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UTILITY OF GOLD NANOPARTICLES AS QUICK, NAKED EYE COLORIMETRIC PROBE FOR DETERMINATION OF CEFPIROME SULPHATE

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ABSTRACT

The present study introduced a simple and sensitive visual and spectrophotometric method for the determination of cefpirome sulphate. Gold nanoparticles were utilized for quick detection of visual color change in the presence of cefpirome sulphate that observed by the naked eye and monitored by UV-Vis spectrophotometer. The change of color was quantitatively correlated with the quenching effect of cefpirome sulphate on the absorption band of gold nanoparticles. The experimental variables, including pH, reaction time, the effect of drug amount, the effect of the gold nanoparticles amount and temperature, etc. were investigated and optimized. Under optimal conditions the linear concentration range was found to be 0.02-120 µg mL^{-1} with a correlation coefficient (r = 0.9996). The calculated lower limit of detection was 0.005 µg mL^{-1} and quantification limit 0.017 µg mL^{-1}. The interference of some common species was investigated. The proposed method was successfully employed for quick and facile determination of the investigated drug in pure form, its pharmaceuticals and biological fluids.

KEYWORDS: Cefpirome sulphate; Colorimetric probe; Spectrophotometry; Gold nanoparticles; Biological fluids.

INTRODUCTION

Cefpirome sulphate (CPS) is a highly stable beta lactamase fourth-generation cephem antibiotic, which has a broad spectrum anti-bacterial activity against gram-positive and gram-negative bacteria.[1] It is chemically known as 1-[[(6r,7r)-7-(2-amino-4-thiazolyl) (methoxyimino)acetyl]amino]-2-carboxy-8-oxo-thia1azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5h-cyclopenta[b] pyridinium sulphate "Figure 1". CPS is a semi-synthetic cephalosporin which is intravenously administered to treat bacterial infections such as bronchopneumonia, pyelonephritis, cellulitis and wound infections, in intensive care patients.[2] There were few studies investigating CPS, including high performance liquid chromatography, [3-5] spectrophotometry[6] and voltammetry.[7]

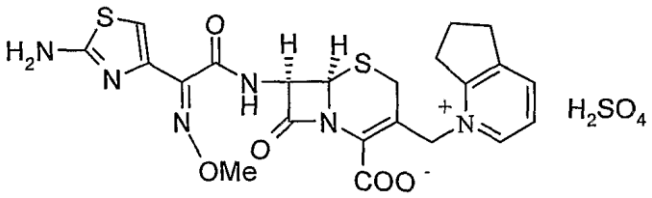

Figure 1: Chemical structure of cefpirome sulphate

The utility of nanomaterials in a wide variety of applications have been grown by synthesis the nanoscale materials with novel and improved properties. Noble metals such as gold, silver and copper, etc. have promising physico-chemical properties which focused recently by many researchers.[8-10] One of the most remarkable properties of the nanoparticles is the surface-volume that provides the optical activity which is not available in the bulk materials.[11]

In the previous studies, many works focused on the applications of nanoparticles in the fields of electronics[12] catalysis,[13, 14] medicine[15] and pharmaceutical analysis.[16] With the development of nanotechnology, nanoparticles based colorimetric methods have been reported for detection of metals[17] cancer cells,[18] bacteria[19] and DNA.[20]

The present study demonstrates to develop a new visual spectrophotometric method based on the quenching effect of CPS on the absorbance band of AuNPs. The analytical method has been developed to detect the drug of interest in pure bulk drug, pharmaceutical formulations and biological fluids.

EXPERIMENTAL

Materials and reagents

All reagents were of analytical grade and were used without further purification. Deionized water was used throughout the experiment. Pure grade of cefpirome sulphate (CPS) and its product for injection (Cefpirome® 1 g /vial) were kindly supplied by (EVA-PHARMA Co., Cairo, Egypt). Gold nanoparticles 0.1 mmol L^{-1} were purchased from (Sigma-Aldrich, Hamburg, Germany). Also, other chemicals such as sodium acetate purity ≥ 99.0 %, acetic acid ≥ 99.7 %, sodium citrate dehydrates ≥ 99.0 %, citric acid ≥ 99.5 %, sodium dibasic phosphate ≥ 99.0 %, sodium monobasic phosphate ≥ 99.0 %, boric acid ≥ 99.5 % and sodium tetraborate decahydrate ≥ 99.0 % were purchased from (Sigma-Aldrich, Hamburge, Germany). Acetonitrile, zinc sulphate and sodium hydroxide (WinLab, Market Harborough, UK) were used to deproteinated the serum samples. Urine samples were obtained from healthy volunteers and the serum samples (Multi-Serum Normal, Randox Laboratories, UK) were obtained from commercial sources. Informed consent was obtained from all volunteers prior to the start of the study, and the medical ethics committee in the College of Medicine, King Saud University, approved this study.

Apparatus

The absorption spectra of AuNPs were measured at room temperature using ULTROSPEC-2100 pro, UV-Visible spectrophotometer with matched 1 cm quartz cells. The image and diameter of AuNPs were measured using the Transmission Electron Microscope (TEM). HANNA microprocessor pH-meter model 211, (Cluj, Romania) was used for pH adjustments.

Standard drug solution

A stock CPS solution of 100 µg mL^{-1} was prepared by dissolving 10 mg of the drug in 100 mL deionized water. Working solutions 0.02-120 ug mL-1 were freshly prepared by serial dilutions using the same solvent.

Preparation of CPS injection solution

The contents of two Cefpirome® 1.0 g vial were mixed well. Accurate weight of CPS powder equivalent to contain 10 mg of CPS drug was transferred into a 100-mL volumetric flask and dissolved in 50 mL deionized water, sonicated for 3.0 min and filtered, then complete to volume to obtain a solution labeled to contain 100 µg mL^{-1}. The working solutions were

prepared by serial dilutions in the range of 0.02-120 µg mL^{-1} and subjected to analysis using the developed method. The mean % recoveries were calculated using a calibration graph. Spiking technique was used to prepare the human serum and urine samples. Approximately 1.0 mL of serum was spiked with a CPS standard drug solution to contain 10 µg mL^{-1} and deproteinated to remove the most interfering species mainly the proteins. The previously reported method[21] was used by adding 1.0 mL acetonitrile followed by 0.1 mL of NaOH (0.1 mol L^{-1}) and1.0 mL of $ZnSO_4.7\ H_2O$. The prepared solution was centrifuged at 2500 rpm for 5 min. The treated sample was diluted with deionized water to obtain a concentration of CPS in the range of 0.02-120 µg mL^{-1}. No further pre-condition was required for urine samples. AuNPs-CPS colorimetric detection method was employed and the absorptions of these concentrations were recorded at λ_{max} = 530 nm and the % recovery was calculated using the calibration graph.

Gold nanoparticles solution and its characterization

The applied AuNPs (0.1 mmol L^{-1}) solution was purchased from (Sigma-Aldrich, Hamburg, Germany). The colloidal dispersion of gold nanoparticles in the solution was investigated using UV-Vis spectroscopy to determine the absorption band which recorded at λ_{max} 530 nm. The size of the particles was detected using TEM which showed that the size of particles was about 50 nm as shown in figures 2 and 3.

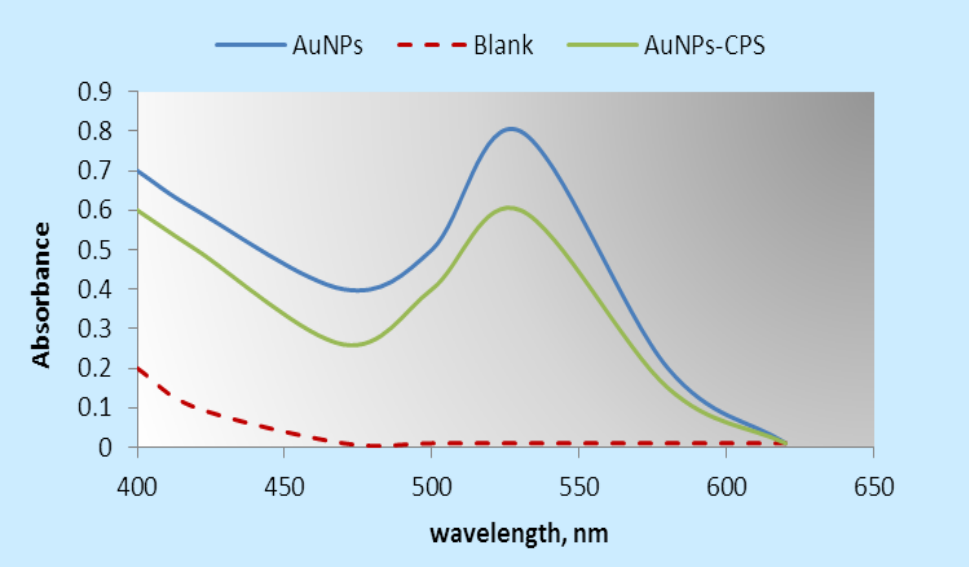

Figure 2: Absorption spectra of AuNPs at λ_{max} 530 nm in the presence and absence of CPS drug against blank solution

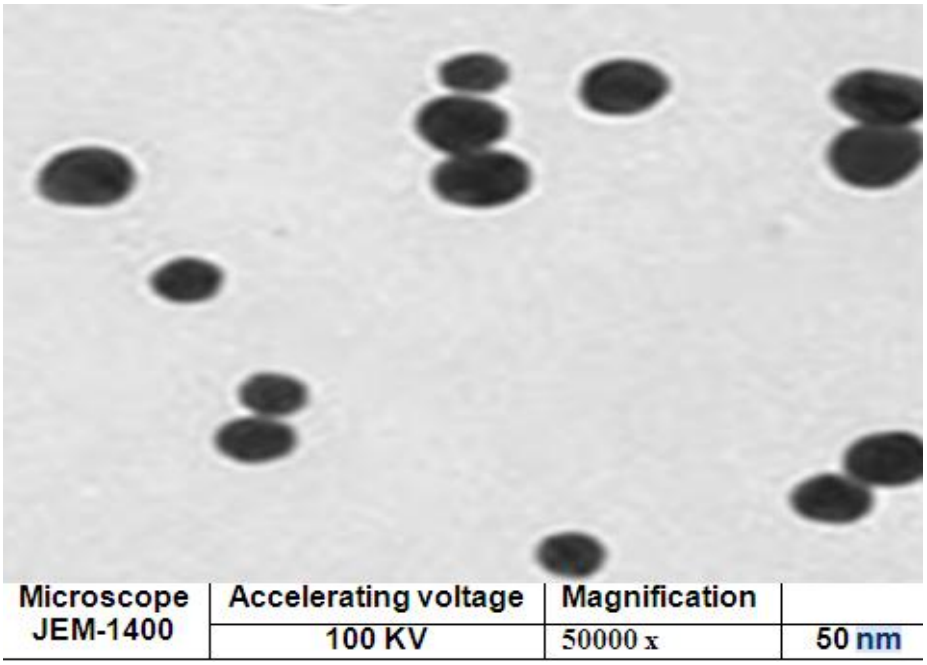

Figure 3: TEM image of gold nanoparticles 50 nm

Experimental procedure

The overall experimental study was performed under optimum conditions. A standard solution of CPS was prepared after adjusting all relevant conditions such as pH, amount of AuNPs, buffer type and its amount, temperature and process time with total volume 5.0 mL by adding deionized water. The absorbance of each solution was recorded against the blank solvent at λ_{max} 530 nm.

RESULTS AND DISCUSSION

Optimization of analytical conditions

Effect of buffer types

To stabilize the pH at optimum value in the determination of CPS, approximately, 0.2 mol L^{-1} of each acetate, phosphate, borate and citrate buffers were investigated with the same experimental variables. The high absorption value of AuNPs was recorded by using an acetate buffer as clarified in Figure 4. Therefore, 0.2 mol L^{-1} acetate buffer is used as a suitable buffer to finish this study.

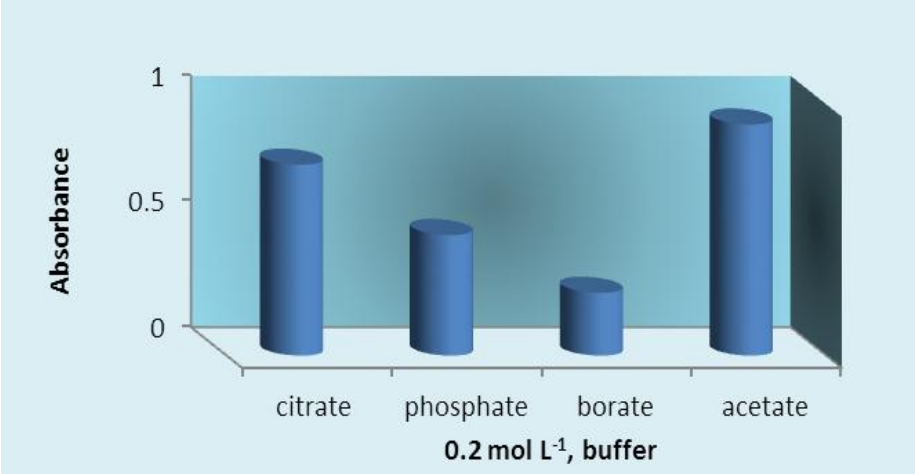

Figure 4: Effect of different kinds of buffers on the absorption of AuNPs at λ_{max} 530 nm, 50 µg mL^{-}CPS and 0.5 mL AuNPs

Effect of pH

The effect of pH on the absorbance of AuNPs was studied by investigating the absorbance at pH ranges from 1-9. As indicated in Figure 5 the maximum absorption was recorded at pH 4. At pH less than 4, AuNPs were aggregated and the absorption was declined. While, at pH above 4 the absorption was decreased because of deprotonation of an amine group of CPS and more aggregation of AuNPs.[22]

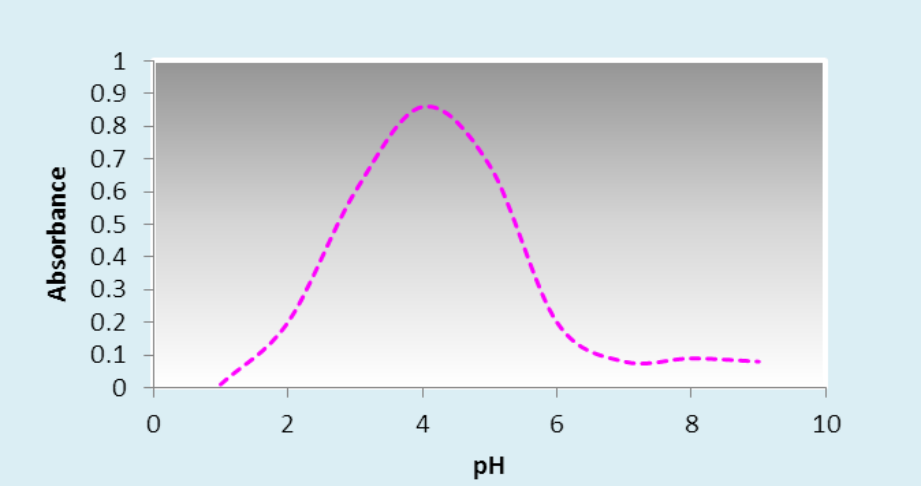

Figure 5: Effect of pH on the absorption spectra of AuNPs at λ_{max} 530 nm, 50 µg mL^{-1} CPS and 0.5 mL AuNPs

Effect of buffer volume

The influence of buffer volume was also studied by testing various volumes of acetate buffer in the range of 0.1-1.0 mL. As seen in Figure 6, AuNPs recorded maximum absorption by adding 0.6 mL of acetate buffer solution.

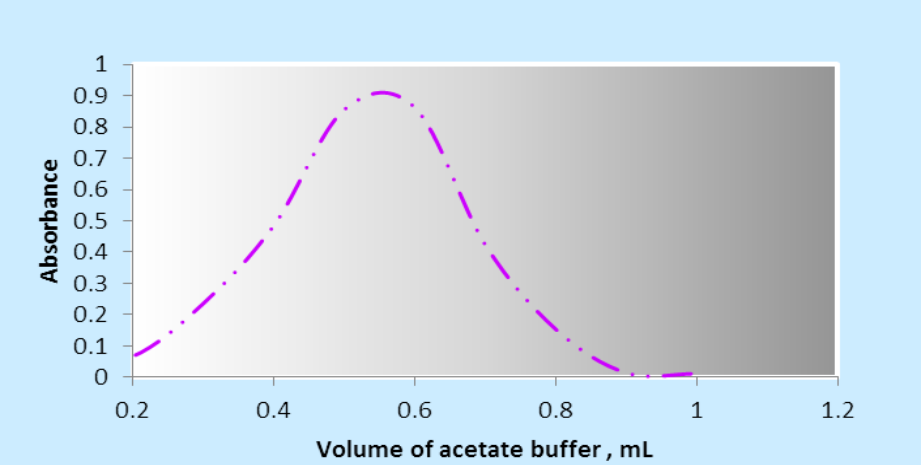

Figure 6: Effect of acetate buffer volume on absorption of AuNPs at λ_{max} 530 nm, 50 µg mL^{-1} of CPS and 0.5 mL of AuNPs

Effect of AuNPs volume

The amount of AuNPs should be adjusted during the determination of CPS. Hence, the volume of AuNPs was investigated by varying the amount of AuNPs in the range of 0.2-0.8 mL. As clarified in Figure 7, the addition of 0.5 mL of AuNPs to the solution gave the maximum absorption. Below 0.5 mL the quantity of AuNPs in the solution was not adequate while, using amounts above 0.5 mL may cause aggregation of AuNPs and resulted in low absorption and sensitivity.[23]

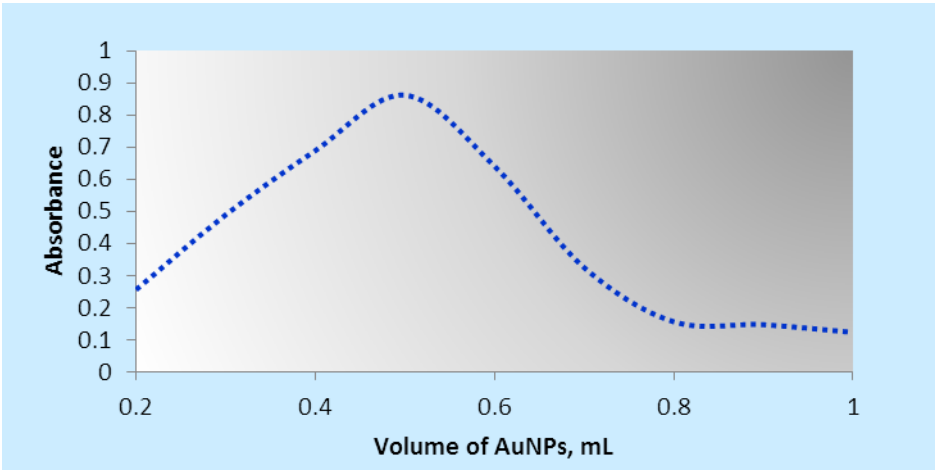

Figure 7: Effect of AuNPs volume on absorption of AuNPs at λ_{max} 530 nm, 50 µg mL^{-1} of CPS and 0.6 mL acetate buffer

The quenching effect of the drug

The quenching effect of the drug concentration was studied under optimal conditions using 1.0 mL of different drug concentrations in the range of 10-120 µg mL^{-1}. The visual color changed can be seen by the naked eye. As shown in Figure 8, it was found that increasing the

concentration of CPS resulted in the decline of the color of AuNPs solution due to the aggregation of AuNPs in the presence of high concentrations of the drug. [24]

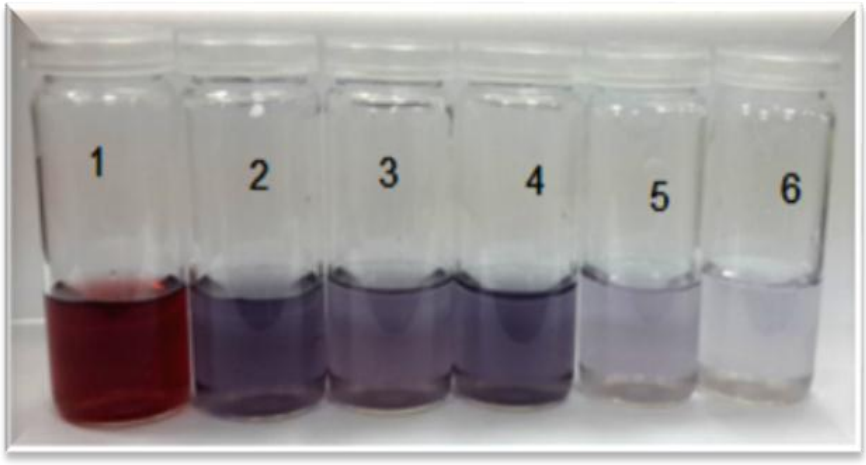

Figure 8: The quenching effect of the drug concentration on AuNPs solution using 1.0 mL of different concentrations of CPS: (1) AuNPs control, (2) 10 µg mL^{-1}, (3) 40 µg mL^{-1}, (4) 80 µg mL^{-1}, (5) 100 µg mL^{-1} and (6) 120 µg mL^{-1}

Effect of temperature and time of the process

The temperature and the process time were investigated using different temperatures and reaction times. It was found that the process time and temperature did not influence the absorbance of AuNPs at the same experimental variables for the determination CPS. The reaction of AuNPs with CPS was stabilized at room temperature for at least 24 h.

Method validation

To confirm that the analytical method is suitable for its intended use, method validation should be employed with respect to linearity, lower limit of detection, quantification limit, accuracy, precision, specificity and robustness. This can be carried out according to ICH guidelines.[25]

Linearity

The linearity of the proposed method was recorded by plotting absorbance as a function of the tested drug concentrations. The regression equation was calculated using the least square statistical method and was found to be Y = 0.0068 *x* + 0.1717, (r = 0.9996). The linear relationship was in the range of 0.02-120 µg mL^{-1}, which indicated wide range for detection of the investigated drug "Table 1".

Table 1: Performance data obtained from the determination of CPS using AuNPs

Analytical characteristics	Value
Linear range, µg mL^{-1}	0.02-120
Detection limit, µg mL^{-1}	0.005
Quantification limit, µg mL^{-1}	0.017
Regression equation	Y= 0.0068 *x* + 0.1717
%RSD (n=9)	0.8 %
Correlation coefficient, r	0.9996

Lower limits of detection and quantification

The lower limit of detection of the proposed method was calculated according to the equation S/N=3 and it was found to be 0.005 µg mL^{-1}. While the quantification limit was evaluated according to the equation S/N = 10 and it was found to be 0.017 µg mL^{-1}.

Accuracy and precision

The accuracy of the developed AuNPs-CPS colorimetric method for the determination of CPS was investigated using standard addition method. The accuracy was calculated in terms of mean percentage recovery. The absorption of CPS standard solutions was measured and the calculated % recovery was found to be 99.17±0.6.

The method precision of the proposed colorimetric AuNPs-CPS detection method for determination of CPS was evaluated using intra-day and inter-day terms. Nine replicates were carried out in this study and the obtained results were calculated as % RSD values. The precision of the developed method was found to be 0.5% and 0.6% for intra-day and inter-day, respectively as shown in Table 2. The above values of % RSD were less than 2% indicating good precision.

Table 2: Accuracy and precision studies of the proposed method for the determination of CPS

Taken µg mL^{-1}	Intra-day		Inter-day	
	Found (µg mL^{-1})	Recovery% ±RSD[(a)]	Found (µg mL^{-1})	Recovery% ±RSD[(a)]
0.02	0.0199	99.5±0.6	0.0197	98.5±0.4
0.08	0.0798	99.8±0.2	0.0788	98.5±0.7
1.0	0.98	98.0±0.9	0.99	99.0±0.8
5.0	4.96	99.2±0.5	4.98	99.6±0.3
10.0	9.95	99.5±0.3	9.93	99.3±0.9
50.0	49.89	99.8±0.7	49.93	99.8±0.2
80.0	79.93	99.9±0.6	79.87	99.8±0.6
100.0	99.94	99.9±0.5	99.89	99.9±1.1
120.0	119.92	99.9±0.2	119.93	99.9±0.7

(a) Values are mean of three determinations for intra and inter-day determination

Specificity

To study the specificity of the proposed method, CPS was determined in the presence of some foreign substances. Such substances as Na^{+}, K^{+}, Mg^{2+}, Cl^{-}, NO_3^{-}, NH_4^{+}, EDTA and SO_4^{2-}, heavy metals, ascorbic acid, uric acid, sugars and amino acids. Table 3 demonstrated that the maximum tolerable value of the investigated species was determined when the absorbance value did not exceed ±5% on addition of each of them. It can be seen that there is no influence on the determination of CPS in pharmaceutical dosage forms. While, for serum samples main possible interference was observed from ascorbic acid, uric acid, urea, heavy metal ions such as Al^{3+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+} and Ni^{2+}. The latter ions can be eliminated by the addition of EDTA. After 1000 fold dilution the interference from such substances could be greatly minimized to negligible levels.

Table 3: Determination of CPS using AuNPs in the presence of some foreign substances

Interferents	Tolerable level µg m L^{-1}
Na^{+}, K^{+}, Mg^{2+}, Cl^{-}, NO_3^{-}, NH_4^{+}, EDTA and SO_4^{2-}	1000
Glucose, sucrose, lactose, talc, starch, magnesium stearate, citric acid	800
Uric acid, ascorbic acid and urea	150
Adrenaline, dopamine, cysteine, histamine, tyrosine, glucosamine	500
Al^{3+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+} and Ni^{2+}	50

Robustness

The robustness of the proposed method for determination of CPS were tested by introducing a small change in method parameters such as pH, volume of AuNPs and volume of buffer. The calculated % recovery of the proposed method was 99.14±0.7%. The obtained results were closely in agreement with those obtained from standard drug solutions.

Analytical applications

AuNPs was employed for determination of CPS. It was evident from obtaining results that it gave satisfactory results for the determination of CPS in pure forms and its dosage forms as presented in Table 4. Statistical treatment in terms of t-test and F-test [26] was applied for the obtained results and compared with those obtained from spectrophotometric method which based on diazotization of primary amine group of cefpirome with sodium nitrite and hydrochloric acid followed by coupling with α-naphthylamine, the absorbance measured at λ_{max} 512 nm.[6] The results did not reveal any significant difference between them at 95% confidence level proving similar accuracy and precision.

Table 4: Determination of CPS using AuNPs colorimetric detection in pure samples and dosage forms in comparison with a reported method [6]

Pure samples			Cefpirome® 1 g /vial			Reported method [6]		
Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %
0.02	0.02	100.0	0.02	0.02	100.0	5.0	4.96	99.2
10.0	9.95	99.5	10.0	9.92	99.2	8.0	7.99	99.8
50.0	49.87	99.7	50.0	49.68	99.4	10.0	9.93	99.3
80.0	79.99	99.9	80.0	80.00	100.0	20.0	19.85	99.3
100.0	100.0	100.0	100.0	99.95	99.9	30.0	30.0	100.0
120.0	119.92	99.9	120.0	120.01	100.0	40.0	40.0	100.0
Mean%±SD		99.8±0.2	99.7±0.4			99.6±0.3		
n		6	6			6		
Variance		0.04	0.16			0.09		
%SE*		0.08	0.16			0.12		
%RSD		0.20	0.40			0.30		
t-test		1.39(2.23)**	0.50(2.23)**			-----		
F-test		2.25(5.05)**	1.78 (5.05)**			-----		

**%SE= $SD/\sqrt{n}$ ** Figures in parentheses are the tabulated values of t-and F-testes at 95% confidence limit [26]*

On the other hand, the proposed method was employed to determine the investigated drug in biological fluids such as human urine and serum as shown in Table 5. The obtained results indicated that the proposed method is simple, sensitive less time consuming and gave more wide linear concentration ranges than other reported methods. Also, it is time consuming and cost effective for reagents and samples.

Table 5: Determination of CPS using AuNPs colorimetric detection in serum and urine samples

Serum samples			Urine samples		
Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %
0.02	0.02	100.0	0.02	0.019	95.0
10.0	9.96	99.6	10.0	9.98	99.8
50.0	49.55	99.1	50.0	49.78	99.6
80.0	79.84	99.8	80.0	80.01	100.0
100.0	100.0	100.0	100.0	99.9	99.9
120.0	119.84	99.8	120.0	119.68	
Mean%±SD		99.7±0.3	99.2±0.8		
n		6	6		
Variance		0.09	0.64		
%SE*		0.12	0.30		
%RSD		0.30	0.80		

**%SE= $SD/\sqrt{n}$*

CONCLUSION

In the present study, gold nanoparticles were utilized for a simple and rapid determination of cefpirome sulphate by visual color change that observed by naked eye and detected by UV-visible spectrophotometer. This method was based on the decline of absorption of gold nanoparticles due to their aggregation as a consequence addition of CPS. After optimization of experimental variables, including pH, buffer type and amount, CPS and AuNPs amounts, calibration plot was depicted at λ_{max} = 530 nm in the range of 0.02- 120 μg mL^{-1} with adequate correlation coefficient of 0.9996, with lower limits of detection and quantification. The proposed method was successfully applied for determination of CPS in its pharmaceutical dosage forms and biological fluids.

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