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Extraction and Preconcentration of Indomethacin with Magnetic Nanoparticles adsorbent prior to its Spectrophotometic determination

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Abstract : A novel, fast and sensitive method based on cetyltrimethyl ammonium bromide coated Fe_3O_4 nanoparticles adsorbent was developed for solid-phase extraction of indomethacin. The unique properties of magnetic nanoparticles (MNPs) including high surface area and super paramagnetism give the advantages of high extraction capacity, fast separation and low consumption of the adsorbents to the method. The main parameters affecting the extraction and desorption efficiently, such as the amount of surfactant, pH of sample solution, desorption conditions, breakthrough volume, amount of MNPs, extraction and desorption times, and ionic strength were investigated and optimized. Under optimum conditions, the method was successfully applied to the determination of the analyte and good linearity in the range of 0.09-25 μg mL^{-1} (r^2=0.9951) was achieved with the low detection limit of 161 ng mL^{-1}. The relative standard deviations (RSD%) 2.16 and 1.33% (for concentration of 0.9 and 0.09 μg mL^{-1} respectively) and a good enrichment factor of 98 were obtained. The method was successfully applied to the determination of indomethacin from human plasma and urine samples and good recoveries in the range of 64-67% were obtained.
Keywords: Indomethacin, Magnetic Nanoparticles Adsorbent..

Introduction

Indomethacin 2-{1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl}acetic acid (Fig. 1) is in a group of drugs called no steroidal anti-inflammatory drugs (NSAIDs). Indomethacin works by reducing hormones that cause inflammation and pain in the body. Indomethacin is used to treat pain or inflammation caused by many conditions such as arthritis, gout, ankylosing spondylitis, bursitis, or tendinitis. Commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling. It works by inhibiting the production of prostaglandins, molecules known to cause these symptoms. It is marketed under more than twelve different trade names [1].

The extensive clinical use of indomethacin triggered our interest for the determination of this drug by a sensitive, simple and rapid technique. Several methods have been reported for the determination of indomethacin in pharmaceutical preparations or biological fluids, such as High performance thin layer chromatography HPLC[2], spectrophotometry [3], capillary zone electrophoresis [4] and electrochemical detections [5-6]. Most of the reported methods suffer from disadvantages such as laborious, time-consuming, long response time, requirement of expensive instrument and low detection capability. On the other hand, a sample pretreatment procedure is usually needed when traces of drug must be determined in complex matrices such as biological fluids.

In the past few years, a new solid phase extraction (SPE) method based on the adsorption of surfactant at solid-liquid interface was developed [7]. Surfactant molecules form bilayer aggregates named mixed hemimicelles (hemimicelles and admicelles) under certain conditions which are similar to Langmuir-Blodgett films; but this aggregates are stable equilibrium structures that are easily formed on a wide variety of surfaces, even porous or heterogeneous materials [8]. Adsolubilization is a phenomenon that species at low concentrations in an aqueous solution dissolve within the organic interior of admicelles. Since, the outer surface of hemimicelles is hydrophobic where as that of admicelles is ionic, they can provide tow-fold mechanisms for the retention of analytes [9-10]. This technique has many advantages, such as high extraction efficiency, easy elution of analytes, high breakthrough volume and no clean-up steps [11-13].

Recently, magnetic nanoparticles (MNPs) have been recognized as new adsorbents with large surface area and small diffusion resistance [14]. These nano-structured materials possess high surface area and superparamagnetic properties, which can provide higher capacity, rapid extraction and ease of separation for large volume samples by applying a strong external magnet [15]. So far, only a few SPE procedure based on the surfactant coated Fe_3O_4 nanoparticles has been reported for the analysis of drugs in different matrices [16-20] and to the best of our knowledge, neither magnetic separation nor mixed hemimicelles SPE method have not yet been applied for the determination of indomethacin in biological samples.

In this work, we established a mixed hemimicelles SPE procedure based on the adsorption of cetyltrimethyl ammonium bromide onto the surface of Fe_3O_4 magnetic nanoparticles. The adsorbents possess the advantages of both mixed hemimicelles and magnetic nanoparticles and were used for the determination of indomethacin from biological fluids. The predominant factors affecting the extraction efficiency were studied and the method was successfully applied to the extraction and precancentration of indomethacin from plasma and urine samples.

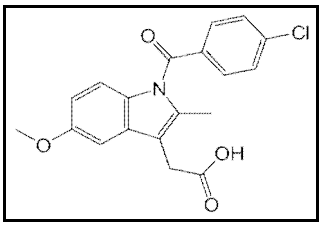

Fig. 1 Structure of indomethacin

Materials and Method

Chemicals and reagents

All reagents were of analytical grade and used as supplied. Ferrous chloride tetra hydrate ($FeCl_2.4H_2O$), Ferric chloride hexahydrate ($FeCl_3.6H_2O$), sodium chloride, cetyltrimethyl ammonium bromide and glycerol was purchased from Merck (darmstadt, Germany). Indomethacin standard was obtained from the Center of Quality Control of Drug, Tehran, Iran.

A stock standard solution of the drug (20 µg mL^{-1}) was prepared by dissolving appropriate amount of indomethacin in deionized water. This solution was replaced every week in order to prevent decomposition of the drug. The working standard solutions were prepared daily by appropriately diluting the stock solution with deionized water.

Instrumentation

The UV-Vis spectra of the solutions were carried out using UV-240 Shimadzu (Tokyo, Japan) UV-Vis spectrophotometer equipped with 0.5 cm ×1 cm quartz cell. Spectra recording were carried out in absorbance mode in absorption wavelength of 318 nm. A Metrohm 827 pH meter (Herisau, Switzerland) with a combined glass electrode was used for the pH measurements. The liquid chromatographic system was carried out on a Acme 9000 Young Lin, (Anyang, Korea) equipped with a Young Lin SP930D pump, a UV-Vis 730D detector. A L1-ODS-1 column (5 µm, 250 mm × 4.6 mm) was utilized with the injection volume set to 20 µL. The mobile phase was consisted of methanol-water containing 0.1% glacial acetic acid (50:30 v/v) at a flow rate of 2

mL min^{-1} with the isocratic elution. The UV detection of indomethacin was performed at 280 nm. A CM30 Philips (Amsterdam, Netherlands) transmission electron microscope (TEM) was used to characterize of particle size and morphology of the nanoparticles. Phase characterization of the magnetic nanoparticles was performed by an APD-2000 X-ray diffractometer (XRD) (Riva Del Garda, Italy) using Cu Kα radiation source (λ= 1.54059 Aº). The FT-IR spectrum of the prepared MNPs was carried out by a Rayleigh WQF-510A FT-IR spectrometer (Beijing, China) in the range of 400-4000 cm^{-1} for sample dispersed in KBr.

Synthesis of magnetic nanoparticles

Magnetic nanoparticles were synthesized according to the procedure described previously [19]. Brifly, 11.68 g $FeCl_3$. $6H_2O$ and 4.30 g $FeCl_2$. $4H_2O$ were transferred in 250 mL round bottom flask and dissolved in 200 mL deionized water under nitrogen atmosphere at 85 ˚C. Then, 45 mL of 25% (v/v) ammonia solution was added. The orange color of the solution turned to black immediately. After cooling down to the room temperature, the suspension was collected in the bottom of the flask by using an external magnate and rinsed sequentially with deionized water (3×200 mL), 0.02 M NaCl (2×100 mL) and again deionized water (2×200 mL). The prepared magnetic suspension was then stored at the concentration of 20 mg mL^{-1} at 4˚C until use.

Recommended SPE procedure

The mixed hemimicelles SPE procedure was carried out in a batch mode as follows: a 200 mL of sample solution containing 0.9 µg of indomethacin was placed in a 250 mL beaker and the pH was adjusted to about 10 with 1.0 M NaOH solution. To prepare CTAB coated MNPs assemblies, 2.5 mL of magnetic nanoparticles (20 mg mL^{-1}) was added to 5 mL of 5 mg mL^{-1} CTAB solution. The mixture was stirred for 2 min and added into the sample solution. The suspension was diluted to 200 ml and stirred for 5 min to facilitate the adsorption of the target analyte. Then the adsorbents were isolated by applying an external supermagnet on the bottom of the beaker and the supernant was poured out. The adsorbed analyte was eluted with 2×1 mL methanol. The concentration of indomethacin was determined using the UV absorbance of the eluted at 318 nm. A blank solution was also run under the same conditions without adding the drug.

Sample preparation

Human plasma (obtained from Iranian Blood Transfusion Organization) and urine samples were collected from different healthy volunteers and stored at -18 ˚C. For analysis, the samples were placed in oven at 37 ºC for 3 h and then centrifuged at 3000 rpm for 10 min. 20 mL of each sample was spiked with appropriate amounts of indomethacin stock solution and diluted to 200 mL after adjusting the pH. Then the proposed mixed hemimicelles SPE procedure was performed under the optimum conditions.

Results and Discussion

Characterization of Fe_3O_4 nanoparticles

Fig. 2 shows the TEM image of the prepared Fe_3O_4 nanoparticles and indicated the particles are relatively uniform with a mean diameter of 7.4 ± 1.6 nm. The XRD pattern of MNPs was represented in Fig. 3.

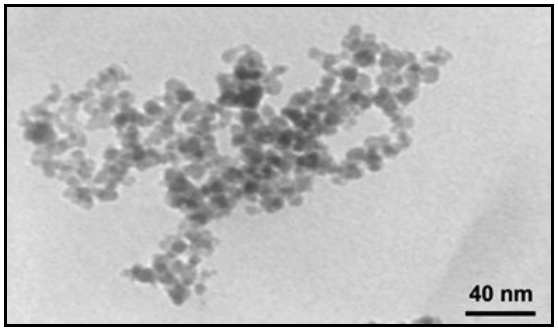

Fig. 2 TEM image of the prepared MNPs adsorbents.

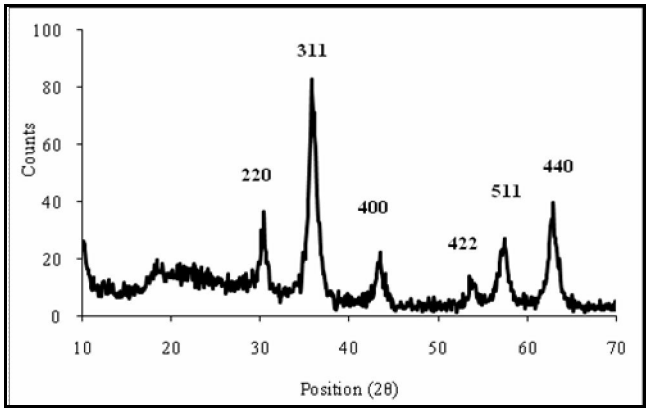

Fig. 3 XRD pattern of the prepared MNPs adsorbents.

As can be seen, the pattern displays reflection peaks in peak positions (2θ) of 30.4, 35.7, 43.4, 53.5, 57.3 and 62.7 which were related to the diffraction planes of 220, 311, 400, 422, 511, and 440. This pattern is match well with those from Joint Committee on Powder Diffraction Standards (JCPDS card, file No.19-0629) for magnetite.

Effect of the amount of surfactant

The surface of Fe_3O_4 nanoparticles are generally covered with hydroxyl groups which vary in form at different pHs. Magnetite nanoparticles are negatively charged when the pH was higher than the pH of point of zero charge (pH_{pzc}) which is reported to be 6.05 previously [16-20] for Fe_3O_4 MNPs. Therefore, the adsorption of ndomethacin ions onto the adsorbents surface dose not occurs. On the other hand cationic surfactants such as CTAB will adsorb onto the surface of MNPs through the positive ammonium moiety in these pH and make the surface of adsorbent hydrophobic. Further addition of CTAB can form a mixed hemimicelle aggregates and thus the adsorption of analytes. So the effect of the amount of surfactant on the adsolubilization of indomethacin was studied in batch mode. As can be seen from Fig. 4a, the adsolubilization of the analyte was increased remarkably with increase in the amount of CTAB in the range of 15 mg and then, it increased gradually till 25 mg. When the added amount of CTAB was exceeded 25 mg, adsolubilization was decreased gradually. This can be explained by the gradual formation of micelles which causes the analyte to redistribute in the bulk solution. Therefore, the amount of 25 mg of CTAB was used for the next experiments.

Effect of solution pH

The surface charge of Fe_3O_4 nanoparticles is pH-dependent. Hence, pH is one of the important influencing factors on the adsolubilization behavior of a mixed hemimicelles system. In this study, the effect of pH was studied by varying pH in the range of 4-12. As can be seen from Fig. 4b, the CTAB-coated MNPs do not exhibit obvious adsorption when the pH value was below 6.This may be explain by the fact that the MNPs have positive charge below the pH_{pzc} and efficient interaction do not occur between the MNPs and CTAB molecules. When the pH value reaches above the pH_{pzc}, the adsorption efficiency of the analyte was increased dramatically and reached maximum in pH 10. Therefore, the pH value of 10 was chosen as an optimum value for subsequent experiments.

Desorption conditions

Organic solvents are known to disrupt the surfactant aggregates (such as mixed hemimicelles system). Thus, the desorption of indomethacin was investigated using different solvents including methanol, acetonitrile, acetone and 1.0 M HNO_3. The experimental results were revealed that 1.0 M HNO_3 was oxidized the Fe_3O_4 nanoparticles and lost magnetization. On the other hand, CTAB-coated MNPs exhibited a relatively low degree of dispersion in acetone causes less analyte desorption. Satisfactory recoveries (60%) were obtained using 2×1 mL methanol as a desorbing solvent and higher volumes of acetonitrile (about 8.0 mL) was required for desorbing the analyte with the same recoveries. Thus, 2 mL of methanol was selected for the subsequent experiments.

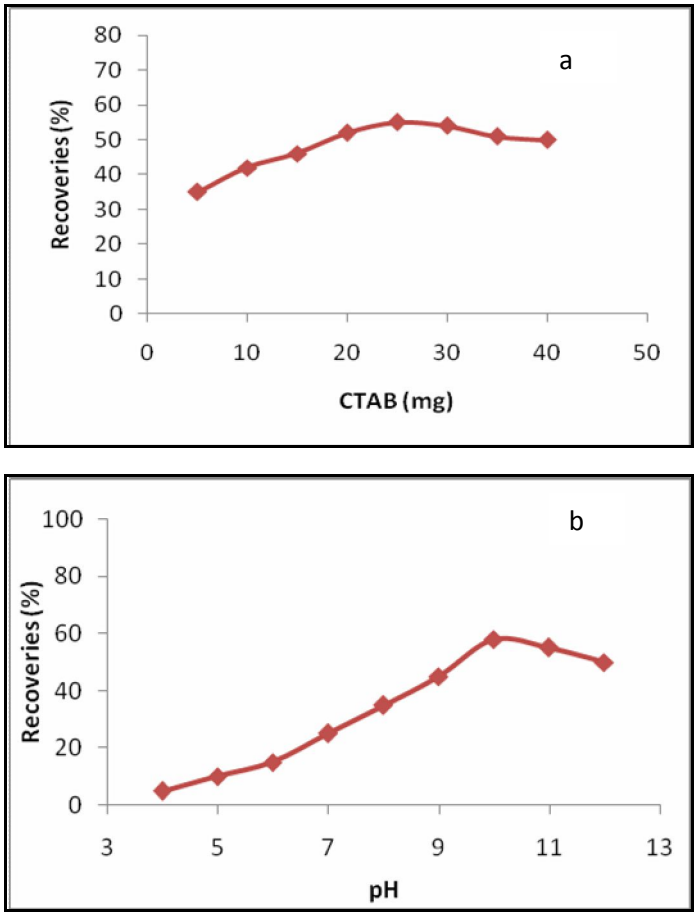

Fig. 4 Effect of the amount of CTAB (a) and pH (b) on the recovery of the analyte. Sample volume; 200 mL, indomthacine concentration; 180 microgram mL^{-1}, amount of the MNPs; 50 mg.

Sample volume

Breakthrough volume is a key parameter in any SPE procedure. The breakthrough volume for indomethacin was determined using a series of the different volumes (50-300 mL) of aqueous solution containing 100 µg of the analyte and 50 mg Fe_3O_4 nanoparticles and 25 mg CTAB were added to the each sample. The Results indicate that the quantitative recoveries were obtained with the sample volumes up to 200 mL and above this amount, it was decreased because the higher samples volume causes the analyte loss from the adsorbents surface. Thus, sample volume of 200 mL was selected as the optimum volume for the next studies.

Extraction and desorption time

The extraction and desorption time profiles were investigated by varying the mixing time of magnetic adsorbent with sample solution/desorbing solvent in the range of 1-20 min. The experimental results reveal that 5 min was sufficient for the satisfactory extraction and 3 min was enough for the complete desorption of the analyte. The high surface area of MNPs, rapid dynamic process and hemogeneous distribution of nano-adsorbent throughout the sample can be the possible reasons for obtaining such a fast SPE process. On a word, a complete SPE process can perform in less than 30 min.

Effect of the MNPs amount

The required amount of MNPs for complete separation and recovery of indomethacin in 200 mL solution containing 100 µg of the analyte at pH 10 was studied. The experimental results were indicated that maximum recoveries were obtained when the amount of MNPs was 50 mg. This lower amount of adsorbent compared with the traditional SPE sorbents is due to the high surface area of nanoparticles and the amount of 50 mg of MNPs was used for the subsequent experiments.

Effect of ionic strength

The ionic strength of the working solution was studied by varying the NaCl concentration in the range of 0-0.02 M. The results indicate that the extracted amount of indomethacin was decreased with the increasing in the ionic strength of the solution this observation indicates that the. electrostatic interaction has an important role in the adsorption process and competition of sodium ions with CTAB cations for negative surface of MNPs can cause to abate the formation of mixed hemimicelles aggregate on the surface of MNPs since the recommended procedure was followed without salt addition.

Analytical performance

Quantitative parameters such as linear range, limit of detection (LOD), precision (as relative standard deviation (RSD%)), and enrichment factor were evaluated. Calibration curve was constructed by diluting indomethacin stock solution to 200 mL with deionized water. The developed method exhibits a good linearity in the range of 0.09-25 μg mL^{-1} with correlation coefficient r^2 of 0.9951. The limit of detection (LOD), which is defined as $LOD=3S_b/m$, where S_b and m are the standard deviation of the blank and the slope of calibration curve respectively, was obtained 161 μg mL^{-1}. The precision of the method was calculated using seven replicate experiments using 200 mL of aqueous sample solution containing 0.09, 0.9 μg mL^{-1} of indomethacin, and the RSD(%) of 2.16 and 1.33 % was obtained respectively. The enrichment factor (EF), defined as $EF=V_s/V_e \times R\%$, where V_s is sample volume, V_e is elution volume and R% is percent recovery, was obtained 98. As the results reveal, the proposed method exhibited good linearity, low LOD and good enrichment factor for the determination of the analyte.

Table 1 Determination of Indomethacin in human plasma and urine samples (n=5, ±RSD%).

sample	Amount added (μg mL^{-1})	Amount found (μg mL^{-1})	Recovery (%)
Plasma	0	-	-
	0.09	0.0598 ± 0.0022	66.4
	0.9	0.505 ± 0.0065	64.5
	1.8	1.1502 ± 0.0166	63.9
Urine	0		-
	0.09	0.0586 ± 0.019	65.1
	0.9	0.5931± 0.0069	65.9
	1.8	1.2024± 0.0148	66.8

3.4 Application of the method:

The proposed mixed hemimicelles-SPE method was applied for determination of indomethacin in biological fluids including human plasma and urine samples. The results are given in Table 1. As seen, good recoveries in the range of 64-67% were obtained which indicate that the complex matrices of biological fluids do not interfere with the analysis of indomethacin.

4. Conclusion:

In this study, the CTAB-coated Fe_3O_4 nanoparticles were successfully synthesized and applied for convenient, fast and efficient extraction and preconcentration of indomethacin from human plasma and urine samples. This method combines the advantages of mixed hemimicelles and magnetic nanoparticles and possesses several advantages like simplicity, low cost and high enrichment factor, especially when more sophisticated techniques such as HPLC or spectroflourimetry are not available. Good recoveries and precision of the method indicate that the proposed method has analytical potentials for the extraction and preconcentration of other drugs from biological fluids.

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