Factors affecting phase behavior of microemulsions comprising *Cymbopogon citratus* oil

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INTRODUCTION

Cymbopogon citratus (Family: Gramineae) is an extensively used herb in tropical countries and known as a source of ethnomedicines (Puatanachokchaia R. 2002). The essential oil of *C. citratus* is widely used in food, cosmetic, and pharmaceutical industries (Negrelle RRB. 2007). However, its low aqueous solubility leads to a limitation in application. In order to increase its solubility, microencapsulation has been used (Jackson LS. 1991). Microemulsion has been shown to be able to protect labile drug, control drug release, increase drug solubility, increase bioavailability and reduce patient variability (Chai JL. 2008, Lawrencea MJ. 2000). The aim of this study was to investigate factors influencing phase behavior of *C. citratus* oil microemulsion.

MATERIAL AND METHODS

Plant material and essential oil isolation

C. citratus was collected from Chiang Mai, Thailand. The fresh overground part was cut into small pieces and subjected to hydrodistillation for 3 h. The essential oil obtained was stored in a refrigerator and protected from light until further use.

Chemical materials

Polyoxyethylene 10 oleyl ether (Brij 97) was of Aldrich (Steinheim, Garmany). Polyoxyethylene sorbitan monolaurate (Tween 20) was from Namsiang Co., Ltd. (Bangkok, Thailand). Octylphenoxy polyethoxy ethanol (Triton X-114) and polyethylene glycol sorbitan trioleate (Tween 85) were from Acros Organics (New Jersy, USA). Sodium dihydrogen orthophosphate dehydrate, sodium chloride and calcium chloride were from Fisher Chemicals (Loughborough, UK). Di-Sodium hydrogen orthophosphate dehydrate was of Ajax Finechem. (NSW, Australia). Ethanol was from J.T.Baker (Selangor, Malaysia). Hexan-1-ol was of Unilab-APS (Asia Pacific Specialty Chemical Limited ABN, Australia).

Construction of pseudoternary phase diagram

Pseudoternary phase diagram of *C. citratus* oil was constructed using water titration method. Surfactants including Brij 97, Triton X-114, Tween 20 and Tween 85 were separately mixed with a co-surfactant, ethanol or hexanol, at a weight ratio of 1:2, 1:1 and 2:1 to obtain the surfactant mixture. The essential oil of *C. citratus* and the surfactant mixture were then mixed at various weight ratios. These mixtures were titrated with water, under moderate agitation. The samples were classified as microemulsions when they appeared as clear liquids. The dropwised water was replaced by phosphate buffer pH 4.0, 6.0 and 8.0 to investigate pH effect and by 0.1 M, 0.5 M and 1.0 M monovalent salt (NaCl) and divalent salt ($CaCl_2$) to investigate ionic strength effect.

RESULTS AND DISCUSSION

Effect of surfactant type

Among 4 surfactants used, the results indicate that Brij 97, Triton X-114, Tween 20 possess higher ability to create the microemulsion. However, Tween 20 was selected for further experiment because it has been reported to have minimal toxicity (Lawrencea MJ. 2000).

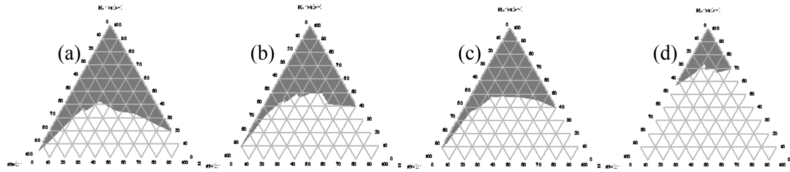

Fig 1. Phase Diagrams of of *C. citratus* oil comtaining Brij 97 (a), Triton X-114 (b), Tween 20 (c) and Tween 85 (d). The dark area represents the region of microemulsion.

Effect of surfactant and cosurfactant ratio

To investigate this effect, ethanol and hexanol were used as co-surfactants. The result reveals that ethanol has more microemulsion forming ability than hexanol as shown in Fig 2. Hence ethanol was selected to use as a co-surfactant in the further study.

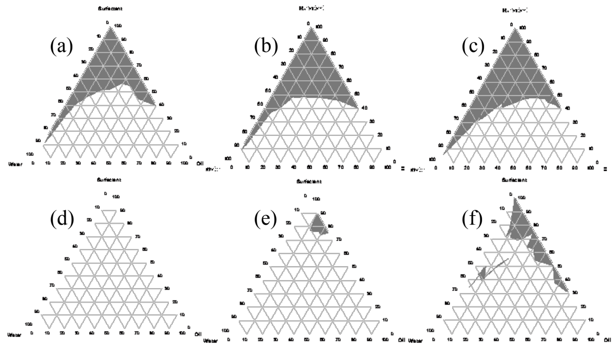

Fig 2. Phase diagrams of *C. citratus* oil using surfactant system of Tween 20 : ethanol of 1 : 2 (a), 1 : 1 (b), and 2 : 1 (c) and Tween 20 : hexanol of 1 : 2 (d), 1 : 1 (e), and 2 : 1 (f). The gray area represents the region of microemulsion.

Effect of ionic strength

To determine the influence of ionic strength, NaCl and $CaCl_2$ were employed for monovalent and and divalent electrolytes respectively. The results show that microemulsion regions of the systems containing monovalent salt resembled to that of divalent salt when they were the same concentration. The increase in ionic strength resulted in a decrease microemulsion regions. Normally, ionic strength is expected to affect the phase behavior of microemulsion with ionic surfactant because of decreasing in the effective head group area of ionic surfactants as the double layer shrinks and screening of the head groups allows closer approach(Lawrencea MJ. 2000). This study indicates that ionic strength also influences microemulsion formed by nonionic surfactant (Tween 20).

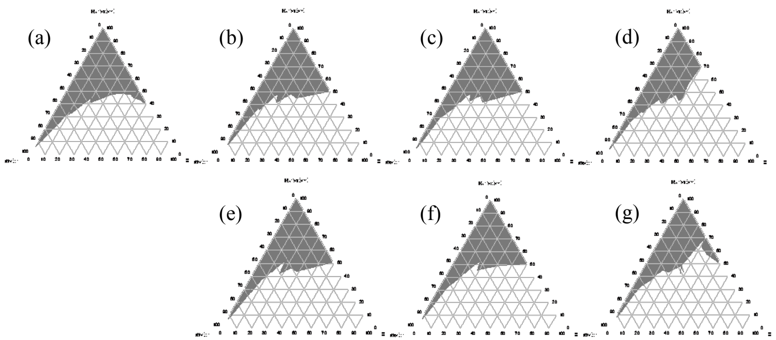

Fig 3. Phase diagrams of *C. citratus* oil using 2:1 of Tween 20 : ethanol surfactant system with the aqueous phase of water (a), 0.1 M NaCl (b), 0.5 M NaCl (c), 1.0 M NaCl (d), 0.1 M $CaCl_2$ (e), 0.5 M $CaCl_2$ (f), 1.0 M $CaCl_2$ (g). The gray area represents the region of microemulsion.

Effect of pH

In this study, the pH of aqueous phase was varied from 4.0 to 8.0. The results indicate that the microemulsion regions of aqueous phase pH 4. 0 and 6.0 were very close and greater than that of pH 8.0 as shown in Fig. 4.

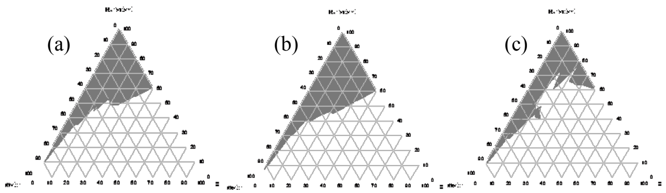

Fig 4. Phase diagrams of *C. citratus* oil en 20 using 2:1 of Tween 20 : ethanol surfactant system with the aqueous pH of 4.0 (a), 6.0 (b), and 8.0 (c). The gray area represents the region of microemulsion

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