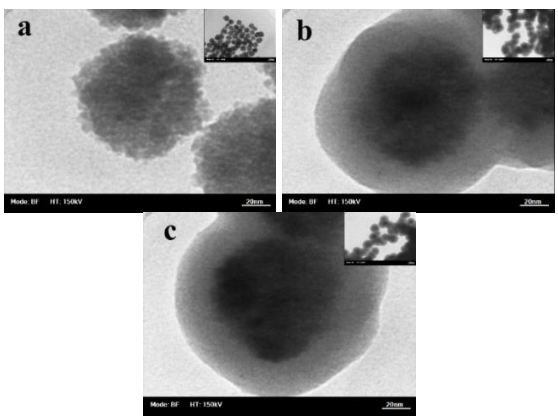


Fig. 5 TEM images of (a) MNSs, (b) SCMNSs and (c) Try-MNSs nanospheres.

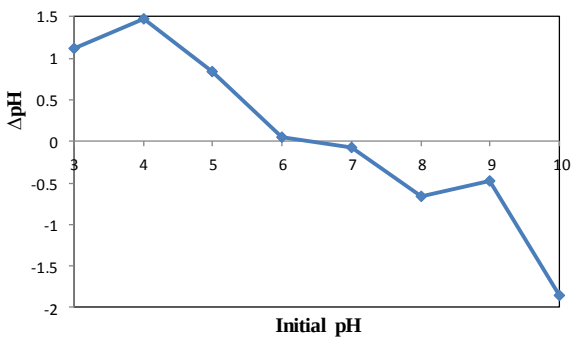


Fig. 6 Point of zero charge (pH_{pzc}) of Try-MNSs nanospheres.

Point of zero charge (pH_{PZC}) of Try-MNSs

The pH_{PZC} of Try-MNSs was determined in degassed 0.01 mol L^{-1} $NaNO_3$ solution at room temperature. Aliquots of 30.0 mL 0.01 mol L^{-1} $NaNO_3$ were mixed with 0.03 g of the nanospheres in several beakers. The initial pH of the solutions were adjusted at 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 using 0.01 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. [20] The pH_{PZC} for Try-SCMNSs was determined using the above procedure and was obtained as 6.4. The results showed in Fig. 6.

Effect of various factors affecting the procedure (I-II) efficiency

Precision limitation of this procedure arises from combination of the precision limitation of route I (E_I) and route II (E_{II}). E_I origin from common random and systematic errors in spectrofluorometrically concentration determination and can be minimized using common consideration. E_{II} origin from both spectrofluorometrically concentration determination limitation and S-Nap removal efficiency. In order to E_{II} minimization, the surface of MNSs have been modified with L-(-)-Tryptophan (Try-MNSs). Selectivity evaluation test cleared that the modified nanospheres have good selectivity toward S-Nap comparing to R-Nap. But it was not specific for S-Nap removal and so E_{II} cannot be zero. However in order to E_{II} minimization various factors, that can potentially affect S-Nap removal efficiency (i.e. pH, contact time and nanospheres dosage), were optimized using "*one-at-a-time*" method.

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 S-Nap and R-Nap removal was investigated in the range 3.0-11.0 using an initial Nap concentration of 2.0 mg L^{-1} and a stirring time of 45.0 min, where the pH was adjusted with Britton-Robinson buffer. Figure 7 indicates that the adsorbent provides highest affinity to S-Nap at pH 4.5. But, maximum enantioselectivity occurs in pH 6.0 (curve c).

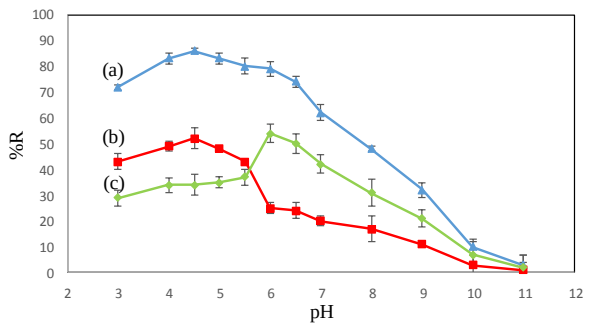


Fig. 7 Effect of pH on the enantioselectivity of the adsorbent: (a) S-Nap removal efficiency, (b) R-Nap removal efficiency and (c) net difference of the enantiomers removal efficiencies vs. pH (curve c= curve a – curve b) (condition: 0.01 g of Try-MNSs, 25.0 mL of 2.0 mg L^{-1} of Nap, agitation time of 45.0 min, n=3).

These results are reasonable because at pH<5.9, according to pH_{PZC} of the nanospheres, the surface of the nanospheres is positively charged. Two functional groups, i.e. primary amine groups of tryptophan and hydroxyl groups of SiO_2 core-shell layer, can be protonated at this pH. It is predicated that both of the functional group are protonated at these pHs (isoelectric point of tryptophan is 5.90 [21]). At the other hand, the Nap acidic groups at pH range of 4.2 to 5.9 are in their deprotonated form (pK_a of naproxen is 4.2 [3]) and non-enantioselective electrostatic interaction is responsible for high removal efficiency of both the enantiomers. But at pH range of 5.9 to 6.4, the primary amine functional group is in its neutral form and the Nap in its deprotonated form. So, maximum hydrogen bonding interaction can be predicated and maximum enantioselectivity is reasonable (curve c). At pH>6.4, negative charge of the nanospheres prevents approaching the enantiomers. At pH<4.2, both of hydrogen bonding and electrostatic interaction are in their minimum levels. So, in order to providing maximum enantioselectivity and minimization of E_b, we chose pH 6.0 for further optimization process.

Effect of nanospheres dosage

The dependence of the adsorption of S-Nap on the amount of modified nanospheres was studied at room temperature and at pH 6.0 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 S-Nap. The results showed that the percentage removal of S-Nap increased by increasing amount of Try-MNSs due to the availability of higher adsorption sites. The adsorption reached a maximum with 0.02 g of adsorbent and maximum percentage removal was about 98%.

Effect of contact time

The effect of contact time on the adsorption of S-Nap was studied to determine the time needed to remove S-Nap by Try-MNSs from a 2.0 mg L^{-1} solution of the drug at pH 6.0. A 0.02 g of the adsorbent was added into 25.0 mL (containing 10.0 mL of buffer solution of pH 6.0) of the drug solution. Fluorescence intensity of S-Nap 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 the drug was adsorbed (%R>98).

Effect of various factors affecting the procedure (I-III) efficiency

Precision limitation of this procedure arise from precision limitation combination of route I (E_I) and route III (E_{III}). E_{III} origin from both spectrofluorometrically concentration determination limitation and S-Nap removal/desorption efficiency. Parameters such as pH, contact time and nanospheres dosage, that are part in removal section, have been discussed in the previous section. In order to maximize desorption efficiency, various factors such as type and volume of desorbing solvent, desorption time and initial volume of the sample have been optimized.

Desorbing solvent

For desorption studies, S-Nap adsorbed Try-MNSs were first washed by ultrapure water to remove the unadsorbed S-Nap that loosely attached to the vial and adsorbent and then with 0.1 mol L^{-1} NaCl aqueous solution to remove R-Nap that attached to the adsorbent *via* electrostatic interactions. In order to estimate the recovery of S-Nap from Try-MNSs, desorption experiments with different reagents (0.01 mol L^{-1} NaOH, methanol and mixture of methanol: 0.01 mol L^{-1} NaOH (1:1 v/v)) were performed. After

adsorption of S-Nap, the adsorbent was magnetically separated .Then 2.0 mL of the eluate was subjected to spectrofluorometer (λ_{ex}= 271 nm, λ_{em}= 353 nm). Samples were collected after 5.0, 10.0, 20.0, 30.0 and 45.0 min contact times with the eluate to evaluate S-Nap recovery. The results showed that the mixture of methanol with 0.01 mol L^{-1}NaOH is most effective as a back-extracting 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 10.0 min.

Table 1 Adsorption isotherm parameters of Langmuir and Freundlich models for the adsorption of the investigated enantiomers on Try-MNSs.

Isotherm models	Langmuir				Freundlich			
	K_L (L mg^{-1})	q_{max} (mg g^{-1})	R^2	RMS	K_f	1/n	R^2	RMS
S-Nap	0.563	7.638	0.9697	0.373	3.413	0.221	0.8241	0.899
R-Nap	0.788	2.043	0.9501	0.137	2.850	0.235	0.8236	0.792

Initial sample volume

The effect of initial sample volume on the drug adsorption was studied in the range 10.0–300.0 mL; 10.0 mL samples containing 2.0 mg L^{-1} of S-Nap were diluted to 10.0, 20.0, 25.0, 50.0, 75.0, 100.0, 150.0, 200.0, 250.0 and 300.0 mL with DDW. Then adsorption and desorption processes were performed under the optimum conditions (pH 6.0; contact time, 30.0 min; Try-MNSs dosage, 0.02 g) as described in the experimental section. The results showed that the drug content in the volumes up to 150.0 mL was 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 drug, a sample volume of 150.0 mL was selected in order to having highest preconcentration factor.

Effect of various factors affecting the procedure (II-III) efficiency

Precision limitation of this procedure arise from combination of the precision limitation of route II (E_{II}) and route III (E_{III}). The parameters that can potentially affect efficiency of this procedure have been discussed at previous sections.

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 solution. For measuring the adsorption capacity of Try-MNSs, the absorbent was added into S-Nap and/or R-Nap solutions at various concentrations, 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 enantiomers by Try-MNSs adsorbent was modeled using Freundlich [22] and Langmuir [23] adsorption isotherm models. The remained the enantiomers in the supernatants was measured spectrofluorometrically at λ_{em}= 356 nm (λ_{ex}= 271 nm), and the results were used to plot the isothermal adsorption curves as shown in Fig. 8. The equilibrium adsorption data were fitted to Langmuir and Freundlich isotherm models by nonlinear regression. The resulting parameters are summarized in Table 1.

The higher correlation coefficient obtained for the Langmuir model (R^2 > 0.95) indicates that the experimental data are better fitted into this model, and adsorption of Nap enantiomers on Try-MNSs 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 S-Nap and R-Nap, which can be adsorbed by Try-MNSs, was found to be 7.638 and 2.043 mg g^{-1} at pH 6.0, respectively. The relatively high adsorption capacity in the case of S-Nap in comparison with R-Nap, shows that the adsorption of S-Nap molecules takes place at a large number of specific homogeneous sites within the adsorbent (primary amine functional groups), besides non-specific interactions (electrostatic interactions) which are approximately identical for both of the enantiomers.

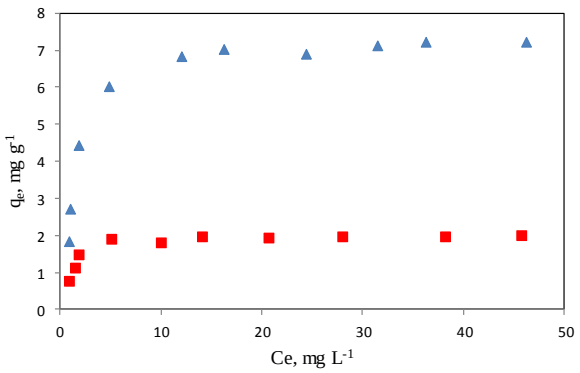


Fig. 8 Isothermal adsorption curves of (▲) S-Nap and (■) R-Nap on Try-MNSs adsorbent.

Reusability and stability of the adsorbent

The reusability and stability of Try-MNSs for the extraction of S-Nap was assessed by performing ten consecutive separations/desorption cycles under the optimized conditions (Conditions: 0.02 g of Try-MNSs, 25.0 mL of 2.0 mg L^{-1} of S-Nap, agitation time of 30.0 min). Desorption of S-Nap from the adsorbent was performed with a mixture of methanol/sodium hydroxide aqueous solution (0.01 mol L^{-1}) 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 Try-MNSs is a reusable and stable solid phase sorbent for the extraction of S-Nap.

Analytical parameters and application

Under the optimum conditions, calibration graph was constructed from spectrofluorometric measurement of various concentrations of S-Nap and/or R-Nap (calibration curve 1) and the desorbed S-Nap after performing its adsorption/separation (calibration curve 2). The results are presented in Table 2. The calibration curve for S-Nap, R-Nap and mixture of the enantiomers were almost equal and implies that the enantiomers have not any synergism effect on their corresponding fluorescence signal. So, in order to simplicity, only one calibration curve have been presented (calibration curve 1). Linear range for S-Nap (calibration curve 2) is apparently lower than that of unpreconcentrated one (calibration curve 1). Because as the S-Nap in 150.0 mL of the sample solution was concentrated into 2.0 mL, a maximum preconcentration factor of 75.0 was achieved in this method.

Table 2 Analytical parameters summaries of the investigated method for determination of Nap enantiomers.

Analytical parameters	Calibration curve 1 (λ_{em}= 356 nm)	Calibration curve 2 (λ_{em}= 353 nm)
Calibration equation (n=8)	IF = 0.5389 C + 3.266	IF = 18.522 C + 9.354
R^2	0.9991	0.9974
Concentration range (ng mL^{-1})	100.0-1800.0	2.0-50.0
LOD (ng mL^{-1})	40.0	0.7
RSD for 10.0 ng mL^{-1} (n=3)	-	0.14
RSD for 30.0 ng mL^{-1} (n=3)	-	0.12
RSD for 150.0 ng mL^{-1} (n=3)	0.18	0.32
RSD for 500.0 ng mL^{-1} (n=3)	0.08	0.36

Table 3 Determination of the naproxen enantiomers mole fraction in synthetic samples.

Theoretical			Experimental					
$X_{S\text{-}Nap}$[a]	$X_{R\text{-}Nap}$[b]		Procedure (I-II)		Procedure (I-III)		Procedure (II-III)	
			$X_{S\text{-}Nap}$	$X_{R\text{-}Nap}$	$X_{S\text{-}Nap}$	$X_{R\text{-}Nap}$	$X_{S\text{-}Nap}$	$X_{R\text{-}Nap}$
0.00	1.00		0.07±0.01[c]	0.93±0.08	0.01±0.01	0.96±0.05	0.01±0.02	1.10±0.08
0.25	0.75		0.28±0.05	0.76±0.07	0.24±0.02	0.74±0.05	0.25±0.05	0.78±0.03
0.50	0.50		0.55±0.10	0.57±0.10	0.51±0.02	0.50±0.02	0.49±0.05	0.52±0.06
0.75	0.25		0.73±0.08	0.20±0.03	0.77±0.07	0.26±0.01	0.73±0.07	0.22±0.02
1.00	0.00		1.02±0.11	0.08±0.01	0.98±0.06	0.01±0.01	1.01±0.12	0.02±0.01
		$\chi^2\times10^3$	9.53	24.83	1.53	2.13	0.83	5.7

[a] Mole fraction of S-Nap

[b] Mole fraction of R-Nap

[c] RSD percent calculated for n=3

Where IF is the fluorescence intensity of eluate at λ_{em}= 356 nm (λ_{ex}= 271 nm) and λ_{em}= 353 nm (λ_{ex}= 271 nm) in the case of calibration curve 1 and calibration curve 2, respectively. C is the concentration of Nap and S-Nap in ng mL^{-1} in the case of calibration curve 1 and calibration curve 2, respectively. 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.

As a feasible test to the proposed procedure, various synthetic samples with different enantiomeric compositions, which were prepared by spiking enantiomer mixtures to the buffer solution, were analysed at optimum condition. In order to evaluate of the precision and accuracy of the measurement using three proposed procedures, the results have been assessed by non-linear Chi-square (χ^2). The Chi-square test measures the difference between the experimental and theoretical data. The mathematical form of this test statistic can be expressed as:

$$\chi^2 = \sum \frac{(X_{exp} - X_{the})^2}{X_{the}} \quad (2)$$

where X_{exp} is the experimental mole fraction data and X_{the} is the theoretical values.

The lower χ^2 values (Table 3) for the procedure (I-III) in comparison with the two investigated procedures, suggest that this procedure is more proper for the special goal of this work. This is maybe due to higher efficiency of affecting factor optimization.

Conclusions

This work report on the synthesis of an enantioselective nanoadsorbent, *via* chemical attachment of L-(-)-Tryptophan onto magnetite nanospheres surface, for the removal and preconcentration of S-Nap in the presence of its enantiomer. Enantioselective removal and preconcentration of S-Nap ability of the adsorbent provided three procedures for Nap enantiomeric composition analysis. The efficiency of each procedure has been investigated and the optimum conditions have been proposed. The results of this work promises a methodology for enantiomeric composition analysis of chiral compound (especially Nap) in concentration limit up to ng mL^{-1} levels.

Notes

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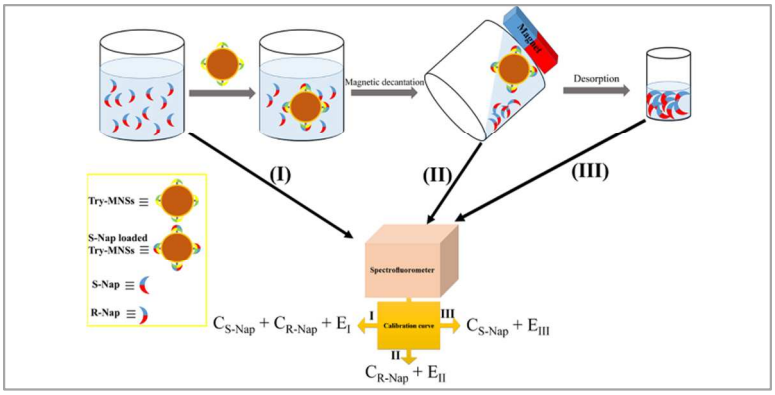

Enantioselective solid phase extraction of naproxen drug enantiomers