Solubilization Behavior of Estriol in an Aqueous Solution of Pluronic L-64 as a Function of Concentration and Temperature[1)]

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The solubilization of estriol was studied in an aqueous solution of a poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock copolymer (pluronic L-64).

The solubility of estriol increased with increasing pluronicL-64 concentrations and temperatures. Two clear transition points were found on the solubility curves at 30 and 40 °C. These two transition points were in good agreement with the 2nd critical micelle concentration and the change points of the micellar structure determined by our previous report. Furthermore, the thermodynamic parameters of estriol solubilization were also discussed.

Keywords pluronic L-64; estriol; solubilization; temperature; micelle formation; micellar structure

Nonionic surfactants are the most commonly used surfactants in the formulation of various pharmaceutical dosage forms, and they increase the solubility of water-insoluble drugs such as vitamins,[2)] steroids,[3)] and barbiturates.[4)] We have previously reported on the micellar formation and the micellar structure of poly(oxyethylene) hydrogenated castor oil (HCO_n) which is widely used in the pharmaceutical industry, and then presented basic information on solubilizing preparations.[5)] However, it has been reported that 12-hydroxystearic acid is formed by the hydrolytic cleavage of HCO_n, which results in the appearance of white cloudy insoluble materials in the solubilizing solution.[6)] Recently, poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock copolymeres (Pluronic®) have been evaluated as a vehicle for a novel dosage form, since these surfactants are low in toxicity and have the ability to form a clear solution in aqueous media.[7,8)] However, there are few reports in the literature on solubility using these pluronic surfactants. As for the solubilization of drugs, Lin *et al.* have reported only on the solubility of indomethacin and diazepam.[9,10)] In this paper, we have investigated the solubilization mechanism of pluronic L-64 (L-64) using estriol as a solubilizate.

Experimental

Materials L-64 was kindly donated by Asahidenka Kogyo K.K. and impurities were removed using *n*-hexane extraction.[11)] It was confirmed by ^{1}H-NMR spectrometry[12)] that the purified L-64 was composed of 45.5 weight % oxyethylene units. Estriol purchased from Wako Pure Chemical Industries, Ltd. was of biochemical reagent grade and was used without further purification. Distilled water per injection JP (Japanese Pharmacopoeia) was obtained from Otsuka Pharmacy Co., Ltd.

Solubility Measurement Excess quantities of the solid estriol were placed in 25-ml glass-stoppered test tubes together with 10-ml aliquots of aqueous solutions of various L-64 concentrations. The solutions were shaken for 24 h at a given temperature and controlled to within ±0.1 °C. This period was found to be sufficient for attaining equilibrium. After the equilibrium was established, sample solutions were filtered through Advantic GC-50 glass filter paper. Then, the samples were suitably diluted with methyl alcohol and assayed spectrophotometrically at a wavelength of 280 nm.

Results and Discussion

Solubility Studies The solubility curves for estriol by L-64 at several temperatures are presented in Fig. 1. The

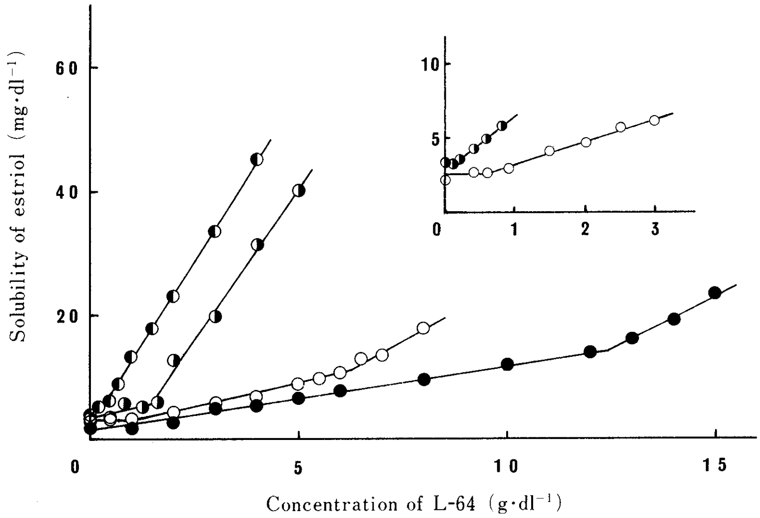

Fig. 1. Solubility of Estriol as a Function of Pluronic L-64 Concentration and Temperature

●, 20°C; ○, 30°C; ◑, 40°C; ◐, 50°C.

solubility of estriol increased with an increase in L-64 concentrations and temperatures. The increase in solubility with increased temperature is thought to be due to an increase in micellar size and wetting of the solubilizate.[13] Furthermore, the transition points of the solubility curves shifted remarkably to lower concentrations with increasing temperatures.

The solubilization of estriol at low L-64 concentrations was closely investigated (see the inset in Fig. 1). At 30 and 40 °C, other transition points were observed in the solubility curves, but they were not observed at 20 or 50 °C. The relationship between the transition point and critical micelle concentration (cmc) of Pluronic surfactants has already been suggested by Lin *et al.*[9,10] Howeveer, the second transition point (at a high concentration) shown in Fig. 1 appeared at a remarkably higher concentration compared to the other polyoxyethylated nonionic surfactants.[13]

Solubilization and cmc Previously, we found two clear discontinuities corresponding to $\mathrm{cmc_I}$ (at a low concentration) and $\mathrm{cmc_{II}}$ (at a high concentration) in the relationship between the surface tension and L-64 concentration in the temperature range of 20—50 °C.[14] In addition, a monomolecular micelle was formed at $\mathrm{cmc_I}$, while a polymolecular micelle appeared at $\mathrm{cmc_{II}}$. Thus, $\mathrm{cmc_I}$ was almost independent of temperature, but $\mathrm{cmc_{II}}$ was influenced greatly. The following equation was obtained from the relationship between $\mathrm{cmc_{II}}$ and temperature (T', °C).

$$\log \mathrm{cmc_{II}} = -0.12T' + 3.7 \qquad (1)$$

Figure 2 shows the relationship between $\mathrm{cmc_{II}}$ obtained from surface tension and the concentrations of the two transition points as a function of temperature. The first transition point of the solubility data corresponded closely to $\mathrm{cmc_{II}}$, and a relationship was observed with polymolecular micelle formation. However, the second transition point was much higher than the $\mathrm{cmc_{II}}$ which was obtained from surface tension measurements. In our previous study,[14] L-64 demonstrated a marked increase in micellar size and volume at remarkably higher concentrations than $\mathrm{cmc_{II}}$. The appearance of the second transition point on the solubility curves may be related to a change in micellar structure since the concentration corresponding to the change in micellar structure is similar to the concentration at the second transition point.[14]

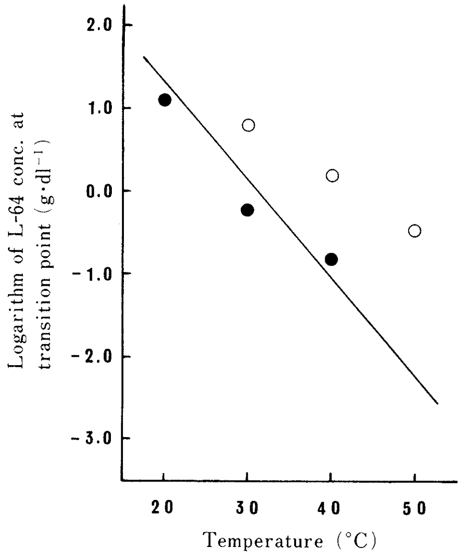

Fig. 2. Relationship between Pluronic L-64 Concentration at Transition Point and Temperature

——, $\mathrm{cmc_{II}}$ obtained from surface tension; ●, 1st transition point; ○, 2nd transition point.

The stability of micelles of a nonionic surfactant in aqueous solution is due to hydration.[15] The solubility curve at 20 °C indicated only the first transition point. This may be because there is no sudden change in the micellar structure at 20 °C[14] since the hydration increases and the stability of micelles increases with decreasing temperature. Also, no transition point corresponding to $\mathrm{cmc_{II}}$ on the solubility curve at 50 °C was observed. This may be because $\mathrm{cmc_{II}}$ at 50 °C is very low, as shown in Fig. 2.

Thermodynamic Studies Micellar solubilization can be treated as a process in which the water-insoluble material is partitioned between an aqueous phase and a micellar phase. The apparent partition coefficient (K_m) associated with this process is expressed by Eq. (2)[16,17]:

$$K_\mathrm{m} = [E_\mathrm{M}]/[E_\mathrm{NM}] \qquad (2)$$

where $[E_\mathrm{M}]$ is the concentration of estriol in the micelle and $[E_\mathrm{NM}]$ is the concentration of estriol in the nonmicellar phase. The slope of the solubilization isomers expressed on a molar basis gives the values for $[E_\mathrm{M}]$. The number of moles of estriol solubilized per mole of water gives the values for $[E_\mathrm{NM}]$. Furthermore, thermodynamic parameters of solubilization were calculated.[18] The standard free energy change ($\Delta G_\mathrm{S}^\circ$), when one mole of estriol transfers from the solution to the micelle, is given:

$$\Delta G_\mathrm{S}^\circ = -\boldsymbol{R}T \ln K_\mathrm{m} \qquad (3)$$

The standard enthalpy of solubilization ($\Delta H_\mathrm{S}^\circ$) is defined by

$$\Delta H_\mathrm{S}^\circ = -\boldsymbol{R} \cdot \frac{\partial \ln K_\mathrm{m}}{\partial (1/T)} \qquad (4)$$

The standard entropy of solubilization ($\Delta S_\mathrm{S}^\circ$) is

$$\Delta S_\mathrm{S}^\circ = (\Delta H_\mathrm{S}^\circ - \Delta G_\mathrm{S}^\circ)/T \qquad (5)$$

where $\boldsymbol{R}$ is the gas constant and T is the absolute temperature.

Thermodynamic parameters together with K_m are given in Table I. The K_m values increased with increased tem-

Table I. Apparent Partition Coefficients and Thermodynamic Parameters for Estriol by Pluronic L-64

Temp. (°C)	K_m ($\times 10^{-3}$)	$\Delta G_\mathrm{S}^\circ$ (kcal·mol⁻¹)	$\Delta H_\mathrm{S}^\circ$ (kcal·mol⁻¹)	$\Delta S_\mathrm{S}^\circ$ (cal·mol⁻¹·K⁻¹)
20	7.43±0.54	−5.19±0.04	4.87±1.26	34.3±4.3·
	(16.9 ±1.1)	(−5.67±0.04)	(7.85±0.82)	(46.1±2.8)
30	9.28±0.38	−5.50±0.03		34.2±4.2
	(24.1 ±1.9)	(−6.07±0.05)		(46.0±2.7)
40	10.3 ±1.4	−5.75±0.09		33.9±4.0
	(44.3 ±3.8)	(−6.65±0.05)		(46.3±2.6)
50	17.1 ±2.4	−6.26±0.10		34.4±3.9
	(54.0 ±1.6)	(−6.99±0.02)		(45.9±2.5)

The parantheses mean the parameters of estriol after the second transition point, respectively.

perature and became remarkably high after the second transition point.[19] ΔG_S° values reflected the results of K_m, and were negative, indicating spontaneous solubilization at all temperatures. The micellar solubilization of estriol was endothermic because the ΔH_S° value was positive. Similarly, positive ΔH_S° is also recognized for the solubilization of indomethacin by pluronic F-68 and F-108.[9]

The ΔS_S° values were nearly constant, irrespective of temperature: ΔS_S° was about 34 $\text{cal}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ before the second transition point and was about 46 $\text{cal}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ after the second transition point. The positive ΔS_S° is considered to be due to that the iceberg of water surrounding estriol molecules is liberated when estriol molecules are transferred from the aqueous phase to the micellar phase.[3] Also, the increase in ΔS_S° after the second transition point is thought to be related to micellar size.[20] Because the increase in micellar size near the second transition point begins as stated above, this may lead to increasing randomness of estriol within micelles.

At this time, further experiments are necessary to explain the usefulness of L-64 as a solubilizing agent. The solubilizing ability of L-64 in comparison to other polyoxyethylated nonionic surfactants will be dealt with in a following paper.

References and Notes

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