

FORMULATION AND PHARMACEUTICAL EVALUATION OF THREE W/O EMULSIONS WITH MYTILUS GALLOPROVINCIALIS LMK. AND RAPANA VENOSA LIPID EXTRACTS

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Abstract

The present paper presents new formulations of three lipid extracts of *Mytilus galloprovincialis* Lmk., *Rapana venosa* molluscs and those mixed (1:1, v/v) through their incorporation in W/O emulsions. These consisted of 4% lipid extracts (active ingredients), 60% liquid paraffin as the oil phase, 3% glycerin, 25.96% water as the water phase, 6% Span 60 as the surfactant and 0.04% nypagin as preservative. The smell was adjusted with 1% *Oleum lavandulae*.

The scope of this study regards the formulation and pharmaceutical evaluation of the three W/O emulsions. Their evaluation consisted of: aspect, colour, smell, type of emulsion by dilution, electric conductivity and total dissolved salts (TDS) methods, dynamic viscosity, stability by heating at 70°C and centrifugation at 3000 rpm, pH and tension determinations.

The results showed a good stability over 90 days of observation period of the three emulsions evaluated through the pharmaceutical parameters.

Rezumat

Lucrarea de față prezintă încorporarea a trei extracte lipidice din moluștele *Mytilus galloprovincialis* Lmk., *Rapana venosa* și amestecul acestora (1:1, v/v) în emulsii de tip A/U. Acestea sunt formate din extracte lipidice (ingredientele active) 4%, parafină lichidă 60% ca fază uleioasă, glicerină 3%, apă 25,96% ca fază apoasă, Span 60 6% ca emulgator și nipagin 0,04% ca și conservant. Mirosul a fost corectat cu *Oleum lavandulae* 1%.

Scopul acestui studiu privește formularea și evaluarea farmaceutică a celor trei emulsii A/U: aspect, culoare, miros, tipul de emulsie prin metoda diluării, colorării, conductibilitate electrică și sărurile totale dizolvate, vâscozitate dinamică, stabilitate prin încălzirea la 70°C și centrifugarea la 3000 rpm, determinarea pH și tensiunii.

Rezultatele arată o bună stabilitate timp de 90 de zile a celor trei emulsii, evaluată prin parametrii farmaceutici.

Keywords: lipid extracts; pharmaceutical evaluation; W/O emulsions; molluscs

Introduction

The lipid extracts from *Mytilus galloprovincialis* Lmk. and *Rapana venosa* molluscs are a mixture of polyunsaturated fatty acids, ω -3, ω -6, especially eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6) and E and D vitamins [1].

Emollients play an important part in the management of patients with dry skin disorders, such as atrophy, allergy, eczema, psoriasis or dryness following chemotherapy or radiotherapy. Their use in the treatment of diseased and sensitive skin requires not only an efficient hydrating and lipid-replenishing effect on the skin, but minimal risk for skin irritation or sensitization. This will be influenced by their formulation and number and type of ingredients and, due to the nature of their application, requires clinical testing to ensure their appropriateness for dermatological rather than cosmetic use. A new generation of emollients has been developed for the care of dry, or very dry, and sensitive skin [4, 5].

The shelf life of a drug in a dosage form is the amount of time that the product can be stored before it becomes unfit for use because of chemical decomposition and/or physical deterioration. In this report we examined the formulation and pharmaceutical evaluation of the lipid extracts incorporated into pharmaceutical ingredients over 90 days period of observation.

Materials and methods

Materials

- Lipid extracts from *Mytilus galloprovincialis* Lmk. and *Rapana venosa* molluscs;
- Liquid paraffin;
- Span 60 (surfactant);
- Glycerine;
- Nypagin;
- *Oleum lavandulae*;
- Distilled water

Formulation of emulsions

The active ingredients from these three emulsions were extracted from *Mytilus galloprovincialis* Lmk. and *Rapana venosa* molluscs according to Christie method [2]. The other ingredients are: liquid paraffin, Span 60, glycerine, nypagin, *Oleum lavandulae* and distilled water.

Based on this, three emulsions were prepared under the same circumstances; the only difference consisted in lipid extracts (active ingredients) concentration.

The first emulsion consisted of *Mytilus galloprovincialis* Lmk. lipid extract, the second of *Rapana venosa* lipid extract and the third of *Mitilus galloprovincialis* Lmk.:*Rapana venosa*= 1:1 (v/v) lipid extracts.

The emulsions containing lipid extracts from molluscs were prepared by the simple dissolving method, stirring the water in lipid dispersion [3]:

- 1st Phase: liquid paraffin, Span 60, nypagin and the lipid extract are mixed together. Afterwards, the prepared phase was allowed to stand 2 h at 70⁰C.
- 2nd Phase: distilled water.
- 3rd Phase: 2nd phase was dispersed in the 1st phase, by continuous mixing, for 3-5 minutes. The glycerine was added at the end.
- 4th Phase: after cooling the emulsion, *Oleum lavandulae* was dispersed for 15 min.

During the preparation of the emulsions, the solution was agitated slowly with a broad, paddle-like stirrer in order to avoid introducing air bubbles, which tend to destabilise the emulsion.

The lipid phase containing lipid extract/liquid paraffin in mass ratio 1:15 (total lipid content 64% w/w) was hydrated for 2 h with an aqueous phase (36% w/w), so the aqueous/lipid phase mass ratio was 1:1,77 (w/w) and active ingredients/aqueous phase mass ratio was 1:9 (w/w).

Pharmaceutical evaluation of the three emulsions

Samples of emulsions were evaluated for aspect, colour, smell, type of emulsion by dilution, electric conductivity and total dissolved salts (TDS) methods, dynamic viscosity, stability by heating at 70⁰C and centrifugation at 3000 rpm, pH and tension determinations [3].

Emulsions examination was performed with Nikon Y-FL (KAPAN) 08003 mycroscope with Nikon Digital Net Camera DN 100.

Electric conductivity and total dissolved salts (TDS) measurements were performed with a SENSION 5, Hach Conductivity Probe device.

Viscosity measurements were performed with an Ubbelohde device, through which the time of flowing was determined.

The pH and tension determination were performed with a 340i/SET, Strabe 1, WTW (Germany) pH-meter.

Results and discussion

Characterization of the three emulsions

All three emulsions presented a homogenous aspect over the 90 days of observation period, in which the colour was characteristic for marine pigments from yellow to brown. These parameters showed a good stability of the three emulsions.

On the other hand, the smell shows a small modification in time, regarding the *Oleum lavandulae* contribution. The diminished smell, at the end of observation period, could be due to the volatilisation of this etheric oil.

The type of W/O emulsion by dilution method shows its miscibility in sun- flower oil. By colour method on the microscope lunette with 1% methylen blue was showed the coloured droplets specifically W/O phase (Fig. 1, 2 and 3).

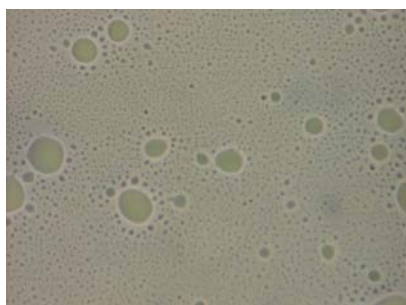


Figure 1
Emulsion with lipid extract from *Mytilus galloprovincialis* Lmk., $\times 40$

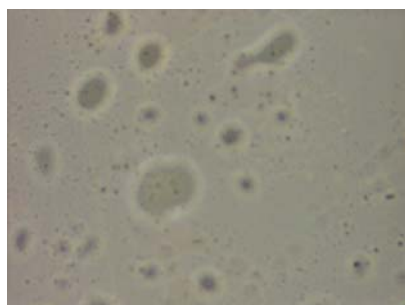


Figure 2
Emulsion with lipid extract from *Rapana venosa*, $\times 40$

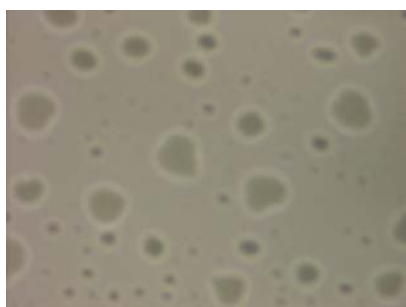


Figure 3
Emulsion with lipid extracts from
Mytilus galloprovincialis Lmk.:*Rapana venosa*= 1:1, $\times 40$

The rate, at which gravitational separation occurs, decreases with increasing droplet concentration because the movement of a droplet is hindered by the presence of the surrounding droplets. At sufficiently high

droplet concentrations gravitational separation may be completely retarded, as it is in many W/O emulsions.

The electric conductivity correlates with the content of total dissolved salts determination which shows that in obtained emulsions the electric undertaken of wearers was high in the first period and then was stabilized on the end of observation period (60-90 days) (Table I).

Table I
The pharmaceutical parameters variation at the beginning (initial) and end (90 days) of the observation period

No.	Pharmaceutical parameters	Emulsion with lipid extract from <i>Mytilus galloprovincialis</i> Lmk.		Emulsion with lipid extract from <i>Rapana venosa</i>		Emulsion with lipid extract from <i>Mytilus galloprovincialis</i> Lmk.: <i>Rapana venosa</i> = 1:1.	
		initial	90 days	initial	90 days	initial	90 days
1.	Electric conductivity ($\mu\text{S}/\text{cm}$)	492	691	865	1015	1688	2324
2.	Total dissolved salts (mg/L)	238	336	423	605	842	1200
3.	Dynamic viscosity (cP)	852.65	1386.85	3900.76	4443.78	3264	3798
4.	pH	6.68	6.89	5.05	5.32	5.54	5.74
5.	Tension (mV)	128.6	149.1	106.7	141.3	78.7	105.4

Dynamic viscosity of the three W/O emulsions was calculated in flowing time as their. This variation in time shows the same growing way in the first observation periods, probably because of some jelly process and it was stabilized after 60-90 days.

The stability test evaluated by homogeneity appreciation of the three emulsions heated at 70°C at different time intervals, showed their thermal resistance. Also, the stability was sustained by the fact that after centrifugation for 30 min at 3000 rpm, was resulted the same number of fractions, independent of conservation time, except the emulsion with lipid extract from *Rapana venosa* which presented three levels, probably because of the lipid component specificity.

The pH and tension variations at different time intervals showed few differences compared to the initial values, which confirm the stability of the obtained W/O emulsions.

CONCLUSIONS

The obtaining procedure of the three emulsions was adapted after preparation methods mentioned in literature. The difference and originally grade results from introduction of lipid extracts obtained from marine organisms which have different characteristics from other oils mentioned in literature. Those stability results was shown and sustained by the evolution in time of the analyzed pharmaceutical parameters.

Novel emulsion delivery system analysed here possesses enormous potential as next-generation smarter carrier system and can be applied into a large area of skin diseases due to its good spreadability and stability demonstrated by pharmaceutical evaluations.

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