Research Article

Use Of Silver – Gelatin Complex For the Determination of Cefoperazone Sodium, Ceftazidime Pentahydrate and Cefotaxime Sodium in Pure and Pharmaceutical Dosage Forms

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Abstract

Simple, accurate, precise, and new spectrophotometric method is developed for the estimation of three third generation cephalosporins, namely, cefoperazone sodium (I), ceftazidime pentahydrate (II) and cefotaxime sodium (III) in both pure and pharmaceutical dosage forms. Gelatin forms a weak colorless complex with silver (I) in alkaline medium. This complex was reduced quantitatively by the sulfide ions produced from the alkaline hydrolysis of the investigated drugs to yellow silver sol. The gelatin prevents the precipitation of the black silver metal and can stabilize the yellow silver sol. This method exhibits maximum absorbance at 348, 350 and 352 nm for the investigated drugs respectively. Different variables affecting the reaction (e.g. NaOH concentration, hydrolysis time, silver – gelatin complex concentration and pH) were studied and optimized. Beer's law was obeyed in the concentration range of (10-50), (14-76) and (24 -76) $\mu g.mL^{-1}$ for drug (I), (II) and (III) respectively .The correlation coefficient (r^2) for the studied drugs were found to be 0.9999. The molar absorptivity (ε), sandell sensitivity, detection (LOD) and quantitation limits (LOQ) were also calculated. The accuracy and precision of the proposed method were satisfactory. The proposed method was successfully applied for the de0termination of certain pharmaceutical dosage forms containing the studied drugs.

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Introduction

Cephalosporins are the most important widely used type of antibiotics. Analysis of these antibiotics is challenge because of their sensitivity and instability to different conditions. Cephalosporins are structurally and pharmacologically related to the penicillins. Like the penicillins, cephalosporins have a β-lactam ring structure that interferes with the synthesis of the bacterial cell wall. They are used for the treatment of infections caused by Gram (+) and Gram (−) bacteria. They are among the safest and the most effective broad-spectrum bactericidal anti- microbial agents and therefore, they are the most frequently prescribed class of antibiotics [1]. Traditionally, cephalosporins are divided into first-, second-, third-, and fourth-generation agents. The studied drugs are three of third- generation agents. Several methods have been reported for cephalo- sporins determination. The official procedures for the cited drugs (I, II ,III) utilize liquid chromatography [2] which is expensive. The hydrolysis of β-Lactum ring, which is the common feature for cephalosporins and penicillins, has been achieved by the sodium hydroxide addition and often used as a preliminary step in the analytical procedure used for their determinations [3-9]. The literature reveals that many spectrophotometric methods were developed for cephalosporins determinations that based on hydrolysis of these drugs using alkaline degradation and subsequent reaction of the formed sulfide ions with chromogenic reagents [4, 5].

Pal.T reported that gelatin forms a colorless complex with Silver (I) in alkaline medium. This complex when reduced yields the yellow silver sol, which can be measured spectrophotometricaly [10]. Silver – gelatin Complex was applied for the determination of either silver (I) [10] or strong reducing agents such as ascorbic acid [11], hydrazine [12] and hydrogen sulphide [13].

In this paper we report a new, simple, sensitive and precise method for the estimation of cefoperazone sodium (I), ceftazidime sodium (II) and cefotaxime sodium (III) in both pure and pharmaceutical dosage forms. The method was based on the the reduction of the colorless silver – gelatin complex to yellow silver sol by the sulfide ions released from the alkaline hydrolysis of the investigated drugs. This yellow silver sol was stabilized by gelatin and was measured spectrophotometricaly at 348, 350 and 352 nm for the investigated drugs respectively.

Experimental

Apparatus

Shimadzu recording spectrophotometer UV 1201 equipped with 10 mm matched quartz cells. Digital analyzer pH meter (USA) was used. MLV type thermostatically controlled water bath (Salvis AG Emmenbruck, Luzern, Germany).

Materials and Reagents

Chemicals of analytical grade and double distilled water were used throughout the work. Cefoperzone sodium, cefozon® vials labelled to contain 1000 mg cefoperzone sodium per vial (Egyptian Int. Pharmaceutical Industries Co., Egypt). Ceftazidime pentahydrate, cefidime® vials: (ceftazidime penta-hydrate – sodium carbonate mixture equivalent to 500 mg ceftazidime per vial). Cefotaxime sodium, cefotax® vials labelled to contain 1000 mg cefotaxime sodium per vial (Egyptian Int. Pharmaceutical Industries Co., Egypt). Gelatin solution was obtained from El-Nasr Chemical Company, Egypt. 1 and 1.5 % was prepared by dissolving 1 and 1.5 g gelatin powder in 100 mL warm distilled water (the solution should be freshly prepared due to the possibility of microbial degradation of gelatin). Silver nitrate solution was obtained from Aldrich Chemical Co. Ltd, (10^{-2} M) was prepared by dissolving 169 mg silver nitrate in 100 mL distilled water. Sodium hydroxide was obtained from El-Nasr Chemical Company, (Egypt).

Working Reagent Solution (Silver–Gelatin Complex)

The solution was prepared by adding 15 mL of silver nitrate solution (10^{-2} M) to 25 mL gelatin solution of the specified concentration in 50 mL volumetric flask. The pH was adjusted, using 0.1 M sodium hydroxide to pH 8, 11, 10 for (I), (II), (III) respectively. The mixture was brought to volume with distilled water and thoroughly mixed. The reagent was freshly prepared.

Standard Drug Solutions

Preparation of cefoperazone sodium and ceftazidime pentahydrate standard solutions: Stock solution was prepared to contain 0.2 mg/mL, by dissolving 10 mg of the pure drug in 50 mL double distilled water.

Preparation of cefotaxime sodium standard solutions: Stock solution was prepared to contain 4 mg/ml by dissolving 20mg of the pure drug in 50 ml double distilled water.

General Procedure

Into three sets of 10 mL volumetric flasks, accurate volumes of standard drug solutions (0.5 – 2.5), (0.7 – 3.8), and (0.6 – 1.9) for (I), (II), and (III) respectively, followed by specific volumes of NaOH of certain molarities (**Table 1**). The solutions were heated in a boiling water-bath for 40, 15, and 25 minutes for (I), (II) and (III) respectively, then cooled to room temperature. To each flask 3 ml of the working reagent solution were added and the mixture was protected from light (wrapping the flasks with aluminium foil). The resulting solutions were mixed and left to stand for 5, 20 and 15 minutes for (I), (II) and (III) respectively at room temperature. The flasks were diluted to volume with double distilled water. The absorbance can be measured directly after dilution except for ceftazidime the solution should be stand for 5 minutes after dilution then measured due to increasing the absorbances in the first five minutes after dilution. The absorbances were measured at 348, 350 and 352 nm for the investigated drugs respectively, against a similarly treated reagent blank.

Table 1 Characteristic parameters for the reaction of studied drugs with silver – gelatin Complex *

I	II	III	Parameters
348	350	352	**λ max (nm)**
10-50	14-76	24 -76	**Beer's law limits (µg mL^{-1})**
40	15	25	**Hydrolysis time (min)**
1M (3 mL)	1 M (3 mL)	2 M (4 mL)	**NaOH (volume)**
8	11	10	**pH of working reagent solution**
10^{-2}	10^{-2}	10^{-2}	**Silver nitrate (M)**
1.5	1	1.5	**Gelatin Concentration (%)**
3	3	3	**Working reagent solution (mL)**
5	20	15	**Time before dilution (min)**
-	5	-	**Time after dilution (min)**
0.0188	0.0153	0.0138	**Slope (b)**
-0.0292	-0.0792	-0.0393	**Intercept (a)**
0.9999	0.9999	0.9999	**Correlation coefficient (r^2)**
1.12	6.68	6.23	**LOD µg/mL**
3.39	20.25	18.88	**LOQ µg/mL**
0.06	0.08	0.08	**Sandell sensitivity µg.cm^{-2}**
1.16	0.83	0.62	**ε (×10^4) L mol^{-1} cm^{-1}**

***Average of three experiments**

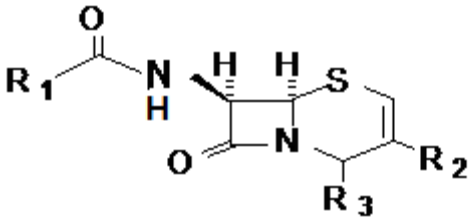
Procedure for Pharmaceutical Formulations

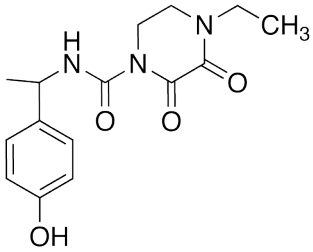
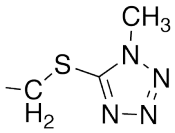
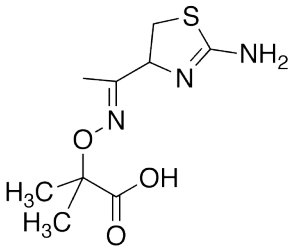
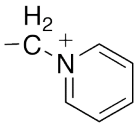
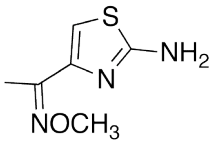
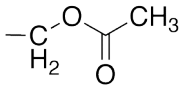
For cefozon ® and cefidime ® vials: Accurate volume of vial equivalent to 10 mg of cefoperazone sodium and ceftazidime pentahydrate was measured, completed to 50 mL with double distilled water and the procedure was completed as under general procedure.

For cefotax ® vial : Accurate volume of vial equivalent to 20 mg of cefotaxime sodium was measured, completed to 50 mL with double distilled water and the procedure was completed as under general procedure.

Recovery experiments were performed by adding known amounts of drugs (**Scheme 1**) to the pre analyzed pharmaceutical vials, results obtained were incorporated in (**Table 2**).

Scheme (1) : Chemical structures of the investigated cephalosporin antibiotics



Name	Chemical structure [2]		
	R_1	R_2	R_3
Cefoperazone sodium (I)			-COONa
Ceftazidime Pentahydrate (II)			-COOH
Cefotaxime Sodium (III)			-COONa

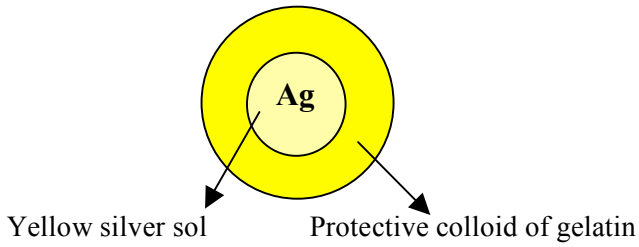
Results and Discussion

The colorless silver – gelatin complex is quantitatively reduced by the sulfide ions released from the alkaline hydrolysis of the investigated drugs to yellow silver sol stabilized by gelatin which exhibits maximum absorbance at 348, 350 and 352 nm for for (I), (II) and (III) respectively (**Figure 1**). The reagent alone exhibits negligible absorbance at the wavelengths of maximum absorbance (Figure 1). The proposed reaction can be simplified in the following equation:

$$S^{2-} + 2\ Ag^{+}\ (gelatin) \longrightarrow 2\ Ag\ (sol) + S^{\circ}$$

Colorless colored

Since the developed method depends on the formation of colored product by the interaction of silver – gelatin Complex with sulfide ions resulted from the alkaline degradation of the investigated drugs (**Scheme 2**) so, optimization studies were carried out to find the optimum conditions for the alkaline degradation and subsequently the optimum yield of sulfide ions and the maximum stability of the chromogen formed. Several parameters such as hydrolysis time, NaOH concentration, pH of silver – gelatin complex, silver nitrate concentration, gelatin concentration, reagent volume and time before and after dilution were optimized to achieve high sensitivity, stability, low blank reading and reproducible results.



Scheme 2. Proposed silver- galatin complex.

Effect of Hydrolysis Time

The effect of hydrolysis time on the absorption intensity was studied using different heating times in a boiling water bath (at 100°C) starting from 5 min until one hour and the reaction was carried out as usual.

Table 2 Application of the standard addition technique to the spectrophotometric determination of the studied drugs I, II, and III with silver-gelatin complex in pharmaceutical dosage forms*.

cefozon® vial				*cefidime ® vial*				*cefotax ® vial*			
Claimed taken µg/ml	**Authentic added µg/ml**	**Found conc. µg/ml**	**Recovery %**	**Claimed taken µg/ml**	**Authentic added µg/ml**	**Found conc. µg/ml**	**Recovery %**	**Claimed taken µg/ml**	**Authentic added µg/ml**	**Found conc. µg/ml**	**Recovery %**
	---	9.90	99.04	14	---	14.20	101.40	24	---	24.22	100.94
	14	13.95	99.62		20.00	20.01	100.07		24	24.22	100.94
	16	16.07	100.47		26.00	26.22	100.85		28	27.92	99.72
	18	18.04	100.24		30.00	30.73	102.44		32	32.05	100.16
10	20	20.06	100.32		32.00	32.56	101.76		34	34.44	101.30
	24	24.05	100.22		36.00	36.75	102.07		36	35.82	99.50
	30	29.90	99.68		40.00	40.08	100.20		40	40.17	100.42
	40	39.90	99.76		42.00	43.02	102.43		42	42.70	101.67
					50.00	50.27	100.55		44	44.15	100.35
									46	46.69	101.50
	Mean		99.92				101.34				100.67
	Variance		0.23				0.78				0.50
	S.D.		0.48				0.88				0.71
	S.E.		0.18				0.31				0.24

* Average of three experiments

The obtained absorbance readings were plotted against hydrolysis time. Heating for about 40, 15 and 25 minutes for (I), (II) and (III) respectively (at 100°C) gives maximum absorption intensity (**Figure 2**).

Effect of NaOH Concentration

The influence of sodium hydroxide concentration on producing the maximum absorption intensity was investigated using 0.1- 3.0 M NaOH keeping other factors constant. Then several volumes of the selected NaOH molarities were tried. It was found that maximum absorption readings were obtained upon using 3 mL 1 M NaOH for (I) and (II) and 4 mL 2 M NaOH for (III) (**Figure 3**).

Effect of pH of Working Reagent Solution

The effect of pH on the reduction of silver – gelatin complex was studied over the pH range 7-11, as gelatin was effectively bound to silver (I) above pH 7. pHs greater than 11 are not recommended as the gelatin solution is hydrolysed above pH 11. The optimum results were obtained at pH 8, 11, 10 for (I), (II), and (III) respectively (**Figure 4**).

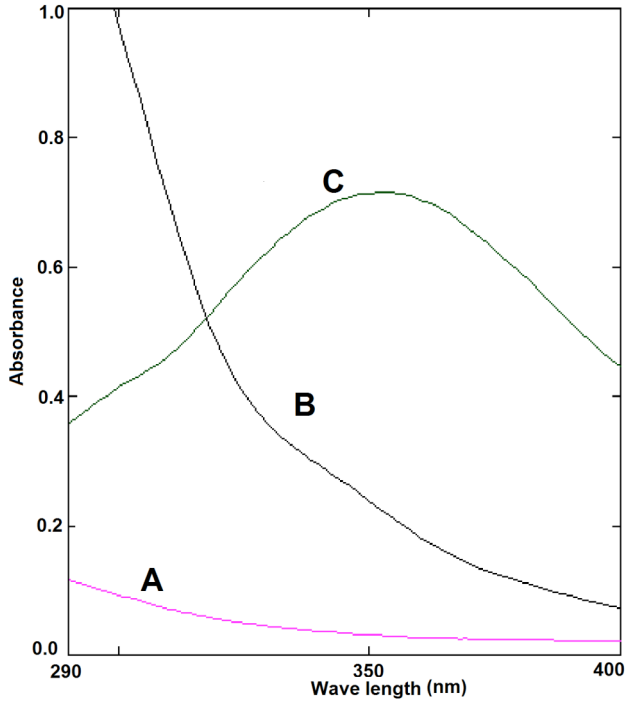


Figure 1 Absorption spectra of **A**, silver-gelatin complex against water; **B**, 50 µg mL^{-1} ceftazidime pentahydrate against water; **C**, The silver sol formed through the reaction with 50 µg mL^{-1} ceftazidime pentahydrate.

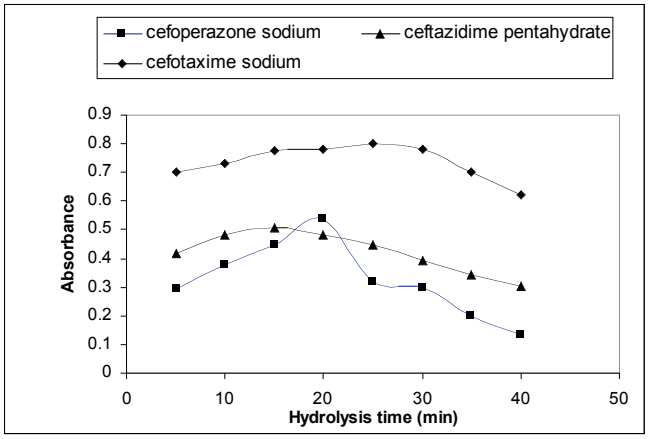


Figure 2 Effect of hydrolysis time on the reduction of silver-gelatin complex using: cefoperazone sodium (30 µg mL^{-1}), ceftazidime pentahydrate (50 µg mL^{-1}), and cefotaxime sodium (64 µg mL^{-1}).

Effect of Silver Nitrate Concentration

Different solutions of silver – gelatin Complex were prepared at the optimum pH by varying silver nitrate concentration (2×10^{-2} – 10^{-3} M), keeping gelatin concentration constant. The optimum concentration was 10^{-2} M for all drugs (Figure 5).

Effect of Gelatin concentration

The role of gelatin is to make a protective colloid to prevent the precipitation of the black silver metal and to stabilize the yellow silver sol. The effect of gelatin

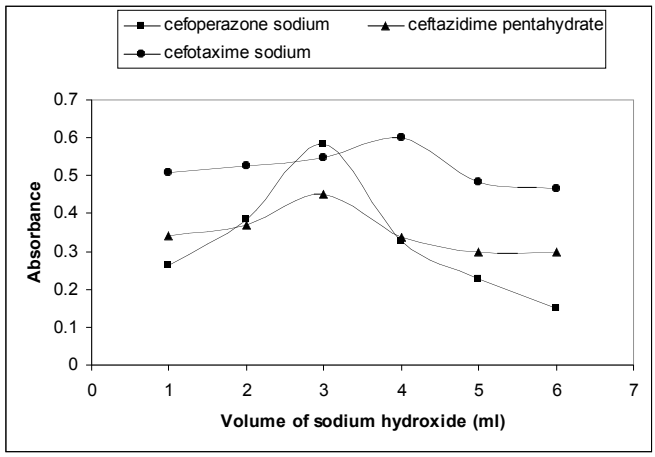


Figure 3 Effect of volume of 1 M NaOH on the absorbance of cefoperazone sodium (30 µg mL^{-1}), and ceftazidime pentahydrat (50 µg mL^{-1}) using 3 ml of silver – gelatin complex as well as 2M NaOH on the absorbance of 64 µg mL^{-1} of cefoperazone sodium using 3 mL of silver – gelatin complex.

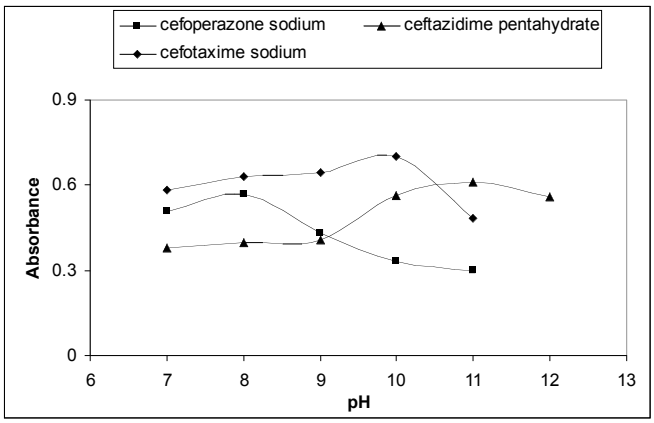


Figure 4 Effect of pH of working silver-gelatin complex on its reduction using 30 µg mL^{-1} cefoperazone sodium, 50 µg mL^{-1} ceftazidime pentahydrate and 64 µg mL^{-1} cefotaxime sodium.

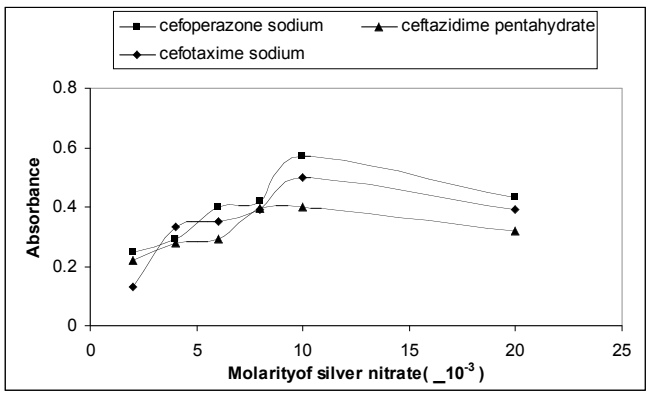


Figure 5 Effect of silver nitrate concentration used to prepare the working silver-gelatin complex on its reduction using 30 µg mL^{-1}cefoperazone sodium, 50 µg mL^{-1} ceftazidime pentahydrate and 64 µg mL^{-1} cefotaxime sodium.

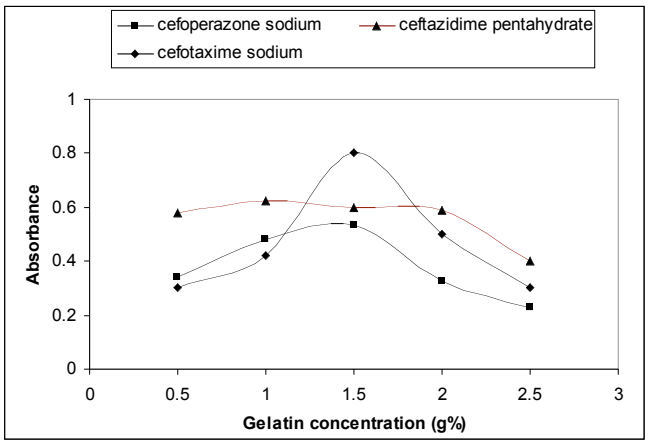


Figure 6 Effect of gelatin concentration used to prepare the working silver-gelatin complex on its reduction using 30 µg mL^{-1}cefoperazone sodium, 50 µg mL^{-1} ceftazidime pentahydrate and 64 µg mL^{-1} cefotaxime sodium.

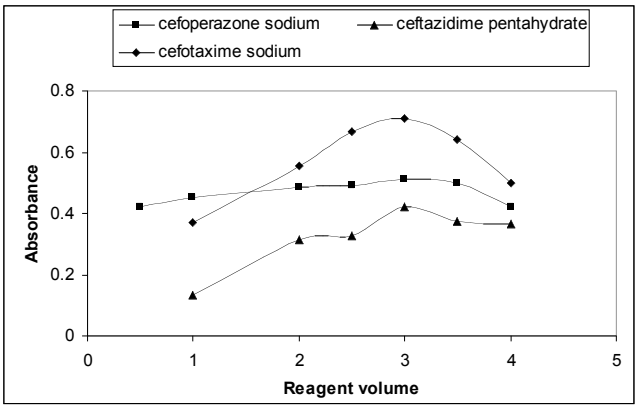


Figure 7 Effect of working reagent volume on its reduction using 30 µg mL^{-1}cefoperazone sodium, 50 µg mL^{-1} ceftazidime and 64 µg ml^{-1} cefotaxime sodium.

concentration was studied by preparing a series of working reagent solutions containing different gelatin concentration (0.5 – 2.5 %) , keeping silver nitrate concentration constant. It was found that maximum absorption readings were obtained upon using 1.5 % for (I) and (III) and 1% for (II) (**Figure 6**).

Effect of Working Reagent Solution Volume

The effect of reagent volume was tested by using varying amounts (0.5 – 4.0 mL) of working reagent solution. The results showed that 3 mL of reagent was sufficient for the production of maximum and reproducible colour intensity for all drugs (**Figure 7**).

Effect of Reaction Time

Time before dilution

The time needed for the reduction of silver – gelatin Complex was studied. It was found that after addition of the working reagent solution , flasks should be stand for 5 , 20 and 15 minutes for (I) , (II) and (III) respectively then dilution with water was made (**Figure 8**).

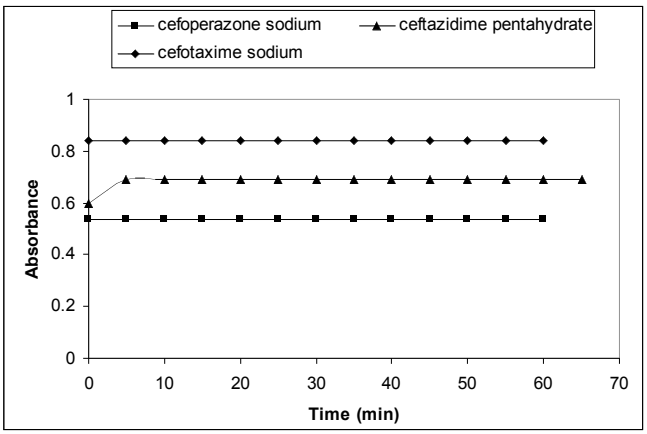


Figure 8 Effect of reaction time on the reduction of silver-gelatin complex using 30 µg mL^{-1}cefoperazone sodium, 50 µg mL^{-1} ceftazidime pentahydrate and 64 µg mL^{-1} cefotaxime sodium.

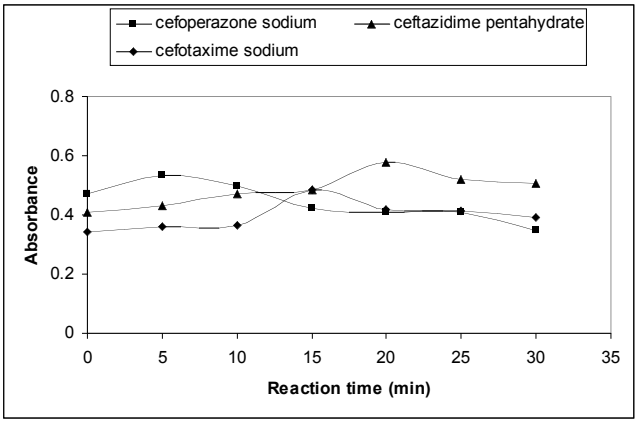


Figure 9 Stability of the formed silver sol in the presence of 30 µg mL^{-1} cefoperazone sodium, 50 µg mL^{-1} ceftazidime pentahydrate and 64 µg mL^{-1} cefotaxime sodium.

Time After Dilution and Stability

Solutions of (I) and (III) can be measured immediately after dilution with water giving stable absorbances for 1 hour (**Figure 9**). For ceftazidime, the solution should be stand for 5 minutes after dilution and then measured. This is due to increasing the absorbances in the first five minutes after dilution then the absorbance become stable for 1 hour (Figure 9).

Effect of Temperature and Sunlight

Direct sunlight and higher temperatures over 50˚C decompose the yellow silver sol into black precipitate, so the flasks were protected from light (wrapping the flasks with aluminium foil) and kept at room temperature through the reaction time.

Method Validation

The method was validated according ICH guidelines on the validation of analytical methods [14].

Linearity

Under the optimum conditions described, standard calibration curves for cefoperazone sodium (I), ceftazidime pentahydrate (II) and cefotaxime sodium (III) with silver – gelatin Complex were constructed by plotting absorbance against concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in (Table 1). Beer's law holds over the concentration ranges (10-50), (14-76) and (24-76) μg mL^{-1} for drug (I), (II) and (III) respectively. The linear regression equation for each drug was listed in (Table 1). The correlation coefficient was 0.9999 indicating good linearity over the working concentration range.

Sensitivity

The detection limit (LOD) for the proposed method was calculated using the following equation according to the ICH [14]

$$LOD = 3.3\ \sigma/S \qquad \text{Eq.1}$$

Where, σ = the standard deviation of replicate blank responses (under the same conditions as for sample analysis).
S = the slope of the calibration curve.

The limits of quantitation, LOQ, is defined as;

$$LOQ = 10\ \sigma/S \qquad \text{Eq.2}$$

According to the previous equations, the LODs and LOQs were calculated as in Table 1.Their values confirm the sensitivity of the proposed method.

Accuracy and Precision

In order to determine the accuracy and precision of the proposed methods, solutions containing one concentration of each drug was prepared and analysed in seven replicates. The relative standard deviation (RSD%) as precision and percentage relative error (Er %) as accuracy of the suggested method were calculated at 95% confidence levels, and can be considered satisfactory. The inter- and intra-day precision and accuracy results are shown in (**Table 3** and **4**). The analytical result for accuracy and precision show that the method proposed has good repeatability and reproducibility.

Analytical Applications

The proposed method was applied to determine the studied drugs in their pharmaceutical dosage forms. Satisfactory results were obtained. To check the validity of the proposed method, the standard addition technique was applied by adding them to the analyzed pharmaceutical dosage forms. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the drug. The results of analysis of the commercial dosage forms and the recovery study as shown in (Table 2). The results obtained were compared with the reported methods [15, 16]. No significant differences were found between the proposed methods and reported methods. Statistical comparison of the results was performed using Student t-test and Variance ratio F-test at 95% confidence level (**Table 5**).

Table 3 The intra-day precision and accuracy data for studied drugs with silver – gelatin Complex

Error %[c]	RSD % [b]	Recovery %	Found conc[a] μg mL^{-1}	Added conc μg mL^{-1}	Drug
- 0.26	0.37	99.74	39.90	40	**I**
0.81	0.91	100.81	50.41	50	**II**
0.54	0.23	100.54	60.32	60	**III**

[a] Average of seven determinations;
[b] RSD%-Relative Standard Deviation;
[c] % of Relative Error.

Table 4 The inter-day precision and accuracy data for studied drugs with silver – gelatin Complex

Error % [c]	RSD % [b]	Recovery %	Found conc[a] μg mL^{-1}	Added conc μg mL^{-1}	Drug
- 0.22	0.48	99.78	39.91	40	**I**
0.61	0.42	100.61	50.30	50	**II**
0.50	0.27	100.50	60.30	60	**III**

Conclusions

The proposed method is advantageous when compared to many of the reported spectrophotometric methods in having higher sensitivity. The data given above reveal that the proposed method is simple, accurate and sensitive with good precision and accuracy. With this method, one can do the analysis at low coast without losing accuracy. No interference from excipients was encountered. Besides, no organic solvents were used .The proposed method can be used as alternative method to the reported ones for the routine determination of the studied drugs in the pure form and in pharmaceutical formulations depending upon the availability of chemicals and the equipment.

Table 5 Determination of for studied drugs with silver – gelatin Complex compared with reported methods [15 and 16].

Reported method	Silver-gelatin method	Analysis	Drug
99.94 ± 0.42 [15]	99.93 ± 0.38	Mean ± R.S.D.	
0.18	0.14	Variance	
-	0.12 (2.145)*	Student t-test	I
-	1.29 (3.69)	F-test	
9	7	n	
99.97± 0.29[15]	100.18 ± 0.45	Mean ± R.S.D.	
0.08	0.20	Variance	
-	1.16 (2.11)	Student t-test	II
-	2.5 (3.14)	F-test	
8	11	n	
100.02 ± 0.44 [16]	99.92 ± 0.39	Mean ± R.S.D.	
0.19	0.13	Variance	
-	0.47 (2.17)	Student t-test	III
-	3.97 (1.46)	F-test	
6	8	n	

* The figures within ( ) are the theoretical values for t- and F-tests (P<0.05)

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