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Stability studies of new cosmetic formulations with vegetable extracts as functional agents☆

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Abstract

A formulation study, using increasing amounts of Sepigel 305 as an emulsifier, has been carried out to find new O/W emulsions and value their stability also in presence of vegetable extracts. Stability results have been compared with those obtained from formulations described in the National Formulary of Italian Pharmacopoeia X and functionalised by us with the same vegetable extracts. By using centrifugation and accelerated ageing tests capable of bringing out the gelling and thermostability properties of Sepigel 305, the study emphasised that the new gel emulsions have a greater stability compared to the other formulations. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Sepigel 305; O/W emulsions; Vegetable extracts; Stability studies; Cosmetics

1. Introduction

Among cosmetic preparations, in recent years emulsions have rapidly evolved thanks to technological advances and research on the properties of raw materials.

A more frequent use of polymers in cosmetics has led to a new approach in the preparation of gel emulsions obtained at room temperature.

The aim of this study was to obtain new Sepigel 305 formulations (O/W skin creams) with high stability also at high temperature. Sepigel 305, also known as Farcosgel polyacrylamide (and) C13-14/isoparaffin (and) laureth-7, appears as a fluid, gelatinous, opalescent, yellowish dispersion with pH 6.5 in a 2% aqueous solution, and is compatible with most materials used in cosmetic preparations. It is classified as a gelling and thickening agent, excellent in the preparation of aqueous gels and emulsions. In this work we set up several formulation studies to obtain O/W gel emulsions with increasing amounts of Sepigel 305 as a main emulsifier, added with vegetable extracts. Furthermore, we compared the stability of these new formulations with those shown by others described in the National Formulary of Italian Pharmacopoeia X (FNFUX) [1], where the same vegetable extracts have been added.

2. Experimental

2.1. Materials

Sepigel 305, propylene glycol, Cetomacrogol 1000, Carbopol 940, PEG-stearate, glyceryl monostearate, glycerine, cetylstearyl alcohol, paraffin wax, mineral oil, Dermol 1 (octanoic acid–decanoic acid triglyceride mixtures), almond oil, olive oil, G.E. *Salvia officinalis*, F.E. *Centella asiatica*, and G.E. *Calendula* were obtained from Galeno Prato, Italy; EDTA-disodium salt, L-ascorbic acid 6-palmitate, and sodium hydroxide were obtained from Aldrich (Milan, Italy); distilled water was further purified with a Milli-Q system (Millipore, Bedford, MA, USA) and preserved with methyl-*p*-hydroxybenzoate–propyl-*p*-hydroxybenzoate 0.2% (Carlo Erba, Milan, Italy).

2.2. Preparation of the O/W skin creams

Preparation of Sepigel 305 O/W gel emulsions. A preserved water solution with propylene glycol (5%)

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and EDTA-disodium salt (0.1%) were added at room temperature (r.t.) to Sepigel 305 [1.5% (**I**), 2.5% (**II**), 5% (**III**), 7% (**IV**)] to obtain the water phase. The oil phase was prepared by melting stearyl alcohol (3%), and adding fluid oil (6%) [mineral oil (**a**), almond oil (**b**), olive oil (**c**), Dermol 1 (**d**)] with L-ascorbic acid 6-palmitate (0.01%). Both phases (the aqueous phase was added to the oil phase) were stirred at r.t. with an emulsifier (by Silverson Mixer/Emulsifier, England) until a homogeneous emulsion was obtained.

In this way 16 gel emulsions were obtained as reported in Table 1.

Preparation of skin creams O/W obtained according to FNFUX [1]: cetomacrogol hydrophilic cream (**V**), carbopol hydrophilic cream (**VI**), amphyphilic hydrophilic non-ionic cream (**VII**), and polysorbate hydrophilic cream (**VIII**) were prepared as described in FNFUX.

Preparation of formulations with vegetable extracts. Each of the skin creams, **IIb**, **IIc**, **IIIb**, **IIIc**, **V**, **VI**, **VII**, and **VIII**, was partitioned into four samples, one of which represented the control while the others were functionalised with a 2% vegetable extract (G.E. *S. officinalis* **1**, F.E. *C. asiatica* **2**, G.E. *Calendula* **3**). In this way, we obtained 24 functionalised samples as reported in Table 2.

2.3. Stability tests

Centrifugation assay. This test was carried out following the recommendations of Merril [2], i.e. the samples were centrifuged at 3000 rpm for 30 min at r.t..

Phase separation rates were reported as percentages referred to the graduated measuring tube (10 ml), i.e. 100 = stable; 0 = total instability.

Table 1
Sepigel 305 O/W gel emulsions [a]

Formulations	% Sepigel
Ia, **Ib**, **Ic**, **Id**	1.5
IIa, **IIb**, **IIc**, **Iid**	2.5
IIIa, **IIIb**, **IIIc**, **IIId**	5
IVa, **IVb**, **IVc**, **IVd**	7

[a] **a** = mineral oil; **b** = almond oil; **c** = olive oil; **d** = Dermol 1.

Table 2
Skin creams functionalised with vegetable extracts

Formulations	Vegetable extracts (2%)
$\mathbf{IIb_1}$, $\mathbf{IIc_1}$, $\mathbf{IIIb_1}$, $\mathbf{IIIc_1}$, $\mathbf{V_1}$, $\mathbf{VI_1}$, $\mathbf{VII_1}$, $\mathbf{VIII_1}$	G.E. *Salvia officinalis*
$\mathbf{IIb_2}$, $\mathbf{IIc_2}$, $\mathbf{IIIb_2}$, $\mathbf{IIIc_2}$, $\mathbf{V_2}$, $\mathbf{VI_2}$, $\mathbf{VII_2}$, $\mathbf{VIII_2}$	E.F. *Centella asiatica*
$\mathbf{IIb_3}$, $\mathbf{IIc_3}$, $\mathbf{IIIb_3}$, $\mathbf{IIIc_3}$, $\mathbf{V_3}$, $\mathbf{VI_3}$, $\mathbf{VII_3}$, $\mathbf{VIII_3}$	E.G. *Calendula*

Temperature stability [3]. Four batches of each sample were stored in triplicate at:

- low temperature ($\sim +5$°C);
- ambient temperature;
- high temperature (42°C);
- in freeze–thaw cycle cabinets (-10 to $+42$°C, two cycles every 24 h) for 2 weeks. Daily checks were performed.

The results were reported as percentages referred to the graduated measuring tube on the grounds of volume loss (exudation), homogeneity, creaming, phase separation, etc.

Rheological measurements. A number of rheological tests were performed with samples at r.t. using a Viscosimeter Brookfield LVDV-II + at 10 rpm.

pH Measurements. Sample pH measurements, after dilution with water and filtering, were carried out at 20°C with a Forlab pHmeter (Carlo Erba).

Microbiological assay. The samples were tested to assess the absence of aerobe microbe strains typically checked for in cosmetics. The method was carried out according to literature [4].

Organoleptic test. The organoleptic features of the samples were examined at the same temperature, lighting, and packaging conditions to assess variations in appearance, colour, smell, and spreadability in time.

All tests were carried out after 24 h, and two, four, and six months after preparation.

3. Results and discussion

Sepigel 305 skin creams have been manufactured with the aim of obtaining products of a simple formulation with precise technical, sensorial, and functional characteristics, to define them as creamy emulsions with a high or medium viscosity. Following the matrix method [5], we prepared aqueous dispersions with increasing percentages of Sepigel 305 [1.5% (**I**), 2.5% (**II**), 5% (**III**), 7% (**IV**)] emulsified with an oil phase made up essentially of fluid oil with different physico-chemical, functional, and organoleptic characteristics for each formulation [mineral oil (**a**), almond oil (**b**), olive oil (**c**), Dermol 1 (**d**)]. The percentage of oil was fixed at 6% and the oil phase was completed by adding stearyl alcohol (3%) as a rheological agent. The formulations were completed by adding adjuvants for stability in time, i.e. propylene glycol (5%) as a humectant, disodium EDTA (0,1%) as a metal chelating agent, ascorbic acid 6-palmitate (0.01%) as an antioxidant and methyl *p*-hydroxybenzoate–propyl *p*-hydroxybenzoate (0.2%) as preservatives. On the whole we obtained 16 different formulations (Table 1).

All gel emulsions were submitted to technical tests to check their physico-chemical and organoleptic characteristics at start and several months after preparation.

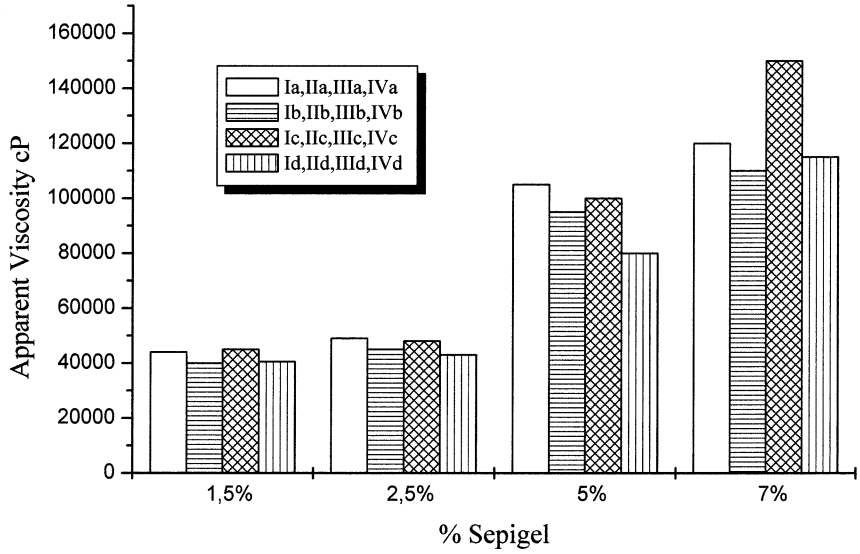

Fig. 1.

As regards centrifuge and thermostability tests, all preparations gave positive results within six months. The pH values showed minimal changes within the 5.5–6.2 range, which remained constant for the entire experimental period.

The rheological characteristics of the skin creams are shown in Fig. 1.

The formulations containing 7% (**IV**) and 5% (**III**) Sepigel 305 show a higher apparent viscosity and therefore greater consistency than those prepared with 2.5% (**II**) and 1.5% (**I**) Sepigel 305 lower apparent viscosity.

The organoleptic and subjective features are reported in Table 3.

The formulations containing vegetable oil were found pleasant as they 'rub in' quickly and give a sensation of freshness and immediate hydration, compared to those made with mineral oil and Dermol 1, which gave an oily sensation on the skin.

As functional agents we chose three vegetable extracts often used in phytocosmetic preparations, i.e. G.E. *S. officinalis* (**1**) as a cell-cleaning agent, G.E. *C. asiatica* (**2**) as a cell-proliferating agent and F.E. *Calendula* (**3**) as an emollient agent. Technologically speaking, vegetable extracts are added to the finished O/W emulsion. When the extract is added to the aqueous phase, it dilutes the system and probably destabilises the cream structure.

To check whether the Sepigel gel emulsions are capable of trapping further aqueous components, we chose medium-to-high apparent viscosity skin cream samples (**II** and **III**), in particular those obtained with almond and olive oils (**IIb**,**c** and **IIIb**,**c**) to be functionalised with vegetable extracts. For the same reason we also prepared another four skin creams formulated according to FNFUX using the same vegetable extracts up to a total of 24 samples as reported in Table 2. These samples were submitted to technological assays to assess their stability, and physico-chemical and organoleptic characteristics 24 h and two, four, and six months after preparation.

The centrifuge assay data are reported in Table 4.

It can be observed that only samples $\mathbf{V_{1,2,3}}$ and $\mathbf{VI_{1,2,3}}$ showed partial creaming four months after preparation. On the other hand, the remaining samples were stable for at least six months, thanks to the emulsion system that can take up further fluid components without phase separation.

Different results were obtained when the 42°C accelerated ageing test was performed (see Fig. 2).

Destabilisation was observed within four months mainly due to a loss in volume of the skin creams $\mathbf{V_{1,2,3}}$,

Table 3
Sepigel 305 gel emulsions organoleptic features

Samples	Appearance	Colour	Smell	Spreadability
Ia	Homogeneous	White	Odourless	Good
Ib	Homogeneous	White pale	Odourless	Good
Ic	Homogeneous	White pale	Olive oil	Good
Id	Homogeneous	White	Odourless	Good
IIa	Homogeneous	White	Odourless	Good
IIb	Homogeneous	White pale	Odourless	Good
IIc	Homogeneous	White pale	Olive oil	Good
IId	Homogeneous	White	Odourless	Good
IIIa	Homogeneous	White	Odourless	Good
IIIb	Homogeneous	White	Odourless	Good
IIIc	Homogeneous	White	Odourless	Good
IIId	Homogeneous	White	Odourless	Good
IVa	Homogeneous	White	Odourless	Good
IVb	Homogeneous	White	Odourless	Good
IVc	Homogeneous	White	Odourless	Good
IVd	Homogeneous	White	Odourless	Good

Table 4
Centrifuge test data of functionalised skin creams

Functionalised creams	Hours	Months		
	24	2	4	6
$IIb_{1,2,3}$	100	100	100	100
$IIc_{1,2,3}$	100	100	100	100
$IIIb_{1,2,3}$	100	100	100	100
$IIIc_{1,2,3}$	100	100	100	100
V_1	100	100	80	75
V_2	100	100	85	80
V_3	100	100	90	85
VI_1	100	100	85	80
VI_2	100	100	80	80
VI_3	100	100	95	90
$VII_{1,2,3}$	100	100	100	100
$VIII_{1,2,3}$	100	100	100	100

$VI_{1,2,3}$, **$VII_{1,2,3}$**, and **$VIII_{1,2,3}$** compared to the control samples. On the contrary formulations **$IIb,c_{1,2,3}$** and **$IIIb,c_{1,2,3}$** were stable for the entire assay. This is probably due to the presence of Sepigel 305, which does not polymerise at high temperatures and can trap free water and oil droplets within its interstices in the O/W emulsions.

As regards the rheological aspect, the skin creams considered showed apparent viscosity values ranging between high (**III**, **VII**, **VIII**) and medium consistency (**II**, **V**, **VI**).

The addition of vegetable extracts in all samples caused a decrease of around 20% in apparent viscosity compared to the basic creams, which remained stable after two months. As an example Fig. 3, shows the change in apparent viscosity in skin creams **IIb**.

The pH values of the prepared formulations are between 5.6 and 6.3 and remain stable for the entire assay period.

Microbiological tests give good results in the entire assay (Table 5), showing that the preservative system used is adequate for a good microbiological quality [6].

The organoleptic features for the final formulations, which are similar throughout the six months of the study period, confirm their durability under normal conditions.

4. Conclusions

The new cosmetic formulations obtained in this study using Sepigel 305 as a main emulsifier have shown that even at low concentrations (**I**) Sepigel 305 stabilises emulsions in which the oil phase is essentially made up of a fluid oil. Addition of vegetable extracts did not compromise the structure of the skin creams, which were confirmed stable both by the centrifuge assay and by the accelerated ageing test six months after preparation.

The same results were not obtained in the formulations according to FNFUX, in which stability has been confirmed. It has been pointed out that addition of vegetable extracts in these formulations can give rise in time to destabilisation processes, which are detected both by the centrifuge assay (samples **V** and

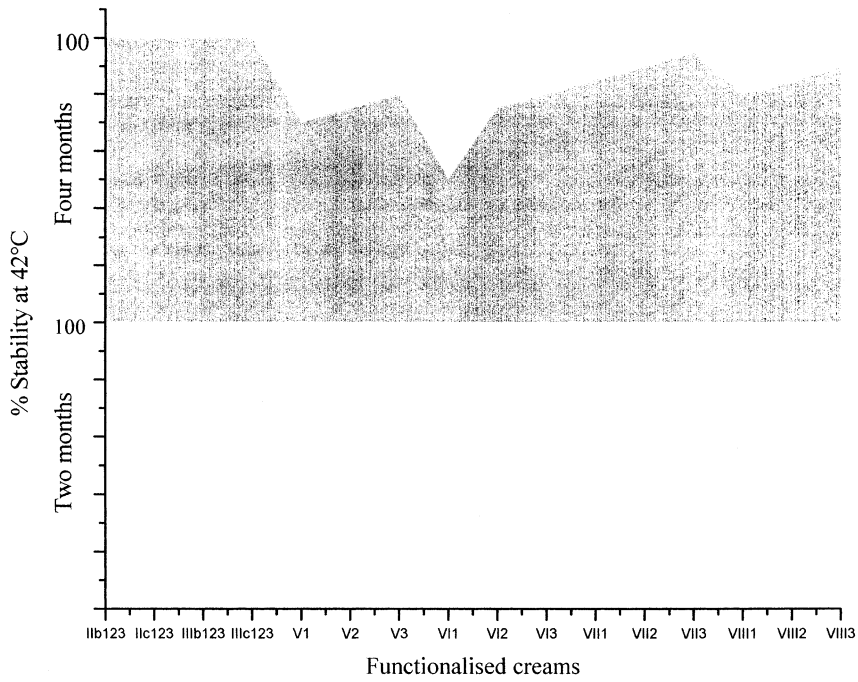

Fig. 2.

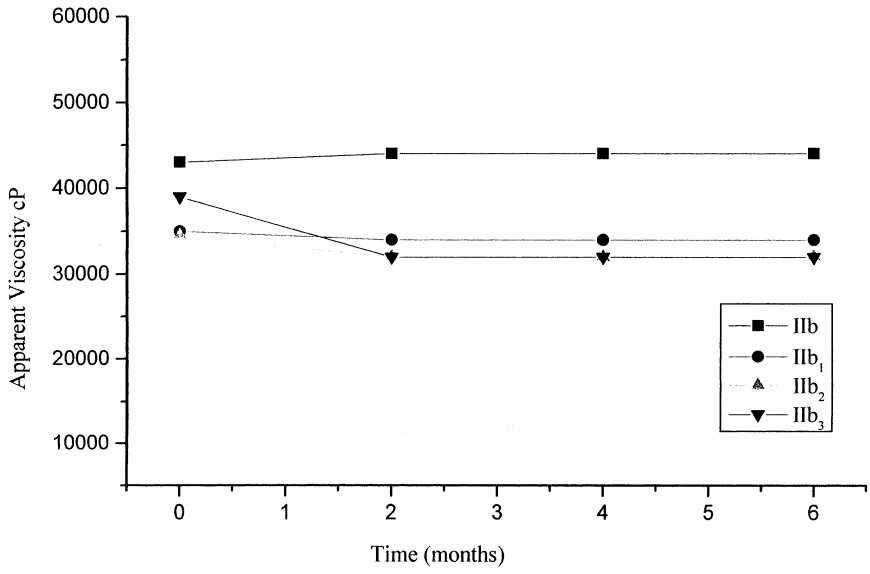

Fig. 3.

Table 5
Microbiological test of functionalised creams at six months after preparations

Functionalised creams	Total viable count	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphiloc. aureus*	*Candida albicans*
	PCA [a] (cfu/g)	Cetrimide (cfu/g)	VRBGl [b] (cfu/g)	BP [c] (cfu/g)	YM [d] (cfu/g)
$IIb_{1,2,3}$	$<10^2$	<10	<10	<10	$<10^2$
$IIc_{1,2,3}$	$<10^2$	<10	<10	<10	$<10^2$
$IIIb_{1,2,3}$	$<10^2$	<10	<10	<10	$<10^2$
$IIIc_{1,2,3}$	$<10^2$	<10	<10	<10	$<10^2$
$V_{1,2,3}$	$<10^2$	$<10^2$	<10	<10	<10
$VI_{1,2,3}$	$<10^2$	$<10^2$	<10	<10	$<10^2$
$VII_{1,2,3}$	$<10^2$	<10	<10	<10	$<10^2$
$VIII_{1,2,3}$	$<10^2$	<10	<10	<10	<10

[a] Plate count agar.
[b] Violet red bile glucose.
[c] Baird parker agar.
[d] Yeast and mould agar.

VI) and by the accelerated ageing test (samples **V**, **VI**, **VII**, **VIII**).

This shows that stability studies can often help overcome problems in the formulation of new cosmetic products, especially when further fluid components must be added to obtain satisfactory functionalisation (i.e. vegetable extracts).

The refinement and balancing of the components of a formulation is a very complex issue that requires a number of carefully chosen steps to obtain a finished product that should be both psycho-rheologically pleasant and stable.

The possibility of obtaining skin creams at room temperature, using Sepigel 305, not only makes the emulsification process easier but also prevents Phase Inversion Temperature (PIT) phenomena.

For these reasons Sepigel 305 and similar compounds can be considered as choice materials in cosmetics formulations.

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