International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Formulation Development of Gentamicin Sulphate Ointment Using Rice Bran Wax as Novel Base for Topical Application

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Abstract: ***The present study aims to investigate the possible use of rice bran wax as novel and cost effective oil in water emulsion (O/W) type of ointment base. Thus the formulations were prepared by incorporating different combinations of Rice bran wax, white petrolatum and cetostearyl alcohol. Gentamicin sulphate was used as a model drug. All the formulations were evaluated for different physicochemical parameters such as, pH, spreadability, skin irritation studies, drug content, rheological studies, in-vitro drug release studies, microbiological studies and stability studies. The optimized preparations were compared with ointment prepared from carnauba wax and also the marketed formulation. The stability studies of prepared ointments revealed that formulation F1 syneresis whereas other formulations were physically stable and syneresis was not observed. No significant changes were observed in pH and viscosity values of stable formulations. The formulations were within the permissible limits of all physical parameters. The microbiological studies revealed that the inhibition zone diameters of prepared formulations were comparable to inhibition zone diameters shown by ointment prepared from carnauba wax and marketed preparation. Thus it can be concluded that rice bran wax can be used as ointment base as well as it can be explored as a cost effective excepient in other pharmaceutical preparations.***

Keywords: Rice bran wax, natural source, ointment base, novel, cost effective excepient

1. Introduction

Natural fats and waxes are biocompatible, biodegradable, safe, cheap and easily available. They have been used in many cosmetic and pharmaceutical preparations and are becoming attractive substitutes for costly synthetic and semi-synthetic excepients.Ointments are used topically for several purposes like protective, antiseptic, emollient, antipruritic, keratolytic and astringent. The base of an ointment is of prime importance if the finished product is expected to function as any of the above categories. Ointment base acts as a carrier for medicaments. The ointment base composition determines not only the extent of penetration but also controls the transfer of medicaments from the base to the body tissues. (1,2)

Rice bran wax is obtained from natural source (*Oryza sativa* -Family Graminae) and abundantly available. It is an important byproduct of rice bran oil industry. Chemically the wax is reported to be chiefly melissylcerotate. Research at Southern Regional Research Laboratory has shown that the properties of refined and bleached rice bran wax are similar to that of the presently imported carnauba wax.(3) It is commercially used as an enteric coating for candy and lozenges, in the preparation of wax emulsion, as a plastisizing material in chewing gum, as a partial substitute for carnauba wax and as an ingredient in manufacturing of carbon paper.(4)It is used in pharmaceutical and cosmetic field as a base for ointment and as a formulation aid in lipstick.(5) V.P. Sable *et al*reported the *in-vitro* and *in-vivo* studies of rice bran wax as skin moisturizer. Further they also evaluated rice bran wax as ointment base.(6,7,8) However not many studies have been reported about the pharmaceutical applications of Rice bran wax with reference to the formulation attributes and drug release characteristic as ointment base.

Gentanicin sulphate chemically designated as (3-deoxy-4-c-methyl-3-(methylamina)-b-L-arabino(1-6)-[2,6-diamino-2, 3, 4, 6-tetradeoxy-a-d-erythro-hexopyranosyl (1-4)-2-deoxy-D streptamine) is potent antibacterial drug known to undergo first pass metabolism, having high skin permeability, not absorbed through gastro intestinal tract appreciably. It is very slowly absorbed when applied topically in an ointment and hypersensitivity reaction occurs.(9) Thus oil in water emulsion type of ointment base was selected for the topical application of Gentamicin sulfate.

The present study aims to investigate formulation attribute and drug release kinetics of rice bran wax as ointment base. The main objective of present study was to formulate rice bran wax as o/w ointment base containing antibiotic drug and compare its characteristics with carnauba wax ointment as standard base and marketed preparation. The studies were also tried to find the release behavior of drug from formulated novel ointment base in presence of microbiological medium against microorganisms which cause topical infections.

2. Materials and Methods

Gentamicin sulfate was obtained as gratis sample from Arco life sciences Hingna Nagpur. Rice bran wax was provided by Maheshwari oil extraction plant, Gondia, it was purified and standardized and used. White petrolatum, cetostrearyl alcohol and propylene glycol were purchased from s.d fine chemicals Mumbai. Sodium lauryl sulfate, methyl paraben, propyl paraben were procured from Loba Chemie Mumbai. Nutrient agar was purchased from Hi- media. All other chemicals used were of A.R. grade. A fabricated diffusion cell, magnetic stirrers from Remi equipment Pvt. ltd and

cellophane membrane (Himedia) were used for drug release studies. For analysis UV spectrophotometer was used.

Purification and standardization of rice bran wax

The crude wax (100 g) was soxhleted with ethyl acetate (300 ml) for 30 min at 85°C. The mixture in thimble was cooled up to 25°Cand was subjected to decolorisation with 2% H_2O_2 at 90°C for 1 h and secondary decolourization with NaOCl 15% at 100°C for 1 h.(10) The purified wax obtained was then used for further study. The rice bran wax obtained after purification was standardized to determine its physicochemical properties.(11)

Preparation of calibration curve

The drug (10mg) was weighed accurately and dissolved in pH 7.2 phosphate buffer. The volume was made upto 100ml(100µg/ml). The aliquots of 0.5ml,1.0ml,1.5ml,2.0ml 2.5ml and 3.0ml were transferred to 10ml volumetric flask. All the flasks were heated in water bath at 100°C for 25 min. Then 1.0 ml of ninhydrin reagent was added to each flask and the volume was made upto 10ml with distilled water. The absorbance of the resulting violet colored solution was measured at 570nm using a reagent blank using UV spectrophotometer. The standard curve was plotted in the range of 5-30 µg/ml and regression coefficient and equation of line was obtained.

Preparation of O/W ointment base using rice bran wax

Rice bran wax ointment containing varying concentrations of rice bran wax (5,7.5,10,12.5,15%w/w) was prepared by melting together white petrolatum, cetostrearyl alcohol and rice bran wax on hot plate at 75°C. Sodium lauryl sulphate, propylene glycol, methyl paraben and propyl paraben were added to purified water and heated to 75°C. The molten phase was then added to aqueous phase by stirring with glass rod until congealed. Drug was incorporated in the prepared ointment base by mixing with glass rod.

Evaluation of formulated ointments

The formulated ointments were evaluated for appearance, pH, viscosity, drug content, spreadability, *in-vitro* diffusion profile, stability testing, antimicrobial activity and skin irritation study using Guinea pigs.

pH and viscosity

The pH of each formulated ointment was measured using a previously calibrated digital pHmeter (Elico, Model no. LI-610). Viscosity was determined using Brookfield digital viscometer (Model no LVDV- I - Prime) with spindle no 62.The sample temperature was controlled at 25±1°C before each measurement.

Spreadability (12)

Spreadability of formulations was determined with a modified apparatus consisting of wooden block provided with two glass slides. Lower slide was fixed on wooden block and upper slide with one end tied to weight pan. Anointment equivalent to 2.5 g was placed between two slides and 1000 g weight was placed over it for5 minutes to press the sample to a uniform thickness. Weight of 80 g was added to pan. The time (in seconds) required to separate the two slides was taken as a measure of spreadability. Shorter time interval to cover the distance of 7.5cm indicates better spreadability.

Drug content uniformity

Formulated ointments 0.5 g were weighed and diluted with about 50 ml of pH 7.2phosphate buffer in a volumetric flask, suitably diluted with the same buffer solution. The resulting solution was then filtered using membrane filters, treated with 1ml of Ninhydrin reagent and absorbance was measured at 570 nm using UV/Vis spectrophotometer.

***In-vitro* diffusion studies through goat skin and cellophane membrane**

In-vitro diffusion studies of formulated ointments (F1, F2, F3, F4, F5, C1 and commercial preparation was carried out using the permeation apparatus as described by Fites *et al* (13). A glass cylinder with both the ends open, 10 cm height and 3.6 cm outer diameter was used as a permeation cell.A cellophane membrane (2.4 nm pore size, cut to suitable size and soaked in phosphate buffer of pH 7.2) was fixed to one end of the cylinder by adhesive tape. One gram of the prepared ointment was taken in the cell (donor compartment)and the cell was immersed in a beaker containing 100 ml of pH 7.2 phosphate buffer (receptor compartment). The cell was immersed in to a depth of 1cmbelow the surface of buffer, which was agitated by a magnetic stirrer and the temperature was maintained at 37°C±1 throughout the experiment. Aliquots of 1ml were withdrawn from the receptor compartment periodically (1, 2, 3, 4, 5, 6, 7 and 8 h). The volume was made upto 10ml with water and treated with 1ml of Ninhydrine reagent. The drug concentration was determined spectrophotometrically at 570 nm. After each withdrawal, the volume of liquid in the receptor compartment was replaced by phosphate buffer of pH 7.2 to maintain sink conditions.

In-vitro drug release studies were also carried out using goat skin in order to optimize the formulations. The abdominal skin of goat was obtained from the nearby slaughter house one day before the experiment. The skin was cut into suitable size and stored at -4°C in formalin solution. The drug release studies through goat skin were carried out by the same procedure as employed for drug release by cellophane membrane.

Microbiological studies(14)

Microbiological studies of prepared formulations were carried out to evaluate antibacterial activity of incorporated drug gentamicin and release characteristics of ointment base.Standard cup plate method was employed using micro-organisms such as Staphyllococcusaureous, Bascillus subtilis and Escherichia coli. Sample containing equivalent amounts of drug from prepared formulations and marketed preparation were applied in the bore of plate prepared using nutrient agar medium and the inhibition zone diameters were measured with the help of a zone reader after incubation at temperature of 37°C for 24 h.

Stability studies

Formulated ointments were filled in well closed glass containers and stored in cooldark place at room temperature for three months. They were analyzed monthlyfor physical characteristics, drug content and synerisis.(15)

Skin irritation study (16)

Guinea pigs (400-500) g of either sex were used for skin irritation study. The animals were kept under standard condition, maintained on standard animal feed and had free access to water. Hair was shaved from back of guinea pigs and area of 4 cm^2 was marked on both the sides. One side served as a control and other served as test. Gel was applied (0.5g/animal) twice a day for 7 days. The site was observed for any sensitivity and reaction if any, was graded 0,1,2,3 for no reaction, slight patchy erythema, slight but confluent or moderate butpatchy erythema and severe erythema with or without edema, respectively

Results and Discussion

In the present study, efforts were made to formulate ointment consisting of o/w emulsion type of ointment base using naturally occurring rice bran wax. Crude rice bran wax was obtained, purified and standardized and then used in the formulation. The ointment prepared from carnauba was also prepared **a**nd used as a standard base for comparison. The prepared formulations were also compared with marketed preparation. In the pre-formulation studies the physicochemical properties of rice bran wax were studied as in the table 2.It was found that rice bran wax complies with standards as specified by Agmark.

Initially the ointment were prepared by using 5-20% w/v of rice bran wax and 5-10% w/v of cetostearyl alcohol but it was found that the ointment with 10% has good consistency and ointment with 15 % w/v of was slightly harder. Hence the ointment with concentration of rice bran wax ranging from 5-15% and cetostearyl alcohol 2.5 to 10% were used for further studies. Thus five formulations were prepared consisting of o/w emulsion type of ointment base to give smooth non-sticky and non oily feel to skin after application.

The physicochemical and rheological studies indicated that all the formulated ointments were cream white in color, smooth, non-sticky, non oily, pliable, homogeneous, elegant in appearance and easily washable with water. When applied on the skin with finger exhibited pseudoplastic flow. The prepared formulations showed good extrudability, spreadability and viscosity. After application on skin the ointments spread easily and immediately vanished which indicated good absorption into the skin. The pH of all the formulations was determined and found to be in the range of neutral pH from7.0 to 7.2. The drug content of all the formulations was within the limits of theoretical values. Hence the ointments were ideal for topical application. The results are shown in table 3.

Stability studies of the formulation indicated appearance of syneresis in formulation F1. This may be due to lower concentration of rice bran wax. The other formulations were physically stable and syneresis was not observed. Significant changes were observed in pH and viscosity values of stable formulations. Therefore formulations F2, F3, F4 and F5 were considered for further studies. Skin irritation studies revealed no sensitivity reaction.

In- vitro drug diffusion studies through cellophane membrane and goat skin revealed that there was no significant difference between the drug release patterns and the cumulative percent drug release profile among the prepared formulations and marketed formulation. It was found that the formulations F2,F3 and F4showed more than 80% of drug release but there was a slight retardation in drug release from formulation F5 may be because of higher concentration of rice bran wax. Thus formulation F2, F3and F4 showed 82.24, 89.99 and 92.56% of drug release whereas formulation F5 showed 65.34% of drug release respectively. *In-vitro* diffusion studies thus indicated ointment prepared using 10 to 20%w/v of rice bran wax were ideal for topical application. The formulation F5 containing 25% of rice bran wax may be evaluated for sustained release of drug required during topical or transdermal application.

From the drug diffusion data, the permeability coefficient for all the batches was calculated using the equation $P_m = (k_{app}. H)/A$, where k_{app} is diffusion rate constant (mg/h) calculated from the slope of the linear drug (d/p) diffusion profiles, H is thickness of the film (cm), A is surface area of the film(cm^2).The rate and the mechanism of release of Gentamicin sulfate through the formulated ointments were analyzed by fitting the diffusion data(17) into zero order equation $Q=Q_0 – k_0t$, where Q is the amount of drug released at time t, and k_0 is the release rate and first order equation, $Q=LnQ-k_1t$, where k_1 is the release rate. The diffusion data was further analyzed to define the mechanism of release by applying the diffusion data following the empirical equation, $M_t/M_a = Kt^n$, where M_t/M_a is the fraction of drug released at time t, K is a constant and n characterizes the mechanism of drug release from the formulations during diffusion process.

Table4 shows the diffusion characteristics of the formulated ointments. The correlation coefficient values (R) depicts that the diffusion profile follows zero order kinetics and the mechanism of drug release was governed by Peppas model. The diffusion exponent of release profiles has a value of 1.0021 –1.0192 (n=1), which indicates Case II transport diffusion.

Microbiological studies indicated that the inhibition zone diameters obtained by prepared formulations were comparable with the inhibition zone diameters shown by marketed preparation.

3. Conclusions

From the present investigation it can be concluded that rice bran wax can be formulated as oil in water emulsion type of ointment base for topical preparations. It possesses good release properties in the lower concentrations but in higher concentrations the release of drug decreases. This property can be exploited in the preparation of sustained release or controlled release formulations such as sustained release ointments or creams or microspheres or tablets. Thus there is a lot of scope for the further studies to the budding researchers and students to find newer uses of rice bran wax. It can also be studied for using rice bran wax as economical excepient in the pharmaceutical formulations.

4. Acknowledgement

The authors are thankful to Dr. S. P. Timande for providing bacterial cultures for the above study. We also thank Mr. J.

G. Thakrey, Chief Chemist, Maheshwari Oil Extraction Plant, Gondia for providing Rice bran wax.

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Table 1: Composition of optimized formulations using O/W emulsion type ointment base

Ingredients	Formulations					
	F1	F2	F3	F4	F5	C
White petrolatum	25	25	25	25	25	25
Cetostrearyl alcohol	10	7.5	5.0	2.5	--	5.0
Propylene glycol	20	20	20	20	20	20
Rice bran wax	05	10	15	20	25	--
Carnauba wax	--	--	--	--	--	--
Methyl paraben	0.025	0.025	0.025	0.025	0.025	0.025
Propyl paraben	0.015	0.015	0.015	0.015	0.015	0.015
Purified water	q.s	q.s	q.s	q.s	q.s	q.s

Table 2: Standardization of Rice bran wax

Melting range	Solubility	Refractive index	Specific gravity	Saponification value	Iodine value	Unsaponifiable matter
77-85°C	Soluble in Chloroform Petroleum ether Insoluble in acetone and water	1.46 at 70°C	0.95	66-72	9-12	62

Table 3: Physicochemical and rheological parameters of Gentamicin sulfate ointments using rice bran wax

Formulation	Appearance	pH	Apparent Viscosity (Cps)	Spreadability	Drug content
F1	Cream white color	6.98	54,600	20	98.43
F2	Cream white color	7.09	54,970	21	98.05
F3	Cream white color	7.07	55,600	22	99.17
F4	Cream white color	7.12	55,925	19	99.37
F5	Cream white color	7.14	56,847	19	98.96
C	Cream white color	7.06	56,894	20	99.28
MP	Cream white color	7.05	54,284	21	100.58

Table 4: Diffusion characteristics of Gentamicin sulphate from different ointments

Formulation	Diffusion Rate Constant K values	Diffusion Exponent (n)	Permeability Coefficient
F2	1.714	1.7555	0.001572448
F3	3.155	1.7142	0.003219387
F4	4.876	1.2359	0.004975510
F5	1.714	1.5944	0.001748976
C	3.892	1.3228	0.003971428
MP	5.935	1.1847	0.006056122

Table 5: Antimicrobial activity of Gentamicin sulfate Ointments

Formulation	Inhibition zone diameter, mm*		
	Staphyllococcus aureous	Bascillus subtilis	Escherichia coli
F1	32.82	31.22	31.91
F2	35.13	34.41	31.75
F3	39.71	42.62	41.56
F4	40.43	39.23	42.87
F5	42.10	40.71	43.54
C	39.38	42.58	42.98
MP	40.25	43.56	43.18

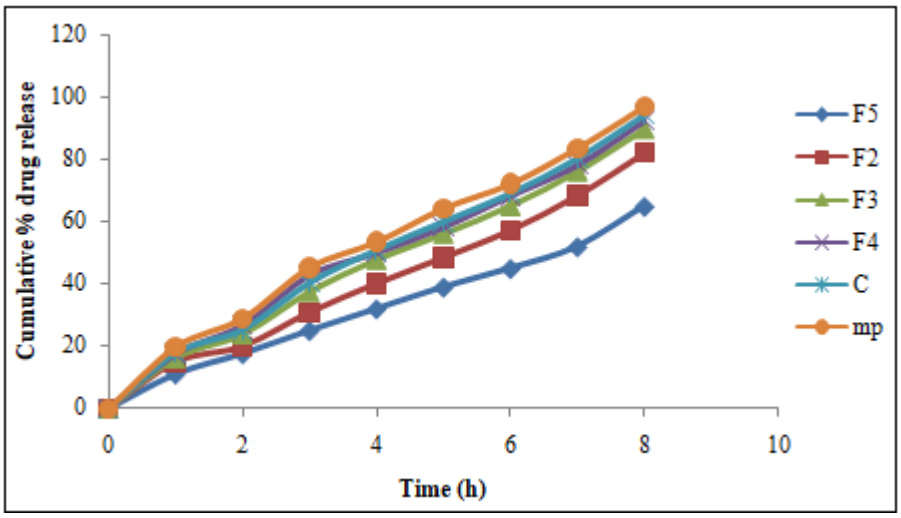

Figure 1: Cumulative % drug release from different ointment formulations using rice bran wax through cellophane membrane.

List of abbreviations:

S .No	Abbreviations	Meaning
1	w/w	Weight by weight
2	v/v	Volume by volume
3	w/v	Weight by volume
4	mm	milimeter
5	λg	is geometric mean diameter
6	xi	is mid-point of range
7	ni.	is number of particles inrange.
8	N	is the total number of particles
9	gm/cm^3	Gram per centimetre cube
10	%	percentage