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Optimization of experimental parameters for the determination of amoxicillin by sensitive spectrophotometric method using synthesized gold nanoparticles

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Abstract

In this study, a sensitive and simple spectrophotometric method has been developed for determination of amoxicillin (AMX) using gold nanoparticles (AuNPs) synthetized by chemical reduction method. This method is based on addition of AMX to AuNPs and nanoparticles aggregation, consequently absorption band of nanoparticles decreased. The optimization of experimental variables was investigated by examining pH, temperature, buffer type and amount, AMX and AuNPs amounts and process time. In the desired condition, the linear calibration graph in the range of 10-250 µg/l was obtained with proper correlation coefficient (R^2=0.99). Limit of detection (LOD) and relative standard deviation (RSD %) were calculated as 0.12 µg/l and 0.61%, respectively. The proposed method was successfully applied to the AMX measurement in pharmaceutical, real water and biological samples.

Key words: Spectrophotometry, Amoxicillin, Gold Nanoparticles

1 Introduction

Amoxicillin (AMX) is one of the amino penicillin's classified as β-Lactam group [1, 2]. It is utilized widely in pharmaceutical industry for production drugs to treat various infections owing to its effectiveness, less side effects and low production cost [3]. Determination of AMX is significant for control of its production in biotechnological processes and also in the quality control of its preparation procedure [4]. Moreover, the presence of antibiotics in wastewater of hospital or pharmaceutical industries is a serious environmental threat due to the possibility of antibiotic-resistant strains formation in pathogenic bacteria [5]. Hence, monitoring of these species is very essential in aquatic environment [6]. However, AMX determination is not easy owing to the lack of any significant chromospheres in its structure. Among various methods for determination of AMX, such as liquid chromatography-mass spectrometry [7,8], high performance liquid chromatography (HPLC) [9], high performance thin layer chromatography[10], derivative spectrophotometry[11,12], voltammetry[13], and capillary electrophoresis[14], HPLC is the most reported technique which needs large amount of pure solvents, derivatizing treatment and long equilibration time. Therefore, application of a rapid, accurate, low cost and selective technique like spectrophotometric method for determination of AMX is essential from practical point of view [15].Gold nanoparticles (AuNPs) have been applied widely and successfully for determination of pharmaceutical andbiological compounds by varying of AuNPs absorption because of aggregation of nanoparticles in the presence of various analytes [16].

In this research work, simple and sensitive spectrophotometric process was developed for determination of AMX in aqueous solutions. First AuNPs were prepared with chemical reduction method [17]. Then, they were applied for determination of AMX amount by changing of experimental variables to find the optimal conditions. Eventually, results were used to find the AMX concentration in real samples including AMX capsule, spring water and biological samples.

2 Materials and methods

2.1 Chemicals

Table1 represents chemical structure and other characteristics of AMX, which is named chemically (2S, 5R, 6R)-6-[(R)-(-)-2-Amino-2-) p- hydroxyphenyl) acetamidol] 3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid trihydrate [18, 19].

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Table1: Characteristics of amoxicillin

structure	Cass number	Molecular formula	λ_{max}	Mw (g/mol)
	26787-78-0	$C_{16}H_{19}N_3O_5S.3H_2O$	220 nm	419.45

Standard reference of AMX and AMX capsules were provided from Dana Co. Iran. Other compounds which were used in this study purchased from Merck, Germany.

2.2 Synthesis of gold nanoparticles

Chemical reduction method was applied to prepare of AuNPs. Briefly, 250 ml of gold salt precursor (tetrachloroauric(III)acid trihydrate, 1.5 %v/v) was heated to 95 ^{o}C, then, 5 ml of trisodiumcitrate was added to the solution. Citrate is soluble in water acting as reluctant agent of Au(III). It has negative charge preventing aggregation AuNPs in solution. The formation of pink solution with maximum absorbance λ_{max} at 520 nm confirmed the production of AuNPs [20, 21].

2.3 Experimental procedure

All experiments were performed in a batch mode. A solution with certain amount of AMX and AuNPs (A), (by pH, buffer type, amount of gold nanoparticles, amount of buffer, temperature, and process time) was prepared with total volume of 3ml by addition of double distilled water. Then, the absorbance of each solution was recorded against the blank solvent (A_o) and (A-A_o) determined at λmax of 520 nm. The pH of solution was measured by pH meter (pH211, Hanna, Romany) and hydrocholoric acid (0.1 M) or sodium hydroxide (0.1 M). The effect of different buffers at The influence of various volume of acetate buffer in the range of 200-800 µl was investigated. As can be seen in Fig.2, addition of 200 µl of acetate buffer solution resulted in maximum absorption of AuNPs.

3.1.1 Effect of pH

The influence of pH on the absorbance of AuNPs was investigated in the range of 3-9 (Fig.1). The maximum absorption was observed at pH of 5. In pHs less than 5, AuNPs aggregated and the absorption declined. Above desired pH the absorption decreased because of deprotonation of amin group of AMX and more aggregation of AuNPs [22].Place the cursor at the beginning of the first line in either of the columns and press the required button.

3.1.2 Effect of buffer types during experiment

pH stabilization at optimum value is essential in determination of AMX. In order to study the role of buffer, acetate and phosphate buffers was selected with concentration of 0.5M in the same experimental variables with Fig 2. Results revealed that AuNPs had more absorption using of acetate buffer (0.61) in comparison of phosphate (0.42) due to closer pKa to optimum pH [23].

3.1.3 Effect of buffer volume

The influence of various volume of acetate buffer in the range of 200-800 µl was investigated. As can be seen in Fig.2, addition of 200 µl of acetate buffer solution resulted in maximum absorption of AuNPs.

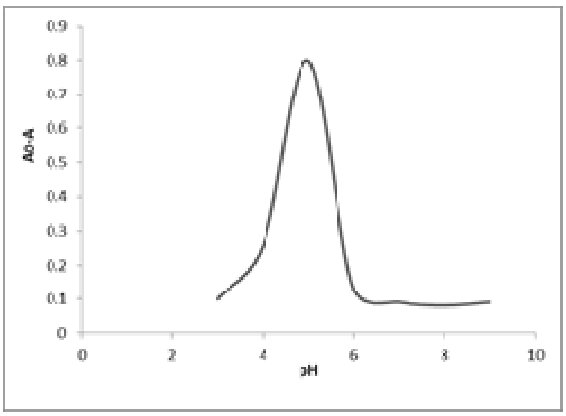

Fig. 1. Effect of pH on AuNPs absorbance at λ_{max} = 520 nm, AMX = 60µg/L, AuNPs = 1000 µl and T =25ºC

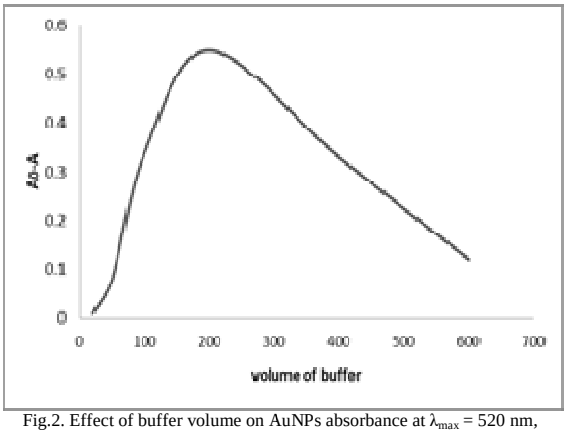

Fig.2. Effect of buffer volume on AuNPs absorbance at λ_{max} = 520 nm, AMX = 60 µg/l, AuNPs = 1000 µl, buffer volume = 200 µl and T = 25 ºC

3.1.4 Effect of AuNPs volume

The AuNPs amount in the solution is key parameter for determination of AMX. Hence, the influence of AuNPs amount was investigated by varying AuNPs volume in the range of 200-900 µl (Fig. 3). Addition of 700 µl of AuNPs to the solution leaded to desired absorption. Below 700 µl there were not adequate AuNPs in the solution and above this value, more aggregation took place resulting in less absorption and sensivity [24].

3.1.5 Effect of temperature and process time

Process time and temperature showed negligible influence on absorbance of AuNPs at the same experimental variables for determination of AMX. Moreover, the product of AuNPs with AMX was stabilized instantly at 25ºC and remained stable after 30h.

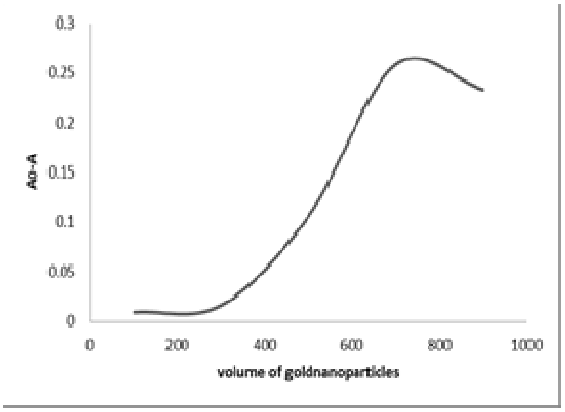

Fig. 3. Effect of AuNPs volume on AuNPs absorbance at λ_{max}= 520 nm. AMX = 60 µg/L, AuNPs = 700 µl, buffer volume = 200 µl and T = 25 ºC

3.1.5 Effect of amoxicillin concentration

The absorbance of AMX with different concentrations at optimal conditions was demonstrated in Fig. 4. The absorbance declined with increasing of AMX amounts due to more aggregation of AuNPs in the presence of drug [25].

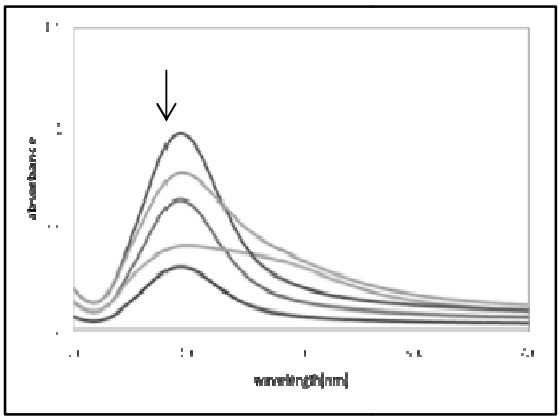

Fig. 4. The UV–Vis absorption spectra of AuNPs in the absence and presence of AMX with concentrations of 10 µl , 100 µl ,200 µl and 250 µl . AMX = 60 µg/L, AuNPs = 700 µl, buffer volume = 200 µl and T = 25 ºC

3.2 Validation

3.2.1 Calibration graph

After selecting desired experimental variables the calibration graph was plotted at AMX concentration range of 10-250 µg/l (Fig.5). The obtained straight line with high correlation coefficient of 0.99 which was obtained based on bear-lambert law indicated appropriate linearity.

3.2.2 Limit of detection and precision

The limit of detection (LOD) was calculated by Eq. (1) as 0.21.

$$LOD=(3\ S_0)/b \quad (1)$$

Where S0 is standard deviation of blank solution and b is the slope of calibration graph. The precision of the suggested method was evaluated by relative standard division at optimized conditions (Eq. (2)) for AMX amount of 60 µg/l, which was repeated for six times as 0.61% .

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$$RSD\%=(S\times100)/m \quad (2)$$

Where S and m are standard deviation and average of AuNPsabsorbance, respectively.

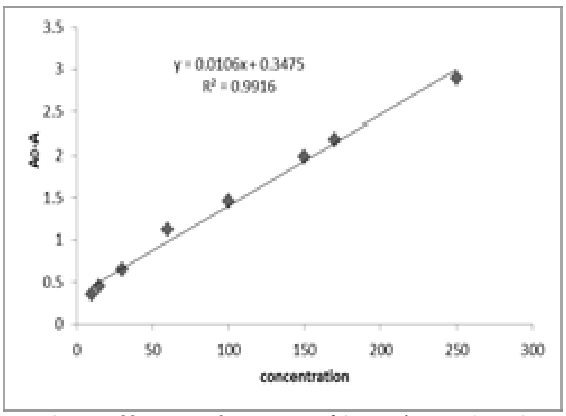

Fig. 5.AuNPs calibration graph in presence of AMX at λ_{max} = 520 nm. AMX = 60 µg/L, AuNPs = 700 µl, buffer volume = 200 µl and T = 25 ºC

3.2.3 Specificity

The determination of 60 µg/l of AMX was studied in presence of various foreign species. Table 2 demonstrates the maximum tolerance value of the investigated species, which was determined when absorbance value did not exceed ± 5% on addition of them.

Table 2: Determination of AMX in presence of various foreign species at λ_{max} = 520 nm.

Sample No.	Foreign species	Maximum tolerance limit (µg/l)
1	Mg^{2+}, Ca^{2+}, k^{+}, Cl^{-}, Ni^{2+}, Na+	1000 ≤
2	Co^{2+}, Ba^{2+}, Sr^{2+}	500
3	I^{-}	250
4	EDTA	100
5	Bi^{3+}	10
6	Cu^{2+},Fe^{2+}	1

AuNPs = 700 µl , acetate buffer = 200 µl , pH = 5, AMX = 60 µg/l and T = 25 ºC

3.3 Analytical application

To investigate the ability of the proposed method for measuring the drug in real samples, it was utilized to determine AMX in pharmaceutical, spring water and biological samples. Recovery experiments for spiked samples solution were performed for evaluation of the method. Recovery ranged from 99.8-100.4% revealing adequate accuracy, which was calculated by Eq. (3). The obtained data presented in Table 3.

$$Recovery\ \% = (m\text{-}c)/spiked \times 100 \quad (3)$$

Table 3: Real sample recovery at λ_{max} = 520 nm

sample	Added mount	Amax	Recovery %
Capsule	10µl	1.064	99.8%
Spring water (sardrood area)	10µl	1.062	97.5%
Biological sample (urine)	10µl	1.01	99.3%

AuNPs = 700 µl , acetate buffer = 200µl , pH = 5, AMX = 60 µg/l and T = 25 ºC

4 Conclusions

In this research, synthetized gold nanoparticles with chemical reduction method were utilized for determination of amoxicillin by the sensitive spectrophotometric method. This method was based on the aggregation of gold nanoparticles as a consequence of amoxicillin addition to them; hence, nanoparticles absorption declined. After optimization of experimental variables including pH, temperature, buffer type and amount, AMX and AuNPs amounts and process time, calibration plot was depicted at λ_{max} = 520nm in the range of 10-250µg/l with adequate correlation coefficient of 0.99. Limit of detection and relative standard deviation of proposed method were calculated as 0.12µg/l and 0.61%, respectively. Amoxicillin amount could be successfully determined in pharmaceutical, real water and biological samples.

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