ORIGINAL ARTICLE

DEVELOPMENT AND OPTIMIZATION OF NEW CAPSAICIN MICROEMULSIONS FOR TOPICAL ADMINISTRATION

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Abstract

In this paper, microemulsions were formulated using pharmaceutically acceptable components for topical administration of capsaicin: isopropyl myristate (oil), Tween 80 (surfactant) and n-butanol/n-octanol (cosurfactants). The purpose of this study was to develop a new method for screening the optimal composition of microemulsions based upon the investigation of the minimum surfactant:cosurfactant ratio and the concentration of tensioactive reagents at which microemulsion formation occurs, by determining the interfacial tension at the liquid-liquid interface between pepper tincture and oil phases. Investigations were conducted for each surfactant-cosurfactant mixture. Also, a n-butanol/n-octanol-free system was considered. The results obtained from the interfacial tension measurements were compared with the ones generated by the titration method, proving the similarity between the two.

Rezumat

În această lucrare, au fost formulate microemulsii folosind componente acceptate din punct de vedere farmaceutic pentru administrarea topică a capsaicinei: miristat de izopropil (ulei), Tween 80 (surfactant) şi n-butanol/n-octanol (cosurfactanți). Scopul studiului îl reprezintă dezvoltarea unei noi metode de *screening* a compoziţiei optime a microemulsiilor bazată pe stabilirea raportului minim surfactant:cosurfactant şi a concentraţiei minime de tensioactivi la care se formează microemulsia, prin măsurarea tensiunii interfaciale la interfaţa tinctură de ardei iute-ulei. Determinările au fost făcute pentru fiecare amestec surfactant-cosurfactant. De asemenea, a fost studiat sistemul formulat în lipsa n-butanolului/n-octanolului. Rezultatele obținute în urma măsurătorilor tensiunii interfaciale au fost comparate cu cele generate prin metoda titrării, evidențiindu-se similaritatea celor două metode.

Keywords: microemulsion, capsaicin, interfacial tension

Introduction

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a naturally occurring alkaloid. It is a well-known phyto-therapeutic substance with important pharmacological implications on cardiovascular, nervous and respiratory systems [21]. It is a member of capsaicinoides class found in plants belonging to *Capsicum* species, along with dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin [3]. Capsaicin stimulates the release of vasoactive neuropeptides (e.g. substance P, a chemical mediator of pain, and CGRP or calcitonin gene-related peptide) from nerve terminals [14, 15].

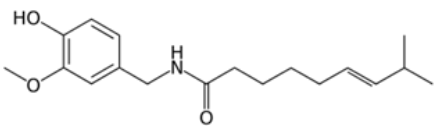

Figure 1.
Capsaicin structural formula

The primary medical use for capsaicin is as an alternative for standard therapy in rheumatic diseases, such as rheumatoid arthritis and osteoarthritis or algia from various causes (diabetic neuropathy, post-herpetic algia, cluster headache, post-mastectomy syndrome etc.) [9]. Its action mechanism is composed of two pharmacological processes: first, neurons excitation by cutaneous nociceptors binding and initial increased sensitivity due to favouring of peptide P secretion, followed by nociceptors desensitization and reduction of susceptibility to pain [4].

Due to its intense irritating action and burning sensation, the topical administration of capsaicin pharmaceutical formulations is limited to skin regions and contact with mucous membranes should be avoided [6].

From a chemical point of view, capsaicin has very poor water solubility and these results in difficulties in designing effective topical and/or transdermal pharmaceutical preparations. Most commonly used

formulations are gels and creams, but lately more studies involving nanosized carriers are being studied as topical delivery of capsaicin, of which microemulsions rise a particular interest [1, 11, 18, 23]. Another advantage of such formulations is that they also cause less irritation and burning sensation on the skin compared to conventional capsaicin formulations [19].

A microemulsion is a pseudoternary system formed when admixing appropriate amounts of water (or any hydrophilic phase), oil and surfactant. Microemulsions are isotropic, transparent, solution-like preparations, characterized by thermodynamic stability and spontaneous formation [5].

For the past decades, microemulsions have been widely studied as topical drug vehicles due to their net advantages over conventional and other nanostructured systems, such as the ease of preparation, enhanced drug solubilization (both hydrophilic and lipophilic), long shelf life and, more importantly, increased drug permeation rate through *stratum corneum* [10]. The last may be attributed to the fact that many of microemulsion's components are well-known penetration enhancers [13].

For the spontaneous formation of microemulsions, an ultralow interfacial tension is required between aqueous and oil phases [22]. Some surfactants (generally, multichain surfactants) can produce by themselves low values for the interfacial tension (10^{-2} to 10^{-5} mN/m), but in most cases, such low values cannot be achieved by a single tensioactive agent, the carboxymethylcellulose (CMC) being reached before the low value of $\gamma_{w/o}$ required for microemulsification is attained [17]. Therefore, almost in any case, a second agent with surface (interface) activity is included (a cosurfactant), typically being another surfactant or a small- or medium-chain alcohol [2]. Alcohols are being adsorbed at the water-oil interface, modifying the interfacial membrane flexibility and favouring microemulsion formation.

The objectives of this study were to develop new biocompatible microemulsion formulations for topical administration of pepper tincture based on determining the minimal concentration of surfactant and the optimal surfactant:cosurfactant ratio at which microemulsion formation occurs, using both titration method and interfacial tension measurements at the hydrophilic-lipophilic interface.

Materials and Methods

Materials

Isopropyl myristate (IPM), purchased from Titolchimica, was used as oil component. Tween 80 was kindly gifted by Actavis. n-butanol and n-octanol were purchased from Merck. The pepper tincture was kindly gifted by Hofigal Export Import S.A. and was obtained by admixing accurately weighed 20 g of ground *Capsici fructus* with 80 g of ethanol (70% v/v) for 100 g solution. The properties of the pepper tincture are given in Table I below.

Table I

Manufacturer's specifications for capsaicin tincture

Characteristics	Acceptance criteria
Appearance	yellow to reddish brown clear liquid
Density (d_{20}^{20})	0.88-0.92 (g/mL)
Ethanol content (% w/w)	50 %
Dry residue	min. 2%
Assay:	
- total capsaicinoids expressed as capsaicin	min. 0.01%
- nonivamide from total capsaicinoids	max. 5%

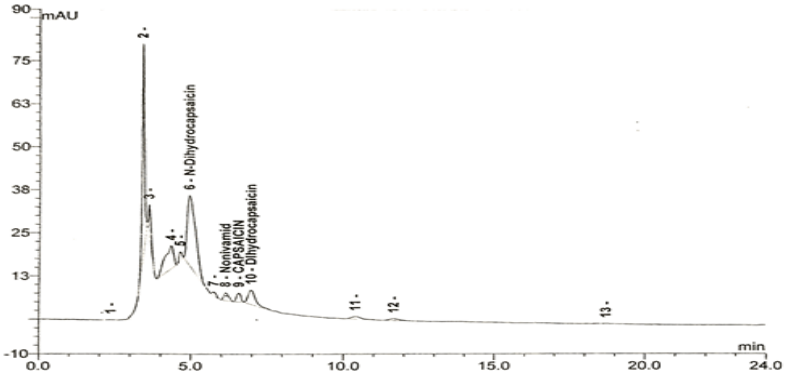

Figure 2.

HPLC chromatogram of the pepper tincture used in this study (courtesy of Hofigal Export Import S.A.)

According to the manufacturer, for the quantitative determination of the tincture composition, a Dionex chromatograph system was used, equipped with a UVD 340U diode array detector (at λ = 225 nm) and using a chromatographic column with L = 0.25 m and Φ = 4.6 mm. The phenylsilyl silica gel for chromatography (5 μm) was used as stationary phase. The mobile phase was composed of acetonitrile (R) and 1 g/L solution of phosphoric acid (R) (40:60 v/v), at a flow rate of 1 mL/min and 30°C temperature. The injected volume was 20 μL. The elution order was: nordihydrocapsaicin, nonivamide, capsaicin, dihydrocapsaicin. The HPLC results sent by Hofigal for the capsaicin tincture are given in Figure 2 and revealed that the concentration of capsaicin in the tincture used in this study was 0.0006 mg/mL (or 0.02% w/w).

Methods

Preparation of microemulsions using the titration method

Three types of systems were investigated: a) tincture/IPM/Tween 80, b) tincture/IPM/Tween 80/n-butanol and c) tincture/IPM/Tween 80/n-octanol. In the case of each system, accurately weighed amounts of pepper tincture and IPM (corresponding to a 1:1 ratio) were titrated either with Tween 80 or with previously prepared mixtures of Tween 80 and n-butanol or n-octanol (equivalent to 3:1, 2:1, 1:1, 1:2, 1:3 weight ratios), using 100 μL incremental steps. Next, each sample was vigorously stirred for homogenization and sonicated for approximately 15 minutes to remove air bubbles entrapped during stirring. The samples were let to equilibrate for 24 hours before further analysis. The obtained systems were classified as either single phase microemulsions or multiphase systems, based on visual inspections against strong light and microscopic appearance under cross-polarized light, using a Motic B1 microscope [7].

The point of titration at which each mixture became a clear single phase microemulsion was noted. The accuracy of the boundary line between single-phase microemulsion and other types of systems is dependent on the titration mixture volume, the smaller the volume, the higher the number of samples and thus the more accurate the determination. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous types.

Interfacial tension measurements

The interfacial tensions were determined by du Noüy ring method (as shown in Figure 3), using a KSV Sigma 703D tensiometer. All measurements were conducted at 30°C, using a 50 mm Boro 3.3 glass vessel and a platinum/iridium ring (R_{ring} = 9.545 mm; R_{wire} = 0.185 mm). The reported values were automatically corrected by the apparatus software using Huh-Mason correction. Throughout the experiments, the tincture:IPM ratio was maintained constant at 1:1 (w/w). Before any measurements, the two phases were allowed to equilibrate and saturate one into each other for 24 hours. Afterwards, Tween 80 and/or n-butanol/n-octanol, respectively, were added into the appropriate phase (hydrophilic or lipophilic), using incremental titration steps, in order to determine the concentration of surfactant and the surfactant:cosurfactant ratio at which the minimal interfacial tension values are attained. The measurements were conducted in triplicate.

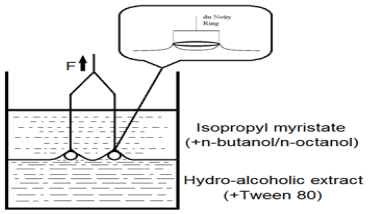

Figure 3.
Graphical representation of the du Noüy ring method

Based on this data, the quantitative composition of each of the three investigated systems was determined and the corresponding samples were prepared. Whether the resulted systems were in fact microemulsions or not was assessed as previously described. The interfacial tension experimental data was compared to data obtained using the titrating mixture method.

Stability

The physical stability of microemulsions was investigated during six months storage at room temperature (25 ± 3°C), after being inserted into sealed glass vials. Samples were monitored for occurrence of phase separation. Additionally, a centrifuge stress test was carried out at 9000 rpm for 20 minutes in order to evaluate the thermodynamic stability of the microemulsions.

Results and Discussion

The same ingredients responsible for the enhanced skin permeation of the active substance are also responsible for the adverse reactions associated with microemulsion topical use, such as producing comedones due to oil component or causing irritation as a result of the high concentration of surfactants often necessary for microemulsion formation. Therefore, reducing the amount of tensioactives to a needed minimum may be the starting point in optimization of a microemulsion formulation. A 1:1 ratio between tincture and IPM was chosen during the experiments in order to assure the formation of microemulsions with maximum solubilization capacity of the two immiscible phases.

Interfacial tension experiments

As Kahlweit et al. showed [12], alcohols behave different when adsorbed at water-air interface

compared to water-oil interface. The interfacial tension of water-air interface is markedly influenced by the addition of an alcohol, causing a sharp decrease in the interfacial tension value, similar to the addition of a non-ionic surfactant with the same number of carbon atoms. On the other hand, the interfacial tension of water-oil interface decreases to a lesser extent upon addition of an alcohol compared to addition of a surfactant, which implies a weaker adsorption of the alcohol to the water-oil interface than amphiphiles. For medium-chain alcohols (n = 3 - 8), this can be the result of alcohol solubilisation in the oil phase, consequence of strong interactions between the hydrocarbon tails and oil, and weak hydration of the alcohol's OH group. This behaviour is opposed to the one showed by surfactants, which, due to their strong hydration of their head groups leads to stronger repulsive interaction with oil, and thus to a greater adsorption at water-oil interface compared to alcohols. In the context of formulating a microemulsion, because of the presence of a surfactant monolayer with an intermediary polarity between the two immiscible phases, it is expected for alcohols to concentrate at this level, which in turn will affect the interfacial tension at the water-oil interface and the interactions between surfactant molecules and between the interfacial film and bulk phases.

The interfacial tension measurements at the pepper tincture-isopropyl myristate interface throughout addition of Tween 80 into the tincture phase, showed that the minimal interfacial tension is achieved at a tincture:Tween 80 ratio of 2.1:1 (Figure 4). The initial interfacial tension between pepper tincture and IPM was determined to be 4.00 ± 0.06 mN/m.

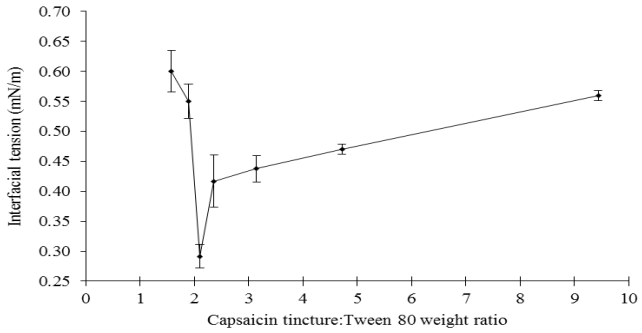

Figure 4.
Interfacial tension measurements between tincture and IPM upon Tween 80 addition

Using this ratio between pepper tincture and Tween 80, two separate experiments were conducted in order to determine the ratio between surfactant and n-butanol/n-octanol corresponding to the minimal interfacial tension between the two immiscible phases. The interfacial tension results obtained for the two experiments are summarized in Table II. The minimum interfacial tension values and the corresponding ratios between Tween 80:alcohol and IPM:alcohol, respectively, are highlighted.

Table II

Interfacial measurements data throughout addition of n-butanol/n-octanol at constant tincture:Tween 80 ratio of 2.1:1 (the minimum is highlighted)

IPM:n-butanol ratio (m/m)	Tween 80:n-butanol ratio (m/m)	Interfacial tension (mean ± SD) [mN/m]	IPM:n-octanol ratio (m/m)	Tween 80:n-octanol ratio (m/m)	Interfacial tension (mean ± SD) [mN/m]
-	-	0.29 ± 0.02	-	-	0.29 ± 0.02
12.35	5.88	0.22 ± 0.00	12.12	5.78	0.18 ± 0.02
6.17	2.94	0.17 ± 0.00	6.06	2.89	0.15 ± 0.01
4.12	1.96	0.13 ± 0.01	4.04	1.93	0.14 ± 0.01
3.09	1.47	0.11 ± 0.01	3.03	1.45	0.11 ± 0.00
2.47	1.18	0.08 ± 0.01	2.42	1.16	0.10 ± 0.01
2.06	0.98	0.03 ± 0.01	1.94	0.93	0.09 ± 0.01
1.76	**0.84**	**0.01 ± 0.01**	**1.67**	**0.80**	**0.05 ± 0.01**
1.54	0.74	0.01 ± 0.01	1.39	0.66	0.05 ± 0.01
1.37	0.65	0.01 ± 0.01	1.21	0.58	0.05 ± 0.01
1.12	0.54	0.01 ± 0.01	1.05	0.50	0.05 ± 0.01

The results showed an optimal Tween 80:n-butanol ratio of 1:1.19, whereas in case of n-octanol, the optimal ratio was determined to be 1:1.25 (Figure 5).

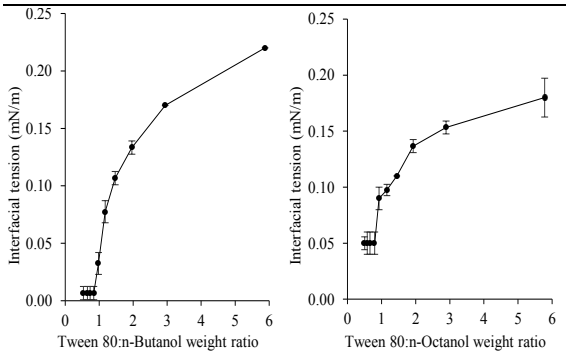

Figure 5.
Interfacial tension measurements at (pepper tincture + Tween 80)-IPM interface throughout addition of n-butanol (left) and n-octanol (right)

Therefore, similar results were reported for both alcohols, although for the n-butanol system the interfacial tension values were smaller compared to n-octanol. A possible explanation is that n-butanol concentrates to a higher extent at the tincture-IPM interface, due to its more pronounced amphiphilic character. As showed by Graciaa et al. [8], when the chain length of the alcohol increases, its interaction on the water side of the interface remains constant (assuming a single OH group), whereas its interaction on the oil side increases. Therefore, compared to n-butanol, n-octanol is less active in the interfacial film and probably concentrates more in the oil layer adjacent to the interface, playing the role of a lipophilic linker.
A schematic representation of the different mechanisms of interaction with the interface of the alcohols used in this study is depicted in Figure 6.

This results in more ordered oil molecules in the layer next to the interface, which in turn results in enhanced interactions between the surfactant and the oil molecules, still favouring the micro-emulsification of the system. Based on these findings, two samples (denominated "optimal") were prepared by admixing accordingly weighed amounts of each component. Both samples led to transparent, single-phase, non-birefringent systems, suggesting their microemulsion nature.

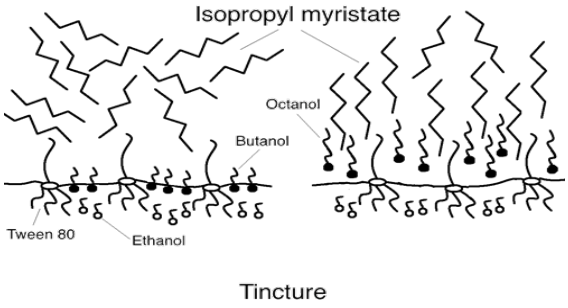

Figure 6.
Schematic representation of the tincture-IPM interface for each type of alcohol used in this study

Titration method experiments
The results from the titration experiments are shown in Figure 7. Samples containing Tween 80:n-butanol/n-octanol at 1:2 and 1:3 ratios, didn't form microemulsions even at a total concentration of nearly 60% and thus were not represented in the graph. Figure 7 also shows the corresponding composition of the amphiphilic mixtures based on the interfacial data measurements.

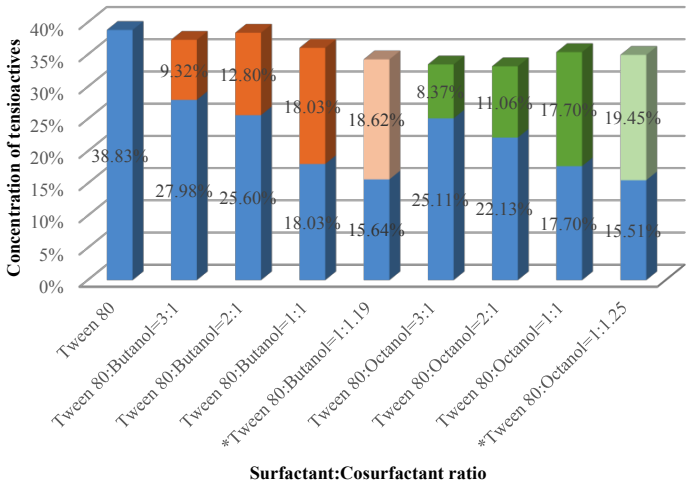

Figure 7.
Minimal concentrations of Tween 80 (blue) and cosurfactants (orange for butanol, green for octanol) at which microemulsion formation occurs (*optimal formulations suggested by the interfacial tension data)

Due to the presence of ethanol (a frequently used cosurfactant in topical microemulsion formulations) in the pepper tincture composition, a microemulsion system was assumed to be generated using only Tween 80. Indeed, as the results show, at approximately 38% Tween 80 (corresponding to a

Tween 80:ethanol ratio of 2.4:1), the system clarifies and a single-phase microemulsion is obtained. This Tween 80 concentration is higher than the one generated by the interfacial tension measurements (approximately 20%, corresponding to a Tween 80:ethanol ratio of 1:1), at which the formation of a microemulsion doesn't occur. This can be explained by the fact that lower concentrations of ethanol favour a decrease in micelle size, while higher concentrations (as it is the case here) disrupt micelle integrity [16]. Therefore, in order to microemulsify the total amount of tincture and isopropyl myristate, a significantly higher Tween 80 content is needed.

The titration method results show that adding a second alcohol (n-butanol or n-octanol) determines a marked reduction of Tween 80 concentration necessary for the microemulsion formation, although the total concentration of tensioactives remains almost the same (around 35%). This is due to the fact that alcohols with longer chains ($n \geq 3$) favour micelle growth and formation of elongated surfactant aggregates [20]. Thus, the minimal concentration at which microemulsion formation occurs is approximately 18% for both n-butanol and n-octanol systems, at a Tween 80:n-alcohol ratio of 1:1. However, below a cosurfactant concentration of 18% (in the total system), the minimum concentration of Tween 80 required for microemulsion formation is lower for the n-octanol formulated system than for the n-butanol system (Figure 8).

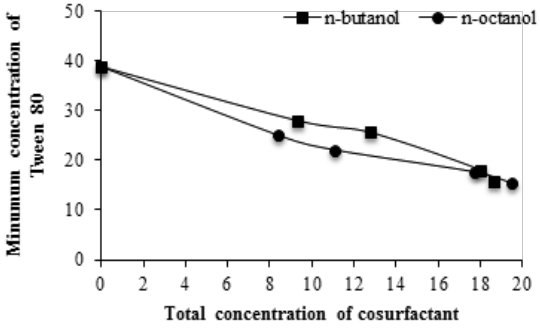

Figure 8.
Variation of minimum concentration of Tween 80 at which microemulsion formation occurs *versus* the total concentration of cosurfactant, for both n-butanol and n-octanol

The same observation applies for the minimum concentration of tensioactives (surfactant + cosurfactant) at which the microemulsion formation occurs, in case of a cosurfactant concentration below 12% (Figure 9). Above these thresholds, a steep decline in the two considered concentrations is observed for the n-butanol formulated system. This suggests that n-octanol is a more efficient cosurfactant compared to shorter-chain alcohols at lower concentrations, while shorter-chain alcohols (including n-butanol) are more efficient when used in higher amounts. This is probably due to the increase of n-butanol partition in oil phase, leading to a polarity increase of IPM and thus to a higher solubilization of Tween 80 in IPM [24].

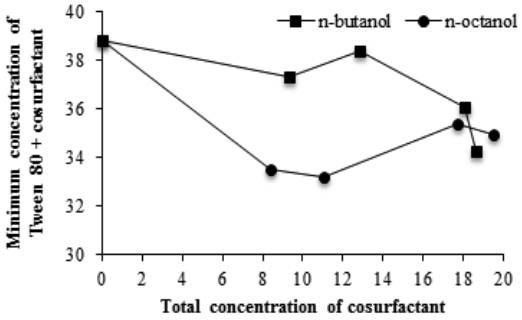

Figure 9.
Variation of minimum concentration of (Tween 80 + cosurfactant) at which microemulsion formation occurs *versus* the total concentration of cosurfactant, for both n-butanol and n-octanol

The results generated by the titration method are very close to the ones generated by the interfacial tension measurements, although smaller concentrations of Tween 80 were achieved using the latter method. Therefore, the process of measuring the interfacial tension between system's components can be considered a more refined and effective approach for screening for optimal microemulsion composition.

Stability

After six months storage at room temperature (25 ± 3°C), all prepared microemulsions remained transparent and no turbidity of phase separation was observed. Also, the centrifuge stress test indicated the thermodynamic stability of the investigated samples, as no phase separation occurred.

Conclusions

In this study, three biocompatible microemulsion systems based on pepper tincture, isopropyl myristate and Tween 80 were developed as vehicles for the topical administration of capsaicin. Two distinct methods (titration method and interfacial tension measurements) were applied in order to optimize the microemulsion composition, assessing the minimal concentration of surfactant required for micro-emulsion formation, while maintaining the maximum solubilization capacity for both pepper tincture and isopropyl myristate. The results were compared and both methods led to similar results. A Tween 80 only microemulsion was obtained at a minimal concentration of surfactant of approximately 38%, the role of the cosurfactant being played by ethanol

contained by the tincture formulation. However, adding a second medium or long-chain alcohol (e.g. n-butanol or n-octanol) determines a marked reduction of Tween 80 concentration necessary for microemulsion formation. Further *in vitro* and *in vivo* studies will be carried out in order to successfully select a candidate microemulsion formulation for the topical delivery of capsaicin.

Acknowledgements

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References

1. Agrawal U., Gupta M., Vyas S.P., Capsaicin delivery into the skin with lipidic nanoparticles for the treatment of psoriasis. *Artif. Cells Nanomedicine Biotechnol.*, 2015; 43(1): 33-39.
2. Alany R.G., Rades T., Agatonovic-Kustrin S., Davies N.M., Tucker I.G., Effects of alcohols and diols on the phase behaviour of quaternary systems. *Int. J. Pharm.*, 2000; 196(2): 141-145.
3. Barceloux D.G., Pepper and Capsaicin (Capsicum and Piper Species), in: Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Toxic Plants, and Venomous Animals, John Wiley & Sons, Inc., Hoboken, New Jersey 2008; 71-76.
4. Căruntu C., Negrei C., Ghiţă M.A., Căruntu A., Bădărău A.I., Buraga I., Boda D., Albu A., Brănişteanu D., Capsaicin, a hot topic in skin pharmacology and physiology. *Farmacia*, 2015; 63(4): 487-491.
5. Danielsson I., Lindman B., The definition of microemulsion. *Colloids Surf.*, 1981; 3(4): 391-392.
6. Fang J.Y., Wu P.C., Huang Y.B., Tsai Y.H., *In vitro* permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types. *Int. J. Pharm.*, 1995; 126(1-2): 119-128.
7. Friberg S.E., Micelles, microemulsions, liquid crystals, and the structure of *stratum corneum* lipids. *J. Soc. Cosmet. Chem.*, 1990; 41(3): 155-171.
8. Graciaa A., Lachaise J., Cucuphat C., Bourrel M., Salager J.L., Improving solubilization in microemulsions with additives. 2. Long chain alcohols as lipophilic linkers. *Langmuir*, 1993; 9(12): 3371-3374.
9. Hayman M., Kam P.C.A., Capsaicin: A review of its pharmacology and clinical applications. *Curr. Anaesth. Crit. Care*, 2008; 19(5-6): 338-343.
10. Heuschkel S., Goebel A., Neubert R., Microemulsions—modern colloidal carrier for dermal and transdermal drug delivery. *J. Pharm. Sci.*, 2008; 97(2): 603-631.
11. Huang Y.B., Lin Y.H., Lu T.M., Wang R.J., Tsai Y.H., Wu P.C., Transdermal delivery of capsaicin derivative-sodium nonivamide acetate using microemulsions as vehicles. *Int. J. Pharm.*, 2008; 349(1-2), 206-211.
12. Kahlweit M., Strey R., Busse G., Effect of alcohols on the phase behavior of microemulsions. *J. Phys. Chem.*, 1991; 95(13): 5344-5352.
13. Karande P., Mitragotri S., Enhancement of transdermal drug delivery *via* synergistic action of chemicals. *Biochim. Biophys. Acta BBA - Biomembr.*, 2009; 1788(11): 2362-2373.
14. Luo X.J., Liu B., Dai Z., Yang Z.C., Peng J., Stimulation of Calcitonin Gene-Related Peptide Release Through Targeting Capsaicin Receptor: A Potential Strategy for Gastric Mucosal Protection. *Dig. Dis. Sci.*, 2013; 58: 320-325.
15. Manzini S., Perretti F., De Benedetti L., Pradelles P., Maggi C.A., Geppetti P., A comparison of bradykinin- and capsaicin-induced myocardial and coronary effects in isolated perfused heart of guinea-pig: involvement of substance P and calcitonin gene-related peptide release. *Br. J. Pharmacol.*, 1989; 97(2): 303-312.
16. Mukerjee P., Mysels K.J., Critical Micelle Concentration of Aqueous Surfactant Systems, NSRDS-NBS 36, Nat. Bur. Stand., 1971; 1-21.
17. Nagarajan R., Ruckenstein E., Theory of surfactant self-assembly: a predictive molecular thermodynamic approach. *Langmuir*, 1991; 7(12): 2934-2969.
18. Popescu M., Chiutu L., Mircioiu C., Dima Ş., Capsaicin microemulsion: Preparation, characterization and *in vitro* release study. *Farmacia*, 2014; 62(1): 58-68.
19. Raza K., Shareef M.A., Singal P., Sharma G., Negi P., Katare O.P., Lipid-based capsaicin-loaded nano-colloidal biocompatible topical carriers with enhanced analgesic potential and decreased dermal irritation. *J. Liposome Res.*, 2014; 24(4): 290-296.
20. Rosano H.L., Clausse M., Microemulsion Systems, Marcel Dekker Inc., New York, 1987, 1-82.
21. Sharma S.K., Vij A.S., Sharma M., Mechanisms and clinical uses of capsaicin. *Eur. J. Pharmacol.*, 2013; 720(1-3): 55-62.
22. Strey R., Microemulsion microstructure and interfacial curvature. *Colloid Polym. Sci.*, 1994; 272(8): 1005-1019.
23. Tavano L., Alfano P., Muzzalupo R., de Cindio B., Niosomes vs microemulsions: New carriers for topical delivery of Capsaicin. *Colloids Surf. B Biointerfaces*, 2011; 87(2): 333-339.
24. Wang W., Weber M.E., Vera J.H., Effect of Alcohol and Salt on Water Uptake of Reverse Micelles Formed by Dioctyldimethyl Ammonium Chloride (DODMAC) in Isooctane. *J. Colloid Interface Sci.*, 1994; 168(2): 422-427.