Rheological Properties and Stability of Squalene Emulsion Prepared with Non-Ionic Emulsifier

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Squalene is natural occurring polyprenyl compound with a linear triterpene chemical structure commonly found in animal and vegetal sources such as skin lipids from human body, liver of certain species of fish, especially sharks, crude vegetable oils (e.g olive, amaranth, palm oil etc.). Recently there has been an increasing interest in studying polymers that can be used as promising emulsions for drug delivery and vaccine applications. Recent studies proved that squalene has several beneficial and unique properties such as natural antioxidant, ensure skin hydration, biocompatibility. This can be used as such or for preparing stable and non-toxic emulsions. Herein we present the preparation and characterization of squalene oil-in-water emulsions stabilized by different non-ionic emulsifiers. Emulsion stability is a primary concern, therefore physical stability of squalene emulsions was also investigated. The major aim of the study was to find the optimal preparation conditions and assessing emulsion stability correlated with their viscosity and composition. In order to establish the connection between their physical stability and viscosity were performed rheological studies.

Keywords: emulsion, physical stability, squalene, viscosity

In the scientific literature emulsions are defined as dispersions obtained from at least two immiscible fluids (such as oil and water) as a result of shearing and dispersion of one phase into the other [1-4]. The disperse phase droplets are dispersed in a continuous medium (liquid phase) [3, 4]. They are several classes of dispersions that can be distinguished: oil-in-water (O/W), water-in-oil (W/O), and oil-in-oil (O/O) [1, 3].

Due to their found use in medicinal and cosmetic applications the formulation of very fine O/W emulsions gained recently an increased interest from the scientific community [4-6]. Finding a matrix suitable for drug delivery represents the main problem regarding the active compound solubility and transportation. Recent studies proved that this challenge could be easily overcome by using O/W emulsions. By associating lipophilic drugs with a hydrophobic oil phase these systems help their solubilization and decrease aqueous instability [4, 5, 7].

In the recent years, polymers have been studied for O/W emulsions formulation due to their favorable properties such as good biocompatibility, easy design and preparation and a large variety of structures [5, 8, 9].

Among the polymeric structures investigated researchers distinguished the class of squalenes, compound synthesized in nearly all human tissues [10, 11]. Squalene has several beneficial and unique properties such as natural antioxidant, ensure skin hydration, biocompatibility, it can be used as such or for preparing stable and non-toxic emulsions [5, 10, 12, 13]. Considering the importance of this molecule, in this study, we analysed the preparation and characterization of squalene oil-in-water emulsions stabilized by different non-ionic emulsifiers [5, 11, 14 -16]. As non-ionic emulsifiers we used polyoxyethylene (20) sorbitan monooleate (Tween 60) and sorbitan monooleate (Span 80) due to their low toxicity, biocompatibility and applicability in food, cosmetics, and pharmaceutical [11, 14, 17].

The major aim of the study was to find the optimal preparation conditions and to assess the emulsion physical stability correlated with their viscosity and composition. In order to have a complete description of the squalene O/W emulsions, through the experimental studies and results reported *herein was also* investigated the kinematic viscosity variation with temperature.

Experimental part

Materials

Squalene (≥98%), Span 80, Tween 60, sodium salicylate (e”99.5%) were purchased from Sigma Aldrich and used as received and Milli-Q water (deionized water purified using a Millipore system). The main physicochemical properties of the non-ionic emulsifiers used here are shown in table 1. The chemical structures of Span 80, Tween 60 are given below in figure 1.

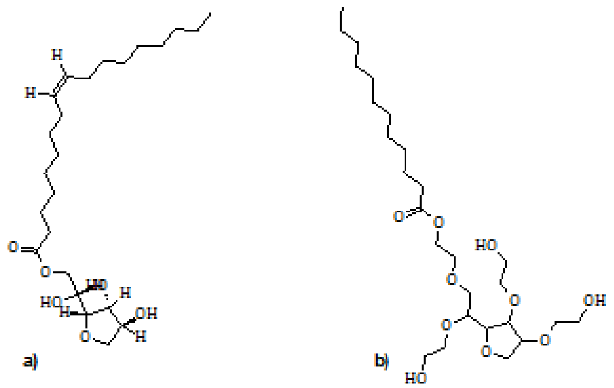

Fig. 1. Chemical structures of Span 80 (a), Tween 60 (b)

Preparation of O/W emulsions

Squalene O/W emulsions were prepared by homogenizing a given amount of oil phase with the continuous aqueous phase at room temperature (25±2 °C). In the first step, aqueous solutions containing a stabilizing agent were prepared by dissolving 2 wt% sodium salicylate in deionized water by agitation using a magnetic stirrer (400 rpm).

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Parameter	Span 80	Tween 60
HLB	4.3	14.9
Molecular weight (g/mol)	429	1312
Density (g/ml)	0.986	1.070
cmc (mM)	0.002	0.0023
Viscosity at 25°C (cP)	1000	550
Phase	Amber colored viscous liquid	Liquid–gel
Water solubility	Partly soluble	Partly soluble
Mineral oil solubility	Soluble	Insoluble

HLB - hydrophilic–lipophilic balance, cmc - critical micellar concentration

Table 1
MAIN PHYSICAL PROPERTIES OF NON-IONIC SURFACTANTS USED IN THIS STUDY [14, 18]

Due to their different solubilities Span 80 (Sp80) was dissolved in the oil phase and Tween 60 (Tw60) in the aqueous phase. In order to enhance the surfactant solubilization and remove the air bubbles the solution thus obtained were sonicated for 5 min.

In the second step, the emulsions were prepared at room temperature under continuous magnetic stirring (500 rpm) by slowly adding the liquid squalene with or without 2 wt% surfactant into the aqueous solution. The mixture was then stirred 30 min in order to obtain a homogenous system. In all experimental studies conducted in this paper was used a constant emulsion mass of 350 g. At an equal amount of emulsion the concentrations of sulfactant and sodium salicylate were *maintained constant* at 2 wt% and the concentration of squalene was varied from 2 wt% to 10 wt% .

Viscosity measurement

The viscosity of squalene emulsions was measured using a Fungilab Expert Series viscometer equipped with a R3 spindle, water bath and temperature sensor. This rotational rheometer is designed to measure kinematic viscosity (ν) and temperature of the sample based on fluid density and using a certain spindle speed. The kinematic viscosity values are expressed in $mm^2 \cdot s^{-1}$ (cSt) and speed of spindle in revolutions/min (rpm).

After previous mixing, the adequate volume (350 mL) of emulsion was put into the Berzelius teflon glass. The viscosity value was determined by heating the sample in a range of temperature from 25 to 60 °C. All the experimental measurement involved using a constant speed of spindle of 200 rpm.

Results and discussions

Emulsion formulation and physical stability measurement

The compositions of squalene O/W emulsions formulation are given in table 2. External conditions (e.g. temperature, chemical or physical factors) and long-term storage of O/W emulsions can affect their stability in time. Due to lower density than surrounding water phase oil droplets tend to move upward into a O/W emulsion, thus leading to phase separation of the system.

In order to evaluate their physical stability were carried out phase separation measurements on the squalene emulsions. Three milliliters of emulsion were transferred into a vial, tightly sealed with a plastic cap, and then stored at room temperature. In the absence of stiring, the emulsions were found to be stable for less than two hours after which the phase separation starts, stability data are also included in table 2.

Emulsion Code	$C_{squalene}$ (wt %)	$C_{sodium\ salicylate}$ (wt %)	$C_{emulsifier}$ (wt %)	Stability (min)
ESp1	2			5
ESp2	4			10
ESp3	6	2	2	20
ESp4	8			30
ESp5	10			60
ETw1	2			15
ETw2	4			30
ETw3	6	2	2	50
ETw4	8			60
ETw5	10			90

Table 2
FORMULATION COMPOSITION AND STABILITY OF SQUALENE O/W EMULSIONS

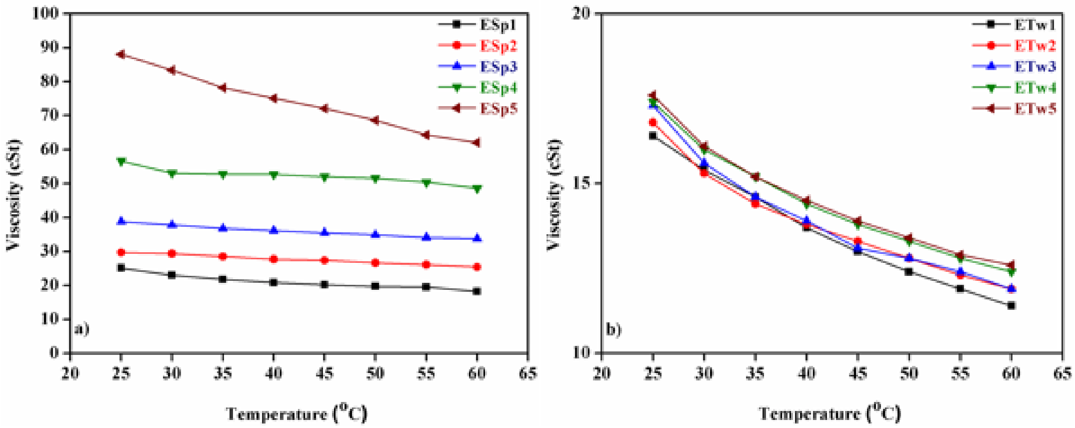

Fig. 2. Plots of viscosity versus temperature at various concentrations constant speed of spindle: a) 2 wt% Sp80, b) 2 wt% Tw60

Table 3
VISCOSITY RATIO ENHANCEMENT OF SQUALENE O/W EMULSIONS

Emulsion / Temperature (°C)	Viscosity ratio enhancement									
	ESp1	ESp2	ESp3	ESp4	ESp5	ETw1	ETw2	ETw3	ETw4	ETw5
25	28.11	33.26	43.45	63.38	98.66	18.37	18.81	19.37	19.48	19.71
30	25.76	32.92	42.33	59.46	93.39	17.25	17.13	17.47	17.92	18.03
35	24.41	31.91	41.21	59.13	87.57	16.35	16.13	16.35	17.02	17.02
40	23.29	31.02	40.43	59.01	84.10	15.34	15.45	15.57	16.13	16.24
45	22.62	30.68	39.75	58.23	80.74	14.56	14.89	14.67	15.45	15.57
50	22.06	29.90	39.08	57.78	76.82	13.89	14.33	14.33	14.89	15.01
55	21.84	29.23	38.19	56.44	72.00	13.33	13.77	13.89	14.33	14.45
60	20.38	28.44	37.85	54.42	69.54	12.77	13.33	13.33	13.89	14.11

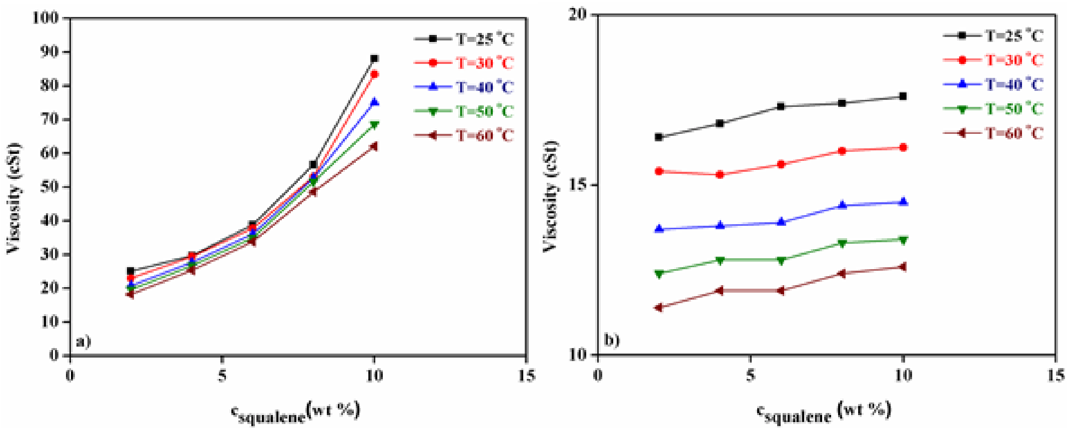

Fig.3. Plots of viscosity versus squalene concentration at various temperatures ans constant speed of spindle: a) 2 wt% Sp80, b) 2 wt% Tw60

The influence of temperature on viscosity

In order to assess the effect of temperature on viscosity of squalene O/W emulsions, the temperature was varied from 25 to 60°C. Under various squalene concentrations and constant speed of spindle the plots presented in figure 2 showed a decrease of emulsion viscosity.

This behaviour can be explained in terms of a lower number of intermolecular forces formed in the system as the emulsion temperature increases. When a stronger heating is inflicted on O/W emulsion the intermolecular forces are weakened, the average speed of the molecules inside the system increases and the contact time between neighboring molecules decreases.

Therefore, a higher temperature of O/W emulsion leads to a decrease of their viscosity due to a lower number of intermolecular forces formed in the system (fig. 2, table 3).

In order to assess the enhancement in viscosity of the obtained emulsions was determined the viscosity ratio by applying *equation 1* given below:

$$\eta_{enh} = \frac{\eta_E}{\eta_{CF}} \qquad (1)$$

where:

η_{enh} - viscosity ratio enhancement,
η_E - emulsion viscosity, cSt
η_{CF} - continuous phase (water) viscosity, cSt.

The viscosity ratio enhancement values obtained for the squalene O/W emulsion are are presented in table 3.

Regarding the influence of surfactant on the emulsions, it is found that the viscosity decrease is higher in the case of emulsions obtained with Tween 60 than those achieved with Span 80.

The influence of squalene concentration on viscosity

In all the cases, we determined that the increase of squalene from 2 wt% to 10 wt% leads to growth the viscosity of O/W emulsion determined against water (continuous phase). This behaviour can be explained in terms of a higher oil concentration available in the system, which might lead to the agglomeration of squalene droplets. The obtained results are confirmed by similar studies based on classic models such as Einstein's [19, 20], Batchelor's [21] and Wang [22] that describe the increase of viscosity as a function of concentration. In order to dissipate the oily component into the aqueous phase will be required a greater force, thus leading to the increase of the emulsion internal shear stress and therefore an increase in viscosity.

Conclusions

We successfully obtained squalene O/W emulsions using various concentrations of oil and constant concentrations of surfactant and sodium salicylate. We evaluated their physical stability in terms of phase separation measurements. The conducted studies showed a low stability of the obtained emulsions, the fluid systems were found to be stable for less than two hours.

The assessement of temperature and squalene concentration upon of O/W emulsion was also debated. In all the cases, we determined that the increase temperature leads to a decrease of O/W viscosity. Regarding the influence of surfactant on the emulsions, it is found that the viscosity decrease is higher in the case of emulsions obtained with Tween 60 than those achieved with Span 80. An increase of squalene concentration leads to growth the viscosity in the O/W emulsion system.

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