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Research Article

Colorimetric Determination of Amoxicillin in Pure and some Pharmaceutical Formulations Via Reaction with Potassium Permanganate as Oxidant Reagent

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ABSTRACT
We proposed two simple, rapid, and convenient spectrophotometric methods are described for the determination of Amoxicillin in bulk and its pharmaceutical preparations. They are based on the measurement of the flame atomic emission of potassium ion (in first method) and colorimetric determination of the green colored solution for manganite ion at 610 nm formed after reaction of Amoxicillin with potassium permanganate as oxidant agent (in the second method) in basic medium. The working conditions of the methods were investigated and optimized. Beer's law plot showed a good correlation in the concentration range of 5-45 µg/ml. The detection limits and relative standared deviations were (2.573, 2.814 µg/ml) (2.137, 2.498) for the flame emission photometric method and (1.844, 2.016 µg/ml) (1.645,1.932) for colorimetric methods for capsules and suspensions respectively. The methods were successfully applied to the determination of Amoxicillin in capsules and suspensions, and the obtained results were in good agreement with the label claim. No interference was observed from the commonly encountered additives and expectancies.

Keywords: amoxicillin, potassium permanganate, oxidation, colorimetry, flame atomic emission, determination.

INTRODUCTION

Amoxicillin, a semi synthetic antibiotic drug, an analog of ampicillin, with a broad spectrum of bacterial activity against many both Gram-positive and negative microorganisms. Chemically name, it is (2S,5R,6R)-6-[(R)-(-)-2-amino-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid trihydrate. The structure represented in the figure.

The molecular formula of amoxicillin is $C_{16}H_{19}N_3O_5S(3H_2O)$, and the molecular weight is 419.45 is a white or almost white, crystalline powder , molecular weight 365.4 g/mol. It is soluble in water, particularly insoluble in alcohol and ether pKa 5.2, 7.3, UV Maxima 260 nm. Percent Composition: C 52.59%, H 5.24% ،N 11.5%, O 21.89%,S 8.78%[1].

Many analytical methods reported were used for determination of amoxicillin in different matrixes such as derivative spectroscopic method[2-5], diazotization method[7,8], colorimetric methods[9-11], spectrophotometric method[12-15], kinetic method[16], titermetric method[17,18], voltametric method[19,20] flow injection method[21], fluorimetric method[22,23] and chromatographic method[24-30]. The aim of this study is to use the precise and accurate colorimetric and flame atomic emission methods for the determination of amoxicillin content in some drug formulation in Iraq to comparison information of these products with standard method or other official methods.

Expermental parts

Instruments and Equipment:-

Flame emission spectrophotometer (Jenway PFP7 / UK) was used for absorbance measurements.

Double-beam UV-Visible spectrophotometer: Varian Gary 100 UV-Vis spectrophotometer.

Analytical balance: DENVER Instrument Max 220 gm, d=.0001g.

Centrifuge, Hermle Laborti, 6000rpm.

Water Bath with temperature controller: BS-11 Lab companion, Jero Tech, CE.

pH/mV(Jenway 3020pH).

Chemicals

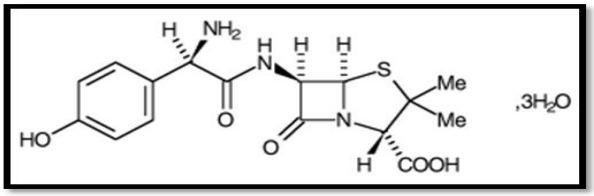

Figure 1: Chemical structure of amoxicillin trihydrate.

Amoxicillin monohydrate standard material, Amoxicillin 250 mg and Amoxicillin 250 mg powder for suspension formulations were supplied from the State Company for

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Drug Industries and Medical Appliances (Samara-IRAQ-SDI). All other chemicals and reagents of analytical grade were obtained from Fisher, Fluka and BDH Companies.

Preparation of Solutions

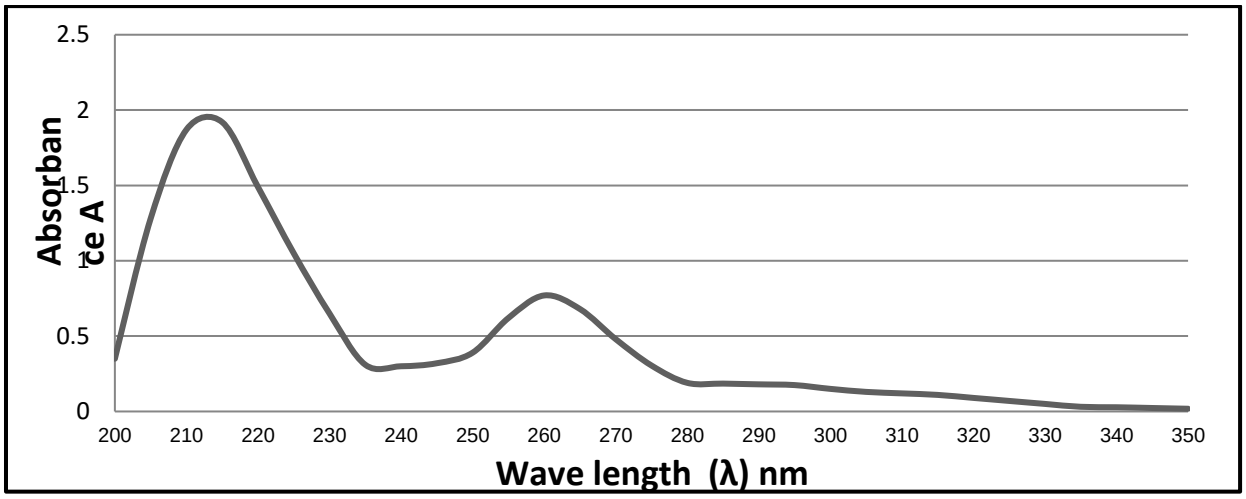

Figure 2: Molecular spectrum of 50 (µg/ml) Amoxicillin solution.

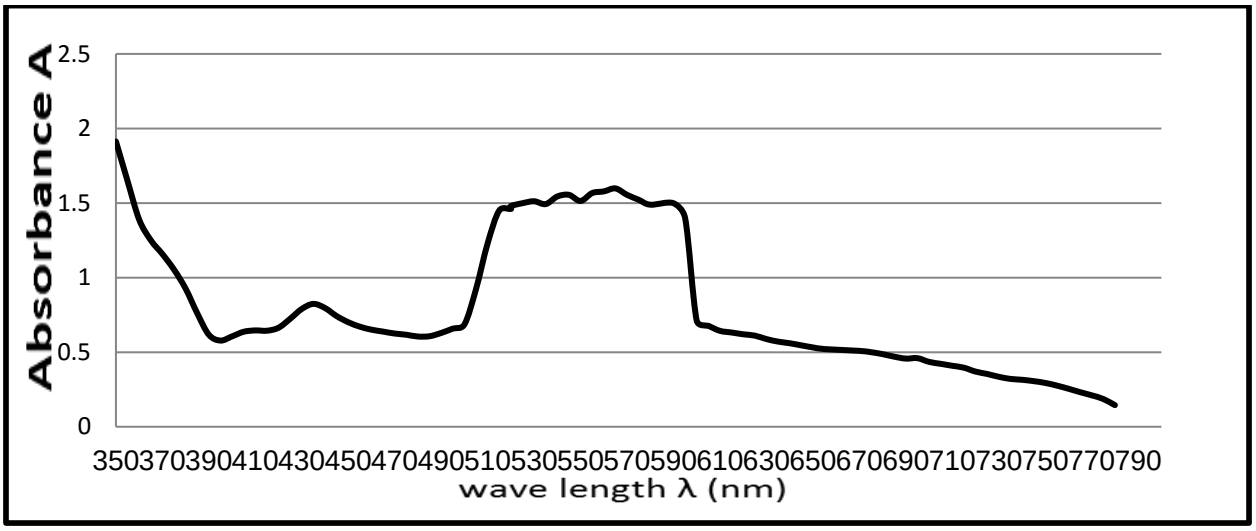

Figure 3: Molecular spectrum of 0.01mol/l $KMnO_4$ in alkaline medium.

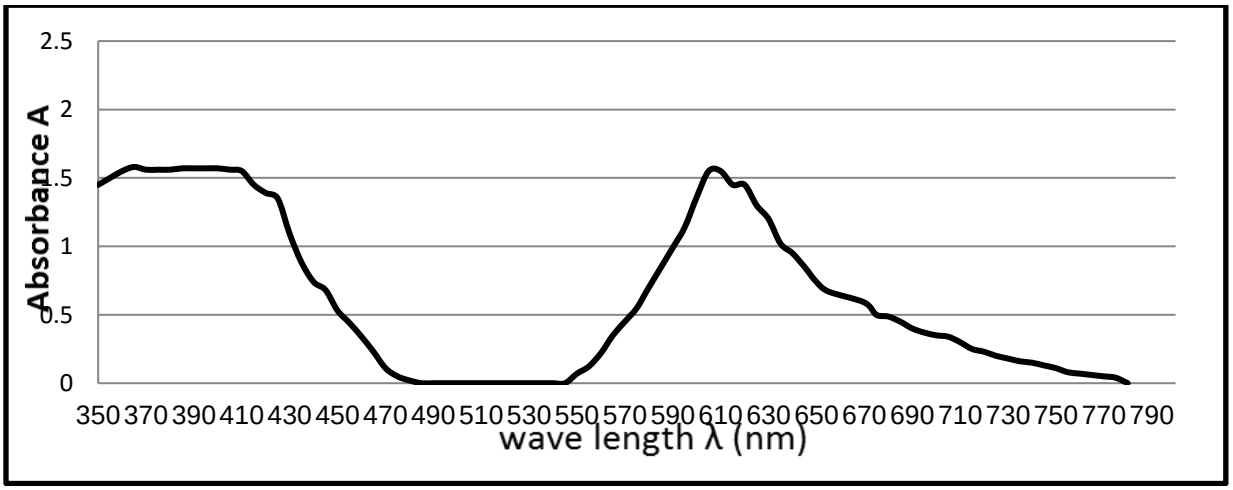

Figure 4: Molecular spectrum of 0.01mol/l $KMnO_4$ with 50 (µg/ml) Amoxicillin in alkaline medium.

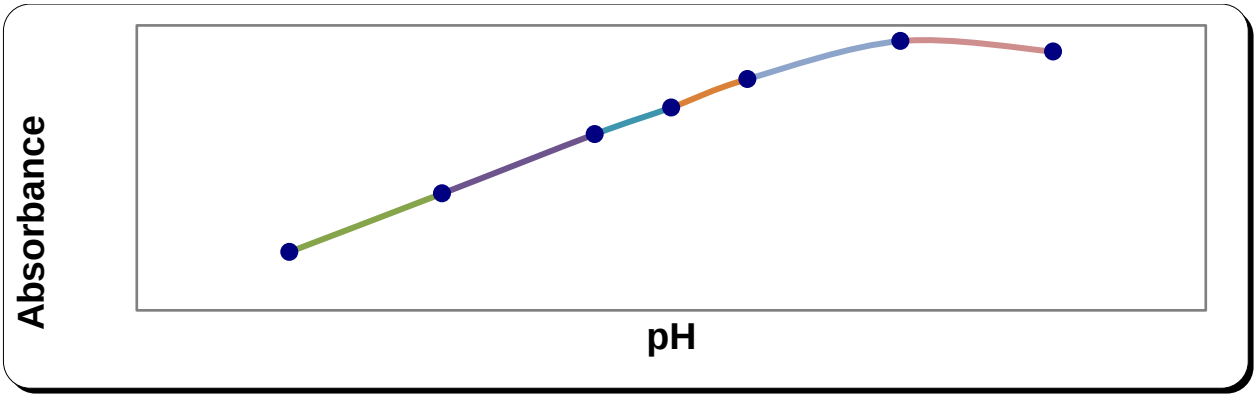

Figure 5: Relation between pH and absorbance of manganite ion.

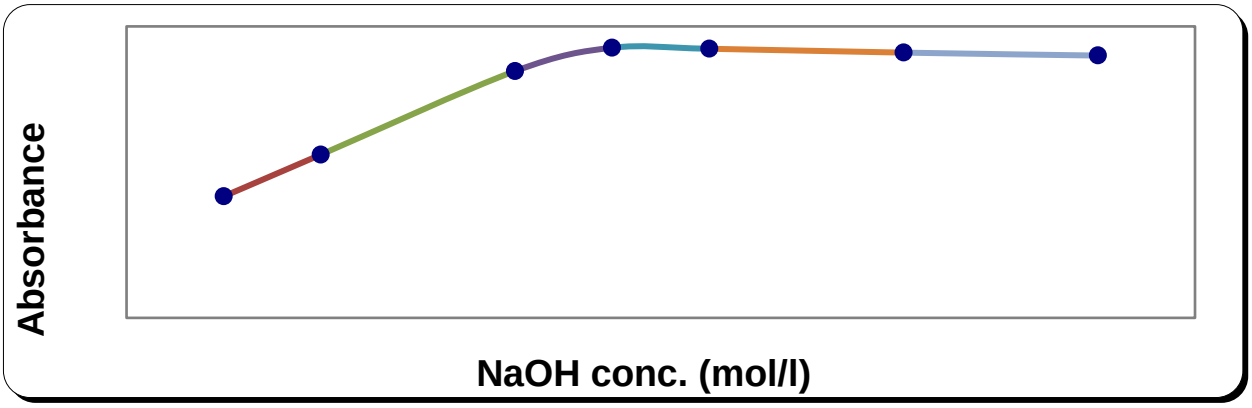

Figure 6: Relation between Sodium hydroxide and absorbance of manganite ion.

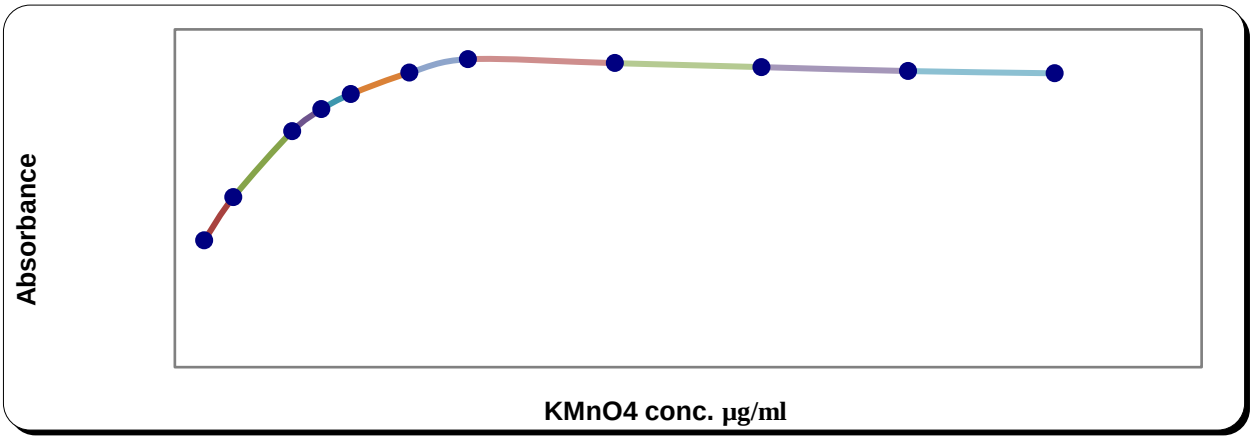

Figure 7: Relation between potassium permanganate and absorbance of manganite ion.

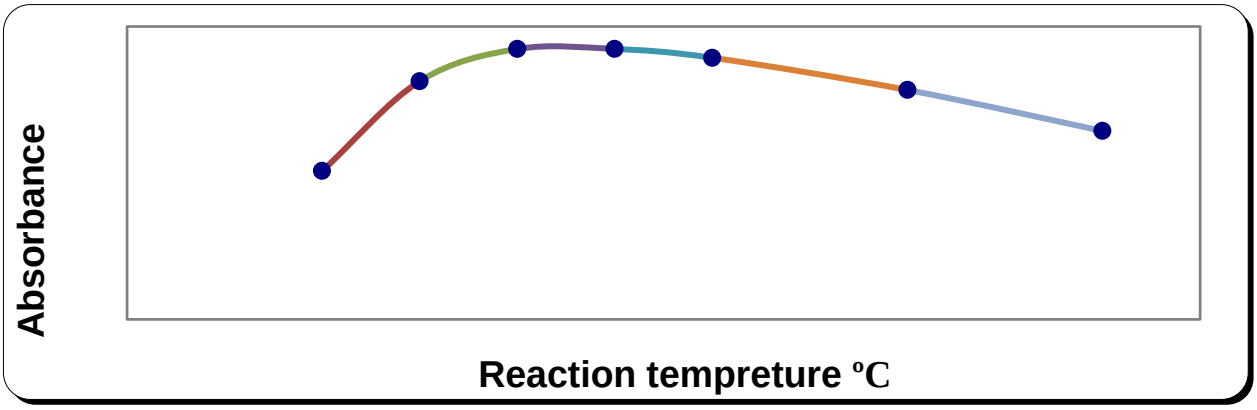

Figure 8: Relation between reaction temperature and absorbance of manganite ion.

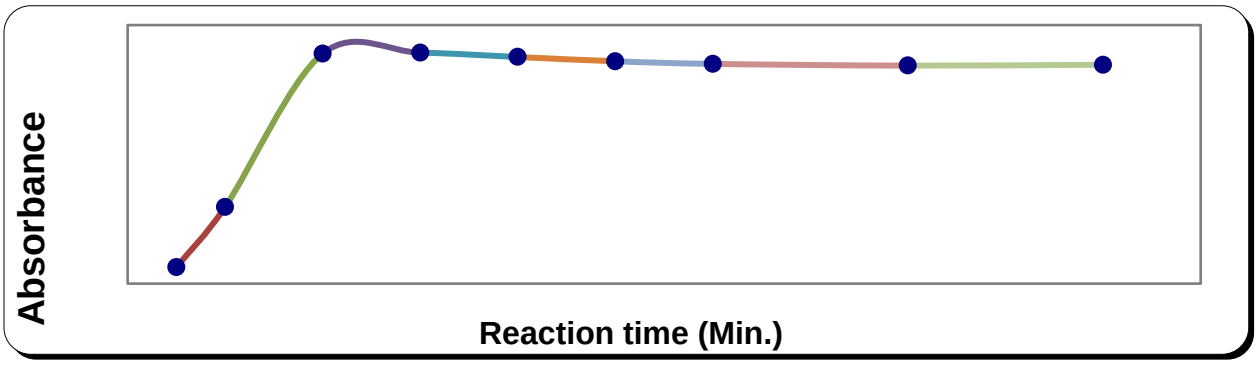

Figure 9: Relation between reaction time and absorbance of manganite ion.

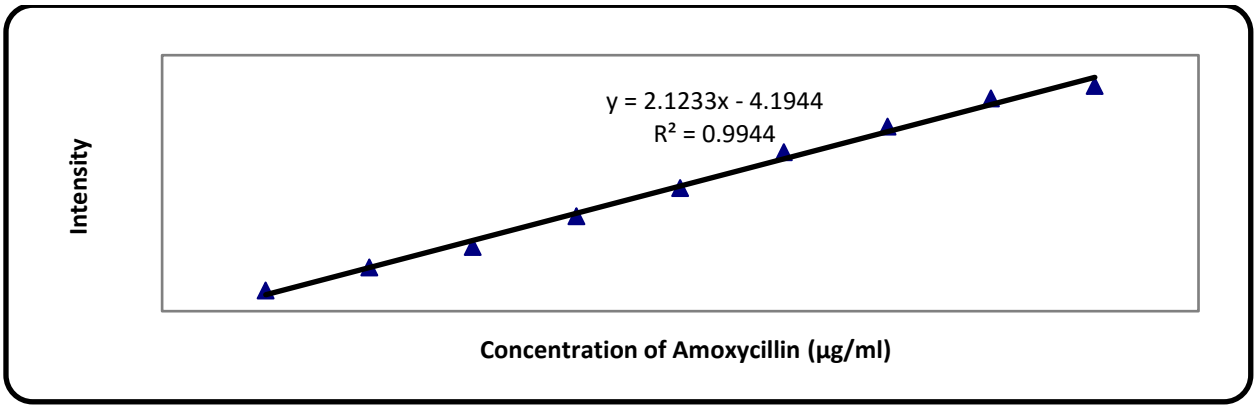

Figure 10: Calibration curve for Amoxicillin with $KMnO_4$ in alkaline medium by Flame Photometry.

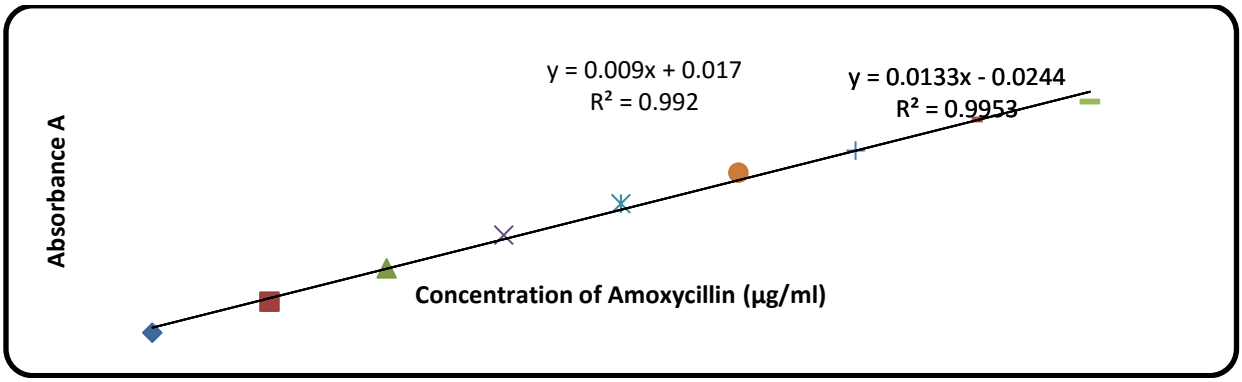

Figure 11: Calibration curve for Amoxicillin with $KMnO_4$ in alkaline medium by colorimetry.

Table 1: The calculated recoveries for the determination of amoxicillin by applying two methods.

	Molecular Absorption (Colorimetry)				
Taken(µg/ml)	Found(µg/ml)	% Recovery		% Error	R.S.D(n=3)
10	9. 871	98.71	Mean	-1.29	1.104
20	19.587	97.935	=97.846	-2.065	2.986
30	29.068	96.893		-3.107	3.472
Flame Atomic Emission (Flame Photometry)					
Taken(µg/ml)	Found(µg/ml)	% Recovery		% Error	R.S.D(n=3)
10	9.817	98.17	Mean	-1.83	1.673
20	19.669	98.345	=97.935	-1.655	3.192
30	29.187	97.29		-2.71	3.889

Stock solution of Amoxicillin 1000 µg/ml was prepared by dissolving 0.1 g of Amoxicillin monohydrate standard material in 100 ml distilled water distilled water. Other standard solutions were prepared by subsequent dilution of stock solution.

Amoxicillin solution 100 µg/ml was prepared by diluting 10 ml of Stock solution to 100 ml DW in volumetric

Table 2: The analytical data for determination of Amoxicillin by colorimetry.

Formula-tion type	Found (µg/ml)	linearity (µg/ml)	Regression equation	correlation coefficient	Recovery %(Rec.%)	Detection Limit DL (µg/ml)	RSD %	Relative Error RE%
Capsules (250mg)	244.400	5-45	Y=0.009 X+0.017	0.992	97.760%	1.844	1.645	2.240
Suspension (250mg)	242.445	5-45	Y=0.009 X+0.017	0.992	96.978%	2.016	1.932	3.022

Table 3: The analytical data for determination of Amoxicillin by Flame Atomic Emission Photometry.

Formula-tion type	Found (µg/ml)	Linearity (µg/ml)	Regression equation	correlation coefficient	Recovery% (Rec.%)	Detection Limit DL (µg/ml)	RSD %	Relative Error RE%
Capsules (250mg)	245.107	5-45	Y=2.123 X-4.194	0.994	98.043%	2.573	2.137	1.957
Suspension (250mg)	244.717	5-45	Y=2.123 X-4.194	0.994	97.887%	2.814	2.498	2.113

flask, diluting this solution to prepare 50 µg/ml for recorded UV-Vis spectrum.

Standard solutions for calibration curve were prepared by diluting Amoxicillin solution 100 µg/ml to (5-40 µg/ml)**.**

Potassium permanganate 0.01 mol/l, dissolve 0.158g of its analytical reagent in 100 ml volumetric flask.

Sodium hydroxide 0.5 mol/l, prepared by dissolving 2.0 g of pure NaOH in 100 ml distilled water.

Hydrochloric acid 0.1mol/l, diluting 1.17ml of 36%, specific gravity 1.19 to 100 ml.

Procedure for Amoxicillin capsules

Empty the contents of 10 capsules (250 mg), and mix well. Transfer a weighed quantity of the powdered capsules equivalent to 10 mg of Amoxicillin into 100 ml volumetric flask and made up to the mark with distilled water. The content of the flask was stirred magnetically for 10 minutes, then transferred 10 ml of this solution into 100 ml volumetric flask, complete to mark with distilled water, pipit 5ml from last solution and proceed as described under "Recommended Procedure".

Procedure for Amoxicillin suspension

Five containers of Amoxicillin suspension (250 mg) after dissolving in 100 ml warm distilled water were mixed. An accurately 2 ml of this solution transferred into a 5 ml test tube, add 3.0 ml of 0.5 mol/l NaOH and then centrifuged at a rate 4000 rpm for five minutes. The residue was washed at least three portions with alkaline solution, then was quantitatively transferred into 100 ml volumetric flask and after the complete dissolution in 0.6 mol/l HCl, diluted to the mark with distilled water, checking in water bath at 60 C^o for 10 min. then transferred 10 ml of this solution into 100 ml volumetric flask, complete to mark with distilled water, pipet 5 ml from last solution and proceed as described under "Recommended Procedure".

Recommended procedures

Transfer aliquot volumes of Amoxicillin standard solution covering the working concentration range from 2.0 to 60.0 µg/ml into 25 ml volumetric flasks; add 3.0 ml of 0.01 mol/l potassium permanganate followed by 3.0 ml of 0.5 mol/l NaOH and shake well, then make up to the mark with water. Allow the reaction mixture to stand for 20 min. In first procedure molecular absorption spectrophotometry, measure the absorbance of the resulting solution at 610 nm against a reagent blank prepared simultaneously. Plot the values of the absorbance against the final concentration in µg/ml to get the calibration curve. Alternatively, derive the corresponding regression equation. The flame emission photometry second procedure involves measure the intensity of potassium emission at 766 nm.

RESULTS AND DISCUSSION

The molecular absorption spectra of Amoxicillin shows two bands small band at 110 nm and broad band at 260 nm fig. 2, $KMnO_4$ in basic medium shows an absorption bands at 510, 530 and 550 nm fig. 3. The addition of aqueous solution of tetracycline to $KMnO_4$ solution in basic medium causes a change in the absorption spectrum of $KMnO_4$, with new characteristic bands at 610 nm fig. 4.

Optimization of Variables

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. These factors include pH, concentration of the reagents ($KMnO_4$) and reaction media (NaOH) , temperature and time of reaction.

Effect of pH

The study of pH effect involves recording absorbance of manganite ion producing after reaction Amoxicillin with potassium permanganate in many reaction media prepared by adding 0.1mol/l HCl and 0.1mol/l NaOH solution was proffered exactly pH close to 10.

Effect of NaOH Concentration

With preparing many solutions with different concentrations, and measuring the absorbance for it after reacting Amoxicillin with potassium permanganate,0.5mol/l NaOH considered optimal value for this study.

Effect of $KMnO_4$ Concentration

After measuring absorbance of many solutions with different concentrations of potassium permanganate, It was found that 0.01 mol/l $KMnO_4$ considered optimal value.

Effect of reaction temperature

The reaction temperature effect involves recording absorbance of manganite ion in many temperatures, we obtained the 20-25 ºC preferred temperatures.

Effect of reaction time

The reaction time effect includes recording absorbance of manganite ion at different time, two minutes was the optimum time for reaction.

Recoveries calculation

Table (1) show the calculated recoveries for the determination of amoxicillin by applied two proposed methods, Molecular Absorption Spectrophotometry and Flame Atomic Emission using three standard solution with different concentrations, the absorbance were recorded three times for any solutions that used.

Constructing calibration curves

Figures (10,11) demonstrated normal calibration graphs for the determination of amoxicillin in two methods by plotting the concentrations and its absorbances.

DISCUSSION

The optimum conditions of concentrations for oxidant reagent $KMnO_4$ and reaction medium sodium hydroxide were studies in this work were (0.01 mol/l, 0.5 mol/l) respectively, after increase concentrations of $KMnO_4$ and NaOH, the absorbance remaining constant in first state but decreased in second state.

When Amoxicillin reacting with $KMnO_4$ in basic medium the three bands (510, 530, 550 nm) were disappeared with appearance of one peak at 610 nm with changing purple color of permanganates to blue for manganite ion[31,32].

$MnO_4^{-1} + OH^- + e \Longleftrightarrow MnO_4^{-2}$ E=+ 0.564 V

Potassium permanganate considered strong oxidant reagent in reaction of Amoxicillin, it ether oxidized sulfur atom to sulfoxide group, and with carbonyl β-lactam ring to carboxyl group, or primary aliphatic amine to form an oxide amine[33]. We applied a new colorimetric method for determination of Amoxicillin in bulk and its pharmaceutical preparations ,we obtained the concentration of Amoxicillin in dosage form was closely for the value recorded (labeled) on the Amoxicillin capsule and suspension (245.107, 244.717 µg/ml) respectively in Flame Atomic Emission Photometry, and in molecular absorption spectrophotometry (244.400, 242.445µg/ml).

CONCLUSION

The proposed study involved simple, fast, precise and accurate methods for the determination of Amoxicillin in pure and dosage form by molecular absorption spectroscopy via oxidation reduction reaction in solutions. They obtained law Percentage errors, detection limits and good linearity.

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