

Micro Spectrophotometric Determination Streptomycin Sulfate by Cloud point Extraction in pure form and pharmaceutical preparation.

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Abstract

A new method for the determination of the drug Streptomycin Sulfate in some Pharmaceuticals using (UV Vis). Ag (I) should forms a chelating complex with Streptomycin Sulfate (STR- Ag I) at pH (1- 12) and the best pH for the formation of chelating complexes was pH 12 . The method based on cloud point extraction (CPE) using Triton X-114 as surfactant. The product was stabilized and measured at 404 nm. Beer's law is obeyed in the concentration range of 2.5-30 $\mu\text{g}\cdot\text{ml}^{-1}$. Sandell's sensitivity was 0.284738041 $\mu\text{g}\cdot\text{cm}^{-1}$, the detection limit was 0.274576 $\mu\text{g}\cdot\text{ml}^{-1}$, and the limit of quantitation was 0.915254 $\mu\text{g}\cdot\text{ml}^{-1}$. All variables including the metal concentration, reaction time, color stability period, and mole ratio were studied in order to optimize the reaction conditions. The composition of the product was (1:1). The method was effectively useful to determine Streptomycin Sulfate in pharmaceutical dose form, and the attained results were in good agreement with the official and other method in literature. No interference was observed from the commonly encountered additives and excipients. The complex was extracted with ethanol. The mole-ratio method has been used to determine the structure of chelate (STR- Ag I) found to be 1:1 L:M (Ligand : Metal) .

Keywords: Antibiotic drug, Streptomycin, Cloud Point Extraction, Spectrophotometry chelating complex

INTRODUCTION:

Antibiotics are the chemotherapeutic agents that kill or inhibit the growth of microorganisms. These chemical agents are used to treat disease by destroying pathogenic microorganisms or inhibiting their growth at concentration low enough to avoid undesirable damage to the host. Antibiotics are drugs preparations which contain some chemical substances that are produced by microorganisms and by chemical synthesis. These substances at very low concentrations are known to totally destroy or partially inhibit microorganisms . Antibiotics have wide spread application in the treatment of bacterial disease ⁽¹⁾ Streptomycin sulphate chemically is sulphate(STR),5-(2,4-diguanidino-3,5,6-trihydroxycyclohexoxy)-4-[4,5-dihydroxy-6-(hydroxymethyl)-3-methylamino tetrahydropyran -2-yl]oxy-3-hydroxy-2- methyl tetrahydrofuran-3-carbaldehyde⁽²⁾ Molecular formula of Streptomycin sulphate is $\text{C}_{21}\text{H}_{39}\text{N}_7\text{O}_{12}$ Molecular Weight (1457.376 g/mol) Melting point 12°C (54°F) , It is sparingly soluble in water , white to off-white powder , STR is one of the most widely used aminoglycoside antibiotics. It is used to treat infections in humans, veterinary medicine, as well as in plant agriculture. Which must be determined and all impurities must meet specified limits before a manufactured lot is used clinically. The current United States Pharmacopeia (USP 30, NF 25) compendial method for streptomycin sulfate measures streptomycin A as the primary antibiotic ⁽³⁻⁴⁾ The structures of drugs are shown in (Fig.1)

The cloud point procedure (CPE) is based on the following phenomenon: an aqueous solution of some surfactant becomes turbid and separates into two isotropic phases if some condition such as temperature or pressure is changed or if an appropriate substance is added to the solution ⁽⁵⁾ This salts can be extracted by used cloud point extraction method⁽⁶⁾. Cloud point extraction (CPE) is based on the phase behavior of non-ionic surfactants in aqueous

solution⁽⁷⁾, which exhibit phase separation after an increase in temperature or the addition of a salting out agent ⁽⁸⁾. Separation and pre concentration based on (CPE) are becoming an important and practical application of surfactant in analytical chemistry⁽⁹⁾. This method is easy, sensitive , experimental conditions are free as heating and environmental friendly because use a small particular for analysis⁽¹⁰⁾.

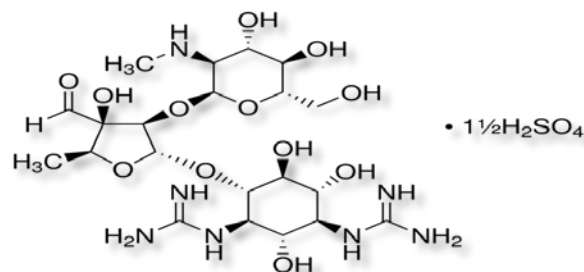


Figure (1) The structure of Streptomycin sulphate

Apparatus

- UV-Visible recording spectrophotometer (1986) Shimadzu Model (160A) (Japan) with a response time of 0.1s ,was used for spectrophotometric determination A quartz cell of 5 ml internal volume and 1cm path length was used for absorbance measurements
- Hotplate Stirrer (Hotplate stirrer Model L-81 Labinco bv)
- Electric Balance (Sartorius, 4digitals, made in Germany)
- Oven (Memmert , maximum temperature 250,made in western Germany.)
- Water Bath (A thermostat water Bath, model Unitemp)
- Centrifuge (Triup International corp ,TRIU 800 Centrifuge ,made in Korea).
- PH-meter (model BP 3001).

Materials

- A pure grade of Streptomycin Sulfate was obtained from Drug Industries and Midical Appliance (SID) Samarra/ Iraq
- All the chemical stock solution were prepared from analytical grade BDH

Preparation of Standard Solutions:

All glassware used was cleaned with distilled water and dried at 50°C for 30 minute prior to use. Batch experiments were carried out in to ensure the reproducibility of results and the average value. All metal used were of the highest purity and most solutions were prepared in distill water.

- 250µgml⁻¹ Stock solution of Streptomycin sulphate was prepared by dissolving 0.025g from Streptomycin sulphate (C₂₁H₃₉N₇O₁₂) in distilled water and diluting to the mark in 100ml volumetric flask
- A 10% (v/v) of Triton X-114 was prepared by diluting 10 ml with water in a 100 ml volumetric flask
- A solution of 500 ppm of Ag⁺ was prepared by dissolving 0.7874 gm of AgNO₃ in small amount of Water and complete the volume to 1000 ml by using volumetric flask .
- A standard stock solution of sodium hydroxide NaOH (1M) was prepared by dissolving (4g) of the solid product in 100 ml of distill water Then 10 ml of the stock solution was diluted to 100 ml with distilled water to Prepare 0.1M solution.

Interference Solutions of 1000 ppm

An amount of 1000 µg ml⁻¹ stock solution of interferences is prepared by dissolving 0.1g of the different organic compound such as [Lactose, Starch, Arabic Gum, Glucose and Talc] and inorganic compound such as of Ca₃(PO₄)₂ and CaCO₃ by 0.2579g and 0.2500g respectively in distilled water and diluting them to the mark in 100 ml volumetric flask

General procedure for CPE

A typical experiment of cloud point include the following steps: In 10ml volumetric flask and added the optimum condition of complex [0.5ml Streptomycin sulfate , 1.4 ml, 1ml Ag 1.2 ml buffer pH 12 and 10%(v/v) Triton X114 then completed to the mark by distilled water . The content of volumetric flask was transfer to centrifuge test tube then added the mixture in water bath 75°C at 30 min and separated by centrifugation 3000 rpm at 10 min. Test tube taken in ice bath to increased viscosity micelles layer 1min. then become easily separated . The separated sediment s dissolved by 1ml of ethanol and measured the absorbance by UV-VIS. And the maximum wave length show in fig:(1)

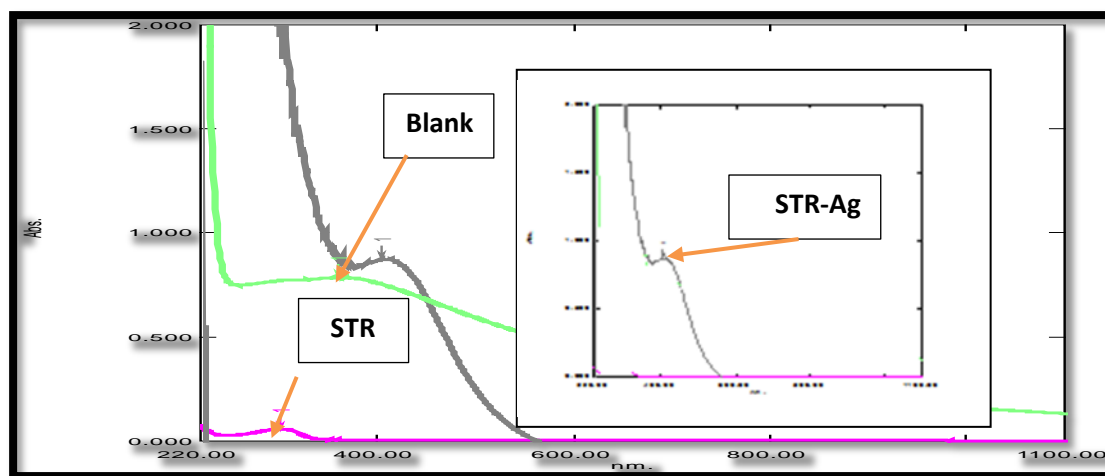
RESULTS AND DISCUSSION

Absorption Spectra

The spectrum complex product of 0.5ml of (12.5 ppm) of Streptomycin sulphate with of 0.5ml of (25ppm) Silver metal ion which was adopted of CPE for the drug . The absorption spectrum of the complex product formed was also recorded against the corresponding metal blank between 220 to 1100 nm before obtaining optimum conditions according to the recommended CPE procedure show an absorbance at a wave length at 404nm. The molar absorptivity value is 3.4308×10⁻⁴Lmol⁻¹cm⁻¹. The value of molar absorptivity enables to carry out the quantitative analysis of Streptomycin sulphate in Pharmaceuticals directly as shown in the figure (2).

Optimization of CPE Methodology

A series of experiments has been conducted to study the effect of several variables that affect the extraction efficiency of the CPE and maximize the sensitivity of the detection system for drug under study using a classical optimization. The variables such as the concentration of metal ion , best of pH ,best of buffer ,best of volume buffer , Triton X-114 amount, equilibration temperature and incubation time.



Figure(2) : Absorbance spectra of the Resulting complex product

Effect of metal ions concentration

The effect of Silver ion concentrations upon the absorbance values of the extracted complexes using (250µg/ml) of drug solution . The optimum concentration of the metal ions that gave maximum absorbance was 500µg/ml of the optimum concentration of Ag(I) ions were for complex The absorbance is measured and the absorbance results to Optimum Volume of (0.4 ml) metal ion.

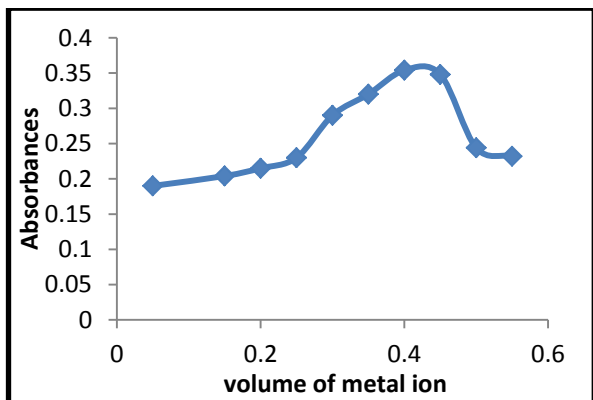


Figure 3 : Effect of Optimum concentration on absorbance of (STR-Ag I) complex

It is obvious that absorbance increase with increase the volume of metal , suddenly the absorbance decrease

Effect of pH

Cloud point extraction yield plays a unique role on metal-ligand formation and subsequent extraction, and is proved to be a main parameter for CPE the described procedure The maximum sensitivity for CPE was obtained at pH12 to find the best acidic function of the ion extraction process different value of pH 1-14 The results are shown in Fig (4), the best separation was achieved at pH =12 for Ag(I) show the value of absorbance intensity for the complexes drug- Ag against the value of pH , the best values of pH recorded for the highest absorbance values were Plotting of the absorbance values versus the value of pH is shown in Fig (4)

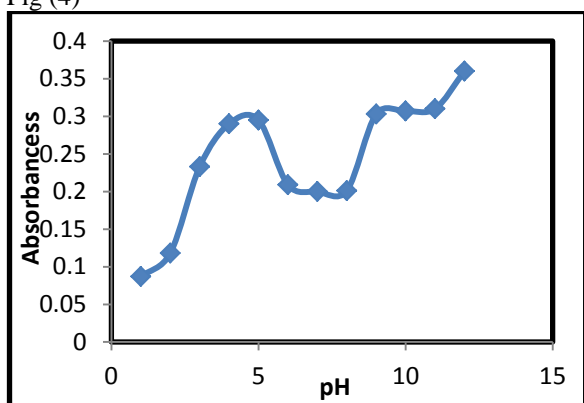


Figure 4 : pH effect on absorbance of (STR-Ag I) complex

Plotting of the absorbance values versus the value of pH is shown in fig (4). Cloud point extraction yield plays a unique role on metal a set of similar experiments in the pH range of 1.0 the described procedure The maximum

sensitivity for CPE was obtained at pH 12 . In more basic solutions, deteriorate ion of the signal occurs due to the ligand protonation,

Effect of buffer solutions

The best values of buffer pH12 recorded for the highest absorbance values were ,The absorbance is measured the absorbance results are shown in table (1).for complexes (Ag+ STR)

Table 1: buffer pH 12

Preparation buffer pH 12	Absorbance
Sodium hydrogen ortho phosphate	0.052
Potassium chloride buffer solutions	0.392

Effect of Volumes buffer solutions

Fig (5) show the value of absorbance intensity for the complexes drug- Ag against the value of buffer solutions , the best value of Potassium chloride buffer solutions recorded for the highest absorbance values

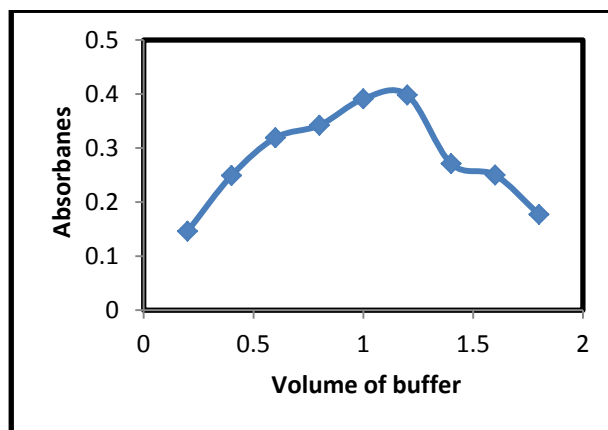


Figure 5: buffer of pH effect on absorbance of (STR-Ag I) complex

It is evident that absorbance increase with increase the volume of buffer , but suddenly decrease the absorbance because the decomposition happen when increase basicity

Effect Type of Surfactant with metal

The type of surfactant plays very substantial role in cloud point extraction process where each surface owns spectral properties depend on practical basis of Micelles .Aliquots of 10ml of a solution contains [0.5ml Streptomycin sulphate ,0.4ml Ag , 1.2ml buffer pH 12] for Silver metal in 10ml volumetric flask and use different surfactant for each drug [Tween 20, Tween80, CTAP, SDS, Triton X-100, Triton X-114] at 50°C for 10 min for complex incubation time then it centrifuged at 3000 rpm for 10min , separated the surfactant- rich phase and dissolved in 1ml ethanol then measured by UV-Vis at λmax = 404nm for Ag results shown in Table (2) Plotting the absorbance values of the cloud point versus the type of surfactant is shown in Fig (6)

Table (2) Data of Absorbance to Type of Surfactant with Ag (I)

No	Surfactant	Absorbance at λ_{\max} =404 nm for Ag (I)
1	Tween 20	0.192
2	Tween80	0.132
3	SDS	0.225
4	CTAP	0.14
5	Triton X-100	0.031
6	Triton X-114	0.559

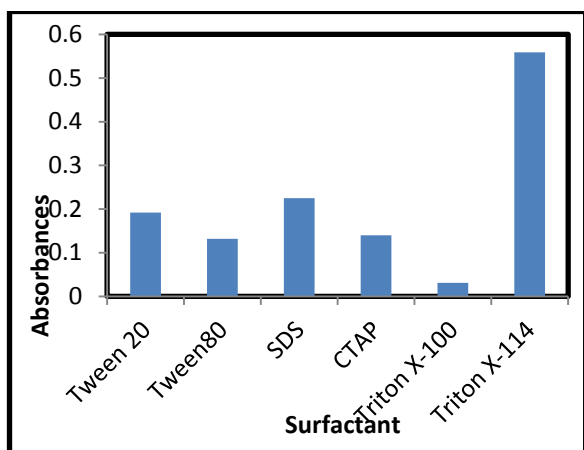


Figure (6) : Effect of surfactant type on absorbance of (STR-Ag I) complex

It was observed that Triton X-114 which have maximum absorbance at 404 nm. It is clear from the results that the nonionic surfactant Triton X-114 is of high absorbance and this surface increases the efficiency of the extraction process in cloud point extraction.

Effect of Triton X-114 Amount

Most studies confirm that the amount of a nonionic surfactant type TX-114 as an extracting medium plays an important role for maximizing the extraction efficiency by minimizing the phase volume ratio (V_s/V_a) and therefore improving the pre-concentration factor of the CPE procedure. Therefore, the amount of TX-114 was investigated by varying the volume of 10% TX-114 between (0.2-2.0 mL) for STR. The results are presented in Fig (7). It was noticed that the absorbance values of STR drug continued to increase dramatically and reached maximum at 1.4 mL of 10% TX-114 (i.e. 1.4% TX-114 in 10 mL solution) for Ag metal. These values were selected as optimal amount and used in the proposed methods for the detection of STR. Plotting the absorbance values of the cloud point versus the volume of Triton X-114 is shown in Fig (7).

Effect of the Equilibration Temperature and Time

In order to optimize the method, it was necessary to examine the effect of the temperature on cloud-point extraction. Temperature that enhances a higher range of (35 – 90)°C and (5 - 50) min, respectively, while keeping all other parameters constant. Excellent absorbance was found at temperature 75°C as shown in fig (8); therefore, choose 75°C higher than is probably due to the decomposition of the complex.

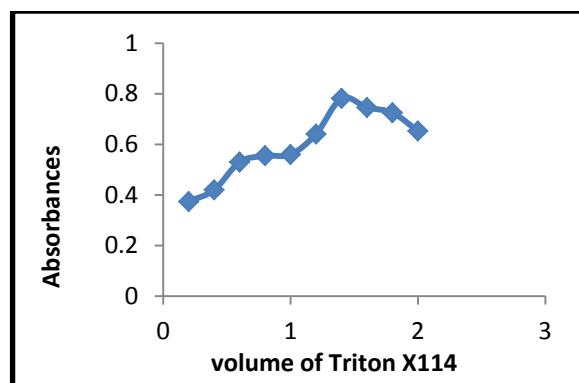


Figure 7: Effect of the TX-114 amount on absorbance of (STR-Ag I) complex

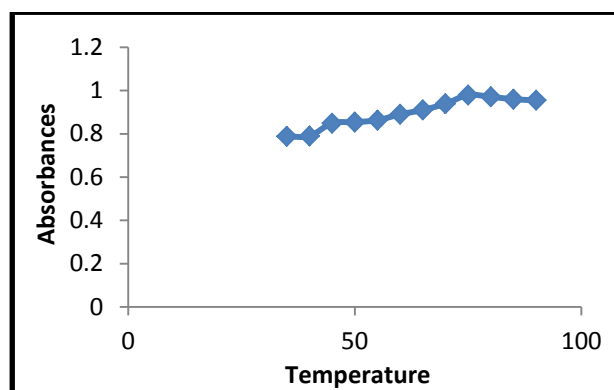


Figure 8. Effect of temperature on absorbance of (STR-Ag I) complex

Incubation time was also investigated in the range of (5-50) min (fig (9)). Excellent absorbance was found at 35 min. The time for 35 min was selected to fulfill efficient separation conditions.

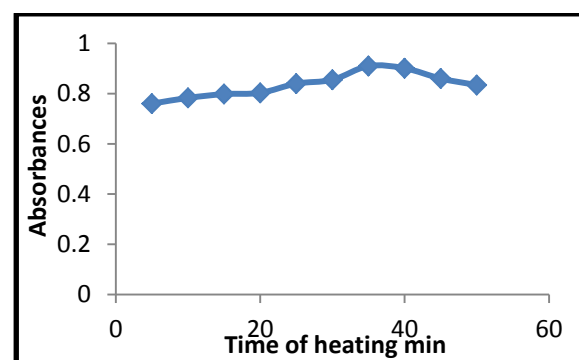


Figure 9: .Effect of time on absorbance of (STR-Ag I) complex

Order of Additions

The effect of order for additions of the metal on the absorbance of each analyte by the general CPE was tested. Fig (10) shows that the best order of addition is the number 1 for target analytes due to giving a highest absorption signal among the others. The absorbance is measured and the absorbance results are shown in table (3).

Plotting of the absorbance values versus the order additions is shown in Fig (10)

Table (3) Data of Absorbance to Order Additions

No	Order Additions	Absorbance at λ_{max} =404 for Ag(III)
1	<i>D+M+B+T</i>	0.98
2	<i>M+D+B+T</i>	0.63
3	<i>D+B+M+T</i>	0.577
4	<i>M+B+D+T</i>	0.367

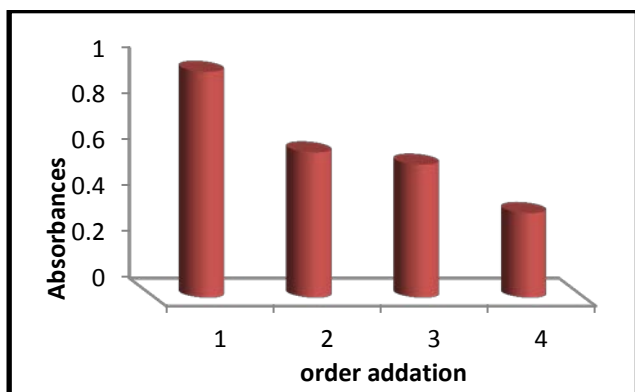


Figure 10 : Effect of Order Additions. on absorbance of (STR-Ag I) complex

It is noted that the best addition is the first order of Ag (I) because if it's another order gets lost in the intensity of color and this order fixed in subsequent experiment

Effect of organic solvents

Different organic solvents (water , Ethanol, Methanol, Acetonitril, H₂O₂, chloroform, Acetyl acetone, Dimethyl formamide, Dimethyl phthalate, Dimethyl malonate) are examined to evaluate their effects on the intensity of the resulting complex and Plotting of the absorbance values versus the solvent is shown in fig (11)

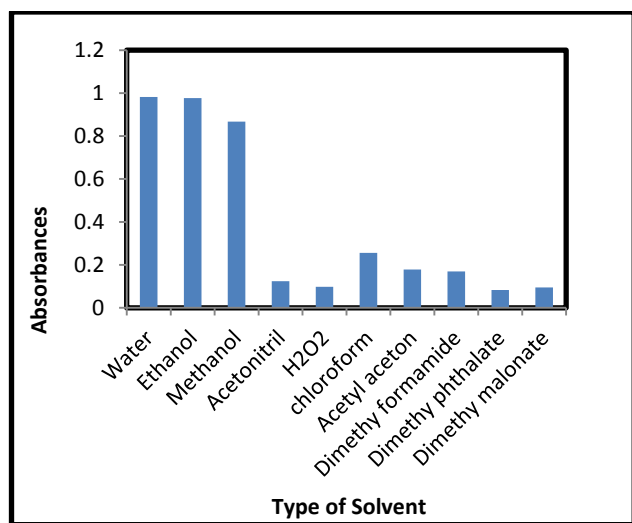


Figure 11 : Effect of Solvents on absorbance of (STR-Ag I) complex

It has been shown that water is the optimum solvent , economically , sensitivity method ,cheap price, to provide and nontoxic. This solvent is fixed in subsequent experiment

Effect of Interference

The effect of some foreign organic compounds and Inorganic compounds, which often found in environmental , were studied by adding 1ml of (100ppm) Equal amounts organic compounds, Inorganic compounds to 1ml of (100ppm) of complex . The color was developed following the recommended procedure described earlier

Table 4: Effect of Interference

100ppm interference	Absorbance at λ_{max} =404 for Ag
With out	0.981
Lactose	0.055
Starch	0.231
Arabic Gum	0.093
Talc	0.045
Glucose	0.0001
Ca ₃ (PO ₄) ₂	0.145
CaCO ₃	0.166

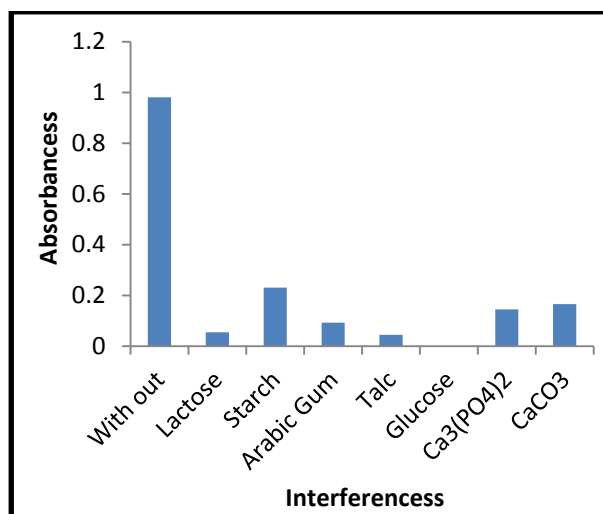


Figure 12 : Effect of organic and inorganic Interferences on absorbance of (STR-Ag I) complex

It was observed that the table (4) were not interfering with the determination at levels found in complex form.

Selected Optimum Conditions

After the study of the effect of different physical and chemical conditions on the absorbance intensity of the colored product, The optimum conditions for the proposed procedure were summarized in (Table 5) and were used in all subsequent experiments.

Calibration Graph

Employing the optimum conditions described in the procedure of cloud point extraction,

liner calibration graph of STR with Silver was obtained Fig(13), which show Beer law obeyed over the concentration range of $2.5\text{-}30\mu\text{g ml}^{-1}$ with correlation coefficient equal to 0.9989. All other analytical characteristics data are summarized in table(6) .then it is completed to the mark by distilled water, are mixed ,heated at optimum temperature in the thermostat water bath at optimum incubation time, to form cloud point then aqueous phase is separated by centrifugation at 3000 rpm for 10min ,1ml ethanol is added to the surfactant-rich phase to dissolve it then is measured by UV-Vis at $\lambda_{\text{max}} = 404\text{ nm}$ for Sliver, triplicate manner The absorbance measurements are illustrated in table 6

Table 5: The optimum conditions for the determination of STR

Optimum	Concentrations	Range selected	Optimum quantities of complex (STR-Ag)
λ_{max} (nm)	----	220-1100	404
Effect of volume of metal ion required	500 ppm	0.05-0.55ml	0.4
Effect of PH	0.1M(Na OH)	1-14	12
Buffer pH	----		
Effect of volume of Buffer	----	0.2-1.8ml	1.2
Effect of volume of triton x114 required	10%(v/v)	0.2-2 ml	1.4
Effect of time heating	----	5-50min	35min
STR solution required	250 ppm	0.1-1.2ml	0.5

Table 6 : The absorbance measurements of standard solutions of complex (STR-Ag)

Conc. ppm	Mean Absorbance	RSD%	Found	Recovery%
2.5	0.465	0.151903	2.2478	89
5	0.587	0.240513	4.8326	96
7.5	0.698	0.101087	7.1843	95
10	0.829	0.085245	9.9597	99
12.5	0.979	0.288615	13.1377	105
15	1.079	0.065503	15.2563	101
17.5	1.201	0.058852	17.8411	101
20	1.315	0.214926	20.2563	101
22.5	1.421	0.248719	22.5021	100
25	1.533	0.092191	24.875	99
27.5	1.645	0.042998	27.2478	99
30	1.767	0.199917	29.8326	99

The calibration curve was . Plotting the mean absorbance values of the cloud point versus the concentration (ppm) of (STR-Ag) as shown in Fig (13)

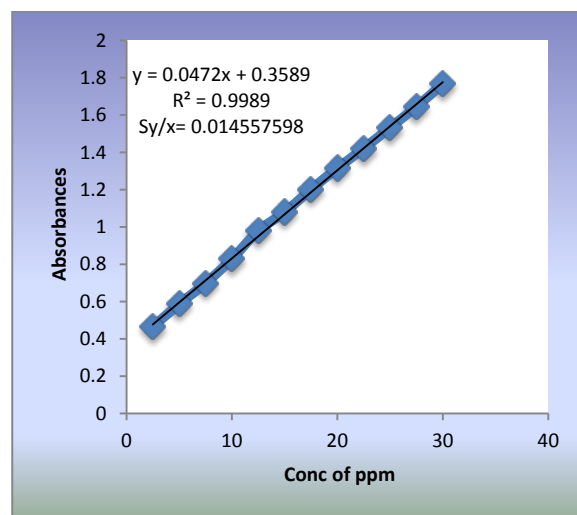


Figure 13 : Calibration Curve of complex (STR-Ag)

Optical characteristics Features of the calibration curve

Table (7) .shows the main features of the calibration curve and measuring the absorbance at 404 nm

Table 7 : Optical characteristic features of calibration curve

Parameter	Complex (STR-Ag)
Wave length λ_{max} (nm)	404nm
Concentration rang ($\mu\text{g ml}^{-1}$)	2.5-30 $\mu\text{g ml}^{-1}$
Regression equation	$y = 0.0472x + 0.3589$
Correlation coefficient(r)	0.9994
Correlation coefficient (r^2)	0.9989
Variation coefficient (%)	99.89
Limit of Detection ($\mu\text{g ml}^{-1}$)	0.274576 $\mu\text{g ml}^{-1}$
Limit of Quantitation ($\mu\text{g ml}^{-1}$)	0.915254 $\mu\text{g ml}^{-1}$
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.284738041
Slope (m)	0.0472
Intercept (C)	0.3589
Molar absorptivity(L.mol ⁻¹ .cm ⁻¹)	2.5×10^3
Composition of product	1:1
C.L for slope (b \pm tSb) at 95 %	$0.0472 \pm 1.0858 \times 10^{-3}$
C.L for intercept (a \pm tSa) at 95 %	0.3589 ± 1.93298
C.L for Conc. 5 $\mu\text{g ml}^{-1}$ at 95%	$0.587 \pm 4.9653 \times 10^{-3}$
C.L for Conc. 15 $\mu\text{g ml}^{-1}$ at 95%	$1.079 \pm 2.4826 \times 10^{-3}$
C.L for Conc. 25 $\mu\text{g ml}^{-1}$ at 95%	$1.533 \pm 4.9653 \times 10^{-3}$

Calculation of the stability constant (K) of complexes: Stability constant of reaction product

The conditional or apparent stability constant of the 1:1 (Drug and metal) product was evaluated and described as shown A series of solution were prepared containing three different concentration of metal and Streptomycin Sulfate (1:1) the concentration (1×10^{-4}) molL⁻¹ for each (Sliver with Streptomycin Sulfate) . are shown in Table (8)

Table 8 : Stability Constant of the complex(Ag+ Streptomycin Sulfate) formed

Vol of Streptomycin Sulfate	Absorbance at $\lambda= 404\text{nm}$				K (Average) (l.mol^{-2})
	A_s	A_m	α	K (l.mol^{-2})	
0.3	0.590	0.597	0.01172	7×10^7	2.7×10^9
0.5	0.768	0.769	1.30039	5.9×10^9	
0.7	0.925	0.927	2.15749	2.1×10^9	

Stoichiometric Determination of Color complex : Continuous Variation Method (Job's method)

A series of (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9) ml of (1×10^{-4}) mol L⁻¹ of the solution that contain Streptomycin Sulfate was pipette into each of 10ml volumetric flask then(0.9,0.8,0.7,0.6,0.5,0.4,0.3,0.2,0.1) ml of (1×10^{-4}) mol L⁻¹ of metal the absorbance of the solution was measured by UV-Vis Spectrophotometer at λ_{max} 404nm the stoichiometric ratio between Streptomycin Sulfate with metal 1:1 results are shown in the Table (9) Plotting the value of absorbance versus the VD / VT is shown in Fig (14)

Table 9. : The continuous variation method of Streptomycin Sulfate with metal (Silver) complex.

V D mL	V M mL	VD / VT	Absorbance at $\lambda= 404$ for Color compound
0.1	0.9	0.1	0.0245
0.2	0.8	0.2	0.078
0.3	0.7	0.3	0.14
0.4	0.6	0.4	0.187
0.5	0.5	0.5	0.262
0.6	0.4	0.6	0.209
0.7	0.3	0.7	0.175
0.8	0.2	0.8	0.111
0.9	0.1	0.9	0.056

VD: values of the compound (Streptomycin Sulfate)
 V M: The values of the metal (silver).
 VT: Total (V M+V D)

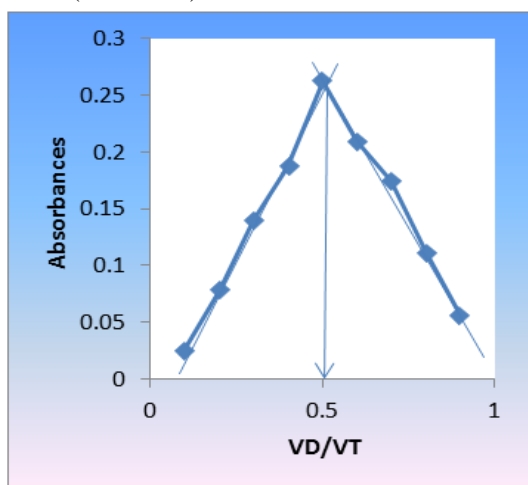


Figure 14: Continuous variation method plot

Mole – Ratio Method

Aliquots of 10 mL solution containing (1×10^{-4}) molL⁻¹ of (1mL) Streptomycin Sulfate and increasing concentrations (1×10^{-4}) mol L⁻¹ of (0.2,0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8,2) mL of (Ag) silver (2×10^{-6} -- 2×10^{-5}) mol L⁻¹ metal . The absorbance of the solutions were measured by UV-Vis spectrophotometer versus blank at $\lambda_{\text{max}}= 404$ nm the stoichiometric ratio between 1:1 results are shown in the Table (10).

Table 10 : The Mole - Ratio Method of the Streptomycin Sulfate with Silver

CL	CL / CM	Absorbance at $\lambda=404$ nm for Color compound
2×10^{-6}	0.2	0.053
4×10^{-6}	0.4	0.098
6×10^{-6}	0.6	0.159
8×10^{-6}	0.8	0.208
1×10^{-5}	1.0	0.234
1.2×10^{-5}	1.2	0.231
1.4×10^{-5}	1.4	0.222
1.6×10^{-5}	1.6	0.214
1.8×10^{-5}	1.8	0.211
2×10^{-5}	0.2	0.201

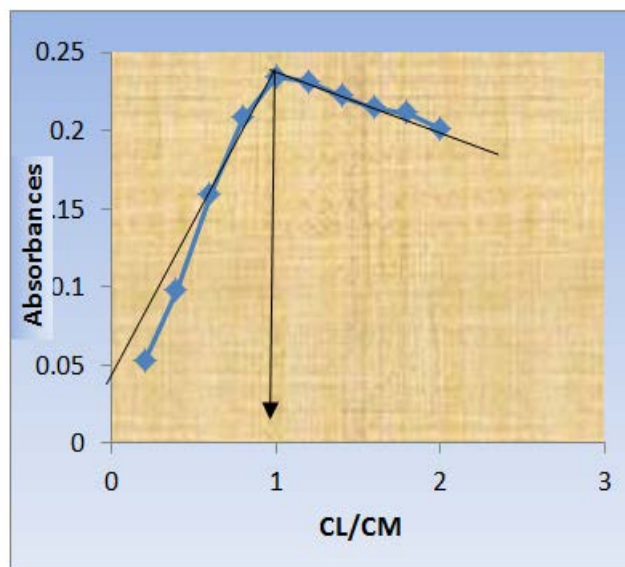


Figure 15: Mole - Ratio plot of Streptomycin Sulfate with Silver complex

Analytical Application

The suggested methods was applied to the quantitative determination of Ag (I) in some Pharmaceuticals it was gave good accuracy and precision Table (11).Application of proposed method for determination of Ag(I)

Table 11 : Data for Determination STR with Ag in the Pharmaceutical Preparation (STR) by CPE.

Amount of STR / $\mu\text{g ml}^{-1}$	Mean absorbance	Relative stander deviation (RSD)	*Found	Recovery%	Average Recovery%	Erel%	Average Erel%
7.5	0.680	0.328834	6.8029	90	97	-9.333	-7.64419
12.5	0.792	0.15464	11.2944	90		-9.68	
17.5	1.279	0.22795	19.4936	111		11.3714	

[*]= Average of Five

CONCLUSION

CPE preconcentration is an easy, safe and inexpensive methodology for separation and Preconcentration of trace metals in aqueous solutions .The ligand was successfully to formed complex with the some metals ion by cloud point extraction. Is a stable, sensitive and selective complexion successfully to determination Ag (I) in some Pharmaceuticals ,the method gives a very low limit of detection and good R.S.D. values and green chemistry.

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