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A COMPARISON OF THE PROPERTIES OF SOME CHLORAMPHENICOL AND NEOMYCIN EYE OINTMENTS, COMMERCIALLY AVAILABLE IN THE U.K.

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ABSTRACT

The rheology, particle size distribution and drug release, measured by dissolution and agar diffusion techniques, of five B.P. Chloramphenicol Eye Ointments and three B.P. Neomycin Eye Ointments have been examined. All ointments showed structural breakdown during continuous shear rheology. Three chloramphenicol ointments displayed spur values whilst one neomycin ointment displayed a bulge on the up-curve of the rheogram. Mass median particle sizes ranged from 6.0 to 13.0 and 5.4 to 9.6 μm for the chloramphenicol and neomycin ointments respectively. Drug release similarly varied. By dissolution techniques the quantity of chloramphenicol that dissolved in 60 minutes ranged from 22 to 41 μg whilst agar diffusion studies produced drug releases which varied from 2.4 to 4.7 and 0.8 to 2.3 μg for the chloramphenicol and neomycin results respectively.

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INTRODUCTION

Many factors, both physiological and formulation, have been identified as influencing the availability of drugs topically applied to the eye. Physiological influences include the pH of the drug solution (1), the relative lipid-water solubility of the drug (2), the nature of the corneal surface (3) and drug interactions with the lachrymal fluid proteins (4).

The formulation of a drug in an eye ointment will also modify further its availability and other factors, especially drug particle size, vehicle viscosity, rheological type, and the partitioning between the lachrymal fluids and ointments may influence drug availability. Konning and Mital (5) demonstrated that as the particle size of benzoic and salicylic acids decreased,their release correspondingly increased. It was postulated (6) that delivery by ointment rather than by eye drops should prolong ocular contact time, thereby increasing absorption. Mindel (1) recognised that bioavailability is improved by increasing viscosity and corneal contact time. In a discussion (7) on the effect of rheological properties of ointment bases on corneal contact time, it was shown that the use of Newtonian vehicles could lead to a loss of solution through tear drainage that was inversely proportional to vehicle viscosity. Pseudo-plastic and thixotropic systems would undergo shear during blinking and would consequently thin and drain from the eye, reducing corneal contact time. Mixing problems between ointments and tears have been reported (8,9) and consequently a partitioning parameter may further affect drug release between ointments and tears.

In order to minimise many of the effects on availability, it is essential that commercial products should demonstrate similar rheological and drug release profiles. Apart from suggesting Simple Eye Ointment B.P. as a suitable base, the British Pharmacopoeia (1980) offers little guidance on the choice of a suitable eye ointment base. No tests are used to estimate drug

release from eye ointments. Recently (11) the rheology, particle size distribution and drug release of sulphacetamide eye ointments have been examined. The B.P. products released their drugs more slowly than other, non-pharmacopoeial ointments.

In this study, two further B.P. eye ointments containing chloramphenicol and neomycin have been studied. The particle size of the dispersed drug has been determined by image shearing microscopy; rheological properties by cone and plate viscometry and drug release by dissolution and agar diffusion techniques.

MATERIALS AND METHODS

Five Chloramphenicol Ointments B.P., each 1.0% w/w (A_1, A_2, B, C and D) were examined as manufactured by 4 companies. A_1 and A_2 were of different batches but prepared by the same manufacturer. Three Neomycin Ointments B.P., each 0.5% w/w (X, Y and Z) and manufactured by different companies were also examined. Chloramphenicol B.P. (Thornton and Ross Ltd.) and Neomycin Sulphate B.P. (Ayrton-Saunders Ltd.) were used for calibration and standards.

Particle Size Analysis

Small quantities of the ointments were placed on microscope slides and gently streaked out using a clean camel-hair brush. Cover slips were not used in order to minimise particle distortion and aggregate breakdown. Size ranges were estimated using a Double Image Shearing Microscope (Fleming Instruments Ltd.). The lower limit of resolution was 0.2 μm and a minimum of 625 particles were counted for each ointment. Aggregates were defined as containing more than 3 individual particles. Each aggregate was considered as one large particle for the purpose of counting.

Rheology

A cone and plate viscometer (Ferranti-Shirley Ltd.) was used at 37°C with a 1200 g.cm spring and a cone of 7 cm diameter

(0.006173 radians). A maximum rate of shear of 100 rev min^{-1} was employed with a total sweep time of 2 minutes.

In Vitro Release

(a) Agar Diffusion Technique

Chloramphenicol and neomycin releases were estimated against Staphylococcus aureus (NCTC 7447) and Bacillus subtilis (NCTC 6276) respectively. For assay, nutrient agar (Oxoid CM3) plates were seeded, 2% v/v, with 18 hour cultures of the control organisms grown in nutrient broth (Oxoid CM1). Either 150 mg of the ointments or 0.05 ml of the standard drug solutions were placed into wells, 9 mm in diameter, cut into the agar. Standards, equivalent to 0.5 and 5 µg neomycin sulphate and to 2 and 10 µg chloramphenicol were used.

After application of the ointments and controls, the agar plates were held at room temperature for 2 hours to allow diffusion of the drug before incubation at 37°C for 18 hours. The zones of inhibition were measured and drug release calculated in µg against the standards.

Dissolution Studies

Because of difficulties in assaying solutions of neomycin sulphate, only the chloramphenicol ointments were evaluated by this method. The ointments were placed into aluminium vial covers (20 mm internal diameter) and the excess was removed to leave a flat surface, level with the cover edge. The covers were mounted, upturned, onto the lower end of the basket shaft of the B.P. dissolution apparatus and centrally positioned in a 150 ml flat bottomed flask and rotated at 100 rev min^{-1} approximately 2 mm below the surface of the dissolution fluid (100 ml of 0.9% w/v aqueous sodium chloride) maintained at 37°C. Chloramphenicol content was determined spectrophotometrically at 278 nm using flow through facilities.

RESULTS

Chloramphenicol Ointments

The particle sizes were near log-normally distributed (figure 1), although deviations were apparent for ointments C

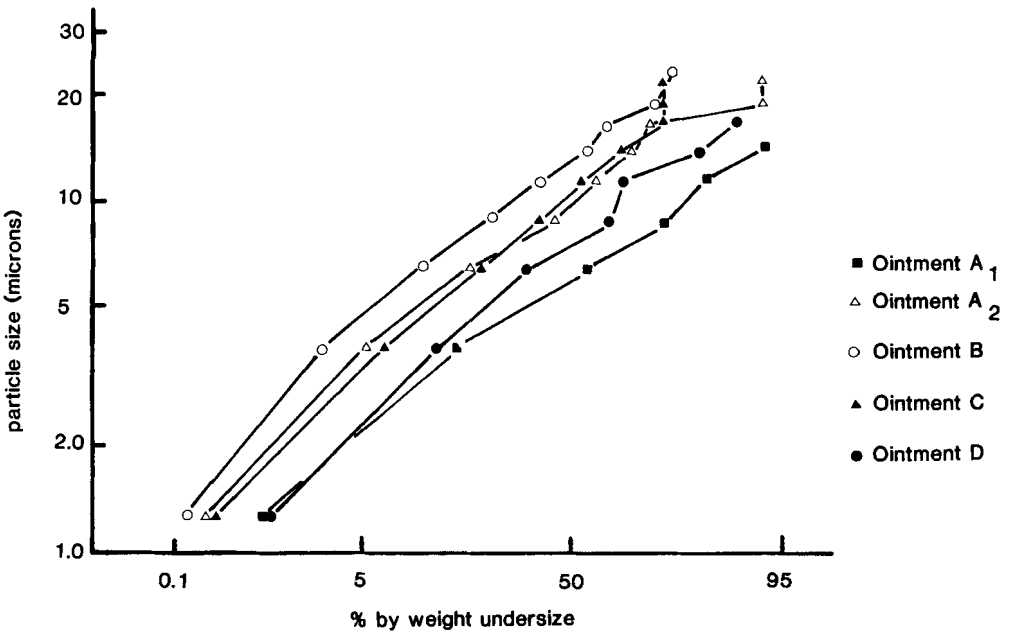

Fig. 1. Particle size distribution of chloramphenicol in Chloramphenicol Eye Ointments B.P., determined by image shearing microscopy.

TABLE 1

The particle size and release characteristics of B.P. Chloramphenicol Eye Ointments.

Ointment	Mass median particle size (μm ± S.D.)	$D_{60\ min}$ * (μg)	Mean release ** of chloramphenicol (μg ± S.D.) by agar diffusion
A_1	6.0 ± 1.8	38	2.7 ± 1.3
A_2	9.6 ± 1.7	32	4.3 ± 0.8
B	13.0 ± 1.9	22	2.4 ± 0.6
C	10.6 ± 2.0	39	4.0 ± 1.2
D	7.9 ± 2.0	41	4.7 ± 1.3

* Mean of 3 determinations.

** Mean and standard deviation of 9 determinations (except B, 11 determinations) assessed against Staph. aureus.

and D. Ointments A_1 and A_2 each contained aggregates which consisted of particles each less than 2.5 μm in diameter. The aggregates consisted of up to 5 particles in ointment A_1 but up to 23 particles in ointment A_2. Ointments B, C and D contained aggregates made up of 3-5 particles which were of various size ranges. The percentages of aggregates to the total number of particles sized were 60.1, 27.2, 20.5, 20.5 and 15.0% respectively for ointments A_1, A_2, B, C and D. The median particle sizes are given in table 1.

Typical rheograms obtained at 37°C are shown in figures 2 and 3. Ointments A_1, B and D gave similar rheograms, each showing spur values on their up-curves. The rheograms of A_2 and C did not show spur values but only yield values, and ointment C was the most viscous ointment (figure 3). All rheograms were anticlockwise hysteresis loops indicative of structural breakdown induced by shear.

The release, assessed by agar diffusion, is given in table 1. Although the ointments may be ranked in decreasing order of

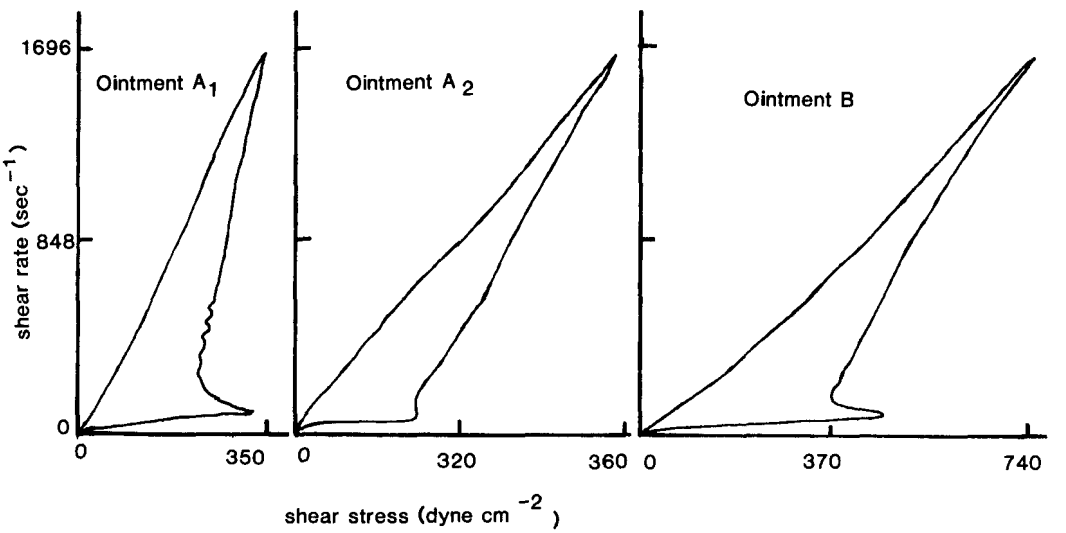

Fig. 2. Continuous shear rheograms of Chloramphenicol eye ointments A_1, A_2 and B.

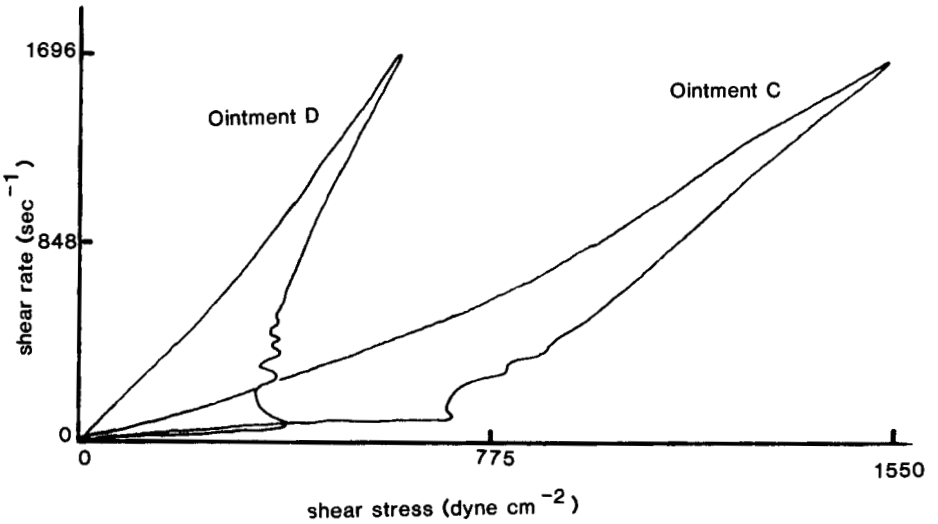

Fig. 3. Continuous shear rheograms of Chloramphenicol eye ointments C and D.

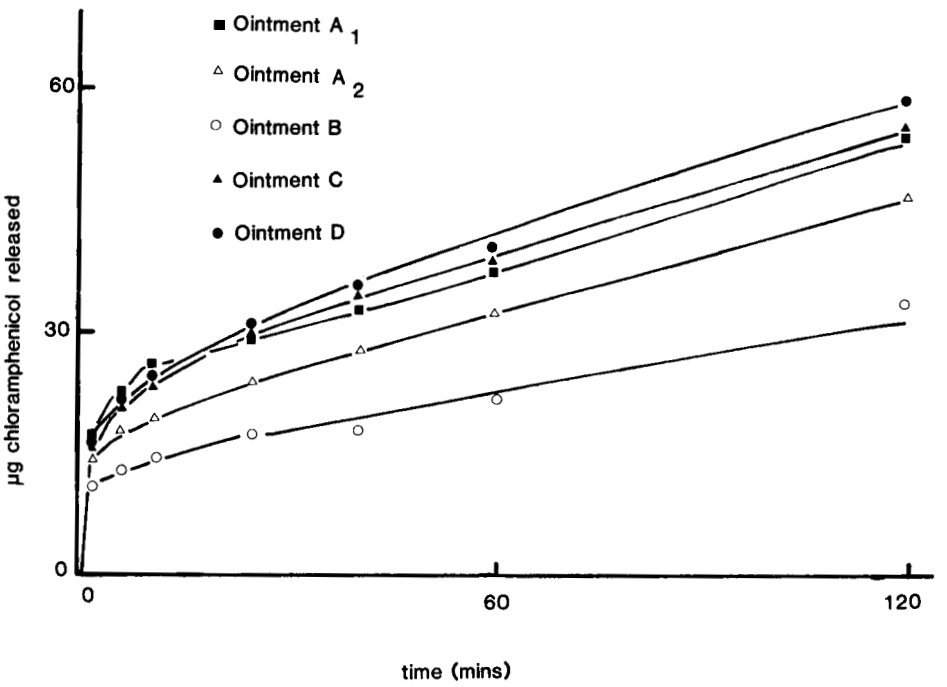

Fig. 4. Dissolution profiles of Chloramphenicol Eye Ointments. B. P. into 0.9% NaCl and 37 C.

release as D > A_2 > C > A_1 > B, statistically ($p \leq 0.05$), only two groups could be discerned; D, A_2 and C having higher releases than A_1 and B. There was no statistical difference ($p \geq 0.5$) between the amounts released from the ointments in each group.

The dissolution of chloramphenicol from the ointments is displayed in figure 4. The amount of drug released after 60 minutes ($D_{60\ min}$) is given in table 1. The release profiles for D, C and A_1 were similar and higher than A_2 and B, the latter possessing the poorest release. Similarly the $D_{60\ min}$'s were in the range 32-41 µg for all ointments except ointment B where the $D_{60\ min}$ was 22 µg.

Neomycin Ointments

The particle size distributions are shown in figure 5 and their calculated mean particle size in table 2. No agglomerates were found in the ointments studied.

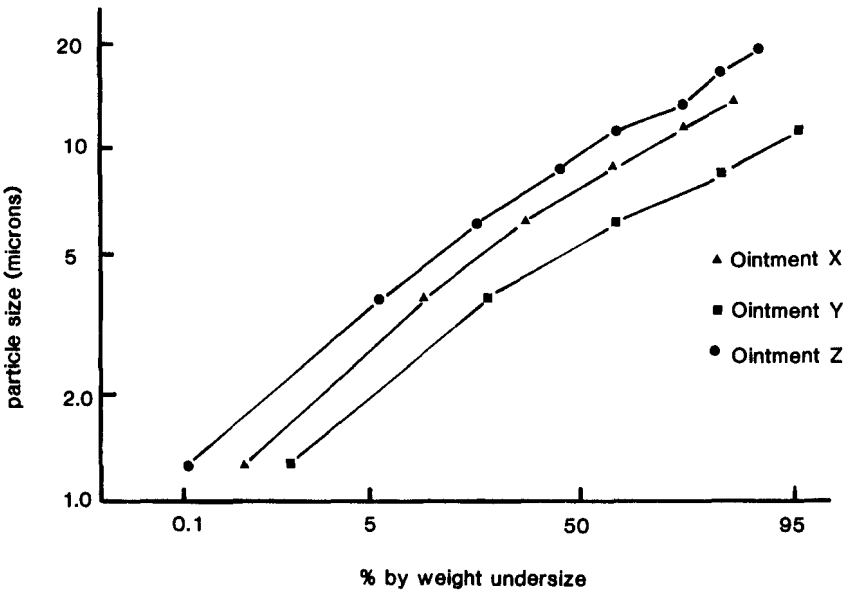

Fig. 5. Particle size distribution of neomycin sulphate in Neomycin Eye Ointments. B.P. determined by image shearing microscopy.

TABLE 2

The particle size and release characteristics of B.P. Neomycin Eye Ointments.

Ointment	Mass median particle size (μm ± S.D.)	Neomycin release * (μg ± S.D.) by agar diffusion
X	7.8 ± 1.8	1.2 ± 0.4
Y	5.4 ± 1.7	2.3 ± 1.0
Z	9.6 ± 1.7	0.8 ± 0.4

* Mean and standard deviation of 24 determinations, assessed against Bacillus subtilis.

The rheograms of ointments X and Z (figure 6) were similar; each displaying pseudoplastic, anticlockwise hysteresis loops with yield values. However the rheogram of ointment Y (figure 6) possessed a bulge on the up-curve indicative of an increase in ointment structure during shear. Again an anticlockwise hysteresis loop was obtained. Ointment Y was the most viscous.

The agar diffusion release (table 2) indicated differences in drug release. The order of release was Y > X > Z; each ointment possessing a release statistically different from those of the other ointments ($p < 0.001$). Thus from table 2 it would appear that an inverse relationship between drug release and particle size may exist.

GENERAL DISCUSSION

The release of drugs from ointments has been shown to be particle size dependent (5). Although for chloramphenicol little correlation was found between the two methods of assessing drug release, ointment B gave the poorest performance in both the diffusion and dissolution releases. It is probably significant that this ointment possessed the highest mean drug particle size (table 1).

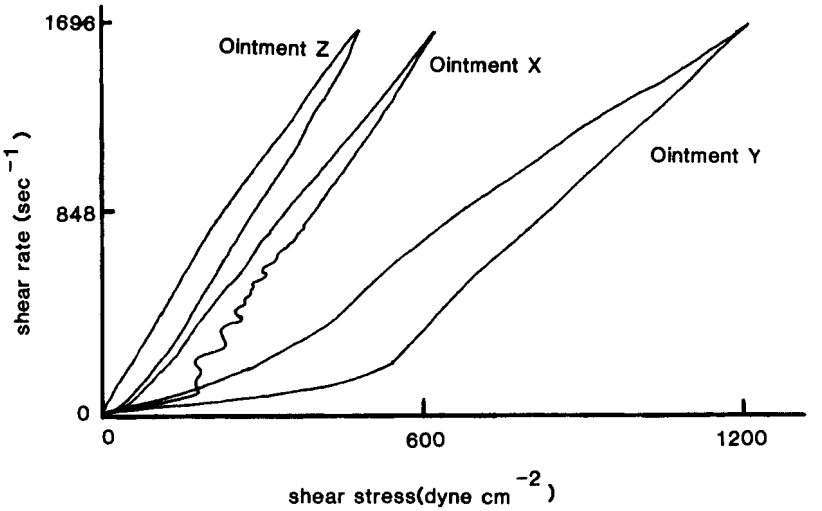

Fig. 6. Continuous shear rheograms of neomycin eye ointments X, Y, and Z

It is generally recognised that application to the eye of particles above a certain size may cause corneal damage. Estimations of this critical size include 10 μm (9) and 20-25 μm (12). Only B and C of the chloramphenicol ointments studied (figure 1) possessed considerable quantities of particles (> 20% by weight) above 20 μm in diameter. No neomycin ointment possessed more than 10% by weight of their particles in excess of 20 μm diameter (figure 5). If 10 μm is taken as the critical size that causes corneal irritation, chloramphenicol ointments A_2, B and C and neomycin ointment Z contained 40% of their particles by weight in excess of 10 μm.

Table 2 shows that for the neomycin ointments the microbiological release increased as the particle size decreased. Since there is an increased likelihood that the ointments containing the largest size particles may cause corneal irritation and that such ointments also release their drugs more slowly than ointments containing smaller particles, the order of suitability of the neomycin ointments could be ranked as Y > X > Z.

It may be argued that passive in vitro dissolution and agar diffusion bear little comparison with the ophthalmic in vivo situation, since both only measure release from an unsheared ointment surface. However only the crystals at the ointment-tear interface would go directly into solution and be available for ocular absorption (13). Similarly Sieg and Robinson (8) postulated that drugs in an ointment dosage form would, irrespective of the diffusion characteristics of the drug in the vehicle, have to partition from the ointment to the tear film (or the epithelium directly) in order for corneal absorption to occur.

Ointment rheological properties may further influence drug availability (9). Sulphacetamide eye ointments have previously been examined (11) and found to be pseudoplastic. Consequently, on shear (eg. blinking), these may thin and drain from the eye resulting in a decreased corneal contact time. The rheograms of the chloramphenicol eye ointments (figures 2 and 3) all showed either spur values or yield values. Interpretation of such phenomena would mean that the ointments may all be difficult to extrude from the tube until that spur or yield value had been exceeded, or that the ointment would not spread across the eye surface on blinking, resulting in a small area of ointment-tear contact and consequently poor drug release. Once the yield or spur value had been exceeded the ointments would shear and thin and consequently drain more rapidly from the eye (7). Since ointment C was the most viscous (figure 2), this ointment should possess the longest corneal retention time.

The rheograms of the neomycin ointments X and Z displayed anticlockwise hysteresis loops and yield values (figure 6). Patton and Robinson (7) stated that the ocular effects of such systems are good provided the yield value was not exceeded when the ointment would again behave pseudoplastically and drain from the eye. However the rheogram of ointment Y (figure 6) displayed a bulge on the up-curve, characteristic of a build up of structure with shear, which may form a three dimensional gel-like

structure (14). Such differences in rheological properties may result in different retention properties and resistance to spreading induced by blinking.

Ideally for eye ointments their in vitro properties should be consistent in order to minimise differences in ocular availability. With regards to diffusion and dissolution properties, 4 of the chloramphenicol ointments were similar (A_1, A_2, C and D). However ointment B possessed both the largest particle size and the poorest release. Similarly differences were observed in the particle size distribution and release from the neomycin eye ointments studied. Such differences, as have been found, need to be investigated in the clinical situation.

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