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Dispersive liquid-liquid microextraction using silver nanoparticles as electrostatic probes for preconcentartion and quantitative analysis of terazosin

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Abstract

An efficient dispersive liquid-liquid microextraction using tetraalkylammonium bromide coated silver nanoparticles (DLLME-AgNPs) prepared in chloroform has been successfully applied as electrostatic affinity probes to the microextraction and preconcentration of terazosin prior to spectrofluorimetry analysis. This technique is based on a ternary system of solvents, where appropriate amount of microextraction containing tetraalkylammonium bromide coated silver nanoparticles (AgNPs), and disperser solvents are directly injected into an aqueous solution containing terazosin. A cloudy mixture is formed, and terazosin in the aqueous matrix is extracted into the fine droplets of microextraction solvent containing AgNPs. The settled phase is collected and transferred into a micro-cell of fluorimeter for the determination of terazosin at excitation/emission wavelengths of 330/376 nm. The obtained results demonstrated that electrostatic attraction forces caused by AgNPs were much stronger than the hydrophobic attraction forces. Various factors influencing microextraction efficiency were studied and optimized. Under the optimum conditions, the method provided a relatively broad linear dynamic range of 0.25 to 100 mg mL−1, a detection limit of 0.075 mg mL−1 and a relative standard deviation of 1.9%. Finally, the method was successfully applied to terazosin determination in actual pharmaceutical formulations and human urine sample.

1. Introduction

Terazosin is a highly selective potent α1 adreno-receptor antagonist. It is an effective drug for hypertension [1-3] and benign prostatic hyperplasia [4-6].

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Terazosin (THD) is rapidly and almost completely adsorbed from the gastro-intestinal tract after oral administration; the bioavailability is reported to be about 90%. In addition, it is metabolized in the liver and extracted in feces via the bile, and in the urine as unchanged drug and metabolites [7].

Previous methods for the measurement of THD in bulk, pharmaceuticals or biological fluids have included methods based on spectrophotometry [8], spectrofluorimetry [9], X-ray fluorescence spectrometry [10], high performance liquid chromatography (HPLC) with UV detection [11], HPLC with fluorescence detection [12,13], HPLC with electrospray ionization mass spectrometry [14], and voltammetry [15]. However, most of these methods are relatively expensive and are not accurately reliable for the determination of trace amounts of THD. In addition, due to matrix effects and low concentrations of THD in different samples, using separation and preconcentration steps are still necessary and coupling of them with simple and less expensive determination techniques such as spectrofluorimetry is very attractive. The use of separation and preconcentration steps based on dispersive liquid-liquid microextraction (DLLME) offers conventional alternative to more conventional extraction systems and permits the design of extraction schemes that are simple, cheap and efficient. DLLME methodology has been successfully applied for preconcentration and extraction of a wide range of organic compounds and metallic ions in different samples [16-18]. To the best of our knowledge, up until now, no attention has been paid to the extraction, preconcentration and determination of drugs based on DLLME-AgNPs. DLLME-AgNPs, as electrostatic affinity probes, is a modified solvent extraction method and its acceptor-to-donor phase ratio is greatly reduced comparing with other extraction methods. Simplicity of operation, rapidity, low sample volume, low cost and high enrichment factor are some advantages of DLLME-AgNPs. In the present work, an efficient dispersive liquid-liquid microextraction using tetraalkylammonium bromide coated silver nanoparticles (DLLME-AgNPs) prepared in chloroform has been successfully applied as electrostatic affinity probes to microextraction and preconcentration of terazosin (THD) prior to spectrofluorimetry analysis in pharmaceutical and urine. Several experimental variables affecting the method sensitivity and stability were investigated in detail. This technique permits analysis and quantification of THD in real samples with simple spectrofluorimetric method instead of time-consuming and tedious HPLC methods. The obtained results demonstrated that electrostatic attraction forces caused by tetraalkylammonium bromide coated silver nanoparticles, were much stronger than the hydrophobic attraction forces. As a result high extraction recoveries, short extraction time, excellent selectivity and appreciable reproducibility were obtained.

2. Materials and methods

2.1. Materials

All fluorescence measurements were made using a Perkin-Elmer LS 50 spectrofluorimeter equipped with xenon discharge lamp, and quartz micro-cell with a path length of 10 mm and a volume of 55 μL. Instrument excitation and emission slits both were adjusted to 15 nm. A centrifuge from Hettich (Tuttlingen, Germany, www.hettichlab.com) was used for centrifuging. The pH-meter model 692 (Herisau, Switzerland, www.metrohm-ag.com) supplied with a glass-combined electrode and universal pH indicator (pH 0-14) from Merck (Darmstadt, Germany, www.merck.de) were used for the pH measurements. An adjustable sampler (10-100 μL) was prepared from Eppendorf (Hamburg, Germany). A 1 mL syringe was prepared from Hamilton.

All used chemicals were of analytical-reagent grade. All aqueous solutions were prepared using ultra pure water from a Ghazi (Tabriz, Iran) water purification system. Carbon disulfide, carbon tetrachloride, chloroform, acetonitrile, $AgNO_3$, tetraoctylammonium bromide, methanol and acetone were obtained from Merck (Darmstadt, Germany). The standard solution of THD (1000 µg mL−1) was prepared by dissolving of pure drug in ultra pure water and diluting it, and stored in a dark bottle at room temperature. The working standard solutions were obtained by appropriate dilution of the stock solution. THD tablets (labeled as containing 2 and 5 mg THD per tablet) were purchased from commercial sources.

2.2. Synthesis of silver nanoparticles

AgNPs coated with tetraoctylammonium bromide (TOAB) in chloroform were prepared. An aqueous solution of 30 mM $AgNO_3$ (30 mL) was mixed with chloroform containing 50 mM TOAB (80 mL) and 50 mM dodecanethiol. The biphasic mixture was stirred vigorously for 30 min, and then $NaBH_4$ (25 mL) was added dropwise. The transfer of AgNPs coated with TOAB can be observed a change in the color of the solution. The organic extracts containing AgNPs were washed with sulfuric acid followed by deionized water in order to remove the excess of TOAB and other impurities present in the solution.

2.3. SEM, UV-visible and Fourier transform infrared (FT-IR) measurements

The UV-Vis absorption spectra (Figure 1a) of AgNPs capped with tetraalkylammonium bromide in chloroform were recorded using a model U-3501 spectrophotometer (Hitachi, Tokyo, Japan). The wavelength of the plasma absorption maximum at 400nm confirmed the size of AgNPs in chloroform were <50 nm. This is consistent with our previous report, preparation of hydrophobic AgNPs coated with dodecanethiol in chloroform, where the size of NPs was found to be <50 nm.6. The morphology of the modified AgNPs was confirmed by using a scanning electron microscope (SEM) (Figure 1b).
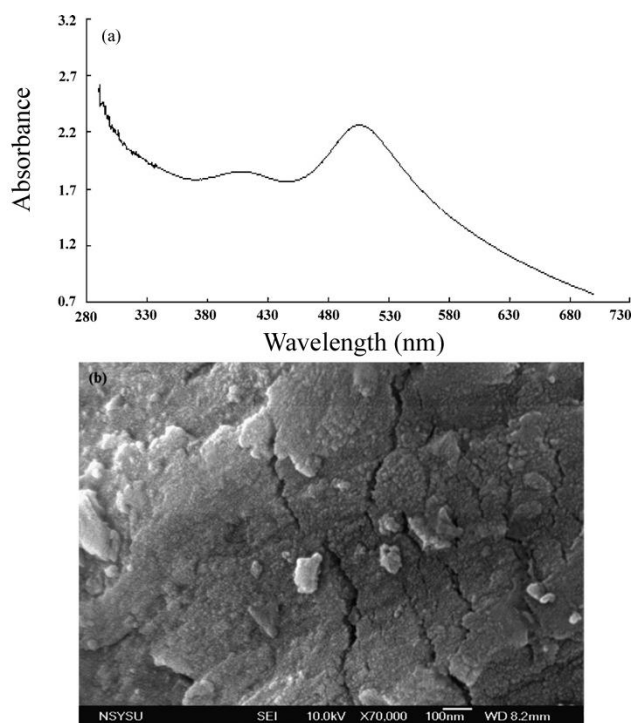
Fig.1. UV-Vis spectrum of AgNPs coated with tetraalkylammonium bromide in chlroform and (a) and (b) SEM image of modified Ag NPs.

The surface of AgNPs coated with tetraalkylammonium bromide was investigated by FT-IR spectroscopy using a Spectrum-100 (Perkin Elmer, USA). The bands observed at 2918.12 and 2850.20cm^{-1} were confirmed by the presence of the CH$_2$ group of tetraalkylammonium bromide on the surface of AgNPs. The band at a 1343.32 cm^{-1} was assigned as C–N stretching which associated with the substitution of nitrogen with a carbon atom in tetraalkylammonium bromide (Figure 2).
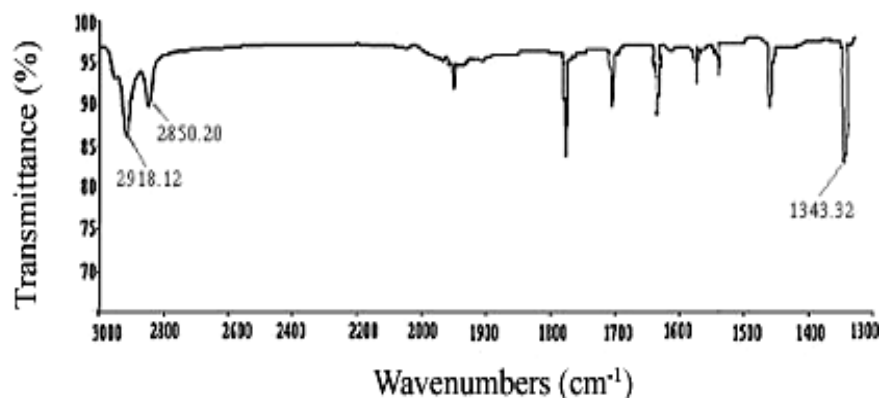

Fig. 2. FT-IR spectrum of AgNPs capped with tetraalkylammonium bromide in chloroform.

2.4. Assay procedure for tablets

Five THD tablets, labeled as containing 2 mg THD each, were weighed and the average mass per tablet was determined. An amount of the powder equivalent to 2 mg of THD was accurately weighed and dissolved in 50 mL ultra pure water. The solution was sonicated for 5 min and filtered into a 100 mL volumetric flask. Further dilutions were made up with ultra pure water to achieve a final concentration of 20 μg mL-1. In addition, appropriate dilution was performed for THD analysis.

2.5. Preparation of spiked human urine samples

Urine samples, obtained from healthy volunteers, were collected and mixed. Aliquot of 10 mL from this mixture were placed in graduated centrifuge tubes. These solutions were centrifuged for 5 min at 6000 rpm and 2 mL of supernatants were transferred into new test tubes and stored frozen until assays. Aliquots of centrifuged human urine samples (each of 200 μL) were spiked with different amounts of THD (1.5 to 10.0 ng mL−1). Each of these samples was put in a centrifuged tube and then the analysis was followed up as indicated in the general procedure.

2.6. General procedure

For DLLME-AgNPs under optimum conditions, sample solution (10.0 mL) containing the analyte (pH = 10) was transferred into a glass test tube with conic bottom. 0.50 mL of acetone (disperser solvent) containing 120 μL of chloroform (microextraction solvent), which involves tetraalkylammonium bromide coated silver nanoparticles, was injected rapidly into the sample solution by using a 1 mL syringe. A cloudy solution (water, acetone and chloroform) was formed in a test tube. In this step, THD extracts into fine droplets of microextraction solvent. The mixture was then centrifuged for 2 min at 4000 rpm. After this process the dispersed fine droplets of microextraction solvent were sedimented at the bottom of conical test tube (60 μL) and then this sedimented phase was removed using a sampler and injected into a 55 μL micro-cell. Subsequently,

the micro-cell was located in spectrofluorimeter to obtain related spectra. The fluorescence intensity was measured at 376 nm with the excitation wavelength set at 330 nm.

2.7. Reference voltammetric procedure

A stripping voltammetric procedure was applied as the reference method [15]. In this procedure, a 10-ml volume of Britton–Robinson buffer was introduced into a dark micro-electrolysis cell, and then de-aerated with pure nitrogen for 10 min. A selected accumulation potential was then applied to the hanging mercury drop electrode for a selected preconcentration time period, while the solution was stirred at 400 rpm. At the end of the accumulation period, the stirring was stopped and a 5-s rest period was allowed for the solution to become quiescent. Then, the background voltammogram was recorded by scanning the potential toward the negative direction using the selected waveform. After recording the background voltammogram, an aliquot of the reactant solution was introduced into the micro electrolysis cell and the voltammogram was then recorded at a new mercury drop. All the data were obtained at room temperature.

3. Result and discussion

In this work, dispersive liquid-liquid microextraction using tetraalkylammonium bromide coated silver nanoparticles (DLLME-AgNPs) was combined with spectrofluorimetry for the first time and THD was chosen as an example analyte to investigate the feasibility of this joining. THD is trapped in a low volume of microextraction solvent containing tetraalkylammonium bromide coated silver nanoparticles, and separated from aqueous phase or biological matrix. The excitation and emission spectra of THD are given in Figure 3 with maxima at 330 ± 3 and 376 ± 3 nm, respectively.
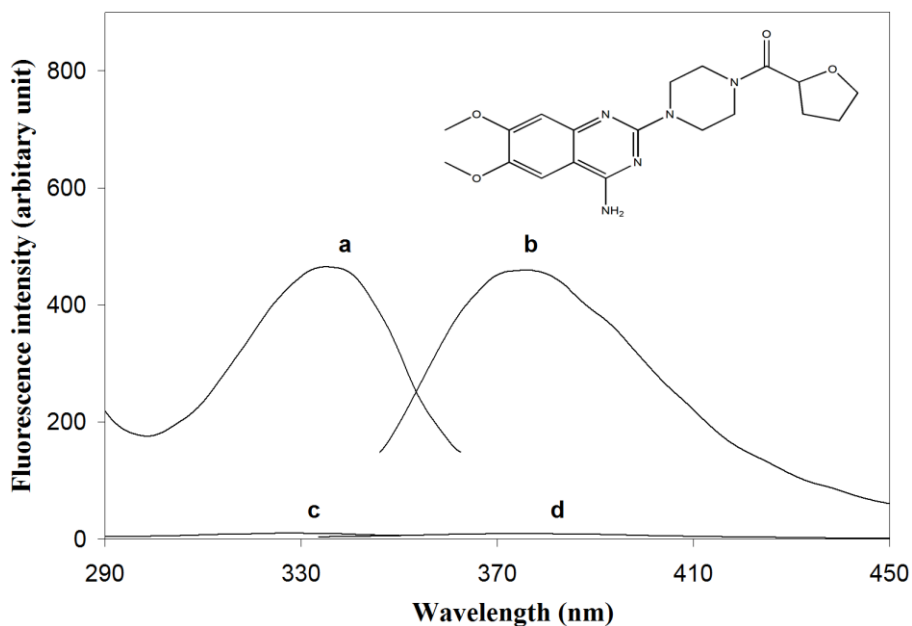
Fig.3. Excitation and emission spectra of THD treated as in the general analytical procedure; λ_{ex} 330 nm, slit width 15 nm; λ_{em} 376 nm, Slit width 15 nm. (a) Excitation and (b) emission spectra of THD (40 ng mL^{-1}); (c) excitation and (d) Emission spectra of the blank of reagent. Inset: Chemical structure of THD.

The influence of different factors affecting extraction conditions, such as kind of microextraction and disperser solvent and their volume, pH, ionic strength and extraction time were studied and optimized to obtain a compromise between preconcentration factor, sensitivity, simplicity and reproducibility. Preconcentration factor (PF) and percent extraction recovery (ER%) as analytical responses were calculated based on the following equations:

$$PF = \frac{C_{sed}}{C_0} \qquad\qquad ER\% = C_{sed} \times V_{sed} / C_0 \times V_{aq} \times 100$$

Where C_{sed} and C_0 are concentration of the analyte in the sedimented phase and initial concentration of the analyte in the aqueous sample, respectively. V_{sed} and V_{aq} are the volume of the sedimented phase and volume of the aqueous sample, respectively. C_{sed} calculation was conducted by direct injection of the THD standard solutions in each microextraction solvent.

3.1. Selection of microextraction solvent

The selection of an appropriate microextraction solvent is critical for the DLLME-AgNPs process. The microextraction solvent has to meet some requirements: it should have a higher density than water, a low solubility in water, and high extraction efficiency of the compound of interest; it also should have no interference with the analyte peak when directly transferred into a micro-cell of fluorimeter for analysis. In the present study, chloroform (density, 1.48 g mL^{-1}), carbon tetrachloride (density, 1.58 g mL^{-1}) and carbon disulfide (density, 1.26 g mL^{-1}) were selected as extractant solvents. ER% using chloroform, carbon tetrachloride and carbon disulfide were 58.8%, 32.4% and 43.8% respectively. The results revealed that chloroform has the highest extraction recovery in comparison with the other tested solvents. It is probably because of higher solubility of THD in chloroform in comparison with carbon tetrachloride and carbon disulfide. Therefore, chloroform was selected as the optimum solvent. The obtained results demonstrated that electrostatic attraction forces caused by silver nanoparticles were much stronger than the hydrophobic attraction forces.
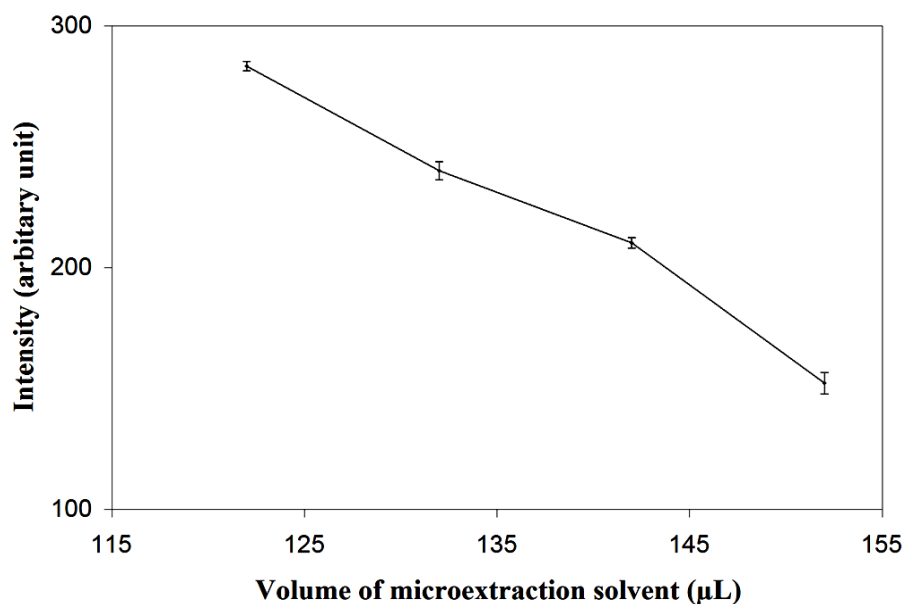
3.2. Selection of disperser solvent

The miscibility of the disperser solvent in the organic phase (microextraction solvent) and the aqueous phase (sample solution) is the main point for the selection of the disperser solvent in the present preconcentration technique. Acetone, acetonitrile and methanol, which show this ability, were chosen as disperser solvents. A series of sample solutions were studied by the injection of 0.50 mL of each disperser solvent containing 120μL chloroform (as microextraction solvent) into the test tube including sample solution. Considering the sedimented phase volume, it was found that with combination of chloroform-acetonitrile, the sedimented phase volume was very higher than 60 μL and the cloudy state was not formed well, whereas in the case of chloroform-methanol, and chloroform-acetone, the sedimented volume was about 60 μL. Thus, acetone and methanol could be selected as disperser solvents for further studies. Further experiments revealed that the PF in the presence of acetone and methanol were 98 and 81, respectively. According to the results, acetone has the higher per-concentration factor, lower toxicity and lower cost in comparison with methanol. Therefore, acetone was used for further studies.

3.3. Influence of microextraction solvent volume

The recovery of about 60 µL of the sedimented phase after DLLME-AgNPs is necessary because of the use of a 55 µL micro-cell in all experiments. Also, it is necessary to add an excess amount of the microextraction solvent due to its solubility in water. In order to evaluate the effect of extraction volume on the extraction efficiency, additional experiments were done using solutions containing different volumes of chloroform (120, 130, 144 and 152 µL) and fixed volume of acetone (0.50 mL). With the increase of the extraction solvent volume from 120 to 152 µL, the volume of the sedimented phase increased (60-95 µL). Using less than 120 µL volume of chloroform decreased the volume of the sedimented phase to less than 60 µL, thus, removing the sedimented phase for injection into a 55 µL micro-cell of fluorimeter would be too difficult and accompanies systematic error.

Figure 4 displays the variation of fluorescence intensity versus volume of the microextraction solvent (chloroform). As can be seen, signal intensity decreases as the volume of microextraction solvent increases due to increase in the sedimented phase volume. So, 120 µL of chloroform was selected in order to achieve a compromise between simplicity, sensitivity and reproducibility.


Fig.4. Influence of microextraction solvent (chloroform) volume on the fluorescence intensity of THD. Experimental conditions: $\lambda_{ex} = 330$; $\lambda_{em} = 376$ nm; sample volume, 10 mL; disperser solvent (acetone) volume, 0.50 mL; concentartion of THD; 20 ng mL^{-1}. Fluorescence intensity values indicated are the average of three independent measurements and error bars correspond to their standard deviations.

3.4. Influence of disperser solvent volume

Volume of the dispersive solvent is one of the important factors to be considered. At lower volumes of the disperser, tiny droplet formation may not be effective thereby lowering the extraction efficiency. At higher volumes of the dispersive solvent, the solubility of THD in aqueous solution increases; thus, lowering the partition of THD into chloroform leading to a decrease in efficiency. The variation of the acetone (as disperser solvent) causes changes in the volume of the sedimented phase. To prevent this problem, and in order to acquire a constant volume of the sedimented phase, the volumes of the acetone and chloroform were changed, simultaneously. Other experimental conditions were kept constant and included the use of different acetone volumes, 0.25,

0.50, 1.0 and 2.0 containing 100, 120, 142, 165 µL chloroform, respectively. In this step, the volume of the sedimented phase was constant (60±3 µL). As can be seen in Figure 5, a growth in the volume of acetone up to 0.50 mL leads to an increase in the fluorescence intensity and then to a decrease. It seems that, in the lower volumes of acetone, a cloudy state is not formed well, thereby, the preconcentration factor is low. In higher volumes of acetone, solubility of THD in aqueous solution increases. Therefore, the extraction efficiency decreases due to the decrease of distribution coefficient. A 0.50 mL of acetone was chosen as optimum value.
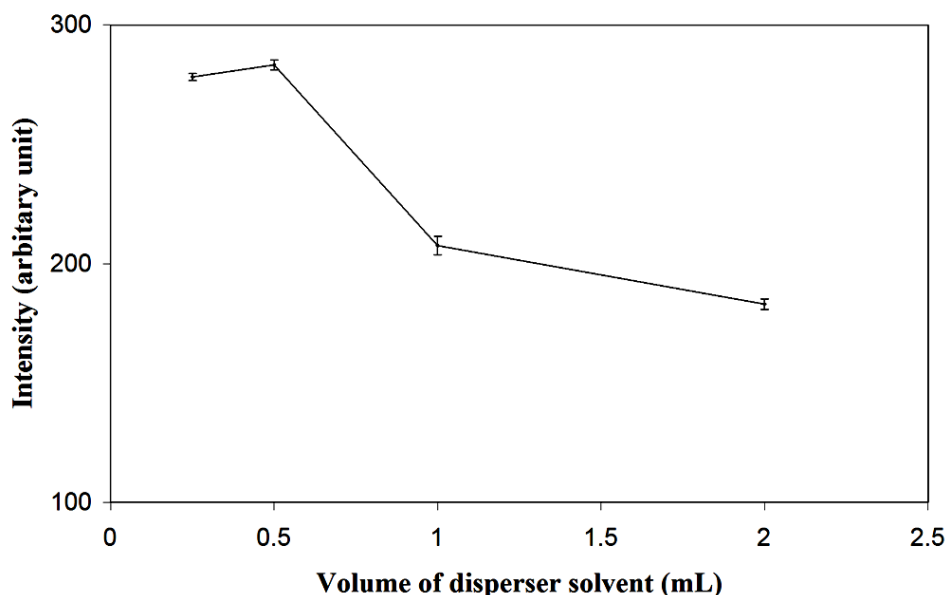
Fig.5. Influence of disperser solvent (acetone) volume on the spectrofluorimetic responses. Experimental conditions: as indicated in Figure 3 with a sedimented phase volume of 60 ± 3 µL. Fluorescence intensity values indicated are the average of three independent measurements and error bars correspond to their standard deviations.

3.5. Influence of pH

The solution pH of the sample is an important factor which affects the chemical structure of THD. The effect of pH on the extraction of THD from aqueous samples was studied over the range of 5.5 to 11.5 using HCl and NaOH. For organic molecules, especially for ionizable species, maximum extraction efficiency is achieved at pH values where the uncharged form of the analyte prevails, and therefore, target analyte is favored to be partitioned into the organic phase. The results illustrated in Figure 6 reveal that analytical signals, obtained for THD, depend on pH. The best conditions of THD extraction were achieved at alkaline pH values due to dissociation of the aromatic amine group, which acts as a weak base. This approach has been supported in concordance experimental results with reported pK_a value of the THD (pK_a=7.1). Based on the results obtained pH 10 was selected as the optimal value. A series of supplemental experiments were carried out by using different buffer systems. In this study, pH of a series of solutions containing analyte and other reagents was adjusted in 10 by using OH^- or different buffer systems and solutions were subjected to the DLLME-AgNPs and spectrofluorimetric determination. The results showed that higher analytical signals were obtained by using OH^- as buffering agent. So, the pH adjustment was not carried out by using buffer systems and addition of proper amount of OH^- was sufficient for adjustment of pH and achievement of higher analytical signal.
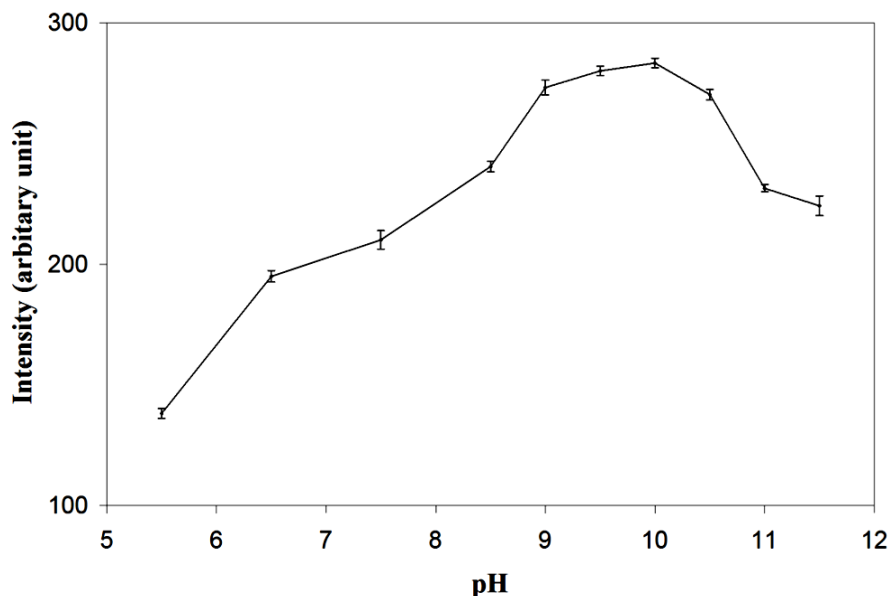
Fig.6. Influence of pH on the analytical signals obtained for THD. Experimental conditions: as indicated in Figure 3 with an extraction solvent volume of 120 μL. Error bars correspond to three independent measurements.

3.6. Influence of salt addition

The impact of ionic strength on extraction efficiency and subsequent measurement was evaluated by adding different amounts of NaCl (0-10% (w/v)). Other experimental conditions were kept constant. Based on the results obtained in this study salt addition has no significant and benefic effect on the extraction efficiency. On the other hand, the enrichment factor is nearly constant by increasing the amount of sodium chloride. Thus, this electrolyte was not used through the rest of the work.

3.7. Influence of microextraction time

Microextraction time is one of the major parameters affecting the extraction efficiency, especially in microextraction methods such as SPME and LPME. In the DLLME-AgNPs method, the extraction time is defined as an interval between the injection of the mixture of disperser solvent (acetone) and the microextraction solvent (chloroform), and starting centrifuge. The dependence of extraction efficiency upon extraction time was investigated within a range of 5-300 sec. Based on the results obtained in this study signal variations versus extraction time were not significant. It was revealed that after formation of cloudy solution, the surface area between extraction and aqueous phase was infinitely large. Therefore, the transfer of THD from aqueous phase to microextraction solvent was very fast. Subsequently, the equilibrium state was obtained quickly and the extraction time was very short. In this method, the most time-consuming step was the centrifuging of the sample solution in the extraction procedure, which was about 2 min. Thus, this method is very fast and this is the most distinct advantage of the DLLME-AgNPs technique. Optimum experimental conditions of DLLME-AgNPs-spectrfluorimetry for the determination of THD are shown in Table 1.

Table 1. Optimum experimental conditions of the dispersive liquid-liquid microextraction using tetraalkylammonium bromide coated silver nanoparticles (DLLME-AgNPs).

Parameter	Value
Extraction solvent (chloroform) volume	120 μL
Disperser solvent (acetone) volume	0.50 mL
Sample pH	10
Ionic strength	NE[a]
Extraction time	<3
Excitation wavelength	330 ± 3 nm
Emission wavelength	376 ± 3 nm
Slit width[b]	15 nm

[a]No effect.
[b]For both excitation and emission slits.

3.8. Excitation and emission spectra of THD

It is well known that a great majority of the organic compounds which exhibit fluorescence possess cyclic, conjugated structures involved π-electron system. As can be seen in Figure 3, the maximum excitation and emission wavelength of THD treated as in the general procedure were 330 and 376 nm (lines a and b). As it can be ascertained, the excitation and emission of the blank of reagent (lines c and d) has no effect on the determination of THD. So, the mentioned wavelengths were selected as excitation and emission conditions, respectively. Optimum experimental conditions of DLLME-AgNPs-spectrfluorimetry for the determination of THD are shown in Table 1.

4. Application

4.1. Analytical characteristics

Under the optimum experimental conditions, the relationship between the analytical signal and concentration was studied over the range 0.1 to 400 ng mL^{-1} and found to be linear from 0.25 to 100 ng mL^{-1}. The corresponding fitted equation was I=106.33+8.8512C with r^2=0.9994, where A is the fluorescence intensity at 376±3 nm and C is the THD concentration in ng mL^{-1}. The limit of detection was determined by using the criterion, LOD=ks$_{bl}$/m, where k is a factor (=3), s$_{bl}$ is the standard deviation of the blank measurements and m is the calibration slope. The value thus found was 0.075 ng mL^{-1}. The repeatability of the system was determined from seven consecutive insertions of a 20 ng mL^{-1} standard solution of THD; the relative standard deviation (R.S.D.) thus obtained was 1.9%.

Initially, the accuracy of the proposed method was evaluated by means of recovery experiences. For this purpose, different amounts of authentic THD were added to the diluted sample solutions, obtained from THD tablets, and subsequently assayed by the developed procedure. The results of this recovery study are shown in Table 2. As can be ascertained, in all cases, the quantitative recoveries (94.0-109.2%) were obtained.

Table 2. Recoveries obtained for THD in pharmaceutical samples by the proposed method.

Sample	Endogenous value [a] (ng mL^{-1})	THD added (ng mL^{-1})	THD found [b] (ng mL^{-1})	Recovery (%)
THD tablet 1 [c]	20.0	0	19.6 ± 0.6	-
		5	27.3 ± 0.9	109.2
		10	32.0 ± 1.3	106.7
		15	34.1 ± 0.8	97.4
		20	42.0 ± 1.6	105.0
THD tablet 2 [c]	10.0	0	10.7 ± 0.2	-
		5	14.1 ± 0.6	94.0
		10	19.0 ± 0.7	95.0
		15	25.6 ± 1.4	102.4
		20	28.1 ± 1.1	93.7

[a] Correspond to a dilution of the sample solution.
[b] The standard deviation values correspond to three independent measurements.
[c] THD tablets 1 and 2: sample solutions with different dilutions.

4.2. Quantitative analysis of THD in pharmaceutical formulations using DLLME-AgNPs

The developed procedure was applied to the determination of THD in the commercial pharmaceutical preparations. The sample solutions, obtained from THD tablets, were treated as mentioned in the general procedure to calculate the amounts of THD in the samples using simple calibration line. Table 3 presents results obtained by applying the present combined methodology and those obtained by the reported voltammetric method [15]. Until date no official method has been reported for THD. These results indicate the accuracy of the proposed method, and its viability for the analysis of THD in this type of samples.

Table 3. Results of analysis of THD tablets by the proposed procedure and by a reported voltammetric method [15].

Claimed (mg/tablet)	Proposed method (mg)[a]	Reference method (mg)[a]	Error (%) [b]	Error (%) [c]
2	1.97 (±0.08)	2.05 (±0.10)	-1.5	-3.9
5	5.06 (±0.21)	5.37 (±0.37)	+1.2	-5.8

[a] Values in parenthesis give the standard deviation based on three replicates.
[b] Error against the declared value.
[c] Error against the reference method.

### 4.3. Quantitative analysis of THD in spiked human urine using DLLME-AgNPs

The accuracy of the present method was proven by determination of THD in spiked urine samples. Aliquots of 200 μL urine samples were spiked with four different concentrations of THD at 1.5, 3.5, 5.0 and 10.0 ng mL^{-1} and recovery experiments were conducted for these samples as well. The results are summarized in Table 4 and calculated amounts of recoveries varied between 91.4-109.0%. In Figure 7, spectra of urine sample with and without DLLME-AgNPs were compared, demonstrating the efficacy of the recommended method to eliminate interference from

urine, which is a highly fluorescent matrix. In addition, a calibration curve was made using spiked urine sample to compare the results obtained in this supplemental experiment with those obtained by the simple calibration curve of aqueous standard solutions. Based on the results obtained in this study, no significant difference was observed between these results, and the calibration curve achieved from spiked urine sample shows good correlation compared to that for aqueous standard solutions. On the other hand, one of the main advantages of the present combined methodology is that the determination of THD in urine can be made by direct comparison with aqueous standard solution. In addition, it is avoided the need of employing a high performance separation instrumental for the treatment of urine previous measurement, which in most cases are necessary for eliminating interferences species.

Table 4. Results of recoveries of spiked urine samples using the dispersive liquid-liquid microextraction using tetraalkylammonium bromide coated silver nanoparticles (DLLME-AgNPs).

Sample	THD added (ng mL^{-1})	THD found (ng mL^{-1})^a	Recovery (%)
urine	1.5	1.6 ± 0.2	106.7
	3.5	3.2 ± 0.7	91.4
	5.0	5.4 ± 0.5	108.0
	10.0	10.9 ± 0.7	109.0

^{a} The standard deviation values correspond to three independent measurements.
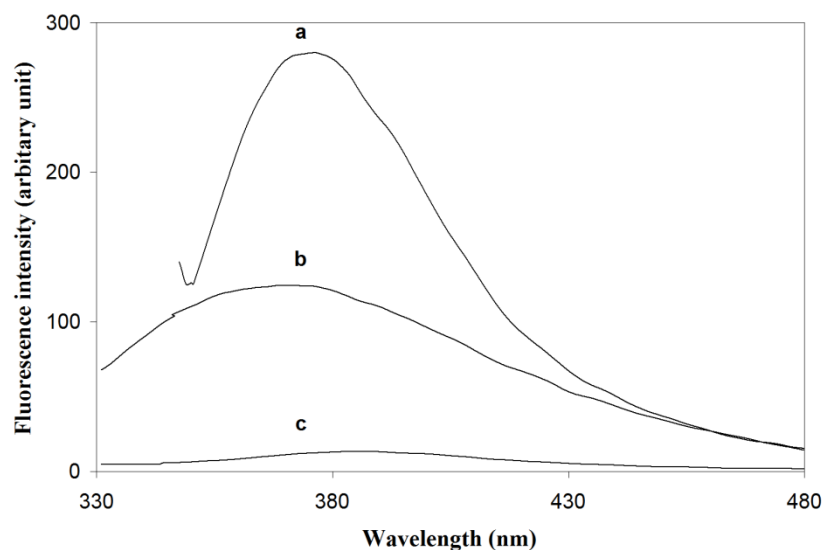
Fig.7. Urine sample spectra with and without DLLME-AgNPs procedure; λ_{ex} 330 nm, slit width 15 nm; λ_{em} 376 nm, slit width 15 nm. (a) Spectrum of THD (20 ng mL^{-1}) in urine treated by DLLME-AgNPs; (b) spectrum of urine alone; (c) Spectrum of urine alone treated by DLLME-AgNPs.

4.4. Comparison with other reported methods

A comparison of the main analytical characteristics (i.e., LOD, LR and RSD) of the proposed DLLME-AgNPs-spectrofluorimetry method for the determination of THD with those of some of the best previously reported methods for this purpose is showed in Table 5.

Table 5. Comparison characteristics performance data obtained by using DLLME-AgNPs spectrofluorimetry and other techniques in determination of THD.

Method	Sample	LOD (ng mL^{-1})	RSD (%)	LR (ng mL-1)	References
Spectrofluorimetry	Serum, urine	210	2.5	Up to 3486.9	[12]
X-ray fluorescence spectrometry	Drug	732	-	732-843000	[14]
HPLC with fluorescence detection	Plasma	0.25	< 7	Up to 100	[17]
HPLC with electrospray ionization mass spectrometry detection	Plasma	0.0625	-	-	[18]
Square-wave adsorptive cathodic stripping voltammetry	Plasma, drug	0.0058	< 1.1	0.39-11.62	[19]
DLLME-AgNPs-spectrofluorimetry	Urine, drug	0.075	1.9	0.25-100	[as optimized in this study]

LOD: limit of detection, LR: linear range, RSD: relative standard deviation.

As can be seen, analytical characteristics of the proposed DLLME-AgNPs-spectrofluorimetry method, with a sample volume of only 10.0 mL, are significantly improved over most of the previously proposed methods. In addition, the extraction time is very short, which indicates the fact that DLLME-AgNPs is a very sensitive, rapid and reproducible technique that can be used for the preconcentration and determination of THD from real samples.

5. Conclusion

An efficient dispersive liquid-liquid microextraction using tetraalkylammonium bromide coated silver nanoparticles (DLLME-AgNPs) prepared in chloroform has been successfully applied as electrostatic affinity probes to microextraction and preconcentration of terazosin prior to spectrofluorimetry analysis. The method developed in this work permits the fast, accurate and reliable determination of THD in commercial pharmaceutical preparations and human urine. There are some advantages of the present method, including: (i) compared with HPLC methods for determination of THD in biological fluids, which require a previous step of clean-up, the proposed procedure allows carrying out the analysis in a simple and quick way and with out long and tedious clean-up steps, (ii) the proposed method improves sensitivity through the preconcentration step, and (iii) the method represents a good approach in the area of pharmaceutical and urine monitoring with low operation cost and simplicity of instrumentation. The obtained results demonstrated that electrostatic attraction forces caused by AgNPs were much stronger than the hydrophobic attraction forces. As a result high extraction recoveries, short extraction time, excellent selectivity and appreciable reproducibility were obtained.

Acknowledgment

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