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Magnetic solid phase extraction of gemfibrozil from human serum and pharmaceutical wastewater samples utilizing a β-cyclodextrin grafted graphene oxide-magnetite nano-hybrid

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ABSTRACT

A magnetic solid phase extraction method based on β-cyclodextrin (β-CD) grafted graphene oxide (GO)/magnetite (Fe_3O_4) nano-hybrid as an innovative adsorbent was developed for the separation and pre-concentration of gemfibrozil prior to its determination by spectrofluorometry. The as-prepared β-CD/GO/Fe_3O_4 nano-hybrid possesses the magnetism property of Fe_3O_4 nano-particles that makes it easily manipulated by an external magnetic field. On the other hand, the surface modification of GO by β-CD leads to selective separation of the target analyte from sample matrices. The structure and morphology of the synthesized adsorbent were characterized using powder X-ray diffraction, Fourier transform infrared spectroscopy, and field emission scanning electron microscopy. The experimental factors affecting the extraction/pre-concentration and determination of the analyte were investigated and optimized. Under the optimized experimental conditions, the calibration graph was linear in the range between 10 and 5000 pg mL^{-1} with a correlation coefficient of 0.9989. The limit of detection and enrichment factor for gemfibrozil were 3 pg mL^{-1} and 100, respectively. The maximum sorption capacity of the adsorbent for gemfibrozil was 49.8 mg g^{-1}. The method was successfully applied to monitoring gemfibrozil in human serum and pharmaceutical wastewaters samples with recoveries in the range of 96.0–104.0% for the spiked samples.

1. Introduction

Applying a simple and selective sample preparation procedure prior to instrumental analysis is the most important and crucial step in an analytical process. Up to now, various sample preparation techniques based on solid phase extraction (SPE) systems have been developed to isolate various types of analytes from different matrices. However, in spite of the whole advantages of SPE, it can still be tedious, time consuming, and relatively expensive [1]. Recently, a new mode of SPE called magnetic solid-phase extraction (MSPE) has been developed [2]. MSPE is based on the combination of magnetic inorganic material and non-magnetic adsorbent material [3]. By taking advantages of the combined benefits of both the materials, the MSPE technology exhibits excellent adsorption efficiency and rapid separation from the crude sample matrix by an external magnetic field [3,4].

It is obvious that the excellent absorbent materials must have high specific surface area, chemical stability, and a lot of adsorption sites [5]. Carbon-based nanomaterials, which have unique π-electronic structure, have been used as excellent adsorbents in cleanup procedures [6]. Recently, Graphene, as a newly found carbon-based nanomaterial with fascinating two-dimensional atomic thickness structure and large theoretical specific surface area, has attracted wide attention and become a hot research tide [7]. However, there are main drawbacks in the usage of graphene as a sorbent material. Graphene nanoparticles tend to aggregate, which may lead to great reduction in the surface area and its adsorption efficiency. Moreover, graphene is an ultralight material and it is usually hard to retrieve from a suspension even by high-speed centrifugation [8,9]. Therefore, chemical modification of graphene is imperative. Graphene oxide (GO), a chemically modified graphene sheet with a giant aromatic macromolecule containing reactive oxygen functional groups on its basal planes and edges such as epoxide, hydroxyl and carboxylic acid, is a unique structure with remarkable properties such as superior dispersibility, and facile modification via its reactive groups which further enhance the selectivity of GO as a sorbent material [10]. Moreover, introducing magnetic properties into graphene or GO can combine the high adsorption capacity of these carbon based nanomaterials and the separation convenience of magnetic materials through a MSPE methodology [4,11,12]. On the other hand, the surface modification of

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magnetic GO can lead to selective separation of analytes from samples with complicated matrices.

β-cyclodextrin (β-CD) is a macrocyclic compound composed of seven D-glucose units linked together by α-(1,4)-glycosidic bonds in a torus shaped structure with a hydrophobic inner cavity, and a hydrophilic outer side [13]. β-CD can selectively bind with various organic, inorganic and biological guest molecules into its cavity to form stable host–guest inclusion complexes by a series of forces such as hydrophobic and van der Waals interactions [14,15]. Consequently, the combination of β-CD and GO simultaneously possesses the unique properties of GO (large surface area and high dispersilbility) and β-CD (high supramolecular recognition capability), providing a good opportunity for application in sample pretreatment methodology [14,16].

Gemfibrozil, 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, is a benzene derivative of valeric acid belonging to a drug group known as fibrates [17]. It is clinically effective in reducing serum cholesterol and triglyceride levels. It has also been demonstrated that this drug lowers the incidence of coronary heart disease in humans [18]. Several methods have been developed for the determination of gemfibrozil in biological samples, environmental substances and pharmaceutical formulations, including high performance liquid chromatography with fluorescence detection, liquid chromatography–tandem mass spectrometry, gas chromatography–mass spectrometry and spectrofluorometry [17–22].

The host–guest inclusion complex between β-CD and gemfibrozil has been previously proven [22], and as mentioned above, the adsorption properties and selectivity of the GO regarding the target analytes could be significantly improved in the presence of β-CD. Therefore, in this work, we report on the first application of β-CD grafted graphene oxide-magnetite nanohybrid as a novel sorbent for MSPE of gemfibrozil from human serum and pharmaceutical wastewater samples prior to spectrofluorometric determination at λ_{em}=304 nm after excitation at 276 nm.

2. Experimental

2.1. Apparatus and instruments

Fluorescence spectra and intensity measurements were carried out using a FP-6200 JASCO Corporation (Tokyo, Japan) spectrofluorometer with a wavelength range of 220–730 nm (with 1 nm intervals) for excitation and emission. The instrument equipped with a 150 W xenon lamp, 1.0 cm quartz cell, Peltier thermostatted single cell holder (model ETC-272), and supported with PC-based Windows® Spectra Manager TM software for JASCO Corporation version 1.02. The slit widths for both excitation and emission were set at 5 nm and the fluorescence spectra were recorded at a scan rate of 250 nm min^{-1}.

In order to structural study of the nano-particles, XRD measurements were performed on a Bruker AXS model D8 Advance (Karlsruhe, Germany) instrument with Cu-K_α radiation source (1.54 Å) between 2 and 70° generated at 40 kV and 35 mA at room temperature. Samples for XRD were ground into powder and then pressed flat in the sample slot. In addition, FT-IR spectra (4000–400 cm^{-1}) were recorded on a Bruker model Vector 22 (Ettlingen, Germany) Fourier transform infrared spectrometer using the KBr disk method with a ratio sample/KBr of 1:100 by mass. A scanning electron microscope (SEM), model LEO1430vp (Carl Zeiss, Geramany), was additionally used to examine the morphological characteristics of the sorbent. An ultrasonic bath (SONICA, Italy) was used to disperse the adsorbent in sample solution vials. A shaker (Pars Azma Co., Iran) was used for controlled stirring the sample solution vials in adsorption and desorption steps. The pH values were measured with a Metrohm digital pH-meter model 827 (Herisau, Switzerland) supplied with a glass-combined electrode. An electronic analytical balance, Mettler Toledo model PB303 (Greifensee, Switzerland) was used for weighting the solid materials.

2.2. Standard solutions and reagents

All chemicals used were of analytical reagent grade and all solutions were prepared with high purity deionized water obtained from Shahid Ghazi Co. (Tabriz, Iran). Graphite flakes (99.99%) $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, $NH_3 \cdot H_2O$ and other chemical reagents was purchased from Merck (Darmstadt, Germany). β-cyclodextrin was purchased from Acros organics (Geel, Belgium). A stock standard solution of 400 mg L^{-1} gemfibrozil (Sigma-Aldrich, St. Loius, MO, USA) was prepared in a 100 mL volumetric flask by dissolving 40.0 mg of gemfibrozil in approximately 10 mL of 0.1 mol L^{-1} sodium hydroxide and diluting to the mark with deionized water.

2.3. Preparation of the nano-sorbent

GO was prepared by oxidizing graphite with acid by a modified Hummers' method [23]. GO/Fe_3O_4 nano-hybrid was synthesized by the *in situ* chemical precipitation of Fe^{2+} and Fe^{3+} in alkaline solution in the presence of GO. For this purpose, 80 mg $FeCl_2 \cdot 4H_2O$ and 216 mg $FeCl_3 \cdot 6H_2O$ were added to 20 mL deionized water containing 40 mg well-distributed GO suspension at 50 °C under a nitrogen atmosphere. After the solution was ultrasonicated for 20 min, 1 mL solution of $NH_3 \cdot H_2O$ was added dropwise into the mixture with vigorous stirring and then heated to 50 °C for 40 min under a nitrogen atmosphere. After cooling to room temperature, the precipitate was isolated by a commercial magnet and washed several times with the deionized water. The resulting product was dispersed in 20 mL water and the homogeneous product of 4 mg mL^{-1} GO/Fe_3O_4 suspension was obtained. For the grafting of GO/Fe_3O_4 nano-hybrid with β-CD, 10 mL of 4 mg mL^{-1} GO/Fe_3O_4 suspension was mixed with 10 mL of 4 mg mL^{-1} β-CD aqueous solution. After being vigorously shaken, the vial was put in a water bath at 60 °C for 3.5 h [10]. The resultant material was precipitated and separated by a magnetic field for several cycles to remove excess β-CD.

2.4. Sample preparation

2.4.1. Human serum samples

All experiments on humans were performed in compliance with the relevant laws and institutional guidelines approved by the Medical Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran. Required consent was obtained. Human serum samples were selected as real samples for analysis by the presented method. To precipitate and remove interfering proteins, the serum samples were diluted 1:4 with acetonitrile and centrifuged for 10 min at 4000 rpm [22]. Then, 1 mL of the supernatant was diluted 200 times and subjected to extraction and determination by following the procedure described in Section 2.5.

2.4.2. Pharmaceutical wastewater samples

Pharmaceutical wastewater samples were collected from Pharmaceutical Manufactory effluents in Tehran, Iran. These samples were filtered through Black band filter paper and centrifuged to remove any suspended particulate. Then, aliquots of 200 mL from samples were analyzed within 24 h of collection without other treatments.

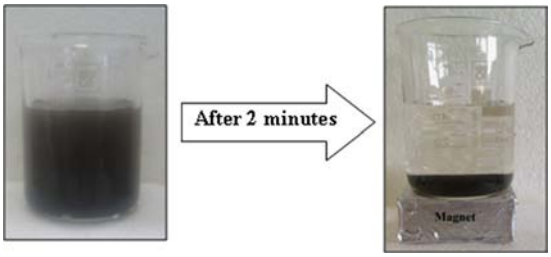

Fig. 1. The photographic pictures of sample solution containing GO/Fe_3O_4/β-CD before and after being exposed to external magnetic field.

2.4.3. Pharmaceutical preparations

In order to find the average mass of each capsule, five capsules of gemfibrozil were weighed. Then, the contents were powdered and mixed. A portion of 10.0 mg of the powder was accurately weighed and dissolved in 10 mL of 0.1 mol L^{-1} sodium hydroxide solution and filtered into a 100 mL volumetric flask [18]. The residue was washed several times with deionized water and the flask was then made up to the mark with the water. A suitable aliquot of this solution was diluted stepwise and taken for magnetic solid phase extraction-spectrofluorometric determination of gemfibrozil.

2.5. General procedure

150 mg of the nano-sorbent was placed in a 250 mL glassware beaker. Then, 200 mL portion of the standard or sample solution containing gemfibrozil in the range of 0.01–5 μg L^{-1} was transferred into a beaker. To disperse the nano-sorbent homogenously through the whole solution, the beaker was placed in an ultrasonic bath for 1 min. The adsorption of gemfibrozil on the sorbent was performed under continuous mechanical stirring of the mixture by a shaker for 5 min at room temperature. Finally, the sorbent was gathered at one side of the beaker under an external magnetic field (Nd–Fe–B, 10,000 Gs) (Fig. 1) and the clear supernatant was directly decanted. The isolated sorbent was eluted with 2 mL of ethanol to desorb the analyte. Desorption process was accelerated by stirring on a shaker for 5 min. This step was also completed with the help of a magnet. The clear solution of the eluent containing gemfibrozil was transferred into a spectrofluorometric cell and fluorescence intensity of the analyte was measured at λ_{em}=304 nm after excitation at 276 nm.

3. Results and discussion

3.1. Characterization of GO/Fe_3O_4/β-CD nano-hybrid

The powder X-ray diffraction (XRD) is a very powerful technique for characterizing the structure of materials. To demonstrate the formation of Fe_3O_4 NPs on the GO surface and the crystal structure of GO/Fe_3O_4, the XRD patterns of the as-prepared GO and GO/Fe_3O_4 were collected. Fig. 2 shows XRD patterns of GO and GO/Fe_3O_4 magnetic nano-particles. As shown in Fig. 2a, the diffraction peak at 2θ=10° is assigned to the (0 0 2) reflection of GO. For the synthesized GO/Fe_3O_4 (Fig. 2b), new diffraction peaks appeared at the Bragg angles of about 30.2°, 35.5°, 43.2°, 53.6°, 57.1° and 62.9° are respectively ascribed to the (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0) facets of the cubic spinel crystal planes of Fe_3O_4 (JCPDS No. 19-0629). So, the existence of Fe_3O_4 NPs on graphene is confirmed, while the (0 0 2) reflection peak of layered GO almost disappeared. It may be due to the fact that after covering with Fe_3O_4 NPs, the GO sheets cannot stack with each other anymore to form crystalline structures [10,16].

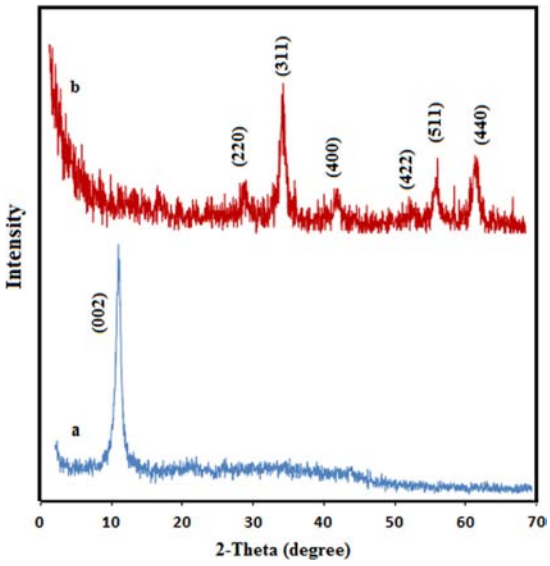

Fig. 2. XRD patterns of (a) GO, and (b) GO/Fe_3O_4.

The infrared absorption spectroscopy was used to further investigate the formation of GO/Fe_3O_4/β-CD nano-hybrid. Fig. 3a–d shows the FT-IR spectra of GO, GO/Fe_3O_4, GO/Fe_3O_4/β-CD and β-CD, respectively. As mentioned before, there is a wide range of oxygen functional groups including hydroxyl, epoxy, and carboxylic acid groups in the GO layered structure. In the FT-IR spectrum of the synthesized GO (Fig. 3a) the broad and strong peak at 3430 cm^{-1} corresponds to the stretching vibration of O–H and the band around 1733 cm^{-1} is ascribed to the stretching vibrations of C=O from carbonyl and carboxylic groups. The bands around 1223 cm^{-1} and 1059 cm^{-1} are ascribed to C–OH stretching vibrations and C–O stretching vibrations, respectively. Moreover, the carbon-carbon double characteristic bond is appeared at 1626 cm^{-1}. FT-IR spectrum of GO/Fe_3O_4 (Fig. 3b) reveals the characteristic peak around 580 cm^{-1} corresponding to the shifted stretching vibration of Fe–O bond compared to that of bare Fe_3O_4 (Fe_3O_4 spectrum is not shown) illustrating that Fe_3O_4 is successfully bound to the GO. It is known that β-CD is covalently attached on the GO surface *via* reaction of –OH groups present in β-CD with the oxygen functional groups present on the GO surface. It could be seen that the FT-IR spectrum of the GO/Fe_3O_4/β-CD (Fig. 3c) exhibits typical β-CD absorption features (Fig. 3d) of the coupled C–O–C stretching-/O–H bending vibrations at wavenumber region of 1000–1150 cm^{-1}, and the peak at 1400 cm^{-1} is attributed to the C–H/O–H bending vibrations. While the peaks at 1028 cm^{-1} and 1076 cm^{-1} arise from the coupled C–O/C–C stretching/O–H bending vibrations. These results clearly confirm that β-CD is attached to the surface of GO/Fe_3O_4 nano-hybrid [10,16].

Field emission scanning electron microscopy was employed to explore the morphology of the synthesized GO and GO/Fe_3O_4 nano-hybrid. Fig. 4A shows the SEM image of GO. The morphology reveals that the large two-dimensional GO with layered-structures exhibits face-to-face stacking of sheets. After *in situ* deposition of Fe_3O_4 NPs onto the surface of the GO, it was obvious that the distribution of Fe_3O_4 NPs on the surface of GO is uniform and narrowly distributed Fe_3O_4 NPs were densely covered on the surface of GO and no large vacancy is observed (Fig. 4B). The obtained results are in good agreement with the results reported by other researchers [10,16].

3.2. Optimization of magnetic solid phase extraction conditions

To evaluate the capability of the presented MSPE method for separation and pre-concentration of gemfibrozil, several variables

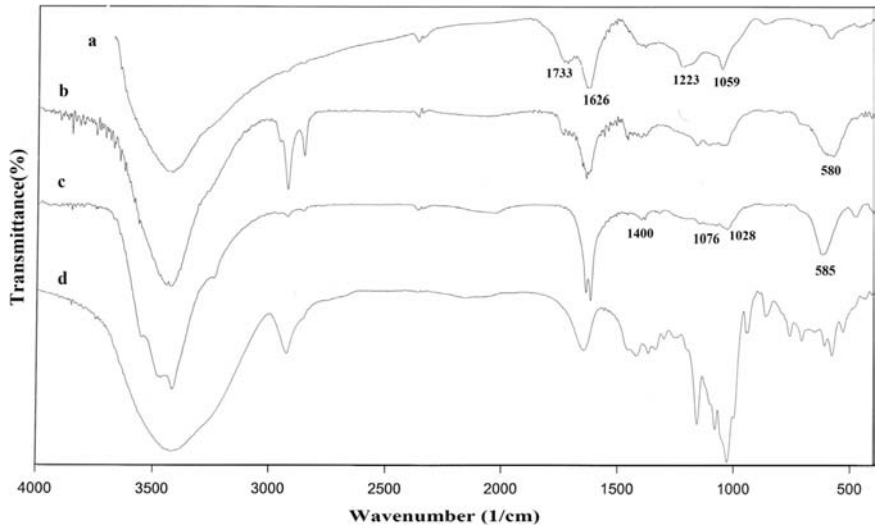

Fig. 3. FT-IR spectra of (a) GO, (b) GO/Fe_3O_4, (c) GO/Fe_3O_4/β-CD, and (d) β-CD.

affecting the extraction efficiency, including pH, the amount of magnetic nano-sorbent, the sample volume, and the type of eluent on the extraction efficiency were studied and optimized. Each parameter was varied individually to optimize the experimental conditions. A 1 μg L^{-1} solution of gemfibrozil was used for all measurements and each experiment was performed three times.

3.2.1. Effect of pH

The influence of the pH value on the separation/pre-concentration of gemfibrozil was studied by adjusting the pH values of sample solution in the range of 4–12 using diluted HNO_3 or NaOH solution, or both. Solutions of pH <4 were not tested because of the probability of the Fe_3O_4 nano-particles dissolving in strongly acidic media. According to the obtained results (results are not shown), the retention of gemfibrozil on the GO/Fe_3O_4/β-CD nano-hybrid is not affected by pH and recovery value is almost constant in the wide pH range. The possibility of the formation of host–guest inclusion complex between gemfibrozil and β-CD in a wide pH range has been reported previously [22] and there is a good agreement between the obtained and reported results. This fact enables the application of the synthesized nano-sorbent in many aqueous medium for extraction and pre-concentration of gemfibrozil without any pH adjustment.

3.2.2. Optimization of elution conditions

The nature of the eluent is of prime importance and should optimally meet three criteria: efficiency, selectivity and compatibility. In addition, it may be desirable to recover the analytes in a small volume of solvent to ensure a significant enrichment factor. In this work, elution of the retained gemfibrozil from GO/Fe_3O_4/β-CD surface was examined using various reagent solutions and the results are shown in Fig. 5. As can be seen, the best recovery was achieved when ethanol was used as an eluent. The effect of elution volume (0.5–4.0 mL) on the recovery was also investigated. The recovery of gemfibrozil increased by increasing the volume of ethanol up to 2 mL and remained constant afterward. So, to achieve the highest pre-concentration factor, 2 mL of the eluent was chosen as the optimum value.

3.2.3. Effect of the amount of GO/Fe_3O_4/β-CD nano-hybrid

The effect of the amount of GO/Fe_3O_4/β-CD nano-hybrid on the sorption of gemfibrozil was examined in the range of 50–500 mg. The results demonstrated that quantitative recoveries ($>95\%$) of the working analyte were observed when the synthesized nano-hybrid was used above 100 mg. Therefore, in the presented procedure, 150 mg of GO/Fe_3O_4/β-CD was recommended.

3.2.4. Effect of sample volume

The possibility of enriching low concentrations of gemfibrozil from large volumes of samples was examined by studying the effect of sample volume on the recovery of the analyte. For this aim, the volumes of sample solutions containing 1 μg L^{-1} gemfibrozil were diluted to 25–400 mL and the analyte was pre-concentrated on GO/Fe_3O_4/β-CD nano-hybrid by applying the presented MSPE procedure. As shown in Fig. 6, a quantitative recovery of gemfibrozil was obtained with up to 200 mL of sample solution; above 200 mL, the recovery decreased. So, by analyzing 2 mL of the final solution obtained after the pre-concentration of 200 mL of sample solution, a pre-concentration factor of 100 was obtained.

3.2.5. Effect of adsorption/desorption time

Due to the superparamagnetic property of the GO/Fe_3O_4/β-CD, the sorbent could be separated rapidly from the sample solution using an external magnetic field instead of filtration or centrifugation. Therefore, the effect of adsorption/desorption time on the recovery of analyte was investigated as analysis time. Both the adsorption and desorption time was varied in the range of 1–20 min. According to the obtained results (results are not shown), 5 min was sufficient for each step.

3.2.6. Regeneration, reusability and adsorption capacity of the nano-sorbent

The potential regeneration and stability of the sorbent were also investigated. The nano-hybrid could be reused after regenerating with 2 mL of ethanol and 20 mL deionized water, respectively. Moreover, based on the obtained results, the prepared GO/Fe_3O_4/β-CD nano-hybrid can be reused for 50 times without obvious loss of analytical performance and magnetic properties. The adsorption capacity (q_e, mg g^{-1}) of the nano-sorbent for gemfibrozil was calculated using the following equation:

$$q_e = \frac{V(C_0 - C_e)}{W} \quad (1)$$

where, C_0 (mg L^{-1}) and C_e (mg L^{-1}) are the initial and equilibrium concentrations of the analyte in aqueous solution, respectively, V (L) is the volume of sample solution, and W (g) is the mass of

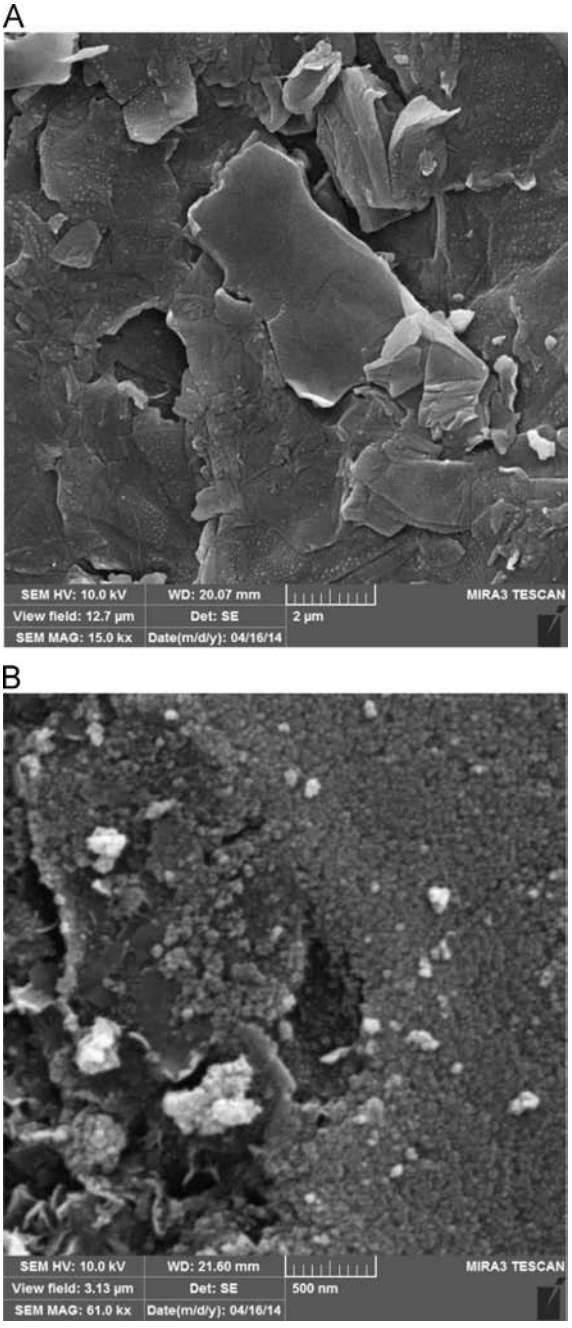

Fig. 4. SEM images of (A) GO, and (B) GO/Fe_3O_4 nano-hybrid.

used GO/Fe_3O_4/β-CD nano-hybrid. For this aim, 300 mg of the nano-sorbent is added to 100 mL of solution containing 15 mg L^{-1} of the analyte and sonicated for 1 min to obtain a fully dispersed nano-hybrid particles throughout the sample solution. The extraction process was continued 45 min by stirring the solution on a mechanical stirrer. Then, the magnetic nano-hybrid was isolated from the solution by a magnet. Finally, the concentration of the analyte in the supernatant was determined by spectrofluorometric approach. As a result, the adsorption capacity of the nano-sorbent for gemfibrozil was found to be 49.8 mg g^{-1} according to Eq. (1).

3.3. Robustness and selectivity of the method

The robustness of the presented method was determined by altering the experimental conditions such as pH, temperature, irradiation and so on and evaluating spectrofluorometric characteristics. According to the obtained results, no significant changes of fluorescence intensity as a function of pH were observed. Also, the fluorescence intensity of gemfibrozil decreased with a temperature coefficient of only 0.8% per °C when the temperature increased from 10 to 50 °C. Moreover, the effect of irradiation with spetrofluorometer xenon lamp (λ_{ex}=276 nm) on the fluorescence emission of gemfibrozil (λ_{em}=304 nm) over 1 h was studied. The results showed that fluorescence intensity was practically stable and unaffected by irradiation. Consequently, the robustness of the presented method is good and small changes in parameters have no significantly effect on the fluorescence intensity of the analyte.

To demonstrate the selectivity of the developed MSPE method for the pre-concentration and determination of gemfibrozil, the influence of some alkali and alkaline earth metals, some commonly used excipients in pharmaceutical formulations and some drugs on the determination of 5 μg L^{-1} gemfibrozil was studied. A 3000-fold mass excess of them over gemfibrozil was tested as the maximum ratio. The criterion for interference was fixed at a ± 5.0% variation of the average fluorescence intensity calculated for the established level of gemfibrozil. The tolerance limits are given in Table 1. According to the obtained results, the developed method was free from the interference of the metal ions, usual excipients and the serum compositions. Regarding the selectivity of the method two aspects should be considered; the extraction process and the detection stage. It was proven that β-CD can selectively bind with gemfibrozil into its cavity to form a stable host–guest inclusion complex with an apparent association constant of 7.57×10^2 L mol^{-1} [22]. On the other hand, the selectivity could be provided by a detection technique for whole analysis process. It is obvious that spectrofluorometry is a selective method in many cases, and selection of proper excitation and emission wavelengths can remove the interfering effects.

3.4. Analytical merits of the method

Optimized experimental parameters and analytical characteristics of the method are given in Table 2. Under these experimental conditions, the analytical features of the presented method, such as the linear range of the calibration graph, the limit of detection (LOD) and limit of quantification (LOQ), the accuracy and the precision were examined. The calibration graph was linear in the range between 0.01 and 5 μg L^{-1}, with a correlation coefficient of 0.9989. The regression equation was I_F=257.03 C+16.66, where I_F is the fluorescence intensity and C is the concentration of gemfibrozil in the sample solution in μg L^{-1}. The LOD and LOQ, defined as 3 S_b/m, and 10 S_b/m (where S_b is the standard deviation of the blank and m is the slope of the calibration curve) were 0.003 μg L^{-1} and 0.010 μg L^{-1}, respectively. The precision of the method was evaluated by repeated analysis of gemfibrozil during the course of experimentation on the same day and on different days under the optimized experimental conditions. For both intra-day and inter-day variation, solutions of gemfibrozil at concentrations of 1 μg L^{-1} were determined in triplicate. The % RSD (n=6) for intra-day and inter-day analysis were found to be 1.09% and 2.67%, respectively.

3.5. Method validation and analysis of real samples

To explore the reliability of the presented method, it was successfully applied to determine gemfibrozil in various samples including pharmaceutical wastewaters and human serum samples along with one pharmaceutical product (gemfibrozil capsules with a nominal content of 300 mg). The results are given in Table 3. Due to unavailability of certified reference materials for gemfibrozil to test the validity of the method, recovery experiments were carried out by spiking the samples with different amounts of gemfibrozil

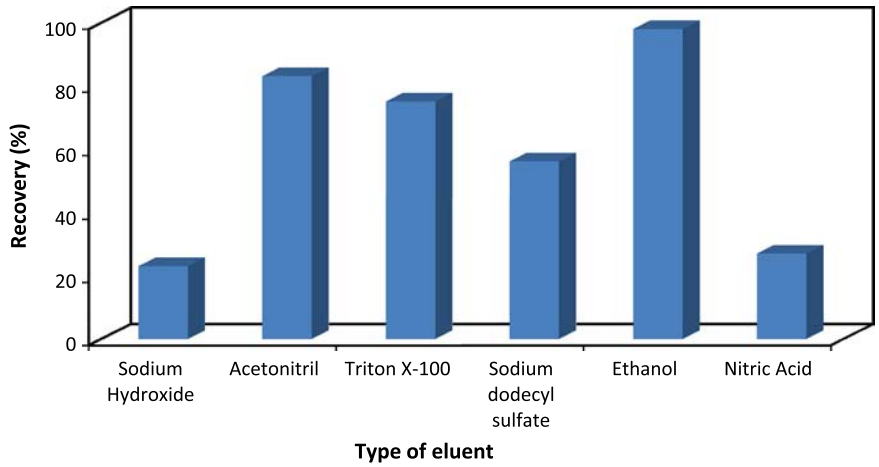

Fig. 5. Effect of eluent type on the elution of gemfibrozil from the surface of the sorbent. ([Gemfibrozil]=1 μg L^{-1}, and other conditions are given in Table 2).

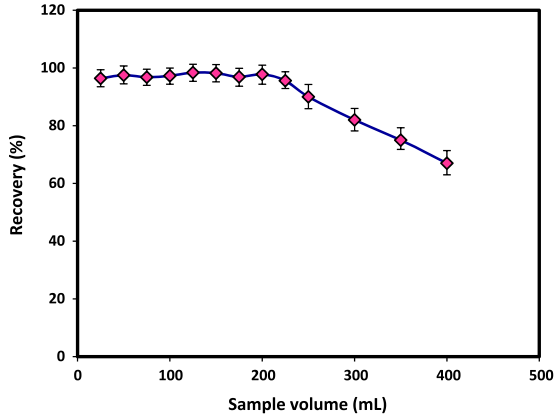

Fig. 6. Effect of sample volume on the recovery of gemfibrozil. (Conditions are as Fig. 5).

Table 1
Tolerance limits of potential interfering ions in the determination of 5 μg L^{-1} of gemfibrozil.

Potentially interfering ions or compounds	Interferent to analyte ratio
Na^+, K^+, Ca^{2+}, Mg^{2+},Fe^{3+}, Cl^-, SO_4^{2-}, NO_3^-, CH_3COO^- CO_3^{2-}, PO_4^{3-}	3000:1
Sodium chloride, glucose, tryptophan, glycin	2500:1
Starch, benzoic acid, lactose	2000:1
Mefenamic acid, diclofenac, ibuprofen	1500:1

before sample preparation and determination by spectrofluorometry. As can be seen in Table 3, relative recoveries between 96.0 and 104.0% were obtained, which confirm the accuracy of the presented method.

3.6. Comparison of the presented method with other pre-concentration procedures

Table 4 compares analytical data from this method with other techniques reported previously for the pre-concentration and determination of gemfibrozil. As it can be seen, the limit of detection and other parameters of the presented method are better than of the other procedures. This method has some advantages, such as a lower detection limit, a higher pre-concentration factor, simplicity, and low cost, and it is environmentally friendly. Accordingly, the presented method can be introduced for versatile and ultra-trace analysis of other pharmaceutical compounds in biological and environmental samples.

Table 2
Optimized experimental parameters and analytical characteristics of the presented method for gemfibrozil determination.

Parameter	Unit	Value
Experimental conditions		
Amount of the sorbent	mg	150
Sample volume	mL	200
Eluent volume	mL	2
Determination conditions		
Excitation wavelength	nm	276
Emission wavelength	nm	304
Slits bandwidth	nm	5
Analytical parameters		
Linear range	μg L^{-1}	0.01 – 5
Intercept	–	16.55
Slope	–	257.03
Limit of detection[a]	μg L^{-1}	0.003
Correlation coefficient	–	0.9989
Relative standard deviation (RSD) (n=6)	%	1.09 (1)[b]
Enrichment factor[c]	–	100

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

4. Conclusions

In summary, graphene oxide–magnetite nano-hybrid grafted with β-CD was prepared using a facile fabrication method. Then, the prepared nano-hybrid was used for magnetic solid phase extraction of gemfibrozil prior to spectrofluorometric determination. Due to the selectivity of the synthesized nano-hybrid and the sensitivity of the spectrofluorometric approach, the whole methodology resulted in a separation/pre-concentration and determination of gemfibrozil with high percent of relative recoveries (96.0–104.0%) and enrichment factor (1 0 0) from biological, environmental and pharmaceutical matrices. The presented method possesses some advantages such as; simplicity, high kinetic sorption of the target analyte, low detection limit (3 pg mL^{-1}), good precision (RSD=1.09%), excellent accuracy, and high sorption capacity (49.8 mg g^{-1}). To the best of our knowledge, this is the first application of the synthesized GO/Fe_3O_4/β-CD nano-hybrid as an adsorbent through a coupled magnetic solid phase extraction–

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

Samples	Added gemfibrozil ($\mu g\ L^{-1}$)	Found gemfibrozil ($\mu g\ L^{-1}$)	Recovery (%)
Wastewater samples[a]			
	–	0.48 ± 0.06	–
Sample 1	0.5	0.96 ± 0.09	96.0
	2.0	2.53 ± 0.09	102.5
	–	1.21 ± 0.08	–
Sample 2	0.5	1.73 ± 0.07	104.0
	2.0	3.24 ± 1.00	101.5
	–	–	–
Sample 3	0.5	0.52 ± 0.07	104.0
	2.0	1.97 ± 0.09	98.5
Serum samples[b]			
	–	–	–
Sample 1	0.5	0.48 ± 0.87	96.0
	2.0	2.04 ± 1.03	102.0
	–	–	–
Sample 2	0.5	0.51 ± 1.0	102.0
	2.0	2.06 ± 1.1	103.0
Gemfibrozil capsule[c]	Nominated amount (mg per capsule)	Found amount (mg per capsule)	Recovery (%)
	300	294.7 ± 8.2	98.2

[a] Collocated from Pharmaceutical Manufactory effluents, Tehran, Iran.
[b] Obtained from Ali-Nasab hospital, Tabriz, Iran.
[c] Obtained from Dr. ABIDI Pharmaceutical Co., Tehran, Iran.

Table 4
Comparison of the proposed method with other pre-concentration methods.

Method	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	EF[a]	Sample volume (mL)	Reference
DLLME–HPLC[b]	0.1–100 ($mg\ L^{-1}$)	12.3 ($mg\ L^{-1}$)	9.3	10	[19]
SBSE–HPLC–DAD [c]	6.4–64	1.7	–	25	[24]
SPE–HPLC–UV [d]	0.03–257	0.025	–	10–20	[25]
QuEChERS–UPLC–MS/MS [e]	5–250 ($ng\ g^{-1}$)	5 ($ng\ g^{-1}$)	–	10 (g)	[26]
MIP–SPE–MS/MS [f]	0.15–200	0.05	–	50	[27]
MSPE–Spectrofluorometry	0.01–5	0.003	100	200	This work

[a] Enrichment factor.
[b] Dispersive liquid–liquid microextraction–high performance liquid chromatography.
[c] Stir bar sorptive extraction–high performance liquid chromatography-diode array detector.
[d] Solid phase extraction–high performance liquid chromatography-ultraviolet detection.
[e] Quick, easy, cheap, effective, rugged and safe method–ultra high liquid chromatography–tandem mass spectrometry.
[f] Molecularly imprinted solid-phase extraction–tandem mass spectrometry.

spectrofluorometry method for ultra-trace analysis of gemfibrozil in human serum and pharmaceutical wastewater samples.

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