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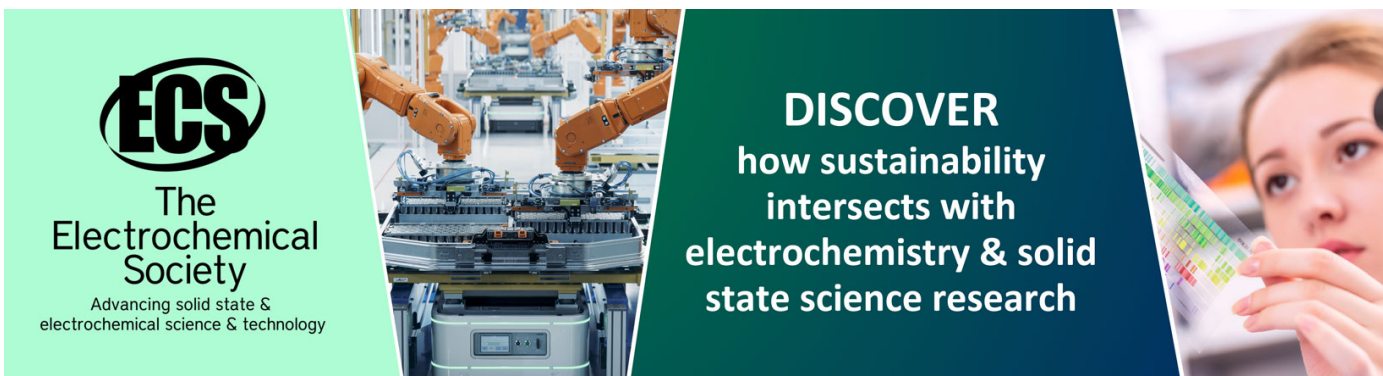
## Spectral estimation and biological activity study of Amoxicillin by using modified nanoparticles and application to some of their pharmaceutical preparations

To cite this article: Mohammed Hasan Barrak *et al* 2020 *J. Phys.: Conf. Ser.* **1660** 012036

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## Spectral estimation and biological activity study of Amoxicillin by using modified nanoparticles and application to some of their pharmaceutical preparations

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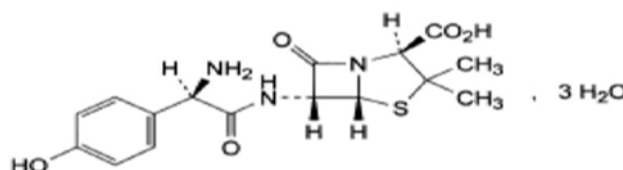
**Abstract.** This study included a simple, fast and sensitive method for estimating Amoxicillin (AMOX) in its pure state and in some of its pharmaceutical preparations, this method depends on the modified nanoparticles through oxidation and reduction reactions, With polyvinylpyrrolidone as a stabilizer in a base medium of sodium hydroxide, the (AMOX) drug act as effective reducing agents to dilute the ore mineral salt from silver nitrate ( $\text{Ag}^+$ ) to silver nanoparticles, the highest absorption of the oxidation-reduction reaction product of amoxicillin appears at 420 nm. the calibration curve was measured and the following information was calculated and showed compliance with the Beer-Lambert Law within the focus range 0.75-57.5 parts per million. Molar absorptivity was  $9.3526 \times 10^{-3} \text{ L / mol.cm}$ , and Sandal's sensitivity was  $0.0448 \mu\text{g/cm}^2$ , the standard deviation rate was 0.016%, and the correlation coefficient is 0.9994. The biological effect on some Gram-positive and Gram-negative bacteria was studied and the results were positive in the efficacy of the samples prepared on these bacteria.

**Keywords.** Amoxicillin, Spectral estimation, biological activity, modified, nanoparticles

### 1. Introduction

(AMOX) which belongs to the amino penicillin subcategory in the classification of penicillin [1] (AMOX), a semi-synthetic ampicillin derivative, is a commonly used, wide-spectrum antibiotic, It mainly works against Gram-positive bacteria, but it also works against some Gram-negative bacteria [2]. The antibiotic is commonly used to treat infectious diseases in humans and animals and to improve growth and development in agriculture, (AMOX) destroys bacteria by interfering with bacterial cell wall synthesis. As a result, the bacterial cell wall is weakened, the cell swells and then ruptures, staphylococcal penicillinase (AMOX) is readily hydrolyzed [3]. The name of the index for Chemical Abstracts is 4-thia-1-azabicyclo [3.2.0] heptane-2-Carboxylic Acid, 6-[[Amino(4-Hydroxyphenyl)acetyl] Amino]-3,3-Dimethyl-7-oxo-, [2S-[2c(5a,6P(S\*))]] [4] Figure 1.





**Figure 1.** Chemical structure of Amoxicillin tri hydrate

To describe the mechanism of amoxicillin's synergistic antibacterial activity with the Nano silvers, Nano silvers and (AMOX) will destroy bacteria with another pathway, if one of them is immune to bacteria, another antibiotic agent can bacteria kill very differently, when the bacteria have no resistance to antimicrobials, a bonding reaction of (AMOX) with nanosilver may trigger the synergistic effect, (AMOX) molecules have several active groups, these groups easily react with Nanosilver.

In the end, the group of antimicrobials that consist of a Nano silver center and the surrounding (AMOX) molecules come into being, whenever groups of antimicrobials act on the bacterial cell surface at one point, they cause the most destruction. So the forming process antimicrobials, in fact, it is that of increasing the concentration of antimicrobial agents[5].(AMOX) was previously estimated by (Chromatographic LC-MS/MS)[6], (Chromatographic LC with fluorescence detection)[7], (Chromatographic UPLC-MS/MS)[8], (Chromatographic Micellar HPLC-UV)[9], (Chromatographic HPLC) [10], (Chromatographic RP-HPLC) [11], (Electrochemical Volumetric)[12], (Near infrared spectroscopy)[13], (U.V-VIS Spectrophotometric) [14], (U.V-VIS Spectrophotometric) [15].

## 2. Experimental Part

### 2.1. Materials and reagents

Either of the substances included in this analysis were affordable with the highest purity and utilized without further disinfection deionized water double was utilized throughout, the  $\text{AgNO}_3$  solution with a concentration of 0.01 M was newly formulated by breaking down 0.4246 g in deionized water in a volumetric vial of 250 ml together with standardized, sodium hydroxide 0.001M that was preparing by dissolving 0.0199 g in 500 ml deionized water as well as polyvinylpyrrolidone solution (0.2 %) by breaking down 0.2 g in deionized water in a volumetric vial of 100 ml. and completed all the volumetric vial volumes to the mark .

And they were delivered together from the company Reagent grade BDH.A 250 ppm and employed standard solution Amoxicillin as prepared by breaking down 25 mg bulk drug in 100 ml of water from SDI (State Drug Industries and Medical Appliances Company) (Iraq) five various forms of drug formulations have been used to apply the studied strategy to Amoxicillin these types are illustrated in the table. (1):-

**Table 1.** The studied pharmaceutical preparations

Drug Formulations samples	Declared composition	Company
Rivamox(Amoxicillin) Powder for oral suspension	Per tablet 250 mg/5ml	RIVA PHARMAS.A.E Free Zone Nasr City, Cairo, Egypt
AMITRON Amoxicilline/Amoxicillin Vial flacon	Per tablet 500 mg Amoxicillin	LDP Spain
Glomox Amoxicillin Capsules (10)	Per tablet 500 mg Amoxicillin	Globalpharma A SANOFI UAE
Co-Amoxicillin Tablets (10)	Per tablets 500 mg/125mg	Bluefish Sweden

	Film-coated tablet	
	Amoxicillin and clavulanic acid	
Amoxicillin capsules (10)	Per tablets	Samarra-Iraq
	500 mg Amoxicillin	

## 2.2. Collection and diagnosis of bacterial isolates

The following pathogenic bacterial isolates with multidrug resistance (MDR): tow gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) while two gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) were isolated from various clinical samples like burns, stool, synovial fluids, wound, blood and urine [16, 17, 18, 19, 20]

Additionally later recently confirmed utilizing automatic bacterial recognition instrument Vitek-2 compact device GP and GN card, all bacterial isolates were stored on BHI broth supplemented with (15%) glycerol at (-20 °C). The isolates were sub-cultivated on BHIA and incubated to 37 ° C for 24 hours before utilize, In the laboratories of the Biology Department of the College of Science at the University of Kufa.

## 2.3. The following chemical samples were prepared for application

Test tube 1: Silver nitrate (1.5 mL), polyvinylpyrrolidone (0.1 mL), amoxicillin (1 mL), then sodium hydroxide (2 mL), then dilution by distilled water to the mark (10 mL). Test tube 2: Silver nitrate (1.5 mL), then dilution by distilled water to the mark (10 mL). Test tube 3: Silver nitrate (1.5 mL), polyvinylpyrrolidone (0.1 mL), then sodium hydroxide (2 mL), then dilution by distilled water to the mark (10 mL). Test tube 4: amoxicillin (1 mL), then dilution by distilled water to the mark (10 mL). Synthesis of compound (RU1) Melamine (2 g, 0,016 mmol) was added to 10 mL of acetic acid in a 150 mL beaker, then add 1,5 g, 0,016 mole of  $\text{ClCH}_2\text{COOH}$  and 17 mL of saturated aqueous sodium acetate is stirred, then stirred, then added with continuous stirring, 40 ml of cold water is added to the sample. The product was then filtered, and then dried.

## 2.4. Antibacterial activity experimental

The preparation of bacterial suspensions was as Ramalivhana, et al. [21] explained. Agar well diffusion method was utilized to evaluate test tubes antibacterial activity versus bacterial isolates [18,22] MHA medium was utilized to evaluate the biological activity of the test tubes versus bacterial isolates.

## 2.5. Agar well diffusion assay

Suspensions of bacterial isolates were prepared to match 0.5 McFarland standard, Distribution of 100 µl of bacterial suspensions BHIB on the surfaces of the MHA plate using the micropipette, wells were punctured in all the culture plates using a sterile cork borer. One of the wells was a perforation in the middle of the plate adding 100 µl of Gentamicin as a positive control; 100µl of (DMSO) was adding as a negative control in the other well; 100µl of test tubes were alone adding in the residual wells five wells to the test tubes. The cultivation plates were then incubated at 37 ° C for 24 h. The clear zone of inhibition around wells has been determined in mm. The tests were done in triplicate [17, 23].

## Apparatus

- T80 UV-Visible Spectrophotometer, PG Instruments Ltd. (Double beam).
- 303 PD UV-Visible Spectrophotometer, Apel, Japan (Single beam).
- UV-1650PC UV-Visible Spectrophotometer, SHIMADZU, Japan (Double beam)
- Electric Balance, Matter Toledo, Switzerland
- Shaking water bath, Model : vs-1205 wl, scientific CO, LTD

## 2.6. Procedure for calibration curve

A volume of (1.5 mL) of silver nitrate was added in volumetric flasks of capacity (10 mL) and then a volume of (0.1 mL) of PVP was added to these volumetric flasks, then different volumes of (AMOX)

were added ranging from (0.01 mL to 3 mL) In these volumetric flasks, then a volume of (2 mL) of sodium hydroxide was added to these flasks, then complete the volume with distilled water to the mark, the colored component is spectrophotometric-ally tracked towards a reagent blank at 420 nm after 30 minutes, the blank reagent is designed in the same way, but without (AMOX) drug.

#### *2.7. Tablet samples solution*

10 from (AMOX) tablets were weighed, then calculated the average of one tablet, the tablets were ground and powdered, and a fine 0.025 g fraction was dissolved in distilled water, filtered to separate the dissolved components and impurities, transferred to a volumetric flask of 100 mL and diluted to the mark then, was done different sizes were taken from them, In these samples, the suggested procedure for evaluating (AMOX) was adopted and implemented. Based on the construction calibration curve, the process was successfully used for the reliable determination of (AMOX) components in these samples.

#### *2.8. Syrup sample solution*

The syrup containing (40 mg/5mL) of (AMOX) was taken (0.5 mL), Transferred in (100 mL) volumetric flask and diluted by a distilled water to the mark, then Taking different volumes and previously treating them in (AMOX) measurements, depending on the design calibration curve, the tested method was effectively utilized for the precise calculation of (AMOX) components in this sample.

#### *2.9. Injection Samples*

The vial contains (500 mg) a weight of (0.0272 g) of (AMOX) dissolved in a beaker and transferred to 100 ml volumetric flask and diluted with distilled water to the mark. Various volumes were taken and treated in (AMOX) measurements previously.

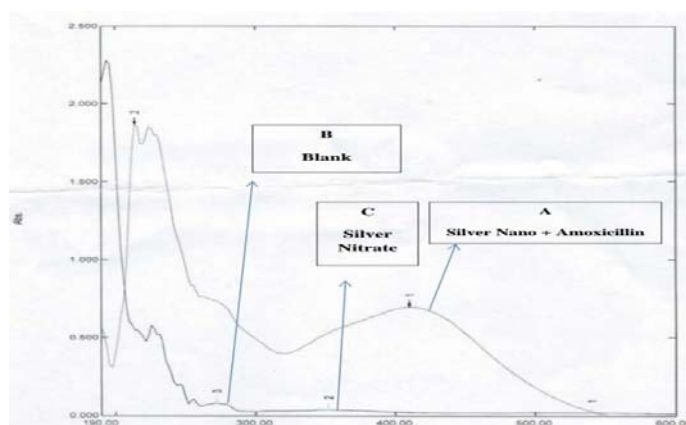
#### *2.10. Capsules Sample as Samarra-Iraq*

Ten capsules of (AMOX) (500 mg) were separately weighed in powder form, the average weight of one capsule was (0.6119 g), (0.03 g) is extracted and Disbanded in a volumetric flasks beaker and condensed to a 100 milliliters volumetric flask, then taking different volumes and treating them in the previous way in (AMOX) measurements.

### **3. Results and Discussion**

#### *3.1. Absorption spectra*

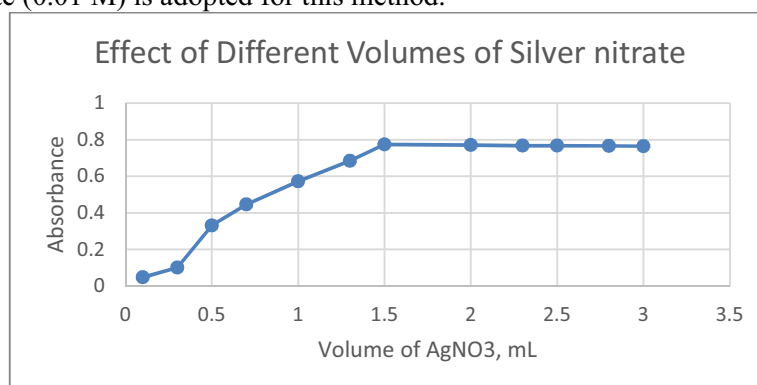
When the Colorless (AMOX) solution (C), Blank (B) (AgNO<sub>3</sub>, PVP and NaOH) colorless solution and sample (A) of (AgNO<sub>3</sub>, PVP, (AMOX) and NaOH), to emphasize the reaction, the red colored product as well as to the reactants are scanned in UV-VIS spectrophotometer the region is within range of 190-800 nm, figure (2) A, B and C shows the spectra of the aqueous solution of pure (AMOX), blank solution and coloured product, from this figure, it is obvious that the color red product, which has maximum absorption at 420 nm is significantly different from the maximum absorption of both reactants, the usefulness of this redshift for a product may be used as an appropriate (AMOX) assay procedure.



**Figure 2.** Absorption Spectrum of the Sample (Silver Nano with (AMOX) antibiotic (A), Blank (All reagents without antibiotic (AMOX) (B) and Amoxicillin Pure (C).

### 3.2. Optimization of reaction conditions Effect of Different Silver Nitrate Volumes

The effect of differing silver nitrate volumes required to obtain maximum absorbance is investigated. The experiment is performed in the range 0.1 mL to 3 mL of  $\text{AgNO}_3$  (0.01 M), figure (3), the maximum absorbance is reached to when 1.5 mL was added, therefore, 1.5 mL of silver nitrate (0.01 M) is adopted for this method.



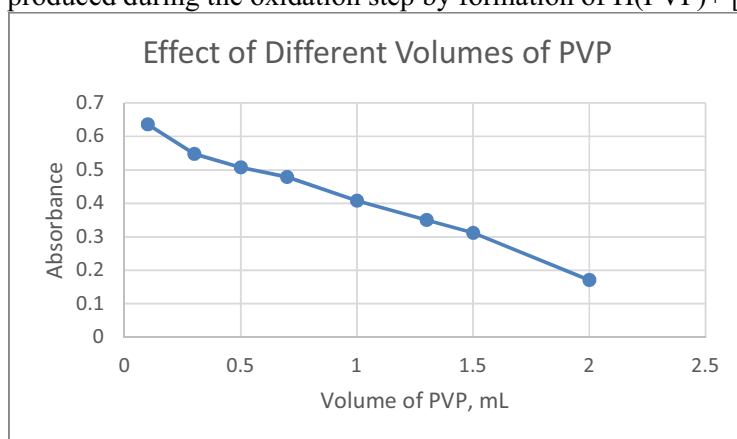
**Figure 3.** Effect of Silver nitrate

### 3.3. Effect of polyvinylpyrrolidone in different volumes

Different polymers (Polyvinylpyrrolidone PVP, Polyurethane PU and Polyvinyl chloride PVC) were used to investigate their effects on color product formation. 1 mL of (0.2 %) concentration was added to determine the effect of the polymers used on the formation of (AMOX), PVP was the best polymer that given highly absorbance for the color solution. After that the best volume of the base type was identified. Different volumes of PVP: 0.1, 0.3, 0.5, 0.7, 1 ... and 2 mL respectively were used to determine their effects on the formation of (AMOX).

Figure (4), the maximum absorbance is reached to when 0.1 mL was added. Therefore, 0.1 mL of Polyvinylpyrrolidone (0.2 %) is adopted for this method, we selected PVP as a stabilizer for preventing of silver nanoparticles agglomeration, and the  $\text{H}^+$  ions are produced by analytes in the silver nitrate reaction. Removal of  $\text{H}^+$  can thus stimulate Ag-NP formation. When adding

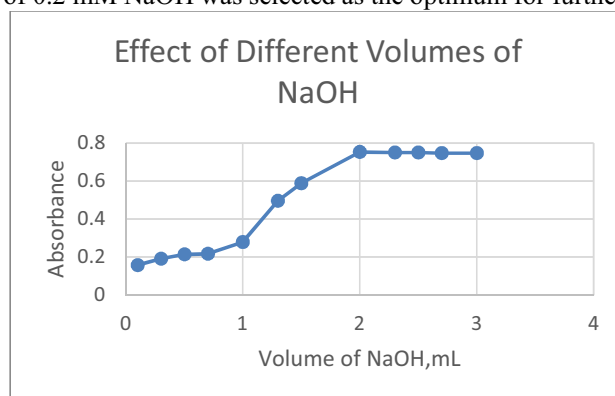
to the solution PVP, it stabilizes the silver ions by forming the complexes of  $\text{Ag(PVP)}^+$  and the remove the  $\text{H}^+$  produced during the oxidation step by formation of  $\text{H(PVP)}^+$  [24].



**Figure 4.** Effect of Polyvinylpyrrolidone

#### 3.4. Effect of Different Volumes Sodium hydroxide

Different bases ( $\text{NaOH}$ ,  $\text{KOH}$ ,  $\text{NH}_4\text{OH}$  and  $\text{Na}_2\text{CO}_3$ ) were used to investigate their effects on color product formation. 1 mL of (0.001 M) concentration was added to determine the effect of the bases used on the formation of (AMOX),  $\text{NaOH}$  was the best base that given highly absorbance for the color solution, after the best volume of the base type was identified. Different volumes of  $\text{NaOH}$ : 0.1, 0.3, 0.5, 0.7, 1, ... and 3 mL respectively were used to determine their effects on the formation of (AMOX), figure (5), the maximum absorbance is reached to when 2 mL was added, therefore, 2 mL of Sodium hydroxide (0.001 M) is adopted for this method, The removal of  $\text{H}^+$  can promote the formation of the Ag-NPs, so the effect of the solution's alkalinity on the reaction was studied by varying the  $\text{NaOH}$  concentration. As it is shown, the signal of silver nanoparticles peak intensity increases by an increase in  $\text{NaOH}$  concentration and then it decreases. This decrease may be due to the formation of the  $\text{Ag}_2\text{O}$ . Thus, a concentration of 0.2 mM  $\text{NaOH}$  was selected as the optimum for further studies [24].



**Figure 5.** Effect of Different Volumes Sodium hydroxide

#### 3.5. Sequence of Addition

The addition sequence of solutions used in the reactions of formation of the silver nanoparticles under investigation greatly influences the intensity of the color of the resulting compounds, therefore several experiments were conducted with a sequence of different additions and for all the studied interactions to choose the best addition sequence that gives the highest absorption of the resulting compounds as shown in the Table (2)

It is found from the table (2) that the order of addition of reagents is by mixing Silver nitrate, then PVP, then (AMOX), then Sodium hydroxide (M+C+D+B) then Diluted by distilled water to 10 mL giving the highest absorbance

Table (2): Effect of Addition sequence on product absorption with (AMOX) M= AgNO<sub>3</sub>; C=PVP; D=(AMOX); B=NaOH.

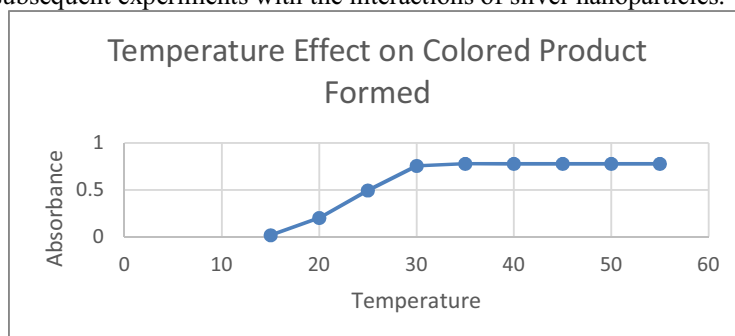
**Table 2.** The Sequence of Addition effect

Sequence	M+C+D+B	D+C+B+M	M+D+C+M	B+C+D+M	B+M+D+C
Absorbance	0.786	0.628	0.616	0.667	0.641

Through the table, it was found that the best addition sequence is (M+C+D+B) for all silver nanoparticle reactions, so it was used in subsequent experiments.

### 3.6. Temperature Effect on Coloured Product Formed

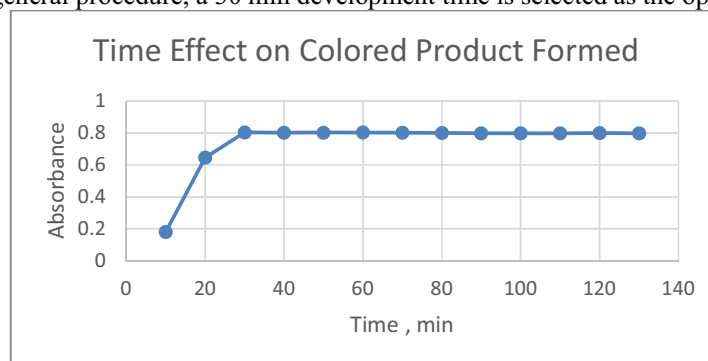
The effect of temperature on the speed at which silver nanoparticles form was studied, as the temperature range used, was from (5-15) Celsius and Figure (6) illustrates this. As absorbance has been found to increase with rising temperature and up to a degree (35) Celsius after, Stability in absorption occurs, and this can be attributed to the possibility of stability in the formation of silver nanoparticles, so temperature (35 °C) was considered the preferred for reactions of formation Silver nanoparticles. These temperatures were chosen in subsequent experiments with the interactions of silver nanoparticles.



**Figure 6.** Effect of temperature on colored product

### 3.7. Time Effect on Coloured Product Formed

Time effect on the formation of silver nanoparticles has been studied, and under the best conditions that have been proven in previous experiments and for periods of time ranging between (10 - 130) minutes and by measuring every ten minutes, through this study, the resulting nanoparticle has a high stability of more than one hours or more, so these interactions can be studied easily and Figure (7) shows that, therefore, in the general procedure, a 30 min development time is selected as the optimum.



**Figure 7.** Time Effect on Colored Product Formed

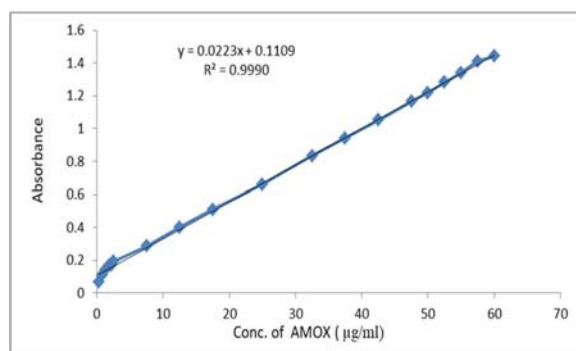


### 3.8. The effect of Time after 72 hours on the coloured product formed

The effect of time on the speed of nanoparticles formation was studied after 72 hours, the speed of nanoparticles formation remained constant at absorption of 0.804.

### 3.9. Calibration Curve

Under the optimum condition studied above, the standard calibration curve has been constructed for the coloured product. However, other analytical parameters are calculated and Figure (8), the results shown in table (3) make this analytical method of good performance for (AMOX) determination at low concentrations.



**Figure 8.** Calibration curve of Amoxicillin

**Table 3.** Analytical Parameter for Determining Amoxicillin

Parameter	Value
beer's law limit (ppm)	0.75 - 57.5
Molar Absorptivity (L / mol.cm)	$9.3526 \times 10^3$
Sandell's sensitivity ( $\mu\text{g}/\text{cm}^2$ )	0.0448
Limit of Detection (LOD) $\mu\text{g}/\text{mL}$	0.1345
Limit of Quantitation (LOQ) $\mu\text{g}/\text{mL}$	0.4484
Correlation Coefficient	0.9994
Determination Coefficient	0.9996
Slope (b)	0.0223
Intercept (a)	0.1109

### 3.10. Precision and accuracy

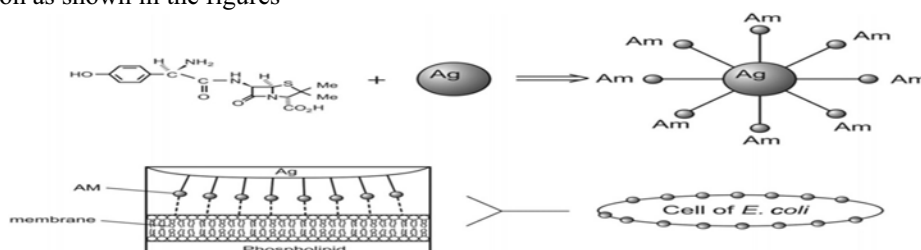
To check the precision and accuracy of proposed method, Precision first measured using nine replicates at 2.5, 25 and 37.5  $\mu\text{g} / \text{mL}$  Amoxicillin concentrations. Three specific concentrations of Amoxicillin are calculated with respect to accuracy, the results shown in Table (4) indicates that the method for Amoxicillin determination is accurate and satisfactory [25].

**Table 4.** Value Accuracy and Precision for the product compound of Amoxicillin

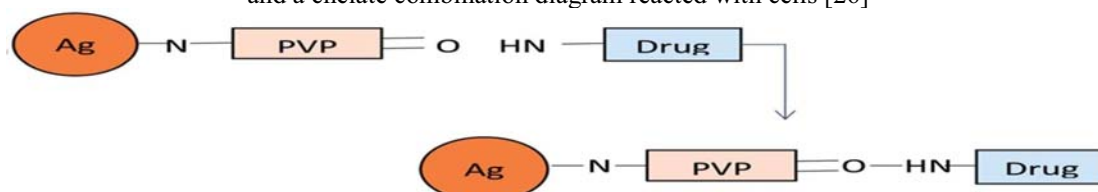
NO	Concentration of Amoxicillin (ppm)		Relative % Error	% Recovery	% R.S.D
	Present	Found			
1	2.5	2.470	- 1.200	98.800	0.602
2	25	24.668	-1.327	98.673	0.151
3	37.5	37.269	-0.616	99.384	0.106

### 3.11. Mechanism of the product

The mechanism of reaction can suggest between the drugs under study and the reagents used in its estimation as shown in the figures



**Figure 9.** Amoxicillin structure and bonding path for chelated silver nanoparticles with amoxicillin, and a chelate combination diagram reacted with cells [26]



**Fig 10.** Schematic representation of the synthesis of drug loaded Ag NPs [27]

### 3.12. Interferences Effect

To ensure selectivity of the method used, with a view to its application in routine analyzes on different samples, especially pharmaceutical preparations containing the pharmaceutical Amoxicillin, He studied the effect of excipients (interferences), as it was done by performing a spectral estimate of the estimated pharmacological compounds and by adding these substances separately to the studied solutions, and these substances are at a concentration of ten times more than the studied drug compound and using the standard method used in the calibration curve. the effects of interference is acceptable if the error ratio is not greater than ( $\pm 2\%$ ) if compared to measurements when there are no overlaps (each value is the rate of three reading)[28], The process was making by the same method in the Calibration Curve 1ml of 250ppm AMOX additionally 1ml For each form of additives, table (5) shows the effect of the presence of additives on the absorption of the colored compound resulting. and through the pursuit of values % Error and % Recovery, note the effect of such additives on the estimation method of (AMOX).

**Table 5.** AMOX 25 ppm measurement for the presence of additives

Interference	Relative Error %	% Recovery
Sucrose	-1.030	98.970
Lactose	-0.540	99.460
Benzoic acid	-0.930	99.070
Glucose	-0.440	99.560
Sodium Strate	-1.140	98.860
Starch	-0.770	99.230
Titanium di oxide	-1.220	98.780
Twin 80	-0.090	99.910
Magnesium Stearte	-0.680	99.320
Acacia	-1.320	98.680
Talc	-1.110	98.890
clavulanic acid	-0.340	99.660

#### 4. Application of the Methods

To determine the success of the methods proposed, A variety of pharmaceutical preparations containing (AMOX) in pharmaceutical solutions needed to be applied according to the methods used, prepare a diluted solution (250 ppm), take three different volumes of each prepared solution, and apply the same steps of the work followed when preparing the calibration curve and then calculate accuracy the analytical method followed by using these prepared solutions and all the studied reactions is consistent the results obtained for a number of pharmaceutical preparations, as shown in table (6), show Effectiveness and Success of the proposed method in applying to pharmaceutical preparations (each value in the table is the average of three readings). And To test efficacy and success of proposed analytical methods, the results should be compared with the results of a well-known and reliable method (found within the British and American pharmaceutical) of substance pure drug and its various forms of pharmaceutical preparations available in the market, the value theoretical for F, T equal 3.44, 2.306 respectively [29, 30, 31, 32], through the results of the measured value, we note that it is smaller than the theoretical value, and this indicates the reliability of the method

**Table 6.** Applications reaction (AMOX) on types from pharmaceutical preparations and F, t compare the accuracy & reliability of the proposed process with the standard nanoparticles composition reaction method Between (AMOX) and silver ion nanoparticles [32].

Preparation (AMOX) Containing	Deliberated process				Official process			
	Conc. of (AMOX) (ppm)		Re %	R.S.D %	Conc. of (AMOX) (ppm)		Re%	R.S.D %
	Present	Found			Present	Found		
Powder for oral suspension, 250 mg/5ml,	2.5	2.515	100.6	0.598	2.5	2.450	98.000	0.941
PHARMAS.A.E	25	24.623	98.492	0.303	25	24.480	97.920	0.822
Free Zone Nasr City, Cairo, Egypt	37.5	37.179	99.144	0.106	37.5	37.440	99.840	0.660
Vial flacon, 500 mg Amoxicillin, LDP Spain	2.5	2.470	98.80	0.602	2.5	2.440	97.600	0.613
	25	25.071	100.284	0.149	25	24.720	98.880	0.523
	37.5	37.448	99.86	0.106	37.5	37.820	100.85 3	0.321
Capsules, 500 mg Amoxicillin, Globalpharma A SANOFI UAE	2.5	2.426	97.04	0.606	2.5	2.470	98.800	0.291
	25	25.026	100.104	0.149	25	25.200	100.80	0.450
	37.5	37.49	99.98	0.105	37.5	37.420	0 99.423	0.610
Tablets, 500 mg/125mg Film-coated tablet Amoxicillin and clavulanic acid, Bluefish Sweden							97.200	
	2.5	2.381	95.24	0.609	2.5	2.430	100.60	0.313
	25	24.982	99.928	0.149	25	25.150	0	0.470
	37.5	37.403	99.742	0.1058	37.5	37.430	99.814	0.280
capsules, 500 mg Amoxicillin, Samarra-Iraq	2.5	2.51	100.40	1.197	2.5	2.490	99.600	0.825
	25	24.93	99.72	0.149	25	24.464	97.856	0.445
	37.5	37.53	100.08	0.1054	37.5	37.920	101.12 0	0.340

Pure	2.5	2.470	98.800	0.602	2.5	2.466	98.640	0.270
(AMOX)	25	24.668	98.673	0.151	25	24.890	99.560	0.770
	37.5	37.269	99.384	0.106	37.5	37.770	100.720	0.680
F- value	0.933							
t-value	0.532							

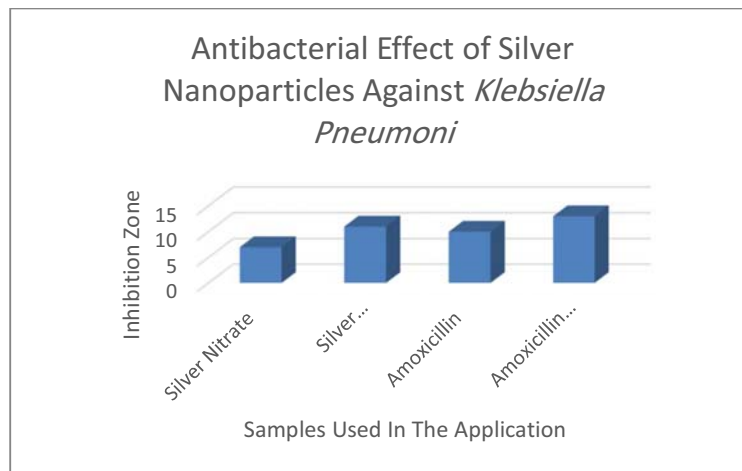
### 5. Biological Activity

Nanoparticles Antibacterial Activity and antibiotics was tested against known human pathogens by an assay for the diffusion of disks. The inhibition zone shown by antibiotics with NPs was comparatively larger than the antibiotics and NPs alone. This demonstrates the likelihood of nanoparticles and antibiotics taking synergistic action [33] as in the following Table (7).

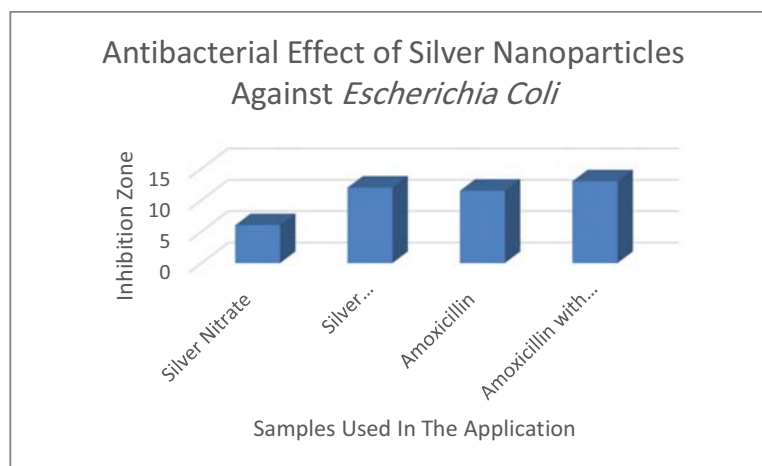
**Table 7.** Effect of Antibacterial of Silver nanoparticles against Gram-negative and Gram-positive pathogenic bacteria.

Types of Bacteria	Antibiotics	Silver Nitrate (inhibition zone)	Silver Nanoparticles (inhibition zone)	Antibiotics (inhibition zone)	Antibiotics with Silver Nanoparticles (inhibition zone)
Klebsiella pneumoniae	Amoxicillin	7 m.m	11 m.m	10 m.m	13 m.m
Escherichia coli	Amoxicillin	6 m.m	12 m.m	11.5 m.m	13 m.m
Staphylococcus aureus	Amoxicillin	7 m.m	12 m.m	11.5 m.m	14 m.m
Enterococcus faecalis	Amoxicillin	5 m.m	9 m.m	8 m.m	10 m.m

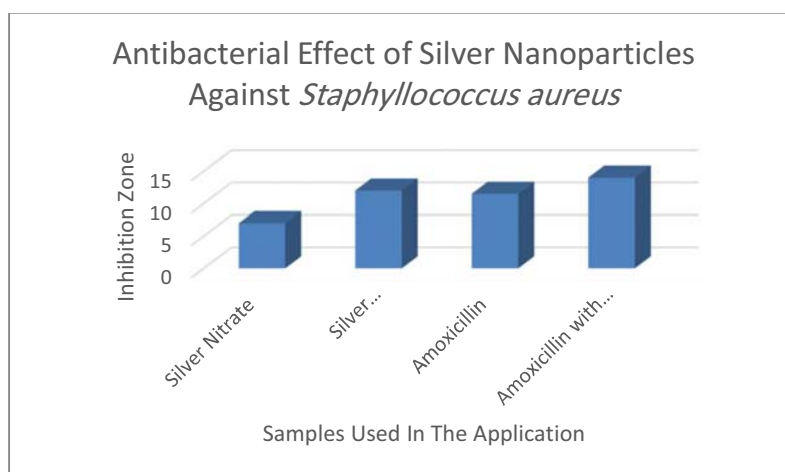
The results indicated that silver nanoparticles had antibacterial effects and synergistic activity, silver nanoparticles were also investigated on their own or in combination with antibiotics [34], the comparative analysis focused on the susceptibility of microorganisms to silver nanoparticles, antibiotics and their combined effects; the diameter of the inhibition zone was increased by a minimum of 2 to 4 mm when nanoparticles and antibiotics were administered together [35, 36] AgNPs is used to inhibit biofilm development, the dose-dependent capacity of AgNPs to inhibit the activity of biofilms produced by human pathogens identified under in vitro conditions. Such findings suggested that the biologically synthesized AgNPs inhibited the function of biofilms for all the bacterial strains tested [37] as in the following figures.



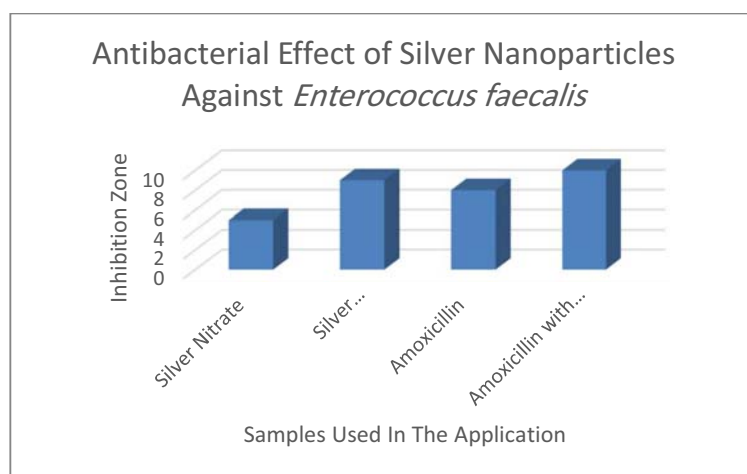
**Figure 11.** Effect of Antibacterial of Silver nanoparticles against *Klebsiella pneumoniae*



**Figure 12.** Effect of Antibacterial of Silver nanoparticles against *Escherichia coli*



**Figure 13.** Effect of Antibacterial of Silver nanoparticles against *Staphylococcus aureus*



**Figure 14.** Effect of Antibacterial of Silver nanoparticles against *Enterococcus faecalis*

## 6. Conclusion

Simple and rapid spectrophotometric method of quantitative-based on the direct assessment of amoxicillin developed both pure form and also in their pharmaceutical preparations, based on modified nanoparticles as colour sensors by the interaction of the oxidation and reduction of amoxicillin with silver nitrate. The suggested spectral method used to estimate Amoxicillin has given high sensitive, low detection, and good linear range. This method has good accuracy and precision, and coloured product are characterized by their high stability in the water medium. The method does not require initial treatments of the model, no the use of solvent extraction.

The method has been effective in estimating Amoxicillin in their pharmaceutical preparations and the findings were well consistent with their original content. No significant difference in the accuracy and reliability of the method and the validity of the analytical application of this method was found in the statistical results t, F test of the proposed spectral method compared to the standard method. The prepared sample was applied to some bacteria, and their effectiveness was clear in reducing the resistance of the bacteria wall.

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