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Synthesis and characterization of core–shell magnetic molecularly imprinted polymer nanoparticles for selective extraction of tizanidine in human plasma

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Abstract. **In this study, simple, effective and general processes were used for the synthesis of a new nano-molecularly imprinted polymers (MIPs) layer on magnetic Fe_3O_4 nanoparticles (NPs) with uniform core–shell structure by combining surface imprinting and nanotechniques. The first step for the synthesis of magnetic NPs was co-precipitation of Fe^{2+} and Fe^{3+} in an ammonia solution. Then, an SiO_2 shell was coated on the magnetic core with the Stöber method. Subsequently, the C=C groups were grafted onto the silica-modified Fe_3O_4 surface by 3-(trimethoxysilyl) propyl methacrylate. Finally, MIPs films were formed on the surface of Fe_3O_4@SiO_2 by the copolymerization of C=C end groups with methacrylic acid (functional monomer), ethylene glycol dimethacrylate (cross-linker), 2,2-azobisisobutyronitrile (initiator) and tizanidine (template molecule). The products were characterized using techniques that included Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), thermo gravimetric analysis (TGA), scanning electron microscopy (SEM), UV spectrophotometry, transmission electron microscopy (TEM) and vibrating sample magnetometer (VSM). Measurement of tizanidine through use of the core–shell magnetic molecularly imprinted polymers nanoparticles (MMIPs-NPs) in human plasma samples compared to the paracetamol showed that the synthesized nanosized MMIP for tizanidine has acted selectively.**

Keywords. **Magnetic nanoparticles; molecularly imprinted polymers; tizanidine; paracetamol; human plasma.**

1. Introduction

Tizanidine hydrochloride or 5-chloro-N-(2-imidazolin-2-yl)-2,1,3-benzothiadiazol-4-ylamine hydrochloride (1-3), is a centrally acting skeletal muscle relaxant. It is a α2-adrenergic agonist that acts mainly at spinal and supraspinal levels to inhibit excitatory interneurons. It is used for the symptomatic relief of spasticity associated with multiple sclerosis or with spinal cord injury or disease. It is also used in the symptomatic treatment of painful muscle spasm associated with musculoskeletal conditions [1]. The United States Pharmacopoeia (USP) recommends HPLC method for determination of tizanidine (I) in the raw materials and tablets. Additionally, a number of methods like spectrophotometer [2], voltammeter [3], GC [4], TLC [5] and HPLC [6], have been reported in the literature for the determination of tizanidine hydrochloride.

Magnetic solid phase extraction (MSPE) by solid nanoparticles as the adsorbents has recently attracted much attention among the scientific community. In 1973, Robinson and his colleagues [7] first used magnetic separation methods in the biotechnology industry. In 1987, Wickstrom and colleagues [8,9] stated that the liquid/liquid extraction methods are comparable with other methods using additives such as iron and iron oxide and quickly lead to phase separation. However, MSPE was first introduced by Šafaříková and Šafařík [10] in 1999 for analytical purposes. In this procedure, MPs are added into the sample solution and the analyte is adsorbed on the surface of the magnetic beads which are separated from the aqueous solution by means of an external magnetic force. Then, the analyte is desorbed by the eluent for further diagnosis. Compared with conventional SPE, sample pretreatment is greatly simplified through the use of MSPE. In batch mode operation, packing of the column with the sorbent is not necessary, since phase separation can be quickly and easily accomplished by applying an external magnetic field [11]. In 1998, MMIP was produced for the first time with a mean diameter of 13 μm using a magnetic iron oxide from the polymerization of monomers in liquid perfluoro-chlorine [12]. Development of this method and the use of special functional monomers have resulted in the production of MMIP to which some stimuli respond including thermal stimuli [13], optical stimuli [14] and PH stimuli [15]. In addition, the use of magnetic nanoparticles, nanocapsules, nanowires and nanotubes that have a high surface-to-volume ratio can also be connected to the MIP, increasing binding capacity and kinetics [16,17].

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Applications of MMIP have broadened in recent years and now include cell separation, stabilization of proteins, enzymes and other molecules; very quick concentration of target molecules in the sample; solid-phase extraction techniques; and chromatography and catalytic applications. Specific applications that are detailed in published studies include identification and extraction of antibiotics [18,19], identification and removal of water and aqueous solutions [20,21], recognition of low molecular weight templates in biomimetic sensors [22], extraction and identification of certain compounds from the urine [23], extraction and identification of hormones [24] or chemical compounds [25] and colour identification [26]. In general, the most important feature of MMIPs is that one can separate and recover them from the solution by the aid of an external magnetic field, as MMIPs do not lump after removal of the external field and it is possible to reuse them.

The aim of this study was to prepare core–shell magnetic molecularly imprinted polymer nanoparticles (MMIP-NPs) for selective extraction of tizanidine. Measurement of tizanidine obtained from MMIP-NPs showed that significant differences exist in the absorption of tizanidine and paracetamol extracted from MMIP-NPs. The imprinted polymer showed good selectivity for tizanidine.

2. Experimental

2.1 *Reagents and solutions*

The drugs used in this study were obtained from Daroupakhsh Co. (Tehran, Iran). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2,2-azobisisobutyronitrile (AIBN), acetic acid (HOAC), methanol (MeOH), tetraethoxysilane (TEOS). $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, hydrogen chloride (HCl) and anhydrous toluene were purchased from Merck chemical company, 3-(trimethoxy silyl) propyl methacrylate 98% was purchased from Sigma Aldrich company, all other chemicals used in this study were of analytical reagent grade and obtained from Merck. Double-distilled water was used throughout the experiments. A stock solution of target analyte (namely, tizanidine (template)) was prepared from methanol containing 1000 mg l^{-1} of the drug. The working solution (5 mg l^{-1}) was prepared daily with the appropriate dilution of tizanidine stock.

2.2 *Instrumentation*

The template was separated by UV (Lambda 25 dual-beam; Perkin Elmer, Waltham, Massachusetts, USA). The FT-IR spectra in KBr were recorded using a Spectrum RXI (Perkin Elmer, USA), a scanning electron microscope (LEO 1430VP; Leo Pharma UK, Berkshire, UK), transmission electron microscopy (TEM), X-ray diffraction (XRD, D8 Advance, Bruker-AXS, Karlsruhe, Germany; Madison, Wisconsin, USA), thermogravimetric analysis (TGA, model PL, UK), magnet 1.3 T dimensions 20 × 40 × 50 cm were used to characterize the core–shell nonmagnetic molecularly imprinted polymer.

The magnetic properties were analysed with a vibrating sample magnetometer (VSM) (LDJ 9600-1, USA).

2.3 *Synthesis of (Fe_3O_4@SiO_2–C=C–MIP) NPs or MMIPs-NPs*

There are four basic steps in the synthesis of MMIPs-NPs according to figure 1, as described in this section.

2.3a *Magnetic Fe_3O_4 nanoparticles (MNPs)*: 11680 mg $FeCl_3 \cdot 6H_2O$ and 4300 mg $FeCl_2 \cdot 4H_2O$ were dissolved in 200 ml of deionized water with a nitrogen atmosphere with vigorous stirring at 70–85°C. Then, 20 ml of 30% aqueous ammonia was added to the mixture rapidly; the colour of the bulk solution immediately changed from orange to black during this step. After magnetic separation via an external magnetic field, MNPs were washed with deionized water and ethanol. Finally, MNPs were dried under a vacuum at 70°C [27].

2.3b *Silica-coated magnetic Fe_3O_4 nanoparticles*: 300 mg of Fe_3O_4 was dispersed in 40 ml of ethanol and 4 ml of ultrapure water by ultra-sonication for 15 min, followed by the addition of 5 ml of $NH_3 \cdot H_2O$ (28%) and 2 ml of TEOS. The mixture was reacted for 12 h at room temperature with stirring at 400 rmp. The products were collected by magnetic separation, washed with diluted HCl and ultrapure water, and dried under vacuum [28].

2.3c *Surface modification of silica-coated magnetic Fe_3O_4 nanoparticles*: 250 mg of Fe_3O_4–SiO_2 particles was dispersed in 50 ml of anhydrous toluene containing 5 ml of 3-(trimethoxysilyl) propyl methacrylate (98%) and the mixture was allowed to react at 70°C for 12 h under dry N_2. The products (Fe_3O_4–SiO_2–C=C) were obtained after magnetic separation, washing with water and drying under vacuum.

2.3d *Polymerization*: In this step, five reagents are used: magnetic nanoparticles coated with silica, cross-linking agent EGDMA, monomers containing functional group MAA, initiator AIBN and tizanidine as target. Tizanidine (58.04 mg) was stirred with 40 ml ethanol and 110 mg monomer MAA for 12 h. With the addition of these materials while stirring, the temperature rises gradually and the reactions ensue. Then, 520 mg EGDMA and 50 mg AIBN were added and the mixtures sonicated for 15 min. Finally, the system was reacted at 62°C [29] for 24 h purge with N_2 gas.

2.3e *Synthesis of MNIPs-NPs*: MNIPs were prepared in a way that was similar to the synthesis of MMIPs without the addition of template molecule.

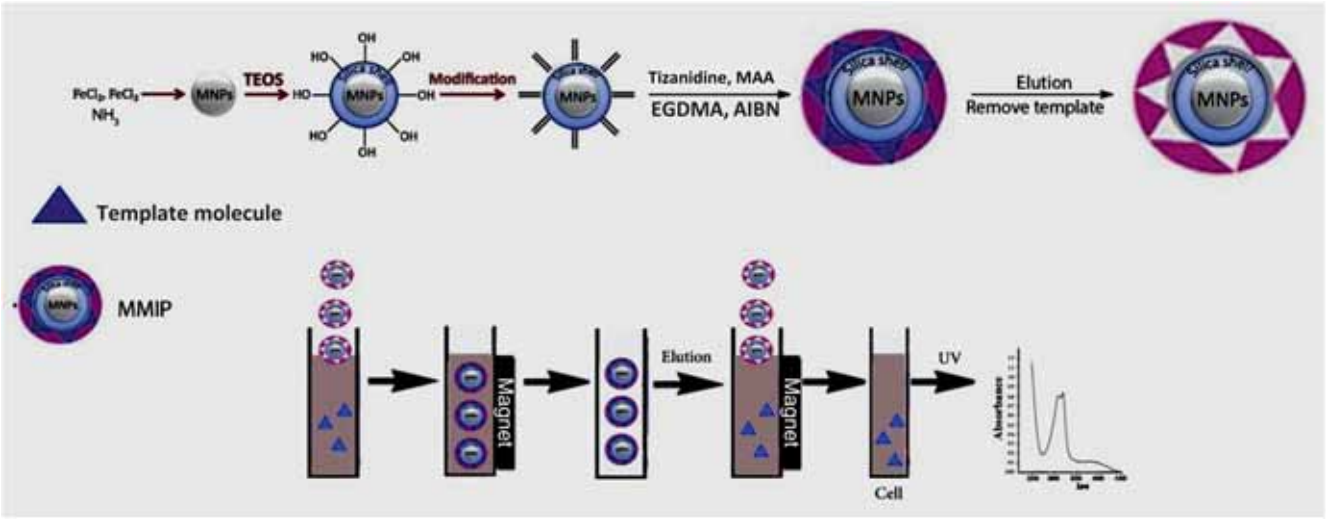

Figure 1. Schematic diagram of synthesis of MMIPs-NPs and their application for extraction of tizanidine by an external magnetic field.

3. Results and discussion

Figure 2 shows X-ray diffraction (XRD) patterns for the synthesized Fe_3O_4, $Fe_3O_4@SiO_2$ and MMIPs. In the 2θ range of 20–70°, six characteristic peaks for Fe_3O_4 (2θ = 30, 35, 43, 53.5, 57 and 63°) were observed for the three samples, and the peak positions at the corresponding 2θ value were indexed as (220), (311), (400), (422), (511) and (440), respectively, which matched well with the database of magnetite in the JCPDS-International Center for diffraction data (JCPDS card: 19-629) file. The XRD patterns show the presence of specific diffraction peaks of the synthesized particles, which are highly crystalline. However, it is insufficient to exclude the presence of γ-F_2O_3; there are probably two types of iron oxide particles in this dispersion, maghemite and magnetite [29–32]. The trace amount of maghemite could be attributed to the oxidation of Fe_3O_4 to γ-F_2O_3 during the coprecipitation and silanization processes [33]. As they have similar magnetic properties, identification is not important in the present study.

Figure 3 shows FT-IR spectra for (a) unleached MMIPs, (b) leached MMIPs and (c) MNIPs. In all three spectra, the peak at 590 cm^{-1} is attributed to the stretch of Fe–O. In comparison with the infrared data of pure Fe_3O_4, the characteristic peaks for the Si–O–Si group at about 1150 cm^{-1} and for the Si–O group at about 800 and 470 cm^{-1} indicate the formation of silica coating on the surface of Fe_3O_4. The strong vibrational band (about 1720 cm^{-1}) is due to the –C=O of the carboxylic acid group of methacrylic acid, which is typically located at the surfaces of the synthesized polymeric particles. This band can be observed in all the examined polymers, including unleached MMIPs, leached MMIPs and MNIPs. In spectrum (a) for unleached MMIPs, there is a band at about 1647 cm^{-1}, which is the result of the –C=O, via coordination bonding. These observations and examined evidence proved the presence and efficient interaction of the selective recognition sites in the MIP particles,

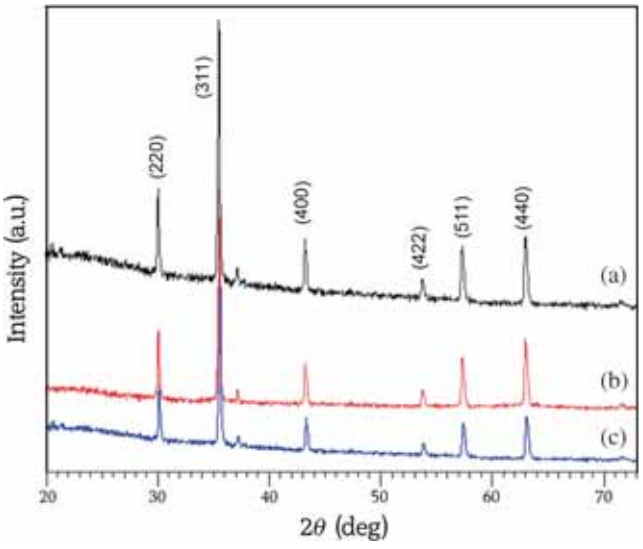

Figure 2. The XRD pattern of (**a**) magnetic nanoparticles Fe_3O_4, (**b**) $Fe_3O_4@SiO_2$ and (**c**) MMIPs-NPs.

which were produced in the course of the imprinting procedure. After the polymers were eluted, the FT-IR spectrum of MIP is very close to that of NIP. The results show that the template molecule has been removed from the MIP.

Figure 4 shows the transmission electron microscopy (TEM) images of Fe_3O_4 and $Fe_3O_4@SiO_2$. The mean diameter of Fe_3O_4 was about 30 nm (figure 4a) and the core–shell structure of $Fe_3O_4@SiO_2$ NPs with silica coating was successfully prepared (figure 5b). After coating with SiO_2, the diameter of the $Fe_3O_4@SiO_2$ increased to approximately 40 nm.

Figure 5 shows the scanning electron microscopy (SEM) images of MMIPs-NPs. With tizanidine as the template, the average diameter of MMIPs-NPs relatively increased to about 80 nm.

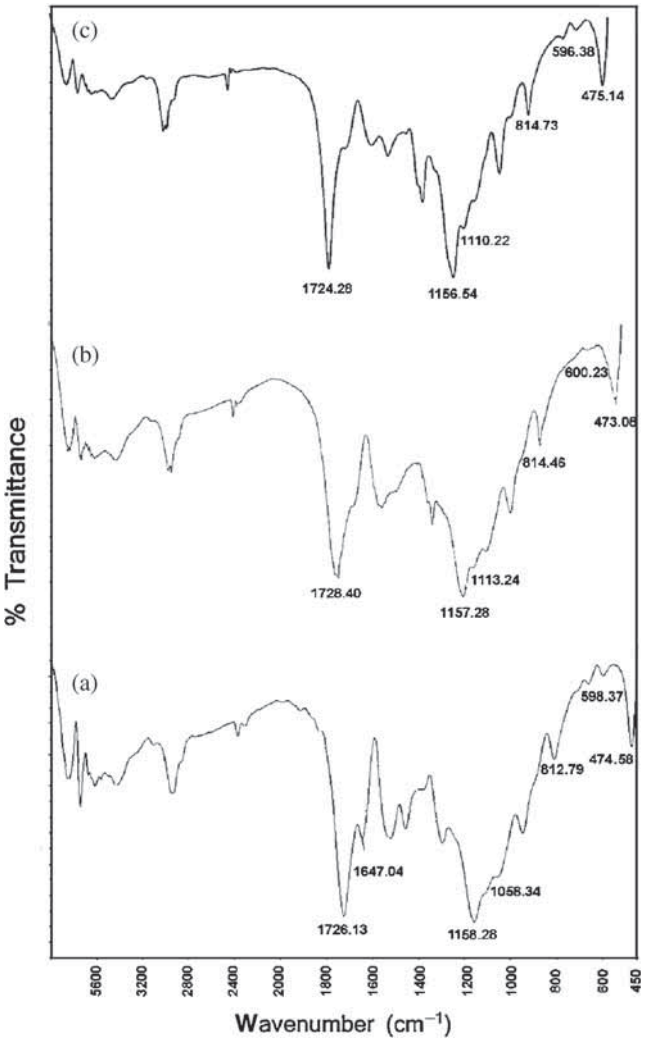

Figure 3. FT-IR spectra for (**a**) unleached MMIPs, (**b**) leached MMIPs and (**c**) MNIPs.

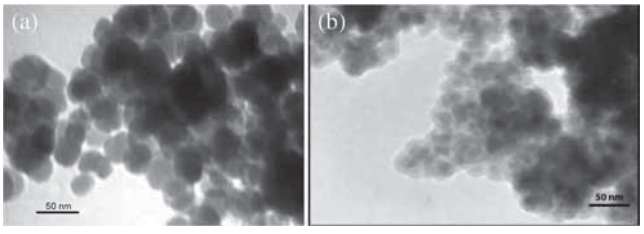

Figure 4. Transmission electron microscopy image of (**a**) Fe_3O_4 and (**b**) Fe_3O_4@SiO_2.

Figure 6 clearly shows the TGA plot for the imprinted polymer. TGA plot for MMIP-NPs can prove the thermal stability of the synthesized polymer. The thermal stability is due to the presence of Fe_3O_4 and SiO_2 in the core–shell system. Therefore, the primary mass removal (about to 13%) can be related to the polymeric portion in this system; however, no mass removal was experienced with respect to thermal stability.

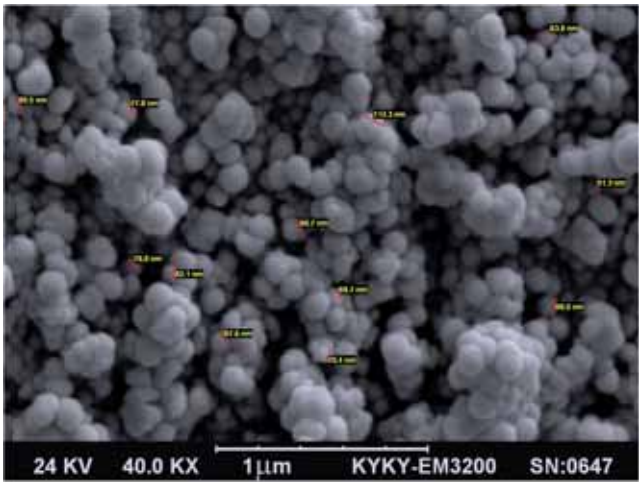

Figure 5. Scanning electron microscopy image of MMIPs-NPs.

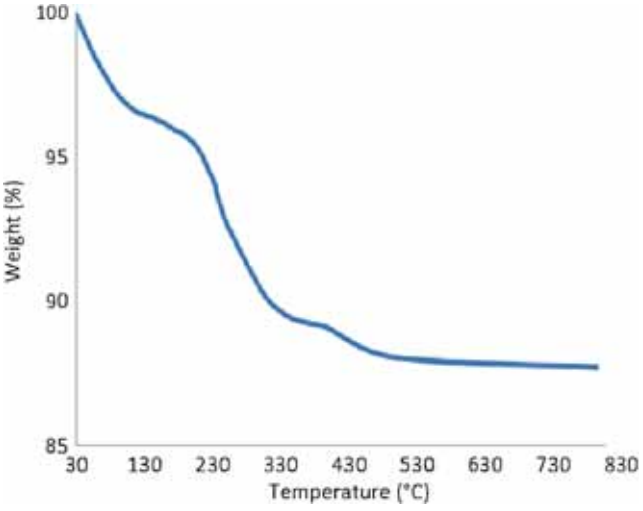

Figure 6. Thermogravimetric analysis of MMIPs-NPs.

Figure 7 shows the employment of vibrating sample magnetometry (VSM) to study the magnetic properties of the synthesized magnetic NPs, and the magnetic hysteresis loop of the dried samples at room temperature. There is no hysteresis and both remanence and coercivity are zero, suggesting that the samples are super paramagnetic. The saturation magnetization values obtained at room temperature were about 40, 20 and 10 emu g^{-1} for Fe_3O_4, Fe_3O_4@SiO_2 and MMIP, respectively. The theoretical value of saturation magnetization for bulk magnetite is reported to be 92 emu g^{-1} [34,35]. The decrease in magnetization value can be attributed to the small particle surface effect, such as a magnetically inactive layer containing spins that are not collinear with the magnetic field [36]. The saturation magnetization of MMIP was reduced to 10 emu g^{-1} in comparison with the pure Fe_3O_4, but remained strongly magnetic at room temperature and allowed to work as effective magnetic separation carrier. Figure 7a shows the separation and redispersion process of MMIP. In the absence of an external magnetic field, a dark homogeneous dispersion exists. When an external magnetic

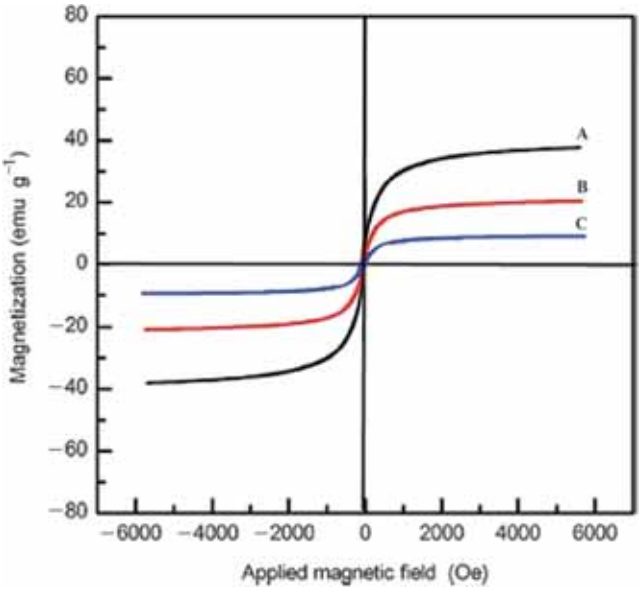

Figure 7. VSM images of (A) Fe_3O_4, (B) Fe_3O_4@SiO_2 and (C) MMIP.

field was applied, the black particles were attracted to the wall of the land via the dispersion became clear and transparent. The super paramagnetism of MMIP prevents MIPs from aggregating and enables them to redisperse rapidly after removal of the magnetic field (figure 7b).

4. Optimization of the MSPE procedure

4.1 *Effect of pH*

The pH value of the sample solution plays a significant role in analyte adsorption by nano-MIP as a result of the chemical structure of the analytes and functional groups existing in the nano-MIP. Therefore, the extraction efficiency of the analyte can be affected by the pH value [36]. To evaluate the effect of pH, a working solution with different pH in the range of 2–10 was studied. As shown in figure 8, the highest adsorption of tizanidine by MIP-NPs was almost constant at pH 8–10. Thus, the pH 8 was selected as optimized pH according to the findings from the present results.

4.2 *Choice of eluent*

This step involves the examination of several solvents. The results have been presented in table 1. From the results, acetic acid in methanol (10 : 90, v/v%) exhibited better recovery compared with the other solvents. Thus, imprinted tizanidine molecules were displaced from the polymeric structure using acetic acid in methanol (10 : 90, v/v%).

4.3 *Effect of elution solvent volume*

To evaluate elution solvent volume on the recovery of the tizanidine from MIP-NPs, various volumes (from 2 to 5 ml)

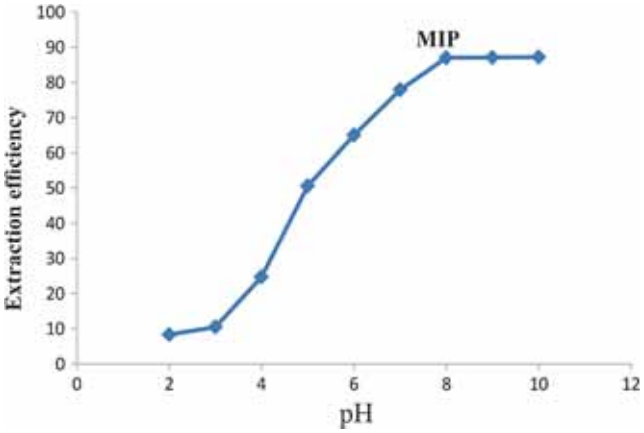

Figure 8. The effect of solution's pH on the extraction efficiency of tizanidine. Experimental and extraction conditions (tizanidine concentration: 5 mg l^{-1}, sample volume: 5 ml).

Table 1. The effect of elution solvent type (the volume for each elution solvent was 5 ml) on the recovery of the tizanidine from MMIP-NPs (the obtained results are the mean of three measurements).

Elution solvent	v/v (ml)	$R^a \pm S^b$
Water	100	68.38 ± 0.17
Methanol	100	98 ± 0.43
Acetic acid : methanol	10 : 90	99.12 ± 0.2
Acetic acid : methanol	80 : 20	98.78 ± 0.31
Acetic acid : methanol	70 : 30	98.47 ± 0.48
Acetic acid : methanol	60 : 40	97.5 ± 0.61
Acetic acid : methanol	50 : 50	96.4 ± 0.8

[a]Recovery (%); [b]standard deviation ($n = 3$).

Table 2. The effect of eluent solvent volume on the recovery of the tizanidine from MIP-NPs (the obtained results are the mean of three measurements).

Acetic acid : methanol	v/v (ml)	$R^a \pm S^b$
10–90	5	99.12 ± 0.2
10–90	4	98.70 ± 0.57
10–90	3	98.43 ± 0.92
10–90	2	98.26 ± 1.17

[a]Recovery (%); [b]standard deviation ($n = 3$).

were studied. As shown in table 2, 5 ml of acetic acid in methanol (10 : 90, v/v%) was used as optimum elution solvent volume.

4.4 *Effect of amount of nano-sized MMIP*

To investigate the optimum amount of MIP-NPs on the extraction efficiency of tizanidine, various amounts were studied from 50 to 200 mg, thus, a 100 mg amount used as the optimum amount of MIP.

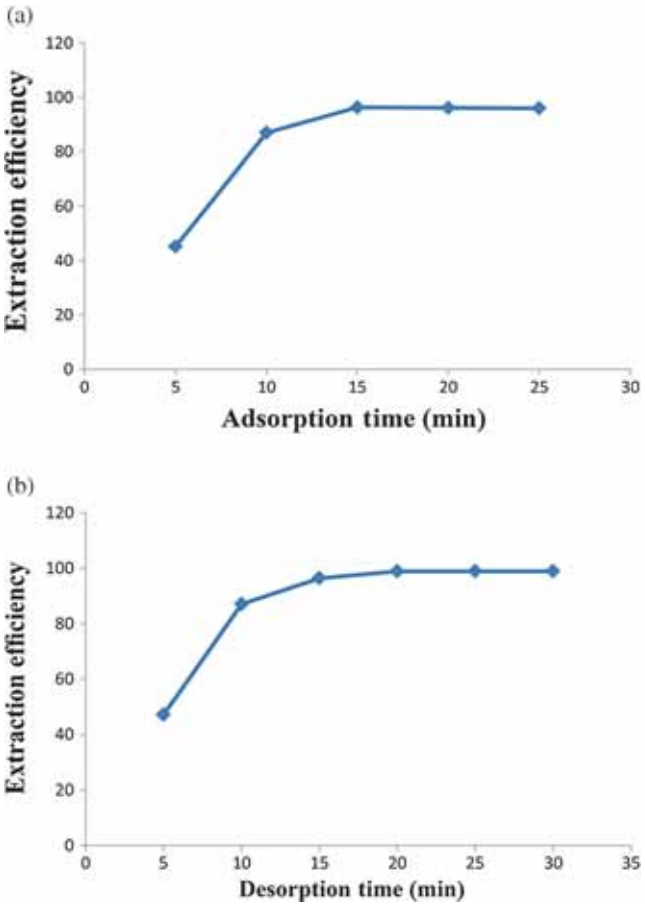

Figure 9. (**a**) The effect of adsorption time and (**b**) desorption time on the extraction efficiency synthesized magnetic molecularly imprinted polymer nanoparticles.

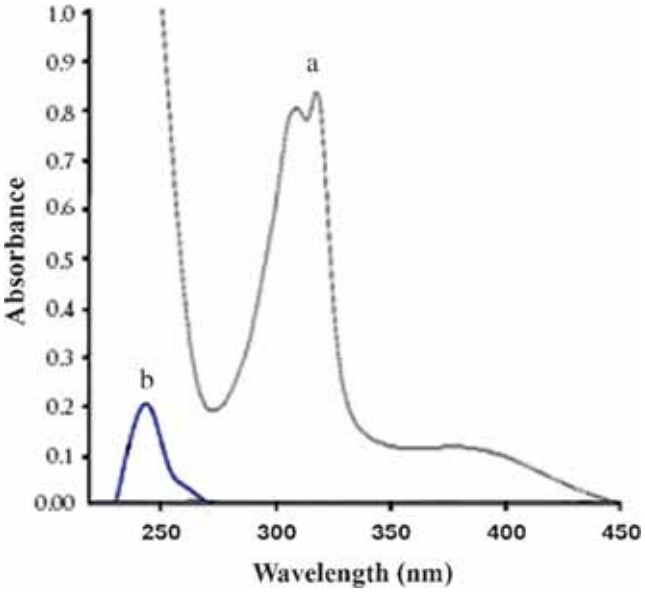

Figure 10. Absorption spectra of (a) tizanidine and (b) paracetamol by MMIPs.

4.5 *Effect of adsorption and desorption times*

To investigate the optimal time for adsorption and desorption, time intervals from 5 to 30 min were studied, with other parameters being kept in optimum conditions. As shown in figure 9, 15 and 20 min time intervals were chosen as the optimum adsorption and desorption times, respectively.

5. Measurement of tizanidine in real sample

Human plasma samples were collected from healthy volunteers, and the tizanidine was extracted and analysed by means of MMIP-NPs in their optimized conditions with the subsequent determination by UV–Vis spectrophotometer. All precipitated materials were removed by centrifuging the samples at 3800 rpm for 20 min. Figure 10a and b illustrates the UV spectra tizanidine and paracetamol after the extraction from plasma samples at the concentration level of 2 mg l^{-1} of tizanidine and paracetamol by nanosized MMIP, respectively. As shown in figure 10a and b, synthesized nanosized MMIP for tizanidine has acted selectively.

6. Conclusion

In this study, simple, effective and general processes were used for the synthesis of a nano-MIPs layer on magnetic Fe_3O_4 NPs with a uniform core–shell structure by combining surface imprinting and nanotechniques. By choosing the right conditions for the synthesis of MMIPs-NPs, this method can also be used to identify other drugs. Moreover, MMIPs-NPs provide conditions for control and separation using a magnetic field and (as an overall technique) provide an alternative to the use of a centrifuge and different kinds of filtration due to the fact that they are available and economical. Other advantages of the suggested method include low consumption of organic solvent, simplicity and selectivity. Synthesized MMIPs-NPs can be used repeatedly with no significant decrease in binding affinities. Measurements of tizanidine obtained from MMIP-NPs in human plasma showed that there were significant differences in absorption between tizanidine and paracetamol extracted from MMIP-NPs. The results showed that synthesized nanopolymers acted selectively.

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