The enhancement of neomycin stability using propylene glycol and sodium metabisulphite

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Neomycin, in common with other members of the aminoglycoside group of antibiotics, is relatively thermostable (Swart, Hutchinson & Waksman, 1949; Baker, 1955; McGinity & Brown, 1975). Autoclaving solutions at 120° gave inconclusive results for stability (Simone & Popino, 1955). The bioassay was subject to an error of ±15% and values within 10% of the initial potency were considered to represent no activity loss. Neomycin has since been claimed to be autoclavable but no supporting data were presented (Wornick, 1967).

On heating, neomycin may undergo hydrolysis to yield the less antimicrobially active molecule neamine, (Tsuji & Robertson, 1969). Oxidative degradation may also occur and sodium sulphite, sodium metabisulphite or ascorbic acid have variously been reported to suppress this (Simone & Popino, 1955; DeLuca, Plains & others, 1969; McGinity & Brown, 1975). The stabilization of other aminoglycoside antibiotics by disodium edetate (Makino, Hayashi & Okamura, 1960) and polyhydric alcohols (Takenaga & Okada, 1972), has also been described.

Certain official preparations of neomycin permit the inclusion of disodium edetate as a stabilizer in the formulation, but reducing agents and polyhydric alcohols are not used for this purpose. The intention of this work was to determine the stability of neomycin at the temperatures normally used for heat sterilization, and in addition, to examine the effects of sodium metabisulphite and propylene glycol on neomycin stability.

Neomycin sulphate (Sigma, stated to be not less than U.S.P. potency) was assayed by the procedure of McGinity & Hill (1975). In the form described below this is suitable for neomycin sulphate solutions in the concentration range 0·2–2·0 mg ml^{-1}.

A sample (1·0 ml) was added to potassium hydrogen phthalate (2·0 ml, 5% w/v pH 4·0), amaranth (B.P.C. 1954) (2·0 ml, 0·2% w/v) plus water (5·0 ml) in a boiling tube. The contents were mixed, filtered (Whatman No. 50 filter paper) and 1·0 ml of the filtrate diluted to 25 ml. The absorbance of this solution was measured at 520 nm. A linear calibration plot was produced in which the neomycin concentration (0·2–2·0 mg ml^{-1}) was inversely proportional to the absorbance of the filtrate (mean slope −0·1903 s.d. 0·0040 n = 7, intercept corresponding to an absorbance of 0·531, s.d. 0·003, correlation coefficient, 0·9986, df = 7). Corresponding values for neomycin solutions containing disodium edetate were −0·1862 (0·0043) and 0·535 (0·015); those for solutions containing sodium metabisulphite were −0·1914 (0·0038) and 0·541 (0·015) (all n = 7). Comparisons were made for both slope and intercept values between the calibration plots for solutions of neomycin alone and those containing disodium edetate and metabisulphite. Calculated Student t values for the slope and intercept of the calibration plot resulting from the solutions containing disodium edetate were 1·85 and 0·53 respectively; those for the metabisulphite-containing solutions were 0·53 and 1·34. These values do not exceed the tabulated value at the 5% level, of 2·18 (d.f. = 12), thus indicating that there are no real differences between the corresponding means. The mean slope and intercept values for solutions containing 5% v/v propylene glycol were −0·1933 (0·0039), t_{calc} = 1·42 and 0·572 (0·016) t_{calc} = 5·29. Because this intercept was significantly different from that for neomycin alone, assay values for solutions containing 5% v/v propylene glycol were determined by reference to this plot.

The degree of correlation between the colorimetric assay and the agar diffusion assay for neomycin was examined. Solutions of neomycin sulphate in 0·003 M phosphate buffer pH 7·0 were heat-degraded such that their concentrations ranged from approximately 100% to 10% of the initial value of 2·0 mg ml^{-1}. These solutions were assayed colorimetrically after which each was diluted 100 fold in 0·01 M phosphate buffer pH 8·0 for the agar diffusion assay. In this, molten Oxoid Diagnostic Sensitivity Test agar (150 ml) was inoculated with an aqueous suspension of spores of *Bacillus pumilus* N.C.T.C. 8241, to give a concentration of approximately 5×10^7 spores ml^{-1} and poured into a 30 cm square glass assay plate (to a thickness of 5 mm) and 0·1 ml sample and standard solutions were placed in cavities (8 mm diam.) in a Latin Square design. After 24 h at 37° the inhibition zones were measured. Results from an undegraded neomycin solution measured colorimetrically were a mean 1·100 mg ml^{-1} (100·0% theory) s.d. 0·0216 (1·96%) (n = 6): the corresponding values for a second solution using the bioassay were 5·7 μg ml^{-1} ±0·495 (8·7%).

The correlation between the colorimetric and the bioassay was determined on three occasions. The combined values for the three experiments resulted in a line with slope, intercept and correlation coefficient values of 0·975, 0·0337, and 0·996 (d.f. = 16) respectively (Fig. 1). Analysis of variance gave a calculated F value (df = 4/11) of 1·65 which is less than the tabulated value at the 5% level of 3·59 showing that the three lines could be represented by a common line.

The loss of neomycin in a range of heated solutions containing different concentrations of propylene glycol was examined to determine the most suitable concentration for subsequent use. Three ml of the appro-

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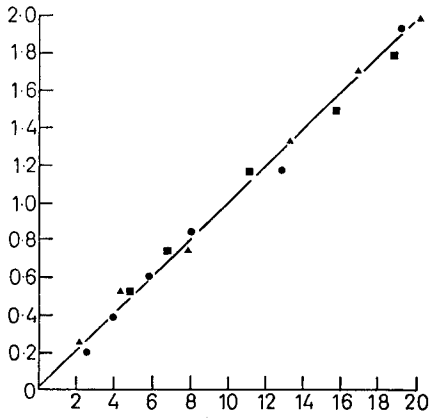

FIG. 1. Correlation of colorimetric and agar diffusion assays for neomycin. ● ▲ ■ Replicate experiments. Ordinate: Potency by colorimetric assay (mg ml^{-1}). Abscissa: Potency by bioassay (μg ml^{-1}).

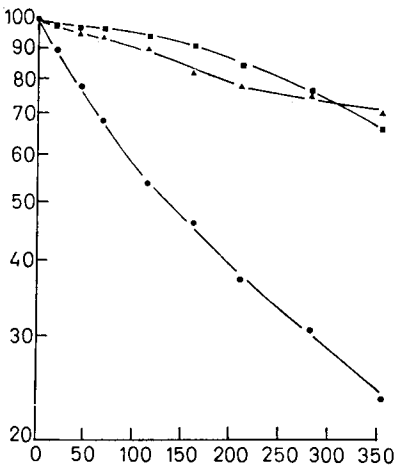

FIG. 2. Influence of sodium metabisulphite and propylene glycol on neomycin degradation. ● 0·2% w/v neomycin; ▲ 0·2% w/v neomycin + 0·5% w/v sodium metabisulphite; ■ 0·2% w/v neomycin + 5% v/v propylene glycol. Ordinate: % initial concentration. Abscissa: Time (h) at 81°.

priate solution was sealed in a 5 ml glass ampoule, leaving 2 ml airspace, and the ampoule was then completely immersed in a water bath at 81 ± 0·05°. This temperature allowed neomycin degradation at conveniently measurable rates and good thermal stability in the bath. The plotted points in Fig. 2 each represent the mean of the assays performed on 3 replicate ampoules from the bath at suitable times. Samples which could not be assayed immediately were stored at <4°.

Inclusion of propylene glycol in the sample solutions resulted in a small increase in the intercept value of the calibration plot of the colorimetric assay. Consequently individual calibration plots were prepared for all concentrations of propylene glycol used in this experiment. When log concentration-time plots were constructed for unsupplemented neomycin solutions or those with varying propylene glycol concentration it was observed that the loss of neomycin did not follow exactly either zero order or first order kinetics. For this reason the percentage of neomycin remaining after 100 h heating was used as a basis for selecting the most suitable propylene glycol concentration (Table 1). Estimations of this parameter gave a mean of 53·6% neomycin remaining, s.d. of 3·0 n = 6. It may be seen from Table 1 that the rate of degradation is much reduced by addition of propylene glycol up to concentrations of 5% v/v. 5% was therefore selected. The concentration of sodium metabisulphite (0·5% w/v) was selected on the basis of its current use in official formulations, e.g. phenylephrine eye drops B.P.C. The stabilizing effects of 5% v/v propylene glycol and 0·5% w/v sodium metabisulphite are illustrated in Fig. 2, which shows the fall in neomycin concentration in solutions heated at 81°.

Discoloration, which has previously been used as a stability guide for neomycin and related antibiotics (Simone & Popino, 1955; Takenaga & Okada, 1972), was also recorded as the absorbance at 450 nm. No peaks could be found in the ultraviolet and visible absorption spectra between 200–800 nm; thus it was not possible to record at a wavelength specific to neomycin decomposition products. In solutions containing 0·5% w/v metabisulphite up to 30% of the neomycin was lost without any discoloration. This activity loss may have been due to hydrolysis. The presence of metabisulphite is unlikely to protect against hydrolytic degradation and may, under certain circumstances, increase the rate of hydrolysis of some materials (Higuchi & Schroeter, 1959; Davies, Meakin & Moss, 1970).

The protective effect of propylene glycol shown is in agreement with the effects of polyhydric alcohols on aminoglycoside stability which were described by Takenga & Okada (1972). It is possible that the effect is due to the preferential oxidation of propylene glycol to the corresponding aldehyde and acid. There is an increase in the slope of the plot for neomycin degradation in the presence of propylene glycol (Fig. 2). The reason for this is unknown but it occurred particularly in

Table 1. *The effect of propylene glycol concentration on neomycin stability.*

Propylene glycol concentration % v/v	Percentage neomycin remaining after 100 h at 81°
0	51
2·5	76
5	96
10	97
15	97

Table 2. *Fall in concentration and discoloration of neomycin solutions on heating.*

	100° for 30 min		115° for 30 min	
	% activity loss	A450	% activity loss	A450
Neomycin	6·4	0·206	10·2	0·280
Neomycin + EDTA 0·01% w/v	4·4	0·094	8·9	0·216
Neomycin + propylene glycol 5% v/v	<2	0·02	2·8	0·040
Neomycin + metabisulphite 0·5% w/v	<2	0·01	<2	0·01
Neomycin + propylene glycol 5% v/v + metabisulphite 0·5% w/v	<2	0·01	<2	0·01

propylene glycol concentrations of less than 10% v/v after a variable period of heating in excess of 100 h at 81°. In the other two solutions containing neomycin and neomycin + 0·5% w/v sodium metabisulphite the slope of the plot was reduced on prolonged heating.

The effects of propylene glycol and metabisulphite were examined further by heating neomycin solutions at sterilization temperatures. This provided a check that the relative effects of the two agents were not substantially changed by an increase in temperature. In this case the basic composition of the heated solutions was identical to that of neomycin eye drops B.P.C. Disodium edetate 0·01 w/v (the inclusion of which is optional in the B.P.C. eye drops), 0·5% w/v metabisulphite or 5% v/v propylene glycol were added as appropriate. 3 ml of each solution was heated in a 5 ml glass ampoule in an oil bath thermostatically controlled to ±0·1°. On removal from the bath, samples were rapidly cooled to room temperature (20°). The results shown in Table 2, are the means of 3 replicate determinations in each case. On heating at 100° or 115° for 30 min none of the solutions containing metabisulphite showed discoloration. Acidic oxidation products of the metabisulphite did not change the pH of the buffered solutions during heating. In all cases the pH of the heated solutions changed by no more than 0·2 pH units. Solutions of neomycin alone, solutions containing disodium edetate or propylene glycol all darkened to an extent which was indicative of the fall in neomycin concentration. The presence of disodium edetate resulted in a small improvement in stability at both tempratures. The inclusion of disodium edetate in several B.P.C. formulae suggests that the chelation of metal ions improved neomycin stability. The presence of calcium has been reported to reduce the antibacterial activity of neomycin. This effect may be related to the calcium binding properties of the antibiotic (Price, Zolli & others, 1957).

The results in Table 2 suggest that buffered neomycin solutions may be autoclaved with a concentration loss of approximately 10%. This figure is much reduced by the inclusion, in these eye drop formulations, of 0·5% w/v sodium metabisulphite. This may be advantageous since the results here show that some discoloration of unsupplemented neomycin solutions may occur after only 30 min exposure at 100°.

Although propylene glycol would be unsuitable for eye drops it is commonly used in topical preparations to increase the activity of some steroids which are often present together with neomycin (Barrett, Hadgraft & others, 1965). Its presence would enhance the stability of neomycin in these products which are particularly susceptible to loss of antibiotic activity due to the incompatibilities which may occur between aminoglycosides and steroids (McGinity & Brown, 1975). Autoclaving neomycin solutions in the presence of propylene glycol might also be convenient for the preparation of sterile topical products which are required in some markets.

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