FORMULATION AND CHARACTERIZATION OF ANTI-AGING COSMETIC EMULSIONS BASED ON COLLAGEN HYDROLYSATE AND CAFFEINE

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The goal of this work was to formulate and characterize some O/W emulsions, designed as skin anti-aging creams. The cosmetic formulations based on collagen hydrolysate, caffeine and natural ingredients (essential and vegetable oils) were evaluated organoleptically, in terms of pH, morphological, superficial and rheological properties. The rheological measurements were carried out at 23 and 32°C, and the shear stress versus shear rate ascending and descending rheograms were built, as well as the flow patterns viscosity versus shear rate. All emulsions obtained are stable at different temperatures and the pH values correspond to the skin physiological one, indicating that cosmeceuticals can be safely applied to the skin. Results from the optical microscopy analysis show that all emulsions presented a creamy and foamy appearance. The superficial profiles, quantified through contact angle at solid/liquid interface, indicated a high emulsions hydrophilicity degree. The emulsions showed a pseudoplastic and thixotropic behaviour, facilitating the flow formulations and the topical application. The flow properties were quantified according to the Power law model, and the thixotropic analysis was performed using specific descriptors as thixotropic area and thixotropic index. The designed emulsions presented physico-chemical properties adequate for cosmetic skin care product formulations.

Keywords: collagen hydrolysate, anti-aging cosmetic cream, physico-chemical characteristics

INTRODUCTION

Skin aging is characterized by a progressive deterioration of the skin functional properties, linked to alterations of dermal connective tissue due to the changes at the cell, gene and protein levels. Skin aging can be divided into two basic processes: intrinsic aging and photoaging (Zhou *et al.*, 2020; Jung-Won *et al.*, 2019).

Collagen, being an important protein for the skin, is essential in the structure and functioning of the extracellular matrix in the dermis (Albu, 2011). Visibly thin skin, fine lines and wrinkles are the first signs of collagen reduction in the skin due to the aging process. In addition to the anti-wrinkle effect, collagen is used in cosmetics also for the hydration it offers by forming a film on the skin surface that prevents the loss of transepidermal water and protects skin by external factors (Aguirre-Cruz *et al.*, 2020).

The most popular systems employed in the manufacture of cosmetics are emulsions (Arrieta-Escobar *et al.*, 2019). To incorporate protein (like collagen) into cosmetic

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products (e.g. emulsions), it is appropriate that the proteins are water-soluble and re-aggregation of peptides is avoided. Hydrolysates of proteins are often added into cosmetic products (Dănilă *et al.*, 2019b; Li *et al.*, 2008).

The aim of this study was to formulate and evaluate some O/W emulsions based on collagen hydrolysate and other natural ingredients such as vegetable and essential oils, and caffeine, that can be used as anti-age creams.

MATERIALS AND METHODS

Materials

Vegetable oils and butter (mango butter, almond oil, rice oil, coconut oil), emulsifiers (glyceryl stearate; cetearyl olivate and sorbitan olivate), floral waters (linden, neroli, melissa, hammamelis), xanthan gum, caffeine, allantoin, essential oils were purchased from a local pharmacy. Type I collagen hydrolysate was obtained by acidic hydrolysis as we previously described (Dănilă *et al.*, 2019b).

Preparation of the O/W Emulsions

According to Table 1, the ingredients of phase A (oils and emulsifier) and phase B (floral water, distilled water, collagen hydrolysate, caffeine) were heated in a water bath in two heat-resistant Berzelius beakers, periodically homogenizing the composition. When both phases reach a temperature of about 70-75°C, they were removed from the water bath; and phase B was slowly added over phase A under continuous stirring. The mixing continued for 10 minutes, avoiding as much as possible the aeration of the emulsion. The beaker was placed in a cold-water bath under continuous stirring for 3 minutes. In the cooled composition, the ingredients of phase C (allantoin, xanthan gum, essential oils) were added and mixed slowly. The obtained emulsion was transferred to a sterile container.

Table 1. Composition of the dermatocosmetic O/W emulsions

Phase	Ingredients, %	Emulsion 1	Emulsion 2	Emulsion 3	Emulsion 4
A	almond oil (mL)	10	20	-	-
A	rice oil (mL)	-	-	10	15
A	mango butter (g)	12.6	11.1	-	-
A	coconut oil (g)	-	-	12.6	16.1
A	Bella emulsifier (g)*	5	5	-	-
A	Olliva emulsifier (g)**	-	-	5	5
B	floral water (mL)***	10	10	10	10
B	distilled water (mL)	60	50	60	50
B	collagen hydrolysate (g)	1	2	1	2
B	caffeine (g)	0.5	1	0.5	1
C	allantoin (g)	0.4	0.2	0.4	0.2
C	xanthan gum (g)	-	0.2	-	0.2
C	essential oil (mL)****	0.5	0.5	0.5	0.5

* Bella emulsifier: glyceryl stearate; **Olliva emulsifier: cetearyl olivate, sorbitan olivate

*** Linden, neroli, melissa, hammamelis; **** Eucalyptus, pine, rosemary, oregano

pH Determination

The pH of dermatocosmetic emulsions pH was evaluated using an inoLab pH meter.

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Optical Microscopy Analysis

The morphology of the designed emulsions was carried out using a LEICA optical microscope model S8AP0, with increase power of 20-160x.

Goniometric Evaluation

The superficial properties were performed with CAM 101 (KSV Instruments), using the *contact angle routine*: liquid drop (water) was spread on a solid surface (emulsion-coated slide test), analyzing the modifications of liquid drop shape on this surface.

Rheological Analysis

The flow properties of the emulsions were conducted with a rotational viscometer Multi-Visc Rheometer (Fungilab), equipped with TR 8 and TR 10 standard spindles, and a ThermoHaake P5 Ultrathermostat to ensure a constant temperature ($23 \pm 0.1^\circ\text{C}$ and $32 \pm 0.1^\circ\text{C}$). The operational conditions were previously reported (Ghica *et al.*, 2016).

RESULTS AND DISCUSSION

All emulsions are homogeneous, stable, with no phase separation and with a smell specific to the ingredients. The W/O emulsions presented a pH between 5.5 and 6, compatible with the skin pH. The morphology of the emulsions was performed by optical microscopy analysis and the images obtained are presented in Figure 1.

Figure 1. Optical microscopy images of W/O dermatocosmetic emulsions

From Figure 1 it can be noticed that all emulsions present a “foam-like” aspect. The differences between the microscopic images are due to various vegetable oils incorporated in the oil phase of the emulsions, with a different content of fatty acids.

The superficial properties of the emulsions were quantified through contact angle (CA°) values. The drop shape (Figure 2) was monitored with a digital camera and mathematically described by the Young equation:

$$\gamma_{\text{SG}} = \gamma_{\text{SL}} + \gamma_{\text{LG}} \cdot \cos \theta \quad (1)$$

where γ_{SG} is the interfacial tension S/G, γ_{SL} – the interfacial tension S/L, γ_{LG} – the superficial tension L/G and θ – the contact angle.

Figure 2. Images of the drop shape for the W/O dermatocosmetic emulsions:

a) Emulsion 1; b) Emulsion 2; c) Emulsion 3; d) Emulsion 4

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It can be remarked that contact angle values are about 1.3-2.3 times higher for the Emulsions 2 and 4 compared to the Emulsions 1 and 3. The CA recorded values indicated a marked hydrophilicity of the emulsions, a quality requirement important in their design, with direct implications in skin hydration capacity.

The rheograms – viscosity as a function of shear rate – were built (Figure 3) and the Power law model was used to quantify the flow behaviour of the formulations:

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (2)$$

where m parameter represents the viscosity obtained for the shear rate value of $1 \cdot s^{-1}$, and n is the flow behaviour index (Dănilă *et al.*, 2019a).

Figure 3. Plots of viscosity versus shear rate, evaluated at 23°C and 32°C, for: a) Emulsion 1 and Emulsion 3; b) Emulsion 2 and Emulsion 4

As can be seen from Figure 3a-b, the emulsion viscosity decreased at shear rate increase showing a pseudoplastic behaviour at both working temperatures. The pseudoplastic behaviour is a requirement needed for the dermatocosmetic emulsions, both conditioning aspect and spreading as a continuous film at skin level.

The parameters n and m, specific to the Power law model, are listed in Tables 2 and 3 for both working temperatures.

Table 2. Values for the determination coefficient and parameter specific to the Power law model, and for the thixotropic descriptors determined at 23°C

Formulation	R ²	m	n	S _{asc} (Pa·s ⁻¹)	S _{thix} (Pa·s ⁻¹)	T _{hyst} %
Emulsion 1	0.9964	4.414	0.523	1423.334	91.303	6.41
Emulsion 2	0.9976	44.794	0.508	1706.082	163.295	9.57
Emulsion 3	0.9936	7.211	0.517	1763.773	123.175	6.98
Emulsion 4	0.9981	39.813	0.606	1189.556	132.985	11.47

Table 3. Values for the determination coefficient and parameter specific to the Power law model, and for the thixotropic descriptors determined at 32°C

Formulation	R ²	m	n	S _{asc} (Pa·s ⁻¹)	S _{thix} (Pa·s ⁻¹)	T _{hyst} %
Emulsion 1	0.9931	2.435	0.525	819.094	43.105	5.26
Emulsion 2	0.9991	29.991	0.529	1179.270	121.677	10.32
Emulsion 3	0.9976	3.135	0.481	963.843	61.379	6.37
Emulsion 4	0.9987	30.061	0.631	916.505	96.107	10.48

The highest values obtained for the determination coefficients R^2 (between 0.9936 and 0.9981 at 23°C, 0.9931 and 0.9991 at 32°C respectively) indicated that the experimental data well fitted the Power law rheological model. The most fluid formulation is Emulsion 1, the m parameter having the lowest value. The maximum values for m parameter are recorded for the Emulsions 2 and 4, about 6-10 times higher comparing to emulsions 1 and 3 at 23°C and about 10-15 times at 32°C.

The forward and backward rheograms – shear stress as a function of shear rate – were also recorded (Figure 4) and the thixotropic behaviour was quantified through thixotropy area (S_{thix}) and thixotropy index ($T_{hyst\%}$).

$$T_{hyst\%} = (S_{thix} / S_{asc}) \cdot 100 \quad (3)$$

where S_{asc} is the area enclosed under the forward rheogram. Thixotropic systems are those with $T_{hyst\%}$ values greater than 5% (Dănilă *et al.*, 2019b). The values for S_{asc} , S_{thix} and $T_{hyst\%}$ are given in Table 2 and Table 3 for both working temperatures.

Figure 4. Forward and backward rheograms, shear stress versus shear rate, evaluated at 23°C and 32°C, for: a) Emulsion 1 and Emulsion 3; b) Emulsion 2 and Emulsion 4

Figure 4a-b indicates that backward curve is placed under forward curve, indicating that for the same shear rate, the shear stress for the return curve is smaller. The thixotropic character is highlighted by the thixotropy index higher than 5% for both temperatures. The thixotropic behaviour is also a quality requirement targeted in emulsions design, allowing the transformation of a viscous product in a more fluid one, easy to spread.

Aside, the emulsions composition that influenced both their viscosity and thixotropic descriptors, another influence factor is related to the temperature. Thus, for

temperature increase it can be noticed a m parameter decrease for all emulsions, about 1.3- 2.3 times, and a decrease of thixotropic parameters about 1.3-2.1 times.

CONCLUSIONS

All emulsions obtained are stable and the pH values correspond to the skin physiological one, indicating that cosmeceuticals can be safely applied to the skin. The optical microscopy analysis shows that all emulsions presented a creamy and foamy appearance. The superficial profiles, quantified through contact angle, indicated a high emulsions hydrophilicity degree. The emulsions showed a pseudoplastic and thixotropic behaviour, facilitating the flow formulations and the topical application. It can be concluded that the designed emulsions presented physico-chemical properties adequate for cosmetic skin care product formulations.

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