The Effect of Liquid Crystalline Phase Formation on Preservation

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SUMMARY

The effect of liquid crystalline phase formation on preservation in ternary and O/W emulsion systems was investigated.

Antimicrobial activity of methyl paraben in tested systems was more enhanced with the increasing concentration of fatty alcohols such as myristyl, cetyl, stearyl and cetostearyl alcohol except lauryl alcohol.

With the increasing addition of such fatty alcohols, the increasing quantity of free methyl paraben in tested systems was determined by equilibrium dialysis method.

The liquid crystal formed in presence of fatty alcohols was observed by a microscope under polarized light. However, liquid crystal could not be observed only in lauryl alcohol.

From these results, we could conclude that the formation of liquid crystalline phase affects decrease of the micelle quantity and suppresses inactivation of methyl paraben.

1. INTRODUCTION

Since the nonionic surfactants have been introduced in cosmetic fields as emulsifier and stabilizer, cosmetics with better stability and feeling could be obtained. However, the phenomena of most nonionic surfactants support in microorganism growth and their interaction with common preservatives such as parabens has increased the risk of microbial contamination.

Preservatives inactivation by nonionic surfactants has been well investigated for a long time and many related articles have been published (1-3). It is generally known that the main reason for the inactivation of preservatives is from the solubilization of preservatives by nonionic surfactant micelles (4,5), or from the formation of hydrophobic complex between preservatives and nonionic surfactants (6). Although many works have been devoted to the study of the interaction of nonionic surfactants and phenolic preservatives, very few studies have been made to reduce such an interaction.

Our experiments sometimes revealed that some emulsions with better stability require less quantity of preservatives to obtain a well balanced preservation in O/W emulsions. It was supposed that such phenomena were attributed to the liquid crystalline phase formation in emulsion.

It is well known that such fatty alcohols as cetyl and cetostearyl alcohol increase the stability and consistency of O/W emulsions with the presumed effects due to the formation of liquid crystalline phase (7-9). The rheological properties of the ternary and O/W emulsion systems prepared with fatty alcohols and surfactants have been investigated, and the thickening effect of fatty alcohols has resulted from the formation of network structure of liquid crystalline phase (10,11). On the other hand, the influences of liquid crystalline phase on emulsion stability could be suggested as the reduced van der Waals pontential for coalescence between droplets covered with liquid crystal (12).

In other words, the emulsions with better stability and desired consistency require the formation of liquid crystalline phase. Very little information, however, on the effect of liquid crystalline phase formation on preservation was available. The present investigation was undertaken to elucidate the liquid crystalline phase formation by addition of various fatty alcohols on preservation in ternary and O/W emulsion systems.

2. EXPERIMENTAL

2-1 Materials

Polyoxyethylene (20) sorbitan monostearate (I.C.I.), Polyoxyethylene (10) cetyl ether (Nikko Chem.) and sorbitan monostearate (I.C.I.) were used as surfactant, and lauryl, myristyl, cetyl, stearyl alcohol (Tokyo Kasei) and ce-

tostearyl alcohol (Henkel) as fatty alcohol. Methyl paraben (Danil Chem.) and imidazolidinyl urea (Sutton Lab.) were used as preservatives, and liquid paraffin (Witco Chem.) as oil. Lauryl, myristyl, cetyl, stearyl alcohol were reagent grade, and others were cosmetic grade without further purification.

2-2 Sample preparation

Ternary and liquid paraffin in water emulsion systems were prepared with the composition as Table 1.

Table 1. Composition of system

	Ternary	Emulsion
Liquid paraffin	–	25.0 g
Surfactant	0.5-2.0	2.0-2.5 g
Fatty alcohol	0-4.0	0-4.0 g
Methyl paraben	0.2-0.25	0.1-0.32 g
Water	100.0	75.0 g

Ternary systems were made with ultrasonic homogenizer and agitator at 70 °C, and emulsions were made by putting water into oil phase while stirring with 1000 rpm. at 70 °C.

All samples were kept for 1 day at 30 °C for examination.

2-3 Organism

E. coli ATCC 8739 was grown on slant of nutrient agar for 48 hours at 37 °C. The growth medium was washed and centrifuged 3 times with sterile saline and the density of the suspension was adjusted to give final population of 10^8 cells/ml.

2-4 Determination of extinction time

The extinction time of ternary systems was determined by inoculating 0.2ml of standardized suspension of E. coli to 30g of each system. The test systems were then incubated at 35 °C.

With suitable time intervals, aliquot of 0.5g from each system was transferred to 10ml of nutrient broth containing appropriate amount of neutralizer.

One ml of mixture was further transferred to 10ml of nutrient indicator broth, incubated at 35 °C for two days, and then visually examined for growth.

2-5 Determination of minimum required concentration

Minimum required concen tration was determined by inoculating 0.2ml of standardized suspension of E. coli to 20g of test O/W emulsion systems containing a series of methyl paraben differed by 0.02%. After incubating at 35 °C, for 7 days, and the surviving cells were counted by viable count technique (13). The minimum required concentration was determined by taking the range between the lowest methyl paraben concentration to kill 99.9% of initial inoculum and the next higher concentration.

2-6 Equilibrium dialysis

The equilibrium dialysis technique was essentially the same in principle as that of Patel & Foss (14) with the exception that the membrane employed in our studies was Silicone rubber (Sialastic sheeting non-reinforced 0.01cm (0.005 in.) Dow Corning Corp.). Both chambers were stirred with a magnetic bar to obtain rapid equilibrium. The sample with high consistency was centrifuged, and the aqueous phase was used for dialysis.

3. RESULTS AND DISCUSSION

3-1 Antimicrobial activity of methyl paraben in ternary systems

It was found that antimicrobial activity of methyl paraben was affected by the addition of fatty alcohols in the ternary systems consisting of nonionic surfactant, fatty alcohols and water.

Fig. 1 shows the change of extinction time of E. coli in the ternary systems with 0.25% methyl paraben, 0.5% POE (20) sorbitan monostearate and various concentrations of fatty alcohols. As shown in Fig. 1, the addition of below 1% such fatty alcohols as myristyl, cetyl, stearyl and cetostearyl remarkably decreased the extinction time. The extinction time, however, was hardly changed in the case of the above 1% concentration. In case of lauryl alcohol, the extinction time was slightly decreased at 0.5%, but a drastic increase of extinction time was noted above 1%. Cetostearyl alcohol was proved to be the most effective in decreasing the extinction time among the tested fatty alcohols.

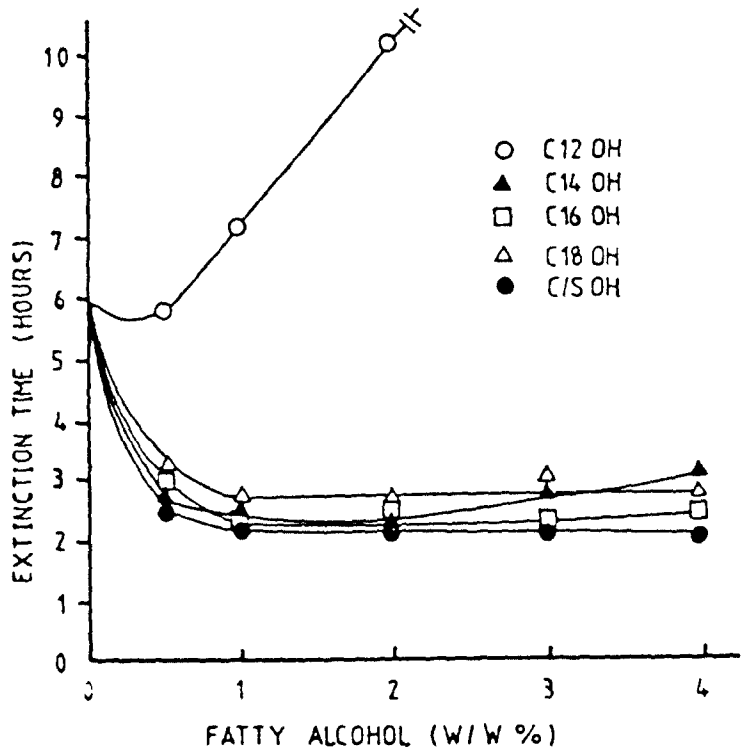


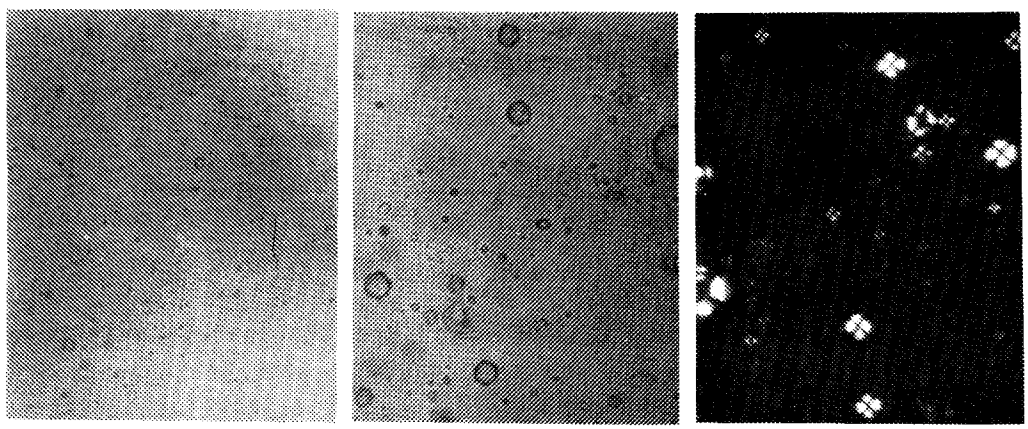
Fig. 1 Change of extinction time of E. coli in ternary systems containing 0.25% methyl paraben and 0.5% POE (20) sorbitan monostearate.

It is well known that fatty alcohols such as cetyl and cetostearyl alcohol increase the consistency by forming liquid crystal with surfactants. In order to ascertain the effects of consistency of ternary systems on antimicrobial activity, the extinction time under the same systems containing water soluble preservatives called imidazolidinyl urea, instead of methyl paraben, was determined. In case of imidazolidinyl urea, the extinction time was not changed by addition of fatty alcohols. It demonstrated that the change of consistency in tested systems from the addition of fatty alcohols has no relationship with the antimicrobial activity. Different from the imidazolidinyl urea, however, the antimicrobial activity of methyl paraben was changed according to the fatty alcohols addition as shown in Fig. 1. This results explained that the difference in methyl paraben antimicrobial activity is due to the interaction reduction between methyl paraben and nonionic surfactant by forming liquid crystal of fatty alcohol with surfactant.

A microscope under ordinary and polarized light was employed to certify the liquid crystal formation by fatty alcohols.

Microscope observation proved that fatty alcohols addition, except lauryl alcohol, could make liquid crystal.

Fig. 2 shows the photomicrographs of ternary systems with respective addition of 2% lauryl alcohol and cetostearyl alcohol. The fact that lauryl alcohol existing as droplets rather than liquid crystal in Fig. 2 well demonstrates the extinction time increased with the addition of lauryl alcohol. Since the large portion of methyl paraben was partitioned into lauryl alcohol droplets, the concentration of methyl paraben in water phase decreased accordingly.



A B C

Fig. 2. Photomicrographs of ternary systems containing 2% fatty alcohols and 0.5% POE (20) sorbitan monostearate (x 800).
A: Lauryl alcohol (ordinary light)
B: Cetostearyl alcohol (ordinary light)
C: Cetostearyl alcohol (polarized light)

Fig. 3 shows the change of extinction time of E. coli in ternary systems prepared with another nonionic surfactant, POE (10) cetyl ether, instead of POE (20) sorbitan monostearate. Similar results as in Fig. 1 were made. The only difference was the little longer extinction time than in Fig. 1. This indicates that the methyl paraben is more severely inactivated by POE (10) cetyl ether than POE (20) sorbitan monostearate.

Fig. 4 shows the extinction time change with cetostearyl alcohol addition under different concentration of POE (20) sorbitan monostearate. As expected, the higer concentration of POE (20) monostearate, the longer extinction time needed.

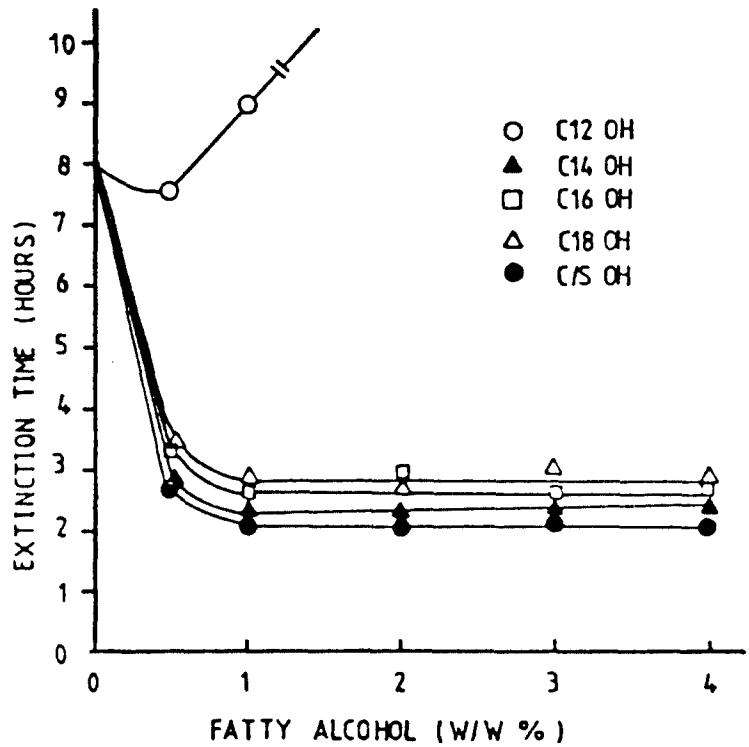


Fig. 3. Change of extinction time of E. coli in ternary systems containing 0.25% methyl paraben and 0.5% POE (10) cetyl ether.

Regardless of the POE (20) sorbitan monostearate concentration, extinction time was decreased by cetostearyl alcohol addition and nearly the same minimum extinction time was recorded under the more of less 2:1 ratio of cetostearyl alcohol and POE (20) sorbitan monostearate. This illustrated the liquid crystal formation of cetostearyl alcohol reduced methyl paraben inactivation by POE (20) sorbitan monostearate.

Fig. 5 and 6 show the variation of free methyl paraben determined by dialysis method in ternary systems prepared with 0.2% methyl paraben, various concentrations of fatty alcohols and 0.5% nonionic surfactants, POE (20)

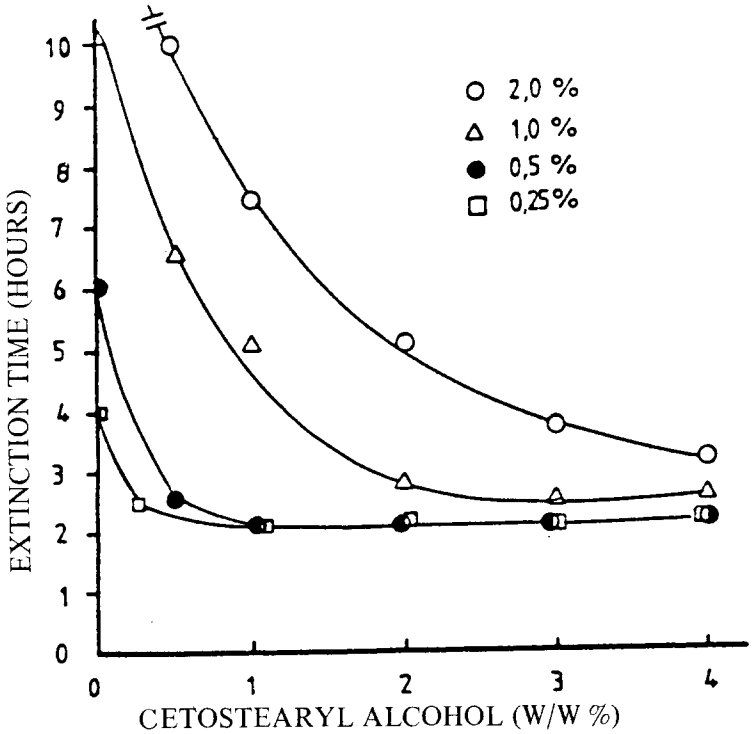


Fig. 4. Change of extinction time in ternary systems containing 0.25% methyl paraben and various concentration of POE (20) sorbitan monostearate.

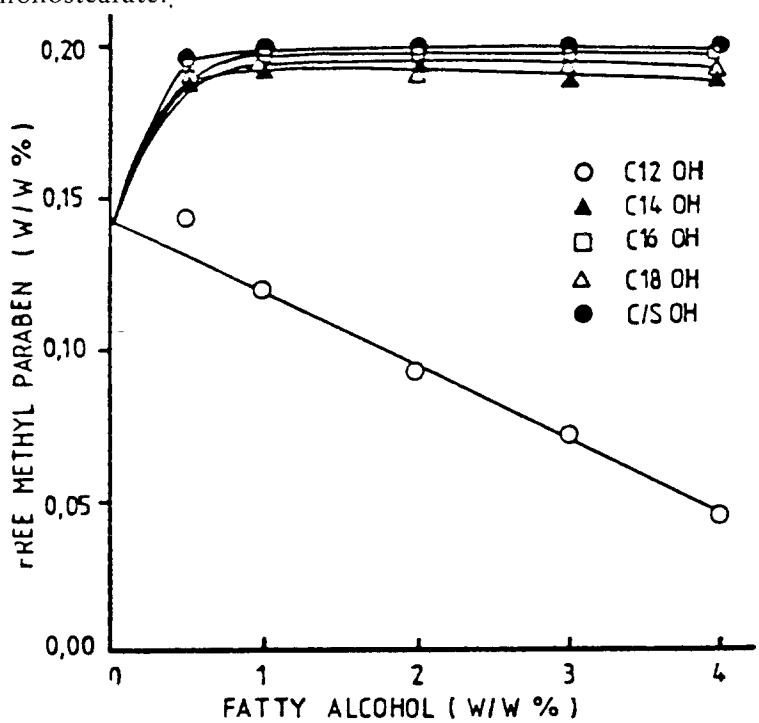


Fig. 5. Change of free methyl paraben quantity in ternary systems containing 0.2% methyl paraben and 0.5% POE (20) sorbitan monostearate.

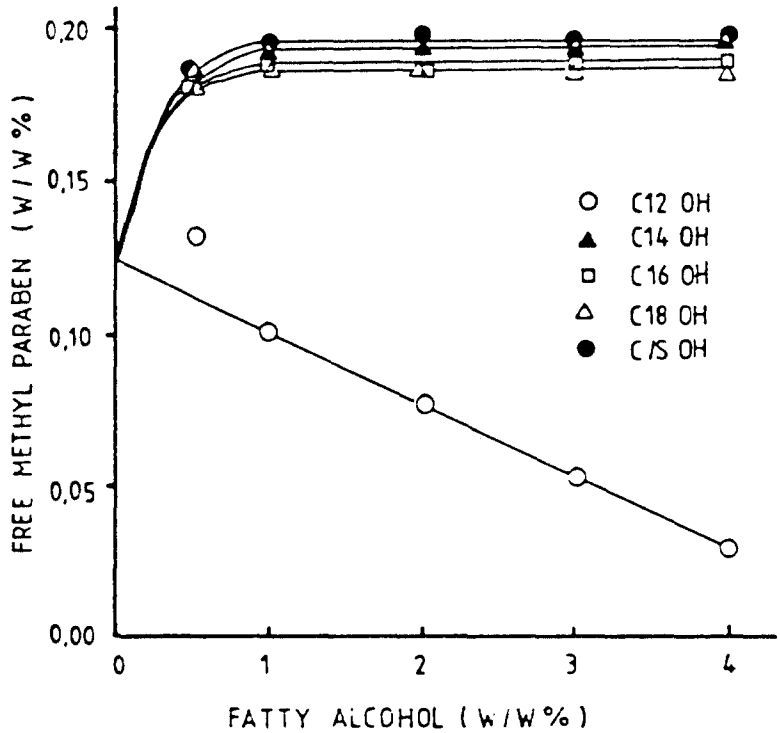


Fig. 6. Change of free methyl paraben quantity in ternary systems containing 0.2% methyl paraben and 0.5% POE (10) cetyl ether.

sorbitan monostearate and POE (10) cetyl ether, respectively. The free methyl paraben quantity increased sharply with the addition of 0.5% fatty alcohols such as myristyl, cetyl, stearyl and cetostearyl alcohol, and above 0.5% addition nearly all of the methyl paraben in tested ternary systems was existed in free state. In case of lauryl alcohol, the free methyl paraben quantity slightly increased at 0.5%, buy decreased linearly over 0.5%. These results agreed with the extinction time change in Fig. 1 and Fig. 4.

It is clearly established that the antimicrobial activity of methyl paraben is largely related to the free methyl paraben concentration, and the main reason for methyl paraben inactivation was the solubilization of methyl paraben into nonionic surfactant micelles. It, therefore, could be assumed that the free methyl paraben quantity is directly reated with the micelle quantity of nonionic surfactants, witch means the more free methyl paraben increase is the more micelle quantity decerase.

These results concluded that liquid crystal formation of fatty alcohols except lauryl alcohol affects the nonionic suravtants micelle quantity decrease and ultimately reduces the methyl paraben inactivation by nonionic surfactants.

3-2 Antimicrobial activity of methyl paraben in emulsion systems

The antimicrobial activity in simple system is sometimses not applied in actual formulation (16). Experiment was performed to investigate the same potentiating action of fatty alcohols in O/W emulsion systems with liquid paraffin in water emulsions as in ternary systems. The preservative aciveity in O/W emulsion is significantly influenced by such factors as type and concentration of surfactants (17), oil/water ratio (18) and the oil/water paration coefficient of preservatives (19).

To get the final preservative activity in O/W emulsion, only actual challenge test enables to determine the precise concentration of necessary preservatives and meet the desiable criteria (20).

Table 2 and 3 show the minimum required concentrations of methyl paraben for adequate preservation of liquid paraffin in water emulsions with 2% POE (20) sorbitan monostearate and 0.5% sorbitan monostearate combined, or POE (10) cetyl ether as surfactants, and various fatty alcohols with diffetent concentrations.

Table 2. Minimum required concentration (W/W%) of methyl paraben in O/W emulsion systems containing 2.0% sorbitan monostearate and o.5% sorbitan monostearate.

Fatty alcohols (W/W%)	0	1	2	3	4
Lauryl		0.24-0.26	0.28-0.30	0.28-0.30	0.30-0.32
Myristyl		0.24-0.26	0.22-0.24	0.22-0.24	0.20-0.22
Cetyl	0.22-0.24	0.24-0.26	0.20-0.22	0.18-0.20	0.14-0.16
Stearyl		0.24-0.26	0.22-0.24	0.18-0.20	0.16-0.18
Cetostearyl		0.22-0.24	0.18-0.20	0.16-0.18	0.12-0.14

Table 3. Minimum required concentration (W/W%) of methyl paraben in O/W emulsion systems containing 2.0% POE (10) cetyl ether.

Fatty alcohols (W/W%)	0	1	2	3	4
Lauryl		0.26-0.28	0.30-0.32	0.30-0.32	0.32<
Myristyl		0.28-0.30	0.26-0.28	0.28-0.30	0.26-0.28
Cetyl	0.26-0.28	0.28-0.30	0.24-0.26	0.20-0.22	0.14-0.16
Stearyl		0.26-0.28	0.26-0.28	0.22-0.24	0.16-0.18
Cetostearyl		0.26-0.28	0.24-0.26	0.20-0.22	0.14-0.16

As shown in Table 2 and 3, the minimum required concentrations of methyl paaben in tested emulsions did not change in presence of any fatty alcohol of concentration 1.0%. The minimum required concentrations of methyl paraben, however, were decreased with the increasing concentration of cetyl, stearyl and cetostearyl alcohol over 2% without remarkable difference. When 4% of such fatty alcohols was added to the tested emulsions, the minimum required methyl paraben concentrations were nearly in half than the same emulsion without fatty alcohol. Lauryl alcohol, on the other hand, has an adverse effect on minimum required concentration of methyl paraben with the increasing concentration.

But, the higher concentration of myristyl alcohol did not affect the antimicrobial activity of tested emulsion systems.

To elucidate the antimicrobial enhancement effect from fatty alcohol addition, free methyl paraben concentrations were measured in emulsion systems by equilibrium dialysis technique. Fig. 7 and 8 display the free methyl paraben change in the same emulsion systems as used in challenge test.

In both emulsion systems, about 1% of fatty alcohol addition makes slight decrease of free methyl paraben and further addition makes the free methyl paraben concentration higher. As fatty alcohol increase in concentration, free methyl paraben tends to increase in quantity accordingly.

Only exceptions are lauryl alcohol with decreasing tendency, and myristyl alcohol with no liberation effect of free methyl paraben.

Under the microscope, liquid crystalline phase formation could be observed (Fig. 9) at the same emulsions used in challenge test with over 2% of myristyl,

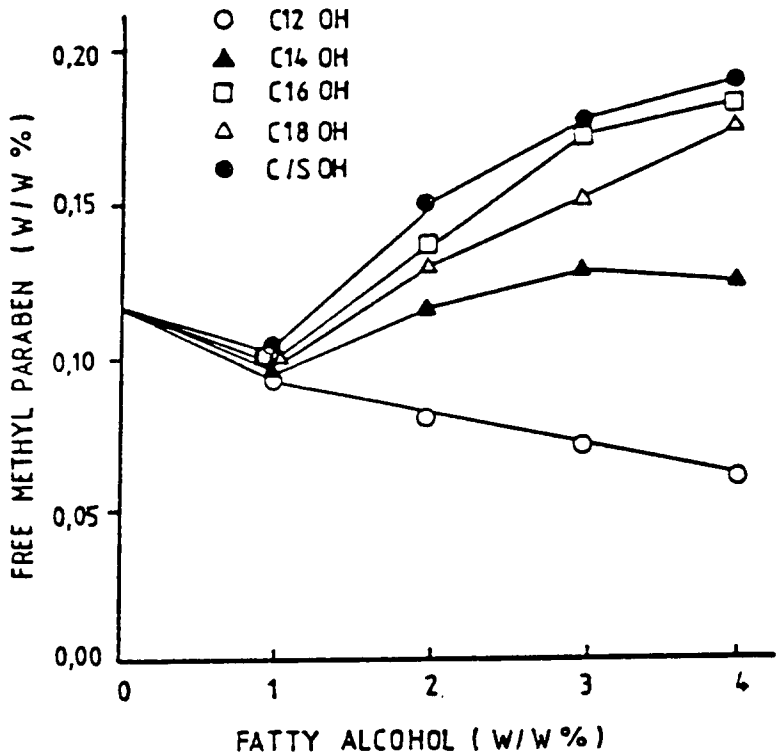


Fig. 7. Change of free methyl paraben quantity in O/W emulsion systems containing 0.2% methyl paraben, 2.0% methyl paraben, 2.0% POE (20) sorbitan monostearate and 0.5% sorbitan monostearate.

cetyl, stearyl and cetostearyl alcohol.

But with lauryl alcohol, liquid crystalline phase could not observed. These results agreed with the challenge test data in Table 2 and 3. These also support the assumption of liquid crystalline phase formation, which decreases the nonionic surfactant micelle quantity and increases the free methyl paraben quantity in tested systems.

The necessary concentrations of fatty alcohols to enhance the antimicrobial activity in emulsion systems were much higher than those in ternary systems. Despite the below 1% fatty alcohols for remarkable enhancement of ternary systems, emulsion systems required above 1% of fatty alcohols to exert the same antimicrobial effect.

The difference of fatty alcohol requirement between ternary and emulsion systems is due to the minimum quantity variance of fatty alcohol in order to

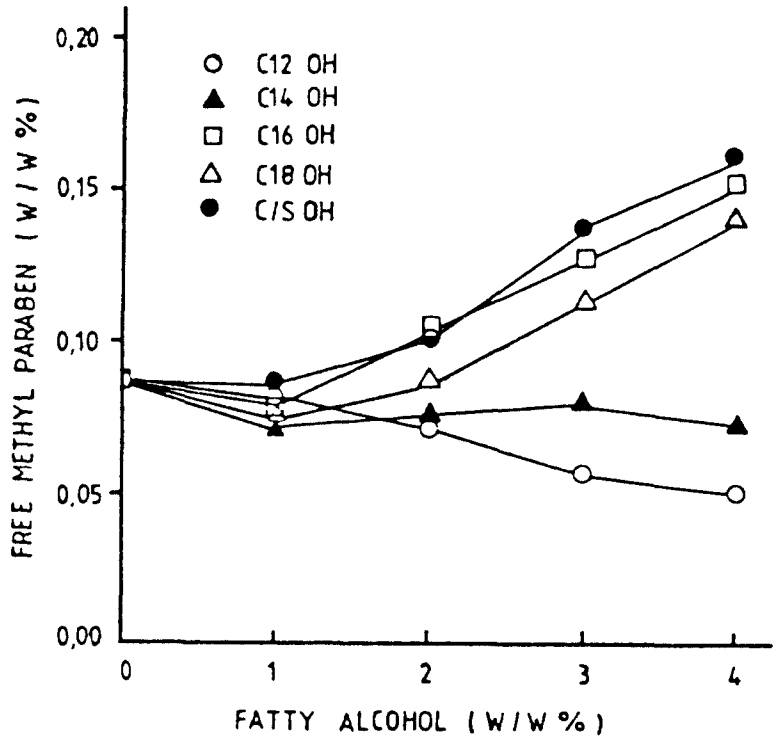
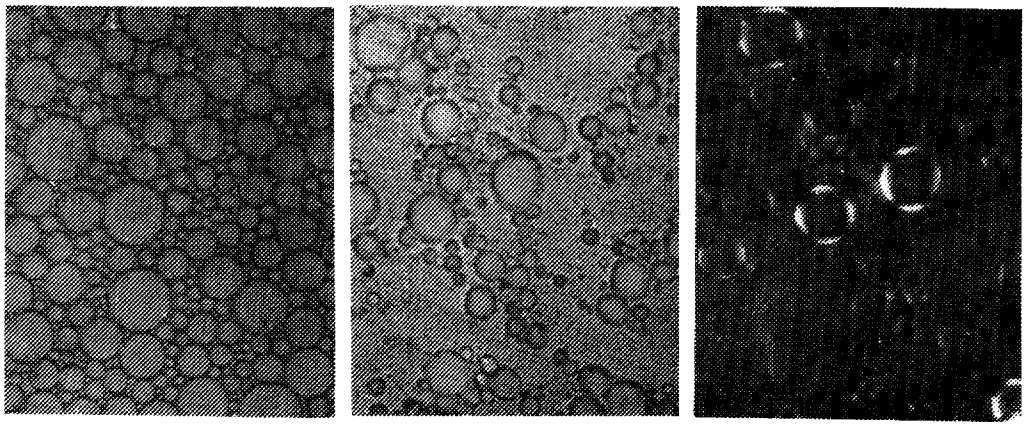


Fig. 8. Change of free methyl paraben quantity in O/W emulsion systems containing 0.2% methyl paraben and 2.0% POE (10) cetyl ether.

form liquid crystal. In emulsion systems, sizable quantity of fatty alcohol added was firstly dissolved in oil phase or existed at the oil/water interface without forming liquid crystalline phase with nonionic surfactants. If the concentration of fatty alcohol goes over the saturation level, the liquid crystal began to form. The adverse effect of lauryl alcohol could be explained by its high solubility in liquid paraffin and its inability to form liquid crystal. As lauryl alcohol is miscible in all proportion with liquid paraffin (21), which increases the oil/water ratio and partition coefficient, eventually the methyl paraben would be partitioned in favor of the oil phase and decreases the antimicrobial activity of O/W emulsion systems.

The negligible difference of methyl paraben activity by myristyl alcohol addition was supposed to be from its solubility in liquid paraffin, which causes the appreciable quantity of methyl paraben partitioned into oil phase together with myristyl alcohol, and helps offset the increasing availability of methyl paraben with liquid crystal formation.



A B C

Fig. 9. Photomicrographs of emulsion systems containing 4% fatty alcohols, 2.0% POE (20) sorbitan monostearate and 0.5% sorbitan monostearate (x 800).
A: Lauryl alcohol (ordinary light)
B: Cetostearyl alcohol (ordinary light)
C: Cetostearyl alcohol (polarized light)

However, the other fatty alcohols such as cetyl, stearyl and cetostearyl alcohol forming the liquid crystalline phase with nonionic surfactants in emulsion enabled to prevent inactivation of preservatives due to nonionic surfactants, and strengthened the antimicrobial activity.

The effects of fatty alcohol on preservation of O/W emulsion can be considered into two aspects. One is the partition coefficient. The fatty alcohol with high partition coefficient seems to cause adverse effect on the preservative activity in O/W emulsion. The other can be called liquid crystal formation. It is explained that the fatty alcohol with lower solubility in oil phase and higher tendency to form liquid crystal causes potentiating effect on the preservative activity in O/W emulsion systems.

It could also be expected that the other fatty amphiphilic materials having the liquid crystal formation tendency with nonionic surfactants prevent the inactivation of preservatives and afford the antimicrobial activity enhancement in each system.

Reviewing all results, it could be concluded that the liquid crystalline phase formation reduced the nonionic surfactants micelles quantity and increased

free methyl paraben, which enhanced the antimicrobial activity of methyl paraben. Under these conditions, the emulsion products with excellent preservative activity and stability could be formulated by liquid crystalline phase formation using appropriate quantity of fatty alcohol, especially cetostearyl alcohol.

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