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PHYTOSTEROL STABILIZED EMULSIONS: INTERFACIAL COMPLEXATION AND STRUCTURAL INVESTIGATIONS

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ABSTRACT

Mechanisms operative in phytosterol (Generol 122®) stabilized oil-in-water emulsions have been described by studying the behavior of Generol 122® in the presence of an amphoteric surfactant, Deriphat 160C® (sodium lauriminodipropionate). Interfacial intermolecular complexation of Generol 122® as cosurfactant with the amphoteric Deriphat 160C®, has been proposed from NMR and IR studies. Such association complexes appear to form liquid crystalline phases at the oil-water interfaces. A ternary phase

diagram of Generol 122®:Deriphat 160C®:water was constructed and the presence of various liquid crystalline phases has been demonstrated.

INTRODUCTION

Oil-in-water emulsions are widely used in pharmaceuticals and cosmetics and are generally stabilized with hydrophilic surfactants in combination with hydrophobic substances such as fatty alcohols or cholesterol. Complexation between two or more emulsifiers or the presence of a cosurfactant tend to increase emulsion stability as compared to the effect of one of the emulsifiers alone at a similar concentration (1). Ternary systems in which surface active substances associate into micelles and liquid crystalline phase have been widely investigated (1-3).

Friberg, et al. (4, 5) have discussed the contribution of liquid crystals to emulsion stability on the basis of changes in Van der Waals energy of interaction. The results suggested that the energy of coalescence was reduced by the adsorbed liquid crystalline phase which functions as a "mechanical" barrier to coalescence.

Previous studies on formulations with phytosterols (especially Generol 122®), which are structurally similar to cholesterol, produced unusually stable emulsions particularly in the presence of the amphoteric surfactant, Deriphat 160C® (sodium lauriminodipropionate) (6-8). Several alternatives for the mechanism of the stabilization can be proposed: electrostatic bonding due to the zwitterionic nature of Deriphat 160C®, hydrogen bonding due to the presence of

3β-OH groups in Generol 122® and -COOH groups in Deriphat 160C® molecules, and lastly hydrophobic bonding due to the long hydrocarbon chains present in both compounds. The objective of the present study was to determine the orientation of molecules in the interfacial phase and to identify interacting groups responsible for emulsion stability.

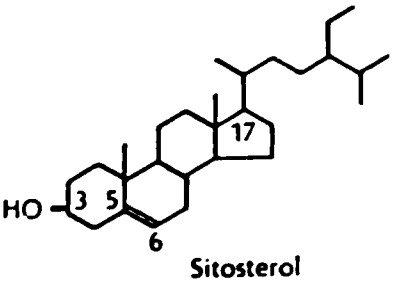
EXPERIMENTAL

Materials

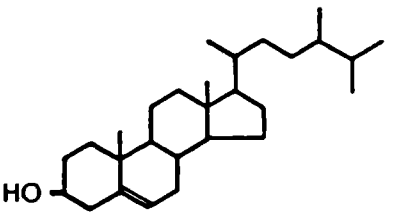
Phytosterol (Generol 122®) and sodium lauriminodipropionate (Deriphat 160C®) were used as received from Cosmedia, Henkel Corporation, Minneapolis. Generol 122®, which is derived from soybean oil is about 55% sitosterol with lesser amounts of campesterol and stigmasterol and minor amounts of associated plant sterols. The chemical structures of Generol 122® and Deriphat 160C® are shown in Figure 1. Water was distilled and then treated in a Millipore Milli-Q2® system.

Methods

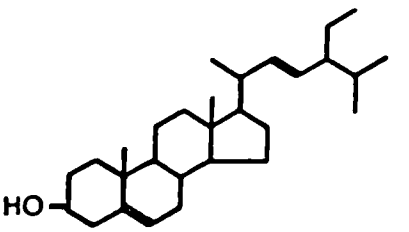
Nuclear Magnetic Resonance Spectroscopy: A Brucker Model HX-90E NMR spectrometer was used to obtain proton magnetic resonance spectra. The solvents were $CDCl_3$ (100.0 atom % D; Aldrich Chemical Co.) and D_2O (99.8 atom % D; Sigma Chemical Co.). Tetramethylsilane (TMS) was used as the internal spectral reference standard. In order to eliminate any shifts due to temperature fluctuations, spectra were taken as rapidly as possible. Fourier transform technique increased the intensity of the signals which were then analyzed by a microcomputer.



Sitosterol

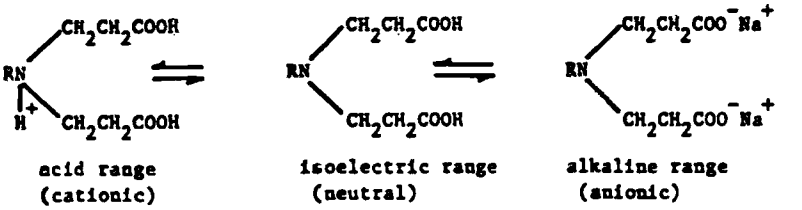


Campesterol



Stigmasterol

A



B

Figure 1: Structures of the main constitutents of Generol 122® (A); and Deriphat 160C® (B), the R of which is a fatty alkyl group derived from lauric acid.

Infrared Spectroscopy: A Beckman spectrophotometer, Model IR 4230, operated at a scanning speed of 600 cm^{-1}/min was used. The samples were triturated with a drop of Nujol prior to their application to sodium chloride crystals.

Phase Equilibria Studies: Ternary systems, consisting of different ratios of Generol 122®, Deriphat 160C® and water were heated and agitated to a homogeneous solution. They were then cooled to room temperature and equilibrated for 24 hours prior to the study. Rosevear classification (9) by polarized light microscopy (Leitz Labolux microscope) was used to identify different types of phases. The fine structure of the various regions of the phase diagram was determined with a Zeiss EM 9S electron microscope using the freeze-fracture electron microscopy technique developed by Moor, et al. (10). Particle size was determined with an Elzone microcomputerized 128 channel particle size analyzer (Model 80 XY-ACD, Particle Data, Inc.) which utilizes the electrozone method. For x-ray diffraction studies, an x-ray generator (Philips PW 1730) equipped with a copper-nickel filter and a Kratky-compact camera system (KCLC, Paer/Philips) was used. The samples were held in an adjustable capillary sample holder, type K-PR, with temperature controller, under an atmosphere of argon/methane in the ratio 90:10. The instrument was operated at 60 KV and 54 mA current.

RESULTS AND DISCUSSION

Nuclear Magnetic Resonance Spectroscopy

In NMR spectra of a Generol 122® solution in $CDCl_3$ 3β-OH peak is observed at 1.55 ppm. This peak was

confirmed by adding a drop of D_2O to the sample when the -OH peak disappeared.

When Deriphat 160C® and Generol 122® were mixed in a 1:1 w/w ratio in $CDCl_3$, a suspension formed. The spectra of this system is shown in Figure 2. The peak at 1.55 ppm due to the 3β-OH group, as observed in Generol 122® spectrum in $CDCl_3$, has apparently "interacted" with the -COOH group of the Deriphat 160C® to give a broad singlet at 3.06 ppm. This peak disappeared after addition of a drop of D_2O. Hansen (11) also observed a single hydroxyl resonance due to rapid exchange of hydroxyl at the interface resulting in a single averaged chemical shift.

As observed from the computer tabulated intensities of peaks, the multiplet between 3.47 ppm and 3.61 ppm is due to -H (the presence of the multiplet at 3.47 ppm in the spectra of Generol 122 in $CDCl_3$ was assigned to -H) present in the 3β-OH group of Generol 122®. This -H causes a heavy shielding effect which would inhibit -COOH bonding with 3β-OH.

Infrared Spectroscopy

Generol 122® had peaks at 1060 cm^{-1} due to the C-O stretch and at 3400 cm^{-1} resulting from the absorption band of intramolecular hydrogen bonding due to the presence of the 3β-OH groups (Figure 3). The spectrum of Deriphat 160C® (Figure 4) has a sharp peak at 1605 cm^{-1} due to $(C=O)_2$ and a peak at 1670 cm^{-1} due to the C=O stretch of the -COOH groups. The peak at 3400 cm^{-1} can be assigned to the unassociated -OH of the -COOH moiety. Saturated solutions of Generol 122® and Deriphat 160C® in chloroform were mixed to form a precipitate which was freeze-dried and scanned by IR.

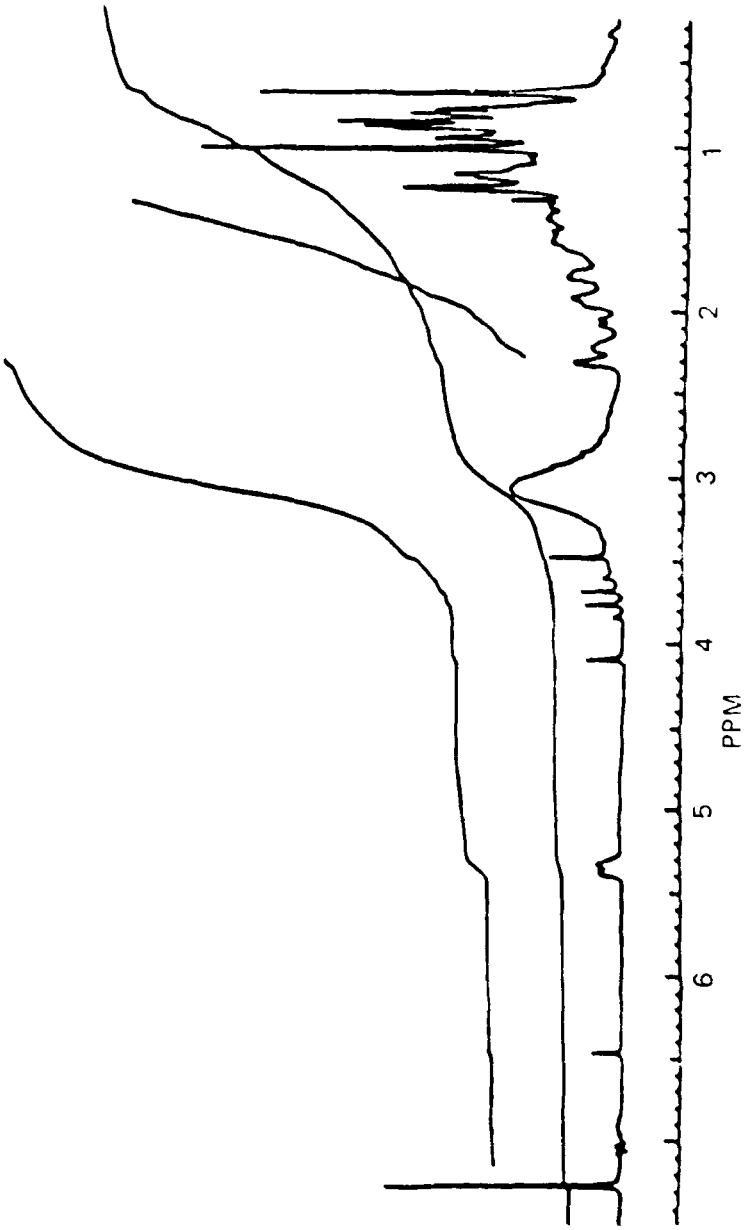


Figure 2: Proton magnetic resonance spectrum at 90 mc/s of a 1:1 w/w Deriphat 160C®:Generol 122® mixture in $CDCl_3$ taken immediately after sample preparation.

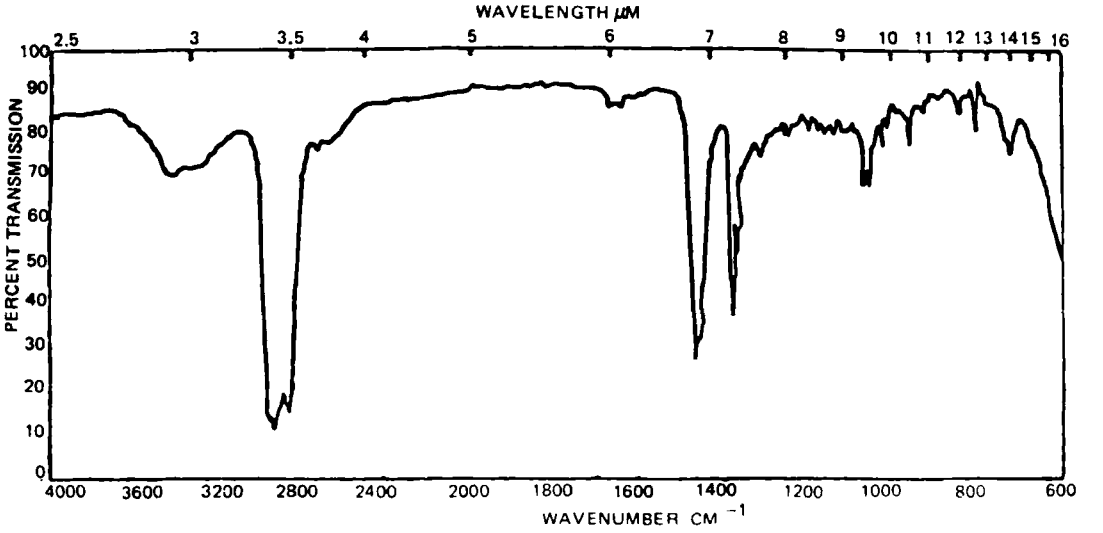


Figure 3: Infrared spectrum of Generol 122® in Nujol.

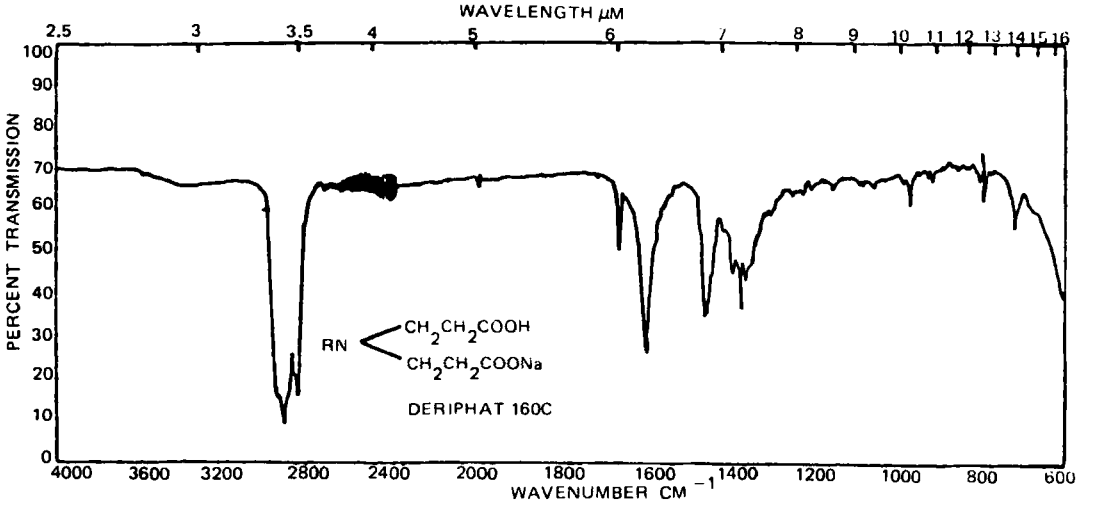


Figure 4: Infrared spectrum of Deriphat 160C® in Nujol.

The spectrum (Figure 5) shows a peak at 3200 cm^{-1} which is assigned to intermolecular hydrogen bonding. This shift in the appearance of the absorption band suggests certain structural changes. The peak at 3400 cm^{-1}, due to the original intramolecular and unassociated 3β-OH, is still present because there is an excess of Generol 122® which is unassociated with Deriphat 160C®. The peaks at 1607 cm^{-1} and 1605 cm^{-1} in the Deriphat 160C® spectrum (Figure 4) caused by -C=O and $(C=O)_2$ have shifted to 1640 cm^{-1} and 1575 cm^{-1} respectively. It is important to note that the shifts in this system are of same magnitude, each one corresponding to 30 cm^{-1} which is indicative of the nature and position of the "complex" formed. Furthermore, the C-O stretch of the C-OH group in the Generol spectrum (Figure 3) originally at 1060 cm^{-1}, shifted to 1045 cm^{-1} (Figure 5) apparently due to resonance and induction changes in the bond order of the C-O group. This change in the bond order decreases due to the bulky nature of the association between Generol 122® and Deriphat 160C® which is probably responsible for causing shifts in wavelength to lower values.

Hydrogen bond energies usually range from 4-8 kcal/mole. Since carbonyl oxygen, C=O, is a strong hydrogen bond acceptor, Generol 122®-Deriphat 160C® hydrogen bonding will be favored over Generol 122®-Generol 122® bonding. A relevant literature example of this is cholesterol-triglyceride bonding which is favored strongly over cholesterol-cholesterol bonding (12). It must be assumed also that the C=O groups of the Deriphat 160C® form hydrogen bonds with water since they are accessible to water and no other hydrogen donors are available. In addition, the partial

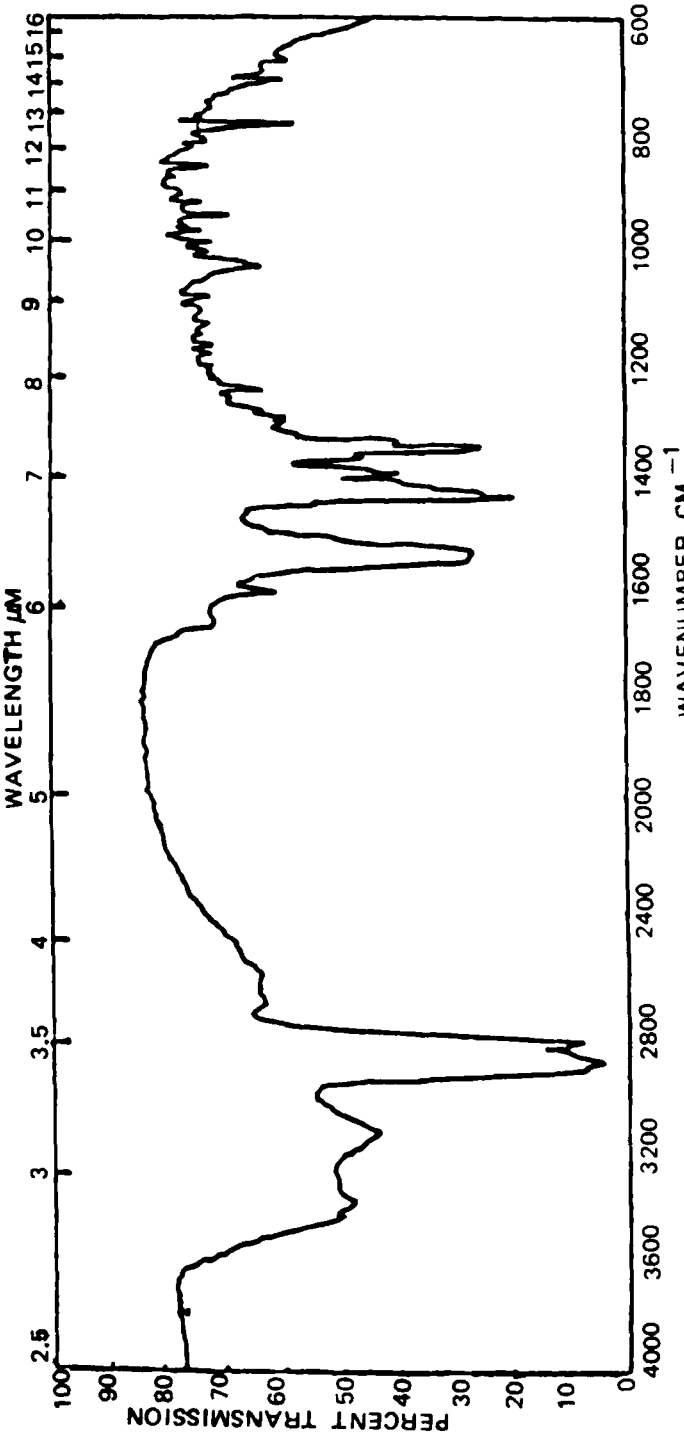


Figure 5: Infrared spectrum of Deriphat 160C® and Generol 122® complex in Nujol.

negative charge on carbonyl groups, due to the zwitterionic nature of Deriphat 160C®, makes them better hydrogen bond acceptors.

Based on these reasons and experimental observations, a hypothetical alignment of Deriphat 160C®, Generol 122® and water is proposed as shown in Figure 6. The possibility of a strong Generol 122®-Deriphat 160C® hydrogen bond does not by itself guarantee its existence and thus may not be necessarily the sole reason for the stability of emulsions formed with these surfactants. The C-O...H-O bond in Figure 6 has to compete with C-O...H_2O hydrogen bond. These results indicate that the carbonyl groups, as well as 3β-hydroxyl groups, must participate in some form of hydrogen bonding, and that they are sterically and energetically in a position to bind to each other at an oil-in-water interface. In addition, water molecules may undergo hydrogen bonding to sterol oxygen while the sterol is donating its hydroxyl proton to a neighboring Deriphat 160C® carbonyl group. This would increase the amount of bound water. However, addition of the phytosterol to the Deriphat 160C® should release some bound water molecules at the site of carbonyl oxygens, resulting in dehydration.

Phase Equilibrium of Ternary Systems:

A ternary phase diagram of Generol 122®:Deriphat 160C®:water is shown in Figure 7. A minimum of 45% water is necessary to form an emulsion. At low concentrations of water, the existence of a middle phase was observed. The phase diagram shows emulsion plus neat phase (A), neat phase (B), viscous neat phase (C), and middle phase (D) regions. The presence of the

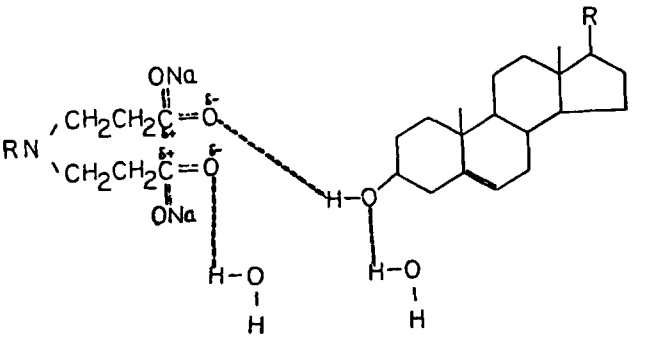


Figure 6: Hypothetical alignment of Generol 122®, Deriphat 160C®, and water at an oil/water interface.

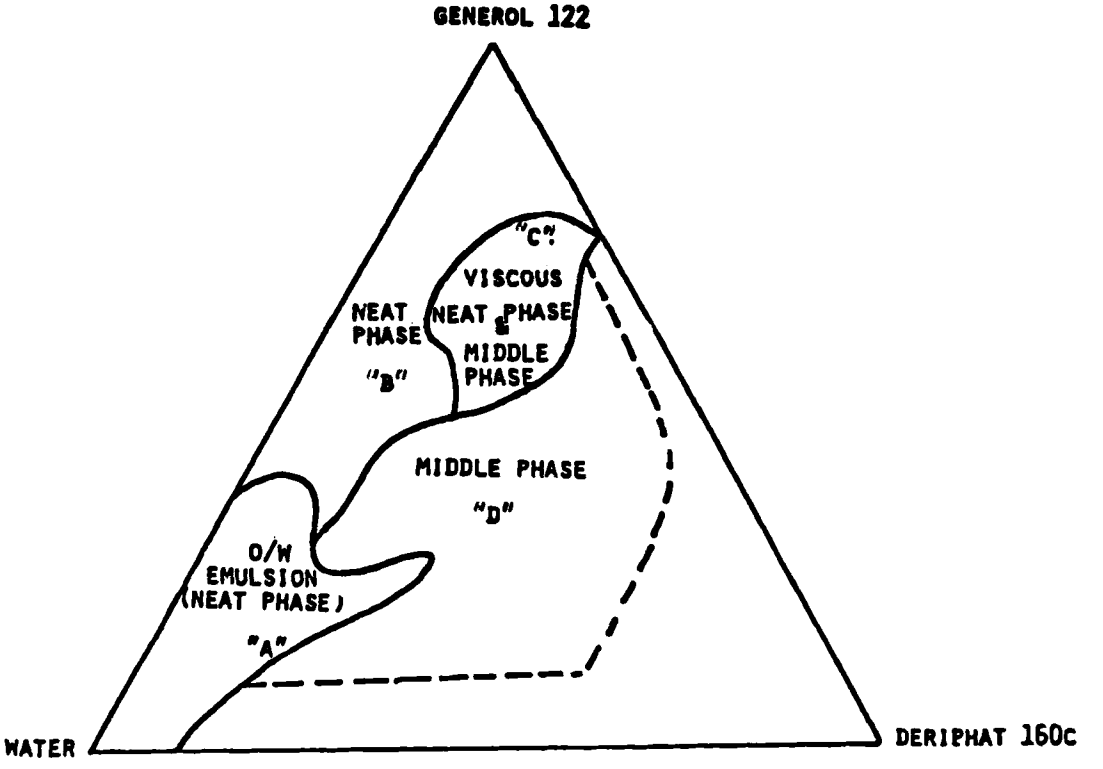


Figure 7: Phase diagram of Generol 122®, Deriphat 160C® and water systems (25°C).

neat phase in the region "A" was confirmed by small-angle x-ray diffraction measurements. Systems of Generol 122®:Deriphat 160C®:water in the ratio of 1:2:8 gave interferences with interlayer spacings of $d_1:d_2d_3$= 1:1/2:1/3. Bragg spacing of 61.58 Å was obtained for the liquid crystals in this region. This region is therefore critical for formulation development of liquid crystal stabilized emulsions.

Recently, Rydhag, et al. (13) determined that the presence of an ionic surfactant stabilized a liquid crystalline phase containing lecithin. In a similar manner, the presence of the zwitterionic Deriphat 160C®, stabilizes the system with Generol 122®. The ionic species conveys a charge to the interface, further enhancing the stability of conventional electric double layer compression, as well as giving rise to lamellar liquid crystals with a considerably higher capacity for solubilizing water (14,15).

A representative sample from region "A", an o/w emulsion with neat phase, (Generol 122®:Deriphat 160C®:water::1:1:2), gave a particle size distribution with mean, mode and median of 2.55 μm, 3.91 μm, and 3.18 μm respectively. These small sized droplets suggest a strong interaction between Generol 122® and Deriphat 160C®, creating the necessary low interfacial free energy for stabilization. This indicates that droplets of the liquid crystal stabilized emulsions in this region are rather small and relatively stable.

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