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Note

Physicochemical characterization and evaluation of a microemulsion system for antimicrobial activity of glycerol monolaurate

Xiaowei Fu [a,*], Fengqin Feng [b], Bin Huang [b]

[a] *College of Life Science, University of China Jiliang, Zhejiang, Hangzhou 310018, PR China*
[b] *Department of Food Science and Nutrition, Zhejiang University, Hangzhou 310029, PR China*

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Abstract

The purpose of this study was to improve the depression, enhance the bioavailability, hence strengthen the antimicrobial ability of poorly water-soluble glycerol monolaurate (GML) by loading it in microemulsion system. Microemulsions were prepared with GML as oil, tweens as surfactant, and medium-and-short chain alcohols at different ratio as cosurfactants. The effect of the ratio of surfactant to cosurfactant on the stability of microemulsion was tested. And the effect of the composition and ratio of cosurfactant and the effect of potassium sorbate dissolved in water at different concentration on the area of O/W microemulsion region in pseudo-ternary phase diagrams were also investigated. The results showed that the microemulsion is most stable when the ratio of surfactant to cosurfactant was 3:2, the suitable cosurfactant is pentanol to dodecane at 2:1, the area of O/W microemulsion region in pseudo-ternary phase diagram increased with increasing content of potassium sorbate. The conclusion of this study was that GML loaded in microemulsion had much higher anti-microbial activity than GML alone.

Keywords: Glycerol monolaurate; Microemulsion; Pseudo-ternary phase diagram; Anti-microbial activity

1. Introduction

Glycerol monolaurate (GML), which is one of the commonly used surfactants in the US, had been warranted as one of GRAS food additives by FDA in 1977. In addition to its use as quality improver in food bakery, it is well-known as surfactant like lipid with the greatest overall antimicrobial activity of all lipids evaluated as anti-microbials (Shibasaki, 1982).

However, the unique physical properties of GML that include high melting point and poor solubility in water, glycerol and other common used solvents in food industry lead to difficulties in its use as an anti-microbial.

Different approaches have been tried to improve the emulsification ability of GML, to form GML-based dispersions for possible cosmetic and food application, and to increase its antimicrobial activity (Kabara, 1978, 1979, 1980). In recent years, enhanced solubility such as oral administration has been reported by using microemulsion system (Ritschel, 1991; Sarciaux et al., 1995). These nano-structured fluids have attracted in the last decade considerable attention in many applications such as foods, pharmaceuticals, cosmetics, pesticides and coating materials. They are thermodymanically stable, isotropically clear dispersions of two immiscible liquids such as oil and water, stabilized by an interfacial film of surfactant molecules (Eccleston, 1992). The advantages of microemulsions as GML systems are the improvement of GML dispersion, the potential for enhanced absorption due to surfactant-induced permeability changes and then strengthen GML antimicrobial and emulsified abilities (Shibasaki, 1982). Tweens were selected as surfactants in this study because non-ionic surfactants like tweens are known to be less affected by pH, ionic strength changes and also can enhance the permeability of GML into cell membrane (Zhong-Gao Gao et al., 1998). Short and medium chain alcohols were also needed to form stable microemulsion, and they are commonly called cosurfactant. Normally, saline can increase the surface activity, and the solution of weak acid salt has strong emulsifying function (Eccleston, 1992). Therefore, potassium sorbate, which is one of the commonly used food antimicrobial, was added to the GML microemulsion system to increase the loading amount of GML in microemulsion system.

* Corresponding author.
E-mail address: fxw13@163.com (X. Fu).

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In this study, various microemulsions were prepared to investigate the effects of alcohol type and potassium sorbate content on the physicochemical characteristics of these systems.

After optimization of the microemulsion system, the antimicrobial ability of GML loaded in the microemulsion systems was also compared to pure GML.

2. Materials and methods

2.1. Materials

Glycerol monolaurate (90% monoester content), was presented by Ultra Chemical (The Galleria, 2 Bridge Ave.Red BankUltra, USA); ethanol, pentanol; octanol; dodecane were all analytical-purity grade purchased from market. Tween 20 (polyoxyethylene (20) sorbitan monolaurate) and Tween 80 (polyoxyethylene (20) sorbitan monooleate) (Wenzhou Qingming chemical ingineeering company, PR China) and potassium sorbate (Sanhe Food Additive Company, PR China) were all food grade additives. The water used was double-distilled.

2.2. Determination of the weight ratio of the surfactant to the cosurfactant (S_{mix})

When GML microemulsion was prepared, a mixture of Tween 20 and Tween 80 at a ratio of 2:1 was used as surfactant, pentanol and dodecane at a ratio of 1:1 as cosurfactant, GML (0.5 g) as oil. The total amount of the system was 7 g. According to the weight ratio of surfactant to cosurfactant in Table 1, the GML was melted when heated to 65 °C, then surfactant and cosurfactant were added in and kept in thermostat bath at 30 °C, and then water was increasingly added to this mixture system under slightly shaking. The clarity of the system was observed, and the amounts of water which were added between the two points from turbid to clear, and from clear to turbid defined as the length of microemulsion domain (LMD) (Friberg, 1977; Schechter and Bourrel, 1988).

2.3. Construction of pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were constructed for glycerol monolaurate/surfactant/cosurfactant/water (aqueous solution of potassium sorbate) systems in which the weight ratio of surfactant to cosurfactant was maintained constant. Titrimetric method was employed to learn the phase behavior of these systems. Either water or aqueous solution of potassium sorbate was increasingly added to GML, surfactant, and cosurfactant mixture under stirring condition at 30 °C. The system will be from turbid to clarity, and then from clarity to turbid. The physical states were represented on a pseudo-ternary phase diagram with one axis representing water (W), one representing oil (O) and the third representing the weight ratio of the surfactant to the cosurfactant (S_{mix}). The influence of the types of cosurfactants (alcohol) and the contents of potassium sorbate on the area of O/W microemulsion region were investigated on the pseudo-ternary phase diagram.

Table 1
The length of microemulsion domain with the different ratio of surfactant to cosurfactant

The weight ratio of surfactant to cosurfactant	The length of microemulsion domain (ml)
9/4	0.6
2/1	0.85
7/4	1.1
3/2	2.05
9/8	1.3
7/8	0.65
5/8	0.35
3/8	0

2.4. Test of the antimicrobial ability of GML microemulsion

The antimicrobial experiments were carried out on potato and beef paste medium with pH of 6–7. The antimicrobial efficacy of GML microemulsion was tested and compared to that of pure GML. The microbials used were *A. niger*, *Bacillus subtilis*, *E. coli* which are all the most typical microbials in food. The ratio of inhibited microbial is the percentage of the number of reduced microbials divided by that of the control blank (without being added any anti-microbial agents).

3. Results and discussions

3.1. The weight ratio of surfactant to cosurfactant

Different ratio of surfactant to cosurfactant were tested. As shown in Table 1, the length of microemulsion domain (LMD) increased with decreasing ratio of surfactant to cosurfactant at a ratio greater than 3:2. In the contrast at a ratio less than 3:2, the LMD decreased with decreasing ratio. When the ratio of surfactant to cosurfactant decreased to 3/8,clear microemulsion wasn't able to form due to insufficient amount of surfactant. This result is in accordance with that of Fortney (1981).

The LMD indicates the area of the O/W microemulsion. The area increases with increasing LMD value. When the weight ratio of surfactant to cosurfactant is too high or too low, LMD will become shorter, which shows that the area of microemulsion is decreased. At the same time, the optimal amount of cosurfactant is good for solubility of GML and then increasing the LMD. Therefore, the mixture of surfactant to cosurfactant at the ratio of 3:2 was selected when the effect of different alcohols and the contents of potassium sorbate on the LMD were studied.

3.2. Phase studies with the different cosurfactants

Different microemulsion were prepared using different cosurfactants to investigate the effect of cosurfactants on the LMD and the area of the O/W microemulsion.

Differences in the ability of alcohols to act as cosurfactants are a consequence of their influence on the nature of the interfacial film around the oil droplets. The presence of alcohol in the interfacial region causes a reduction in the rigidity of the otherwise condensed GML film, allowing the curvature necessary

Table 2
The LMD of microemulsion with different alcohols

Cosurfactant	LMD (ml)
Ethanol/dodecane (1/1)	0
Octanol/dodecane (2/1)	0.7
Pentanol/dodecane (2/1)	1.95
Pentanol/dodecane (1/1)	1.25

for droplet formation. The distribution of the alcohol between the interface and the aqueous continuous phase depends on its hydrophilicity (Salager, 1977). The low interface/water partition coefficient of ethanol which results from its high water solubility implies that larger quantities of this alcohol are required to produce similar effects on the interfacial layer as the less hydrophilic alcohol such as pentanol, octanol, dodecane (Anan Yaghmur et al., 2002).

When ethanol was used as cosurfactant, the LMD was approximately zero. However, the 2:1 ratio of pentanol to dodecane could form the longest LMD (Table 2). In addition, the sequence of the area of O/W microemulsion region from the largest to the smallest was B, C, and A (Fig. 1). That means pentanol and dodecanol at a ratio of 2:1 is the most suitable cosurfactant when GML microemulsions were prepared within the testing range. Therefore this combination of cosurfactants was adopted when the effect of potassium sorbate content on the formation of O/W microemulsion was studied.

Different concentration of potassium sorbate solutions were tested, and the results were shown in Fig. 2.

The areas of isotropic regions of O/W microemulsion systems increased with the increase of the potassium sorbate concentration (Fig. 2). It indicates that the maximum proportions of oil (GML) incorporated in microemulsions increased significantly with the increasing amount of potassium sorbate. From a formulation viewpoint, the increased oil content in microemulsions may provide a greater opportunity for the dispersion of poorly water-soluble GML and enhance the antimicrobial activity of GML (Friberg, 1977; Schechter and Bourrel, 1988; Lisker and Poster, 1982; ter steeg et al., 2001).

3.3. The result of the antimicrobial activity test

Microemulsion contained 8% GML and no potassium sorbate was used when the antimicrobial experiments were carried out. Different concentrations of GML microemulsion were tested

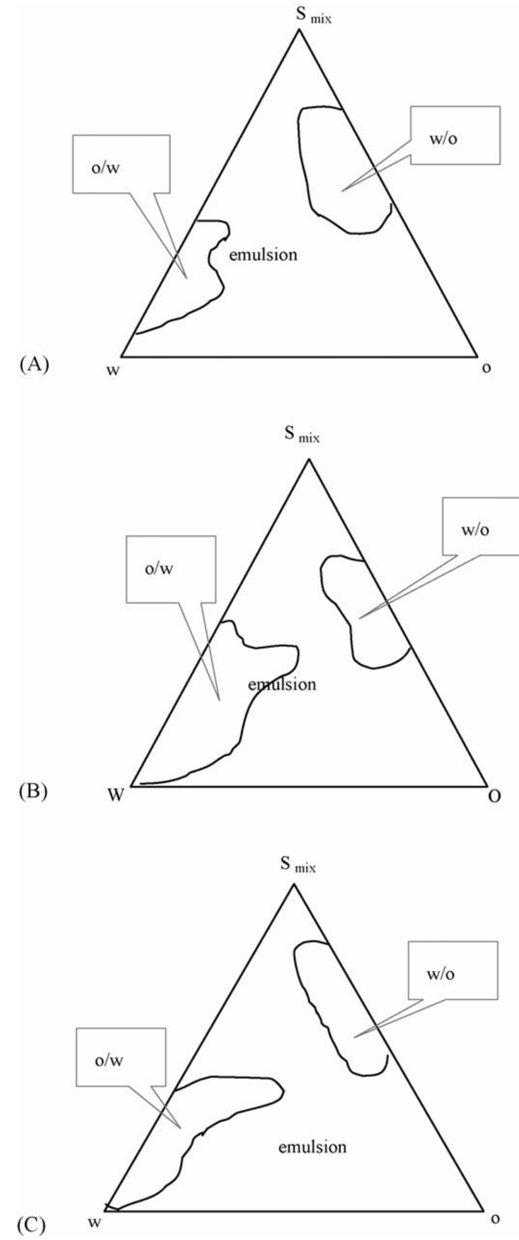

Fig. 1. The area of the O/W microemulsion region with the different types of cosurfactants. The phase diagram were prepared at 30 °C the effect of potassium sorbate content on the formation of O/W microemulsion. *Notes*: (A) Octanol/dodecane (2/1), (B) pentanol/dodecane (2/1) and (C) pentanol/dodecane (1/1).

Table 3
The antimicrobial effect of the O/W GML microemulsion

		The ratio of inhibited microbial (%)		
		A. niger 1.5×10^6 (5 d)	*Bacillus subtilis* 2.57×10^6 (48 h)	*E. coli* 3.15×10^6 (48 h)
GML[a] (%)	0.25	31	22.53	0
	0.5	67	53.17	16.7
Microemulsion (%)	0.05	78	92	77
	0.125	100	99	86
	0.2	100	100	95

[a] The status of GML is solid, and it will be solved by heating when using.

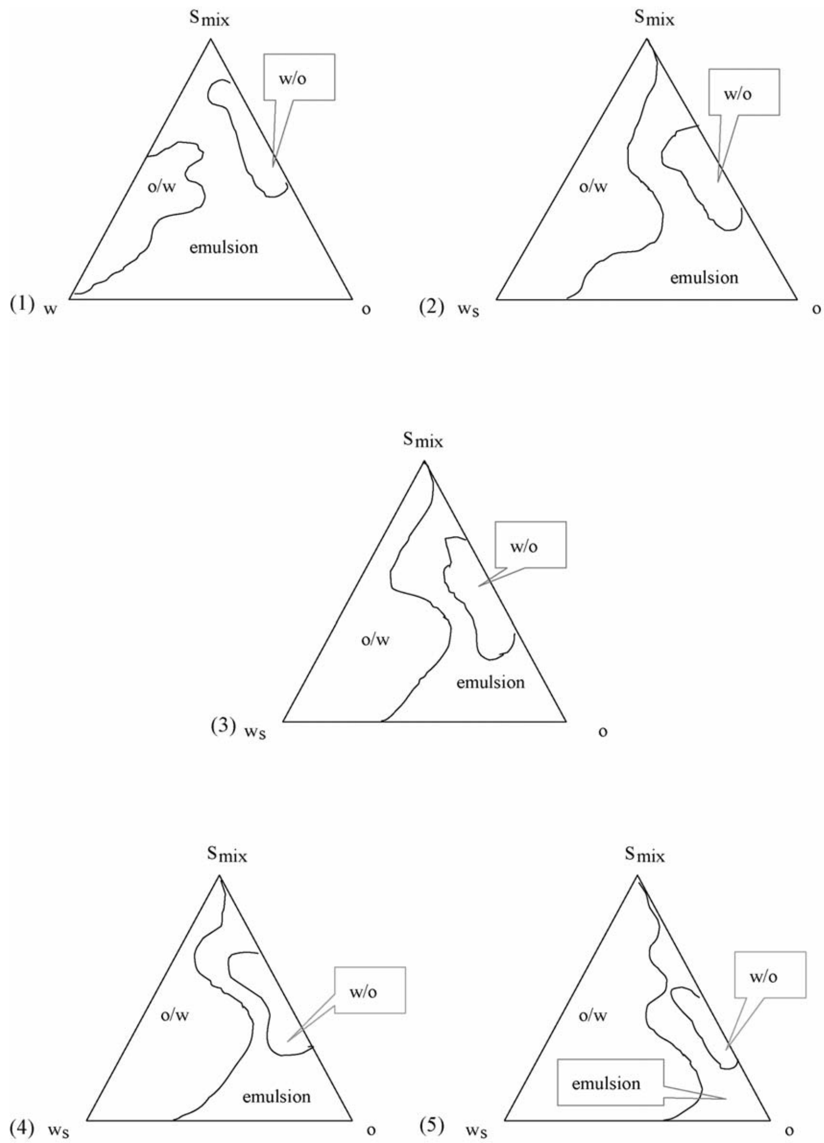

Fig. 2. The area of the O/W microemulsion region with the different contents of potassium sorbate. The phase diagrams were prepared at 30 °C. The concentration of potassium sorbate is as follows: (1) 0%; (2) 10%; (3) 15%; (4) 25%; and (5) 30%.

on *A. niger*, *B. subtilis*, *E. coli*. The results (Table 3) showed that when GML was loaded in microemulsion system, its anti-microbial activity was increased greatly. As for *A. niger*, the ratio of inhibited microbial of pure GML with the usage of 0.5% was 67%, and hence that of GML microemulsion put up to 78% with the one-tenth usage of GML (Table 3). As for *B. subtilis* and *E. coli*, it's the trend, different but bigger increase of the ratio of inhibited microbial were obtained with GML microemulsion than with pure GML. Therefore, GML loaded in microemulsion had much higher anti-microbial activity than GML alone.

4. Conclusions

The enhancement of bioavailability (inhibited microbial ability) of GML by using O/W microemulsion optimized in this study is thought to mainly be due to the GML solubilization effect.

From the pseudo-ternary phase diagrams, we can see that numerous formulation with different concentration and ratio of GML and potassium sorbate can be prepared. Because both GML and potassium sorbate are commonly used food additive, and each of them has a special antimicrobial spectrum, it can be expected that GML microemulsion incorporating potassium sorbate would have higher antimicrobial activity and wider antimicrobial spectrum than each of them. Therefore further study need to be done on this aspect.

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