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ROLE OF NEW ANTIOXIDANTS IN THE STABILIZATION OF OPHTHALMIC AND EAR DOSAGE FORM PREPARATION OF HAMYCIN

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ABSTRACT

The study involves the development of eye-ointment and ear-drop dosage form of hamycin, a polyene antifungal antibiotic, using anti-oxidants and stabilisers to enhance the stability of these dosage forms. It has been found that incorporation of hydroquinone (HQ), butylated hydroxy anisole (BHA), nordihydroguiaretic acid (NDGA), sodium deoxycholate (DSC) and ascorbyl palmitate (AP) in varying combinations in the dosage preparation have enhanced the stability and preserved physicochemical properties of the ointment for a period of 12 months period under refrigerated temperature

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($8+2^0C$) conditions. It was further observed that this dosage preparation is more stable in polyethylene glycol bases than in lanolin bases. Ear-drops dosage preparation has shown stability for 12 months period in the presence of BHA and in combination of other antioxidants under refrigerated temperature. However, ear-drops dosage form was found to be stable only for a period of 6months in ambient condition. It is therefore, recommended that these dosage preparations be stored in refrigerated conditions.

INTRODUCTION

Hamycin, a potent anti-fungal antibiotic belonging to polyene heptaenic macrolide group was discovered and developed at Antibiotics Research Centre, Hindustan Antibiotics Limited, Pune, India (1). It was isolated as the metabolic product of Streptomyces Pimprina Thirum[1] and shown to be active against a large number of fungi, pathogens as well as Saprophytes, the yeast like fungi being especially sensitive[2]. Further, hamycin has also been shown to be effective in the treatment of otomycosis, chronic or subacute infection of external auditory meatus and ear canal (3,4,5). In addition, hamycin has been found to be very effective in the ophthalmic fungal infections especially mycotic corneal ulcers (6,7).

Like other macrolide polyene antibiotics, Hamycin also faces stability problems primarily due to its inbuilt chemical nature. It has been reported that polyenes, on exposure to light and oxygen, liberate peroxides because of th oxidative degradation. It could be minimised by adding certain antioxidants and storing at low temperature (5^0C) (8). Keeping

in mind these available informations, studies have been conducted to know the effect of individual as well combination of antioxidants and stabilizers on the stability of hamycin eye and ear dosage preparation. On the basis of these experiments successful development of hamycin dosage preparation having shelf-life of 12 months period was carried out.

MATERIALS AND METHODS

Butylated hydroxy anisole (BHA) (May & Baker, UK), Hydroquinone (S.D.Fine Chemicals, India), Ascorbyl palmitate (AP) (Lobochem, India), Nordihydroguiaretic Acid (NDGA) (Buch's,Switzerland), Sodium deoxycholate (S.D.Fine Chemicals, India), Benzalkonium Chloride, Indian pharmacopoeal grade (Sarabhai Chemicals, India), Microcrystalline wax, Yellow soft paraffin, Lanolin, Liquid paraffin (Metropolitan Drug & Chemical Co. India), Glycerol and Ethanol, analytical grade, were used in the study.

Eye-Ointment: Two principal bases were selected, viz. Paraffin base (oleagenous in nature) and polyethylene glycol (water soluble) base as the case may be, were heated separately on a water bath to 55^0C and then antioxidants and stabilisers were incorporated either individually or in combination. This mixutre was sterilised by heating at 150^0C for 1 hr and allowed to cool. Hamycin was then incorporated into cool molten matrix. The whole mixture was stirred vigorously until congealing takes place. The ointments thus obtained were passed through tripple roller mill (Erweka, Germany) to achieve homogeniety. The ointment is prepared with utmost aseptic precautions and it

must be free from large particles having more than 10u size. The whole experiment was carried out with minimum exposure to light and air. Finally, the ointment was filled in collapsible tubes and stored at refrigerated 8 ± 2^0C and ambient conditions $30+2^0$C for stability studies.

Ear-Drops: Hamycin powder was solubilized in a mixture of glycerol : ethanol (60%) 3:2 ratio. Anti-oxidants and stabilisers were then incorporated either individually or in combination for different dosage preparations. The pH of each dosage preparation was adjusted to neutral using dilute sodium hydroxide. The preparations wer filled in amber coloured glass bottles and stored at refrigerated 8 ± 2^0C as well as ambient termperature $30+2^0$C for determining stability.

Antifungal activity of dosage form preparation was determined employing cup plate method using Paecilomyces variotti as test organism (9).

Eye irritancy studies in animals: Chronic application of hamycin eye-ointment and ear-drops formulation was undertaken to test for an increased vascularity, lacrimation, effect on pupillary size, reaction to light and sensation or any other toxicity.

RESULTS AND DISCUSSION

Hamycin eye-ointment preparation in PEG base: The results in Table 1 indicate that ointment with PEG bases containing no antioxidant or stabiliser is stable for not more than 3 months in refrigerated temperature and ambient temperature.

TABLE 1

HAMYCIN EYE OINTMENT DOSAGE PREPARATION WITH PEG BASES

Biological potency at different time intervals (months) in lakh units/mg.

	Under refrigerated temperature						Under ambient temperature			
B.No.	Initial	3	6	9	12	15	3	6	9	12
1	2.30	2.20	1.88	-	-	-	2.00	1.50	-	-
2	2.32	2.40	2.01	2.05	1.85	-	2.24	2.00	2.00	1.20
3	2.46	2.45	2.36	2.31	2.04	-	2.36	2.15	2.10	1.24
4	2.44	2.40	2.36	2.18	2.10	-	2.40	2.10	2.00	1.56
5	2.51	2.56	2.52	2.36	2.10	1.99	2.50	2.20	2.05	1.68
6	2.33	2.36	2.30	1.88	1.65	-	2.35	2.20	1.88	1.60
7	2.33	2.40	2.35	1.89	1.89	1.69	2.10	2.18	1.65	-
8	2.33	2.33	2.45	1.88	1.50	-	2.20	2.25	1.80	-

Potency below 2,00,000 unit/mg ± 15% variation is considered as loss of antimicrobial activity.

The Batch No. listed in table 1 can be identified as follows:

1. Contains 4000 : 400 PEG base + hamycin (no antioxidant or stabilisers)
2. Sodium sulphite 0.1% + hydroquinone 0.1% + 1
3. Sodium sulphite + BHA 0.1% each
4. Sodium sulphite + BHA + hydroquinone (0.05% each)
5. SDC 0.1% 6. NDGA 0.1% 7. AP 0.1% 8. SDC + NDGA 0.05% each

Sodium sulphite alone increases stability of eye ointment to 9 months if stored either in refrigerated or ambient temperature.

Sodium sulphite in combination with hydroquinone further increased stability of the ointment to 12 months in refrigerated temperature and upto 9 months in ambient temperature. Similar stability was also observed when sodium sulphite was combined with BHA. The eye ointment when prepared with Sodium sulphite, BHA and hydroquinone showed a stability upto 15 months in refrigerated and 9 months in ambient temperature. It was found that Sodium deoxycholate, NDGA and AP when added individually did not show significant improvement in stability either in refrigerated or ambient temperature. Preparation of eye ointment with SDC and NDCA showed stability upto 15 months period, when stored in refrigerated temperature and 9 months in ambient temperature.

Hamycin ointment preparation in Lanolin bases: Ointment containing no stabiliser or antioxidant showed stability upto 6 months in refrigerated temperature and 3 months in ambient conditions.

Addition of BHA alone in hamycin ointment gave a stability of 6 months only in refrigerated and 3 months in ambient conditions.

Hamycin ointment containing BHA and hydroquinone showed a stability of 9 months and 6 months period in refrigerated and in ambient conditions respectively. Addition of SDC, NDGA and

AP individually in the ointment did not improve the stability and shelf-life remained 9 months in refrigerated and 3 months in ambient conditions.

Combination of antioxidant BHA + NDGA + AP and NDGA + AP + SDC gave a stability of 12 months and 6 months in refrigerated and ambient conditions respectively. The results of both Table 1 and Table 2 showed stability of 15 months for PEG based (Table 1) and 12 months (Table 2) for lanolin based dosage preparation.

Hamycin ear-drop dosage preparation: Hamycin ear-drop preparation containing no antioxidant and stabiliser was stable only for 3 months in refrigerasted conditions as well as ambient conditions.

Addition of buffer in hamycin ear-drop preparation to maintain pH around 7 did not help much as it degrades the preparation both in refrigerated as well as ambient conditions also formation of crystals was observed.

Addition of hydroquinone along with BHA and Benzalkonium chloride stabilised the ear-drop dosage preparation for a period of 12 months in refrigerated condition and 6 months in ambient condition.

Increasing the quantity of hydroquinone from 0.05% to 1% did not improve the stability; it was reduced to 9 months in the refrigerated condition. Dark coloration was observed in the ear-drop solution preparation after 3 months in ambient condition.

TABLE 2

HAMYCIN EYE OINTMENT DOSAGE PREPARATION WITH LANOLIN BASES

Biological Potency at different time intervals (months) in lakh units/mg.

	Under refrigerated temperature					Under ambient temperature			
B.No.	Initial	3	6	9	12	3	6	9	12
1	2.40	2.25	2.30	1.90	-	2.30	1.70	-	-
2	2.30	2.33	2.10	1.80	1.70	2.30	1.90	1.60	-
3	2.60	2.54	2.30	2.23	1.50	2.54	2.20	1.50	-
4	2.36	2.20	2.30	2.10	1.50	2.10	1.80	-	-
5	2.40	2.20	2.10	2.00	1.85	2.20	1.70	1.50	-
6	2.40	2.20	2.10	2.00	1.74	2.20	1.75	-	-
7	2.40	2.10	2.00	1.99	1.98	2.00	1.98	1.68	1.25
8	2.40	2.10	1.98	2.00	2.00				

Potency below 2,00,000 units/mg ± 15% variations is considered as loss of antimycrobial activity.The batch No. listed in Table 2 can be identified as follows:

1 contains Lanolin liquid paraffin, yellow soft paraffin and paraffin wax in suitable proportion with hamycin but no additional preservatives or stabiliser.2.contains BHA = 0.05% +1 3.BHA + hydroquinone = 0.1% each 4.SDC = 0.1% 5.NDGA=0.1% 6.AP = 0.1% 7.BHA + NDGA + AP =0.05% each

8NDGA + AP + SDC = 0.05% each

On addition of individual antioxidants viz. ascorbyl palmitate, NDGA and SDC, the shelf-life of dosage preparation remained 6 months in refrigerated and 3 months in ambient conditions. Combination of antioxidants,e.g. SDC + NDGA, NDGA + AP+ SDC, resulted a shelf-life of 12 months in refrigerated condition and 6 months in ambient condition. The animal studies involving daily application of all these formulations for 10 days did not elicit any adverse effect or reaction. (like local irritation readening or edema formation) there by indicating that these formulations are safe for application.

The structure of hamycin(see figure 1)is complex because the molecules consists of many reactive functional groups; there by making hamycin very sensitive to light, oxygen and oxidising agents. It has also been observed that hamycin is comparatively more stable in the bulk form than in its dosage form. This is primarily due to the presence of exvepients with which the reactive functional groups of hamycin tend to react. These reactions lead to highly reactive free radicals and set off a chain reaction by providing electrons to react with the free radicals (13). It is also likely that the molecules are subjected to auto-oxidation, which is generally initiated by ultraviolet radiation in the presence of trace amounts of oxygen. In the presence of R.(Free radicals formed due to radiation and oxygen) the following auto oxidation reaction can takes place.

$$R.' + R''- CH_2-CH = CH - R''' \; K1 \; R''- CH-CH = CH-R'''+ R'H \quad ---(i)$$

$\longleftrightarrow$

Where R' is Radical from hamycin molecule whereas R" $-CH_2$

TABLE 3

HAMYCIN EAR-DROP FORMULATION

Biological potency at different time intervals (months) lakh units/mg.								
Under refrigerated temperature						Under ambient temperature		
B.No.	Initial	3	6	9	12	3	6	9
1	2.07	2.10	1.80	1.50	-	1.98	Colour becomes brown	
2	2.09	2.14	1.98	2.00	1.99	1.99	1.95	-
3	2.10	2.00	2.05	1.98	1.45	1.98	immediate darkening	
4	2.09	1.98	1.95	-	-	1.95	1.55	-
5	2.28	1.95	1.95	-	-	2.00	1.86	-
6	2.39	1.98	1.90	1.50	-	1.95	1.45	-
7	2.24	2.20	2.20	2.10	1.99	2.00	1.95	-
8	2.25	2.10	2.00	1.99	2.00	2.20	2.05	1.50
9	2.10	2.00	-	-	-	1.98	Crystallization starts	

Potency fall below 2,00,000 units/mg ± 15% considered loss of antimycrobial activity.

The Batch No.listed in Table can be identified as follows:

1) Contains basic vehicle of ethanol 60% glycerol (2:3) ratio with no antioxident or preservative.
2) Hydroquinone (0.05%) + Benzalkonium chloride (0.02%) + BHA
3) BHA+ hydroquinone(1.00% each)+ Benzalkonium chloride (0.02%)

TABLE 3. Continued

4) A.P. 0.1% + Benzalkonium Chloride (0.02%)

5) NDGA 0.1% + Benzalkonium Chloride

6) SDC 0.1% + Benzalkonium Chloride

7) SDC + NDGA (0.05% each) Benzalkonium chloride (0.02%)

8) SDC + NDGA + AP + Benzalkonium Chloride (0.05%)

9) Phosphate buffer pH 7.0

Note: Samples form 5 different batches were taken randomly for analysis and the mean vaalues were mentioned. The standard deviation is found to be not more than 3% of mean value in each case. it is pplicable to all other tables.

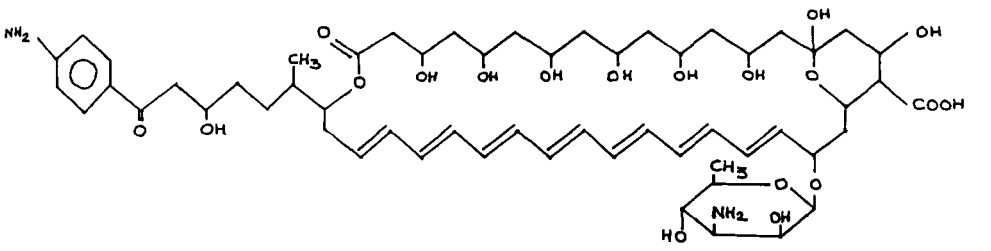
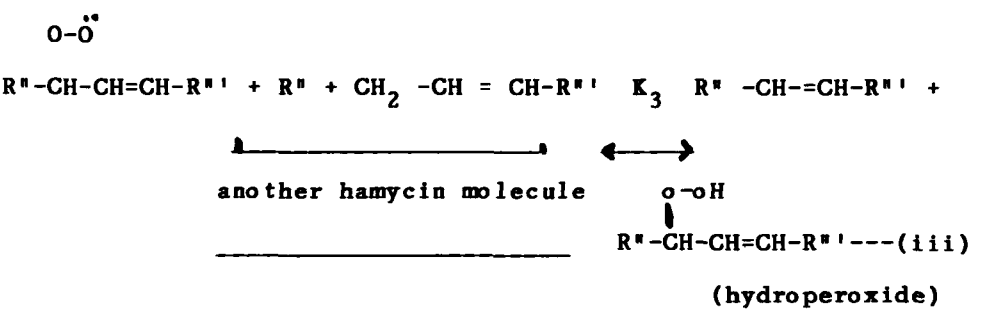


FIGURE 1.

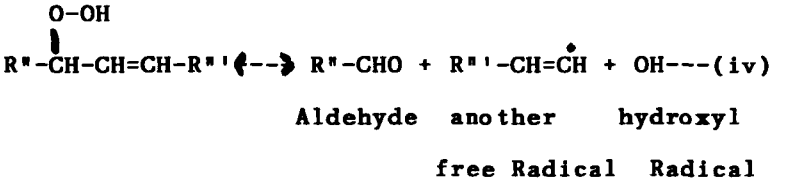
CH=CH-R"' is double bond moiety of hamycin molecule.(Only one double bond is considered for illustration purpose). This can happen to any of the seven conjugated doubel bonds in hamycin (refer the structure).

$$R''- CH-CH=CH-R''' + O2 \xrightarrow{K_2} R''- CH-CH= CH-R''' \quad ---(ii)$$

from (1)



This hydroperoxide of (iii) Step can be broken down to give rise multiple radicals.



This free radical can again oxidise another hamycin molecule. Thus, these reactions form a continuous process and lead to hamycin instability primarily due to lability of carbon-carbon double bonds of the polyene moiety towards auto oxidation which is also observed by the earlier workers (8)if these molecules of hamycin are protected with the help of anti-oxidants, the autooxidation can partly be prevented. Therefore it is possible that primary antioxidants act by interfering with the propagation step of autooxidation process. To mentain propagation of chain free radical e.g R or Roo. are required.Antioxidant AH has the ability to react with such radicals which results in the formation of another free radical A.Which is not sufficiently reactive to substain a chain reaction (See the illustration).

First antioxidant

$$R. + AH \longrightarrow RH + A. \quad ---(v)$$

(Free radical) (Antioxidant)(Stable molecule) (antioxidant free radical)

$$ROO. + AH \longrightarrow ROOH + A. \quad ---(vi)$$

(Hydroperoxideradical)(Antioxidant)(Stablemolecule)(Antioxidant free radical)

Subsequently the antioxidant free radical is annihilated by reacting with another antioxidant radical or another free radical in the following manner which is an indication of effective stablisation.

$$A. + A. \longrightarrow AA \quad (vii)$$

$$A. + R. \longrightarrow AR \quad (viii)$$

In case of combintion of various antioxidants. The second antioxidant reaction proceeds as follows:

Second Antioxidant

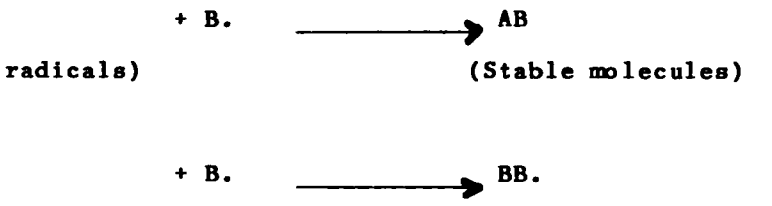
$$R. + BH \longrightarrow RH + B. \quad (ix)$$

(Free radical)(Second antioxidant)(Stablemolecule) (Antioxidantradical)

$$A. + BH \longrightarrow AH + B. \quad -----(x)$$

(Antioxidant radical)(Second antioxidant)(first antioxidant)

The generation of first antioxidant in the above reaction (X) will make the first antioxidant reinitiate the reactions (v) and (vi). Simultaneously B. also take in the reaction mentioned herein below in order to maintain stabilisation.



Further it has been reported that the antioxidants like - tocoferol, BHA, BHT and NDGA etc also block the chain reaction in similar manner. (14) It is found that ascrobyl palmitate generally act as chain terminator as it is less prone to further oxidation. Under such situations combination of ascorbyl palmitate with antioxidants like NDGA. BHA etc.act synergistically in the stabilization process and there by facilitate shelf-life of hamycin dosage preparation.

In conclusion the antioxidants ascorbyl palmitate, NDGA in combination with BHA or SDC used in the present study have increased the stability of eye and ear Hamycin dosage form preparation.

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