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FORMULATION OF PROPOLIS EXTRACT EMULSIONS. I. O/W CREAMS BASED ON NON-IONIC SURFACTANTS AND VARIOUS CONSISTENCY AGENTS

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ABSTRACT

The formulation of propolis extract O/W emulsions, a material of therapeutic value in wound healings, is described. Type and optimal concentration of excipients (non ionic surfactants, consistency agents, solubilising factors) are chosen to obtain stable emulsions of desirable macroscopic properties, satisfactory consistency and pH compatible with cutaneous application.

INTRODUCTION

In previous work we standardised a procedure of manufacturing propolis extract (1). The wound healing properties of this extract, which is particularly rich in flavonoids (2) were demonstrated in the *albino* Rabbit and *hairless* Rat (3,4). We recently undertook the development of dosage forms of this extract, and formulated hydrophilic and hydrophobic ointments suitable for the treatment of bed sores (5). The work described here concerns the development and physical stability testing of O/W creams based on propolis extract.

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MATERIALS AND METHODS

Raw Materials

Active ingredient

Propolis extract was prepared by extraction with a Soxlhet extractor, using 70% (v/v) ethanol (1). The extractive solution was concentrated under reduced pressure at 40°C to a stiff consistency(dry extract 80%).

Excipients

We used a thick paraffin oil associated with a moistening additive, glycerol (Coopération Pharmaceutique Française) and a cutaneous penetration factor isopropyl palmitate (Unichema).

The other excipients, classified according to their major function, are given in table1.

Methods

Preparation of the Emulsions

The additives of the lipophilic phase were fluidified using a water bath at 70°C. The softened propolis extract was blended into the melted blending. The aqueous phase was warmed to 70°C and mixed into the lipophilic phase with an IKA RW20 helical stirrer. The stirring rate, initially at 240 r.p.m., was gradually increased during addition of the aqueous phase and also during cooling of the preparation. Finally this rate was raised to 500 or 600 r.p.m. for semi-fluid creams, and 700 or 800 r.p.m. for semi-stiff creams. Stirring was maintained until cooling was complete. These preparations were placed under a vacuum in a Stephan apparatus to obtain a homogeneous deaerated emulsion.

Tests after fabrication

The following tests were performed 24 hours after preparation :

Macroscopic stability

The distribution of the propolis extract and the homogeneity of the emulsions (no creamage or sedimentation) were checked by visual inspection (6).

Determination of Spreadability

One gram of emulsion was pressed between two horizontal plates 20 cm square, upper one of which weighed 125 g, and its diameter (∅) was measured after one min.

Under the experimental conditions, the following classification was adopted : semi-stiff creams $\varnothing \leq 50$ mm and semi-fluid creams $50 < \varnothing \leq 70$ mm (7).

Penetrating Power

Penetrating power (P) was measured using a Prolabo penetrometer fitted with the penetration cone for lubricating grease (cone weight 47.5 g). The cone fall was automatic and timed at 5 seconds. Under these conditions, two types of creams can be distinguished : semi-stiff creams $P \leq 300 \pm 5$ 1/10 mm and semi-fluid creams $P > 300 \pm 5$ 1/10 mm.

Rheological Study

The rheological study of the emulsions was carried out at 21°C using a Brookfield RVTD V2 instrument fitted with an SC4-28/13R small adapter.

TABLE 1
Excipients used for the Formulation of Propolis Extract O/W Emulsions

Type of excipients	Chemical denomination	Commercial name (laboratory)
Consistency Agents	cetylalcohol	(Cooperation Pharmaceutique Française)
	cetearylalcohol	Lanette O® (Henkel)
	Mono and diglyceryl stearate/palmitate	Cutina MD®(Sidobre Sinnova) Tegin 90® (Goldschmidt)
Non ionic Surfactants	Sorbitan mono-oleate	Arlacel 165® (I..C.I)
	Polyethylene(25) sorbitan mono-oleate	Tween 85® (I.C.I.)
	Polyethylene(12 or 20) cetearyl alcohol	Eumulgin B1® Eumulgin B2® (Sidobre Sinnova)
Solubilising Factors	Caprylic/capric triglyceride	Miglyol 812® (Dynamit Nobel)
	Polyethylene(7) glyceryl oleate	Cetiol HE® (Henkel)

pH Determination

We used a CG 837 Schott pHmeter (6).

Stability Tests

The emulsions were packed in opaque tubes and stored at room temperature (23 ± 2 °C) (6). For each formulation, the parameters previously described were checked at regular time intervals (3, 6 and 12 months) : macroscopic stability, spreading diameter, penetrating power, rheological study and pH determination.

RESULTS AND DISCUSSION

Formulation

The aim of this work was the formulation of O/W creams suitable for the treatment of bed sores, creams being the commonest dosage forms in this field. Application of compresses impregnated with healing creams is recommended at the budding stage ; the consistency of these emulsions has therefore to be semi-stiff. When skin is reformed improving tissue elasticity and preventing cheloid formation is essential in this case, semi-fluid creams that spread well and have high hydrating ability are preferable. Such preparations are also suitable for preventive trophic massages.

Concentrated propolis extract is the active ingredient. It is used at its optimally effective concentration (5 % w/w) (4).
We used as lipid base a thick paraffin oil (5 % w/w), an excipient with hydrating properties due purely to occlusive effects. It was associated with a moisturising agent, glycerol (5 %, w/w). The activity of glycerol on skin elasticity and suppleness has been fully demonstrated both *in vitro* and *in vivo* (8). In addition, this hygroscopic excipient incorporated in a cream at a concentration between 3 and 10 % w/w prevents drying of the preparation. We also used a skin penetration factor, isopropyl palmitate (6 % w/w) ; this excipient, which possesses a high emulsionability, also confers excellent spreadability and a high degree of creaminess (9).
Dissolution of propolis extract in the paraffin oil-glycerol-isopropyl palmitate mixture is only possible in the presence of a fat with solubilising properties. Two agents were tested, Miglyol 812 (R) and Cetiol HE(R). The emulsification of all the lipid compounds to form an O/W cream requires surfactants and consistency factors. Among surfactants, stable non-ionic derivatives that are active at all values of pH are particularly useful. These are less irritating than cationic or anionic compounds ; the best tolerated are ester surfactants with long hydrocarbon chains bearing a large number of ethylene oxide groups.
Dosage forms were therefore made up by associating such surfactants with various consistency factors ; cetyl alcohol, cetylstearic alcohol and mixtures of mono- and diglycerides of stearic and palmitic acids. Table 2 gives the compositions of these emulsions and their test results.

For the first formulation we tested a neutral oil based on fractionated medium-length chain saturated fatty acids from coconut, Miglyol 812(R) to solubilise the propolis extract (10).
We used cetyl alcohol as a consistency agent. This mixture of solid alcohols, composed mainly of hexadecanol, is commonly used as a thickener for vaseline oil, and vegetable and animal oils (6).
The emulsification was carried out using a mixture of non-ionic ester surfactants, HLB 11, Tween 85(R) and Arlacel 165(R). The first, still called polysorbate 85, is a polyoxyethylenated sorbitol trioleate (approx. 20 moles of ethylene oxide) (11). Arlacel 165(R) is a polyoxyethylenated glycerol monostearate, of a solid consistency. When used in excess, it acts as both surfactant and thickener (11).
From the technical documentation and initial testing we performed, the optimal concentrations of Tween 85(R) and Arlacel 165(R) were respectively 3 and 6 % w/w. The emulsion thereby obtained displayed no creamage or sedimentation, but was not very homogeneous. This is due to poor solubilisation of the extract in the lipid phase.

Since increasing the concentration of Miglyol 812(R) only slightly improved the homogeneity of the preparation (formulation 2), we turned to another type of solubilising agent, Cetiol HE(R). This mixture of polyoxyethylenated esters of fatty acids of coconut also possesses softening properties (12).

TABLE 2
O/W Emulsions associating non-ionic surfactants and consistency agents

Formulation N° / Raw materials (%w/w)		1	2	3	4	5	6	7	8
Propolis extract		5	5	5	5	5	5	5	5
Thick paraffin oil		5	5	5	5	5	5	5	5
Glycerol		5	5	5	5	5	5	5	5
Isopropyl palmitate		6	6	6	6	6	6	6	6
Solubilisating agent	Miglyol 812®	5	8						
	Cétiol HE®			8	8	8	10	10	8
Non-ionic surfactants	Arlacel 165®	6	6	6					
	Tween 85®	3	3	3					
	Eumulgin B1®				1.5	1.5	1.5	1.5	1.5
	Eumulgin B2®				1.5	1.5	1.5	1.5	1.5
Consistency agent	cetylalcohol	5	5	5	5				
	Lanette O®					10	10	12	
	Cutina MD®								12
Demineralized water qsp		100	100	100	100	100	100	100	100

Macroscopic controls	Homogeneity	-	±	+	+	-	+	+	+
	Coalescence	-	-	-	-	-	-	-	-
	Creamage	-	-	-	-	-	-	-	-
	Sedimentation	-	-	-	-	-	-	-	-
∅ after1 min (mm)		-	-	45	55	-	58	50	50
P (1/10 mm)		-	-	299	318	-	345	295	298
Viscosity at 5 r.p.m. ($m.Pa.s.x10^3$)		-	-	274	14.3	-	75.2	32.4	156
pH		-	-	5.55	5.5	-	6.45	6.1	6.45

Under these conditions the semi-stiff cream obtained was perfectly homogeneous (formulation 3), rheologically, it was pseudoplastic and thixotropic. This formulation was selected for keepability testing.

From this formulation, we tested another association of non-ionic surfactants. Eumulgin B1® and Eumulgin B2®, at prescribed concentrations of under 4 % w/w, i.e. appreciably less than for the surfactants chosen initially.

Eumulgin B1® (HLB 13) and Eumulgin B2® (HLB 15) are two polyoxyethylenated cetylstearyl alcohols, containing respectively 12 and 20 moles of ethylene oxide (13). It is known that using such a pair of fatty acids to emulsify paraffin oil gives it

appreciable hydrating properties, in the absence of any specifically hygroscopic material (14).

In the supplier's technical documentation, it is particularly recommanded to associate these surfactants with fatty alcohols such as cetyl alcohol. The cream formulated with 5 % w/w of this consistency factor and 1.5 % w/w of each of the preceding surfactants (formulation 4) was perfectly homogeneous. This type of pseudoplastic, thixotropic, semi-fluid emulsion was retained for physical stability testing.

Another fatty alcohol, Lanette O® was then tested with the association Eumulgin B1®-Eumulgin B2®. This waxy-looking consistency factor, made up essentially of cetyl and stearyl alcohols, also acts as a skin protector. For the production of creams, it is prescribed at concentrations between 8 and 12 % w/w (12). As shown in table 2, incorporating 10 % w/w of Lanette O® requires a higher percentage of Cetiol HE®, 10 % w/w instead of 8 % w/w (formulation 6). The emulsion obtained displayed satisfactory macroscopic properties, it was pseudoplastic thixotropic.

With a spreading diameter of 58 mm, this formula, which was retained for keepability testing, belongs to the category of semi-fluid creams suitable for skin application in preventive treatment and at the skin reforming stage during healing of bed sores.

Use as a curative measure during healing requires a semi-stiff consistency that allows impregnation of compresses. Accordingly, we formulated an emulsion containing 12 % w/w of Lanette O® (formula 7). This cream, which has a spreading diameter of 50 mm and which is pseudoplastic and thixotropic, was retained for subsequent stability testing.

Besides fatty alcohols, mono- and diglycerides of fatty acids are also recommended in association with Eumulgin® surfactants.

Formulation 8 was made up with Cutina MD®, a mixture of mono- and diglycerides of palmitic and stearic acids. The supplier recommends a 1:4 ratio of surfactants Eumulgin B1®-Emulgin B2® and Cutina MD® (13) ; the percentage of the latter was accordingly set at 12 % w/w. Unlike the emulsions containing Lanette O®, a concentration of 8 % w/w of Cetiol® proved sufficient here. The semi-stiff emulsion obtained displayed the same rheological behaviour as the preceding creams, and was retained for physical stability testing.

Physical stability testing

The physical stability tests were carried out at room temperature. The following parameters were monitored :

- Macroscopic stability : all the creams should conserve a homogeneous appearance, and any creamage or sedimentation should be reversible (6).
- pH : initially between 5.55 and 6.45 depending on the formula, it should remain compatible with skin application.
- Viscosity : a rheological study reveals any modification of viscosity and/or flow behaviour of the emulsion.
- Spreadability and penetrability.

TABLE 3
Physical Stability of O/W Emulsions :
Spreading Diameter, Penetrating Power and Viscosity

Formulation N°		3	4	6	7	8
∅ after 1 min (mm)	3 months	45	55	58	50	50
	6 months	45.5	55	57	48.5	50
	12 months	46	56	56	47	51
P (1/10 mm)	3 months	299	318	345	295	298
	6 months	299	318	345	295	299
	12 months	300	318	345	295	302
Viscosity at 5 r.p.m. ($m.Pa.s.x10^3$)	3 months	269	14.0	83.3	35.5	143
	6 months	257	13.5	96.4	41.5	139
	12 months	238	12.5	97.6	42.1	134

These two parameters allow consistency of preparations to be compared and their stability to be monitored. Two creams were of semi-fluid consistency and two were semi-stiff. No correlation was observed between values of spreading diameter, penetrability and viscosity. The viscosity of formulation 7 was approximately five times lower than that of formulation 8 for the same spreading diameter (50 mm) ; likewise, formulations 3 and 8, with respective viscosities of 274,000 and 156,000 m.Pa.s, had pratically identical penetrabilities.

The stability of these different parameters was studied at regular time intervals ; 3 months, 6 months and one year. After one year's storage in the dark at room temperature, all the creams displayed perfect macroscopic stability and their pH was unchanged. The results of the other tests are given in table 3.

These comparative stability tests show the following constatations.

The spreading diameters and penetrability of the creams associating non-ionic surfactants and cetyl alcohol were practically unchanged after one year's storage (formulations 6 and 7). Their viscosity decreased by only 13 %.

The emulsions based on Lanette O® as consistency agent were appreciably less stable. Though their penetrability did no vary, their spreadability fell and their viscosity rose markedly, by some 30 %.

The spreading diameter and penetrability of formulation 8 containing Cutina MD® was barely modified, and its viscosity fell by only 14 %.

These tests clearly show the superiority of formulations 3, 4 and 8. Further tests are in progress.

Formulation 4, of semi-fluid consistency, is suitable for trophic massages and application at the skin reforming stage. As mentioned above, Eumulgin B1® and B2® confer hydrating properties conductive to tissue softening and prevention of cheloids.

The other emulsions (formulations 3 and 8), of the semi-stiff type, are especially suited to the curative treatment of bed sores, in particular at the scar budding stage.

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