

Original Article

METHYL SALICYLATE MICROEMULSION BASED SYSTEMS. PART I: EFFECT OF ADDITIVES ON THE PHYSICOCHEMICAL PROPERTIES

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ABSTRACT

Objectives: The objective was to determine the use of methyl salicylate, the oily active substance, as oil phase in a given surfactant system of Tween 20-IPA(1:1) in microemulsion preparation and also to investigate the effects of additives in the oil phase as well as their amounts on the physicochemical properties of such microemulsions.

Methods: The pseudoternary phase diagram of methyl salicylate/Tween 20-IPA(1:1)/water system was first constructed as control. Then, other additive-containing systems were investigated to illustrate their phase behaviors. Each additive was incorporated at three different weight ratios of methyl salicylate to additive varying as 3:1, 2:1, 1:1. Afterwards the single phase liquid systems composed of 15% oil phase in the absence and the presence of additives, 50% the Tween 20-IPA (1:1) mixture, and 35% water were prepared and characterized.

Results: Methyl salicylate was successfully used as oil phase in microemulsion preparation. All obtained formulations in the absence and the presence of additives were o/w microemulsions. They showed acceptable physicochemical properties of microemulsion characteristics, which had low viscosity with Newtonian flow. After long term stability for 1 year, the methyl salicylate microemulsion showed good physical stability. The systems containing menthol, isopropyl palmitate or isopropyl myristate as additive at the methyl salicylate-additive weight ratios of 3:1 and 2:1 also showed good physical appearance.

Conclusions: Menthol, IPP and IPM were proper additives in this study at the methyl salicylate-additive weight ratios of 3:1 and 2:1.

Keywords: Methyl salicylate/Tween 20-IPA, Methyl salicylate, Microemulsions

INTRODUCTION

Nowadays most people work hard and then often suffer from minor pains of muscles or joints due to the uncomfortable position during working. Methyl salicylate is the drug of choice used topically to treat minor aches and pains. It appears as oily liquid. Therefore, it was usually prepared in the forms of creams, liniments, and ointments [1,2].

As was known, microemulsion is the optically transparent and thermodynamically stable system. It forms spontaneously after mixing the proper concentration of components together without the need of more energy input and sophisticated machines [3,4]. Many microemulsion researches were carried out in the aspect of its superior transdermal application to solutions or conventional dosage forms [5,6,7,8]. Although many published studies reported the promising data of topical microemulsions as potential drug delivery systems [9,10,11,12] in which the usual pharmaceutical oil excipients were used as oil phase, no study dealt with the use of the active substance with oily liquid characteristic as oil phase. This study would like to make different from already published reports. An attempt was made to gain insights into this point of view. In the present study, Tween 20 and isopropyl alcohol were selected as surfactant and cosurfactant, respectively. The objective of this study was first to determine whether methyl salicylate itself could serve as oil phase in microemulsion preparation and then to investigate the effect of additives as well as their amounts in oil phase on the physicochemical properties of the formulations. The additives were selected on the basis that they are usually found in topical dosage forms either as the minor active substance such as eugenol and menthol or as the basic oil vehicle such as oleic acid [9,13], isopropyl myristate (IPM) [14,15], and isopropyl palmitate (IPP) [16]. Eugenol and menthol were usually found in the methyl salicylate formulation. Eugenol acts on contact to depress cutaneous receptors involved in pain perception [2]. Menthol is effective in topical preparation as a counterirritant at the concentration of 1.25-16% [2]. Oleic acid [17,18,19], IPM [20,21], and IPP [20,22] were reported

to have penetration enhancer property and thus they are useful for rapid delivery of drugs into the skin.

MATERIALS AND METHODS

Chemicals

Methyl salicylate (Ph. Eur. grade, Fluka) and eugenol (purity 99%, Aldrich) were purchased from Sigma-Aldrich (USA). Isopropyl myristate (IPM, Ph. Eur. grade) and isopropyl alcohol (IPA, Emsure[®]) were obtained from Merck KGaA (Germany). Tween 20 was purchased from Merck Schuchardt OHG (Germany). Menthol (BP/USP grade) was purchased from S.Tong Chemical Co., Ltd. (Bangkok, Thailand). Oleic acid was of Ph. Eur. grade obtained from Panreac Quimica S.A.U. (Spain). Isopropyl palmitate (IPP) was obtained from Stearinerie Dubois Fils (France). Water with conductance of 6.39 μ S/cm was used as water phase throughout the experiment. Brilliant blue was purchased from The Government Pharmaceutical Organization (Bangkok, Thailand). All chemicals were used as received without further purification.

Phase behavior study

In order to obtain the concentration range of components for the range of the single phase, the pseudoternary phase diagrams were constructed using water titration method at ambient temperature. Tween 20 and IPA were mixed at weight ratio of 1:1 to obtain the surfactant and cosurfactant mixture. Each additive was added to the methyl salicylate at the methyl salicylate-additive weight ratios of 1:1, 2:1 and 3:1. The oil phase containing methyl salicylate in the absence and the presence of the studied additive was mixed with the mixture of Tween 20-IPA(1:1) at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Water was added dropwise to each mixture under magnetic stirring until the mixture became turbid. The concentrations of components were recorded to complete the pseudoternary phase diagrams. The concentrations of oil phase, Tween 20-IPA mixture, and water in the area of the single phase were selected based on these results for further preparation.

It should be noted that, in the present study, no attempt had been made to distinguish between a microemulsion and other association structures.

Preparation of samples

According to the single phase region in the pseudoternary phase diagrams, the formulations of the single phase liquid mixture designated as microemulsions were defined and prepared. Each formulation was composed of 15% oil phase in the absence and the presence of additive, 50% the mixture of Tween 20-IPA(1:1), and 35% water. The variables were the absence or the presence of the investigated additives. Formulation A without additive was used as control. The letters 'E', 'M', 'O', 'IP', and 'IM' indicated the type of additives added which were eugenol, menthol, oleic acid, isopropyl palmitate, and isopropyl myristate, respectively. The number 3, 2 and 1 following the letter corresponded to the ratio of methyl salicylate to additive as 3:1, 2:1 and 1:1, respectively. The microemulsions were obtained by mixing the all required amounts together under magnetic stirring. All samples were kept in the well-closed glass bottle at ambient temperature for at least 24 hours to achieve equilibrium before further investigation.

Sample Characterization

Appearance observation

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

Particle size measurements

The average droplet diameters of the prepared microemulsions were determined by photon correlation spectroscopy instrument (Delsa™ Nano C Particle Analyzer, Beckman Coulter®, USA) at a temperature of 25°C. Each formulation was run in triplicate.

RESULTS AND DISCUSSION

Pseudoternary phase diagrams

The study of phase diagram was primarily used as the guideline on the microemulsion formation. Methyl salicylate is miscible with the Tween 20-IPA(1:1) mixture. In this study, methyl salicylate was successfully used as oil phase in the phase behavior study. The pseudoternary phase diagram of methyl salicylate/Tween 20-IPA(1:1)/water system was observed and used as control for comparison with those of additive-containing systems. The effects of the addition of each additive, i.e. eugenol, menthol, oleic acid, IPP and IPM into the oil phase of the pseudoternary phase diagram were investigated. Figure 1(A) demonstrated the pseudoternary phase diagram of the systems containing eugenol as additive. The obtained single phase zones were comparable to that of the control system and remained unchanged as

Viscosity measurements

The viscosities of the prepared microemulsions were determined by a Brookfield® Digital Rheometer (Model DV-II+ Visometer, 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 microemulsions.

pH measurements

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

Conductivity measurements

The electroconductivity was measured at 25°C using a conductivity meter (SevenEasy, Mettler Toledo, Germany) which was calibrated using the standard solution of 1413 $\mu\text{S}/\text{cm}$ before testing. The measurement was run in triplicate and reported as average value.

Test for microemulsion type

The qualitative test to determine the type of the prepared microemulsions was carried out using dilution test. The 1% brilliant blue aqueous solution and methyl salicylate were used to qualitatively determine the type of the prepared microemulsions. The miscibility was visually assessed. The o/w microemulsions were able to miscible with 1% brilliant blue aqueous solution but immiscible with methyl salicylate. On other hand, the w/o microemulsions were expected to show the opposite results.

Stability evaluation

Physical appearances were evaluated by visual inspection of the samples after storage at ambient temperature for 1 year. Stable systems were determined as those free of any physical change such as colour, clarity, phase separation and/or precipitation. The mean particle size of microemulsions upon storage was also measured to assess whether droplet coalescence and/or aggregation occurred.

the weight ratio of methyl salicylate to eugenol changed since 3:1 to 1:1. As presented in Figure 1(B), the addition of menthol as additive led to the smaller single phase areas than that of the control system. Incorporation of oleic acid as additive at the methyl salicylate-oleic acid weight ratios of 3:1, 2:1, and 1:1 seemed to slightly enlarge the single phase areas compared with that of control as observed in Figure 2(A). Figure 2(B) obviously elucidated the pronounced effect of the higher weight ratio of IPP (1:1) incorporated on decreasing the single phase area. The similar result was observed in the case of IPM as illustrated in Figure 2(C). IPP and IPM are fatty acid esters. All these results could be explained in part on the basis of the available published observations.

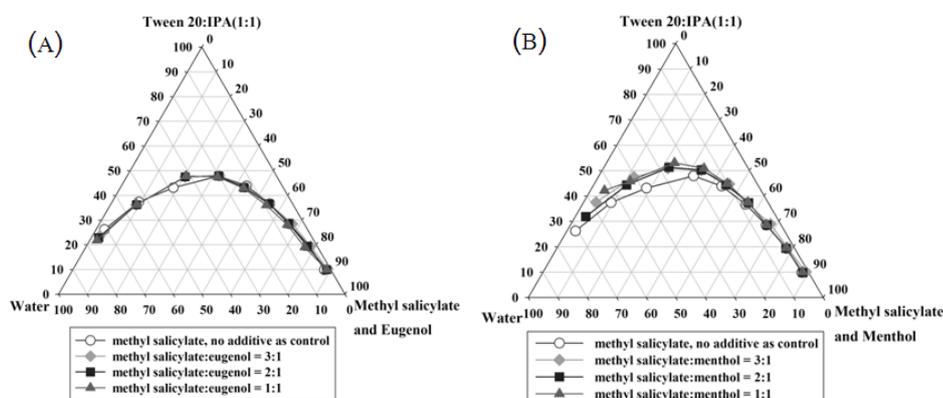


Fig. 1: The effect of eugenol (A) and menthol (B) as additive in the oil phase on the pseudoternary phase diagrams.

In case of long-chain triglycerides, such as vegetable oils, medium chain triglyceride and fatty acid esters generally used as pharmaceutical oil excipients in microemulsion formulation, it was already addressed that the lower the molecular volume of oils, the greater the surfactant efficiency [23]. In the present study, the effect of investigated substances as additive in the oil phase varied considerably from substance to substance. Selected additives are either soluble in or miscible with methyl salicylate. Methyl salicylate, eugenol, and menthol are of comparable low molecular volumes about 215.1, 255.5, and 291.5 cm³/Mol, respectively. In contrast, oleic acid, IPM, and IPP have larger molecular volumes of 523.9, 528.2, and 581.2 cm³/Mol, respectively. Small molecular volume substances should be solubilized completely in the tail of surfactant.

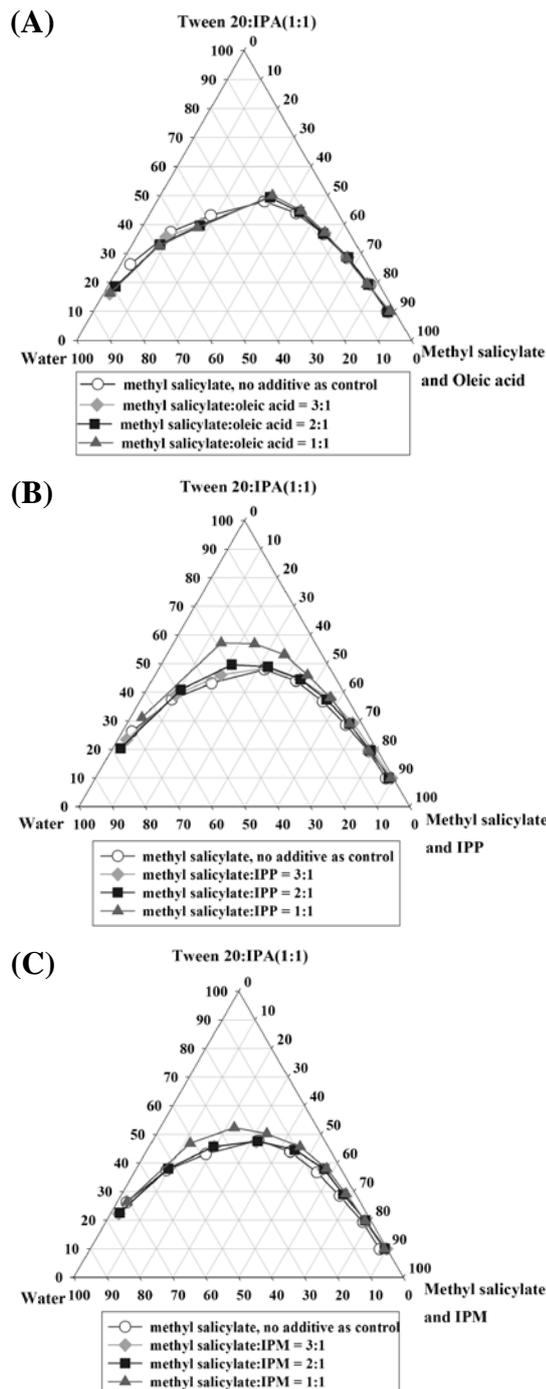


Fig. 2: The effect of oleic acid (A), IPP (B) and IPM (C) as additive in the oil phase on the pseudoternary phase diagrams.

Even though oleic acid, a polar substance, has about twice the molecular volume of menthol, it seemed to show less effect on the single phase region in the pseudoternary phase diagram. These results confirmed that solubilization of substances in a given surfactant mixture depended not only on the molecular volume, but, to a certain extent, on other physicochemical properties of solubilisates as well. In addition, the amount of the additive added into the oil phase also played a role in the solubilization as observed in case of incorporation of IPM and IPP. It was possible that the more addition of IPM or IPP at the 1:1 weight ratio of methyl salicylate to IPM or IPP made less efficient the solubilization of a given Tween 20-IPA (1:1) surfactant mixture for oil phase, thereby resulting in the smaller single phase area.

Based on the pseudoternary phase diagram results, the appropriate concentrations of components to obtain the single phase liquid mixtures were selected and used in the preparation of microemulsions. The desired characteristics of the preparations as well as the maximum and minimum acceptable levels of components for a topical application were considered. For systems containing eugenol, menthol, or oleic acid as additive, the effect of three levels of the methyl salicylate-additive weight ratios (3:1, 2:1, and 1:1) on the physicochemical properties of the formulation were studied whereas the effect of two weight ratios (3:1 and 2:1) were investigated for the system containing IPP or IPM. Therefore, there were 14 microemulsion formulations for further physicochemical characterization.

Microemulsions and physicochemical characterization

All 14 formulations were the transparent homogenous single phase liquids as shown in Figure 3(A). Under the cross-polarized light microscopy, the view of all samples looked dark and no birefringence was found, indicating isotropic property [24]. Isotropic characteristic is one of the important property of microemulsions. After long term stability at ambient temperature for 1 year as presented in Figure 3(B), no phase separation and precipitation were found in all samples. They were still clear homogenous single phase liquids. The colour of 8 formulations including A, M3, M2, M1, IP3, IP2, IM3, and IM2 visually remained unchanged. In contrast, the colour of the formulations containing eugenol changed considerably. In addition, the oleic acid-containing formulations gained yellowish colour. These suggested the physical instability. It should be noted that in this study, no stabilizer was added into the formulations. Even though eugenol was usually found together with methyl salicylate in various topical analgesic formulations, in this study eugenol posed problems in the system, resulting in the drastic change in colour. This might be attributed to the property of eugenol itself which darkens on exposure to air [25]. In case of oleic acid, it oxidized and acquired colour on exposure to air [25].



Fig. 3: The physical appearance of all prepared formulations. (A) after preparation, (B) after storage for 1 year.

The average droplet size of all 14 microemulsion formulations ranged from 67.5 to 163.6 nm as shown in Figure 4(A). All had small polydispersity index values in the range from 0.071 to 0.264, suggesting the narrow size distribution. The addition of different additives as well as different weight ratios of methyl salicylate to additive affected the particle size. Obviously, the incorporation of menthol led to the significant increase in the microemulsion droplet size. The more the menthol incorporated, the bigger the particle size observed. It should be noted that the presence of oleic acid at 1:1 weight ratio of the methyl salicylate to oleic acid resulted in a remarkable increase in microemulsion droplet size up to 120.8 nm.

From the particle size data, these could be explained on the basis of nature characteristic of investigated additives. All additives chosen are water-insoluble hydrophobic compounds. After the microemulsion was formed, the chemical nature of each additive made it possible to locate itself within the core or at surfactant-cosurfactant film of the microemulsion droplet. The non polar compound was surely in the core whereas the molecule of water-insoluble substance with intermediate hydrophobicities was orientated at the interface. The droplet size became larger possibly due to the enlargement of the core or due to an increase in the number of surfactant and cosurfactant molecules per microemulsion

droplet in an attempt to cover the core. In case of oleic acid, our study was in concordance with the finding of Paolino et al. [26]. They reported a significant increase in the microemulsion droplet size due to the presence of oleic acid in the formulation. Upon storage for 1 year, the average droplet diameters of all were shown in Figure 4(B). Taking the 8 formulations, which showed good physical appearances as mentioned above into consideration, most had the mean droplet size below 100 nm and the narrow size distribution (data not shown). The mean particle size of the menthol-containing formulations significantly became bigger. This was probably due to the occurrence of droplet coalescence and/or aggregation.

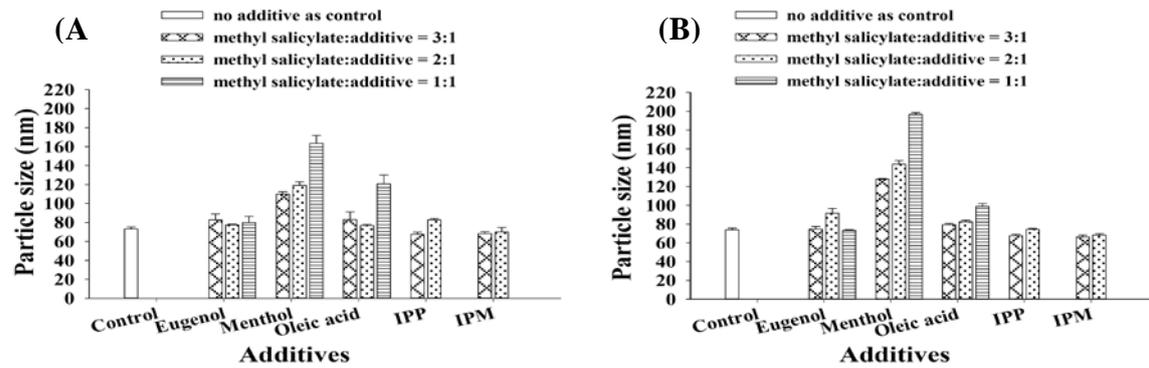


Fig. 4: The mean droplet size of all prepared formulations. (A) after preparation, (B) after long term stability for 1 year.

Table 1 summarized the apparent viscosity, conductivity and pH values of all obtained samples. All 14 formulations showed low viscosities ranging from 12.0–16.1 cPs. It seemed that the addition of additives showed no effect on the apparent viscosity. The correlation coefficients between shear rate and shear stress were high approaching 1.0 (data not shown), suggesting Newtonian flow behavior. The low viscosity with Newtonian flow behavior is typical of microemulsion system [27]. The obtained high conductivity data suggested the formation of o/w structure. It is general for the system with water as external pseudophase. For dilution test, all 14 formulations were found to be miscible with 1% brilliant blue

aqueous solution but turbid after the addition of methyl salicylate, indicating the o/w characteristic. Taking the conductivity data and the dilution results into consideration, it was shown that all prepared formulations were of the o/w structure. For pH measurement, all formulations except for oleic acid-containing formulations showed pH above 6. It was obvious that a noticeable decrease in pH was observed in formulations containing oleic acid. This was because oleic acid is a weak acid substance. The higher the oleic acid content, the more decrease the pH value. However, these obtained pH values were appropriate for further topical formulation study.

Table 1: Viscosity, conductivity and pH values of prepared formulations

Formulations	Viscosity (cPs)	Conductivity ($\mu\text{S}/\text{cm}$)	pH
A	12.0	55.6	6.58
E3	12.2	53.8	6.56
E2	12.7	46.8	6.53
E1	12.7	53.4	6.55
M3	12.9	52.4	6.68
M2	12.9	48.7	6.69
M1	14.4	43.1	6.71
O3	14.0	50.1	5.65
O2	14.0	48.8	5.43
O1	16.1	43.8	5.32
IP3	14.4	48.0	7.03
IP2	15.1	46.7	6.96
IM3	14.2	52.5	6.95
IM2	15.1	49.2	6.91

CONCLUSIONS

Methyl salicylate, the medicative substance with oily liquid characteristic, was successfully used as oil phase in the microemulsion preparation. The single phase liquid mixtures, composed of 15% oil phase in the absence and the presence of various weight ratios of studied additives, 50% the Tween 20-IPA (1:1) mixture, and 35% water, were oil in water microemulsions. These obtained systems showed the acceptable physicochemical properties of microemulsion characteristics. Upon storage for 1 year, the methyl salicylate microemulsion showed the good physical appearance and physicochemical properties.

For additive-containing systems, it was shown that menthol, IPP and IPM were proper additives at the methyl salicylate-additive weight ratios of 3:1 and 2:1. It was noteworthy that in case of menthol-containing systems, the amounts of methyl salicylate and menthol at the weight ratio of 2:1 were comparable to those found in the commercial analgesic cream, Counterpain®. The success in this study is a useful guideline introducing a new alternative delivery system of methyl salicylate.

CONFLICT OF INTERESTS

Declared None.

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