

Properties of the Aqueous Vesicle Dispersion Formed with Poly(oxyethylene)hydrogenated Castor Oil

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Associated properties of the aqueous dispersion of poly(oxyethylene)hydrogenated castor oil ether (HCO-10) were studied by means of polarized microscopy, freeze-fractured electron microscopy, particle size analysis, ESR spin labelling and $^1\text{H-NMR}$ spectra. A stable multilamellar vesicle with an average diameter of ca 0.4μ is formed from the HCO-10 concentration below 20 wt% whose phase shows a concentric lamella.

The hydrophilic layer of the vesicle is more rigid than the hydrophobic core. The fluidity around the spin label 12NS incorporated in the HCO-10 vesicle significantly decreased with temperature; however, both spin labels 5NS and 16NS did not change significantly.

The mechanism of fluidity change with temperature was discussed in terms of the dehydration of ethylene oxide and the breakdown of hydrogen bonding in the HCO-10 vesicle.

Since Bangham and co-workers (1) demonstrated that closed bilayers (vesicles) were formed when lecithins in natural occurrence were dispersed in water, lecithin liposomes gained popularity as membrane models and have been extensively studied in terms of ion permeability (2).

From a medical point of view, liposomes have been considered for drug encapsulation. In order to act effectively, drugs, which are very insoluble in water, can be encapsulated to improve their properties and to control either the rate of decomposition of the drug or the rate of discharge of the drug from our bodies (3,4). However, application in the pharmaceutical field is very limited because of the poor drug content of the capsule, complicated preparation and poor storage stability, which is due to the double bonds in the alkyl chain of the lecithin.

Kunitake *et al.* (5) reported that some ionic surface active agents were able to form closed bilayers. However, ionic surfactants are of very limited use in the pharmaceutical field because of the safety problems associated with their use.

In addition, Handjani-Vila *et al.* (6) reported on the properties of the dispersion of the lamella phase of nonionic lipids in cosmetic products. Since then, nonionic surface active agents have been recognized as safe substances with easily designed molecular structures for synthetic, tailor-made lipids.

However, we have found only limited oligomer typed nonionic surfactants which are able to form vesicles which include both monomer (6) and dimer type (7) surfactants. Therefore, we have searched for vesicle-forming surfactants which are easy to prepare and have a good storage stability. A poly(oxyethylene) (10) hydrogenated castor oil ether was found to have these properties.

In this paper we report on vesicle formation and its associated properties by means of ESR spin labelling in the oligomer typed nonionic surfactant, poly(oxyethylene) (10) hydrogenated castor oil ether.

EXPERIMENTAL

Materials and sample preparations. Poly(oxyethylene)hydrogenated castor oil ether shown in Figure 1 was purchased from Japan Surfactant Co., Ltd. (Tokyo, Japan) and used without further purification.

A vesicle dispersion of poly(oxyethylene) (10) hydrogenated castor oil ether in distilled water was prepared by mechanical stirring (ca 100 rpm) for 30 min.

Measurements. Polarizing microscopy was made using an Olympus BH-type microscope. An electron micrograph of freeze-fractured samples was achieved using a Hitachi Co., Ltd. (Hitachi-shi, Japan) H-600 electron microscope.

Particle size measurements were made using a Brookhaven Instruments Co. (New York, NY) BI-90 Particle Sizer.

ESR measurements were made using various spin probes such as 5-,12- and 16-doxyl stearic acids (5NS,12NS and 16NS, respectively) and methyl 5-,12- and 16-doxyl stearates (5NSM,12NSM and 16NSM, respectively) shown in Figure 2. These spin probes

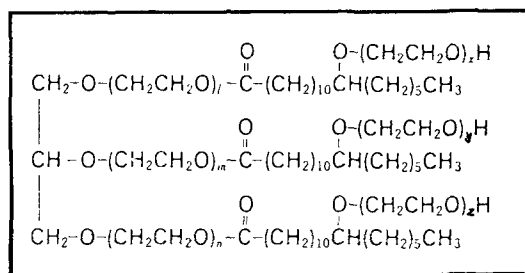


FIG. 1. Chemical structure of poly(oxyethylene)hydrogenated castor oil ether.

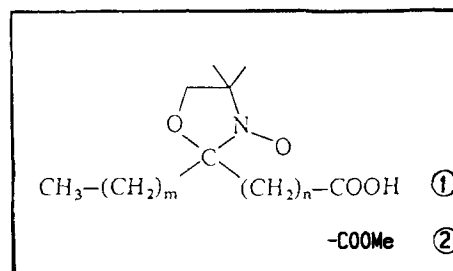


FIG. 2. Spin probes. 1. Doxyl Stearic Acids; 2. Methyl Doxyl Stearates.

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were purchased from the Sigma Chemical Co. (St. Louis, MO) and were used without further purification. The concentration of the probes was always in the order of 10^{-4} M. Each sample dispersion was drawn up in a capillary tube of 0.85ϕ mm. ESR spectra were recorded on a Japan Electron Optics Laboratory (Akishima, Japan) Model JES FE Spectrometer with 100kHz-0.63G modulation.

The order parameter, S_{33} , of the probe (8) was calculated from equation [1];

$$S_{33} = \{(A_{zz} - A_{xx}) / (A_{zz} - A_{xx})\} (aN' / aN') \quad [1]$$

where aN' is a hyperfine splitting constant, $A_{zz} = 33.6$, $A_{xx} = 6.3$ and $aN = 14.1$.

The hyperfine splitting constant (aN') was calculated from equation [2];

$$aN' = 1 / \{3(A_{zz} - 2A_{xx})\} \quad [2]$$

The $^1\text{H-NMR}$ spectra were obtained on a Japan Electron Optics Laboratory Model FX-90Q Fourier Transform NMR Spectrometer. Calibration of the chemical shift was done by using tetramethylsilane in dodecane as the external standard.

RESULTS AND DISCUSSION

Vesicle formation of HCO-10 phase diagram. Figure 3 shows the phase diagram of poly(oxyethylene) hydrogenated castor oil ether (HCO-10). HCO-10 is an oily substance, and is pale yellow at room temperature. When added to distilled water and mixed well, it gave a homogeneous, milky-white dispersed phase. As the HCO-10 concentration increased, it turned into a semi-transparent viscous solution.

Observation by polarizing microscopy indicated that HCO-10 has a neat phase liquid crystal in the concentration range of 5–60 wt% below 40°C . However, the texture of the liquid crystals change according to the HCO-10 concentration. Photo-

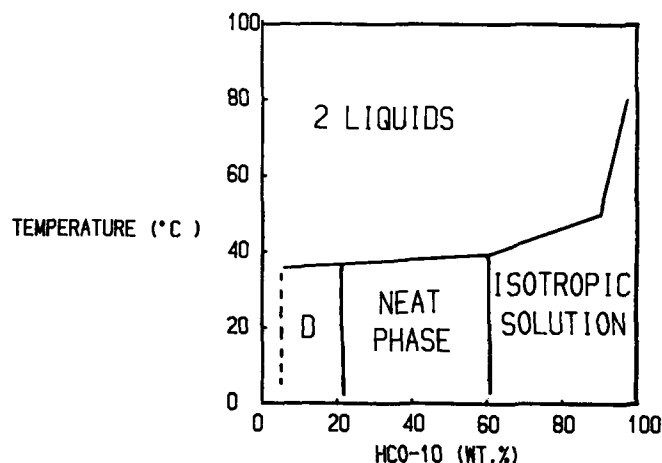


FIG. 3. Phase diagram of poly(oxyethylene)(10)hydrogenated castor oil ether. D: Vesicle Dispersion (Concentric Lamella).

graphs show cross nicols at a concentration below 10 wt%, a mixture of cross nicols and oily streak texture in the neat phase liquid crystals at 10–20 wt%, and an oily streak texture of the liquid crystals at 20–60 wt%.

It is suggested that the aqueous dispersion solution which shows cross nicols under polarized light seems to be able to form vesicles, and we observed freeze-fractured HCO-10 samples using the electron microscope.

Figure 4 shows electron micrographs of freeze-fractured HCO-10 samples at various concentrations. Electron micrographs illustrated the HCO-10 dispersion formed multilamellar vesicles at a concentration of 5–20 wt% and that it swelled until transformation at 20 wt%. With a further increase in the HCO-10 concentration, the multilamellar HCO-10 vesicle collapsed.

Figure 5 shows an electron micrograph of the freeze-fractured HCO-10 samples above the transition temperature (T_c). The micrographs showed that the multilamellar vesicles collapsed to give oily coagulated droplets.

As shown in Figure 3, a stable multilamellar vesicle was formed from the HCO-10 at a concentration below 20 wt%. This phase not only showed a concentric lamellar, but also that factors such as the H.L.B. value, the ratio of surfactant to water and temperature of the solution all play important roles in forming vesicles in HCO-10.

Average particle size of the HCO-10 vesicle. Figure 6 shows the particle size distribution of the HCO-10 vesicles' dispersion by means of a Submicron Particle Sizer. The average diameter of the HCO-10 vesicles was ca 400 nm in size.

Figure 7 shows the average particle size of the HCO-10 vesicles plotted as a function of temperature. The particle size remained constant up to 30°C , but it decreased remarkably at 35°C to ca 50 nm in size. Gradual cooling restored the vesicle to its original particle size. It was found that particle size, which was very sensitive to temperature, was one characteristic of the HCO-10 vesicle.

Associated structure of the HCO-10 vesicle flexibility gradient. In order to obtain a depth profile of the bilamellar structure in the HCO-10 vesicles, we carried out ESR measurements using various spin probes incorporated in them. The spin probes used were 5NS, 12NS and 16NS, along with 5NSM, 12NSM and 16NSM.

Figure 8 shows the order parameter for the doxyl stearic acids calculated from equation [1] vs the position of the carbon labelling above and below the transition temperature in comparison with the results of the methyl doxyl stearates.

Below the transition temperature (T_c), the order parameter (S_{33}) of 5NS, 12NS and 16NS were equal to those of 5NSM, 12NSM and 16NSM, respectively.

Above the transition temperature, the order parameter of 5NS (S_{33}) was much larger than that of 5NSM, while the order parameter of 16NS (S_{33}) was nearly the same as that of 16NSM.

Therefore, the spin label 5NS binds strongly to the hydrophilic sphere of the membrane in the HCO-10

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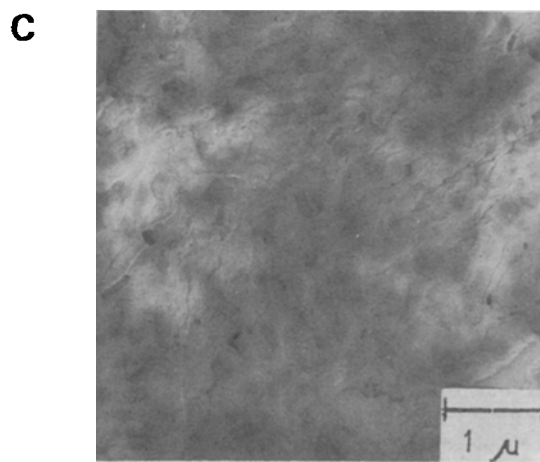
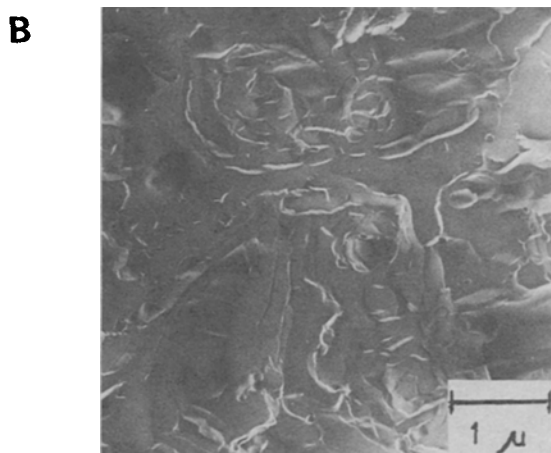
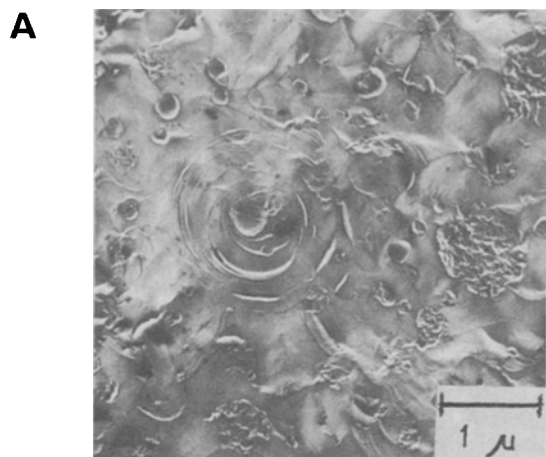


FIG. 4. Electron micrograph of freeze-fractured HCO-10 samples—
a:HCO-10 concentration is 10 wt% below T_c ; b:HCO-10 concentration is 20 wt% below T_c ; and c:HCO-10 concentration is 40 wt% below T_c .

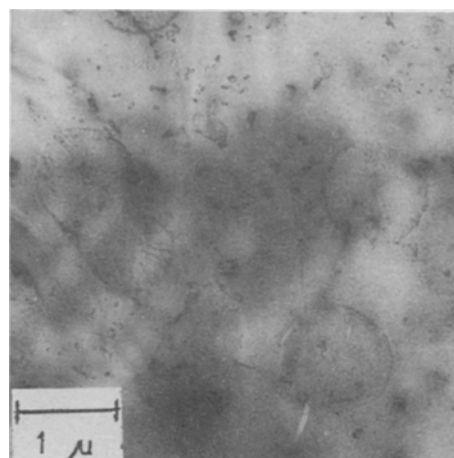


FIG. 5. Electron micrograph of freeze-fractured HCO-10 samples (concn:10 wt%) above T_c .

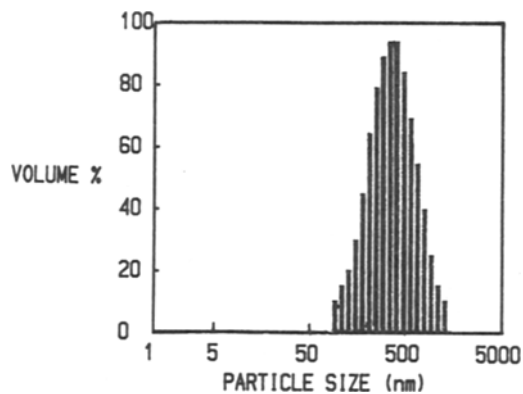


FIG. 6. Particle size distribution of HCO-10 vesicle at 25°C.

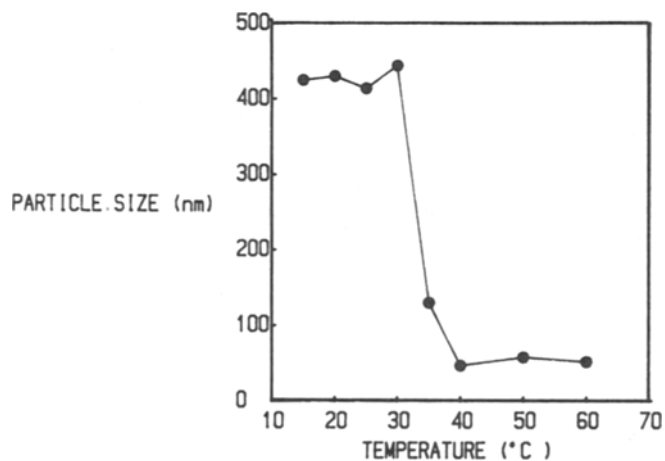


FIG. 7. Relationship between average particle size of HCO-10 vesicles and temperature.

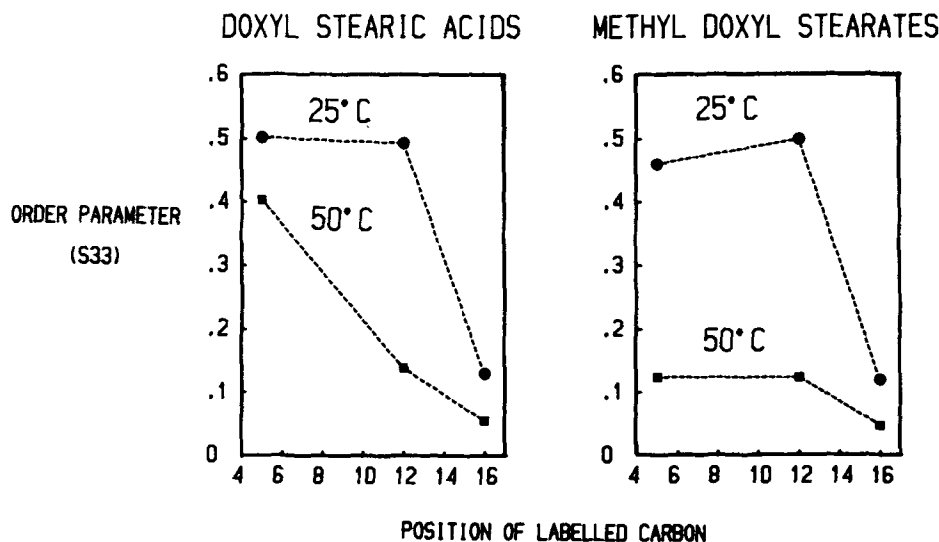


FIG. 8. Plots of the order parameter (S_{33}) against position of labelled carbon of the spin probes.

vesicle. Hereafter, we employed a series of doxyl stearic acids as the spin probes. In a series of spin labels, the order parameter of 5NS (S_{33}) was much larger than that of 16NS. The fluidity around the spin label 5NS was much more restrained.

It was also noted that the hydrophilic layer of the HCO-10 vesicle membrane was more rigid than the hydrophobic core.

Order parameter temperature dependence. Figure 9 shows the order parameter of 5NS, 12NS and 16NS vs temperature when these probes were incorporated into the HCO-10 vesicle.

The order parameter of 12NS decreased remarkably at 30–35°C, but did not change significantly for the 5NS and 16NS probes.

The fact that the fluidity around the spin label 12NS was significantly sensitive to temperature seems to suggest that it depends on either a change in associated structure, which is due to the concentration of HCO-10, or a change in intermolecular conformation which is due to the number of ethylene oxide in the hydrogenated castor oil ether.

Order parameter concentration dependence. Figure 10 shows the order parameter of 5NS, 12NS and 16NS vs the HCO-10 concentration above and below the transition temperature. The order parameter of each spin label was kept constant at an HCO-10 concentration of 5–60 wt% above and below the transition temperature. The displacement of the 12NS order parameter above and below the transition temperature was much larger than those of 5NS and 16NS. Therefore, the significant fluidity change around spin labelled 12NS with temperature was not due to the change in associated structure, which depended on the HCO-10 concentration.

Influence of the number of ethyleneoxides in hydrogenated castor oil ether on the order parameter. Figure 11 shows the plots of the order parameter of 5NS, 12NS and 16NS against the number of ethylene oxides in hydrogenated castor oil ether. The order parameter decreased with an increase in the number of

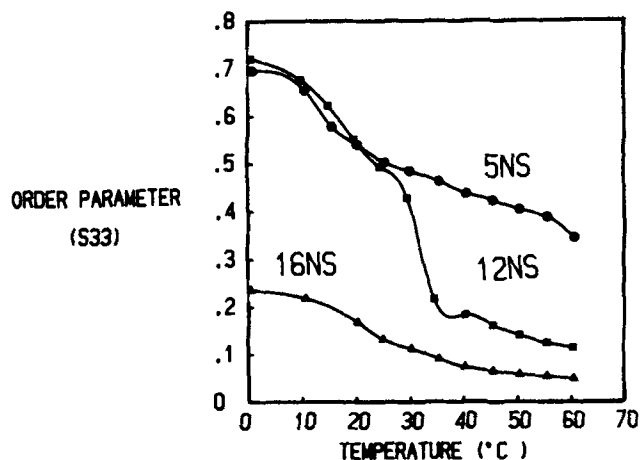


FIG. 9. Plots of the order parameter (S_{33}) of 5NS, 12NS and 16NS vs temperature.

ethylene oxides in hydrogenated castor oil ether. This seemed to be due to the increase in the solubility of the poly(oxyethylene)hydrogenated castor oil ether in water.

Figure 12 shows the plots of the order parameter of 12NS with various ethylene oxide numbers of hydrogenated castor oil ether vs temperature. It showed that the plots of the order parameter vs temperature has an inflection point where the value changed sharply at a given temperature. The temperature where this inflection occurs varied depending on the ethylene oxide content. Examples of this are shown by HCO-5 exhibiting the inflection at 40°C, whereas the HCO-10 to HCO-40 exhibited this inflection at 30–35°C. As the ethylene oxide number increased to 100, there was no clear inflection point.

As a consequence, it was suggested that the ethylene oxide number of hydrogenated castor oil ether was influenced by the associated structure of the HCO-10 vesicle.

Therefore, in order to study how the ethylene

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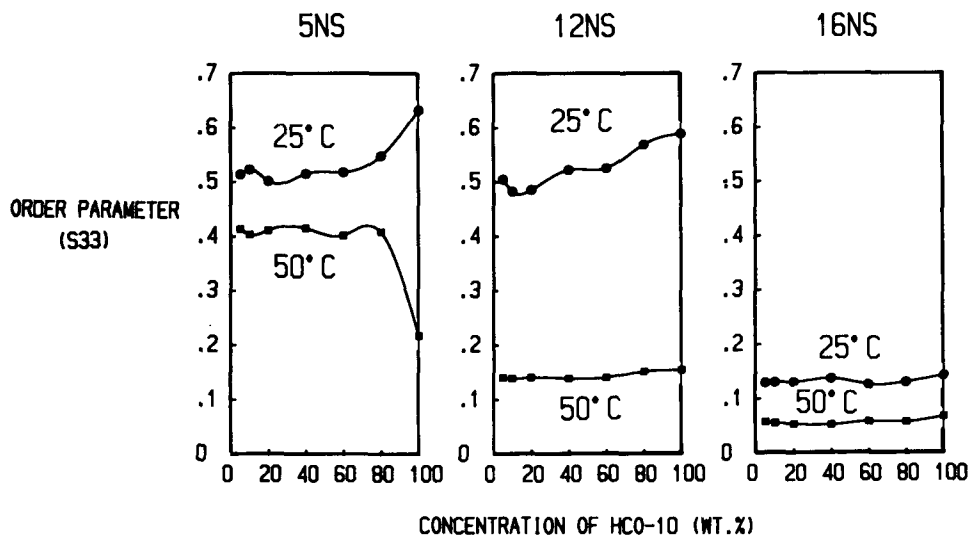


FIG. 10. Plots of the order parameter of 5NS, 12NS and 16NS vs the HCO-10 concentration above and below the transition temperature.

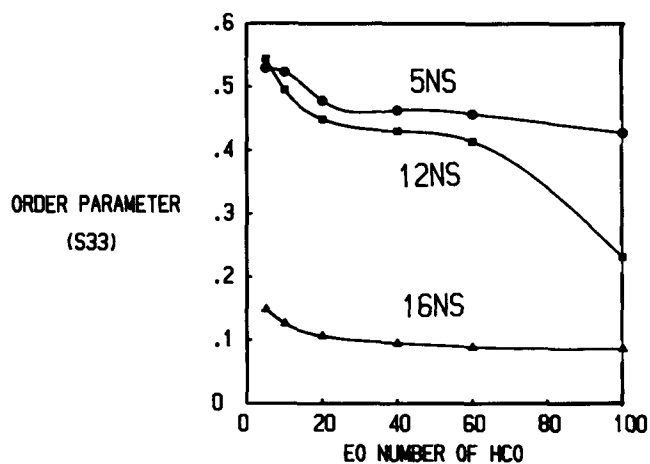


FIG. 11. Plots of the order parameter of 5NS, 12NS and 16NS vs the number of ethylene oxides in hydrogenated castor oil ether.

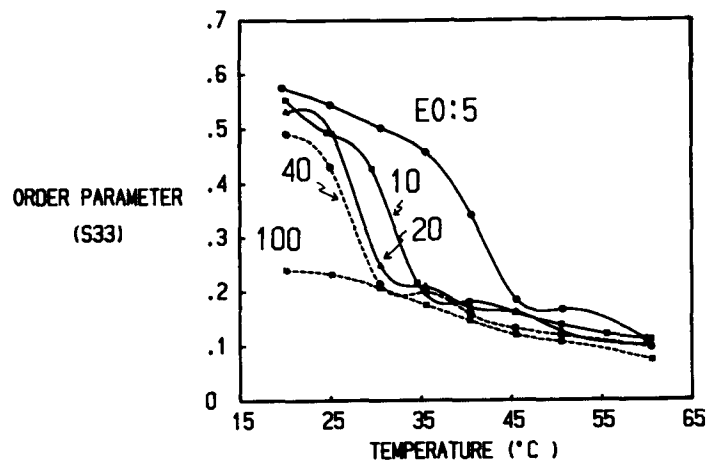


FIG. 12. Plots of the order parameter of 12NS with various ethylene oxide numbers of hydrogenated castor oil ether vs temperature.

oxide chain in hydrogenated castor oil ether influenced the associated structure of the HCO-10 vesicle, we carried out $^1\text{H-NMR}$ measurements for water at 4.6 ppm in the aqueous HCO-10 vesicle dispersion.

Figure 13 shows the plots of the chemical shift of water vs temperature. One peak remained stationary, but the order shifted to a lower magnetic field with increasing temperature. It was suggested that two kinds of water, such as free and bound water, existed in the HCO-10 vesicle and that the free water content increased with temperature.

Figure 14 shows the plots of the hyperfine splitting constant ($a\text{N}'$) of 12NS vs temperature. The hyperfine splitting constant, calculated from equation [2], reflects the polarity around the spin label 12NS incorporated in the HCO-10 vesicle. As can be seen in Figure 14, once $a\text{N}'$, the hyperfine splitting constant, decreased at 35°C , it gradually increased with temperature and was restored to almost its original value.

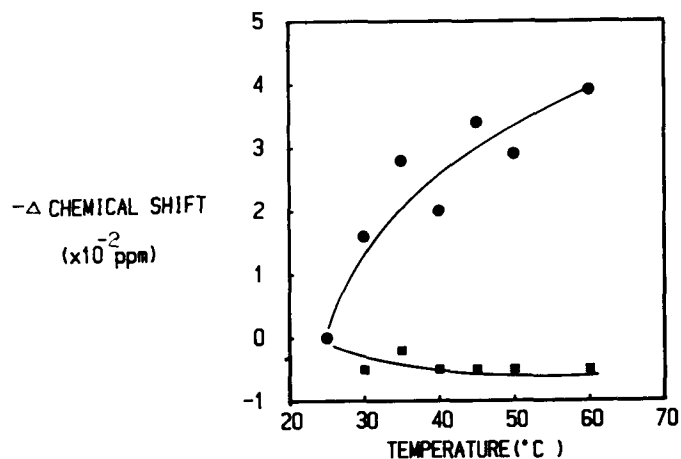


FIG. 13. Plots of the chemical shift of water at 4.6 ppm vs temperature.

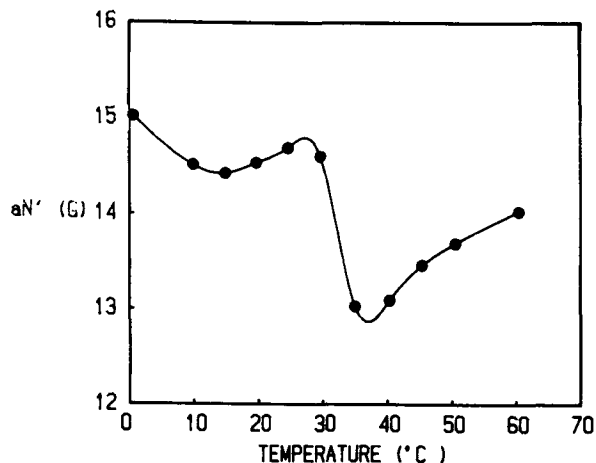


FIG. 14. Plots of aN' , the hyperfine splitting constant of ^{12}NS vs temperature.

The former was due to the reduction of free water between the bilamellars in the HCO-10 vesicle, and the latter due to the penetration of water into the hydrophilic layer of the HCO-10 vesicle owing to thermal agitation.

We combined the ESR results with the $^1\text{H-NMR}$ results and found that the drastic change in the fluidity around the spin labelled ^{12}NS with temperature

was based upon the conformational change of the ethylene oxide groups which were influenced by either the hydrogen bond or the intermolecular hydrogen bridge in the poly(oxyethylene)hydrogenated castor oil ether.

ACKNOWLEDGMENTS

The authors wish to thank A. Mori of the Lion Corporation for permission to publish this paper and K. Tajima of Kanagawa University for technical advice.

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[Received January 20, 1988, accepted August 23, 1989]
[J5393]