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DEGRADATION OF TOBRAMYCIN IN AQUEOUS SOLUTION

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ABSTRACT

The kinetics of degradation of tobramycin (**Ne-De-Ka**) in aqueous solution was studied as a function of pH. Tobramycin hydrolyzes in acidic solution to yield kanosamine (**Ka-OH**) and nebramine (**Ne-De-OH**) with a pseudo first-order rate constant of 2.7 x 10^{-6} s^{-1} in 1 N HCl at 80°C. The activation energy for the acid catalyzed hydrolysis is 32 kcal mol^{-1}. In basic solution, the hydrolysis products are deoxystreptamine (**De-OH**), nebramine (**Ne-De-OH**) and deoxystreptamine-kanosaminide (**HO-De-Ka**). The pseudo first-order rate constant for the hydrolysis in 1 N KOH is 1 x 10^{-8} s^{-1} at 80°C. The activation energy for the base catalyzed hydrolysis is 15 kcal mol^{-1}. Tobramycin is very stable towards hydrolysis at neutral pH; however, it rapidly oxidizes giving several products including **De-OH**, **Ne-De-OH**, and **HO-De-Ka**. In pH 7 phosphate buffer (0.01 M), the t_{90} value is 70 hr at 80°C.

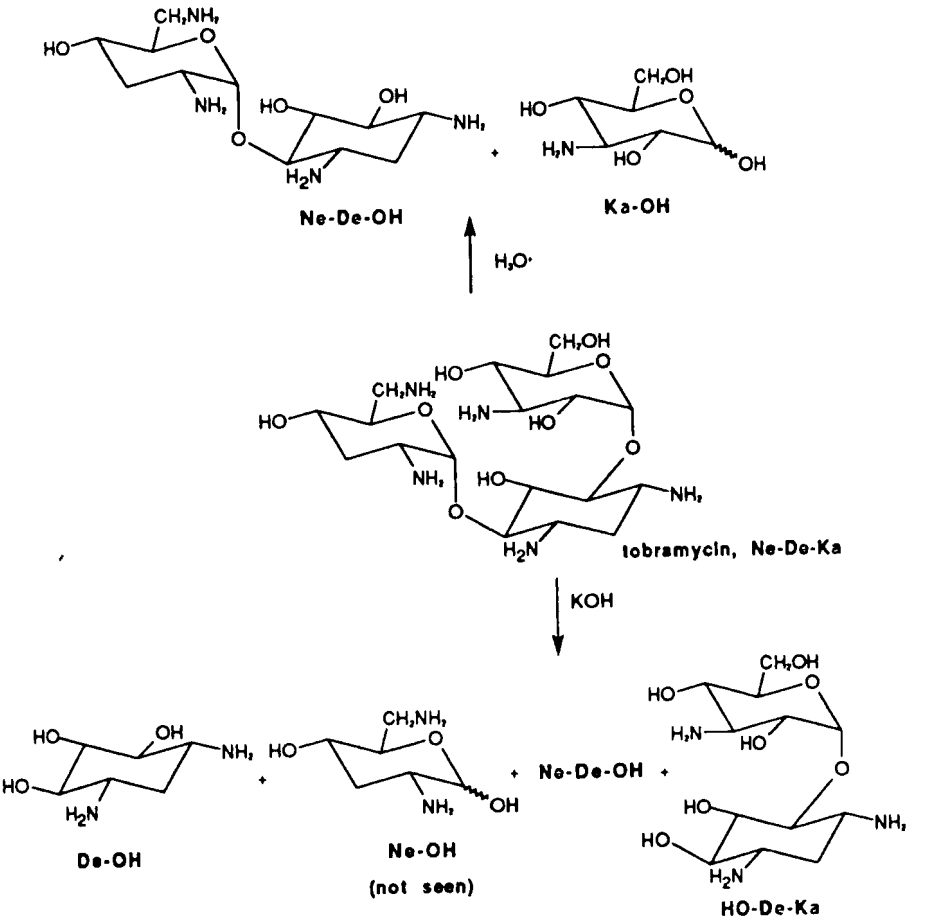
INTRODUCTION

Tobramycin, **Ne-De-Ka**, is an aminoglycoside antibiotic which is produced by *Streptomyces tenebrarius*. It has been used in both parenteral and ophthalmic formulations in either its sulfate form or its free base form[1,2]. The parenteral solution is formulated at drug concentrations of 10 and 40 mg/ml (pH 5.8) and the ophthalmic solution at 3 mg/ml (pH 7.4)[3].

The structure of tobramycin consists of a central deoxystreptamine ring, **De-OH**, which has two of its hydroxyl groups bonded in alpha linkage to kanosamine, **Ka-OH**, and nebrosamine, **Ne-OH** (Scheme 1). Tobramycin hydrolyses in 6 N HCl to the deoxystreptamine antibiotic nebramine, **Ne-De-OH**, and kanosamine, **Ka-OH**[4]. Literature reports on the reactivity of tobramycin in neutral solutions are largely focussed on its interactions with beta-lactam antibiotics and sugar excipients[5,6]. In this study the kinetics and the products from the hydrolysis of tobramycin in acidic and basic solution are investigated in more detail. Additionally, we have identified an auto-oxidation reaction which occurred primarily at neutral pH values.

EXPERIMENTAL

Sigma Chemicals, respectively. Picryl sulfonic acid was obtained from Aldrich Chemical Company and high purity solvents were obtained from American Burdick and Jackson. The degradation products **Ka-OH**, **De-OH**, **Ne-De-OH**, and deoxystreptamine-kanosaminide (**HO-De-Ka**) were prepared by literature



SCHEME 1

Hydrolysis Products of Tobramycin

methods[4,7,8] from tobramycin and kanamycin and isolated using a gravity column of AG1-X4 resin from Bio-Rad. The structures of these products were confirmed by the ^{1}H and ^{13}C NMR (Bruker 300 MHz) and mass spectral data (Finnigan-MAT TSQ-70) and are consistent with the literature[4].

Instrumentation

pH measurements were made with either a Radiometer (Model GK2401C) or a Sensorex (Model SG900C) electrode calibrated with aqueous standard buffer solutions at the indicated temperature. The HPLC system consisted of an HP 1090 pump and autosampler connected to a UV-visible detector from Applied Biosystems (Model 783) or a Spectra Physics 8430 RI detector. The detectors were interfaced to a Macintosh SE computer and the data analyzed with Dynamax software from Rainin.

HPLC Methods

Two HPLC methods were used to analyze tobramycin and its degradation products. Method 1 employed a Waters Spherical silica column and a mobile phase of 8 mM EDTA and 5 mM sodium chloride solution-methanol (90:10). The flow rate was 0.8 ml/min and the eluant was monitored by refractive index detection[9].

Method 2 involved derivatization with picryl sulfonic acid[10] and analyzing the samples on an Altex octyl column at 350 nm. The derivatization procedure involved reacting 100 µl of a tobramycin solution (≈1-10 mM) with a solution of 10 mg picryl sulfonic acid in 1 ml of water and 2 ml of pyridine at 80°C for 4.5 hr. The solution was then diluted 1:7 with 33% acetonitrile-water before

injection onto the HPLC. A flow rate of 1 ml/min was used and a linear gradient between the percentages shown below was used.

t = 0-5 min, 20% CH_3CN / 70% water / 10% THF
t = 25-45 min, 55% CH_3CN / 35% water / 10% THF
t = 60-75 min, 20% CH_3CN / 70% water / 10% THF

Kinetic Methods

Samples of tobramycin (≈1 mg/ml) were reacted in 3 ml borosilicate ampules from Wheaton Scientific except for the reactions in 1 N KOH. For the reactions in 1 N KOH solution, 5 ml of 0.2% solutions of tobramycin were reacted in tightly sealed Teflon containers. Samples were placed into 60, 80 or 100°C ovens. At known time intervals, ampules were removed and refrigerated until they were assayed by HPLC. For reaction solutions at the pH extremes, the solutions were neutralized with 1 M HCl or 1 M KOH. Degassed samples were prepared by sealing ampules that were by purged with N_2 for 5 minutes.

RESULTS and DISCUSSION

Degradation of Tobramycin at Extreme pH Values

The degradation of tobramycin was studied in HCl and KOH (1.0 and 0.1 N) solutions at elevated temperatures using HPLC Method 1. HPLC Method 1 was an isocratic method which used RI detection to observe tobramycin and its degradation products[9]. Figure 1a and 1b show the disappearance of tobramycin in 1.0 N HCl and 1.0 N KOH at elevated temperatures determined by HPLC Method 1. The disappearance of tobramycin was fitted to first-order

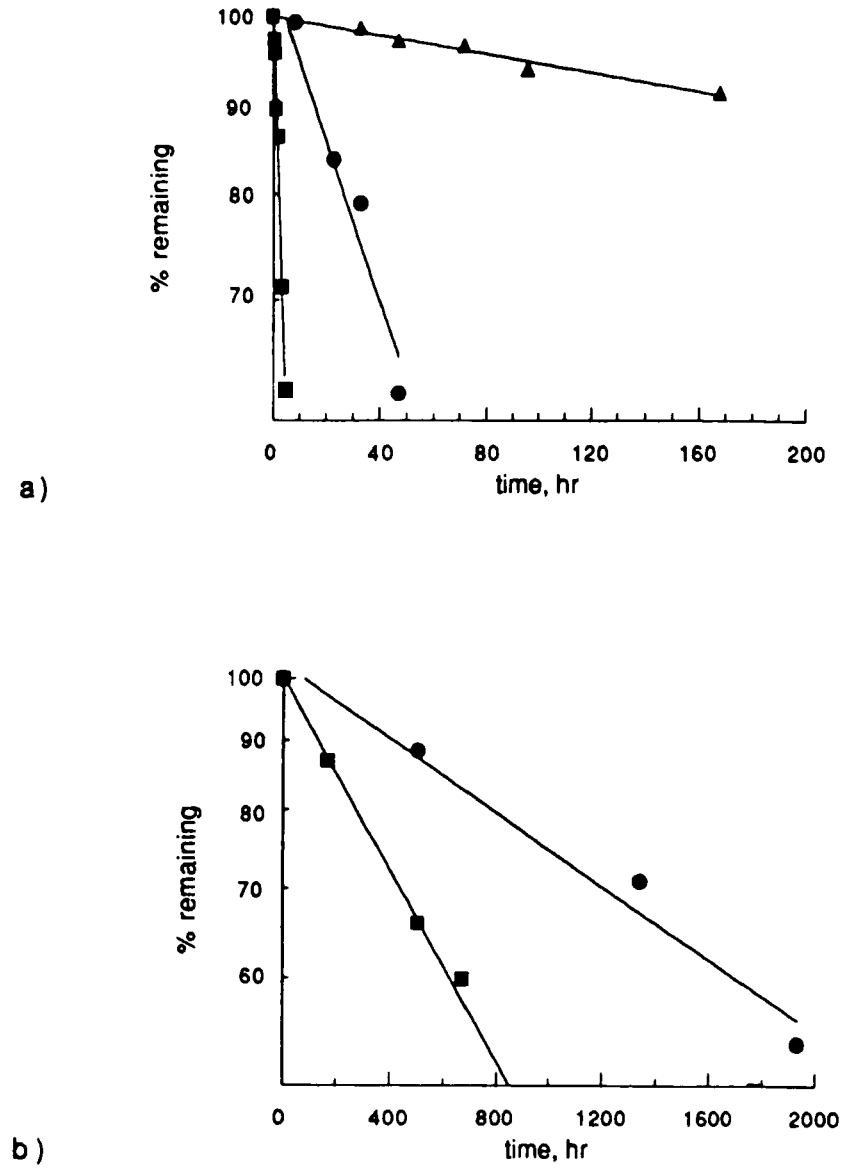


FIGURE 1

Plot of % remaining vs. time for the degradation of tobramycin in a) 1 N HCl at 60 (▲), 80 (●) and 100°C (■) and b) 1 N KOH at 80 (●) and 100°C (■). The loss of tobramycin is fitted with an exponential curve.

TABLE 1.

Kinetic Parameters for the Hydrolysis of Tobramycin.

Solution	60°C (s-1)	80°C (s-1)	100°C (s-1)	E_a (kcalmol-1)
1N HCl	1.7×10^{-7}	2.7×10^{-6}	2.8×10^{-5}	32
0.1 N HCl			1.1×10^{-6}	
1 N KOH	2.0×10^{-8}	1.0×10^{-7}	2.5×10^{-7}	15
0.1 N KOH			5.0×10^{-8}	

kinetics (Figure 1). The first-order rate constants for the acid and base catalyzed degradation of tobramycin at different temperatures and the Arrhenius activation energies are given in Table 1.

The structures of the decomposition products were determined by injecting independently prepared samples of the expected degradation products (see Experimental). With HPLC Method 1, **Ne-De-OH**, **HO-De-Ka** and **De-OH** were observed (Figure 2). Since the expected products, **Ka-OH** and **Ne-OH** were not detected a gradient method that derivatized primary amino groups with picryl sulfonic acid was developed, HPLC Method 2. The optimum conditions for derivatization were found to be 4.5 hr at 80°C. This method allowed the detection of both anomers of **Ka-OH** and **Ne-De-OH** in the samples degraded in acid and **De-OH**, **Ne-De-OH** and **HO-De-Ka**, in the sample degraded in base (Figure 3). The mass balance obtained by area normalization was greater than 95% for both the acid and base catalyzed hydrolysis

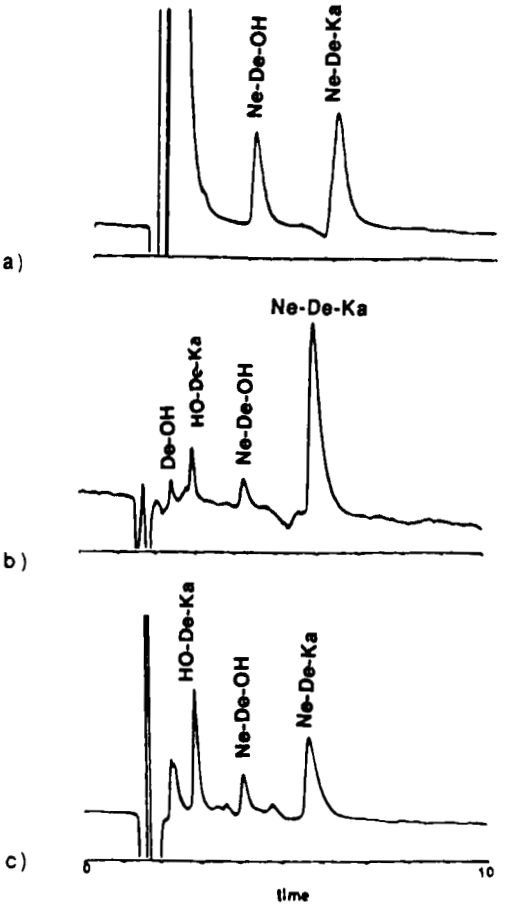


FIGURE 2

HPLC chromatograms of tobramycin solutions using HPLC Method 1: a) tobramycin hydrolyzed in 1 N HCl, 50% degraded b) tobramycin hydrolyzed in 1 N KOH, 50% degraded and c) tobramycin oxidized at neutral pH, 80% degraded.

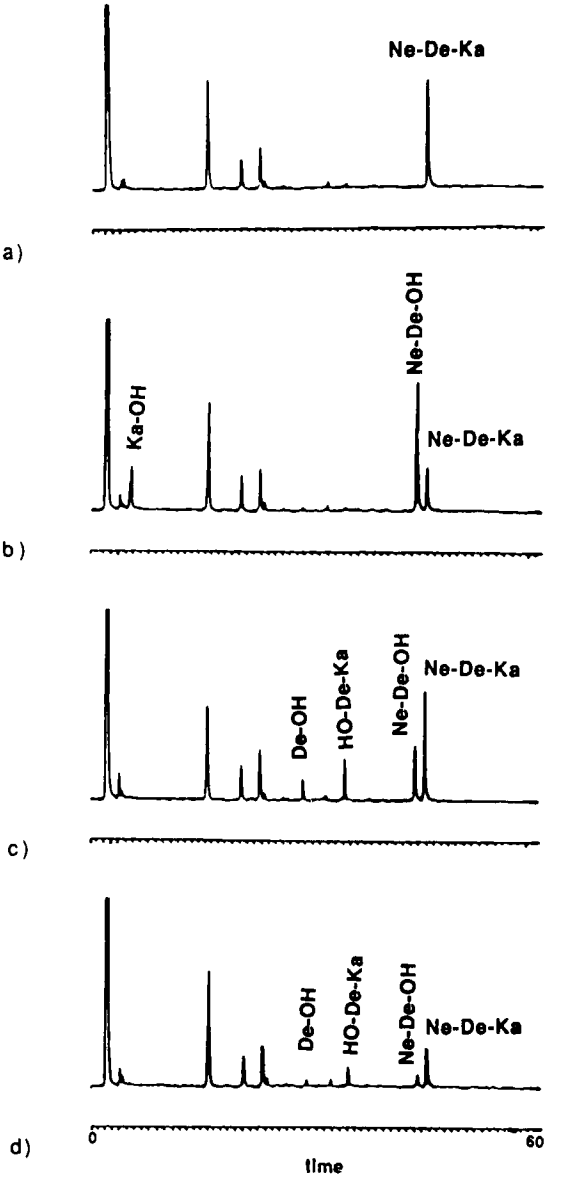


FIGURE 3

HPLC chromatograms of tobramycin solutions using HPLC Method 2: a) tobramycin b) tobramycin hydrolyzed in 1 N HCl, 75% degraded c) tobramycin hydrolyzed in 1 N KOH, 50% degraded and d) tobramycin oxidized at neutral pH, 80% degraded. The additional peaks in a) are present in the absence of tobramycin.

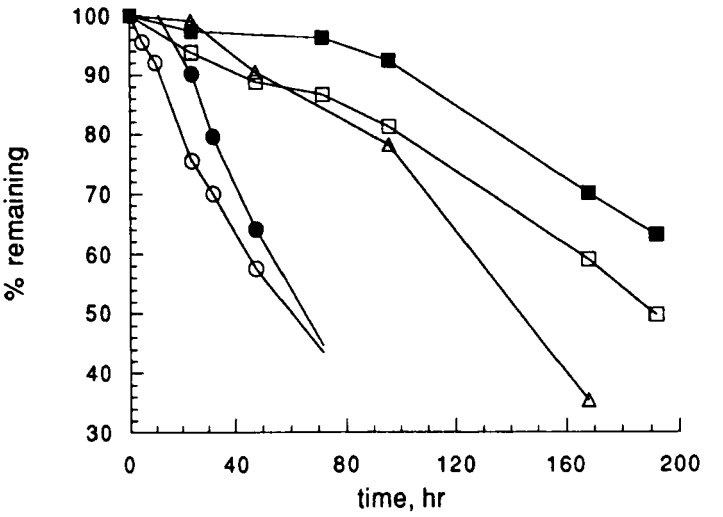


FIGURE 4

Plot of % remaining vs. time for the degradation of tobramycin at 100°C in 0.01 M buffer solutions. Potassium acetate, pH 4 (❑) and 6 (■), and potassium phosphate, pH 7 (○), pH 8 (●) and pH 11 (Δ) were used to prepare the buffers.

using HPLC Method II. **Ne-OH** was not observed as a degradation product and could not be obtained to establish its presence.

At neutral pH values, the degradation of tobramycin was found to be extremely slow in the absence of oxygen. For instance, a degassed solution at pH 7 (0.1 N phosphate buffer) showed less than 7% degradation after eight months at 100°C. The degradation, however, was rapid under ambient air[11]. Figure 4 shows the disappearance of tobramycin with time at 100°C from pH 4-11 (0.01 N phosphate or acetate) under ambient air. A simple kinetic scheme could not be derived to fit the observed kinetics so approximate t_{90} values (time to 10% degradation) were used to compare the data

TABLE 2

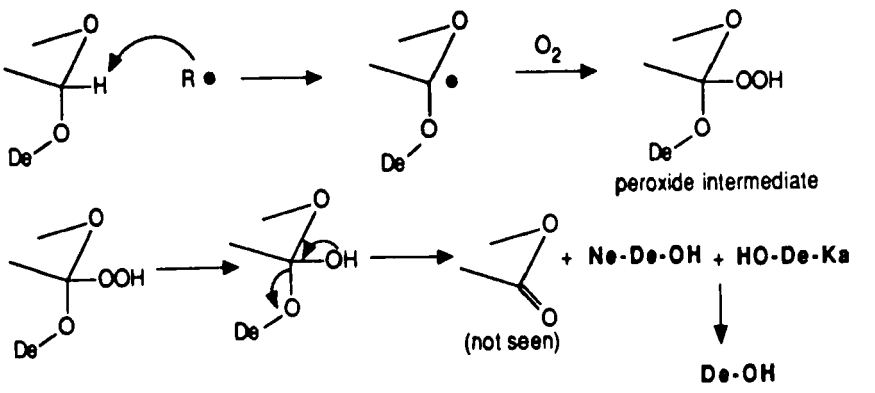
Approximate T_{90} Values for the Oxidation of Tobramycin.

Solution[a]	60°C (hr)	80°C (hr)	100°C (hr)
pH 4		750	100
pH 6	1500	450	50
pH 7	600	70	10
pH 8	650	70	20
pH 11	1400	200	40

[a]pH 4 and 6 samples were in 0.01 M potassium acetate buffer and the pH 7, 8 and 11 samples were in 0.01 M potassium phosphate pH values were measured at room temperature.

(Table 2). Although, the effect of pH on the apparent oxidation of tobramycin is complicated by the presence of buffer, inspection of the data in Table 2 suggests that the auto-oxidation occurred more readily at neutral pH values.

Figures 2c and 3d show chromatograms of a sample of tobramycin degraded at 80°C in pH 7 phosphate buffer using HPLC Method 1 and 2, respectively. The products identified were **De-OH, Ne-De-OH** and **HO-De-Ka,** however, considerable material is left unaccounted. After degradation to 20% remaining, the overall mass balance (HPLC Method 2) of the three products only accounted for 30% of the reacted tobramycin.



SCHEME 2

Possible Scheme for the Oxidation of Tobramycin

A possible mechanism[12] which accounts for the observed products is given in Scheme 2. The auto-oxidation reaction probably proceeds through a peroxide intermediate at either of the anomeric carbons which decompose to break the glycosidic bond. The observed products, **Ne-De-OH** and **HO-De-Ka**, occur through an oxidation reaction since a single oxidation of one of the terminal sugar moieties, **Ne** or **Ka** leaves the other two moieties unaltered. After a rapid cleavage of the oxidized moiety, **Ne-De-OH** and **HO-De-Ka** are formed. Further oxidation at **Ka** or **Ne** and cleavage results in **De-OH**. The mechanism predicts that lactones should be seen as additional products, however, the lactones may react further under the reaction conditions or elute with the solvent front and thus go undetected.

CONCLUSIONS

Two complementary analytical methods were used in this study to investigate the kinetics and degradation products of

tobramycin in aqueous solution. Although hydrolysis of tobramycin occurs at the pH extremes, it is not an important degradation pathway at neutral pH values. The major degradation pathway for tobramycin at neutral pH values where the drug is formulated (pH 5.8-7.4) is oxidation.

ACKNOWLEDGEMENTS

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