

Original Article

## KINETIC SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF GENTAMICIN IN PHARMACEUTICAL FORMS

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### ABSTRACT

**Objective:** The objective of this study is to develop and validate new selective kinetic methods for the determination of Gentamicin in pure and pharmaceutical preparations.

**Methods:** The proposed methods (A and B) are based on the oxidation of Gentamicin drug with alkaline potassium permanganate at fixed temperature. The oxidation reaction was followed spectrophotometrically by measuring the increase in the absorbance owing to the formation of  $MnO_4^{2-}$  at 610 nm. These methods (A) and (B) are based on initial-slope and fixed-time methods respectively.

**Results:** The methods were linear in the concentration ranges 20–125 and 25-140  $\mu\text{g/ml}$  with correlation coefficients of 0.995 and 0.988 and limits of detection LOD of 10.5 and 9.2  $\mu\text{g/ml}$  for the two methods respectively. The proposed methods were applied for the determination of gentamicin in pharmaceutical formulations with recovery and relative standard deviations of  $97\% \pm 8$  and  $98\% \pm 7$  for the two methods.

**Conclusion:** the developed methods were accurate, precise and reproducible compared to the official methods.

**Keywords:** Gentamicin, Spectrophotometric, Determination, Oxidation, Drug formulation.

### INTRODUCTION

Gentamicin, which is a broad spectrum aminoglycoside antibiotic (fig. 1), belongs to the class of medicinal compounds capable of inhibiting the growth of Gram-positive and Gram-negative bacteria [1]. Gentamicin is one of the most effective drugs used in the treatment of serious suppurative and septic processes, especially those that are caused by Gram-negative microorganisms. The advantages of gentamicin over other aminoglycosides (kanamycin, neomycin) are its activity towards *Pseudomonas aeruginosa* and microorganisms of the *Serratia-Klebsiella-Enterobacter* group, a faster bactericidal effect, and the rare development of gentamicin resistant strains. Several methods used for determination of GT including colorimetric [2, 3], amperometric enzyme-immunosensors [4], flow injection chemiluminescence [5], spectrofluorometry [6,7], capillary electrophoresis with UV detection [8], liquid chromatography (LC) [9–14] and high performance liquid chromatography (HPLC) [15–23]. Kinetic methods have many advantages over other spectrophotometric methods due to their high selectivity and elimination of interferences effects especially in pharmaceutical analysis.

In this work, we develop new methods for determination of gentamicin based on its oxidation reaction by permanganate in alkaline medium, producing the green manganate ion with  $\lambda_{\text{max}}$  at 610 nm (fig. 2). All the reaction conditions including the concentration of the oxidant, amount of sodium hydroxide and temperature were studied. The reaction was utilized for developing two direct kinetic spectrophotometric determination of GT by monitoring the increase of the absorbance of manganate ion as a function of time at controlled temperature. These methods (A) and (B) are based on initial-slope (Method A) and fixed-time (Method B), and they were validated for linearity, sensitivity, accuracy and precision. The proposed methods were applied for the determination of GT in pharmaceutical preparations and the results were comparable with standard methods [24].

### MATERIALS AND METHODS

#### Apparatus

Spectro UV-Vis Double Beam (UVD-3500, Labomed. Inco) was used with spectral bandwidth of 1.0 nm, wavelength accuracy  $\pm 0.3$  nm

(with automatic wavelength correction), wavelength range (190 nm–1100 nm), wavelength reproducibility  $\pm 0.2$  nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution.

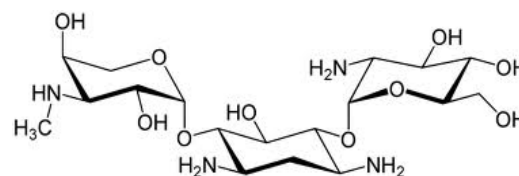


Fig. 1: Chemical structure of gentamicin

#### Materials

All chemicals and reagents used were of analytical reagent grade. Pharmaceutical grade gentamicin sulfate was purchased from Sigma Aldrich. Gentamicin injection vials were obtained from a local pharmacy, potassium permanganate was purchased from sigma Aldrich.

A Stock solution of 200  $\mu\text{g/ml}$  of (GT) was prepared daily by dissolving appropriate amount of gentamicin sulfate in double distilled water, then the solution was used to prepare different concentrations by dilution. Potassium permanganate reagent was prepared by dissolving 0.20 grams in 250 ml volumetric flask with distilled water, standardized, then it was used to prepare different appropriate solutions. A stock standard of 0.2 M sodium hydroxide solution was prepared in distilled water.

#### Construction of calibration curves

The absorbances of manganate ion in solutions containing various amounts of GT was measured at a pre-selected fixed time, calibration plots of the absorbance at 610 nm versus initial concentrations of the drugs were established.

All the prepared solutions were kept at constant temperature in a water bath before reaction starts.

**Method A:** different volumes of 200 µg/ml GT solution were pipette in a series of 10 ml volumetric flasks, then 2 ml of NaOH solution and 1.5 ml KMnO<sub>4</sub> solution were added then the volume were completed to 10 ml by distilled water, the mixture is in a water bath at 40 °C, then the solution mixture was transferred immediately into the spectrophotometer cell for absorbance monitoring at 610 nm during 40 minutes at controlled temperature. The absorbance change with time during the reaction progress is obvious in (fig. 2), and it is clear that the absorbance increase as the initial concentration of GT increases. For the initial-slope method calibration curve was constructed for Gentamicin by plotting the logarithm of the initial rate versus the logarithm of initial concentration of GT, the results shown in (fig. 3) illustrate good linearity in the range of 20-125 µg/ml.

**Method B:** different volumes of 200 µg/ml GT solution were pipette in a series of 10 ml volumetric flasks, then 2 ml of NaOH solution and 1.5 ml KMnO<sub>4</sub> solution were added then the volume were completed to 10 ml by distilled water, the mixture is shaken in a water bath at 40 °C, then the solution was kept then the absorbance was measured after 20 minutes an 610 nm. For fixed-time method, the calibration curve is constructed using the optimized conditions with good linearity in the range of 25-140 µg/ml as shown in (fig. 4). The regression results obtained for determination of GT using the proposed methods are shown in (table 1).

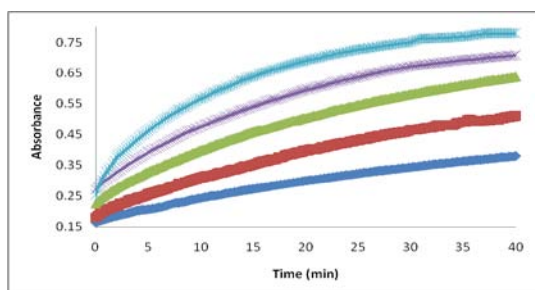


Fig. 2: Absorbance at 610 nm versus time graphs Using different volumes of (GT) with KMnO<sub>4</sub> in alkaline medium

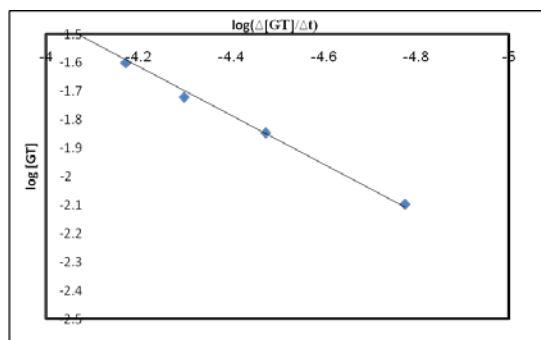


Fig. 3: Calibration plot of logarithm rate against logarithm concentration of GT for initial-slope method

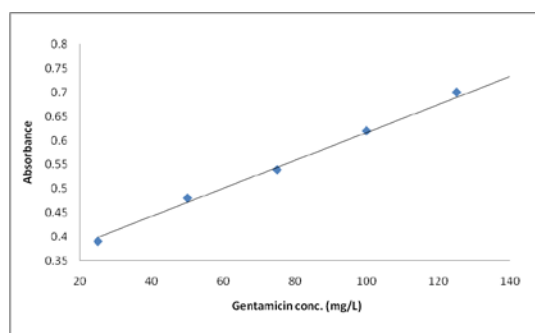


Fig. 4: Calibration plot of GT determination using fixed-time method Different volume of GT, after 20 minutes at 40 °C

**Optimum reaction conditions**

The oxidation reaction of GT in alkaline permanganate is influenced by the concentrations of the oxidant and sodium hydroxide as well as the temperature. To determine the optimum reaction conditions that produce the stable, green manganate ion in a short time, it was decided to study each factor; keeping the other factors constant.

**Effect of KMnO<sub>4</sub> solution concentration (Method B)**

The effect of KMnO<sub>4</sub> solution concentration was studied by using different concentrations of KMnO<sub>4</sub> solution in a series of 10 ml volumetric flasks, keeping the concentration of NaOH solution constant at 0.05 M. GT concentration was kept at 50 µg/ml. The solution mixture was shaken and wormed at 40° C for 20 min, cooled to room temperature and the absorbance was measured at 610 nm. The results are shown in (fig. 5).

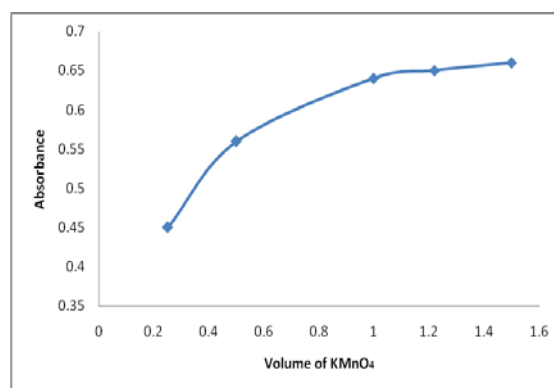


Fig. 5: Effect of KMnO<sub>4</sub> volume on the absorbance at 610 nm as a result of the reaction with 50 µg/ml of GT in NaOH solution

**Effect of NaOH solution concentration (Method B)**

The effect of NaOH solution concentration was studied by using different concentrations of NaOH solution in a series of 10 ml volumetric flasks, keeping the concentration of KMnO<sub>4</sub> solution constant at 3.0X10<sup>-4</sup> M. GT concentration was kept at 50 µg/ml. The solution mixture was shaken and wormed at 40° C for 20 min, cooled to room temperature and the absorbance was measured at 610 nm. The results are shown in (fig. 6) below.

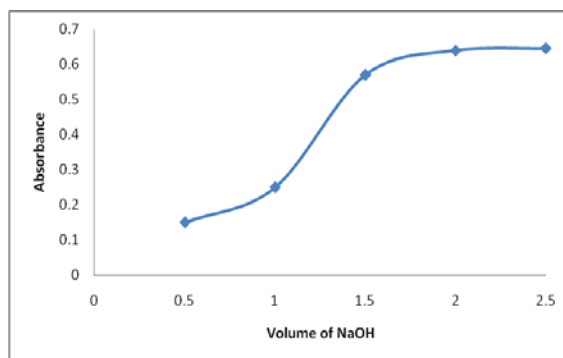


Fig. 6: Effect of NaOH volume on the absorbance

The optimum concentration of reagents for Method (B) was studied in the same manner.

**Procedure for drug formulations**

The content of ten injection vials (Garamicin®) was mixed separately, then solutions were made using double distilled water,

samples equivalent to 50 mg/l were taken, then the same procedures were applied for these samples using the proposed methods, data are shown in (table 2).

## RESULTS AND DISCUSSION

The oxidation of Gentamicin by  $\text{KMnO}_4$  resulted in manganous ( $\text{Mn}^{2+}$ ) and manganate ( $\text{MnO}_4^{2-}$ ) ions depending on the pH of the medium, in acidic medium  $\text{Mn}^{2+}$  predominate while in alkaline medium  $\text{MnO}_4^{2-}$  predominate and stable with time.  $\text{Mn}^{2+}$  absorbs light at 440 nm and  $\text{MnO}_4^{2-}$  absorbs light at around 610 nm while permanganate ion has two strong absorptions at 520 and 540 nm. It is obvious from (fig. 7) that manganate ion is being formed as well as manganous ion during the reaction progress, as a result the fixed-time method can be applied by selecting suitable time and temperature for the quantitative determination of GT using appropriate experimental conditions. According to (fig. 2) it is obvious that the absorbance of the green manganate ion having a maximum absorbance at 610 nm is directly proportional to the amount of GT drug and this represents the base for quantitative method depending on the initial-slope method.

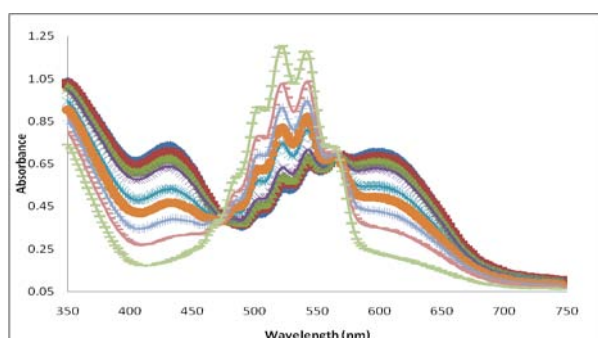


Fig. 7: Absorption spectra of GT solution during the reaction with  $\text{KMnO}_4$  in NaOH solution after five minutes intervals

### Method validation

The initial rate of the reaction would follow a pseudo order rate constant and will obey the following rate equation:  $v = (\Delta A / \Delta t) = \kappa' C^n$ , Where  $v$  is the reaction rate,  $A$  is the absorbance,  $(t)$  is the time,  $\kappa'$  is the pseudo order rate constant,  $C$  is the Molar concentration of GT drug and  $n$  is the order of the reaction. the logarithmic form of the above equation can be written as follows:  $\log v = \log (\Delta A / \Delta t) = \log \kappa' + n \log C$ . The calibration graph was prepared by plotting the logarithm of the initial rate of the reaction ( $\log v$ ) versus the logarithm of molar concentration of the drug ( $\log C$ ), the data shown high correlation over a wide dynamic range as shown in table 1. The value of the slope shows that  $n \approx 1$  which suggests a pseudo-first order mechanism. For the fixed-time method: Preliminary experiments were performed to investigate the progress of the reaction at different temperature, by monitoring the absorbance of the green manganate during 40 minutes periods while keeping the concentrations of all components constant. The regression equations of this method were evaluated according to the correlation coefficient and the linear range. From the data collected it was suggested that a fixed time of 20 minutes at 40 °C conditions is the most acceptable values and the results of this method are shown in (table 1).

### Application to drug formulations

The proposed kinetic methods were applied successfully for the determination of Gentamicin in commercial forms, statistical comparison of the accuracy as recovery compared with a standard method reveals that there is no significant difference in the accuracy or precision between the proposed methods and the standard method as shown in (table 2).

Gentamicin has been determined in pure as well as in pharmaceutical forms using the proposed methods. According to the results obtained it is obvious that both the two methods have wide dynamic range with good accuracy and precision as compared with

other spectrophotometric methods used. Based on the application of the proposed methods on real pharmaceutical forms the recovery results were very high and conform with the requirements of the official pharmacopeia. The proposed kinetic methods which depend on selective oxidation reactions under controlled conditions allow the proposed methods to show a relative freedom from interferences by the usual pharmaceutical excipients. The proposed kinetic methods can be used for routine quality control tests of gentamicin in drug formulations as it offers the advantages of sensitivity, selectivity and low cost compared to chromatographic methods.

Table 1: Regression results obtained by the proposed Methods

Property	Method A	Method B
Concentration range ( $\mu\text{g/ml}$ )	20-125	25-140
Correlation coefficient	0.995	0.988
Slope	0.854	0.072
Intercept	1.961	0.332
Detection limit ( $\mu\text{g/ml}$ )	10.5	9.2
R. S. D %	5.6	7.1

Table 2: Evaluation of the accuracy of the proposed methods

Drug	proposed method		standard method		t
	Amount taken $\mu\text{g/ml}$	Recovery <sup>a</sup> $\pm\text{RSD}\%$	Amount taken $\mu\text{g/ml}$	Recovery $\pm\text{RSD}\%$	
Gentamicin@					
Method A	50	97 % $\pm$ 8	50	98 % $\pm$ 7	1.9
Method B	50	98 % $\pm$ 7	50	98 % $\pm$ 6	1.6

<sup>a</sup>Average of five determinations. Tabulated t-value at 95% confidence level and 5 degrees of freedom (2.57)

## CONCLUSION

Gentamicin was determined in simple kinetic spectrophotometric methods that were based on oxidation of the drug by potassium permanganate in alkaline medium. The absorbance of the resultant manganate ion was monitored at 610 nm using initial-slope and fixed-time kinetic methods. The proposed methods were simple, rapid, accurate and were validated and compared to standard methods.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTERESTS

Declared None

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