Solid Phase Extraction of Theophylline in Aqueous Solutions by Modified Magnetic Iron Oxide Nanoparticles as an Extractor Material and Spectrophotometry Technique for the Determination

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Abstract

A new, simple and fast solid-phase extraction method for separation and preconcentration of trace theophylline in aqueous solutions was developed using magnetite nanoparticles (MIONPs) coated with aluminium oxide (AMIONPs) and modified with palmitate (P) as an extractor (P@AMIONPs). It has shown that the developed method has a fast absorbent rate of the theophylline at room temperature. The parameters that affect the absorbent of theophylline in the aqueous solutions have been investigated such as the amount of magnetite nanoparticle, pH, standing time and the volume, concentration of desorption solution. The linear range, limit of quantification (LOQ) and limit of detection (LOD) for the determination of theophylline were 0.05-2.450 µg mL^{-1}, 0.05 µg. mL^{-1} and 0.032 (n=10) respectively.

The PF (Preconcentration factor) obtained for the proposed method in 98% is 75. The intra-day and inter-day precisions were 2.1% and 3.6% (for 1.0 µg mL^{-1}, n=10). The relative standard deviation R.S.D for 1.0 µg mL^{-1} is *1.26 (n=10). The proposed method has been successfully applied for the determination of theophylline in the pharmaceutical preparations.*

Keywords: Soli phase extraction, magnetic nanoparticles, theophylline, preconcentration, pharmaceutical preparation.

Introduction

Theophylline is one of the most important chemical compounds which was naturally found in many of the food products such as coffee, tea, chocolate and beans. It is a type of methyl-xanthine derivatives which exist widely in nature; it is also known as 1, 3-dimethyl-3, 7-dihydro-1H-purine-2, 6-Dione (Fig. 1). For many years, theophylline has been used in medical fields for the treatment of many diseases such as chronic bronchitis and symptoms of chronic asthma[1, 2]. The effective therapeutic concentration of theophylline in serum[3,4] is ranged from 5-20 µg mL. However, theophylline has lots of side effects when its concentration in human body becomes higher than 20 µg mL^{-1}, which can cause fever, insomnia, dehydration, arrhythmia, heartburn tachycardia, anorexia and coma [5]. Therefore, need of the simple, rapid, accurate and low-cost determination method in pharmaceutical formulations has become the main concern of many analytical chemists.

Till now, several of theophylline analytical methods including Raman spectrometry[6], Electrospray ionization mass spectrometry (ESI-MS)[7], Gas chromatography (GC)[8], spectrophotometry [9, 10], HPLC [11-13] and Microchannel electrophoresis [14] have been used in the literature in the determination of theophylline. In addition, electrochemical methods have attracted the attention because of their several advantages like fast response, low cost, cheap instrument, timesaving, high sensitivity and simple operation. Therefore, some material had been used to modify the electrode such as ionic liquid[15], carbon nanotubes[16] and theophylline oxidase[17]. However, their detection limits were not low. All these disadvantages encouraged the researchers looking for novel methods for determination of theophylline in pharmaceutical preparations.

Recently, nanotechnology has strongly entered in the field of analytical chemistry due to the unique properties of nanomaterials such as excellent distribution in the solution and large surface, which offered novel methods for chemical analysis. Nanoparticles, like the magnetic iron oxide (MIO), have several applications in drug delivery, treatment of wastewater, labelling of the biological cell, separation of chemicals, targeting and magnetic resonance imaging. The main reason for using the magnetic iron oxide nanoparticles in the solid phase extraction as an extractor material is that it can be easily collected by applying a magnetic field in any batch system.

Thus, combining the magnetic field with adsorption properties can generate excellent material for separation of chemicals in solid phase extraction. The selectivity of some analytical methods for determination of the drug in pharmaceutical preparations has been markedly increased by applying lots of modifications on the following techniques: solid phase extraction (SPE)[18], liquid-liquid extraction (LLE)[19] and cloud point extraction (CPE)[20].

Because of its high preconcentration factor, low cost, low consumption of solvent, simplicity and flexibility, SPE method is considered as the highly effective preconcentration method amongst the previous methods [6,9,10]. In this study, we present a fast, simple and new method for determination of trace amount of theophylline in aqueous solutions using magnetic iron oxide nanoparticles which

were coated by aluminum oxide and modified with palmitate as a solid phase extractor (P@AMIONPs).

The solid phase extraction of theophylline is based on absorption of theophylline on P@AMIONPs. Then, the desorption of the theophylline is conducted by sodium hydroxide solution and the preconcentrated sample was then determined spectrophotometrically at 280 nm. The proposed method has shown a successful analysis procedure of theophylline without any interference from the other pharmaceutical components. In the literature, there are no previous studies about using the P@AMIONPs as a solid phase for the determination of the theophylline in real pharmaceutical preparation samples. The proposed method shows fast, cheap, shorter time and lower detection limit than other reported methods[1,21-23].

Material and Methods

Solutions and Chemicals: All chemicals and standards were of analytical grade and all dilutions were performed using double distilled water. The stock standard solutions of theophylline (200 µg mL^{-1}) were prepared by dissolving an appropriate amount of theophylline in double distilled water. The working standard solutions were prepared by an appropriate dilution of the stock standard solution. Palmitic acid, ferric chloride ($FeCl_3.6H_2O$, 99,9% m/m), ferrous chloride ($FeCl_2.4H_2O$, 99.9% m/m), aluminum oxide, ammonia (25%), hydrochloric acid (37 %) acetonitrile, ethanol, and thiourea were purchased from Sigma-Aldrich. The pH adjustments were made with NaOH and HCl (0.5-1.0 $mol.L^{-1}$). The buffer solution pH 3.0 was prepared using formic acid (0.5-1.5 $mol.L^{-1}$).

Apparatus: A digital pH meter (Type HANNA Bench) was used to make sure obtaining the required pH. While in the timing experiments, a magnetic stirrer, ultrasonic and shaker were required to shake the samples for a certain time. All of the spectrophotometric measurements were conducted by UV-visible double beam spectrophotometer device (Shimadzu, type 1800, Japan). The functional groups were characterized in the synthesis material by Fourier Transform Infrared spectra (FT-IR, Type 8400, Shimadzu, Japan). The size of magnetic iron oxide nanoparticles was characterized using the Atomic Force Microscopy (AFM) (Type AA3000, Angstrom Advanced Inc). All the figures were plotted using Origin Pro 9.1(Microcal). A magnet (1.3 T, 12 cm x 4 cm x 3 cm) was used to collect the magnetic iron oxide nanoparticles.

Synthesis of alumina coated-magnetic iron oxide nanoparticles (AMIONPs): The alumina coated-magnetic iron oxide nanoparticles (AMIONPs) were prepared in two steps according to common co-precipitation procedure [24]. In a short, at the first step, iron oxide nanoparticles were prepared by mixing equal volumes of ferrous chloride (12 .5 mL, 0.1 $mol.L^{-1}$) with ferric chloride (12.5 mL, 0.2 $mol.L^{-1}$). Then, ammonia solution (25 mL, 2 $mol.L^{-1}$) was added drop wise to the mixture under vigorous stirring. After the reaction, the black iron oxide nanoparticles were collected from the solution by a magnet and rinsed with 250 mL double distilled water to remove all the unnecessary ammonia[25]. The particles size of prepared MIONPs was determined using AFM and found to be 51.36 nm (Fig. 2). Then the product was dried in the oven under 80C for 2 hrs. Secondly, to prepare AMIONPs, 1.0 g of alumina was dissolved in ethanol (60 mL) forming a clear solution.

Then, 1.0 g of iron oxide nanoparticles was dispersed in the alumina solution under the ultrasonic waves for 5 minutes. The solution was standing for 1 hr and then collected, washed with ethanol and dried in the oven. The modified AMIONPs was confirmed by comparing the FT-IR spectra of MIONPs with AMIONPs. The FT-IR spectra of MIONPs show peaks at 644 cm^{-1}, 1602 cm^{-1} and 3497 cm^{-1} which are assigned to Fe-O-Fe, nitrogen atoms on the surface of nanoparticles and for the water molecules respectively. While the FT-IR spectra of modified AMIONPs shows peaks at 638 cm^{-1}, assigned to the overlap of alumina AL-O with Fe-O peak.

Synthesis of palmitate @alumina coated-magnetic iron oxide nanoparticles (P@AMIONPs): 100 mg of palmitic acid was dissolved in 25 mL of sodium hydroxide (0.1 $mol.L^{-1}$) and diluted to the 50 mL with double distilled water. 10 mL of palmitate solution was added to the 10 mL of distilled water which contains 0.1 g of AMIONPs. The mixture was then stirred for 20 minutes, separated by a magnet and rinsed with distilled water. The final product is used as a sorbent for theophylline.

General procedure for extraction and desorption: The extraction process of theophylline was made in a beaker. 300 mL of mixture solution which contains 0.05-2.45 µg mL^{-1} of theophylline, 0.4 mL of formate buffer was stirred vigorously with 0.1 g of P@AMIONPs for 3 min. The nanoparticles were then collected by using a magnet, rinsed with 2 mL sodium hydroxide (1.5 $mol.L^{-1}$) to desorb the theophylline from the nanoparticles. The magnet was used to collect the nanoparticles and the adsorption solution spectrophotometry values were measured at 280 nm. The spectrophotometry values of standard theophylline solution and the blank solution were measured at 280 nm prior to the extraction method (Fig. 3).

Sample preparation: The tablets which were supplied by three different companies were used. 10 tablets from each sample were weighed and powdered. An accurate weight of the theophylline (equivalent to 0.01 g) powder was transferred to the 250 mL volumetric flask. 0.1 N of HCl was added to the powder and shook for 10 min and filtered. Further dilution was made to the filtered theophylline solution in order to obtain 100 µg mL^{-1}. The stock solution was then used in further experiments.

The validation of method: The extraction method of theophylline was validated for the accuracy, precision,

recovery and linearity based on the British pharmacopeia guideline validation of drug methods [26]. In order to validate the proposed method, the absorbances measurements of desorption for 3 samples of quality control were interpolated into the calibration curve and the concentrations calculated at the same day. The obtained results of quality control were used to examine the accuracy and precision of the proposed method. The calibration curve of theophylline was plotted using the absorbances for eight desorption standard theophylline solutions in the range 0.05-2.54 µg mL^{-1}.

The lowest concentration of theophylline in the calibration curve is defined as the limit of quantification. The repeatability, accuracy and intra-day precision were conducted by analysis of three different concentrations of theophylline (low, mid and high) samples at the same day while the inter-day precision and accuracy were conducted by analysis of three samples of theophylline on different days (5 days). The recovery was measured by comparing the obtained absorbance results of the theophylline samples (Pharmaceutical preparations) with those obtained from the standard theophylline solution. The selectivity of the proposed method was also examined by comparing the difference in the absorbance values of the extracted theophylline by P@AMIONPs from different solutions with other solutions which contain interfering species.

Results and Discussion

The pH effect: The pH value of sample solution (Theophylline) for all of extraction and desorption procedures plays the main role. Therefore, the pH ranging from 2-12 was investigated for extraction and recovery of theophylline using many buffer solutions (Formate, sodium acetate and ammonium acetate). Positive charges on the surfaces of AMIONPs can adsorb negative charges of palmitate. Therefore, the adsorption of theophylline on the surface of P@AMIONPs can occur. Based on the obtained results in fig. 4, the absorption of theophylline on the P@AMIONPs was in pH range 3-5. At pH 4, the maximum recovery % of theophylline was obtained, therefore, pH 4 was selected as the optimum pH value and pH value in each sample was an adjustment by adding 0.4 mL of formate buffer solution.

At the higher pH values (more than 4), the recovery % of theophylline was diminished due to the fact that the hydrated surfaces of nanoparticles were decreased. The pH values (lower than 4), lead to decreasing the adsorption of theophylline because nanoparticles can be dissolved in a strongly acidic medium and also to the competition of H^+ and theophylline on the surfaces of nanoparticles.

The effect of AMIONPs and palmitate amounts: In order to determine the required amount AMIONPs for complete separation and adsorption for theophylline in 300 mL (containing 0.2 µg mL^{-1} and 0.8 µg mL^{-1} theophylline, pH 4), different amounts of AMIONPs ranging from 10-140 mg were investigated. The maximum percentage recovery of theophylline was obtained when the amount of AMIONPs was 110 mg. After this amount, the recovery percentage remained constant. 110 mg was chosen as the optimum amount which was used in all further experiments. The negative charges on the surfaces of the palmitate can strongly adsorb to the positive charges on the AMIONPs charges, therefore, various amounts of palmitate (10-100 mg) which are below the critical concentration of forming the micelles (0.001 $mol.L^{-1}$) were examined in order to obtain the optimum concentration. The results have shown that 60 mg of palmitate was chosen as the optimum concentration and was used for further experiments (Fig. 5).

Studying the sample volume effect on the preconcentration factor (PF) and the recovery: In order to conduct the SPE on the aqueous solution to obtain a high preconcentration factor (PF), the volume of the sample needs to be investigated. In this case, the adsorption of the sample on the AMIONPs was studied using various volumes of the sample which ranged from 50-400 mL, each of aqueous sample solution has spiked with a fixed amount of AMIONPs (110 mg) and theophylline (1.5 µg). The maximum recovery percentage (>98%) was observed when the volume of the sample was 300 mL (optimum sample volume which was used in further experiments) (Fig. 6). By applying the sample of volume (300 mL) with the stated previous optimum conditions, the desorbing of theophylline with 2 mL of sodium hydroxide (1.5 $mol.L^{-1}$) and diluting to 4 mL, the preconcentration factor of 75 was achieved.

The loading capacity and regenerating the adsorbent: In order to know how much amount of adsorbent (P@AMIONPs) that is required to add to the sample solution in removing a specific amount of theophylline, the loading capacity needs to be calculated. 110 mg of P@AMIONPs was added to 300 mL of solution containing 5 µg mL^{-1} of theophylline and stirred for 10, 20, 30, 40, 50 and 60 minutes. After each time, the supernatant was removed and determine the concentration of theophylline. The loading capacity was found to be 2.36 $mg.g^{-1}$. To re-generate the adsorbent materials is considered as the main key for evaluating the method. In this study, it was noticed that the adsorbent can be reused four times without losing any of method performance.

The effects of time and eluent solution: In order to obtain the complete extraction, the time factor for both processes (Absorption and desorption) was studied. The experimental results have shown that 4 minutes are quite sufficient to achieve a satisfying adsorption and 2 minutes for the desorption process because of the P@AMIONPs have super magnetic properties and take a short time to be completely collected from the solution. Several of desorption eluents have been used to find the suitable eluent for the complete desorption of theophylline. Among the following eluent solutions (thiourea, acetonitrile, ethanol and NaOH), 1.5 $mol.L^{-1}$ provided a maximum recovery and was chosen to be the best eluent for Further experiments (Fig. 7).

The effect of interfering ions: Prior to applying the proposed procedure on the real pharmaceutical samples, the effect of interfering ions on the absorption and desorption of theophylline was vital to be investigated. In the literature, the tolerance limit is defined as the quantity of foreign ion that can affect on the absorbance reading by ±5%. Under the described optimum conditions, a solution containing 0.1 µg mL^{-1} of theophylline and excess of prepared ions (Ag^{+}, K^{+}, Na^{+}, F^{-}, SO_4^{-2}, NO_3^{-}, CO_3^{-2}, Cl^{-}, Br^{-}, Co^{+2}, Cu^{+2}, Zn^{+2}, Fe^{+2}, Mn^{+2}, Al^{+3}, Fe^{+3}, Cd^{+2}, Mg^{+2}, Ca^{+2}, PO_4^{-2}, Pb^{+2}, Hg^{+2} and Cr^{+3} was prepared. The results have shown that no one of the studied ions interferes with the determination of theophylline in real samples.

Analytical method performance and validation: The calibration curve for the proposed method of theophylline under the optimum conditions theophylline was linear in the range 0.05-2.45 µg mL^{-1}. The equation of calibration curve is $A=0.6422\ C_{\text{Theophylline}} - 0.0116$ ($R^2=0.9832$) where A is the absorbance of the real sample and C is the concentration of theophylline in µg mL^{-1}. The limit of detection (LOD) and Limit of quantification (LOQ) were 0.037 and 0.052 at (n=10) respectively. The relative standard deviation R.S.D for 1.0 µg mL^{-1} is 1.26 (n=10). The intra-day and inter-day precision and accuracy for solid phase extraction of theophylline are tabulated as in table 1.

The application of proposed methods on real samples: To examine the validity of the proposed method, the concentration of theophylline was determined in pharmaceutical preparations by applying the optimum conditions. The reliability and accuracy of the proposed method were examined by using the recovery tests. Different amounts of theophylline were added to the 300 mL of sample and the recoveries of added were determined and evaluated. The obtained results showed the high ability of the proposed method to determine the theophylline in pharmaceutical preparations at very low concentrations without any interference from excipients which are used in the drug (Table 2). The comparison between the obtained results of proposed method and the obtained results from the standard method [26] of theophylline determination was achieved as shown in (Table 3).

Table 1
The intra and inter day accuracy and precision of solid phase extraction of theophylline from the aqueous solution using P@AMIONPs; (n=10 for intra- day and 5 days for inter day)

Theophylline concentration (µg mL^{-1})		R.S.D %	
Added	**Found**	**Intra-day**	**Inter-day**
0.4	0.412 ±0.008	2.18	3.05
1.0	1.090 ± 0.03	2.12	3.6
1.5	1.480 ± 0.06	2.05	3.4

Table 2
Determination of theophylline in pharmaceutical preparation samples using the proposed method (n=6)

Sample	Added Theophylline (µg mL^{-1})	Found Theophylline (µg mL^{-1})	Recovery %
Sample 1	-	0.54 ±0.08	-
Sample 1	0.4	0.96 ±0.04	105
Sample 1	1	1.53 ±0.05	99
Sample 2	-	0.49 ±0.06	-
Sample 2	0.4	0.87 ±0.07	95
Sample 2	1	1.51±0.02	102
Sample 3	-	0.59 ±0.03	-
Sample 3	0.4	0.99 ±0.05	100
Sample 3	1	1.58 ±0.04	99

Table 3
The obtained results of theophylline from the analytical proposed and standard methods

Theophylline samples	Proposed method (µg mL^{-1})	Standard method (µg mL^{-1}) [23]
Sample 1	0.409	0.405
Sample 2	0.410	0.406
Sample 3	0.420	0.412

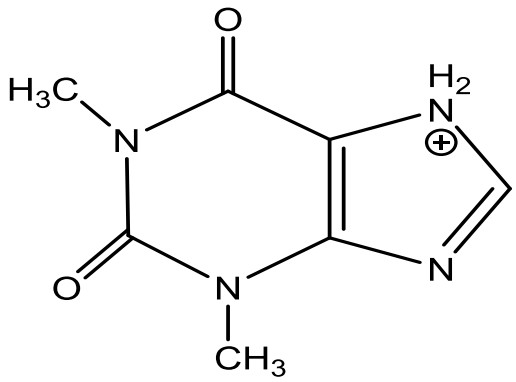

Figure 1: The structure of theophylline in acidic medium

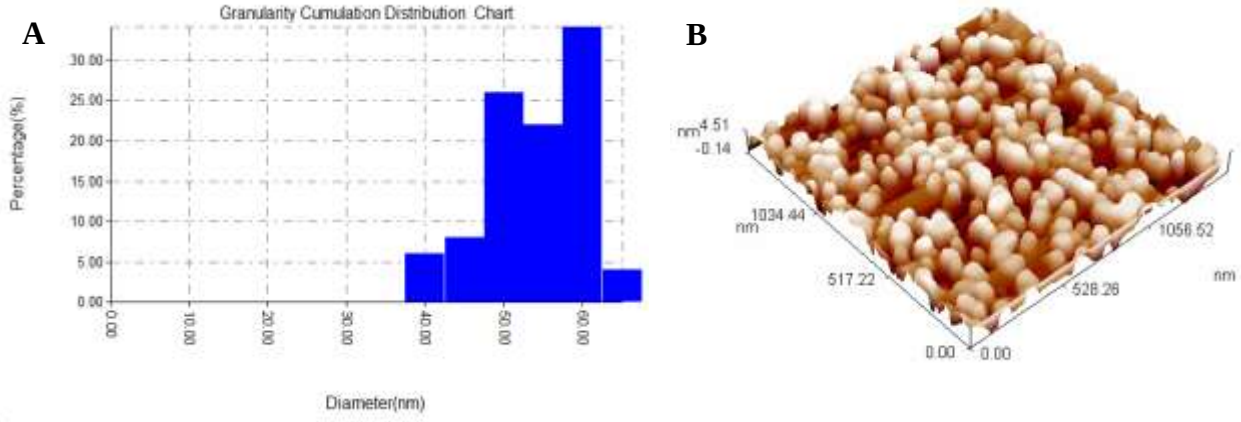

Figure 2: The cumulating distribution of prepared MIONPs (A) and 3D structure (B).

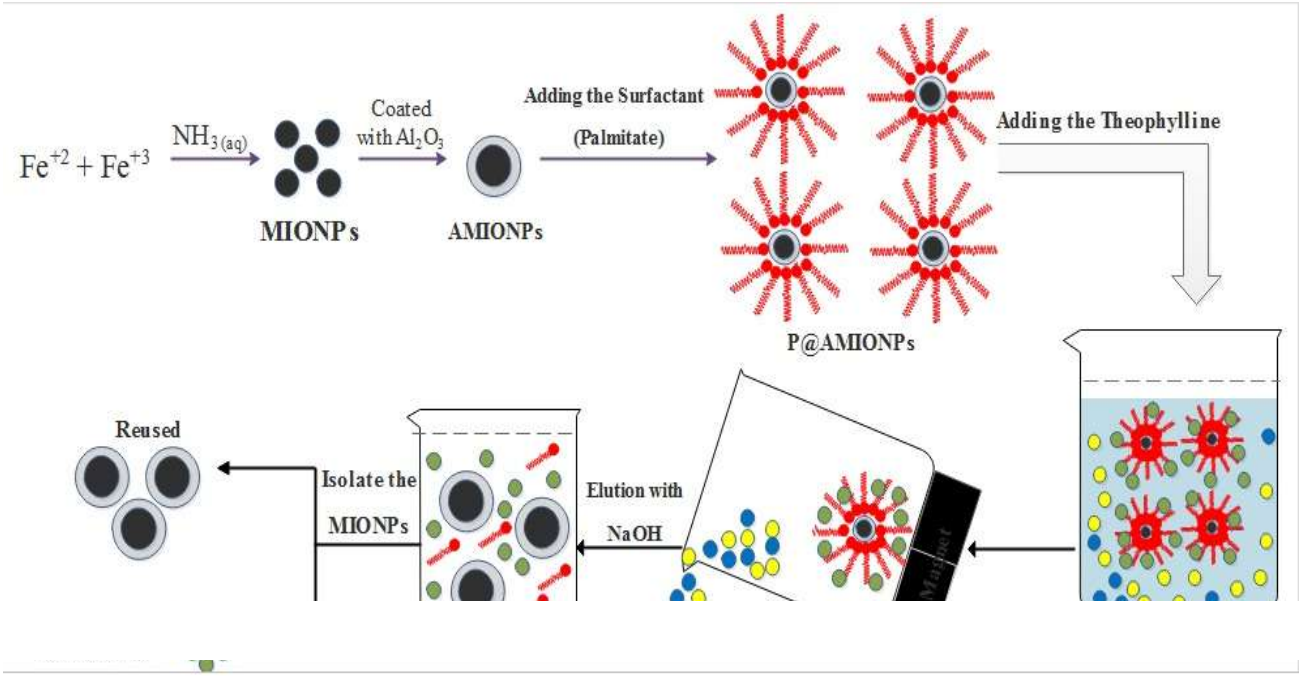

Figure 3: The general procedure of the modified magnetic solid phase extraction method

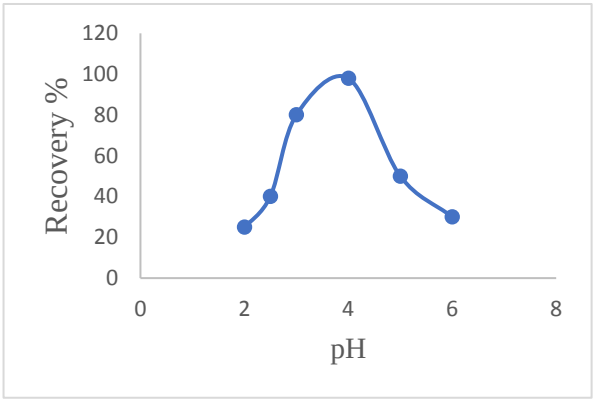

Figure 4: The effect of pH on the recovery % of 1.0 µg mL^{-1}of theophylline (Conditions: sample volume 300mL; amount of P@AMIONPs: 0.11g)

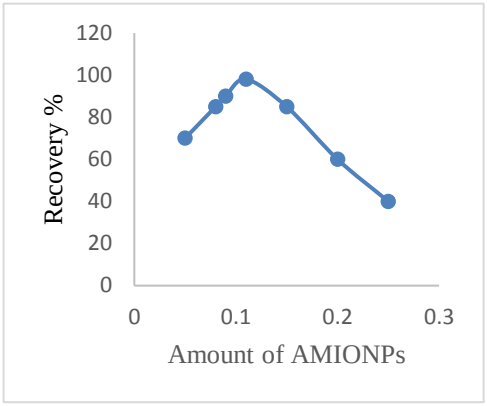

Figure 5: The effect of AMIONPs (absorbent) on the recovery % of 1.0 µg mL^{-1}of theophylline (Conditions: sample volume 300mL; pH: 4)

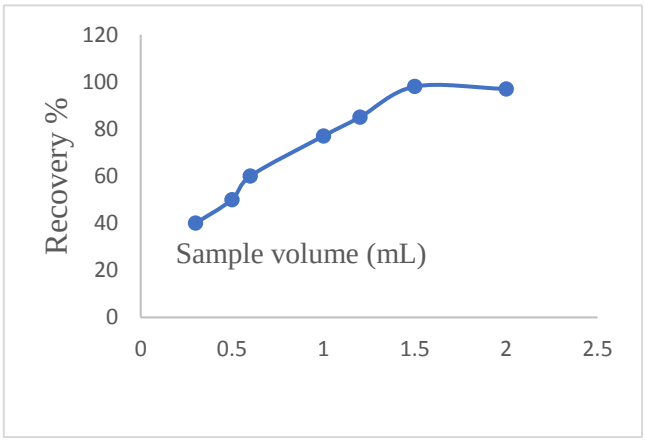

Figure 6: The effect of sample volume on the recovery % of 1.0 µg mL^{-1}of theophylline (Conditions: pH: 4; amount of P@AMIONPs: 0.11g)

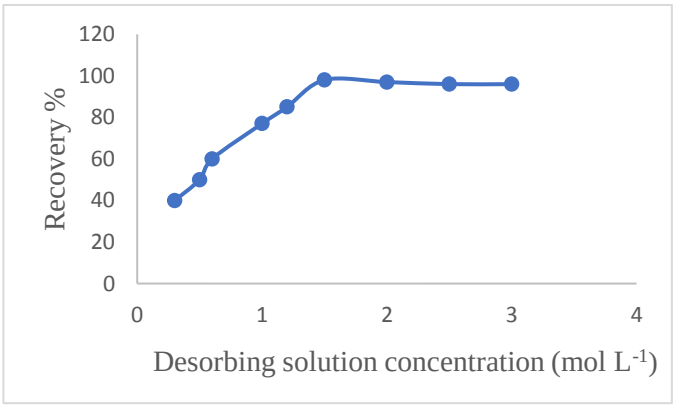

Figure 7: The effect of desorbing solution concentration on the recovery % of 1.0 µg mL^{-1}of theophylline (Conditions: sample volume: 300 mL; pH: 4; amount of P@AMIONPs: 0.11g)

Conclusion

The proposed procedure was fast, simple, cheap, sensitive and highly accurate for determination of theophylline in pharmaceutical dosages. It is also observed that the preconcentration process is very fast and the complete theophylline analysis can be obtained within 6 minutes. The linearity and the limit of detection of the proposed method are better than several reported methods[27-32]. The obtained results of the proposed method are in agreement with the standard methods presented [26]. The obtained results showed the high ability of the proposed method to determine the theophylline in pharmaceutical preparations at very low concentrations without any interference from excipients which are used in the drug.

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