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ANTIFUNGAL ACTIVITY OF UNDECYLENIC ACID EMULSIONS BY MICROBIOLOGICAL METHODS

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A B S T R A C T

Two nonionic surfactants (Simulsol 98 and Simulsol OL 50) alone and I:I mixtures, corn oil-undecylenic acid and water formed emulsions, oily isotropic liquid phases, lamellar and hexagonal liquid crystal phases. The optimum release of undecylenic acid from these phases were controlled microbiologically. The active ingredient undecylenic acid, is released more from emulsion systems containing liquid crystals than only liquid crystalline and oily isotropic liquid phases.

I N T R O D U C T I O N

The emulsion systems prepared from corn oil-undecylenic acid-water and two nonionic emulsifiers (Simulsol 98 and Simulsol OL 50) were compared with the other regions observed in the phase diagram by microbiological method.

The results of this study were reported at the VII.Science Congress of the Scientific and Technological Research Council (TÜBİTAK),September, 1980, Ankara, Turkey.

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The aim of this investigation was, to find the area which optimum releases the active ingredient; undecylenic acid, an antifungal agent. The results were discussed in terms of the effect of the physical properties of an area (in this case, oily isotropic liquid, emulsion plus liquid crystalline and only liquid crystalline areas) on the release of undecylenic acid by microbiological determination.

EXPERIMENTAL

Materials

Simulsol OL 50 : Castor oil - $(O\text{-}CH_2\text{-}CH_2)_{40}$

Simulsol 98 : Oleyl alcohol - $(O\text{-}CH_2\text{-}CH_2)_{20}$

Ethoxylated castor oil and ethoxylated oleyl alcohol have been used as received.

Corn oil : (Adayar Ltd, Turkey, T.S. 888) was used as received. Corn oil has a spesific gravity of 0.9170 and refractive index 1.4735 at 25°C. Acid, saponification and iod indices were found to be 0.22, 190.0 and 120.75, respectively. IR and UV spectra were also taken.

Undecylenic acid : (Sigma Chem. Co. England) Liquid form was used as received. The structure is,

$$CH_2 = CH(CH_2)_8COOH$$

It is not soluble in water. It is already miscible with chloroform, alcohol, ether, benzene and volatile and non-volatile oils. Its specific gravity and refrective index at 25°C were 0.9102 and 1.4486 respectively.

Distilled water: Deionized and distilled in glass vessels and has a surface tension of 72.6 mNm^{-1} at 20°C.

Acetone : Used as received

Sabourraud dextrose agar : Prepared in the laboratory.

Sabourraud dextrose broth : Prepared in the laboratory.

Trycophyton rubrum : provided from Hacettepe University, Institute of Microbiology.

Method

The microbiological method used for determining the antifungal activity was a modified version of the method used by Patel et al.(1). Here, petri dishes containing Sabourraud dextrose agar were inoculated with a culture of Trycophyton rubrum. The inoculated plates were inculated at ambient room temperature of 20-26°C for one month.

Materials tested for antifungal activity included four regions of the triangular phase diagram (Figure 1). These systems were applicated in two ways; one after storing overnight at 37°C ± 1°C thermostated water bath and the other after storing for two months at 37°C ± 1°C water bath. These regions were, oily isotropic liquid, emulsion with lamellar and hexagonal phase liquid crystals and only lamellar phase liquid crystalline areas on the triangular phase diagram.

Plugs of agar containing mycelial growth were cut out using a sterile transfer loop with an outside diameter of 0,6 cm and an inside diameter of 0,5 cm. Each plug was immediately transferred to a test tube containing 5 ml (% 5 concentration of the compound) material. Test tube was agitated by vortexing for two minutes and the plug was transferred to a test tube containing 10 ml sterile sabourraud dextrose broth. This tube was agitated slightly for a period of three minutes to free the mycelia of any soluble or miscible material the plug was then transferred to a test tube containing 10 ml of a 30 % aqueous acetone solution where it was kept for 5 minutes to remove any traces of adhering antifungal compound. The plug was transferred to a test tube of sterile Sabourraud dextrose broth for a two minutes period in order to remove any traces of acetone. Finally this plug was removed from the broth and placed culture side down on the surface of a sterile slant of Sabourraud dextrose agar contained in a test tube. The inoculated slants were incubated at a temperature of 28°C for 13 days. Ten tests for each of the system were run. Controls (systems not containing antifungal agent) were run along with each region being tested.

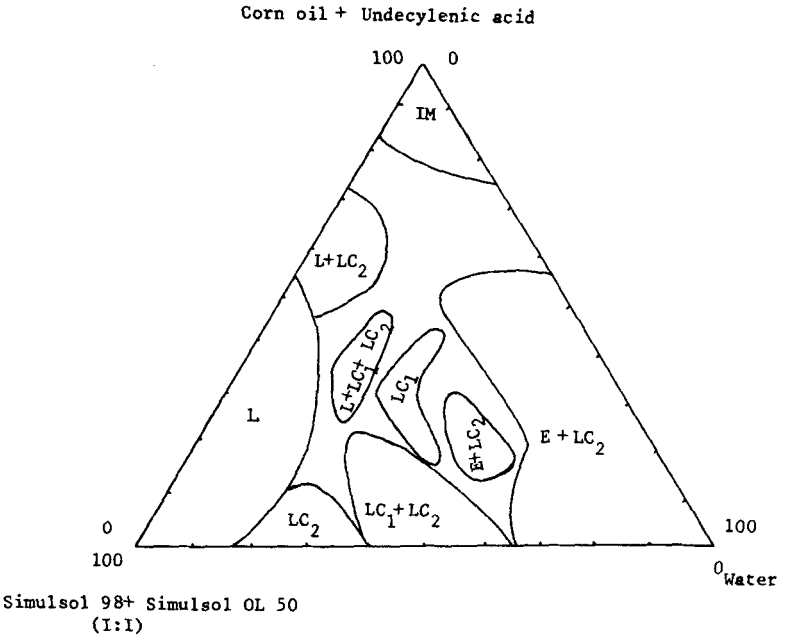

FIGURE 1.

Phase equilibrium diagram of Simulsol OL 50 and Simulsol 98 (I:I)-water-corn oil and undecylenic acid at 37°C.

L- Oily isotropic liquid phase

LC_1- Hexagonal liquid crystalline phase alone.

LC_2- Lamellar liquid crystalline phase alone

$L + LC_2$- Oily isotropic liquid phase plus lamellar liquid crystalline phase.

$L + LC_1 + LC_2$- Oily isotropic liquid phase plus hexagonal and lamellar liquid crystalline phase.

$E + LC_2$- Emulsion plus lamellar liquid crystalline phase

IM - Insoluble Material.

Since the aim of this study was to investigate o/w type of undecylenic acid emulsions, primarily emulsion regions, observed in the phase equilibrium diagrams, were chosen (Fig.1). Emulsion plus liquid crystalline phase were also investigated because the emulsions were not observed alone in the phase equilibrium diagrams. The release of undecylenic acid from liquid crystals and oily isotropic phases were investigated to compare its release from emulsion systems. The samples stored for overnight and the samples stored for two months, were studied to investigate the effect of storage time on the release of undecylenic acid.

RESULTS

1. Simulsol OL 50

The compositions of the systems obtained by Simulsol OL 50-Corn oil-Undecylenic acid and water shown in Table 1. These systems which are investigated microbiologically are equilibrated at 37°C. The microbiological test results of samples stored overnight and 2 months are shown in Table 2.

TABLE 1

The Compositions of Systems Prepared by Simulsol OL 50

Systems	Simulsol OL 50(%)	Corn oil (%)	Undecylenic acid (%)	Dist.water
L	60	15	5	20
$E + LC_2$	10	25	5	60
LC_2	60	5	5	30

L - Oily isotropic liquid phase

$E + LC_2$ - Emulsion plus lamellar liquid crystalline phase.

LC_2 - Lamellar liquid crystalline phase alone.

TABLE 2

Results of Fungicial Testing Using Simulsol OL 50

Systems	Number of tubes exhibiting growth after 13 days	
	Stored Overnight	Stored 2 months
L	4	9
E + LC_2	1	7
LC_2	10	7

L - Oily isotropic liquid phase

E + LC_2 - Emulsion plus lamellar liquid crystalline phase.

LC_2 - Lamellar liquid crystalline phase alone.

TABLE 3

Compositions of The Systems Prepared by Simulsol 98

Systems	Simulsol 98 (%)	Corn oil (%)	Undecylenic acid (%)	Dist.water (%)
L	60	25	5	10
E + LC_1	20	15	5	60
E + LC_2	10	25	5	60
LC_2	70	5	5	20

L - Oily isotropic liquid phase

E + LC_1 - Emulsion plus hexagonal liquid crystalline phase

E + LC_2 - Emulsion plus lamellar liquid crystalline phase

LC_2 - Lamellar liquid crystalline phase alone.

TABLE 4

Results of Fungicidal Testing Using Simulsol 98

Systems	Number of tubes exhibiting growth after 13 days	
	Stored overnight	Stored 2 months
L	10	10
E + LC_1	8	N.G.
E + LC_2	N.G.	N.G.
LC_2	10	7

N.G.: No Growth

L - Oily isotropic liquid phase

E + LC_1 - Emulsion plus hexagonal liquid crystalline phase

E + LC_2 - Emulsion plus lamellar liquid crystalline phase

LC_2 - Lamellar liquid crystalline phase alone.

TABLE 5

Compositions of The Systems Investigated Microbiologically

Systems	Simulsol OL 50: Simulsol 98 I:I (%)	Corn oil(%)	Undecylenic acid (%)	Dist.Water (%)
L	70	15	5	10
E + LC_1	50	15	5	30
E + LC_2	10	25	5	60
LC_2	60	5	5	30

L - Oily isotropic liquid phase

E + LC_1 - Emulsion plus hexagonal liquid crystalline phase

E + LC_2 - Emulsion plus lamellar liquid crystalline phase

LC_2 - Lamellar liquid crystalline phase alone.

TABLE 6

Results of Fungicidal Testing

Systems	Number of tubes exhibiting growth after 13 days	
	Stored overnight	Stored 2 months
L	N.G.	6
$E + LC_1$	N.G.	N.G.
$E + LC_2$	N.G.	N.G.
LC_2	1	1

N.G.: No Growth.

L - Oily isotropic liquid phase

$E + LC_1$ - Emulsion plus hexagonal liquid crystalline phase

$E + LC_2$ - Emulsion plus lamellar liquid crystalline phase

LC_2 - Lamellar liquid crystalline phase alone.

2. Simulsol 98

Test compositions and the microbiological test results of the systems obtained using Simulsol 98 are shown in Tables 3 and 4 respectively.

3. Simulsol OL 50: Simulsol 98 (I:I)

The compositions and the microbiological test results of the systems obtained using I:I mixtures of Simulsol OL 50 and Simulsol 98 are shown in Tables 5 and 6 respectively.

DISCUSSION

In an oil/water emulsion, the factor limiting the release velocity of an oil soluble drug in its release velocity from the oil. The structure of the interfacial film through which the substance has to diffuse has also an influence on the release velocity of the oil soluble drug (2).

The surfactant molecules by their amphiphilic structures effect the drug activity (3). In general, the higher surfactant concentrations have a tendency to reduce the release of the active ingredient (4), whereas the lower surfactant concentrations often favour the release. In this investigation, the release of undecylenic acid, when surfactants were used alone and/or 1:1 mixture, was also reduced at higher surfactant concentrations, as shown in Tables 2,4 and 6.

This was also seen in emulsion systems containing two types of liquid crystals hexagonal and lamellar phases. The release of undecylenic acid was reduced in hexagonal phase liquid crystals where the surfactant concentration was high. The reduction in the release characteristics, could be releated to the entrapment of the active ingredient in the micelles above the CMC (4,5).

When emulsion, isotropic and liquid crystalline regions in the phase diagram were compared, it was found that the release characteristics were increased in the emulsion systems than the isotropic and only liquid crystalline phases. This could also be related to the increase in the surfactant concentration and to the existance of ordered paracrystalline regions.

This relationship could be supported by Friberg's investigations (6).

Less growth was observed in system consisting of emulsion plus liquid crystalline phases than the other regions in the phase diagram (Tables 2,4,6). This may be due to the more flexible and fluid characteristics of liquid crystals when they are in the emulsion system, and also to the low surfactant concentration.

Surfactants showed better results when they were used as I:I mixtures. They could release the active ingredient more easily because of the flexible structure, that could be established by the different ethylene oxide unit (Simulsol OL-50, 40 EO; Simulsol 98, 20 EO) they contain.

When the effect of storage on the release of undecylenic acid from different phases were investigated it was shown that the release was

decreased in the only liquid crystalline phases. This decrease in the release characteristics could be related to the entrapment of the active ingredient inside the micellar structures. The increase in the release could be due to the change in the arrangement of the ordered structures.

According to the in vitro microbiological results, newly prepared and 2 months stored emulsion systems consisting of 10 % I:I Simulsol OL-50 and Simulsol 98, 25 % corn oil, 5 % undecylenic acid and 60 % distilled water, performed the antifungal activity. This needs to be supported by in vivo investigations.

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