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Original Article

OCULAR TOLERANCE AND *IN-VITRO* RELEASE OF CHLORAMPHENICOL IN PROSPECTIVE EYE OINTMENT BASES

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ABSTRACT

Objective: Chloramphenicol is poorly released from official eye ointment (EO) base. This study investigated alternative promising bases, enhanced drug release with surfactant and evaluated ocular tolerance of prototypes.

Methods: Chloramphenicol eye ointment (1 %w/w) was prepared by levigation in five bases: EO, simple ointment (SO), hydrous wool fat (HW), hydrous sheabutter (HS), and neat sheabutter (NS). In-vitro drug release of these and EO and SO formulations containing graded concentrations (0.1–10.0 %w/v) of surfactant (polysorbate 80 or propylene glycol) were studied over 3 h by dialysis technique, and the release kinetics assessed. Water-absorption capacity (WAC) and melting temperature of the bases was determined for probable influence on drug release. Ocular tolerance of the formulations, bases and surfactants was evaluated by *in vivo* irritancy test on albino rabbits.

Results: Total drug released from test formulations differed with base used: NS 12, HS 11, SO 7, HW 5, and EO 4 %. The Higuchi release kinetics was determined. HS and NS demonstrated highest release extent and rate; EO and HW exhibited poor release. WAC and softening temperature of bases showed some correlation with drug release propensity. Propylene glycol in EO and polysorbate 80 in SO formulations showed 3-fold and 2-fold enhanced drug release, respectively. All items for ocular toxicity test gave scores indicating practically no irritation, except neat HW that was mildly irritant.

Conclusion: HS and NS proved best alternatives to EO. Propylene glycol (10 %) in the EO formulation was 3-fold drug-release enhanced. All prototype formulations were non-irritant to eye tissue.

Keywords: Chloramphenicol, Ointment, Base, Surfactant, *In-vitro* Release, Ocular tolerance.

INTRODUCTION

Chloramphenicol is a broad-spectrum antibiotic often applied topically to conjunctiva for treatment of infections caused by susceptible microorganisms. It is the drug of choice in ocular infections because of its high transcorneal penetration and broad spectrum activity [1] and is administered as ophthalmic drops or ointment. The ointment offers an advantage of longer contact time over the solution drug form that suffers rapid and extensive pre-corneal loss due to high lachrymal fluid turn over and drainage rate [2]. The potency of a topical medication is markedly influenced by its vehicle, e. g. an ointment base [3], and the corneal absorption of the administered drug may be enhanced by the presence of a surfactant [4]. Non-ionic surfactants (such as polysorbate 80) are found to be less toxic than anionic and cationic surfactants in predictive ocular irritancy studies [5] and are uniquely suited for topical ocular drug delivery through their potential ability to increase bioavailability by increasing drug solubility, prolonging pre-corneal retention, and enhancing permeability [6]. Polysorbate 80 (aqueous solution) has been used to produce nanoparticles for ocular delivery of hydrocortisone [7] and as a surfactant component of ocular indomethacin microemulsion [8]. Propylene glycol is a water-diffusive liquid with surfactant property and useful as cosolvent and skin penetration enhancer for poorly soluble compounds in topical medicinal products [9]. When tested on rabbit eye, neat (undiluted) propylene glycol showed some measure of ocular irritation [10]. Also, occupational exposure to propylene glycol vapour has been reported to cause ocular irritation in human subjects [11]. However, dilutions of propylene glycol (at use concentrations) are generally non-toxic [12]. *In vivo* Draize eye test in rabbit [13] is the international standard assay for acute ocular toxicity [14].

Eye ointment basis BP (EO) is a British Pharmacopoeial (standard) eye ointment vehicle generally recommended for use in eye ointment preparations [15]. But preliminary study in our laboratory suggested that chloramphenicol is poorly released from EO. Also, a vehicle for eye ointment medication is required to be non-irritant to the conjunctiva [15]. Yet, no information is found in literature on irritancy appraisal or ocular tolerance of EO, which should authenticate its suitability and general acceptability for ophthalmic use. Although the main components of EO (liquid and soft paraffin) are common pharmacopoeial ointment vehicle components which, in earlier ocular irritation studies, had been used as plain diluent or medium [16, 17], the lack of any literature data indicative of the ocular tolerance of EO or its components, even in later report on predictive eye irritation potentials of several substances in current use [18], is quite undesirable.

Other established (pharmacopoeial) ointment bases were having similar components (paraffin, lanolin) to EO namely, simple ointment BP (SO) and hydrous wool fat BP (HW) are, however, for dermatologic use. These and sheabutter bases (hydrous sheabutter [HS] and neat sheabutter [NS]) are investigated in this study, being considered as prospectively useful alternative ophthalmic ointment vehicles for chloramphenicol. Sheabutter, extracted and purified from *Vitellaria paradoxa* C. F. Gaertn (Sapotaceae) nuts, has been earlier studied for use as a dermatologic ointment vehicle and its utility found comparable to that of established bases [19, 20]. However, for lack of satisfactory, unequivocal information on its safety for human use, sheabutter was listed by the European Commission 2006 among substances not exempted from registration [21].

A valuable dermatologic vehicle, nonetheless, holds promise for advantageous ophthalmic application if it is innocuous on the eye tissue. Ocular tolerance testing of promising dermal ointment vehicles could lead to expansion of their utilities. Hence it was adjudged important to ascertain, by testing, the ocular toxicity potential of EO and of the propitious dermatologic bases: SO, HW, HS and NS as necessary premise for recommendation or proscription of their ophthalmic use. Therefore the objectives of this study were to determine ocular irritation potential of the bases and of chloramphenicol eye ointment prepared with each

base in the absence and presence of polysorbate 80 or propylene glycol as drug-delivery enhancement surfactant; and to evaluate drug release propensity of the formulations.

MATERIALS AND METHODS

Materials

Chloramphenicol in powder, anhydrous (SIGMA); cellulose dialyzer tubing (SIGMA); propylene glycol (May and Baker, England); cetostearyl alcohol, lanolin anhydrous (British Drug Houses, England); polysorbate 80, hard paraffin, liquid paraffin, white soft paraffin (William Ransom & Sons. England); neat sheabutter, freshly processed from shea nuts (National Center for Agricultural Mechanization, NCAM, Ilorin Nigeria).

Methods

Preparation of bases for chloramphenicol eye ointment

Five ointment bases (EO, SO, HW, HS, and NS; table 1) were prepared. The EO, SO, and HW compounded according to compendium guidelines [15], and HS produced from NS were prepared sterile. EO and SO were dry-heat sterilized (BP procedure) while HW, HS and NS were oven-melted (≤ 60 °C) and sterilized by membrane filtration aided by suction pump. Freshly sterilized HS and NS base samples having polymorphic property were kept (undisturbed) at the ambient temperature (28±2 °C) for 5 d before use, to allow full restoration and stability of their physical characteristics and semisolid consistency [22].

Table 1: Composition of ointment bases

	Name of base/Ingredient concentrations (%w/w)			
Ingredients	**Eye ointment basis BP**	**Simple ointment BP**	**Hydrous wool fat BP**	**Hydrous sheabutter**
Soft paraffin	80	85	–	–
Wool fat	10	5	75	–
Liquid paraffin	10	–	–	–
Hard paraffin	–	5	–	–
Cetostearyl alcohol	–	5	–	–
Water (purified)	–	–	25	18
Neat sheabutter	–	–	–	82

Key:–Ingredient not contained in base

Determination of softening/melting point and water-absorption capacity of ointment bases

Softening and melting temperatures of the bland bases were determined using the method for suppository bases as described by Adebayo and Akala [23]. The temperature at which the base sample began to liquefy was defined as the softening point, while the temperature of complete liquefaction was the melting point. The mean and standard deviation (SD) of four determinations was recorded. The possible influence of water-absorption capacity (WAC) of ointment base on drug release propensity of the composite (medicated) ointment formulation was assessed. Mean WAC of each base was determined from quadruplicate tests at the ambient temperature, using the pharmacopoeial method described for hydrous wool fat [15].

Preparation of medicated ointment

Chloramphenicol eye ointment (1 %w/w) samples, 20 g each, were prepared at the ambient temperature with each of the five ointment bases by levigation method and aseptic processing, for subsequent eye irritation and drug release testing. The medicated ointment samples in EO and SO containing either propylene glycol or polysorbate 80 (surfactant) at graded concentrations (0.1, 0.5, 1.0, 5.0 or 10.0 % w/v) were also prepared for drug-release enhancement testing. The requisite amounts of the drug (≤ 90 µm particle size) and surfactant were incorporated into each base, respectively. Each preparation was aseptically packaged in sterile disposable 2 or 5-ml capacity hypodermic syringes from which to be dispensed at the point of use, and kept for a minimum of 24 h at the ambient temperature before testing, to permit equilibration with the environment.

In-vitro drug release studies

The release rate of chloramphenicol from each medicated ointment sample was determined using an *in-vitro* dialysis technique described earlier [19]. The release compartment was a 4 g medicated ointment sample enclosed in 8 cm long cellulose dialyzer tube previously hydrated in water for 24 h at room temperature, suspended vertically on the rotary shaft of an Erweka dissolution apparatus (Heusenstamm Kr. Offenbach, Germany) and set to revolve at 50 rpm. The dissolution medium was a phosphate buffer solution (900 ml, pH 7.2) maintained at 37±0.5 °C, simulating lachrymal fluid pH [24]. Samples of the dissolution-medium (5 ml each) were withdrawn at specified time intervals for 180 min and assayed for chloramphenicol. The volume of dissolution medium was kept constant by replacing withdrawn volumes with equal amount of fresh medium maintained at the same temperature. The amount of drug in withdrawn samples was analyzed spectrophotometrically at 278 nm (JENWAY 6305, UK). A calibration graph was generated from a concentration range of the drug (0.5 to 4.0 mg/ml) prepared in pH 7.2 phosphate buffer solution which showed a λmax of 1.239 UV absorbance at 278 nm wavelength, from which the amount of drug in solution at each sampling time was determined.

In vivo study

Animal ethics and experimental conditions

The study was approved by the Health Research and Ethics Committee of Obafemi Awolowo University (OAU) Ile-Ife Nigeria (Ref. no. OAUTHC/CS/232/Vol. V/268), and executed according to the Test Guidelines no. 405 of the Organization for Economic Cooperation and Development (OECD) in adherence to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the use of animals in ophthalmic and vision research. Healthy, New Zealand White albino rabbits (0.7–2.0 kg) of both sexes without pre-existing ocular irritation was selected for the study. The females were nulliparous and non-pregnant. The animals were nurtured in the animal house of the Faculty of Pharmacy, OAU Ile-Ife; housed in 56 × 56 × 46 cm well ventilated stainless steel suspended cages, and were provided with standard feed from the OAU Biological Gardens Unit, occasionally supplemented with herb (*Tridax procumbens* Linn), and water *ad libitum*. The animal room was maintained at temperature 26-30 °C, humidity 50-80 %, and light/dark cycle 12h/12h conditions. Prior to dose initiation, the rabbits were acclimatized to laboratory conditions for 21 d.

Primary eye irritation test on rabbits

The potential of each test item to cause irritation from a single ocular instillation in a group of 3 rabbits as experimental bio-model was assessed as recommended by the OECD, to determine the ocular tolerance profile. The test items were: 0.1 g of each ointment base and of the medicated ointment prepared in each base, and 100 µl of each surfactant solution (10 %w/v) in purified water. Three animals were used per test substance, which was instilled into the conjunctival sac of the right (test) eye of each rabbit by gently

pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for ~ 1 s to avoid loss of the test substance. The left eye of each animal remained untreated as control. Following treatment, ocular irritation was evaluated macroscopically using a high-intensity white light (Mag Lite) at 1, 24, 48, and 72 h post-instillation.

To minimize the number of animals used, any test animal showing no untoward response to applied treatment was reused once only, after a 14-day rest/re-acclimatization period under the laboratory conditions. Twenty-four animals in all (10 males, 14 females) were used in the study. Individual eye irritation scores were recorded for each animal. Absence of corneal damage at 24 h was verified by fluorescein dye evaluation. Ocular lesions were scored according to the Scale for Scoring Ocular Lesions [25]. Any other observed lesions were noted in addition to observations of the cornea, iris and conjunctivae. The rabbits were also monitored at least once daily for signs of gross toxicity or behavioral changes during the test period. The average score for all 3 rabbits per group at each scoring point was calculated. For evaluation of the overall eye irritation scores, the time interval with the highest mean score (Maximum Mean Total Score, MMTS) for all rabbits in the group was used to classify the test substance in accordance with the scoring system of Kay and Calandra [26].

Data analysis and statistics

The extent of drug release was assessed from the total amount of drug present in the dissolution medium at the end of 180 min. The drug release kinetics applicable for the ointment samples was evaluated by analyzing with three mathematical models: zero-order kinetics (Q vs t), diffusion-controlled (or Higuchi) model (Q vs square-root of t), and first order kinetics (log[Qo–Q] vs t), where Q is the amount of drug released at time 't' and Qo is the initial amount of the drug. The model that consistently produced the highest correlation for the ointment preparations was used for assessment of drug release rates, and the slope obtained from linear regression analysis of the plot was determined as the drug release rate constant. The results expressed as mean±SE (standard error of the mean percent drug-released values of pooled data) were generated from triplicate determinations for each ointment formulation. The data were subjected to *t* tests, analysis of variance (ANOVA) and F-test to determine significance of mean drug-released value differences.

RESULTS

Amounts and profile of chloramphenicol release from ointment bases

The total amount of drug released from the chloramphenicol eye ointment formulations over 3 h testing period depended on the ointment base used. Over the period, the neat bases: NS released 12 %, HS 11 %, SO 7 %, HW 5 %, and EO 4 % of the drug (fig. 1). Also, while NS and HS gave gradual increase of the drug released over 3 h, the SO, HW and EO formulations exhibited a drug release peak and no further appreciable release beyond 60, 120 and 120 min, respectively.

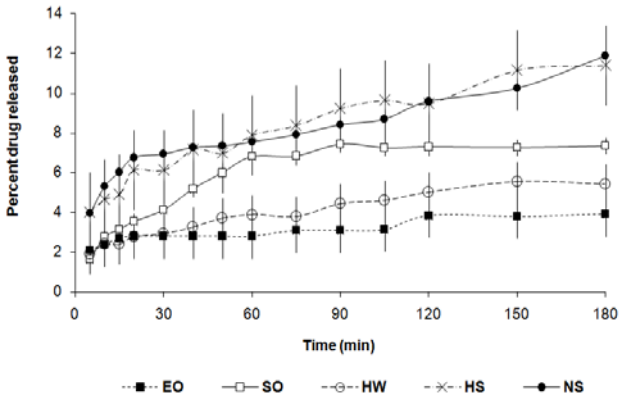

Fig. 1: Release profile of chloramphenicol (1 %w/w) eye ointment in different bases

For determination of chloramphenicol release kinetics, consistent highest correlation of linear plots occurred when the amounts of drug released were plotted against the square root of time in accordance with the Higuchi kinetics. The correlation coefficient values obtained for most data of other kinetic models (zero and first order) were consistently lower (table 2), implying preponderance of the diffusion-controlled release mechanism for the drug.

Table 2: Diffusion-controlled rates and correlation coefficients of chloramphenicol release mechanism profiles from ointment bases

Ointment base	Diffusion-controlled model		First-order model correlation coefficient	Zero-order model correlation coefficient
	Release rate constant (mg. min$^{-½}$)	Correlation coefficient		
Eye ointment basis BP	0.07	0.88	0.88	0.88
Simple ointment BP	0.27	0.87	0.72	0.71
Hydrous wool fat BP	0.17	0.98	0.94	0.94
Hydrous sheabutter	0.34	0.98	0.94	0.94
Neat sheabutter	0.28	0.94	0.93	0.92

The chloramphenicol release rate constant values in the ointment bases ranked in the order: HS (0.34)>NS (0.28)>SO (0.27)>HW (0.17)>EO (0.07 mg. min$^{-½}$). Thus, HS and NS demonstrated greater release potential for chloramphenicol than the pharmacopoeial bases (SO, HW and EO). When analyzed by ANOVA test, the release profile of chloramphenicol in the different ointment groups overall (i.e. by F test) differed significantly from one another (*P>0.05). However, *t* test comparisons showed that the release data of HS compared to NS formulations was not significantly different (*P<0.05).

Effect of water-absorption capacity and softening temperature of base on drug release

The WAC and softening of the bases apparently influenced their drug release propensity. With exception of HW, the bases (HS and NS) softening at ~37 °C and having relatively lower WAC values showed greater propensity for release of chloramphenicol than the bases (EO and SO) with higher softening temperature and WAC values (table 3). 37 °C is mammalian mean body temperature and the *in-vitro* test condition. Inclusion of a surfactant as a component of chloramphenicol eye ointment enhanced drug release from the EO and SO formulations selectively. Thus 10 % propylene glycol increased the amount of drug released in EO ointment from 4 % (over 3 h) in an absence of the surfactant (fig. 1) to 13.5 % (in ≥1.5 h) when the surfactant was present (table 4).

This represents more than three-fold enhancement of the drug release capacity of the base. Similarly, 10 % polysorbate 80 raised the drug released in SO ointment from 7 % (over 3 h) in absence of the surfactant to 18.9 % (in≥2 h) when the surfactant was present,

which represents more than two-fold enhancement of the drug liberating potential of the base. However, the presence of propylene glycol and polysorbate 80 (surfactant) in SO and EO ointments, respectively, did not appreciably improve drug release (table 4).

Table 3: Melting temperatures and water absorption capacity of ointment bases

Ointment base	Heat susceptibility		Water-absorption capacity (ml/10 g)†
	Softening temperature (°C)	Melting temperature (°C)	
Eye ointment basis BP	38.5±0.5	44.0±0.5	35.0 (0.4)
Simple ointment BP	40.0±0.5	46.0±0.5	36.5 (0.4)
Hydrous wool fat BP	37.0±1.0	43.0±2.0	20.5 (0.4)
Hydrous sheabutter	37.5±2.0	42.5±1.5	10.5 (0.2)
Neat sheabutter	37.0±0.5	38.5±0.5	19.0 (0.2)

Key:-Mean values; with SD values in parenthesis

Table 4: Effect of surfactant on release of chloramphenicol from ointments prepared with Eye ointment BP or Simple ointment BP base

Time (h)	ıcentration of surfactant (%w/v)	*Base*: Eye ointment BP		*Base*: Simple ointment BP	
		Surfactant/Mean percent drug released[a]		Surfactant/Mean percent drug released[a]	
		Propylene glycol	Polysorbate 80	Propylene glycol	Polysorbate 80
	0[b]	2.8 (1.1)	2.8 (1.1)	4.1 (0.4)	4.1 (0.4)
	0.1	5.7 (2.0)	0.7 (0.5)	0.9 (1.0)	8.5 (6.0)
0.5	0.5	4.1 (2.0)	2.5 (5.1)	1.9 (2.0)	7.7 (5.0)
	1	3.9 (1.1)	4.7 (2.0)	2.2 (2.3)	8.5 (2.0)
	5	4.8 (0.8)	3.2 (2.0)	3.0 (1.5)	5.1 (1.5)
	10	8.0 (1.0)	4.5 (2.0)	8.1 (1.0)	10.6 (1.0)
	0[b]	2.8 (1.1)	2.8 (1.1)	6.8 (0.4)	6.8 (0.4)
	0.1	5.7 (2.0)	0.8 (0.5)	2.2 (1.0)	11.2 (6.0)
1.0	0.5	5.0 (2.0)	4.8 (5.1)	3.4 (2.0)	9.4 (5.0)
	1	4.3 (1.1)	4.7 (2.0)	3.4 (2.3)	9.3 (2.0)
	5	5.9 (0.8)	4.8 (2.0)	3.9 (1.5)	8.3 (1.5)
	10	13.2 (1.0)	5.1 (2.0)	8.1 (1.0)	14.7 (1.0)
	0[b]	3.1 (1.1)	3.1 (1.1)	7.5 (0.4)	7.5 (0.4)
	0.1	5.7 (2.0)	0.8 (0.5)	2.8 (1.0)	13.9 (6.0)
1.5	0.5	5.0 (2.0)	5.7 (5.1)	4.3 (2.0)	11.7 (5.0)
	1	4.3 (1.1)	4.7 (2.0)	4.7 (2.3)	9.3 (2.0)
	5	6.2 (0.8)	5.9 (2.0)	5.3 (1.5)	9.8 (1.5)
	10	13.5 (1.0)	6.1 (2.0)	8.1 (1.0)	17.4 (1.0)
	0[b]	3.8 (1.1)	3.8 (1.1)	7.3 (0.4)	7.3 (0.4)
	0.1	5.7 (2.0)	0.9 (0.5)	3.0 (1.0)	15.1 (6.0)
2.0	0.5	5.0 (2.0)	6.4 (5.1)	4.9 (2.0)	13.3 (5.0)
	1	4.3 (1.1)	4.7 (2.0)	4.7 (2.3)	9.3 (2.0)
	5	6.2 (0.8)	6.9 (2.0)	6.1 (1.5)	12.1 (1.5)
	10	13.5 (1.0)	6.9 (2.0)	8.1 (1.0)	18.9 (1.0)
	0[b]	3.8 (1.1)	3.8 (1.1)	7.3 (0.4)	7.3 (0.4)
	0.1	5.7 (2.0)	0.9 (0.5)	3.9 (1.0)	15.5 (6.0)
2.5	0.5	5.0 (2.0)	7.2 (5.1)	5.6 (2.0)	15.5 (5.0)
	1	4.3 (1.1)	4.7 (2.0)	4.7 (2.3)	9.3 (2.0)
	5	6.2 (0.8)	6.9 (2.0)	6.6 (1.5)	14.8 (1.5)
	10	13.5 (1.0)	6.9 (2.0)	8.1 (1.0)	18.4 (1.0)
	0[b]	3.9 (1.1)	3.9 (1.1)	7.4 (0.4)	7.4 (0.4)
	0.1	5.7 (2.0)	0.9 (0.5)	4.0 (1.0)	15.8 (6.0)
3.0	0.5	5.0 (2.0)	7.2 (5.1)	5.6 (2.0)	15.8 (5.0)
	1	4.3 (1.1)	4.7 (2.0)	4.7 (2.3)	9.3 (2.0)
	5	6.2 (0.8)	6.9 (2.0)	7.3 (1.5)	14.4 (1.5)
	10	13.5 (1.0)	6.9 (2.0)	8.1 (1.0)	18.9 (1.0)

Key:-[a]Standard error values in parenthesis, [b]0 % surfactant preparations contained no surfactant

Acute ocular irritation results

All the test animals appeared healthy and were active. The triplicate animals eye testing produced no ocular irritation with propylene glycol (10 %) and 7 ointments (namely, four neat bases: EO, SO, HS, NS; and chloramphenicol ointment prepared in SO, HW, and NS). The other test items caused irritation to different measures: Two of 3 animals treated with neat HW showed corneal opacity, observed 1 h after instillation, and partial conjunctiva swelling occurred in 1 of the 2 positive corneal opacity cases. One of 3 animals showed conjunctiva redness within 1 h of administering polysorbate 80 (10 %), chloramphenicol in HS and chloramphenicol in EO ointments. The drug in EO ointment also produced mild swelling of the eye lid. However, in every case, all the irritation signs were absent at the 24 h assessment. Apart from the eye irritation results noted above, there were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. Table 5 shows the individual total and group mean ocular irritation scores. The maximum mean total score of 0.67 was obtained for polysorbate 80 (10 %) solution and for chloramphenicol in HS ointment; the drug in EO gave MMTS of 1.33, while neat HW produced the highest score of 14.0. According to the Kay and Calandra classification system, all the test items proved practically non-irritant except neat HW that was classified as mildly irritant.

Table 5: Individual total scores and group mean scores for ocular Irritation

Test item/Rabbit ID/No; Sex (M/F)	Time-interval of observation/Individual total score			
Polysorbate 80 solution (10 %w/v)	1 h	24 h	48 h	72 h
0061; F	2	0	0	0
0095; F	0	0	0	0
0023; F	0	0	0	0
Group Total	2	0	0	0
Group Mean Score	0.67	0	0	0
Chloramphenicol (1 %w/w) in Hydrous Sheabutter	1 h	24 h	48 h	72 h
0161; F	0	0	0	0
0158; M	2	0	0	0
0164; F	0	0	0	0
Group Total	2	0	0	0
Group Mean Score	0.67	0	0	0
Chloramphenicol (1 %w/w) in Eye ointment basis	1 h	24 h	48 h	72 h
0141; F	4	0	0	0
0088; F	0	0	0	0
0133; M	0	0	0	0
Group Total	4	0	0	0
Group Mean Score	1.33	0	0	0
Hydrous Wool Fat (neat)	1 h	24 h	48 h	72 h
0067; M	0	0	0	0
0052; F	20	0	0	0
0033; M	22	0	0	0
Group Total	42	0	0	0
Group Mean Score	14	0	0	0

DISCUSSION

This study meets the need to conduct ocular tolerance testing of eye ointment basis BP in order to investigate and validate its general acceptance as the standard ophthalmic ointment vehicle. Erstwhile untested probability that selected dermal ointment vehicles may be suitable for ophthalmic drug delivery has also been elucidated. The corneal epithelium is more permeable to medicinal agents than skin epithelium [27]. The *in vivo* rabbit eye test approximates to human physiological and anatomical characteristics and response [28], hence it was appropriate for the study.

The drug release mechanism of chloramphenicol from the bases was determined as Higuchi (diffusion-controlled) model. Similar earlier studies reported fluconazole to be released from water-soluble ointment bases following zero order kinetics [29] and metronidazole released from different ointment base types following the Higuchi kinetics [19]. The selective enhancement of release of chloramphenicol from the EO and SO formulations is not unusual. Earlier studies indicated that inclusion of surfactant in semisolid drug delivery vehicle may sometimes increase [30] or decrease the release of incorporated drug [31, 32]. Characteristics demonstrated by neat HW base in this study were unique and different from those of other bases namely, non-conformity of its drug release potential with the observed trends against WAC and temperature susceptibility, and its (mild) ocular irritancy potential.

CONCLUSION

Chloramphenicol eye ointment formulations in five different bases, with and without surfactant, investigated in this study were all well tolerated and non-irritant to the eye. One test item only (neat HW) gave an overall eye irritation score (MMTS) of mild irritancy. All the four neat bases investigated in parallel as alternatives to EO showed greater propensity for delivering the drug from ointment than EO, with NS and HS demonstrating the highest potentials for both the rate and extent of drug release. WAC and softening temperature of base apparently influenced its drug release propensity. Whereas the presence of polysorbate 80 in chloramphenicol-in-EO formulation did not appreciably improve the drug release from the base, propylene glycol (10 %w/v) in similar formulation caused more than three-fold enhancement of the drug release.

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CONFLICT OF INTERESTS

All authors have none to declare

REFERENCES

1. Blogg JR. Chloramphenicol 4: Responsible topical use in ophthalmology. Aust Vet J 1991;68:8-9.
2. Scruggs J, Wallace T, Hanna C. Route of absorption of drug and ointment after application to the eye. Ann Ophthalmol 1978;10:267-71.
3. Orafidiya LO, Oyedele AO, Shittu AO, Elujoba AA. The formulation of an effective topical antibacterial product containing *Ocimum gratissimum* leaf essential oil. Int J Pharm 2001;224:177-83.
4. Lee M, Hammarlund ER. Corneal absorption of ophthalmic drugs. J Pharm Sci 1974;63:721-4.
5. Cserhati T. Alkyl ethoxylated and alkylphenol ethoxylated nonionic surfactants: Interaction with bioactive compounds and biological effects. Environ Health Perspect 1995;103:358-64.
6. Jiao J. Polyoxyethylated nonionic surfactants and their applications in topical ocular drug delivery. Adv Drug Deliver Rev 2008;60:1663-73.
7. Zimmer AK, Maincent P, Thouvenot P, Kreuter J. Hydrocortisone delivery to healthy and inflamed eyes using a micellar polysorbate 80 solution or albumin nanoparticles. Int J Pharm 1994;110:211-22.
8. Vandamme TF. Microemulsions as ocular drug delivery systems: recent developments and future challenges. Prog Retinal Res 2002;21:15-34.
9. Squillante E, Needham T, Maniar A, Kislalioglu S, Zia H. Codiffusion of propylene glycol and dimethyl isosorbide in hairless mouse skin. Eur J Pharm Biopharm 1998;46:265-71.
10. Conquet P, Durand G, Laillier J, Plazonnet B. Evaluation of ocular irritation in the rabbit: Objective versus subjective assessment. Toxicol Appl Pharm 1977;39:129-39.
11. Wieslander G, Norbäck D, Lindgren T. Experimental exposure to propylene glycol mist in aviation emergency training: acute ocular and respiratory effects. J Occup Environ Med 2001;58:649-55.

12. Flume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler D, *et al.* Safety assessment of propylene glycol, tripropylene glycol, and PPGs as used in cosmetics. Int J Toxicol 2012;31 Suppl 2:245-60.
13. Draize JH, Woodward G, Calvary HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;82:377-90.
14. Wilson SL, Ahearne M, Hopkinson A. An overview of current techniques for ocular toxicity testing. Toxicology 2015;327:32-46.
15. British Pharmacopoeia (BP) Volumes I, II and III British Pharmacopoeia Commission: The Stationery Office, London; 2009.
16. Maurer JK, Parker RD, Carr GJ. Ocular Irritation: Microscopic changes occurring over time in the rat with surfactants of known irritancy. Toxicol Pathol 1998;26:217-25.
17. Brown VKH, Hunter CG. Experimental studies on skin hazard with cyclo octa-I,5-diene and cyclo dodeca-I,5,9-triene. Br J Ind Med I968;25:75-6.
18. Takahashi Y, Hayashi K, Abo T, Koike M, Sakaguchi H, Nishiyama N. The short time exposure (STE) test for predicting eye irritation potential: Intra-laboratory reproducibility and correspondence to globally harmonized system (GHS) and EU eye irritation classification for 109 chemicals. Toxicol *In Vitro* 2011;25:1425-34.
19. Oyedele AO. Delivery of metronidazole from purified Nigerian sheabutter in comparison to standard and modified ointment bases. Ife J Sci 2012;14:253-7.
20. Odusote MO, Ifudu ND. Nigerian shea butter as an ointment base. Nigerian J Pharm 1987;18:31-3.
21. Blainey M, de Avila C, Van der Zandt P. Review of REACH Annex IV–Establishing the minimum risk of a substance based on its intrinsic properties. Regul Toxicol Pharmacol 2010;56:111-20.
22. Oyedele AO. Investigation of ointment bleeding in some absorption and water-miscible base preparations. Nigerian J Pharm Res 2007;6:94-100.
23. Adebayo AS, Akala EO. Kinetics model for the *in vitro* release of an hydrophilic drug (Amodiaquine) from fat-based Suppositories. Int J Arts Technol 2005;2:1-11.
24. Fischer FH, Wiederholt M. Human precorneal tear film pH measured by microelectrodes. Graefe's Arch Clin Exp Ophthalmol 1982;218:168-70.
25. Draize JH, Woodward G, Calvary HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;82:377-90.
26. Kay JH, Calandra JC. Interpretation of eye irritation tests. J Soc Cosmet Chem 1962;13:281-9.
27. Rojanasakul Y, Wang L, Bhat M, Glover DD, Malanga CJ, Ma JKH. The transport barrier of epithelia: a comparative study on membrane permeability and charge selectivity in the rabbit. Pharm Res 1992;9:1029-34.
28. Marulanda AT, Maya JV. Ocular tolerance profile of a cosmetic product for babies *in vivo*. Cuban J Pharm 2012;46:49-60.
29. Mekkawy A, Fathy M, El-Shanawany S. Study of fluconazole release from o/w cream and water-soluble ointment bases. Br J Pharm Res 2013;3:686-96.
30. Kaur IP, Rana C, Singh M, Bhushan S, Singh H, Kakkar S. Development and evaluation of novel surfactant-based elastic vesicular system for ocular delivery of fluconazole. J Ocul Pharmacol Ther 2012;28:484-96.
31. Rafiee-Tehrani M, Mehramizi A. *In-vitro* release of piroxicam from oil-in-water creams and hydroalcoholic gel topical formulations. Drug Dev Ind Pharm 2000;26:409-14.
32. Sieg JW, Robinson JR. Vehicle effects on ocular drug bioavailability. J Pharm Sci 1979;68:724-8.