The Sol-Gel Transition of Sodium Deoxycholate Solutions under High Pressures

Gohsuke SUGIHARA, Toshiaki UEDA, Shoji KANESHINA,* and Mitsuru TANAKA

Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma, Nishi-ku, Fukuoka 814
**College of General Education, Kyushu University, Ropponmatsu, Chuo-ku, Fukuoka 810*

(Received August 11, 1976)

The effect of pressure on the sol-gel transition of sodium deoxycholate (SDC) solutions was investigated by the electric conductivity method up to 2000 atm in the temperature range from 10 to 65 °C. The gelation of SDC solutions is effected more easily at lower temperatures, at higher pressures, and at higher concentrations of SDC. The changes in the volume ($\Delta\bar{V}^{(\alpha\beta)}$), the enthalpy ($\Delta\bar{H}^{(\alpha\beta)}$), and the entropy ($\Delta\bar{S}^{(\alpha\beta)}$) of gelation were calculated thermodynamically. The values for $\Delta\bar{V}^{(\alpha\beta)}$, $\Delta\bar{H}^{(\alpha\beta)}$, and $\Delta\bar{S}^{(\alpha\beta)}$ are all negative under the conditions of this experiment. Therefore, it is concluded that the gel formation of SDC is attributable to intermolecular hydrogen bonds.

One of the most unique characteristics .of sodium deoxycholate (SDC) is its specific hydrolysis, *i.e.*, the pH change in aqueous solutions, and another is its polymer-like aggregation. The relation between these two characteristics has been studied by the present authors[1-3] and by Rich and Blow.[4] The micelle formation of SDC in aqueous solutions is well known. The formation of polymer-like aggregates in the aqueous solution of SDC was described for the first time by Rich and Blow.[4,5] Under suitable conditions, the micelles of SDC aggregate secondarily to form a gelatinous complex with macromolecular dimensions in a solution. X-Ray diffraction studies of the fibers of this complex showed that the molecules assume an elongated helical configuration 3.6 nm in diameter. Botré *et al.*[6] have studied the stability of the polymer-like structure and the effect of cations on the sol-gel transition of deoxycholic acid in aqueous solutions. One of the present authors previously suggested that the polymer-like aggregates of SDC were formed by intermolecular hydrogen bonding between the 3α-hydroxyl group and the carboxyl group of SDC.[1] In spite of these studies, however, there remain many problems to be solved with respect to the gelation of bile salt solutions. In order to make clear the mechanism of gelation, it is useful to investigate the sol-gel transition as a function of the pressure as well as of the temperature and concentration, because the study of the pressure effect affords effective thermodynamic information. In this study we will examine the pressure effect on the sol-gel transition of SDC solutions by means of the electric conductivity method.

Experimental

Materials. The SDC (Merck Co.) was recrystallized from a mixed solvent of methanol and ethanol. All the other chemicals used were of a guaranteed reagent grade. The thrice-distilled water used was previously freed from CO_2 gas by passing the air through a soda-lime column.

Apparatus. The high-pressure apparatus used in the present study consists of a pressure generator and a pressure vessel. A screw- and hand-pump (Hikari Kikai Co.) was used as a pressure generator. The pressure vessel was made of stainless steel (SUS 27) so as to withstand pressures up to 3000 atm; 80 mm o.d., 20 mm i.d., and 250 mm height. The pressure generator and vessel were connected with a piece of flexible stainless steel tubing (1/8 in. o.d. and 0.024 in. i.d.). The prevention of oil leakage from the vessel and the connection between conductivity cell and electrical leads are devised in a manner described before.[7] The conductivity cell fitted with platinized platinum electrodes was made by modifying an injector. The specific conductivity of SDC solutions was measured by means of a Yanagimoto conductivity outfit, Model MY-8. The cell constant was checked with a KCl solution before and after applying high pressures; no change was observed. The pressures were measured within an accuracy of ±3.5 atm by means of a Heise pressure gauge. The thermostatted bath in which the pressure vessel was immersed was regulated within ±0.01 °C. The measurement of the conductivity was carried out in the temperature range from 10 to 65 °C, and in the pressure range from 1 to 2200 atm.

Procedure. The preparation of the SDC solution and of the gel was performed according to the method of Botré *et al.*[6] so as to confirm their results. The SDC gel was obtained by dissolving a known amount of SDC in water and by then adjusting the pH value to 6.85 at 20 °C by adding a small amount of HCl under continuous and con-

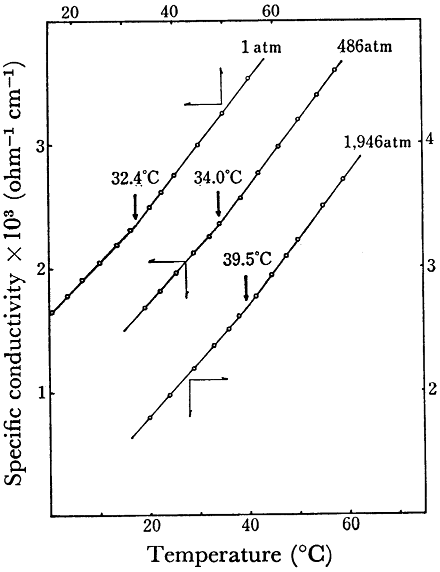

Fig. 1. The specific conductivity of SDC solution (3.180 $\times 10^{-2}$ mol/kg) as a function of temperature. The break in the slope indicates a transition.

stant stirring. All the gels obtained were stored at about 5 °C, and the gels used for measurements were permitted to stand for at least 20 h after the preparation.

The temperature of the sol-gel transition was decided as follows. The specific conductivity of an SDC solution with a given concentration was measured at various temperatures and at a desired constant pressure. The temperature was raised or lowered so slowly that the solution reached a sufficient equilibrium. It was confirmed by the constancy of the conductivity that the equilibrium was established after the thermostatted bath had been regulated at a constant temperature.

The specific conductivity measured at a constant pressure and concentration was plotted against the elevating temperature, resulting in the two straight lines which intersect each other at a certain temperature, as is shown in Fig. 1. The temperature corresponding to the break in these lines indicates the exact transition temperature,[6] which can be determined under various pressures as well as under atmospheric pressure. The strict determination of the transition temperature was performed by computing the intersection point of the two straight lines obtained by the least-squares method. The differences between the transition temperatures determined on elevating the temperature and on lowering the temperature were within 1 °C.

Results

Plotting the transition temperature at various concentrations of SDC against the pressure, straight lines are obtained, as is shown in Fig. 2. This figure shows a sol-gel phase diagram of the SDC–HCl solution system at different concentrations of SDC. The phase on the left-hand side of each curve is of a gel, while the phase on the right-hand side is of a sol. On the curves, the sol and gel phases may coexist in equilibrium.

In Fig. 3, the logarithm of the SDC concentration (in mole fraction) on the gelation at given pressures is plotted against the transition temperatures, which are taken from Fig. 2 by reading interpolated points on the curves. The region above each curve is of a gel, while that below each curve is of a sol.

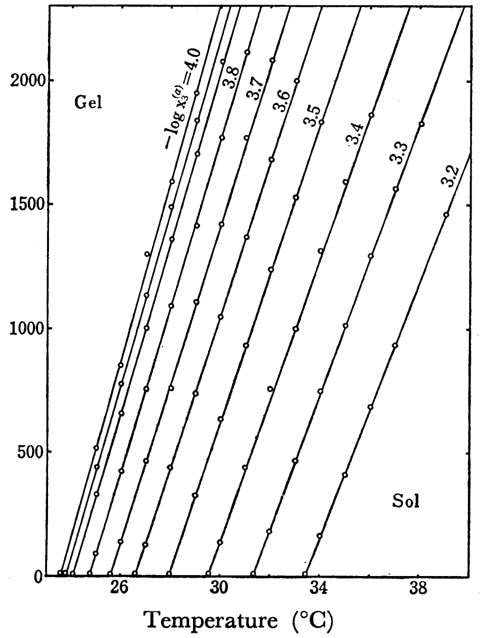

Fig. 2. Sol-gel phase diagram of SDC solutions at various concentrations. Numerical values indicate $-\log X_3{}^{(\alpha)}$. These relations are of Eq. 13 in the text.

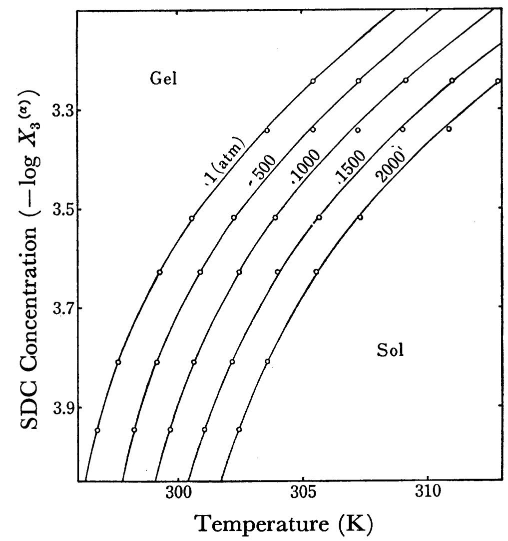

Fig. 3. Sol-gel phase diagram of SDC solutions at various pressures. Numerical values indicate pressure in atm. These relations are of Eq. 14 in the text.

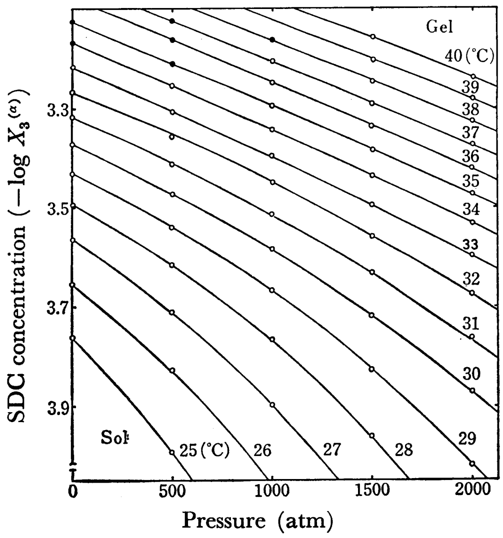

Fig. 4. Sol-gel phase diagram of SDC solutions at various temperatures. Numerical values indicate temperature in °C. These relations are of Eq. 15 in the text. (●: Extrapolated value in Fig. 3).

In Fig. 4 the phase diagram is made by plotting the transition pressure against the logarithm of the SDC mole fraction at given temperatures. The regions above and below each isotherm refer to the gel and the sol phases respectively. It may be seen from Figs. 2, 3, and 4 that the gelation of SDC solutions is caused more easily at the lower temperatures, the higher pressures, and the higher concentrations of SDC.

Discussion

In order to analyze the above results, we will try to treat thermodynamically the gelation of SDC in an aqueous dilute solution of HCl. The system under given conditions is in equilibrium between the sol phase (α) and the gel phase (β) and consists of three components; water (1), HCl (2), and SDC (3). The Gibbs free energy of each phase is expressed as follows:

$$G^{(\alpha)}[T,\ P,\ N_1^{(\alpha)},\ N_2^{(\alpha)},\ N_3^{\mathrm{s}(\alpha)},\ N_3^{\mathrm{m}(\alpha)}] \tag{1}$$

$$G^{(\beta)}[T,\ P,\ N_1^{(\beta)},\ N_3^{(\beta)}] \tag{2}$$

where T, P, and N denote the temperature, the pressure, and the number of moles, and where the superscripts, s and m, refer to singly dispersed SDC and micellar SDC respectively.

In the sol phase the Gibbs free energy is expressed by:

$$\mathrm{d}G^{(\alpha)} = -S^{(\alpha)}\mathrm{d}T + V^{(\alpha)}\mathrm{d}P + \sum\mu_i^{(\alpha)}\mathrm{d}N_i^{(\alpha)} \tag{3}$$

where $\mu_i^{(\alpha)}$ is the chemical potential of the i component in the sol phase. In the system under consideration, HCl is very diluted and the pH of solutions is adjusted to 6.85 at 20 °C. Since co-ions, *i.e.*, chloride ions, scarcely affect the gelation, the $N_2^{(\alpha)}/N_1^{(\alpha)}$ ratio can be regarded as constant. Hence, the aqueous dilute solution of HCl may be regarded as the solvent, and $N_{12}^{(\alpha)}=N_1^{(\alpha)}+N_2^{(\alpha)}$. Therefore, $X_{12}^{(\alpha)}=(N_1^{(\alpha)}+N_2^{(\alpha)})/(N_1^{(\alpha)}+N_2^{(\alpha)}+N_3^{(\alpha)})=1-X_3^{(\alpha)}$. Here, $N_3^{(\alpha)}$ is the total number of moles of singly dispersed and micellar SDC, and $X_3^{(\alpha)}$ is the mole fraction of total SDC in the sol phase.

At equilibrium,

$$\mathrm{d}\mu_3^{\mathrm{s}(\alpha)} = \mathrm{d}\mu_3^{\mathrm{m}(\alpha)} = \mathrm{d}\mu_3^{(\alpha)}. \tag{4}$$

Therefore,

$$\mathrm{d}G^{(\alpha)} = -S^{(\alpha)}\mathrm{d}T + V^{(\alpha)}\mathrm{d}P + \mu_{12}^{(\alpha)}\mathrm{d}N_{12} + \mu_3^{(\alpha)}\mathrm{d}N_3^{(\alpha)} \tag{5}$$

and

$$\mathrm{d}\mu_i^{(\alpha)} = -\bar{S}_i^{(\alpha)}\mathrm{d}T + \bar{V}_i^{(\alpha)}\mathrm{d}P + RT(\partial \ln a_i^{(\alpha)}/\partial \ln X_3^{(\alpha)})_{T,P}\mathrm{d}\ln X_3^{(\alpha)} \tag{6}$$

where

$$\mu_i^{(\alpha)} = \mu_i^{\circ(\alpha)} + RT\ln a_i^{(\alpha)}.$$

It may be seen from Eqs. 5 and 6 that the state of solution could be completely defined by three variables, T, P, and $X_3^{(\alpha)}$, because $X_{12}^{(\alpha)}=1-X_3^{(\alpha)}$.

Next, in the overall gel phase (β) we obtain Eq. 7 from a consideration of the Gibbs-Duhem equation:

$$\bar{S}^{(\beta)}\mathrm{d}T - \bar{V}^{(\beta)}\mathrm{d}P + (1-X_3^{(\beta)})\mathrm{d}\mu_1^{(\beta)} + X_3^{(\beta)}\mathrm{d}\mu_3^{(\beta)} = 0. \tag{7}$$

By analogy with Eq. 6,

$$\mathrm{d}\mu_1^{(\beta)} = -\bar{S}_1^{(\beta)}\mathrm{d}T + \bar{V}_1^{(\beta)}\mathrm{d}P + RT\left(\frac{\partial \ln a_1^{(\beta)}}{\partial \ln X_3^{(\beta)}}\right)_{T,P}\mathrm{d}\ln X_3^{(\beta)}, \tag{8}$$

and

$$\mathrm{d}\mu_3^{(\beta)} = -\bar{S}_3^{(\beta)}\mathrm{d}T + \bar{V}_3^{(\beta)}\mathrm{d}P + RT\left(\frac{\partial \ln a_3^{(\beta)}}{\partial \ln X_3^{(\beta)}}\right)_{T,P}\mathrm{d}\ln X_3^{(\beta)}. \tag{9}$$

At an equilibrium between α and β phases,

$$\mathrm{d}\mu_{12}^{(\alpha)} = \mathrm{d}\mu_1^{(\beta)} \text{ and } \mathrm{d}\mu_3^{(\alpha)} = \mathrm{d}\mu_3^{(\beta)}.$$

Hence, from Eqs. 7, 8, and 9,

$$\Delta\bar{S}^{(\alpha\beta)}\mathrm{d}T - \Delta\bar{V}^{(\alpha\beta)}\mathrm{d}P + RT\{(1-X_3^{(\beta)})(\partial \ln a_{12}^{(\alpha)}/\partial \ln X_3^{(\alpha)})_{T,P} + X_3^{(\beta)}(\partial \ln a_3^{(\alpha)}/\partial \ln X_3^{(\alpha)})_{T,P}\}\mathrm{d}\ln X_3^{(\alpha)} = 0. \tag{10}$$

Here, the changes in the entropy ($\Delta\bar{S}^{(\alpha\beta)}$) and volume ($\Delta\bar{V}^{(\alpha\beta)}$) on the gelation are:

$$\Delta\bar{S}^{(\alpha\beta)} = \{\bar{S}^{(\beta)} - (1-X_3^{(\beta)})\bar{S}_{12}^{(\alpha)} - X_3^{(\beta)}\bar{S}_3^{(\alpha)}\} \tag{11}$$

and

$$\Delta\bar{V}^{(\alpha\beta)} = \{\bar{V}^{(\beta)} - (1-X_3^{(\beta)})\bar{V}_{12}^{(\alpha)} - X_3^{(\beta)}\bar{V}_3^{(\alpha)}\} \tag{12}$$

If the concentration dependence of the activity coefficient in Eq. 10 is negligible in the vicinity of the equilibrium state, the following equations may be derived; these equations enable us to obtain $\Delta\bar{S}^{(\alpha\beta)}$, $\Delta\bar{V}^{(\alpha\beta)}$, and $\Delta\bar{H}^{(\alpha\beta)}$.

At a constant concentration of SDC,

$$\left[\frac{\partial P}{\partial T}\right]_{\ln X_3^{(\alpha)}} = \frac{\Delta\bar{S}^{(\alpha\beta)}}{\Delta\bar{V}^{(\alpha\beta)}} = \frac{\Delta\bar{H}^{(\alpha\beta)}}{T\Delta\bar{V}^{(\alpha\beta)}}. \tag{13}$$

At a constant pressure,

$$RT\left[\frac{\partial \ln X_3^{(\alpha)}}{\partial T}\right]_P = -\Delta\bar{S}^{(\alpha\beta)}. \tag{14}$$

At a constant temperature,

$$RT\left[\frac{\partial \ln X_3^{(\alpha)}}{\partial P}\right]_T = \Delta\bar{V}^{(\alpha\beta)}. \tag{15}$$

It should be noted that the analytical concentration can be used for the evaluation of the above thermodynamic quantity. The left-hand sides in Eqs. 13, 14, and 15 are experimentally determinable by using the results shown in Figs. 2, 3, and 4 respectively. From the slopes of the P *vs.* T curves in Fig. 2, we first obtained $\Delta\bar{S}^{(\alpha\beta)}/\Delta\bar{V}^{(\alpha\beta)}$ or $\Delta\bar{H}^{(\alpha\beta)}/T\Delta\bar{V}^{(\alpha\beta)}$ by using Eq. 13, *i.e.*, the Clausius-Clapeyron relation. The entropy change in the gelation is obtained from the slope of the log $X_3^{(\alpha)}$ *vs.* T curve in Fig. 3 based on Eq. 14. The volume change, $\Delta\bar{V}^{(\alpha\beta)}$, is obtained from the slope of the log $X_3^{(\alpha)}$ *vs.* P curve in Fig. 4. This estimation is based on Eq. 15. Actually, we obtained $\Delta\bar{V}^{(\alpha\beta)}$ by applying Eq. 15 to Fig. 4 and then estimated $\Delta\bar{S}^{(\alpha\beta)}$ and $\Delta\bar{H}^{(\alpha\beta)}$ by the use of Eq. 13 and Fig. 2.

The change in volume on gelation, $\Delta\bar{V}^{(\alpha\beta)}$, is shown as a function of the pressure at various temperatures in Fig. 5. The transition from a sol to a gel is accompanied by a decrease in the volume. The lower the temperature and the higher the pressure, the larger the degree of

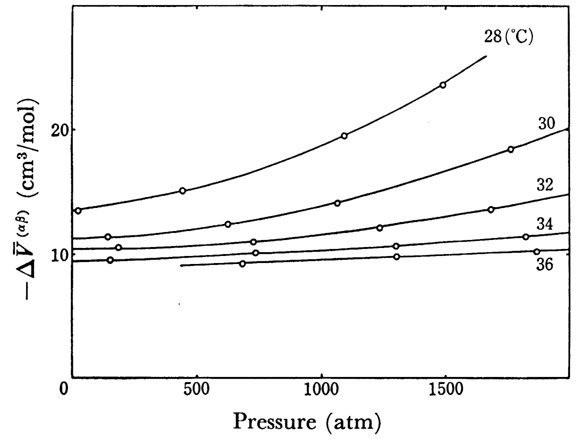

Fig. 5. The volume change of gel formation as a function of pressure at various temperatures. Numerical values indicate temperature in °C.

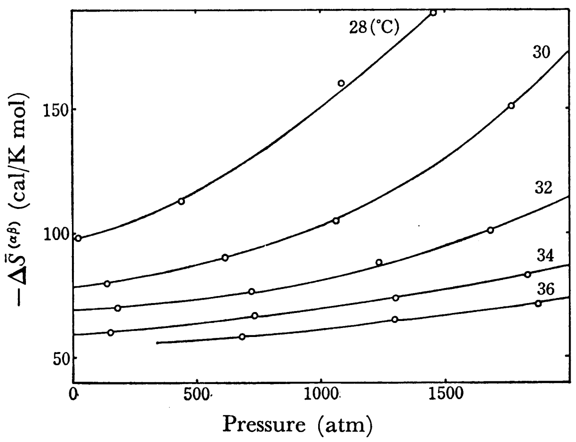

Fig. 6. The entropy change of gel formation as a function of pressure at various temperatures. Numerical values indicate temperature in °C.

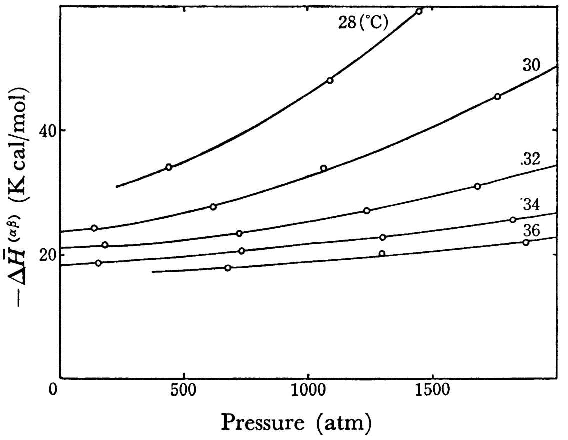

Fig. 7. The enthalpy change of gel formation as a function of pressure at various temperatures. Numerical values indicate temperature in °C.

volume decrease. The changes in the entropy ($\Delta\bar{S}^{(\alpha\beta)}$) and enthalpy ($\Delta\bar{H}^{(\alpha\beta)}$) of gel formation are shown as functions of the pressure at various temperatures in Figs. 6 and 7 respectively. The values of $\Delta\bar{S}^{(\alpha\beta)}$ and $\Delta\bar{H}^{(\alpha\beta)}$ are all negative under the conditions of this study and come to be negatively larger as the temperature is lowered and the pressure is raised.

Suzuki and his co-workers[8] have studied the effect of the pressure on the thermodynamically reversible gelation of gelatin, poly(vinyl alcohol), and methylcellulose in aqueous solutions. They have found that the formation of intermolecular hydrogen-bonded cross-links results in negative values of both the enthalpy and volume change on gelation. Taniguchi and Suzuki[9] have reported the effect of the pressure on the gel formation of 12-hydroxyoctadecanoic acid in CCl_4 and showed the enthalpy and volume changes on gelation to be negative.

In a thermodynamically reversible gel, the cross-links are known to be caused by secondary forces, such as hydrogen bonds, hydrophobic, and electrostatic interactions, rather than by covalent bonds. Of these, the hydrogen-bond formation is characterized by a negative change in the volume and an exothermic change in the enthalpy. On the other hand, the hydrophobic interactions (*i.e.*, the hydrophobic bonds) lead to positive changes in volume and entropy upon the gel formation. Therefore, the negative changes in the thermodynamic quantities on gelation are at least attributable to the cross-links of the intermolecular hydrogen bonds.

The enthalpy change per mole of the hydrogen bond is well known to be within the range from -4 to -4.5 kcal/mol.[10] The volume change per mole of the hydrogen bond has also been found to be -5.5 cm^3/mol in the gel formation of 12-hydroxyoctadecanoic acid in CCl_4,[9] -4.64 cm^3/mol for the association of 1-butanol in CS_2,[11] -3.0 to -3.4 cm^3/mol for the hydrogen-bond formation between phenol and dioxane in hexane,[12] and -7.0 cm^3/mol for the dimerization of formic acid.[13]

A molecule of SDC has, as a maximum, three sites for hydrogen bonds, *i.e.*, the 3α- and 12α-hydroxyl groups and the carboxyl group. Judging from the values of $\Delta\bar{V}^{(\alpha\beta)}$ and $\Delta\bar{H}^{(\alpha\beta)}$ shown in Figs. 5 and 7 and the respective values for hydrogen-bond formation per mole described above, the number of hydrogen bonds per mole of SDC in the gel formation can be roughly estimated to be about 3 or more if the gel formation involves the hydrogen-bond formation alone. The number of hydrogen bonds tends to increase as the temperature is lowered. This is probably attributable to the hydrogen bonds with solvent water.

References

1) G. Sugihara, K. Motomura, and R. Matuura, *Memoirs of Fac. of Sci., Kyushu Univ. Series C. Chem.*, **7**, 103 (1970).

2) G. Sugihara, *Fukuoka Univ. Sci. Reports*, **1**, 23, 29 (1972).

3) G. Sugihara and M. Tanaka, *Bull. Chem. Soc. Jpn.*, **49**, 3457 (1976).

4) A. Rich and D. M. Blow, *Nature*, **182**, 423 (1958).

5) D. M. Blow and A. Rich, *J. Am. Chem. Soc.*, **82**, 3566 (1960).

6) C. Botré, P. A. Ciccnetti, G. Lionetti, and M. Marchetti, *J. Pharm. Sci.*, **56**, 1035, (1967).

7) M. Tanaka, K. Kaneshina, T. Tomida, K. Noda, and K. Aoki, *J. Colloid Interface Sci.*, **44**, 525 (1973).

8) K. Suzuki, Y. Taniguchi, and T. Enomoto, *Bull. Chem. Soc. Jpn.*, **45**, 336 (1972).

9) Y. Taniguchi and K. Suzuki, *J. Phys. Chem.*, **78**, 759 (1974).

10) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman, San Francisco, Calif. (1960).

11) E. Fishman and G. H. Drickamer, *J. Chem. Phys.*, **24**, 548 (1956).

12) K. Suzuki and M. Tsuchiya, *Bull. Chem. Soc. Jpn.*, **48**, 1701 (1975).

13) K. Suzuki, Y. Taniguchi, and T. Watanabe, *J. Phys. Chem.*, **77**, 1918 (1973).