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# Eco-Friendly Method for Determination of Allopurinol Drug in pure form and Pharmaceuticals after Cloud Point Extraction

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**Abstract**

A new spectrophotometric method for the determination of allopurinol drug was investigated. The proposed method was based on the determination of allopurinol in pharmaceuticals. The method relies on the interaction of metal ions with allopurinol in an alkaline medium to form a micelles extracted from a non-ionic surfactant (Triton X-114) and allopurinol-Ag II at a maximum of 906 nm. Experimental variables were performed individually to obtain high extraction efficiency for both compounds. Under optimal conditions, Beer's law was obeyed in the concentration range of 5-35 µg $ml^{-1}$ ( r=0.9997) the detection limits of 0.245951 µg$ml^{-1}$ The proposed method was applied successfully for the determination of allopurinol drug in pharmaceuticals with a good accuracy and precision. The optimum condition for the colour development has also been investigated.

*Keywords:* drug, allopurinol , silver Cloud Point Extraction, chelating complex

## 1. Introduction

Allopurinol (ALLO) was developed in 1946 by Elion, et al. at the Burroughs– Welcome Company. It was discovered together with other molecules using spectrographic techniques when other purines were being considered for the treatment of cancer[(1)] Allopurinol (1H-pyrazolo[3,4-d]pyrimidin-4-ol) (Fig. 1) is a commonly used drug in the treatment of chronic gout or hyper uricaemia associated with leukemia, radiotherapy, antineoplastic agents and treatment with diuretics conditions.[( 2)] Allopurinol is a structural isomer of hypoxanthine (a naturally occurring purine in the body) and acts to inhibit xanthine oxidase. Allopurinol (AP) is one of the most effective and widely used drugs for[( 3)] the treatment of hyper uricaemia and gout. Its main function is to inhibit xanthine oxidase which catalyzes the formation of xanthine from hypoxanthine and further to uric acid [( 4-5)] AP has also been estimated by micelle-stabilized room temperature phosphorescence in urine samples[( 6)] The cloud point procedure (CPE) is based on the following phenomenon: an aqueous solution of some surfactant becomes turbid and separates in to two isotropic phases if some condition such as temperature or pressure is changed or if an appropriate substance is added to the solution [( 7)] over all characterizes of allopurinol are certain in fig (1)

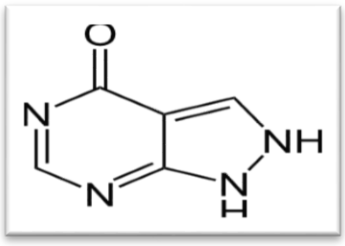


Figure (1) : Structure of Allopurinol

This salts can be extraction by used cloud point extraction method[(8)] .Cloud point extraction (CPE) is based on the phase behaviour of non- ionic

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surfactants in aqueous solution[9], which exhibit phase separation after an increase in temperature or the addition of a salting out agent [10].Separation and pre concentration based on (CPE) are becoming an important and practical application of surfactant in analytical chemistry[11].This method is easy, sensitive , experimental conditions are free as heating and environmental friendly because use a small particular for analysis[12 ,13].

The aim of present work was to develop simple, economical, rapid, precise ,accurate and eco-friendly method for determination of single drug by using Cloud Point Extraction

## 2. Experimental

### 2.1. Instrumentation and apparatus

SHIMADZU UV Visible Spectrophotometer, Dual UV-Vis, Manufactured UV-1800 (Japan) Model with 0.1s Response Time Used to Estimate Optical Spectrum 1ml Indoor Quartz Cell and 1cm Path Length for Measurements Absorbance. Stir plate (Model L-81 Labinco bv). Electric balance (Sartorius, 4 digits, made in Germany) OVEN (Memmert, max temperature 250, made in West Germany). Water bath (water bath thermostat, Unitemp model) centrifuge (Triup International corp, TRIU 800 Centrifuge, made in Korea). pH Meter (Model BP 3001)

### 2.2. *Drug and Materials*

The chemicals used in this work are of high purity and used as received distilled water. They were used in preparing all solutions and final rinsing of glassware. A pure degree of allopurinol was obtained from the pharmaceutical and medical devices (SID) Samarra / Iraq. A solution of 250 µgml $^{-1}$ or ( 1.836× $10^{-3}$ M) of ALLO was prepared by dissolving 0.025 g of the least amount of water and diluting it to place a mark with water in a 100 mL volumetric flask. 0.1M NaOH (BDH, UK) was prepared from a concentrated solution (1M) by converting 10 ml into a 100 ml volumetric flask and diluted to distinguish it with water. Stock solutions (500µg ml $^{-1}$ ) were prepared from silver II (95.5%, Sigma, USA) by dissolving 0.7874g of silver ion in a 1000 ml volumetric class. Triton X-114 (purity> 99.9%) was purchased from AMRESCO LLC (Solon, USA). 10% (v / v) of Triton X-114 was prepared by diluting 10 ml with water in a 100 ml volumetric flask

### 2.3. Recommended CPE Procedure for ALLO Drug

Aliquots 10 ml of solution containing a known amount of allopurinol was mixed with AgII ion and the pH was set using 0.1M NaOH and 10% (V / V) Triton x-114. The mixture was shaken for one minute and left to stand in a thermally mentioned bath at 50 ° C for 10 minutes. Phase separation was achieved by centrifugation at 4000 rpm for 10 minutes, stirring at 5 ° C in an ice bath the remainder of the micellar phase was dissolved by ethanol, and absorption absorption measurements were followed by the ultraviolet visible spectrophotometer using a quartz cell at $\lambda_{max}$ equal to 1.0 cm up to 906 nm of ALLO-Ag II against blank prepared in the same manner but without drug.

## 3. Results and discussion

### 3.1. Absorption Spectra

It was observed that the absorption maximum of the colored complex of allopurinol in 1.0 mL of 10% TX-114 occurred 906nm, giving the molar absorptivity of $3.055\times10^{-4}$ L.mol$^{-1}$.cm$^{-1}$for allopurinol –AgII complex. Thus the wavelength maximum at 906nm for the allopurinol complex was used throughout this study for ppm amounts

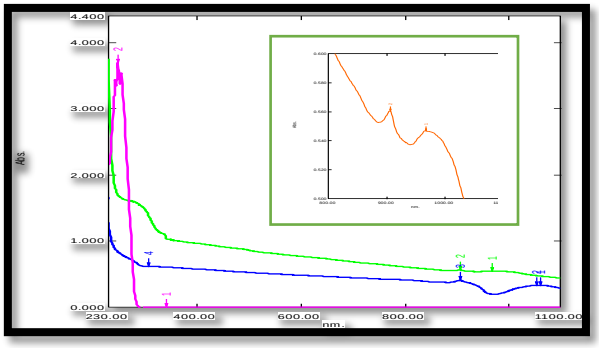


Figure 2:The absorption spectrum of [ ALLO- AgII]ALLO (25µg.ml$^{-1}$)-AgII (50 µg.ml$^{-1}$) versus blank.

### 3. 2. Optimization of CPE Methodology

### 3.2.1 Effect of metal ion concentration

The effect of sliver ion concentrations upon the absorbance values of the extracted complex using (500µgml$^{-1}$) of drug solution. The optimum concentration of the metal ions that gave maximum absorbance was 50µg/ml of AgII for the drug whereas the optimum concentration of AgII ion were 50µgml$^{-1}$for allopurinol . The absorbance is measured and the absorbance results are shown in figure

(3).Plotting of the absorbance values versus the concentration of metal ion

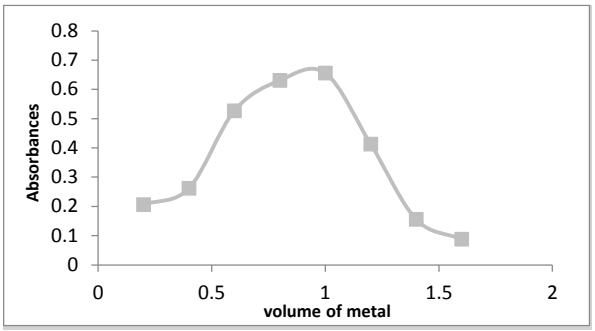


Figure 3 : Effect of optimum vol. AgII ion on absorbance of complex

### 3.2.2 Effect of pH

Figure (4) show the value of absorbance intensity for the complex ALLO- AgII against the value of pH the best values of pH recorded for the highest absorbance values,then absorbance values were found to decrease with increasing pH which may be attributed to the formation of metal

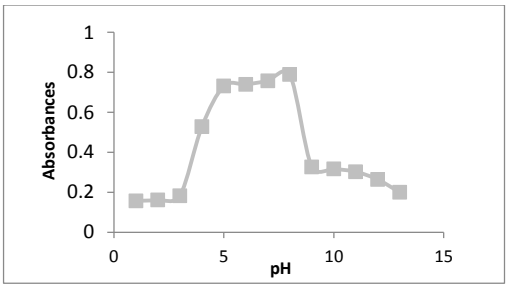


Figure 4 : pH effect on the absorbance of Allo- AgII complex

Plotting of the absorbance values versus the value of pH is shown in figure (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 8. In more acidic solutions, deteriorate ion of the signal occurs due to the ligand protonation,

### 3.2.3 Effect of buffer solutions

The best values of buffer pH 8 recorded for the highest absorbance values were,the absorbance is measured results are shown in table (1) for complex (AgII- allopurinol)

### 3.2.4 Effect of volumes buffer solutions

Figure (5 )show the value of absorbance intensity for the complex drug- Ag against the value of buffer solution ,the best value of potassium di hydrogen phosphate buffer solutions recorded for the highest absorbance values**,** plotting of the absorbance values versus the volume of pH

Table (1) Types of buffer pH 8

| buffer pH 8 | Absorbance |
|---|---|
| Disodium tetraborate(borax) buffer solutions | 0.497 |
| Tris (hydroxymethyl) amino methane buffer solutions | 0.109 |
| Potassium dihydrogenphosphate+(0.1M)NaOH | 0.367 |
| Potassium dihydrogenphosphate+(0.2M)NaoH | 0.856 |

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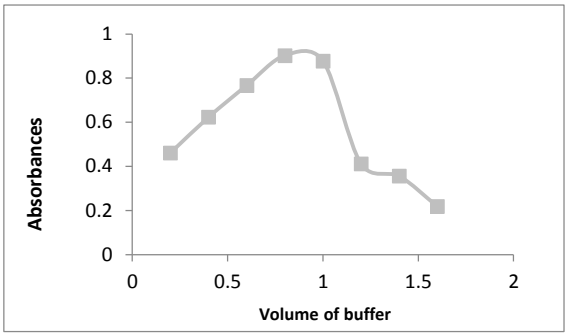


Figure 5**:** volume of buffer of pH effect on the absorbance of Allo- AgII complex

### 3.2.5 -Effect Type of Surfactant

Aliquots of 10ml of a solution [ 1ml allopurinol , 1ml AgII , 0.8 ml buffer pH=8 and 0.5mlTriton ×114 ] in 10ml volumetric flask and use different surfactant for complex [Tween 20, Tween80, CTAB, SDS, Triton X-100, Triton X-114] at $50^0$C for 10 min incubation time then it centrifugated at 4000 rpm for 10min , separated the surfactant- rich phase and dissolved in 1ml ethanol then measured by UV-Vis at $\lambda_{max}$ 906nm for AgII results shown in table (2) and Fig (6)

Table (2) Data of Absorbance to Type of Surfactant

| No | Surfactant | Absorbance at $\lambda_{max}$ 906nm for complex |
|---|---|---|
| 1 | Tween 20 | 0.167 |
| 2 | Tween80 | 0.920 |
| 3 | SDS | 0.031 |
| 4 | CTAB | 0.052 |
| 5 | Triton X-100 | 0.721 |
| 6 | Triton X-114 | 0.950 |

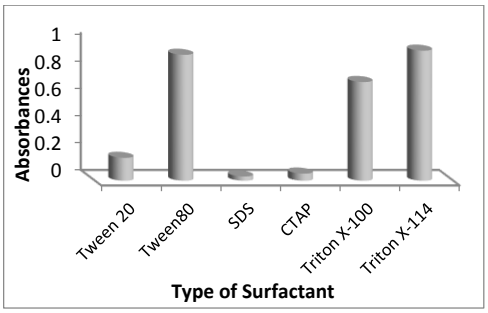


Figure 6: Type of surfactant on the absorbance AgII-Allo complex .

It is clear from the results that the non ionic surfactant Triton X-114 is of high absorbance and this surface increases the efficiency of the extraction process in cloud point extraction

### 3.2.6 Effect of Triton X-114 Volume

Amount of 10ml solution are prepared [1ml allopurinol ,1ml AgII , 0.8 ml buffer pH 8 and ml Triton×114] in 10ml volumetric flask and uses varying volumes of 10%(v/v)TritonX-114(0.2-2.0)ml for complex then it is completed to the mark by distilled water ,are mixed , heated at $50^0$C for 10 min for AgII to form cloud point then centrifugation at 4000 rpm for 10min ,1ml ethanol is added to the surfactant-rich phase to dissolve it then it is measured by UV-Vis at $\lambda_{max}$ 906nm for AgII results shown in Figure (7)

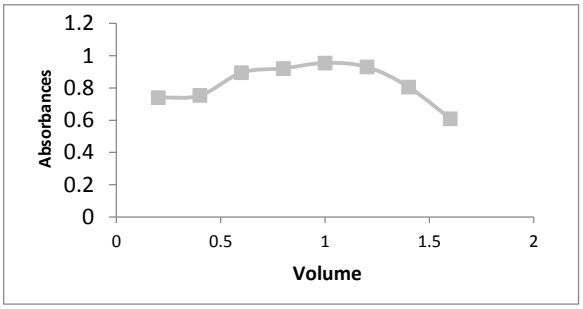


Figure 7: Volume of Triton X-114 on the absorbance of Allo-AgII complex.

It is clear from the result that the absorbance increases with the increase volume of Triton X-114 but suddenly decreases at higher amount. Effect the amount of surfactant on the efficiency of extraction and improve the enrichment factor[13].These represent the optimum volume to reach equilibrium extraction process that give highest efficiency with smaller size and higher density in cloud layer. The decrease in absorbance below the optimum volume is due to insufficient micelles to entrap the hydrophobic product quantitatively Therefore the optimum volume of Triton X-114 ( 0.2- 2) ml for AgII in subsequent experiments to achieve high extraction efficiency

### 3.2.7.Effect of the incubation Time

Cloud point extraction requires enough time to get equilibrium between aqueous phase and surfactant-rich phase by more aggregation the micelles. This time represents the amount of heat accumulated in the solution that allows Micelles lose water molecules in order to give small size hydrophobic with high viscosity easily entrap the product in it. It is clear that the optimum incubation time is 10min for complex Plotting the absorbance values of the cloud point versus the time / min is shown in Figure (8)

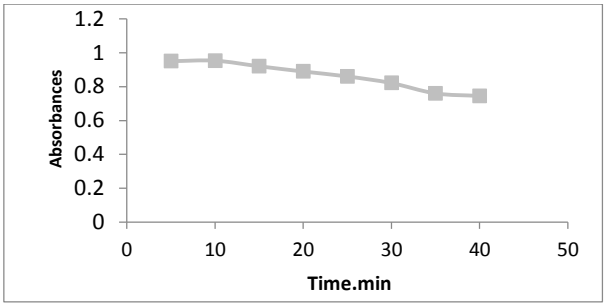


Figure 8 : Absorbance Versus Time on the absorbance of Allo-AgII complex

show the effect of reaction time on the complex formation, by following the variation of absorbance values. Maximum absorbance for all extracted Highest absorbance values of the Allo-AgII complex were obtained on heating the solutions for 10min. Heating for longer time resulted in decreased absorbance values results shown in table (7).

### 3.2. 8 Effect of Temperature

The complexation reactions of the studied drugs with AgII ions were very slow at room temperature. Figure (9)show that highest absorbance values of metal complexes were achieved at 60°C for allopurinol - Ag II complex for 10 min complex . Heating the solutions to higher temperatures led to decrease of absorbance and this may be attributed decomposition of complex

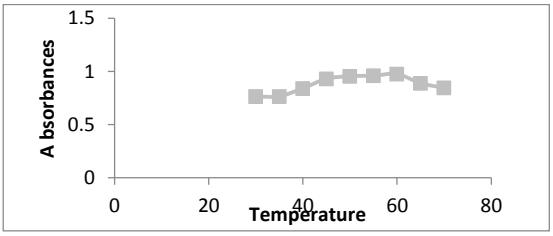


Figure 9: Absorbance Versus Temperature on the absorbance of Allo- AgII complex .

We need to heat the aqueous solution at a certain temperature to form a layer of the cloud point that smaller size and high viscosity due to aggregation the micelles this called (Cloud point temperature -CPT). The results show that the highest absorbency and extraction efficiency of the drug at temperature $60^0$C for Ag II then decreases in absorbance at higher temperature due to decomposition of product which reduces the extraction efficiency. This temperature is fixed in subsequent experiments .

### 3.2.9 Effect of extraction time

Figure (10) The centrifugation time does not have a considerable effect on the analytical characteristics of the CPE method. This parameter was examined in the range of 5-25 min at 4000 rpm. A time of min was selected as optimum, since complete phase separation occurs in this time and no appreciable improvements were

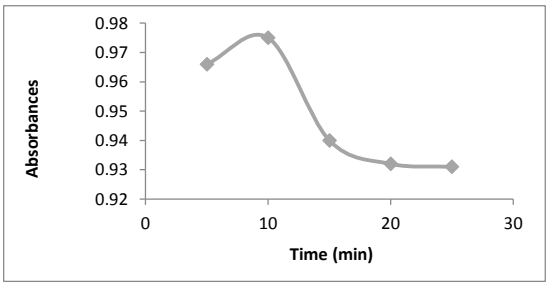


Figure 10: Extraction time effect on the absorbance of Allo- AgII complex

### 3.2.10 Effect of Order Additions.

The effect of order for additions of the metal on the absorbance of each analyte by the general CPE was tested. Fig(11) and table (3) shows that the best order of addition is the number one for target analytes due to giving a highest absorption signal among the others.

Table (3) Data of Absorbance to Order Additions

| No | Order Additions | Absorbance at $\lambda_{max}$ 906nm for complex |
|---|---|---|
| 1 | *D+M+B+T* | 0.974 |
| 2 | *M+D+B+T* | 0.950 |
| 3 | *D+B+M+T* | 0.867 |
| 4 | *M+B+D+T* | 0.831 |

Plotting of the absorbance values versus the order additions is shown in figure ( 11 )

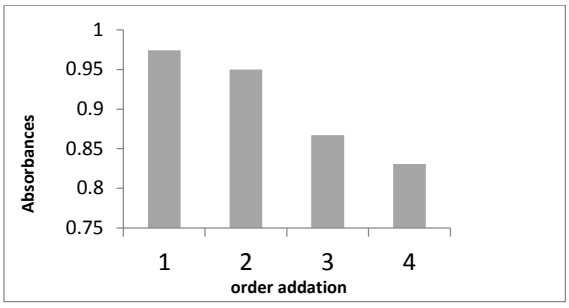


Figure 11: Effect of Order Additions on the absorbance of Allo-AgII complex

### 3.2.11. Effect of Solvents

The absorbance is measured and the absorbance results are shown in table(4).

Table (4) Data of Absorbance to Solvents

| No | Solvents | Absorbance at $\lambda_{max}$ 906nm of complex |
|---|---|---|
| 1 | Water | 0.979 |
| 2 | Ethanol | 0.862 |
| 3 | Methanol | 0.513 |
| 4 | Acetonitril | 0.066 |
| 6 | chloroform | 0.678 |
| 7 | Acetyl aceton | 0.032 |
| 8 | Dimethy formamide | 0.145 |
| 9 | Dimethy phthalate | 0.167 |
| 10 | Dimethy malonate | 0.131 |

Plotting of the absorbance values versus the solvent is shown in figure (12)

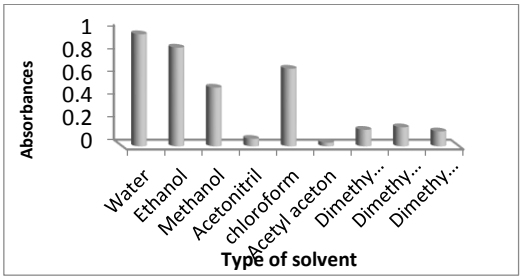


Figure 12: Effect of Solvents on the absorbance of Allo- AgII complex

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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

### 3.2.12. The Effect of Interference

The effect of interference expected present in drug allopurinol has been studied to know method selectivity under study by added 1ml (100 ppm) from each interference [Lactose, Starch, Arabic Gum, Glucose, Talc, $Ca_3(PO_4)_2$, $CaCO_3$] with 1ml(10 ppm) from each drug and the rest of addition are optimal

conditions then diluted with distilled water in 10ml volumetric flask then measured. The interference experiment is performed to estimate the systematic error caused by other materials that may be present in the specimen being analyzed. It must be the size of interference is small for a sample to limit the dilution of sample and use the maximum concentration expected in the sample. The results are shown in Table (5)

Table (5) Data of Absorbance for Interference

| 100ppm interference | Absorbance at $\lambda_{max}$ 906nm for complex |
|---|---|
| With out | 0.977 |
| Lactose | 0.506 |
| Starch | 0.615 |
| Arabic Gum | 0.467 |
| Talc | 0.314 |
| Glucose | 0.052 |
| $Ca_3(PO_4)_2$ | 0.067 |
| $CaCO_3$ | 0.129 |

From the previous tables we notice that there is no expected interference to be present with drug in pharmaceuticals

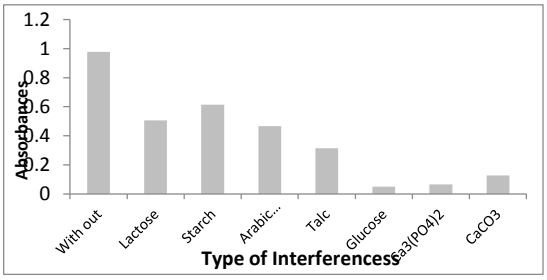


Figure 13: Effect of Interference on the absorbance of Allo- AgII complex

**Selected Optimum Conditions**

The optimization conditions method that was used above to study the effect of variables on the absorbance intensity After the study of the effect of different physical and chemical conditions on the absorbance intensity of the colored product , that gave the optimum conditions shown in Table (6).

Table 6: The optimum conditions for the determination of complex

| Optimum | Concentrations | Range selected | Optimum quantities of complex |
|---|---|---|---|
| λ max(nm) | ---- | 190-1100nm | 906nm |
| Effect of volume of metal ion required | 500 µgml$^{-1}$ | 0.2 -2 ml | 1 ml |
| Effect of pH | 0.1M(NaOH) | 1-14 | 8 |
| Effect of buffer solution volume | | 0.2-1.6ml | 0.8ml |
| Effect of volume of triton x-114 | 10%(v/v) | 0.2 -1.6ml | 1 ml |
| Effect of time heating | | (5-40) min | 10min |
| Allopurinol solution required | 250 µgml$^{-1}$ | 5-45 µgml$^{-1}$ | 0.5ml |
| temperature | | 30-70ºC | 10 min on 60ºC |

The optimum conditions for the proposed procedure were summarized in Table (6) and were used in all subsequent experiments.

**3.2.13. Preparation of Calibration Curve in CPE**

Amount of 10ml solution is prepared containing increasing concentration of drug allopurinol by taking [( 5-35) µg ml$^{-1}$ allopurinol , 1ml AgII , 0.8ml buffer pH=8 , and 1ml 10%(v/v)Triton X-114] 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 4000 rpm for 10min ,1ml ethanol is added to the surfactant-rich phase to dissolve it then is measured by UV-Vis at $\lambda_{max}$ 906nm for complex . Plotting the absorbance values of the cloud point versus the concentration of silver is shown in Figure (14)

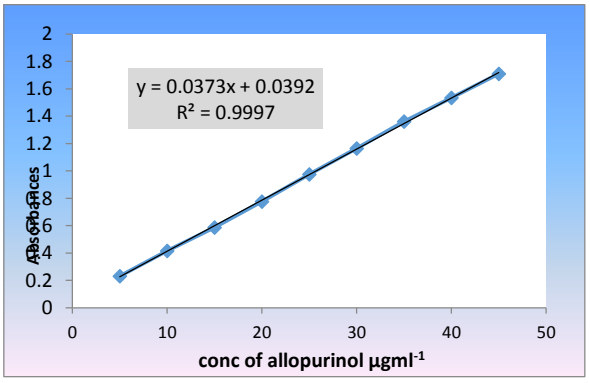


Figure 14: (allopurinol +AgII ) Calibration Curve

Linear calibration graph is established by plotting absorbance versus concentration for Complex allopurinol with Sliver in Figure (14) the extent (5-35) µg ml$^{-1}$ obeys the Beer law , the of complex equals Sandall's sensitivity ($4.456\times10^{-7}$ µg.cm$^{-2}$).

The Detection & Quantitation Limit has been calculated for complex by CPE taking ten replication of blank solution against distilled water with drop of ethanol .LOD = 0.245951 µg ml$^{-1}$ and LOQ = 0.819839 µg ml$^{-1}$ for allopurinol - AgII complex . The results of statistical data analyses of the CPE

determination of Complex by the proposed method are tabulated in table (7).

Table (7) Analytical Data Analysis of the CPE of complex

| Parameter | Complex ( allpurinol-AgII) |
|---|---|
| Color of product | Brown |
| Wave length $\lambda_{max}$ (nm) | 906 |
| Concentration rang (μg $ml^{-1}$) | ( 5-35 μg $ml^{-1}$) |
| Regression equation | y = 0.0373x + 0.0392 |
| Correlation coefficient(R) | 0.9998 |
| Correlation coefficient ($R^2$) | 0.9997 |
| Variation coefficient (%) | 99.97 |
| Limit of Detection (μg $ml^{-1}$) | 0.245951 |
| Limit of Quantitation(μg $ml^{-1}$) | 0.819839 |
| Sandell's sensitivity (μg $cm^{-2}$) | $4.456\times10^{-7}$ |
| Slope (m) | 0.0373 |
| Intercept (C) | 0.0392 |
| Molar absorptivity($L.mol^{-1}.cm^{-1}$) | $3.055\times10^{-4}$ |

### 3.3 .Applications of the Cloud Point Extraction on Pharmaceuticals

CPE has been applied on pharmaceutical allopurinol, the manufacture company [GlaxoSmithKline pharmaceuticals S.A. ,Poznan, Poland] that contains (300mg) from allopurinol . The results are summarized in the table (8) for allopurinol

Table (8) Data for Determination allopurinol –AgII Complex in the Pharmaceutical Preparation table 300mg (allopurinol ) by CPE.

| Amount of ALLO / μg $ml^{-1}$ | *Found | Recovery% | Average Recovery% | Erel% | Average Erel% |
|---|---|---|---|---|---|
| *5* | *5.14* | *102.8* | *102.5* | *2.8* | *2.26* |
| *10* | *10.0* | *100.7* | | *0* | |
| *15* | *15.62* | *104.1* | | *4* | |

Table (9) Data for Determination allopurinol- AgII Complex in table 100mg (allopurinol ) by CPE

| Amount of ALLO / μg $ml^{-1}$ | *Found | Recovery % | Average Recovery% | Erel% | Average Erel% |
|---|---|---|---|---|---|
| *5* | *4.39* | *87.82* | *94.14* | *-12.2* | *-5.87* |
| *10* | *9.51* | *95.1* | | *-4.9* | |
| *15* | *14.92* | *99.5* | | *-0.53* | |

The proposed method is also applied on table allopurinol 100mg the manufacture company is [Novartis].AgII from drug contains (100mg) allopurinol we get good and high reliability results that are summarized in the table (7) for allopurinol by CPE

### 3.4. Calculation of the stability constant (K) of complex

The conditional or apparent stability constant of the 1:1 ( Drug and metal ) product was evaluated and described as shown Complete founding the stability constant [K] colored product Formed imputation of ( metal :drug ) as followed: A series of solution were prepared containing three different concentration of metal and allopurinol (1:1)and the concentration ($3\times10^{-5}$) $molL^{-1}$ for(Sliver with allopurinol) when Formed imputation under this Condition easily to Hydrolysis and the Intensity Absorption was very low . Another series of solution was prepared containing three deferent concentration of metal and allopurinol but with abundance of the metal (the best concentration) The complex was prepared with no decomposition .express of the intensity absorption $A_m$ and application the relationship the value degree of decomposition can be calculated as follows ( α ):

$$\alpha = \frac{A_m - A_s}{A_m}$$

Stability constant [K] as follows ; and calculated the

$$S + R \rightarrow SR$$

$$\alpha c \quad \alpha c \quad (1-\alpha)c$$

$$K = \frac{[SR]}{[S][R]}$$

$$K = \frac{(1-\alpha)c}{(\alpha c)(\alpha c)} = \frac{1-\alpha}{\alpha^2 c}$$

Where: K; stability constant

C; the concentration of the product complex .and it equivalence the concentration of allopurinol . are shown in Table (10 )

Table 10: Stability Constant of the complex( AgII-allopurinol ) formed

| Vol of allopurinol | Absorbance at $\lambda_{max}$ 906nm | | | |
|---|---|---|---|---|
| | $A_s$ | $A_m$ | α | K ($l.mol^{-2}$.) |
| 0.3 | 0.490 | 0.495 | 0.010 | $1.4\times10^{-6}$ |
| 0.5 | 0.501 | 0.563 | 0.110 | 73.7365 |
| 0.7 | 0.621 | 0.732 | 0.151 | 37.2842 |

### 4. Conclusion

The proposed method is simple, sensitive and free from drastic experimental conditions such as heating. It is also accurate, precise enough to be successfully adopted as an alternative to the existing

spectrophotometric methods and evaluation of Allopurinol in an metal Using CPE and in pharmaceutical Preparation samples determination AgII in some Pharmaceuticals ,the method gives a very low limit of detection and green chemistry

## 5. Conflicts of interest

"There are no conflicts to declare"

## 6. Acknowledgments

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## 7. REFERENCES

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