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Kinetic Determination of Tobramycin In Drug Formulations

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ABSTRACT

A kinetic method for the accurate determination of tobramycin has been developed. A solution of tobramycin is heated with $6x10^{-2}$ M ascorbic acid in dimethylsulfoxide at 100C for a fixed time of 30 min, after which the reaction is quenched by cooling and the absorbance of the colored product is measured at 390nm and 532nm. The following equations were used for calculating unknown concentrations of tobramycin at 390nm and at 532nm respectively:

$$A = 0.027 + 0.019\ C$$
$$A = 0.009 + 0.006\ C$$

The method was applied for the determination of tobramycin in drug formulation and a statistical comparison with the BP biological assay method was made. The determination of tobramycin by the fixed-concentration method is feasible with the calibration equations obtained but the fixed time method has been found to be more applicable.

Keywords: Kinetic determination; Tobramycin; Ascorbic acid

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INTRODUCTION

Tobramycin (Figure 1) is an aminoglycoside antibiotic used most widely against gram-negative enteric bacteria [1]. Its systematic (IUPAC) name is 4-amino-2-[4,6-diamino-3- [3-amino-6-(aminomethyl)-5-hydroxy-tetrahydropyran-2-yl]oxy- 2-hydroxy-cyclohexoxy]-6-(hydroxymethyl) tetrahydropyran-3,5-diol.

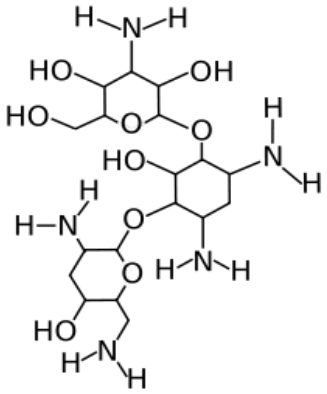

Figure 1. Chemical structure of tobramycin

Methods reported for the quantitative determination of tobramycin in pharmaceutical formulations included chromatography [2, 3] and spectrophotometry [4-9]. In the BP [10] tobramycin is assayed biologically.

Ascorbic acid has been frequently utilized as an analytical reagent in pharmaceutical analysis. Ascorbic acid is reported to have a selective reaction with ammonia, primary aliphatic amines of the type R-CH2-NH2 (λ_{max} 390 and 532 nm) [11], amino acids with α-aminoacyl function (λ_{max} about 400 nm) [12]. Based on these reports, in the method described here tobramycin is assayed kinetically by the fixed-time method in which it is reacted with ascorbic acid at 100°C for 30min and the absorbance of the purple product is measured at 390nm and 532nm after cooling. The method was proposed for the determination of the aminoglycoside, tobramycin in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Reagents

All materials and reagents used were of analytical grade. Tobramycin authentic material (M. Wt 467 and 101% claimed purity) was supplied by Medico lab, Syria. Pharmaceutical formulations containing tobramyin (Tobra® injections 80mg/2ml, MFG 10/06 , exp. 10/09) manufactured by Shifa pharmaceutical industries, Aleppo, Syria ;TOBRADEX® eye drops 0.3%w/v tobramycin, 0.1% dexamethasone, and TOBREX® eye drops 0.3%w/v tobramycin, MGF 4/08,exp. 3/10) manufactured by Alcon-ouvreur, Belgium.

Ascorbic acid reagent was obtained from BDH, Poole, England; a 0.1%w/v of the reagent was freshly prepared in dimethylsulfoxide; dimethylsulfoxide (DMSO) was obtained from Loba Chemie, India; dimethylformamide (DMF) was obtained from Merck KGA, Germany.

Apparatus

JASCO V-530 UV/VIS spectrophotometer,Kyoto Japan

* Water bath, Gesellschaft fur Labortechnik mbH, Germany.
* Balance, Kern ALS 120-4, Germany.
* Hamilton microsyringe 100µl.

Stock solution of standard Tobramycin

0.2g of tobramycin standard was acurrately weighed, transferred into 25ml volumetric flask, dissolved in 10ml distilled water and then the volume was completed to the mark with distilled water.

Stock solution of Tobramycin injection

5ml of tobramycin injection (80mg/2ml) was transferred into 25 ml volumetric flask, dissolved in distilled water, and the volume completed to the mark with water.

Ascorbic acid stock solution

0.01 g of ascorbic acid was dissolved in 10 ml dimethyl sulfoxide to produce 10 ml of 0.1 %w/v ($6x10^{-2}$M).

Procedure

1ml of ascorbic acid stock solution was added to the appropriate amount of tobramycin solution in a stoppered glass tube. The volume was then completed to 10ml with dimethylsulfoxide. The glass with its contents was shaken gently and then thermostated at 100 C in a water bath with a stop watch turned on. At a fixed time of 30 min, the reaction was quenched by cooling under tap water for 3 min and the absorbance was measured at 390nm and 532nm against reagent blank (1ml of ascorbic acid 0.1%w/v was added to 100µl of water and the volume was completed to 10ml with dimethylsulfoxide). The unknown concentration of tobramycin was then calculated from the corresponding equation for the calibration graph for the fixed-tome method.

RESULTS AND DISCUSSIONS

The method is based on the coupling of tobramycin with ascorbic acid as a chromogen. The possibility of the reaction of ascorbic acid with tobramycin was invistigated under various conditions. It was found that the reaction proceeds only in acidic media and at elevated temperature. As a result of the reaction a purple species that absorbs at 390nm and 532nm is

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produced [11]. The extent of formation of this species depends on the concentration of both reactants, solvent and temperature, and therefore the effect of these variables were studied. The reaction rate was found to increase with increasing temperature with a subsequent increase in the slope of the calibration graphs (Table 4a and 4b), indicating higher analytical sensitivity. Ascorbic acid concentration of 0.06M in dimethylsulfoxide and temperature of 100̊C were chosen as most suitable conditions.

To summarise, the optimum working conditions for the kinetic determination of tobramycin are $6x10^{-2}$M ascorbic acid in dimethylsulfoxide and heating at 100̊ C.

The rate of the reaction was also found to be tobramycin concentration dependent. The rates were followed at 100̊ C with various concentrations of tobramycin in the range 16-80µg/ml.

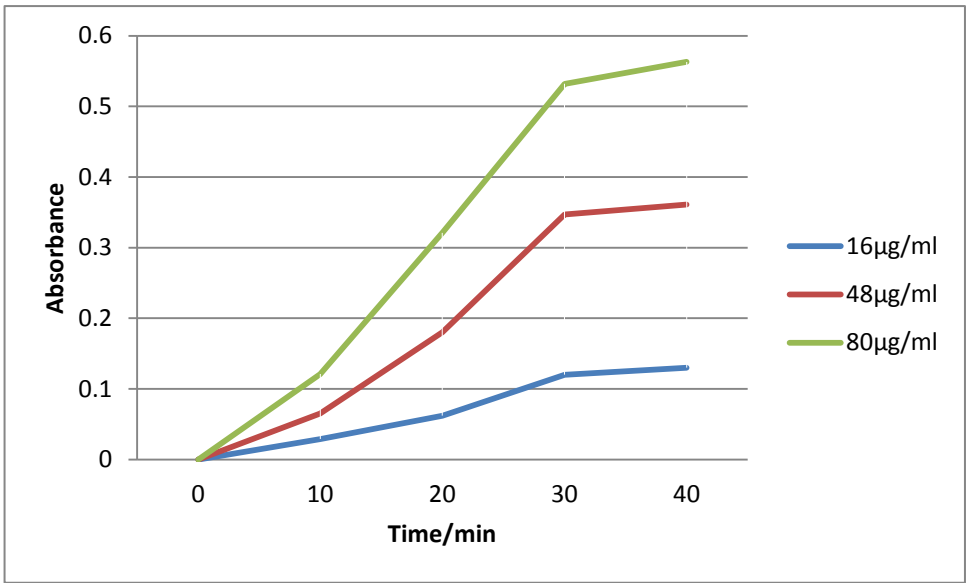

Figure 2. Absorbance vesus time curves for the reaction of ascorbic acid with different concentrations of tobramycin

The graphs shown in Fig.2 were obtained, from which it is clear that the rate increases as the tobramycin concentration increases, following the equation:

$$\text{Rate} = k` [\text{tobramycin}]^{n} \quad \ldots\ldots\ldots\ldots \quad (1)$$

Where $k`$is the pseudo-order rate constant and the n is the order of the reaction. The rate of the reaction may be estimated by the variable time method measurement [13] as $\Delta A / \Delta t$, where A is absorbance and t is time in seconds. Taking logarithims of rates and concentrations (Table 1), equation 1 becomes

$$\text{Log (rate)} = \log \Delta A / \Delta t = \log k` + n \log [\text{tobramycin}] \quad \ldots\ldots (2)$$

log (rate) *versus* log [tobramycin] gave the regression equation (at 390nm)

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Log (rate) = 0.474 + 0.92 log C (r=0.9977)

Hence $k`$= 2.98 s^{-1} and the reaction is first order (n= 0.92).
And at 532nm Log (rate) = 0.056 + 0.93 log C (r=0.998)
Hence $k`$= 1.14 s^{-1} and the reaction is first order (n= 0.93).

Table 1. Logarithms of rates for different concentrations of tobramycin at 100C, 390nm and 532nm

log Δ*A* /Δ*t* at 390nm	log [tobramycin] (M)	log Δ*A* /Δ*t* at 532nm	log [tobramycin] (M)
-3.71	-4.55	-4.18	-4.55
-3.44	-4.25	-3.90	-4.25
-3.25	-4.08	-3.71	-4.08
-3.16	-3.93	-3.61	-3.93
-3.06	-3.85	-3.53	-3.85

Appraisal of Kinetic Methods

The determination of tobramycin under the optimum conditions mentioned above would result in pseudo-first-order with respect to their concentrations where ascorbic acid concentration was at least 214 times the initial concentration of tobramycin. However the rate will be directly proportional to tobramycin concentration in a pseudo-first-order rate equation as follows:

Rate = $k`$ [tobramycin]…….. (3)
Where $k`$is the pseudo-first-order rate constant

Several experiments were then carried out to obtain tobramycin concentrations from the rate data according to equation (3). Rate constant, constant-concentration and constant-time methods [14,15] were used and the best method was chosen on basis of applicability, the slope (S) of the calibration graph, intercept and correlation coefficient(r).

Rate-constant method

Graphs of log (absorbance) versus time for tobramycin concentraion in the range 2.8x 10^{-5} – 14x 10^{-5} M were plotted and all were found to be straight lines. Pseudo-first-order rate constants ($k`$) corresponding to different tobramycin concentrations (C) were calculated from the slopes multiplied by -2.303 and presented in Table 2. Regression of C *versus* $k`$ gave the equation

$k`$= 0.00089- 0.0.21C (r=0.1) at 390nm
$k`$ = 0.00086 + 0.3C (r= 0.245) at 532nm

The poor linearity is probably due to changes in the rate constant with the slight changes in the elevated temperature of the reaction.

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Table 2. Values for *k`* calculated from slopes of logA *versus* t graphs multiplied by -2.303 for different concentrations of tobramycin at 100C

k` / s^{-1} at 390nm	[Tobramycin]/M	*k`* / s^{-1} at 532nm	[Tobramycin]/M
-8.7x 10^{-4}	2.8 x 10^{-5}	-8.6x 10^{-4}	2.8 x 10^{-5}
-8.5x 10^{-4}	5.6 x 10^{-5}	-8.4x 10^{-4}	5.6 x 10^{-5}
9.6x 10^{-4}-	8.4 x 10^{-5}	-9.8x 10^{-4}	8.4 x 10^{-5}
-8.9x 10^{-4}	11.2 x 10^{-5}	9.1x 10^{-4}-	11.2 x 10^{-5}
-8.2x10-4	14 x 10^{-5}	8.6x10-4-	14 x 10^{-5}

Fixed-concentration method

Reaction rates were recorded for the different tobramycin concentrations in the range 2.8x 10^{-5} – 14x 10^{-5} M. A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time *versus* the initial concentrations of tobramycin (Table 3) was plotted and the following equations of the calibration graphs were obtained:

1/t= 0.000045+ 14.5C (r= 0.996) at 390nm
1/t= 0.000065 + 14.4C (r= 0.998) at 532nm

Table 3. Values of reciprocal of time taken at fixed absorbance for different values of variable concentrations of tobramycin

Wave length 390nm (A=0.35)		Wave length 532nm (A=0.12)	
1/t /s^{-1}	[Tobramycin]/M	1/t /s^{-1}	[Tobramycin]/M
4.8 x 10^{-4}	2.8 x 10^{-5}	5 x 10^{-4}	2.8 x 10^{-5}
8.7 x 10^{-4}	5.6 x 10^{-5}	8.8 x 10^{-4}	5.6 x 10^{-5}
1.21 x 10^{-3}	8.4 x 10^{-5}	1.23 x 10^{-3}	8.4 x 10^{-5}
1.6 x 10^{-3}	11.2 x 10^{-5}	1.65 x 10^{-3}	11.2 x 10^{-5}
2.14 x 10^{-3}	14 x 10^{-5}	2.13 x 10^{-3}	14 x 10^{-5}

Fixed-time method

Reaction rates were measured for different concentrations of tobramycin. Each time the reaction was quenched by cooling at a pre-selected fixed time, which was accurately measured. Calibration graphs of absorbance *versus* initial concentrations of tobramycin were obtained at fixed times of 10, 20, 30 and 40 min with the calibration equations shown in Table 4a and 4b. It is clear that both the slopes and intercepts vary with time, and that the best correlation coefficient was obtained for a fixed time of 30 min, which was therefore chosen as the most suitable time for measurement.

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Table 4a.Calibration equations at different fixed times for tobramycin concentrations in the range 2.8 x 10^{-5} - 14 x 10^{-5} at wave length 390nm

Tobramycin concentration (µg/ml)	Absorbance at fixed times			
	10 min	20min	30 min	40 min
2.8 x 10^{-5}	0.084	0.179	0.348	0.377
5.6 x 10^{-5}	0.151	0.365	0.651	0.682
8.4 x 10^{-5}	0.189	0.522	1.024	1.047
11.2 x 10^{-5}	0.255	0.734	1.251	1.288
14 x 10^{-5}	0.351	0.931	1.565	1.633
Calibration equation	A=0.007+ 0.004C	A= -0.007+ 0.011C	A=0.027+ 0.019C	
R	0.933	0.992	0.9986	0.996

Table 4b.Calibration equations at different fixed times for tobramycin concentrations in the range 2.8 x 10^{-5} - 14 x 10^{-5} at wave length 532nm

Tobramycin concentration (µg/ml)	Absorbance at fixed times			
	10 min	20min	30 min	40 min
2.8 x 10^{-5}	0.029	0.062	0.12	0.130
5.6 x 10^{-5}	0.052	0.126	0.224	0.235
8.4 x 10^{-5}	0.065	0.180	0.347	0.361
11.2 x 10^{-5}	0.088	0.253	0.438	0.444
14 x 10^{-5}	0.121	0.321	0.532	0.563
Calibration equation	A=0.002 + 0.001C	A= -0.002+ 0.004C	A=0.009 + 0.006C	A= 0.011+ 0.006C
R	0.9933	0.9992	0.9998	0.9985

Applications

The fixed-time method was applied to the determination of tobramycin in commercially available drug formulations (injections). The proposed chemical method was found to be complementary to the BP bioassay method since the identity, purity and efficacy of the antibiotic can be verified by the two methods. The concentration of tobramycin was calculated using the corresponding calibration equation shown in Table 4 at a fixed time of 30 min.

The results obtained were compared statistically with those obtained by the BP bioassay method [10] (Table 5). The t-values at the 95% confidence level did not exceed the theoratical value of 2.78 [16], indicating no significant difference between the two methods.

Table 5. Statistical comparison of the results obtained by the fixed-time method with those obtained by the official BP method.

Drug	Recovery ± sd* %		t**
	Kinetic method	Official method	
Tobra	99.5 ± 0.59	100 ± 0.01	1.47

*Average of three determinations.

* * Theoratical value = 2.78

The proposed method proved to be simple, precise, accurate and economic. The bathochromic shift obtained by reacting tobramycin with ascorbic acid is considered selective for aminoglycosides with free amino group.

In general physiochemical methods have simplified determination of antibiotics, however, the bioassay methods are equally important as they confirm the required level of activity against susceplible micro-organisms.

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