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Stability Study of O/W Cosmetic Emulsions Using *Rosmarinus officinalis* and *Calendula officinalis* Extracts

Evi-Maria Varka, Eforia Tsatsaroni*, Nikoleta Xristoforidou, Anna-Maria Darda
Department of Chemical Technology and Industrial Chemistry, School of Chemistry, Aristotle University, Thessaloniki, Greece
Email: *tsatsaro@chem.auth.g

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ABSTRACT

Cosmetic emulsions, as all macro emulsions, are inherently unstable systems, from a thermodynamic viewpoint. More specific eco-friendly oil/water (O/W) cosmetic emulsions are usually less stable than conventional ones as milder chemicals or less intense (energy consuming) production processes are involved. In this context, two traditional techniques an optical technique and a volumetric one have been used for the assessment of the stability of cosmetic emulsions and compared to each other. Eco-friendly cosmetic emulsions were produced with different olive oil/water extracts (*Rosmarinus officinalis* and *Calendula officinalis* extracts) and emulsifier (Glycerol monostearate, GMS) concentrations. Emulsions' stability was registered simultaneously by 1) Microscopy photos of samples withdrawn at regular intervals from the test vessel; 2) Global volumetric measurements of the different phases (water/oil/emulsion) inside the test vessel made at regular intervals for determining the evolution of the location of the phases separation interface. Analysis of the results of each technique and comparisons among them are presented and discussed in detail.

Keywords: Cosmetic Emulsions; Olive Oil; Stability Studies; *Rosmarinus officinalis*; *Calendula officinalis*

1. Introduction

Since antiquity, emulsions have been used in cosmetics. In contrast to pharmaceutical ointments which must penetrate deep into the skin, cosmetic products are meant only for the immediate surface of the skin *i.e.* the epidermis. However, many commercial cosmetic products contain artificial chemicals that inevitably penetrate the skin. In the last few years physical products have started to gain the interest of consumers. Because of the inconsiderate use of chemical components and the harmful consequences of them in peoples' health, scientists started to look out for alternative findings to surpass these problems. For this reason herbs' study has gained attention [1-4].

Eco-friendly cosmetics—also known as organic cosmetics-appeared in the early 1900s. The biggest advantage of organic cosmetics is that they are safe for humans and the environment as they do not contain toxic chemicals, they need no animal testing and their results can be as pleasing as those achieved by commercial products.

Rosmarinus officinalis L. (*Lamiaceae*), commonly known as rosemary, is a household plant used worldwide as a food-flavouring agent, widely distributed in the Mediterranean region. A preclinical survey confirmed that rosemary has powerful anti-inflammatory, antibacterial, antidiabetic, antitumor, cytoprotective and hepatoprotective properties [5-10]. Rosemary has one of the highest antioxidant activities of all the herbs and spices that have been investigated [11]. The antioxidant activity of rosemary justifies its use in a broad range of applications, including food preservatives [12], cosmetics [13], phytomedicines etc. These properties attributes can be related to rosemary's high content of polyphenolic compounds, especially rosmarinic acid [14], which is considered a chemical marker of this species.

Calendula officinalis L. (*Asteraceae*), also known as marigold, is an annual herb native to the Mediterranean region. It is cultivated for ornamental and medicinal purposes in Europe and America. More than 35 properties have been attributed to the decoctions and tinctures from the flowers, e.g. anti-inflammatory, analgesic, bactericide, tonic and the healing of wounds and skin eruptions [15]. Previous phytopharmacological studies on the extracts of *Calendula officinalis* flowers have confirmed the presence of bioactive secondary metabolites, flavonol glycosides [16,17] and triterpenoids [18].

From the above we can see that traditional herbs provide an interesting, largely, unexplored source for development of potential new cosmetics. In the present study we tried to combine all this beneficial components of the herbs in order to make O/W emulsions.

The oil being used was olive oil. Olive oil is natural oil

*Corresponding author.

typically used in cosmetics and foods. Apart from being a natural and hypo allergic way to moisture skin, extra virgin olive oil has the extra advantage of containing strong antioxidants, like vitamins A and E. The antioxidants help repairing and renewing skin that has been damaged from overexposure to sun, air pollution and other environmental hazards. The selected emulsifier was GMS which is an eco-friendly emulsifier widely used in lotions, creams powders etc. as it is safe for humans and gives skin a soft and smooth appearance, while the water phase was deionized water.

A series of emulsions was made with different oil or water phase from the above extracts and their stability was studied. Emulsions' stability was registered simultaneously by 1) Microscopy photos of samples withdrawn at regular intervals from the test vessel or determining the evolution of the local droplet size distribution; 2) Global volumetric measurements of the different phases (water, oil) inside the glass tubes for determining the evolution of the phase's separation.Additionally, pH, surface/interface tension measurements were also made. Analysis of the results of each technique and comparesons among them are presented and discussed in detail.

2. Materials and Methods

2.1. Materials

The plant materials *Rosmarinus officinalis* and *Calendula officinalis*, were collected at the bloom stage at the end of May. The plant material was dried at room temperature and then milled. The dry plant material was then packed in paper bag and kept in a dark, dry and cool place.

Greek olive oil was obtained by "Altis S.A." whereas Glycerol monostearate (GMS), an esterification product of glycerine and stearic acid was obtained from Panreac (purity > 98%) and used as emulsifier.

2.2. Plant Material Extractions (Oil/Water Phases)

Plant materials (*Rosmarinus officinalis and Calendula officinalis*), were placed separately in a vessel with olive oil in a proportion of 10%. The material was warmed up for 5 minutes and then was kept in a dry place for 40 days approximately in order to have a complete extraction. The remaining oils were used as oily extracts.

Water extracts, where needed, were taken by inserting herbs (*Rosmarinus officinalis and Calendula officinalis*) into boiled water for 10 minutes in a proportion of 10%.

2.3. Emulsification Process

Emulsification was implemented by mixing water or water extract with olive oil or oil extract using an impeller rotating at the central axis of a glass vessel with internal diameter of 9.5 cm. The impeller was placed 1.0 cm above the bottom of the vessel and not at the traditional (for mixing applications) 1/3 of the vessel height. Four buffles were placed at 90 degrees intervals around the vessel in order to allow high rotation speeds without vortexing and air suction. Emulsions were prepared in the above glass vessel as follows. Initially, proper amount of GMS (**Tables 1**, **2**) was added to the 20 ml of oil and then the mixture was heated to 70˚C (GMS is solid at ambient temperature). The corresponding amount of aqueous solution 80 ml was heated separately to 70˚C. Emulsification started by adding the hot aqueous phase into the hot oil. At that moment heat supply was turned off and agitation was set at 700 rpm. The impeller was running at 700 rpm, for a total 1.5 min. This period was followed by 30 more minutes of agitation at 700 rpm which was sufficient for the emulsion to cool down to ambient temperature (25˚C ± 1˚C).

2.4. Creaming Index from Volumetric Measurements

Direct optical observations were employed to determine the instantaneous heights of the emulsion and of the aqueous phase inside the glass vessel (**Figure 1**) and to estimate the creaming index according to the formula (Equation (1)):

$$CI = \frac{H_{aq}}{H_{tot}} \times 100 \quad (1)$$

Table 1. Oil-to-water types of *Rosmarinus officinalis* emulsions and concentration of GMS in the oily phase used for emulsion preparation.

Type of emulsions	4.5 g GMS	6 g GMS
O/W[a]	√[b]	√
O/R	√	√
R/W	√	√
R/R	√	√

Table 2. Oil-to-water types of *Calendula officinalis* emulsions and concentration of GMS in the oily phase used for emulsion preparation.

Type of emulsions	3 g GMS	4 g GMS
O/W	√	√
O/C	√	√
C/W	√	√
C/C	√	√

a. O/W: always the first letter denotes oily phase whereas the second one the water phase; R: *Rosmarinus officinalis* extracts; C: *Calendula officinalis* extracts. b. √: denotes the emulsions being made.

Creaming index, CI, represents the instantaneous global (*i.e.* the whole liquid volume) volumetric water fraction. It is the most customary measure of emulsion destabilization reflecting phase separation (**Figure 1**).

2.5. Droplets Size from Microscope Photos

Several photos were taken by the microscope from different parts of the withdrawn samples until a population above 300 droplets was collected for each sample in order to ensure statistical significance in the determination of droplets size. A custom made software capable of handling even very dense emulsions was employed to obtain droplet diameter distributions and from them to acquire droplet size distributions (**Figure 2**).

2.6. Additional Measurements

2.6.1. pH Measurements

pH measurements were made by dipping into the emulsion the pH sensor of a WTW pH 535 microprocessor.

2.6.2. Surface/Interface Tension Measurements

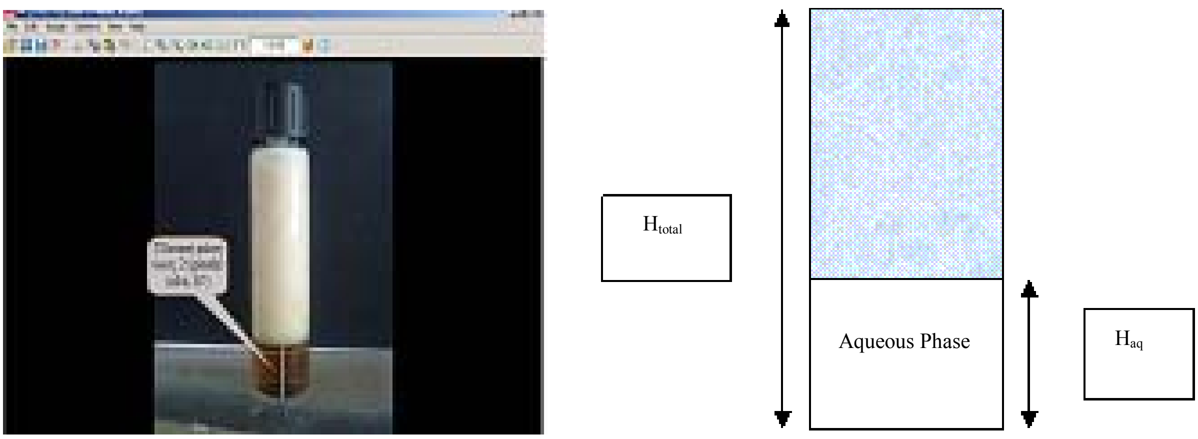
The surface tension measurements were taken with the Wilhelmy plate technique using a KSV Sigma 70 tensiometer. The platinum plate was partially immersed into the oil layer, where it remained to fixed position during the course of the experiment. Thus, the surface tension decreased as a function of time. When the surface tension was stabilized then the experiment was stopped. For the interfacial tension measurement Ring Du Nouy method was used. A ring from platinum was going down and immersed into the water phase. Then carefully the oily phase was being placed from the top of the water one. The ring was pulling up until the lamella from the interface breaks off. At this point the measured force gives interfacial tension value. This method has an easy handling, big accuracy and does not depend on wetting contact angle. All measurements were made at 25˚C ± 0.1˚C.

3. Results and Discussion

3.1. Creaming Index

Figures 3 and **4** present the computed creaming index for *Rosmarinus officinalis*/*Calendula officinalis* oil-in-water cosmetic emulsions respectively.

The higher the creaming index value is, the more destabilized the emulsion is. In our occasion maximum



(a) (b)

Figure 1. (a) Glass vessels being employed for the determination of creaming index value according to scheme (b).

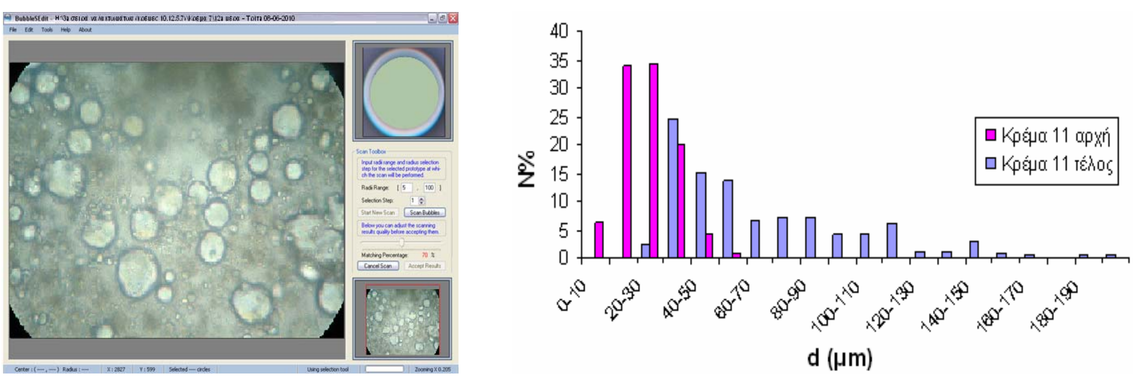


Figure 2. Schematic representation of estimating droplet diameter distributions.

value proportion of water is 80%. There are two general features in the evolution of creaming index: 1) as oil phase is enriched with oil extract, creaming rate decreases; 2) as emulsifier concentration increases, creaming rate decreases.

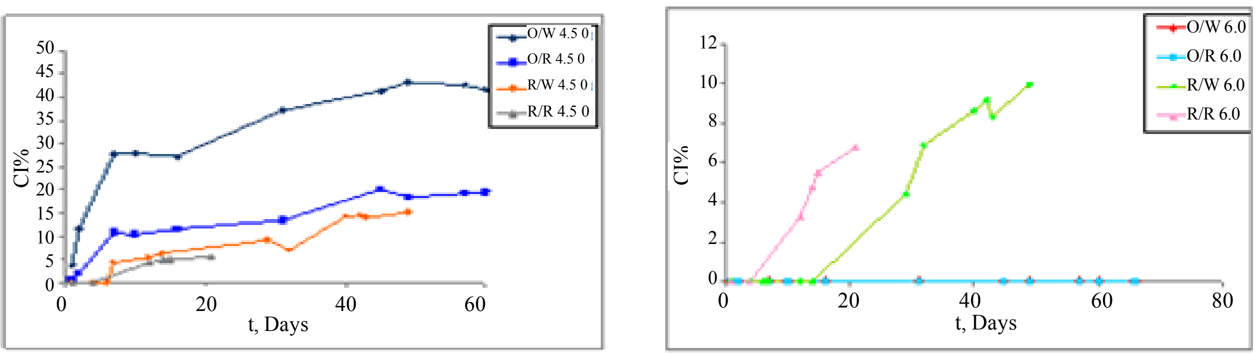
More specific, **Figure 3** represents creaming index value for *Rosmarinus officinalis* emulsions. For an emulsion with 6 g GMS and *Rosmarinus officinalis* oil extract no sign of creaming was observed for the first 60 days after production. Yet, all other examined cases show some variability with time although none of the emulsions reached the value of 80% representing the complete separation. Probably the emulsions were stabilized after having a metastable form. Separation of the two phases was almost complete in less than 20 days in the emulsions with no addition of extracts, regardless GMS concentration.

Same trends are also observed in **Figure 4**. Separation of the two phases was almost complete in less than 5 days in the emulsions with no addition of extracts.

Comparing **Figures 3** and **4** the following comments can be made 1) Emulsions made up with *Calendula officinalis* extracts are more stable than those made with *Rosmarinus officinalis* due to lower values of creaming index (results were not changed up to 30 days for *Calendula officinalis* emulsions) 2) emulsions having oily extracts were the most stable in both cases.

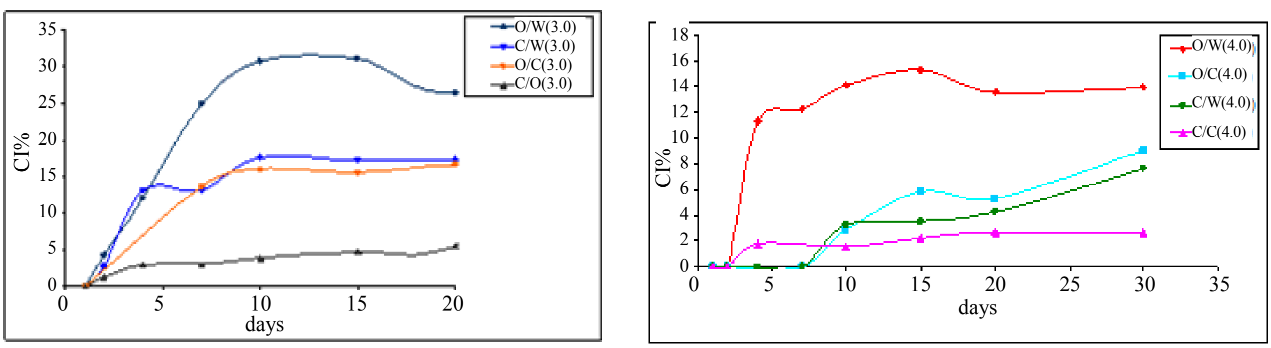
3.2. Droplet Size Distributions

Results obtained from the analysis of the microscope photographs are presented in **Figures 5** and **6**. The mean diameter of the emulsion from samples is shown against the GMS concentration for the two emulsion types. In accordance with stability results from volumetric measurements the following are concluded: 1) droplet size decreases as GMS concentration increases and also as emulsions are enriched with herbal extracts. One would expect that droplet sizes would be smaller for emulsions made up with 6 g GMS instead of 3 and 4 g. Although data points for the two emulsion compositions are close enough and can be considered comparable given the statistical lack of confidence (at a 95% level), on the other hand; 2) different herb components may affect the total results. In terms of droplet size *Calendula officinalis*' emulsions present better stability (smaller droplets) than



(a) (b)

Figure 3. Creaming index versus time for *Rosmarinus officinalis* extract with (a) 4.5 g GMS and (b) 6 g GMS (where O: Olive oil; W: water; R: *Rosmarinus officinalis* oil and water extract).



(a) (b)

Figure 4. Creaming index versus time for *Calendula officinalis* extract with (a) 3 g GMS and (b) 4 g GMS (where O: Olive oil; W: water; C: *Calendula officinalis* oil and water extract).

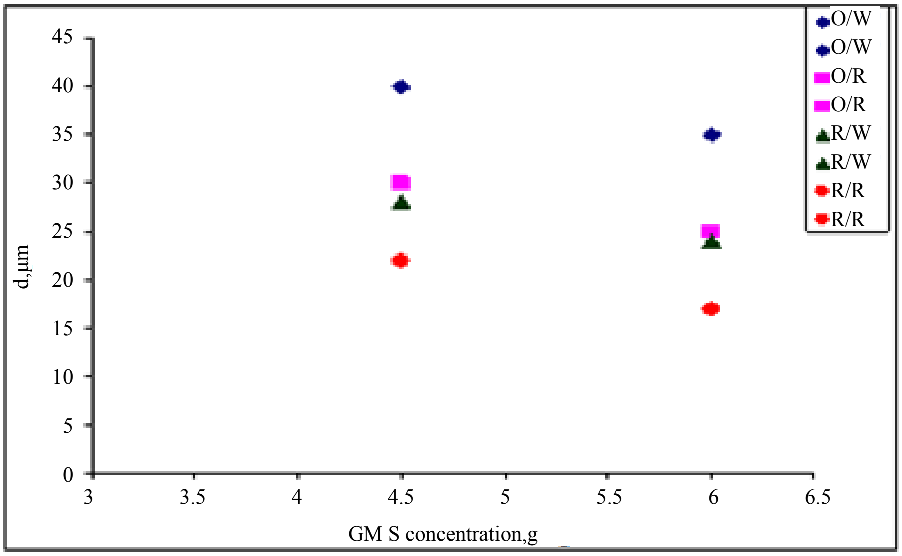


Figure 5. Effect of GMS concentration in mean droplet diameter in *Rosmarinus officinalis* emulsions.

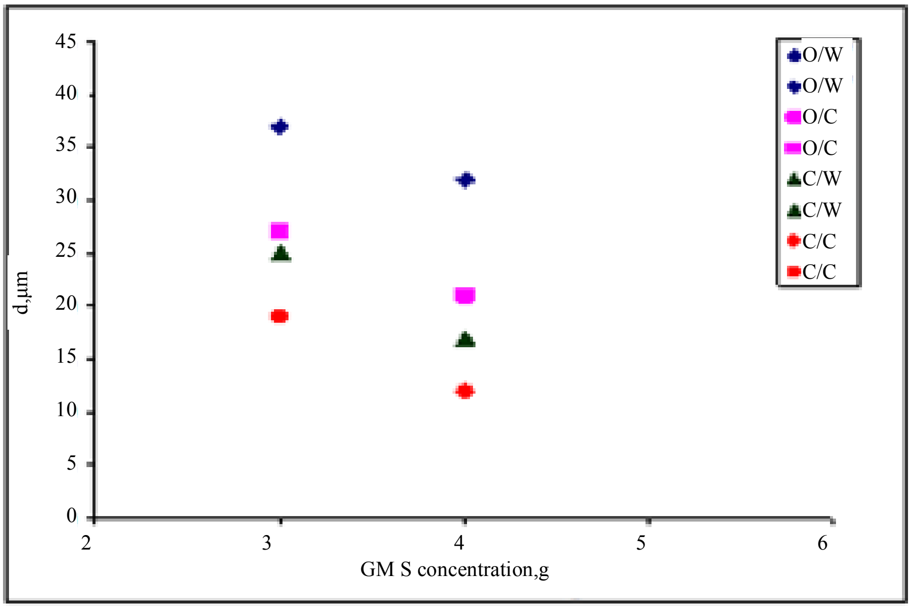


Figure 6. Effect of GMS concentration in mean droplet diameter in *Calendula officinalis* emulsions.

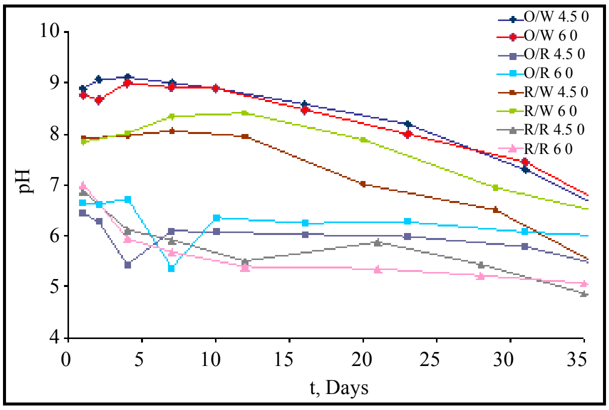
those of *Rosmarinus officinalis*' emulsions. In accordance with creaming index results *Calendula officinalis* emulsions are more stable.

3.3. pH Measurement

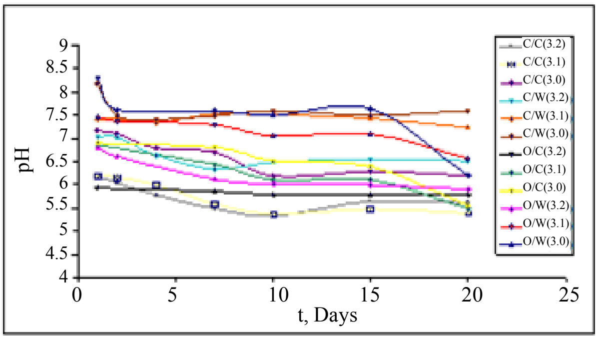
In **Figures 7(a)** and **(b)** the effect of storage on the pH values of the emulsions is given. As expected, in both types of emulsions as oil and/or water extract is added, pH decreases and this due to different active ingredients of herb extracts. The emulsions with *Calendula Officinalis* present a remarkable pH stability with time (**Figure 7(b)**). The emulsions with *Rosmarinus officinalis* extracts are not as pH stable as those with *Calendula officinalis*.

3.4. Surface—Interface Tension Measurements

The low values of interfacial tensions between oil extracts and water extracts (**Tables 3-5**) are in line with results obtained from optical monitoring and volumetric measurements. The lower the interfacial tension is, the more stable the emulsion will be. The droplet size distribution resulting from the emulsification procedure is the outcome of the simultaneous processes of coalescence between droplets and droplet breakage. The presence of



(a)



(b)

Figures 7. Results obtained from pH meter.

Table 3. Surface tensions of olive oil.

	Temperature (˚C)	Surface Tension (mN/m)
Olive oil	25 ± 0.1	20
H_2O	25 ± 0.1	71.8

Table 4. Interfacial tensions olive oil/*Rosmarinus officinalis* oils.

	Temperature (˚C)	Interfacial Tension (mN/m)
Olive oil-H_2O	25 ± 0.1	17
Olive oil-Water extract	25 ± 0.1	6
Olive oil extract-H_2O	25 ± 0.1	14
Olive oil extract-Water extract	25 ± 0.1	6

Table 5. Interfacial tensions olive oil/*Calendula officinalis* oils.

	Temperature (˚C)	Interfacial Tension (mN/m)
Olive oil-H_2O	25 ± 0.1	17
Olive oil-Water extract	25 ± 0.1	5
Olive oil extract-H_2O	25 ± 0.1	13
Olive oil extract-Water extract	25 ± 0.1	6

surface active components in herbal extracts reduces the coalescence process (stabilizing the water film between colliding droplets) and enhances the breakage process due to the reduction of the effective interfacial tension of the droplet. The combination of these effects results in the production of smaller droplets (more stable emulsions), as interfacial tension decreases.

Conclusively, comparison of the two herbs, *Rosmarinus officinalis* and *Calendula officinalis*, results in the following: 1) The effect of *Calendula officinalis* extracts is stronger than those of *Rosmarinus officinalis*. Less amount of GMS result in more stable emulsions; 2) The emulsion stability evaluated from factors such as Creaming Index, volumetric index for *Calendula officenalis* emulsions reveals more stability than those referred to *Rosmarinus officinalis*.

4. Conclusion

In the present work, two experimental techniques were used for the assessment of the stability of oil-in-water cosmetic emulsions produced with different herbs and surfactant concentrations. Results from two classical techniques (the well known volumetric measurement of phase separation and the optical measurement of droplet diameters from withdrawn samples) were examined and compared to each other. The droplet sizes estimated from optical observations of the droplets were in accordance with results obtained by interfacial tension measurements referring that emulsions made up with *Rosmarinus officinalis* are less stable than those of *Calendula officinalis*. The results and their discussion in this work reveal that the addition of herbs in cosmetic emulsions can improve emulsions' stability without further addition of chemical stabilizers.

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