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Optimization of Ampicillin Oxidation Reaction with Hydrogen Peroxide and Potassium Dichromate in Different Media

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Abstract: Three simple and sensitive methods for the determination of Ampicillin are described. The first method is based on investigation of the oxidation reaction of the drug with alkaline potassium dichromate for a fixed time of 70 min., where sodium hydroxide used as an alkaline media. The absorbance of the colored dichromate ions is measured at 369 nm. The second method is based on the oxidation reaction of Ampicillin with acidic potassium dichromate. Spectrophotometric measurement was achieved by recording the absorbance at 340 nm for a fixed time of 70 min., where sulfuric acid used as an acidic media. The third method is based on the oxidation reaction of Ampicillin with alkaline hydrogen peroxide; the absorbance is measured at 302nm for a fixed time of 70 min., all procedures were achieved at room temperature.
All variables that affecting the procedures were investigated and the conditions were optimized. Calibration graphs were constructed; mean and standard deviation were calculated.
Keywords: Optimization of Ampicillin Oxidation Reaction with Hydrogen Peroxide and Potassium Dichromate in Different Media Spectrophotometrically.

Introduction

Ampicillin is an antibiotic in the class of drugs called Penicillin. It is abroad-spectrum semi-synthetic penicillin that is effective in the treatment of gram-positive and gram-negative bacterial infections produced by Streptococcus, Bacillus anthracis, Haemophilus influenzae, Neisseria gonorrhoeae, and Escherichia coli. This antibiotic is used in the treatment of upper respiratory tract infections, genital and urinary tract infections, and otitis media in children [1].

Ampicillin Trihydrate (4-Thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid -6- (2-amino-2-phenyl acetamido) -3 , 3-dimethyl-7-oxo) , $C_{16}H_{19}N_3O_4S.3H_2O$ (Fig 1) , Percent Composition : C 55.00% , H 5.48% , N 12.02% , O 18.32% , S 9.18% , is a white crystalline powder with molecular weight of 403.5, melts in the range (198- 200°C).

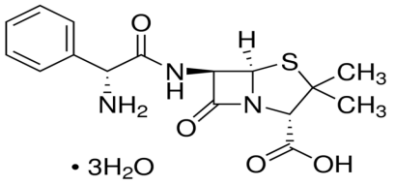

Figure 1 Chemical structure of Ampicillin trihydrate

It is slightly soluble in water, and dissolves in dilute acids and alkaline solutions because of the presence of (NH_2) and (COOH) groups. However, it is insoluble in alcohol, ether and in fatty oils[2]. Ampicillin is one of the important penicillin antibiotics used to treat or prevent bacterial infections. In view of its pharmacological importance, considerable work has been done for its detection and quantification[3].

The literature reported several analytical procedures for the determination of amoxicillin and ampicillin in pure form or in pharmaceutical formulations as well as in biological fluids. Thus, the methods reported included spectrophotometric[4–10], polarographic[10,11], fluorimetric[12,13], flow injection analysis[14,15] and HPLC methods [17–19], capillary electrophoresis [20] and microbiological assay[21,22].

In the present work, spectrophotometrically based methods are proposed for the determination of Ampicillin by measuring the absorbance at (369, 340) nm after oxidation with alkaline, acidic $K_2Cr_2O_7$ respectively and at 302 nm after alkaline oxidation with H_2O_2 solution. All variables affecting the development of the color were investigated and the conditions were optimized. The proposed methods were successfully applied for the determination of Ampicillin in bulk powder. The determination of Ampicillin by the fixed concentration method is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable.

Furthermore, some specific advantages in the application of the kinetic methods can be expected:

1. Selectivity due to the measurement of the evolution of the absorbance with the time of reaction instead of measuring a concrete absorbance.
2. Possibility of no interference from the colored/turbid background of the samples[23,24].

Materials and methods

The experiments conducted at the laboratories of oil technology department - Koya technical institute – Koya – Erbil – Iraq.

Apparatus

All of the spectrophotometric measurements were made with a CECIL CE 7200 DOUBLE - BEAM SERIES spectrophotometer with matched 1 cm quartz cells.

Reagents and solutions

All chemicals were of analytical reagent grade (A.R.) and the water was always double distilled water. Ampicillin trihydrate (99.8 %) was supplied by Sigma Company - India, sodium hydroxide from Sigma - Aldrich (extra pure 100.5%), Germany; Sulfuric acid Sigma –Aldrich (98%), Germany and Hydrogen peroxide Scharlau (extra pure 50% w/w), Spain. It was used without further purification.

A solutions of 0.05 M potassium dichromate , 0.5 M sodium hydroxide, 0.1M sulfuric acid and 3% w/w hydrogen peroxide used when the experiments carried out.

Preparation of standard test solutions:

1. Six concentrations of standard ampicillin trihydrate powder (2, 5, 7, 10, and 15, 20) ppm were prepared (procedure 1). Into a series of 50 ml volumetric flasks, add to each flask 3.5 ml of 0.5 M NaOH and 3.5 ml of 0.05M $K_2Cr_2O_7$, mix well, leave to stand for 70 minutes, take 1.2 ml of each solution and then dilute to 50 ml volume with water. Measure the absorbance of the resulting solution at λ_{max} 369 nm at ambient temperature (25°C) against a blank solution prepared simultaneously.
2. Six concentrations of standard ampicillin trihydrate powder (2, 5, 7, 10, and 15, 20) ppm were prepared (procedure 2). Into a series of 50 ml volumetric flasks, add to each flask 5 ml of 0.1 M H_2SO_4 and 5 ml of 0.05M $K_2Cr_2O_7$, mix well, leave to stand for 70 minutes, take 2 ml of each solution, and then dilute to 50 ml volume with water. Measure the absorbance of the resulting solution at λ_{max} 340 nm at ambient temperature (25°C) against a blank solution prepared simultaneously.
3. Six concentrations of standard ampicillin trihydrate powder (2, 5, 7, 10, and 15, 20) ppm were prepared (procedure 3). Into a series of 50 ml volumetric flasks, add to each flask 7 ml of 3% w/w H_2O_2 and 5 ml of 0.5M NaOH, mix well, leave to stand for 70 minutes, take 0.1 ml of each solution, and then dilute to

50 ml volume with water. Measure the absorbance of the resulting solution at λ_{max} 302 nm at ambient temperature (25°C) against a blank solution prepared simultaneously.

After optimizing the reaction conditions, the fixed time was applied to the determination of Ampicillin in pure form over the concentration ranges (2- 22) ppm for the three procedures, respectively. The value of absorbance plotted against the respective concentrations of ampicillin (ppm) to obtain the calibration curve.

Results and discussion

1-Oxidation with $K_2Cr_2O_7$ in alkaline and acidic medium:

The reaction between Ampicillin and $K_2Cr_2O_7$ in NaOH and H_2SO_4 solutions yields orange color as a result of the dichromate species, which absorbs at λ_{max} of (369 and 340) nm (Fig.2 and 3) respectively. The intensity of the color produced increases gradually reaching its maximum after 70 minutes, when it remains stable.

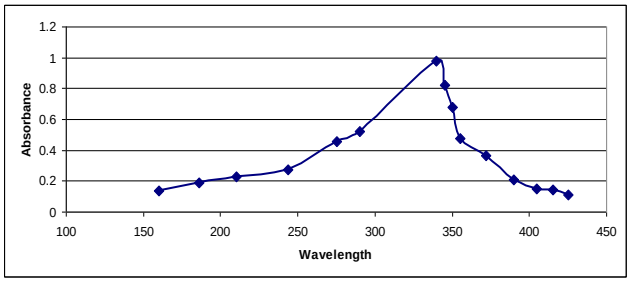

Figure 2 determination of λ_{max} for ampicillin with $K_2Cr_2O_7$ in acidic media

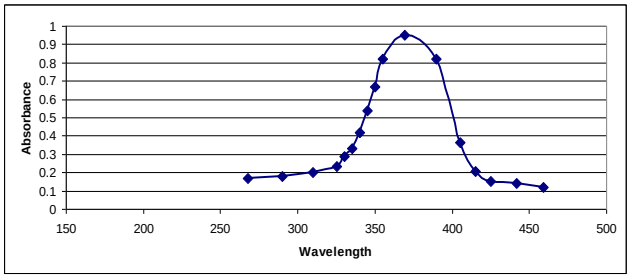

Figure 3 determination of λ_{max} for ampicillin with $K_2Cr_2O_7$ in basic media

At room temperature the reaction increased substantially with time, as revealed by the intensification of the developed color and subsequent increase in the slope of the calibration graphs (Fig 5 and 6) indicating high analytical sensitivity.

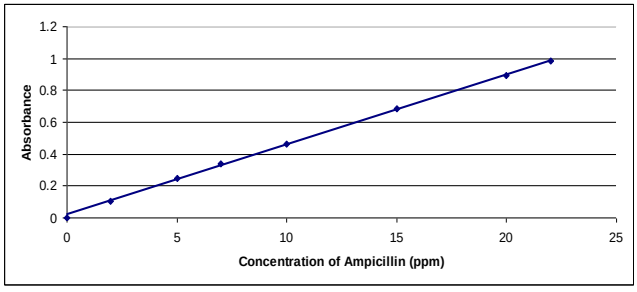

Figure 5 Calibration graph for ampicillin with $K_2Cr_2O_7$ in acidic media

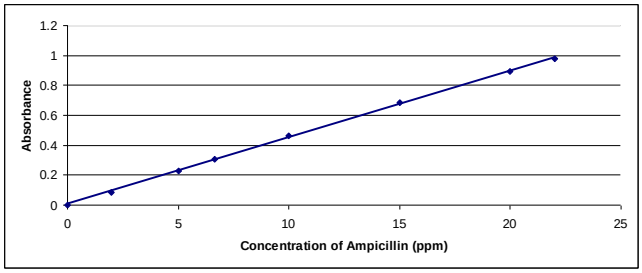

Figure 6 Calibration graph for ampicillin with $K_2Cr_2O_7$ in basic media

2-Oxidation with H_2O_2 in alkaline medium:

The same procedure was repeated using of 3% H_2O_2 solution in NaOH solution, which absorbs at λ_{max} of 302 nm (Fig.4).The calibration graph constructed (Fig. 7).

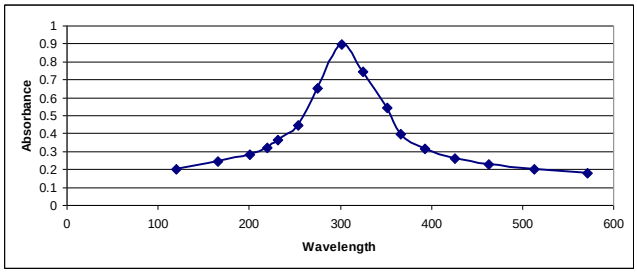

Figure 4 determination of λ_{max} for ampicillin with H_2O_2in basic media

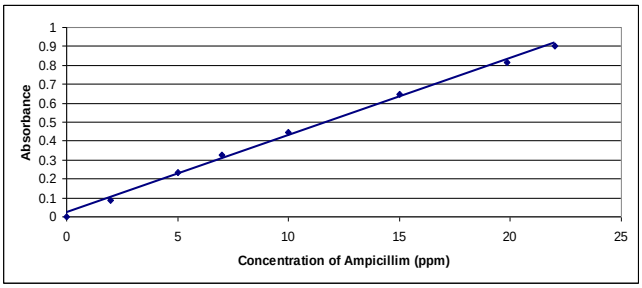

Figure 7 Calibration graph for ampicillin with H_2O_2 in basic media

3-Optimization of reaction conditions

3-1 For procedure1: (in basic media)

The reaction rate and absorbance increases with increasing $K_2Cr_2O_7$ concentration on the formation of dichromate ion.

The absorbance was studied in the range of (0.1 - 5) ml of 0.05M $K_2Cr_2O_7$ keeping all other parameter constant. It was found that 3.5 m l of $K_2Cr_2O_7$ solution is the optimum concentration for the absorbance of Ampicillin in this procedure.

Also, solutions examined at constant concentration of drug, dichromate ion and varying volume (0.1–5) ml of 0.5 M NaOH at 25°C. The optimum absorbance was obtained with 3.5 ml of 0.5 M NaOH, after which increase in volume of NaOH caused no changed in absorbance.

Dilution process carried out by preparing a solutions with the range of (0.1 – 2) ml of a standard solution diluted to 50 ml with double distilled water, it was shown that 1.2 ml is the optimum volume. The mean value and standard deviation of this procedure were calculated (0.5198 and 0.3413) respectively.

3-2 For procedure 2: (in acidic media)

The absorbance was studied in the range of (1 - 7) ml of 0.05M $K_2Cr_2O_7$ keeping all other parameter constant. It was found that 5 m l of $K_2Cr_2O_7$ solution is the optimum concentration for the absorbance of Ampicillin in this procedure.

The effect of the color development was investigated by adding different volumes (1–7) ml of 0.1M H_2SO_4. The optimum absorbance was obtained with 5 ml of 0.1 M H_2SO_4, after which increase in volume of H_2SO_4 caused no changed in absorbance.

Dilution process carried out by preparing a solution with the range of (1 – 7) ml of a standard solution diluted to 50 ml with double distilled water, it was shown that 2 ml is the optimum volume. The mean value and standard deviation of this procedure were calculated (0.53 and 0.3321) respectively.

3-3 For procedure 3: (H_2O_2 in basic media)

The absorbance was studied in the range of (1 - 8) ml of 3% w/w H_2O_2 solution keeping all other parameter constant. It was found that 7 ml of H_2O_2 solution is the optimum concentration for the absorbance of Ampicillin in this procedure.

The effect of the color development was investigated by adding different volumes (1– 6) ml of 0.5 M NaOH. The optimum absorbance was obtained with 5 ml of 0.5 M NaOH, after which increase in volume of NaOH caused no changed in absorbance.

Dilution process carried out by preparing solutions with the range of (0.1 – 1.5) ml of a standard solution diluted to 50 ml with double distilled water, it was shown that 0.1 ml is the optimum volume. The mean value and standard deviation of this procedure were calculated (0.494 and 0.3049) respectively.

Conclusion:

Three different methods for determination of Ampicillin tri hydrate proposed in this work, these methods are direct, simple and sensitive. An optimization for all variables the methods depends on was achieved. The proposed methods could be applied successfully for determination of Ampicillin either in pure form or pharmaceutical preparations.

The optimization of the effect of time on oxidation reactions for all experiments were investigated (Fig. 8), the optimum time was 20 minutes.

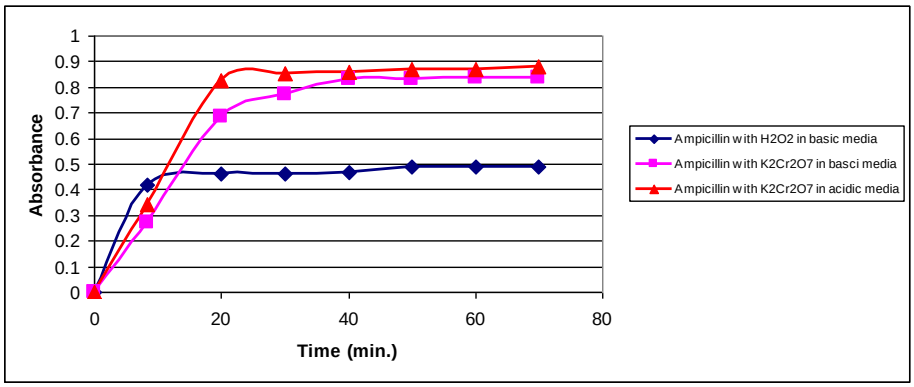

Figure 8 the effect of time on the three procedures

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