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Stability determination of an alternative approach to use of liquid drug substance as oil phase in microemulsion formulations: Methyl salicylate

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ABSTRACT

Objective: The objective of this study was to investigate the stability of microemulsions using methyl salicylate as oil phase in the presence and absence of hydrophobic additives and/or drug after 6-month storage at ambient temperature. **Materials and methods:** The investigated additives were menthol, isopropyl palmitate, and isopropyl myristate. Indomethacin was as a model drug. The surfactant system of Tween®20- isopropyl alcohol (1:1) was kept constant at 50% while the dispersed phase was at 15%, in which weight ratios of methyl salicylate-to-additive were varied. Formulations were prepared and then characterized. **Results and Conclusion:** All samples were transparent homogenous liquids with nano-sized droplets and the O/W characteristic. After storage, all samples visually remained unchanged. The analytical results showed methyl salicylate and indomethacin was stable in the prepared microemulsions. The optimized oil phase comprised menthol as additive at a 3:1 weight ratio of methyl salicylate-to-menthol in the presence and absence of indomethacin.

INTRODUCTION

Microemulsions are the isotropic, transparent, and thermodynamically stable systems. They are low viscosity liquid mixtures which form spontaneously after simply mixing the proper concentration of suitable components together without the need of more energy input and sophisticated machines.[1-3] By virtue of microemulsion properties, they are now of pharmaceutical interest as the promising drug delivery systems. The advantage of microemulsions for the topical use was found not only to increase the solubilization of drugs in the system but also to enhance the permeation of drugs through the skin.[4-11] In traditional method for making microemulsions, medium chain triglycerides, fatty acids, or esters of fatty acids are commonly used as oil phase in which the active drug substance is incorporated.[1,2,12] The oil acts as drug reservoir for delivering the solubilized drug. By this means, it is a very difficult task for the incorporation of a high-dose liquid drug substance because the loading capacity might be limited. Therefore, our question was: Is it possible to use the liquid drug itself as oil phase to form stable microemulsions in the presence and absence of the hydrophobic additive? This is unprecedented which requires detailed investigations. In our study, the experiment was carried out to give proof of this approach. Methyl salicylate was chosen as a model liquid drug substance and used as oil phase in which the investigated hydrophobic additive was incorporated. Investigated hydrophobic additives were menthol, isopropyl palmitate (IPP), and isopropyl myristate. These substances were generally found in the topical formulations and also known as penetration enhancer to facilitate the delivery of drug molecule through skin.[13-16] The presence of the hydrophobic enhancer makes the formulations more appropriate for topical use.[13,15,16] The microemulsion formulation was defined from the single-phase region of the pseudoternary phase diagram of the methyl salicylate-water-surfactant system (Tween®20-isopropyl alcohol [1:1]).[17] In addition, to gain insight into the relative aspect, we extended the experiment to determine the stability of the resultant systems after incorporation of a second hydrophobic drug

substance. Indomethacin was chosen as a model hydrophobic drug incorporated into the counterpart formulations.

Methyl salicylate is a salicylic acid derivative used as a topical analgesic for the relief of pain in musculoskeletal, joint, and soft-tissue disorders. The structure of methyl salicylate is shown in Figure 1a. Several different dosage forms of methyl salicylate are on the market such as liniments, creams, and ointments.[18,19]

Indomethacin is a nonsteroidal anti-inflammatory drug used in the treatment of musculoskeletal and joint disorders.[18,19] Its chemical structure is illustrated in Figure 1b. Adverse effects are more frequent with indomethacin after oral administration. Therefore, indomethacin is formulated into topical dosage forms to avoid the undesirable side effects and now commercially available in the forms of topical spray and gels for external supportive treatment of pain. As methyl salicylate and indomethacin provide clinical benefits of topical pain relief, a combination of methyl salicylate and indomethacin in the formulation is worth studying, and particularly, that both active substances are formulated in the form of topical microemulsions is the novel aspect.

In the present study, the aim was to determine the stability in terms of physicochemical properties and drug stability of the microemulsion systems using methyl salicylate as oil phase in the presence and absence of the investigated hydrophobic additive and/or drug after storage at ambient temperature for 6 months. The important point of view of this paper was to gain insights into the use of the liquid drug substance like methyl salicylate as oil phase in microemulsion preparation. The achievement of this study was demonstrated. This concept helps formulators to complete the formulation with high drug loading, and to some extent, to reduce costs. The obtained systems still have the promising characteristics of microemulsions. This is useful for pharmaceutical product development.

MATERIALS AND METHODS

Materials

Methyl salicylate (Ph. Eur. grade, Fluka) was purchased from Sigma-Aldrich (USA). Isopropyl myristate (IPM, Ph. Eur. grade), isopropyl alcohol (IPA, Emsure®), methanol (high-performance liquid chromatography [HPLC] grade), and acetonitrile (HPLC grade) were obtained from Merck KGaA (Germany). Tween®20 was acquired from Merck Schuchardt OHG (Germany) and menthol (BP/USP grade) from S. Tong Chemical Co., Ltd. (Bangkok, Thailand). Isopropyl palmitate (IPP) was obtained from Stearinerie Dubois Fils (France) and indomethacin from CSPC Ouyi Pharmaceutical Co., Ltd. (China). Water with conductance of 6.39 μS/cm was used as water phase throughout the experiment. All chemicals were used as received without any further purification.

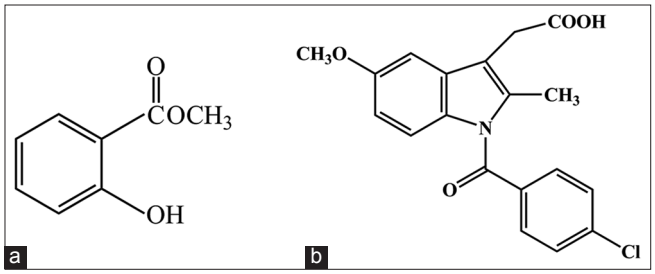

Figure 1: Molecular structures of (a) methyl salicylate and (b) indomethacin

Methods

Preparation of samples

All formulations were only composed of few components which were adequate to form microemulsions. No attempt was made to add any suitable stabilizers such as antioxidant, buffering agent, and so on which could change the actual physicochemical properties of the studied systems. Methyl salicylate was used as oil phase formulated at the commercial concentration. The enhancers as the hydrophobic additive were menthol, IPP, and IPM. The concentration of surfactant system was kept constant at 50%w/w while the dispersed phase was at 15%, in which the weight ratios of methyl salicylate to additive were varied as 1:0, 2:1, and 3:1. Indomethacin as a hydrophobic drug was incorporated at the commercial concentration (0.75%w/w) of indomethacin topical gel. All microemulsion formulations were defined as presented in Table 1 and prepared. Indomethacin was dissolved in the Tween®20-IPA (1:1) mixture. Methyl salicylate and the additive were subsequently added. After mixing the all required components together under magnetic stirring, microemulsions were obtained. All samples were kept in the well-closed glass bottles at ambient temperature (30 ± 2°C) and protected from light for at least 24 h to achieve equilibrium before further investigation.[17,20]

Sample characterization

Appearance observation

The physical appearances including color and clarity as well as the occurrence of phase separation and/or precipitation were observed visually. The optical isotropy of the prepared samples was investigated using the cross-polarized light microscopy (Nikon Microscope, Eclipse 50i, Japan).

Particle size measurements

The average droplet diameters of the prepared samples were measured by the photon correlation spectroscopy instrument (Delsa™ Nano C Particle Analyzer, Beckman Coulter®, USA) at a temperature of 25°C. Each formulation was run in triplicate. The results were recorded as the average value ± standard deviation (SD).

Viscosity measurements

The viscosities of the prepared samples were determined by a Brookfield®Digital Rheometer (Model DV-II+ Viscometer, Brookfield Engineering Laboratory, USA) using a S18 spindle at 60 rpm. The measurement was run in triplicate. In addition, the correlation coefficients (R_{xy}) between shear rate (x) and shear stress (y) were also observed to indicate the flow property of the prepared samples.

pH measurements

The pH values were measured at 25°C using a pH meter (Lab 850, Schott®Instrument, Germany). The results were recorded as the average value ±SD.

Table 1: The compositions of microemulsion formulations (%w/w)

Compositions	A0	A1	A2	A3	A4	A5	A6	B0	B1	B2	B3	B4	B5	B6
Methyl salicylate	15.00	11.25	10.00	11.25	10.00	11.25	10.00	15.00	11.25	10.00	11.25	10.00	11.25	10.00
Menthol	-	3.75	5.00	-	-	-	-	-	3.75	5.00	-	-	-	-
IPP	-	-	-	3.75	5.00	-	-	-	-	-	3.75	5.00	-	-
Isopropyl myristate	-	-	-	-	-	3.75	5.00	-	-	-	-	-	3.75	5.00
Tween®20:IPA (1:1)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Water	35.00	35.00	35.00	35.00	35.00	35.00	35.00	34.25	34.25	34.25	34.25	34.25	34.25	34.25
Indomethacin	-	-	-	-	-	-	-	0.75	0.75	0.75	0.75	0.75	0.75	0.75

Conductivity measurements

The electrical conductivity was measured at 25°C using a conductivity meter (SevenEasy, Mettler Toledo, Germany) which was calibrated using the standard solution of 1.413 μS/cm before testing. The measurement was run in triplicate and reported as the average value ± SD.

Phase separation

The stability of indomethacin-loaded samples was also tested by the centrifugation (Universal 320R, Hettich, UK) at 12,000 rpm for 30 min at 25°C.

Microemulsion morphology

To observe the morphology of the microemulsion droplets, the optimized formulations with and without indomethacin were subjected under transmission electron microscope using a Tecnai T20 G^2 electron microscope (FEI, Netherlands) at an accelerating voltage of 120 kV. The samples placed on a Formvar coated-copper grid (EMS, USA) were negatively stained with 2% uranyl acetate, subsequently dried, and viewed under microscope. The photographs were taken at the magnification of ×29,000.

Stability evaluation

All samples kept in the well-closed glass bottles were stored at ambient temperature (30 ± 2°C) and protected from light for 6 months. Physical appearances such as color, clarity, phase separation, and/or precipitation were evaluated by visual inspection. The mean particle size, electrical conductivity, viscosity, and pH values of all samples on storage were also investigated.

The contents of methyl salicylate and indomethacin were determined by HPLC method. To determine the contents of methyl salicylate and indomethacin in the microemulsion samples, a 0.2 g of microemulsion samples was accurately weighted in 10-mL volumetric flask, diluted to volume with methanol, filtered through a membrane filter (0.45 μm), and injected into HPLC. Each formulation was quantified in triplicate. The amount of drugs present in each microemulsion after preparation accounted for 100%. In contrast, the contents of drugs that remained after 6-month storage were expressed as a mean percentage of drug remaining ± SD.

HPLC assay for quantification of methyl salicylate and indomethacin

Methyl salicylate and indomethacin were quantified using a HPLC system (Shimadzu, Japan) consisting of a CBM-20A (system controller), a LC-20AD (solvent delivery unit), a DGU-20A5R (degassing unit), a SPD-20A (ultraviolet-visible detector), and a SiliaChrom®XDB1 C8 column (4.6 mm × 250 mm, 5 mm). The chromatographic data analysis was performed with the LCsolution Program (Shimadzu, Japan). The injection volume was 20 μL. The mobile phase was prepared by three solvents including A, B, and C: Solvent A was DI water pH 3.2 (adjusted with phosphoric acid), solvent B was acetonitrile, and solvent C was methanol. The separation process followed a gradient elution procedure in which concentration ratios of solvents A and B changed linearly. Flow rate was 1.0 mL/min. The retention times of methyl salicylate and indomethacin were about 15.86 and 22.08 min, respectively. The detection wavelength was 320 nm. The developed analysis method was also validated. The calibration curve for methyl salicylate was in the concentration range of 500.0–4,000.0 μg/mL with the correlation coefficient of 0.9988 and for indomethacin in the range of 25.0–200.0 μg/mL with the correlation coefficient of 0.9984. For precision indicated by the percent relative SD (%RSD), the %RSD for methyl salicylate and indomethacin was 1.13 and 1.62, respectively.

Statistical Analysis

All experiments were carried out in triplicate. Data were expressed as the mean values ± SD. Statistical significance of difference was analyzed by Student's *t*-test at the level of $P = 0.05$.

RESULTS

All samples after preparation were the transparent homogenous liquids. Indomethacin at a concentration of 0.75% w/w was completely soluble in the surfactant system of Tween®20-IPA (1:1). The formulations with indomethacin incorporation, B0–B6, were yellowish. Under the cross-polarized light microscopy, all samples appeared dark, and no birefringence was found, indicating isotropic property.[21] Birefringence is a typical feature of lyotropic liquid crystals (except for cubic phase) which exhibit typical black and white textures.[22] No crystal of drug was observed in indomethacin-loaded formulations, confirming that indomethacin was dissolved completely. For centrifugation test, all indomethacin-loaded formulations remained clear with no evidence of phase separation and drug precipitation, suggesting physical stability. After the samples kept in the well-closed glass bottles and protected from light were stored at ambient temperature (30±2°C) over 6 months, the visual inspection found that all 14 liquid mixtures remained unchanged, being clear and homogenous. No phase separation and precipitation of drug was observed.

The mean particle sizes and the polydispersity index values of all formulations after preparation and after storage for 6 months were shown in Figure 2. The particle sizes of formulations A0-A6 and B0-B6 were in the range of 72.0–119.5 nm and 69.1–90.2 nm, respectively. The low polydispersity index values suggested that all prepared formulations had the narrow size distribution. When the methyl salicylate coexisted with the investigated additive in the dispersed phase in the absence of indomethacin, formulations A1-A6 (white vertical bars in Figure 2a), the presence of the hydrophobic additive enlarged the particle sizes to varying extent depending on the additive type and amounts. Apparently, the particle size was found to increase with increasing the menthol content. In contrast, indomethacin-loaded formulation B0 showed the comparable particle size (71.8 nm) to its counterpart formulation, A0 (72.0 nm). Interestingly, when methyl salicylate, additive, and indomethacin coexisted in the dispersed phase, the effect of indomethacin was obviously observed. Even though the particle size was as a direct result of the interplay of all components, it seemed that the effect of indomethacin predominated over the investigated additive as observed in formulations B2–B6 but not in formulation B1. The particle sizes of formulations B2–B6 were smaller than those of their counterpart systems without indomethacin. Among indomethacin-loaded formulations, the menthol-containing formulations B1 and B2 showed larger particle sizes than others. After 6-month storage at ambient temperature, formulations A2–A6 exhibited the remarkable decrease in both the particle size and the polydispersity index value. In contrast, the opposite results were observed for indomethacin-loaded formulations B0–B6. Formulations B2, B4, and B6 showed the considerable increase in both the particle size and the size distribution. Among them, the similarity was that their dispersed phases possessed a 2:1 weight ratio of methyl salicylate to the additive. Formulation B1 was likely more stable because its mean particle size increased insignificantly ($P > 0.05$) and its particle size distribution changed to a lesser degree. The results obviously suggested that the amount of the investigated additive incorporated in methyl salicylate oil phase should be optimized.

For pH investigation as shown in Table 2, the observed pH values after preparation of all formulations were below 6. The presence of indomethacin remarkably lowered the pH as observed in indomethacin-loaded formulations B0–B6 compared to their counterpart formulations A0–A6. The result also indicated that the coexistence of the investigated hydrophobic additive with other components in the dispersed phase had no influence on the pH of all samples. After 6-month storage, the pH values of indomethacin-free formulations A0–A6 decreased considerably. In contrast, a certain decrease in pH was observed for indomethacin-loaded formulations B0–B6.

The mobility properties of the system expressed with viscosity and electrical conductivity were shown in Table 3. All formulations showed rather low apparent viscosity values with their correlation coefficients (R_{xy}) between shear rate and shear stress approaching 1.0 as shown in Figure 3, for example, suggesting the Newtonian flow behavior.[23,24] The low viscosity and the Newtonian flow are typical of microemulsions.[25] The indomethacin-loaded formulations B0–B6 had slightly higher viscosity and slightly lower electrical conductivity values than their counterpart formulations A0–A6. All formulations showed electrical conductivity values higher

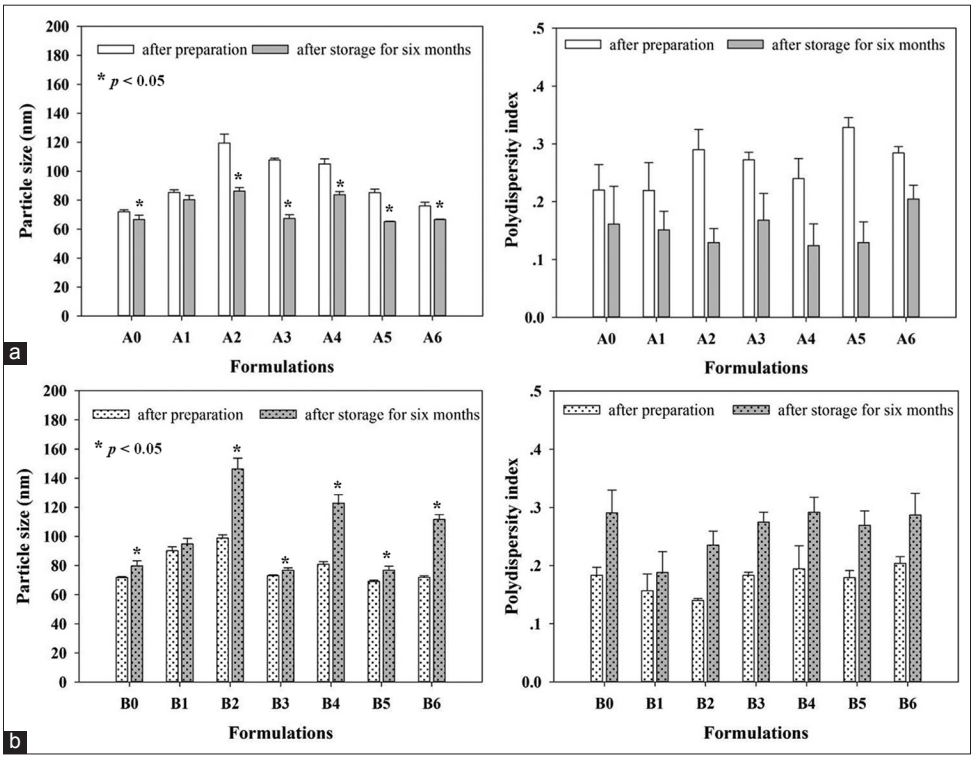

Figure 2: The particle size and polydispersity index after preparation and after storage at ambient temperature for 6 months of (a) indomethacin-free formulations A0–A6 and (b) indomethacin-loaded formulations B0–B6

than 10 μS/cm which is indicative of the water as the external pseudo-phase.[26] Consequently, they all could be classified as O/W microemulsions.

The TEM images of the optimized microemulsions, formulations A1 and B1, revealed the spherical droplets as shown in Figure 4.

To ascertain the stability of drugs in the formulations after 6-month storage at ambient temperature, the stability result was expressed as a percentage of drug remaining as shown in Table 4. The HPLC chromatograms of methyl salicylate and indomethacin in the prepared microemulsions were shown in Figure 5. The HPLC analysis of the methyl salicylate content of all 14 formulations found little change over 6-month storage; however, there might be an error in formulation A5 that made the interpretation unlikely. Formulations B0-B6 retained a high content of indomethacin with little loss after 6-month storage. As a result, it was obvious that methyl salicylate and indomethacin were stable in these prepared formulations. The percentage of indomethacin remaining in formulation B0, as a control in the absence of an additive, was high, suggesting that indomethacin had good compatibility with methyl salicylate. In addition, the data also showed that the presence of the investigated hydrophobic additive in the methyl salicylate oil phase had less effect on the stability of methyl salicylate and indomethacin as observed in formulations A1–A6 and B1–B6.

After screening the formulation stability with regard to the change in the particle size and drug stability after 6-month storage, formulations A1 and B1 were found to be optimum.

Table 2: The pH values of all microemulsion formulations

Formulations	pH	
	After preparation	After 6-month storage
A0	5.709±0.008	5.368±0.009
A1	5.719±0.007	5.422±0.007
A2	5.694±0.021	5.439±0.007
A3	5.710±0.011	5.374±0.004
A4	5.716±0.012	5.407±0.003
A5	5.694±0.030	5.392±0.005
A6	5.693±0.003	5.368±0.005
B0	5.607±0.003	5.537±0.019
B1	5.578±0.005	5.509±0.019
B2	5.572±0.009	5.486±0.023
B3	5.571±0.004	5.461±0.005
B4	5.570±0.002	5.492±0.011
B5	5.558±0.009	5.452±0.008
B6	5.555±0.006	5.494±0.009

DISCUSSION

Menthol, IPM, and IPP as the investigated hydrophobic additives are soluble in or miscible with methyl salicylate; therefore, they can be readily contained in the methyl salicylate oil phase. Indomethacin is a practically water-insoluble drug substance. It was completely soluble in a 50% of the Tween®20-IPA (1:1) surfactant system. The yellowish color of formulations B0-B6 was due to indomethacin itself. Indomethacin appears as the pale-yellow powder.

As was known, the compositional variables such as oil used and the presence of other hydrophobic molecules as well as amphiphilic molecules have an influence on the efficiency of a surfactant system to form microemulsions and thus in turn on the physicochemical properties of the obtained microemulsions. The site of solubilization within microemulsions is closely related to the chemical structure and the physicochemical properties, such as hydrophobicity and molecular volume, of the solute. In this study, as expected,

Table 3: The viscosity and conductivity of all microemulsion formulations

Formulations	Viscosity (cPs)		Conductivity (μS/cm)	
	After preparation	After 6-month storage	After preparation	After 6-month storage
A0	12.3±0.1	11.5±0.1	49.2±0.0	53.1±0.1
A1	12.5±0.1	11.6±0.1	45.2±0.1	48.5±0.1
A2	12.8±0.2	11.9±0.1	43.7±0.0	47.1±0.0
A3	14.4±0.1	13.0±0.2	46.9±0.1	51.0±0.1
A4	14.9±0.1	13.7±0.1	46.9±0.1	49.2±0.1
A5	13.6±0.1	12.6±0.1	47.6±0.1	49.7±0.1
A6	14.2±0.1	13.3±0.1	47.6±0.1	49.9±0.1
B0	14.3±0.0	12.3±0.1	45.4±0.1	47.8±0.2
B1	13.9±0.0	13.2±0.1	43.1±0.3	46.5±0.0
B2	13.6±0.1	13.5±0.1	42.1±0.2	44.8±0.0
B3	14.3±0.1	14.8±0.2	43.4±0.1	47.2±0.1
B4	15.3±0.0	15.4±0.2	43.3±0.0	46.7±0.1
B5	14.4±0.0	14.2±0.1	43.8±0.0	46.6±0.1
B6	15.1±0.0	14.7±0.2	43.2±0.0	47.0±0.0

Table 4: The % drug remaining after 6-month storage at ambient temperature

Formulations	% remaining	Formulations	% remaining	
	Methyl salicylate		Methyl salicylate	Indomethacin
A0	97.7±0.0	B0	99.5±0.7	98.5±1.2
A1	97.9±0.9	B1	99.1±0.4	99.1±0.4
A2	99.9±0.4	B2	98.2±0.5	97.4±0.8
A3	97.7±0.7	B3	98.6±0.3	98.8±0.7
A4	98.1±0.7	B4	99.0±0.6	98.1±1.1
A5	102.7±0.2	B5	100.0±0.1	100.0±0.5
A6	100.0±0.5	B6	96.8±0.9	94.5±1.1

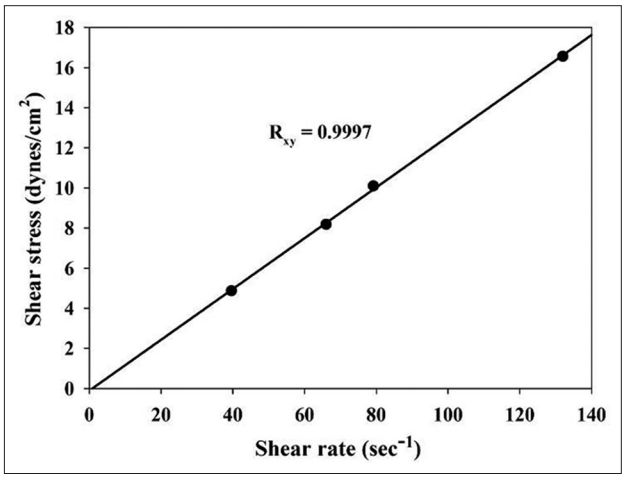

Figure 3: Shear stress as a function of shear rate of formulation A1

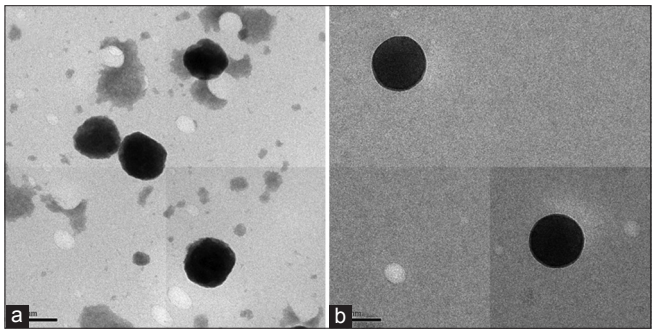

Figure 4: TEM photographs (×29,000) of (a) formulation A1 and (b) formulation B1

the oil-phase components as system variables affected the droplet size of microemulsions. Methyl salicylate as a major component of the dispersed phase is carboxylate ester with intermediate hydrophobicity. Methyl salicylate is a small molecular weight oil. At a 50% of the Tween®20-IPA (1:1) surfactant system, methyl salicylate at a high concentration of 15% was solubilized well, giving small microemulsion droplets as observed in formulation A0. This was because there was the sufficient amount of surfactant molecules for interfacial coverage to emulsify oil and water phases. It should be mentioned from the present data that the use of active oil itself as oil phase is useful. The active oil substance could be loaded into the system as much as its concentration is in the isotropic microemulsion region in the pseudoternary phase diagram. The presence of menthol, IPP, or IPM in the methyl salicylate oil phase had an influence on the particle size of microemulsions as observed in formulations A1-A6. The effect of menthol on the particle size increased with increasing the menthol concentration. The reason was that, due to its small molecule and lipophilic characteristics, menthol entered the droplet core and then was solubilized. Menthol was certainly solubilized as much as possible inside the droplet cores, hence swelled the tails of surfactant layer, and then, enlarged the droplet size. The more the menthol, the larger the particle size. IPP and IPM are fatty acid esters with the molecular volumes of 581.2 and 525.7 cm^3/mol, respectively, which were calculated according to the study of Richardson *et al*.[27] The molecular volume of IPP and IPM is not too large; therefore, the incorporation of IPP or IPM into the investigated systems did not cause phase separation. This also suggested the good efficiency of the Tween®20-IPA (1:1) surfactant system for solubilization of the oil-phase components. Due to its long hydrocarbon chain, IPP or IPM should be buried inside the hydrocarbon core of the surfactant monolayer. As a result, the particle size became larger as observed in formulations A3-A6. It should be concluded here that the presence of the investigated additive, related to types and amounts, in the methyl salicylate oil phase had an impact on the particle size. In contrast, the effect of indomethacin on the droplet size was obviously observed in certain circumstances related to oil-phase components. Indomethacin played a significant role in influencing the particle size when it coexisted with IPP, IPM, or 5% menthol in the methyl salicylate oil phase. It was not clear by which mechanism indomethacin affected the particle size. There are several researches reporting the change in particle size due to the incorporation of the amphiphilic drugs to microemulsion systems.[4,20,28] In fact, the incorporation of amphiphilic drug may result in either an increase or a decrease in microemulsion droplet size depending on the system components. There was the possible explanation in the present study for the effect of indomethacin. Indomethacin is the amphiphilic molecule. Its structure contains a lipophilic and a hydrophilic portion which enables it to locate at the interface and interact with the surfactant monolayer. By this means, indomethacin affected the surfactant-cosurfactant packing and then brought about the loss of flexibility of the interfacial film which in turn restricted the growth of the microemulsion core. As a result, the smaller particle size was observed.

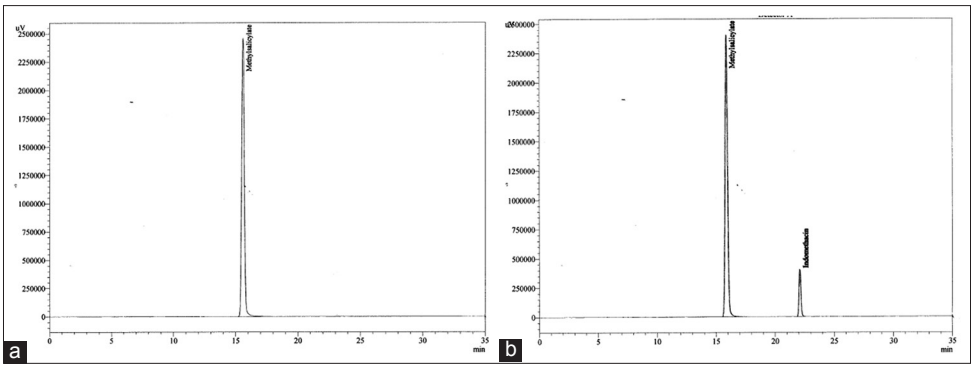

Figure 5: The high-performance liquid chromatography chromatograms of (a) methyl salicylate in formulation A2 and (b) methyl salicylate and indomethacin in formulation B2, for example

One method for assessing microemulsion stability is to follow the particle size and size distribution with time. According to the colloid principles, during storage, any particle droplets of the colloidal size undergo Brownian movement. The particle droplets approach one another. A collision occurs, resulting in either smaller or larger particle droplets. However, an increase in the particle size and a broadening of the particle size distribution are indicative of the occurrence of droplet coalescence and/or aggregation. Taking indomethacin-loaded formulations into consideration, the particle size after storage became larger to varying degree depending on the oil-phase components. The remarkable change was obviously observed when the formulations contained the additive at a 2:1 weight ratio of methyl salicylate to additive. One possible mechanism was considered to explain why the particle size became larger after storage. As mentioned above, indomethacin as the interface-associated solute may affect the size of the microemulsion droplets.[29] The less flexibility of the droplet interface film caused by indomethacin might make the interface layer weakness, thus eventually bringing about the droplet coalescence and/or aggregation after storage. The presence of a large portion of the investigated additive in the methyl salicylate oil phase would further encourage the formation of the droplet coalescence and/or aggregation. Among the prepared formulations, formulations A1 and B1 showed good stability regarding the particle size and the size distribution. The similarity between them was that they comprised menthol as an additive at a 3:1 weight ratio of methyl salicylate–to-menthol. We concluded here that the optimized formulation components were 3.75% menthol, 11.25% methyl salicylate, and 50% the mixture of Tween®20-isopropyl alcohol (1:1) in the presence and absence of 0.75% indomethacin. Tween®20 as a surfactant played a role in covering the droplet cores and simultaneously reduced the interfacial tension between oil phase and water phase. Furthermore, isopropyl alcohol as a cosurfactant helped the surfactant reduce the interfacial tension to very low values to achieve system stability.

All indomethacin-loaded formulations B0-B6 were of lower pH values than their counterpart formulations A0-A6. This was because indomethacin is a weak acidic drug with a pK_a value of 4.5.[30,31] It was expected that, in the experimental condition, an ionization of carboxylic group of indomethacin occurred and led to a decrease in pH of the systems. After 6-month storage, a decrease in pH values of indomethacin-free formulations A0-A6 was attributed to the occurrence of salicylic acid after the intramolecular general base-catalyzed hydrolysis of methyl salicylate. This hydrolysis took place by a water molecule through the participation of the ionized phenolic group which acted as a general base catalyst.[32-34] The degradation products were salicylic acid, methanol, and water. For indomethacin-loaded formulations B0–B6, the subtle change in pH was due in part to the degradation of methyl salicylate and/or indomethacin which caused the weak acidic degradation products. Indomethacin molecules could experience degradation through the hydrolysis of amide moiety, thus generating the ρ-chlorobenzoate and 2-methyl-5-methoxy-indole-3-acetate.[30] However, it should be noted that the observed pH values in the range of 5-7.5 were best for topical administration,[35] and in addition, the O/W microemulsion type was also suitable for the topical use.

Indomethacin-free formulations A0–A6 had higher water content than their counterpart formulations B0–B6. The higher water content reasonably contributed to a slightly higher electrical conductivity and a slightly lower viscosity. A slight increase in the electrical conductivity after 6-month storage might result from the occurrence of the degradation products of methyl salicylate and/or indomethacin.

The data of pH and electrical conductivity were consistent with the analytical results of the active drug substances. A slight decrease in the amount of methyl salicylate after storage resulted from the degradation to some extent of methyl salicylate. It was considered from the published reports[32] that the degradation rate constant of methyl salicylate in rather low alkalinity conditions at 35°C was pH-independent. Therefore, under the condition in the present study, it is reasonable to assume that the pH-independent hydrolysis was responsible for the degradation process of methyl salicylate. However, the analytical results indicated that most methyl salicylate molecules were well preserved from degradation. In case of indomethacin, it is unstable in aqueous solution. Its maximum stability at room temperature occurs near pH 3.75 with a calculated shelf life of about 8.4 days.[36] The little loss of indomethacin after storage revealed that in the prepared systems it was protected to a greater degree against degradation. Our data were in good agreement with other studies that reported on the stability of indomethacin when solubilized in the aqueous surfactant solutions.[37-39] A high retained level

of methyl salicylate and indomethacin after storage could be explained on the principles of micellar solubilized systems, in which the rate of drug hydrolysis is generally small. It was logical to deduce that, in the microemulsion system, the coverage of the surfactant interfacial film had a positive contribution to the reduction in the rate of hydrolysis of methyl salicylate and indomethacin; therefore, the degradation can be minimized and the solubilized drugs should be preserved to a greater degree. Our findings strongly suggested that methyl salicylate and indomethacin solubilized in the investigated system had good stability.

CONCLUSIONS

This study showed the achievement for the use of the liquid drug substance like methyl salicylate as oil phase in microemulsion formulations. The obtained microemulsions had the acceptable physicochemical properties necessary for topical use. They also had proven the capability of preserving solubilized methyl salicylate and indomethacin from hydrolysis to a greater degree over 6-month storage at ambient temperature. The optimized formulation consisted of 11.25% methyl salicylate, 3.75% menthol, and 50% the mixture of Tween®20-isopropyl alcohol (1:1) in the presence and absence of indomethacin. This study gains a useful perspective on product development. From this point of view, many liquid drug substances instead of common esters of fatty acids or fatty alcohols could be used as oil phase in microemulsion preparation.

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