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Original Article

FORMULATION, CHARACTERISATION AND OPTIMISATION OF NATURAL ARGAN OIL MICROEMULSION FOR TOPICAL DELIVERY

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ABSTRACT

Objective: The purpose of this study was to develop, optimize and characterize a stable microemulsion of Moroccan cosmetic Argan oil.

Methods: In this work, microemulsion system was studied by the construction of phase diagrams using titration method. At first, various surfactants (Brij56®, Tween 80®, Solutol®, Tween 20® and Labrasol®) and various weight ratio surfactant/cosurfactant (1:0, 3:1, 2:1 and 1:1) were tested to select the optimal surfactant and concentration to use. The microemulsions with tween80 were evaluated with different techniques using various parameters such as droplet size, transmittance, viscosity and pH. Stability studies of these microemulsions were conducted for 8 w at 5 °C, 25 °C and 40 °C, and underwent centrifugation at 3000 rpm and ultracentrifugation at 10,000 rpm.

Results: The largest microemulsion formation area was achieved for the microemulsions containing Tween 80/PEG 400 at a ratio of 3:1. The obtained microemulsions M1 to M12 were homogeneous. More the percentage of PEG 400 increases, more the pH of the preparations and their viscosity decreases, while preparations with a high oil content have low transmittance. Thermodynamic and physical stability shows that only samples with a minimum of 31.5% of Tween 80 and a maximum of 9% of oil showed good stability.

Among the stable preparations, M11 (9% O, 10% W and 40.5/40.5% S/Cos) was the formula which exhibited properties such as transparency, soft acidic pH and low viscosity, making it suitable for cutaneous use.

Conclusion: The use of pseudo-ternary phase diagrams allows for the development of an optimal microemulsion with perfect stability.

Keywords: Microemulsion, Argan oil, Surfactant, Cosurfactant

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INTRODUCTION

Argan oil is endemic to southwestern Morocco and is produced from the seeds of L. *Argania spinosa*. Its therapeutic benefits have been claimed by natives of Morocco and explorers for more than eight centuries [1]. Cosmetic Argan oil is known for its high content of free-radical scavenging saponins, which may be responsible for its antioxidant properties [2, 3], as well as phenolic compounds and tocopherols. The Antioxidant properties of these compounds seem to protect against aging phenomena for skin [1, 4]. This oil cures acne and protects, restores, revitalizes and hydrates the skin. It reduces the degree of malondialdehyde (MDA) in human fibroblasts induced by UVA rays and thus exhibit a high capacity for reducing harmful effects of oxidative stress on the skin [4]. Due to the therapeutic advantages and complex composition of cosmetic Argan oil, various formulation approaches, including carrier technology such as microemulsions are used.

Because of their properties and great potential, microemulsions containing natural oils have attracted much interest during the last decades in terms of delivery and target potential [5, 6]. Microemulsions are considered as potential drug delivery vehicles because of their simplicity of preparation, their power to enhancing the solubilization of drugs poorly soluble and their ability to increase drug absorption, especially for topical applications [7-9]. They are one of the most promising techniques for enhancement of transdermal permeation *in vivo* and *in vitro* [10]. Microemulsions are homogenous dispersions of two immiscible liquids stabilized by an interfacial film of combination surfactant-cosurfactant. They are thermodynamically stable transparent system located between micellar solutions and simple emulsions with droplet size less than 140 nm [11]. These systems have a higher range of surfactant concentrations and various oil-to-water ratios [12].

The aim of this study was to develop and to characterize a topical microemulsion of Moroccan cosmetic Argan oil. Therefore, to determine stable preparation of microemulsion using lower concentration of surfactant to be suitable for use by cutaneous route.

MATERIALS AND METHODS

Reagents

The microemulsions were formulated using distilled water, cosmetic Argan oil (Arganati Bio, Morocco), polyethylene glycol 400 (PEG 400 Riedel-de Haën, Germany) as cosurfactant and different hydrophylic surfactants such as polysorbate 80 (TWEEN 80®) with an HLB of 15.0 (Riedel-de Haën, Germany), polysorbate 20 (TWEEN 20®) with an HLB of 16.7 (Riedel-de Haën, Germany), polyethylene glycol hexadecyl ether (Brij 56®) with an HLB of 12.9 (Fluka chemika, Switzerland), caprylocaproyl macrogol 8-glycerides (Labrasol®) with an HLB of 14.0 (Gattefossé, France) and polyethylene glycol 15-hydroxy stearate (solutol Hs 15®) with an HLB of 14 (BASF, Germany).

Instruments

The droplet size distribution was determined using Zetasizer nano series (Malvern Instruments, France). The pH was determined by pH meter (BANTE 920, BANTE instruments L. China), and the transmittance was measured by UV-Vis spectrophotometer (JENWAY 6305, UK).

Selection of surfactant

Selection of surfactant is a critical process as an ultra-low interfacial tension ($<10^{-3}$mN/m) is to be achieved at the oil/water interface, which will facilitates dispersion process during the preparation of microemulsion [11]. Concentration of surfactant selected must be quite high to stabilize the microdroplets formed, which requires the use of cosurfactant. So, the interest of using cosurfactant is to facilitate microemulsion formation with a minimum amount of surfactant [12].

The weight ratio of surfactant to cosurfactant (S/Cos) varied as 1:0, 3:1, 2:1 and 1:1. All the surfactants used are nonionic because of their non-toxicity [13]. The formulation of different preparations was conducted by fixation of an equal amount of demineralised water and Argan oil (500 µl: 500 µl). Each mixture thus formed is titrated drop by drop by one of the surfactants mentioned above mixed to PEG 400 using micropipette, until a transparent microemulsion is obtained. During the titration, samples were stirred slowly for a sufficient length of time for homogenization [13].

The selection criterion of the surfactants was their ability to form instant, clear, homogeneous and fluid microemulsions with the minimum amount.

Development of microemulsion system and definition of microemulsion area

The microemulsion system was developed through approaching the procedure of El Alaoui *et al.* [13]. The pseudoternary diagrams were conducted by the titration method using Statistica 13.0 in order to define microemulsion area. Each pseudoternary diagram was prepared by a constant weight ratio of Tween 80 to PEG 400 (1:0, 3:1, 2:1 and 1:1).

In order to optimize the concentration of Argan oil, Tween 80, PEG 400 and distilled water, different samples of various concentrations were prepared (table 1). Each mixture of S/Cos was added into the oil phase at weight ratios of 1:9, 3:7, 5:5, 7:3 and 9:1. Afterwards, each obtained mixture was titrated dropwise with different proportions of distilled water; 10, 30, 50, 70 and 90% under slight agitation at room temperature.

The different samples obtained were visually rated after 72 h [14] in the following areas: microemulsion, gel emulsion, emulsion, and phase separation. Microemulsion area was determined essentially on the basis of two parameters: clarity and fluidity. No test was made to recognize types of microemulsion; oil in water (O/W), water in oil (W/0) or bicontinuous microemulsions.

Table 1: Composition of studied mixtures in % w/w

Formulation code	Argan oil %	S/Cos %	Distilled water %
P1	9	81	10
P2	27	63	10
P3	45	45	10
P4	63	27	10
P5	81	9	10
P6	7	63	30
P7	21	49	30
P8	35	35	30
P9	49	21	30
P10	63	7	30
P11	5	45	50
P12	15	35	50
P13	25	25	50
P14	35	15	50
P15	45	5	50
P16	3	27	70
P17	9	21	70
P18	15	15	70
P19	21	9	70
P20	27	3	70
P21	1	9	90
P22	3	7	90
P23	5	5	90
P24	7	3	90
P25	9	1	90

Characterization and evaluation of obtained microemulsions

Microemulsions were evaluated using different techniques. At the macroscopic level, viscosity and transmittance provide useful information for evaluating of microemulsions [15, 16]. The dynamic light scattering technique, also called photon correlation spectroscopy (PCS) analyses the fluctuations in light scattering to the Brownian motion of globules, thus allowing the determination of their size [13, 17].

Droplet size analysis

Droplet size is an important microemulsion parameter since it affects microemulsion stability and skin penetration [18-20]. The droplet size distribution of microemulsion was measured with PCS using zetasizer at a temperature of 25 °C. The samples were prepared from 1:100 dilution of the microemulsion in demineralized water. The evaluation of droplet size analysis data was insured using intensity distribution.

Transmittance test

The transmittance of microemulsions obtained was measured with UV-Vis spectrophotometer. The microemulsions were diluted 100-fold with 0.1 N HCl and checked at a wavelength of 650 nm [21].

Viscosity determination

The viscosity of microemulsions was evaluated by sensory analysis, which is the most direct method for assessing and understanding the texture [22]. A viscosity score was attributed to each selected preparation by sensory analysis 72h after preparation, according to a scale from 0 to 10. Score 0 corresponds to a very liquid microemulsion; however, score 10 matches with gelled microemulsions.

pH of microemulsions

The pH was determined at room temperature with a glass electrode pH meter. The samples were prepared by dilution of selected microemulsions at 10% (v/v) in demineralised water [23]. Before each usage, the pH meter was calibrated with buffer solutions of pH 4.0, 7.0 and 9.0 [24, 25].

Stability evaluation of microemulsion formulations

Evaluation of thermodynamic stability

The samples were divided into three groups over 2 mo according to temperature: those that were heated to 40 °C±2 °C, those that refrigerated at 5 °C±3 °C and those that were stored at room

temperature 25 °C±2 °C. The appearance, droplet size and pH were investigated every 4 w [21].

Evaluation of physical stability

To evaluate physical stability of microemulsions, the samples were examined by observation after centrifugation at 3000 rpm for 30 min [26, 27] and ultracentrifugation at 10,000 rpm for 10 min. The physical stability was assessed visually by looking for phase separation after centrifugation.

RESULTS AND DISCUSSION

Selection of surfactant

Choice of adequate surfactant is a critical step for the formulation of microemulsion, since it reduces the interfacial tension oil-water during spontaneous emulsification [11, 28]. It also conditions the type of microemulsion; the surfactants with a HLB value greater than 10, such as surfactants used in our experience, are suggested to form O/W [26, 29]. But because of their irritant effect, the ideal surfactant used was that which form a microemulsion with the lowest amount possible [30].

Table 2 reports the minimum concentration of various surfactants needed to emulsify the mixture of equal amount of Argan oil and demineralized water. The order of the proportion of each surfactant was Brij56®<Tween 80®<Solutol®<Tween 20®<Labrasol®. It was found that the Brij 56® and the Solutol formed white preparations, which solidify at room temperature. However, the Tween 80®, the Tween 20® and the Labrasol® formed very fluid and transparent preparations. Therefore, Tween 80® was the surfactant that showed the lowest concentration needed to form clear microemulsions with low viscosity.

Table 2: Solutol®, Labrasol®, Brij 56®, Tween 20® and tween 80® concentrations needed to form microemulsions at different weight ration surfactant/PEG 400

Weight ratio S/Cos	S/Cos 1:0			S/Cos 3:1			S/Cos 2:1			S/Cos 1:1		
	Surfactant % (w/w)	Viscosity	Appearance	Surfactant % (w/w)	Viscosity	Appearance	Surfactant % (w/w)	Viscosity	Appearance	Surfactant % (w/w)	Viscosity	Appearance
Solutol ®	76	10	White and solid at room temperature	57	10	White and solid at room temperature	50	10	White and solid at room temperature	41	10	White and solid at room temperature
Labrasol ®	86	1	Homogeneous, transparent	66	1	Homogeneous, transparent	58	1	Homogeneous, transparent	45	1	Homogeneous, transparent
Brij 56®	47	10	White and solid at room temperature	36	10	White and solid at room temperature	31	10	White and solid at room temperature	27	10	White and solid at room temperature
Tween 20®	80	3	Homogeneous, transparent	60	2	Homogeneous, transparent	53	1	Homogeneous, transparent	42	1	Homogeneous, transparent
Tween 80®	68	3	Homogeneous, transparent	55	2	Homogeneous, transparent	49	1	Homogeneous, transparent	40	1	Homogeneous, transparent

The use of cosurfactant decreases the surfactant levels used in the formation of microemulsions by going from 3:1 to 1:1 S/Cos ratio. It also improves the stability of the interfacial film between oil and water [11, 31].

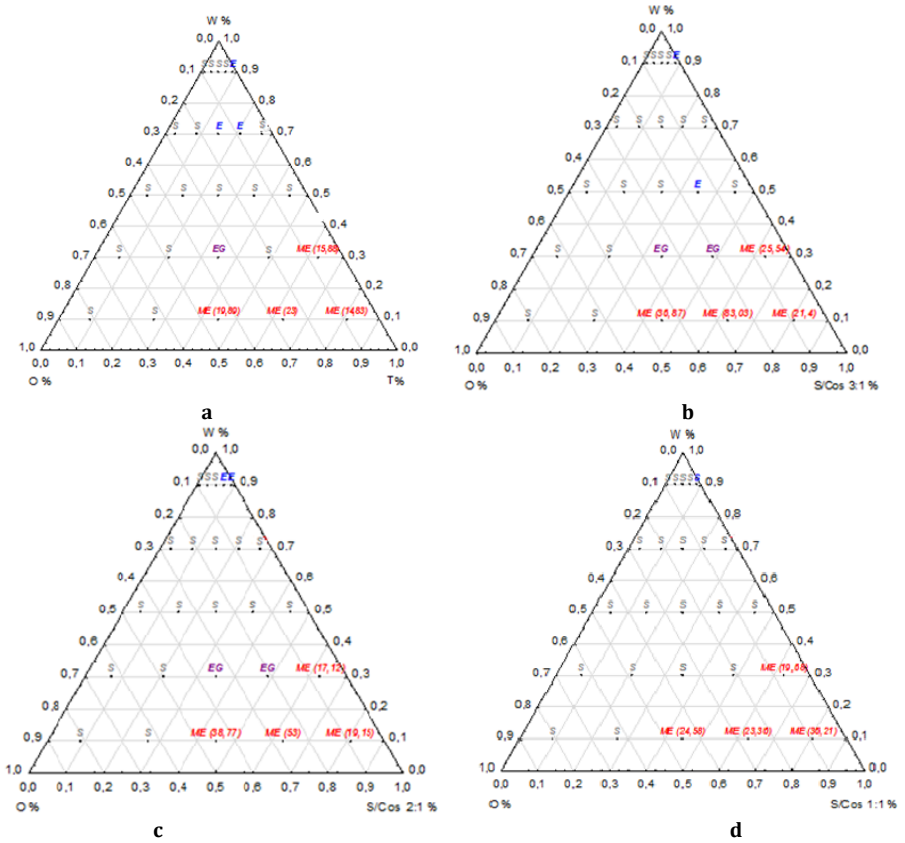

Fig. 1: The Effect of various content of Tween 80, PEG 400, Argan oil and mineralized water on microemulsion area in pseudoternary diagram. (a) Tween 80; (b) Tween 80/PEG 400 = 3:1; (c) Tween 80/PEG 400 = 2:1; (d) Tween 80/PEG 400 = 1:1

Development of microemulsion system and definition of microemulsion area

The pseudoternary diagrams shown in fig. 1 put the accent on the microemulsion area obtained with different weight ratio of Tween 80 to PEG 400.

Abbreviations: ME: microemulsion, EG: gel emulsion, E: emulsion, S: phase separation, W: water, O: oil, S/Cos: surfactant/cosurfactant, T: Tween 80.

The analysis of phase behavior allowed to define four domains: microemulsion, gel emulsion, emulsion, and phase separation [13].

The largest microemulsion formation area was achieved for the microemulsions containing Tween 80/PEG 400 at a ratio of 3:1 (fig. 1b) and with Tween 80 alone (fig. 1a). Moreover, this zone becomes narrower while increasing the content of PEG 400 at a ratio of 2:1 (fig. 1c) and 1:1 (fig. 1d), which can be explained according to D. Attivi *et al.* by the destabilization of the interfacial film due to excessive cosurfactant [7]. Therefore, the microemulsion area was defined by a limited proportion of oil (7-45%), water (10-30%), and Tween 80/PEG (3:1) (45-81%).

Characterization and evaluation of obtained microemulsions

The obtained microemulsions appear as translucent, slightly yellowish and perfectly homogeneous without phase separation.

Table 3: Characteristics of selected microemulsions

Weight ratio S/Cos	Microemulsion code	Composition				Characteristics			
		O%	W%	S %	Cos%	Droplet size (nm)*	Transmittance (%)*	pH*	Viscosity score
1:0	ME1	9	10	81	0	14.8±2.3	93.3±0.9	6.5±0.1	6
	ME2	27	10	63	0	23.0±2.1	21.0±1.9	6.8±0.1	4
	ME3	45	10	45	0	19.9±3.1	1.8±1.2	7.0±0.3	4
	ME4	7	30	63	0	15.9±4.0	96.1±0.2	6.6±0.1	5
3:1	ME5	9	10	61	20	21.4±2.2	98.9±0.4	6.9±0.2	3
	ME6	27	10	47	16	83.0±2.9	51.1±0.6	6.7±0.2	2
	ME7	45	10	34	11	36.9±5.1	20.4±1.4	5.7±0.1	2
	ME8	7	30	47	16	25.5±3.2	99.1±0.3	6.0±0.3	3
2:1	ME9	9	10	54	27	19.2±3.8	96.5±0.7	4.5±0.1	3
	ME10	7	30	42	21	17.1±2.4	95.4±1.3	5.2±0.3	4
1:1	ME11	9	10	40.5	40.5	36.2±1.9	72.3±1.1	5.0±0.1	2
	ME12	7	30	31.5	31.5	19.7±2.2	77.0±0.8	4.1±0.2	2

*Data expressed as mean±SD, n=3

According to the results obtained in table 3, the droplet size of various microemulsions studied varies between 14 and 83 µm. It increases slightly with increasing the oil concentration or decreasing the percentage of surfactant in the formulation [7]. Although, the polydispersity index (PDI) found do not exceed 0.326, which indicates small distribution width and low polydispersity of the system [32, 33]. The globule size intensity distribution of formulation M11 is shown in fig. 2.

It was also observed in table 3 that the transmittance values of microemulsions obtained were variable depending on the amounts of water and oil used, but also depending on the weight ration S/Cos. At a ratio of 1:0, 3:1 and 2:1, the transmittance founded is between 93.3 and 99.1% for samples whose oil and water content does not exceed 9% and 30%, respectively**.**

The viscosity changes mainly due to the change in the percentage of surfactant and cosurfactant. The preparations become more and more fluid with an increasing amount of PEG and a decreasing amount of Tween 80.

Results of pH measurement showed that a higher surfactant concentration led to the higher pH of microemulsions [34].

Stability evaluation of microemulsion formulations

Evaluation of thermodynamic stability

Organoleptic appearance

Based on the organoleptic appearance of microemulsion selected for 2 mo, all preparations showed no change, they preserved their homogeneity and transparency, indicating that these formulas have good stability during storage (table 4).

Determination of pH

The pH of a microemulsion is an important parameter to monitor during a stability test. A variation in pH can itself affect the stability of the system as well as the cutaneous tolerance of the preparation.

According to the results of pH measurement for 2 mo showed a slight decrease in pH during storage (table 4). This probably because the hydrolysis of surfactant or oxidation of oil used in formulation under the storage conditions of microemulsions. The hydrolysis of Tween 80 can lead to a decrease in pH due to the release of fatty acids [35]. The oxidation of Argan oil can also cause a decrease in the pH by production of free fatty acids, such as oleic acid and linoleic acid, which are common degradation products during the oxidation of oils. These free fatty acids can react with the water present in the environment to form carboxylic acids, which can lead to a decrease in pH [36].

Droplet size analysis

The results in table 4 show that the globule size of the different preparations measured during the stability test does not exceed 95.3 nm. This is proved that the microemulsions tested met the droplet size requirements (≤140 nm).

Evaluation of physical stability

The results of the centrifugation test (table 5) conducted at a speed of 3000 rpm for 30 min and 10 000 rpm for 10 min show no change in appearance for low oil concentration microemulsions M1, M4, M5, M8, M9, M10, M11 and M12. These microemulsions remain stable while preserving their homogeneity without any phase separation. However, microemulsions M2, M3, M6 and M7 showed phase separation under centrifugation and ultracentrifugation.

According to the analysis of various parameters studied as well as the results obtained in the literature, it noted that phase diagrams indicate that formation of ME regions depends significantly on the proportions of the constituents, and these regions may be conducted in accordance with the applicability to delivery system development [37]. Low surfactant concentrations were unable to stabilize component mixtures, leading to phase separation. In our study the minimum threshold was 31.5 % of Tween 80 versus 40 % found by Pessoa RS *et al.* when optimizing a babassu oil-based ME system [15].

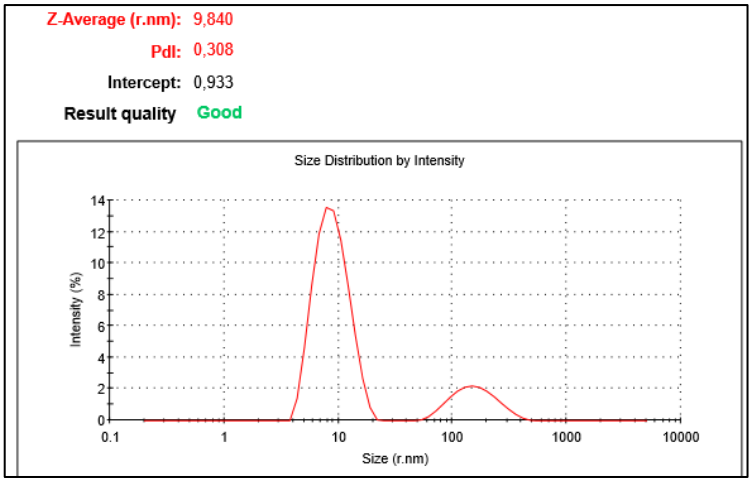

Fig. 2: Size distribution of formulation M11

Table 4: Physico-chemical results for microemulsion stability studies

Microemulsion code	Storage conditions	Phase separation			Transparency			pH*			Globule size (nm)*		
		T0	30 d	60 d	T0	30 d	60 d	T0	30 d	60 d	T0	30 d	60 d
M1	5±3 °C	H	H	H	T	T	T	6.5±0.1	6.4±0.1	6.4±0.2	14.8±2.3	27.1±1.8	18.6±2.9
	Room temperature	H	H	H	T	T	T	6.5±0.1	6.5±0.1	6.3±0.1	14.8±2.3	28.9±2.2	38.5±1.9
	40±2 °C	H	H	H	T	T	T	6.5±0.1	6.2±0.2	6.0±0.1	14.8±2.3	23.0±2.1	19.1±2.0
M2	5±3 °C	H	H	H	T	T	T	6.8±0.1	6.8±0.1	6.8±0.1	23.0±2.1	26.4±3.0	32.1±3.2
	Room temperature	H	H	H	T	T	T	6.8±0.1	6.9±0.3	6.5±0.2	23.0±2.1	25.1±3.0	64.8±3.8
	40±2 °C	H	H	H	T	T	T	6.8±0.1	6.7±0.1	6.3±0.3	23.0±2.1	22.8±2.3	33.0±2.6
M3	5±3 °C	H	H	H	T	T	T	7.0±0.3	6.8±0.1	6.8±0.2	19.9±3.1	36.7±4.1	43.1±4.2
	Room temperature	H	H	H	T	T	T	7.0±0.3	6.8±0.1	6.8±0.1	19.9±3.1	26.4±3.0	22.9±2.9
	40±2 °C	H	H	H	T	T	T	7.0±0.3	6.8±0.2	6.6±0.1	19.9±3.1	24.5±3.9	22.3±2.8
M4	5±3 °C	H	H	H	T	T	T	6.6±0.1	6.6±0.1	6.5±0.1	15.9±4.0	38.6±2.4	25.2±2.8
	Room temperature	H	H	H	T	T	T	6.6±0.1	6.6±0.3	6.3±0.1	15.9±4.0	30.1±2.6	18.8±1.1
	40±2 °C	H	H	H	T	T	T	6.6±0.1	6.3±0.1	6.1±0.1	15.9±4.0	25.9±2.2	26.1±3.0
M5	5±3 °C	H	H	H	T	T	T	6.9±0.2	6.9±0.1	6.5±0.1	21.4±2.2	29.7±3.1	36.7±3.6
	Room temperature	H	H	H	T	T	T	6.9±0.2	6.8±0.3	6.8±0.1	21.4±2.2	18.2±3.1	27.4±2.6
	40±2 °C	H	H	H	T	T	T	6.9±0.2	7.0±0.1	6.8±0.2	21.4±2.2	23.2±2.8	26.7±1.9
M6	5±3 °C	H	H	H	T	T	T	6.7±0.2	6.6±0.2	6.6±0.1	83.0±2.9	27.3±1.9	24.6±3.3
	Room temperature	H	H	H	T	T	T	6.7±0.2	6.7±0.3	6.4±0.4	83.0±2.9	17.6±2.8	22.9±2.9
	40±2 °C	H	H	H	T	T	T	6.7±0.2	6.2±0.3	6.3±0.2	83.0±2.9	27.8±2.3	40.8±4.0
M7	5±3 °C	H	H	H	T	T	T	5.7±0.1	5.7±0.3	5.5±0.2	36.9±5.1	41.9±3.6	43.4±4.2
	Room temperature	H	H	H	T	T	T	5.7±0.1	5.5±0.2	5.5±0.2	36.9±5.1	18.4±3.1	24.7±2.1
	40±2 °C	H	H	H	T	T	T	5.7±0.1	5.4±0.1	5.2±0.2	36.9±5.1	53.8±4.1	95.3±5.0
M8	5±3 °C	H	H	H	T	T	T	6.0±0.3	6.1±0.1	5.9±0.3	25.5±3.2	28.4±2.0	31.5±4.0
	Room temperature	H	H	H	T	T	T	6.0±0.3	6.0±0.1	6.0±0.1	25.5±3.2	28.2±1.8	28.2±3.2
	40±2 °C	H	H	H	T	T	T	6.0±0.3	5.9±0.1	5.7±0.2	25.5±3.2	21.5±2.6	22.4±2.2
M9	5±3 °C	H	H	H	T	T	T	4.5±0.1	4.4±0.1	4.5±0.1	19.2±3.8	41.0±3.1	47.6±3.1
	Room temperature	H	H	H	T	T	T	4.5±0.1	4.4±0.2	4.3±0.1	19.2±3.8	33.1±2.9	44.5±5.8
	40±2 °C	H	H	H	T	T	T	4.5±0.1	4.1±0.1	4.0±0.2	19.2±3.8	50.3±4.2	62.7±4.9
M10	5±3 °C	H	H	H	T	T	T	5.2±0.3	5.1±0.4	5.0±0.3	17.1±2.4	57.3±5.0	32.2±3.1
	Room temperature	H	H	H	T	T	T	5.2±0.3	5.0±0.1	5.0±0.1	17.1±2.4	33.7±2.7	33.3±2.9
	40±2 °C	H	H	H	T	T	T	5.2±0.3	4.9±0.1	4.8±0.1	17.1±2.4	42.4±2.5	58.5±4.1
M11	5±3 °C	H	H	H	T	T	T	5.0±0.1	5.1±0.2	4.9±0.1	36.2±1.9	57.5±3.3	35.0±3.1
	Room temperature	H	H	H	T	T	T	5.0±0.1	4.9±0.1	4.8±0.2	36.2±1.9	28.2±3.7	61.5±3.9
	40±2 °C	H	H	H	T	T	T	5.0±0.1	4.9±0.1	4.7±0.1	36.2±1.9	42.4±2.8	35.8±4.0
M12	5±3 °C	H	H	H	T	T	T	4.1±0.2	4.0±0.1	4.0±0.1	19.7±2.2	42.9±3.2	33.0±2.6
	Room temperature	H	H	H	T	T	T	4.1±0.2	4.0±0.1	4.0±0.1	19.7±2.2	31.6±3.1	32.7±2.9
	40±2 °C	H	H	H	T	T	T	4.1±0.2	3.9±0.2	3.6±0.1	19.7±2.2	28.1±2.8	24.9±2.0

*Data expressed as mean±SD, n=3, d: day, H: Homogeneous, T: transparent.

Table 5: Results of physical stability study

Weight ratio S/Cos	Microemulsion code	Composition				Physical stability	
		O%	W%	S%	Cos%	Phase separation/ Centrifugation at 3000 rpm	Phase separation/ Ultracentrifugation at 10,000 rpm
1:0	ME1	9	10	81	0	-	-
	ME2	27	10	63	0	+	+
	ME3	45	10	45	0	+	+
	ME4	7	30	63	0	-	-
3:1	ME5	9	10	61	20	-	-
	ME6	27	10	47	16	+	+
	ME7	45	10	34	11	+	+
	ME8	7	30	47	16	-	-
2:1	ME9	9	10	54	27	-	-
	ME10	7	30	42	21	-	-
1:1	ME11	9	10	40.5	40.5	-	-
	ME12	7	30	31.5	31.5	-	-

(+): Phase separation; (-): No phase separation.

We can also see that the samples analysed showed good physical and thermodynamic stabilities when the percentage of Argan oil do not exceed 9 %; they remained transparent and homogeneity during the whole period of the test. This percentage may vary depending on the nature of the oil used, You X *et al.* and Chen Y *et al.* found a percentage of 6 % for caprylic/capric triglyceride oil (GTCC), while Pesssoa RS *et al.* found a percentage of 10 % [14, 38, 39]. These results are confirmed by several reports that predict that a larger proportion of oil phase up to 20 % [40]. Which explains the instability of M2, M3, M6, and M7 formulations whose percentage of oil exceed 27 %.

From the above, we may deduce that the M11 was the most fluid-stable microemulsion possessing a soft acidic pH, which incorporates the maximum possible amount of Argan oil and whose formulation requires a surfactant concentration which does not exceed 41 %, which makes it the most suitable for cutaneous use.

CONCLUSION

Topical microemulsions of Moroccan Argan oil were developed using a non-toxic surfactant, Tween 80, which showed the lowest concentration needed to form clear microemulsions with low viscosity. The use of PEG 400 as cosurfactant is with the aim of increasing the stability of the interfacial film between demineralised water and Argan oil.

During this microemulsions formulation, the appropriate components and their optimal content were obtained using pseudo-ternary phase diagrams. Based on the results, it is concluded that the microemulsion M11 containing 9 % oil, 10 % water, 40.5 % Tween 80 and 40.5 % PEG is the most fluid-stable preparation with soft acidic pH and lowest surfactant concentration.

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AUTHORS CONTRIBUTIONS

Ouhaddouch Hind contributed to the bibliographic research, the experimental work, the analysis of the results, and the writing of the manuscript.

Aliat Zineb contributed to the execution of part of the experimental work

El Alaoui Yassir, in addition to contributing to the bibliographic research, served as the supervisor

Laatiris Abdelkader, Cherkaoui Nawal, Fahry Aicha, and Rahali Younes contributed to writing the manuscript in English and revising it.

CONFLICT OF INTERESTS

Declared none

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