The Behavior of Aqueous Solution of Sodium Deoxycholate in Capillary Flow

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Primary micelles form secondary, polymer-like aggregates in a capillary flow of aqueous solution of sodium deoxycholate (SDC). The formation of the polymer-like aggregate is promoted by mechanical action, degradation being simultaneously caused by shearing stress. The viscosity of the SDC solution in the capillary flow attains equilibrium value between polymerization and degradation. The viscosity changes a great deal with the time of observation and pH. Under given conditions the SDC solution behaves as a non-Newtonian fluid obeying a power-law.

The flow of colloid-dispersed systems is of practical and theoretical interest as regards transportation through pipe and humors in a capillary *in vivo*. The motion of material *in vivo* can be classified into two types, permeation through an interface (membrane) and the flow in a capillary. Flow systems accompanied by reactions are of special interest. Attention has been paid to the variation in particle size or particle structure during the course of flow which considerably affects the flowing characteristics.

Sodium deoxycholate (SDC), a bile salt which is a surfactant *in vivo*, was chosen. Vochten and Joos[1] studied the viscosity of SDC solutions with an Ubbelohde type viscometer. They measured the viscosity at $pH=7$ for SDC of constant concentration containing NaCl in different concentrations, and found that the viscosity varies with time. Fontell performed viscosity measurement of aqueous solutions of various bile salts and pointed out that micelles at higher concentrations are voluminous and anisometric, indicating the formation of a secondary structure by the interlinking of primary micelles.[2] However, Fontell ignored the time dependency in the viscosity of the aqueous solution of SDC. We have regarded the viscous behavior in the capillary flow as a process of polymer-like aggregation of primary micelles. The aggregation is accelerated by mechanical action, degradation being caused by shearing stress simultaneously. Blow and Rich[3] observed this phenomenon for the first time by viscosity measurement with an Ostwald-type viscometer. However, they did not carry out analysis.

We have investigated aqueous solutions of SDC in detail, and found that the solution has a rheologically complex character, which might be clarified by means of an improved method of viscosity measurement on non-Newtonian flow.

The present study was carried out with the use of horizontal viscometers in order to clarify the effect of shearing stress on the flow and to correlate the result with SDC concentration, pH, and temperature.

Experimental

Horizontal viscometers were so constructed as to enable us to observe flow volume and flow time of the solution by connecting a 2 cm³ mess-pipet to the capillary. Two viscometers were used: (a) capillary length 10 cm, and inner diam. 1.64×10^{-2} cm and (b) capillary length 10 cm, inner diam. 1.66×10^{-2} cm. The Reynold number was less than 60 under hydraulic pressure of 100 cm. The viscosity measurement was carried out by immersing the viscometer in a thermostated water bath, putting the SDC solution in the viscometer and giving an arbitrary shearing stress. The temperature was kept constant within ±0.01 °C.

A pressure-generator was set up by combination of two water-tanks (20 dm³), U-tube for manometer (180 cm in height) and cocks. This makes it possible to give an arbitrary difference of pressure and observe both forward and backward processes by use of T-cocks. For the simultaneous measurement of viscosity and pH, the horizontal viscometer was fitted with a combination-type electrode (Hitachi-Horiba 6028-10T for test tube) near the exit of capillary (Fig. 1). The variation of pH with time was measured with a Hitachi-Horiba pH meter, model F-5 and recorded automatically with a recorder (QPD-54, Hitachi).

The solutions were prepared as follows. Aqueous SDC and inorganic salt solutions (buffer) were prepared separately with concentration twice higher than necessary for the experiments. Both solutions were kept at a constant temperature. The

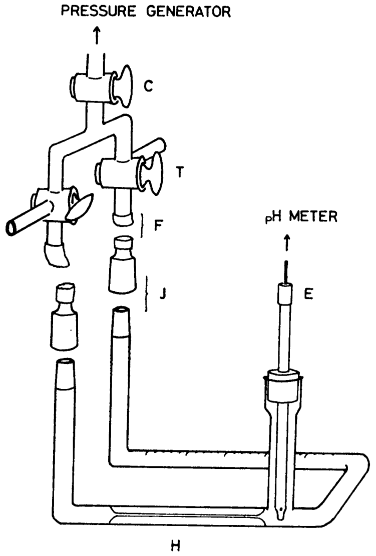

Fig. 1. Horizontal viscometer for the simultaneous measurement of pH and viscosity.
Key C: cock, T: T-cock, F: flexible pipe ,J: glass joint, H: horizontal viscometer, E: combination-type electrode (for test-tube use).

same amount of each was mixed in a vessel, and the mixture was freed from dust by a Millipore 47 mm membrane filter. 5 cm^3 of filtered solution was immediately put into the viscometer immersed in thermostated bath, and then subjected to incessant flow. The pH of the solutions was adjusted to 6.8, 7.0, or 7.2 by the Sørensen buffer solution.

SDC (E. Merck Co.) was thrice crystallized from ethanol and dried at 110 °C *in vacuo* for a day. Twice distilled water was used. Other chemicals were of guaranteed reagent-grade.

Results and Discussion

Correlation of Viscosity with Flow Time. After the initial Newtonian flow the viscosity rises abruptly, showing a characteristic peak and stationary value (Fig. 2). The viscosity is expressed in time (s) required for the flow of the unit volume of liquid. The abrupt rise in viscosity with time seems to be correlated with various factors. The reproducibility is not always good because of sensitivity to the delicate difference of running or existence of impurity. We might interpret the initial part of the curve as follows. It is assumed that the polymer-like aggregation of SDC takes place by crystallization or phase transition. The growth of crystalline nuclei may be hindered by thermal and mechanical motions. The temperature of the whole system is kept constant during the observation, but minute, local variation is unavoidable. The rate of producing nuclei depends largely on the temperature nearer to that of phase transition, increasing rapidly with a lowering temperature. Thus, there is a possibility for effective nuclei to exist whose rate of production and growth exceed that of collapse during the minute, local variation of temperature. Once the effective nuclei are produced, polymer-like aggregation is accelerated in the field of flow. On the other hand, the degradation of the aggregates proceeds in the flow. The curves in Fig. 2 can be regarded as the result of polymerization and degradation.

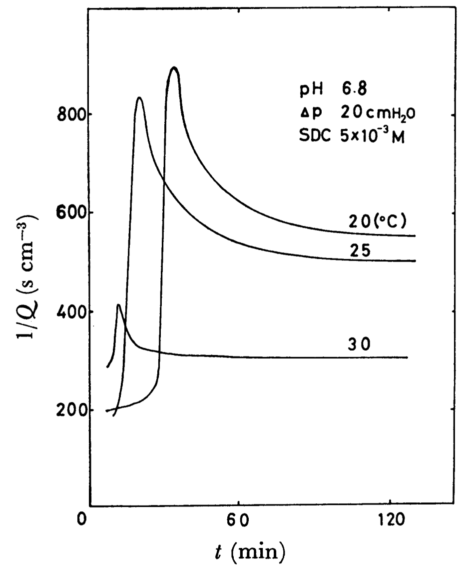

Fig. 2. An example of viscosity change with time in the capillary flow.

The time-dependent equation of the number-average degree of polymerization $P_{(t)}$ at time t is[5]

$$P_{(t)} = \frac{q e^{qkt}}{e^{qkt}-1+qT/(t+T)}, \tag{1}$$

where T denotes the half-life of particle (monomer) number, k the rate constant of degradation of polymer-like aggregates, and $q=2g-1$, g being the limiting degree of polymerization. If we denote the mass of primary particle (monomer) by m and the Avogadro number by N, the time-dependent weight average molecular weight $[M]_{w,t}$ is given by[5]

$$[M]_{w,t} = \frac{2mNq e^{qkt}}{e^{qkt}-1+qT/(t+T)}, \tag{2}$$

where parameters T, q, and k have the same meaning as in Eq. 1. These parameters and m should be determined by separate experiments. No complete analysis can be carried out from only the curves shown in Fig. 2. We see from Eqs. 1 and 2 that $P_{(t)}$ and $[M]_{w,t}$ attain the values q and $2mNq$, respectively, after a sufficiently long time. These two values correspond to the dynamic equilibrium after the peak in Fig. 2.

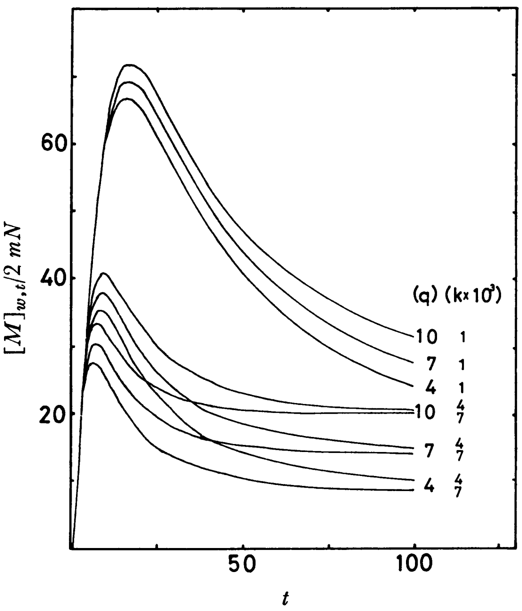

Fig. 3. Simulation curves for the relation between the weight-average degree of polymerization, computed with various values of each parameter and time *t*.

Figure 3 shows simulation curves computed by substituting appropriate values for the parameter in Eq. 2. The equilibrium value varies with concentration of SDC, temperature and pH. This is of dynamic equilibrium related directly to shearing stress or shear rate. The value of dynamic equilibrium does not depend on the history (the half-life T and the rate constant of degradation k) but on only the limiting degree of polymerization g determined by given conditions. Thus only the treatment of dynamic equilibrium is required for the analysis of the characteristics of the flow system.

Apparent and Real Viscosity in Dynamic Equilibrium. The behavior of capillary flow of aqueous SDC solution was investigated by examining the relation between the value of dynamic equilibrium and various conditions. All systems in Fig. 2 behave as non-Newtonian flow.

In the case of non-Newtonian flow the coefficient of viscosity η is a function of shearing stress p_t or shear rate $\dot{e}_t$:

$$\eta = \frac{p_t}{\dot{e}_t} = \frac{g(\dot{e}_t)}{\dot{e}_t} = \frac{p_t}{f(p_t)}. \tag{3}$$

The shearing stress P and shear rate V on the wall-surface of capillary are

$$P = \frac{R\Delta p}{2l}, \tag{4}$$

$$V = \frac{4Q}{\pi R^3}, \tag{5}$$

where R is the radius of capillary, l the length, Δp the pressure difference on the unit area of cross-section of liquid cylinder in the capillary, and Q the amount of flowing liquid per unit time. Q is expressed as a function of P (or Δp) as follows.[6)]

$$Q = (2l/\Delta p)^3 \int_0^P p_t^2 f(p_t)\mathrm{d}p_t = (\pi R^3/P^3)\int_0^P p_t^2 f(p_t)\mathrm{d}p_t. \tag{6}$$

It is desirable to find the relation between the measured Q and Δp, but the type of function $f(p_t)$ is unknown. The function can be obtained, if we use the following equation.[6-9)]

$$f(P) = V(3/4 + (1/4)\ \mathrm{d}\log Q/\mathrm{d}\log P). \tag{7}$$

If we determine the slope of the curve $\log P$-$\log Q$, we obtain the second term in parenthesis. Since P is proportional to Δp, we can use $\log \Delta p$ instead of $\log P$.

In the Newtonian flow $\mathrm{d}\log Q/\mathrm{d}\log P$ is equal to unity and we have $f(P) = V$. While V in Newtonian flow gives a real shear-rate on the capillary-wall, V in non-Newtonian flow gives only an apparent one. Thus, P/V gives the apparent viscosity η_a. The real coefficient of viscosity η is given by $P/f(P)$.

When the non-Newtonian flow is of a power function type, $f = kp_t^n$, we obtain the following equation from Eqs. 6 and 4.

$$Q = \frac{\pi R^3 k}{n+3}P^n = \frac{\pi k R^{n+3}}{(n+3)(2l)^n}(\Delta p)^n. \tag{8}$$

Thus we have $\mathrm{d}\log Q/\mathrm{d}\log P = n$. Hence Eq. 7 gives the relation $f(P) = (3+n)V/4$. The value of real viscosity η is given by

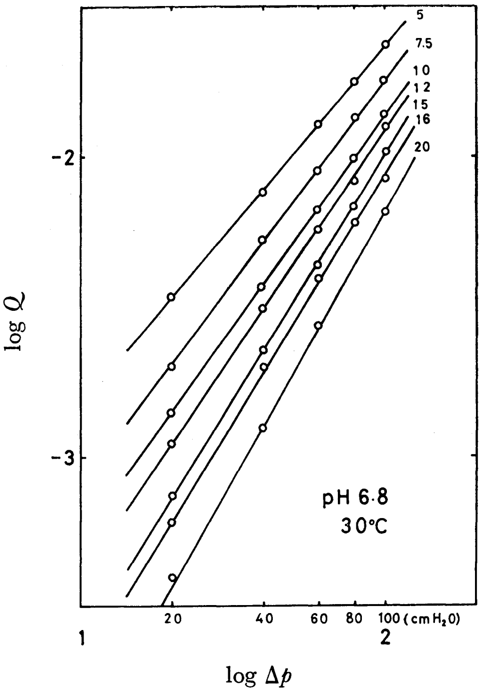

Fig. 4. The plot of $\log \Delta p$ *vs.* $\log Q$ at pH 6.8 and 30 °C. The figures at the right hand of curves indicate the concentration of SDC in mM. The slope of the straight line gives the power n in Eq. 8.

$$\eta = \frac{4}{n+3}\eta_a. \tag{9}$$

Straight lines are obtained by plotting $\log Q$ against $\log \Delta p$ in dynamic equilibrium (Fig. 4). This indicates that the flow of the SDC solution is non-Newtonian and of the power function type. The values n obtained from the slopes of $\log \Delta p$-$\log Q$ curves are given in Table 1. The value of n may give a criterion of the deviation from Newtonian type, or a degree of non-Newtonian. Although the real physical meaning of n is not known, it seems to correspond to the degree of interaction among aggregated particles. We see from Table 1 that the value of n is larger in higher concentration range for any temperature and pH.

Relation between Real Viscosity, Concentration of SDC, and Shearing Stress at Various Temperatures and pH. The dynamic equilibrium value of real viscosity η at pH 7.0 and 25 °C is shown in Fig. 5, in which (a) is the plot of η *vs.* concentration of SDC and (b) the plot of η *vs.* shearing stress, where the pressure difference Δp (cm

TABLE 1. THE POWER VALUE n OF SDC SOLUTION

		20 °C			25 °C			30 °C	32.5 °C	35 °C
	pH	6.8	7.0	7.2	6.8	7.0	7.2	6.8	6.8	6.8
SDC concn (mM)	5	1.25	1.23	1.26	1.20	1.22	1.10	1.20	1.08	—
	7.5	1.37	1.32	1.39	1.32	1.35	1.24	1.35	1.31	1.21
	10	1.49	1.35	1.45	1.42	1.47	1.41	1.41	1.40	1.35
	12	1.58	1.42	1.49	1.50	1.51	1.50	1.49	1.46	1.45
	15	1.75	1.46	1.60	1.57	1.56	1.59	1.63	1.57	1.58
	16	1.77	1.50	1.61	1.59	1.60	1.59	1.70	1.59	1.60
	20	1.88	1.64	—	1.73	1.68	—	1.81	1.80	1.77

TABLE 2. THE REAL VISCOSITY η (C.P.) OF SDC SOLUTION AT DYNAMIC EQUILIBRIUM

SDC concn	Temperature (°C)	pH	Pressure difference between both ends of the capillary Δp in cm H_2O				
			20	40	60	80	100
1.00×10^{-2} M	20	6.8	6.61	4.61	3.63	3.20	2.82
		7.0	5.30	4.15	3.51	3.15	2.77
		7.2	5.11	3.82	3.13	2.76	2.50
	25	6.8	4.58	3.67	2.97	2.76	2.48
		7.0	4.74	3.43	2.82	2.51	2.19
		7.2	3.56	2.73	2.26	2.04	1.87
	30	6.8	3.60	2.76	2.29	2.09	1.80
	32.5	6.8	3.11	2.38	2.00	1.86	1.69
	35	6.8	2.33	1.86	1.54	1.44	1.27
1.60×10^{-2} M	20	6.8	15.56	9.33	6.51	5.33	4.60
		7.0	9.90	7.23	5.87	4.98	4.45
	25	6.8	9.15	6.55	5.31	4.55	4.10
		7.0	9.65	6.37	4.93	4.21	3.75
	30	6.8	7.50	5.02	3.75	3.18	2.80
	35	6.8	5.38	3.23	2.51	2.15	1.84

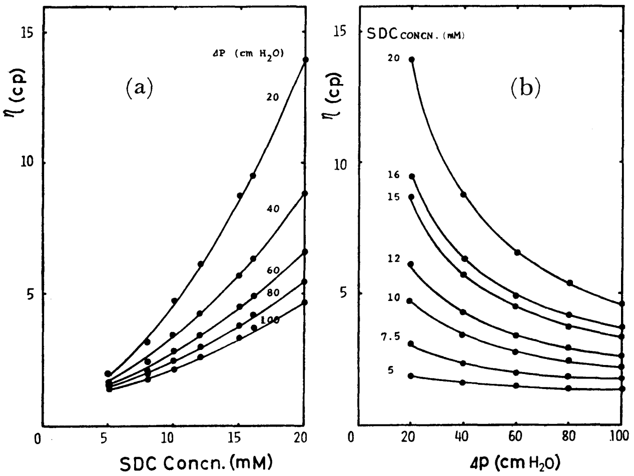

Fig. 5. The correlation of the real viscosity (cp) with SDC concentration (mM) and pressure difference Δp (cm H_2O) at pH 7.0 and 25 °C. (a) The plot of SDC concentration *vs.* η, where numerical values indicate the pressure difference Δp in cm H_2O. (b): The plot of Δp *vs.* η, where numerical values indicate the SDC concentration in mM.

H_2O) is used instead of the real shearing stress. Numericals at the end of respective curves refer to the pressure difference (in cm H_2O) in (a) and to the concentration (in mM** (mol m^{-3})) of SDC in (b). Though the value of η varies with pH and temperature, all the results are similar. Decrease in shearing stress tends to increase the viscosity, and the higher the concentration of SDC, the greater the dependence of real viscosity on shearing stress. The values of η of the 1.00×10^{-2} M** solution and 1.60×10^{-2} M solution in the dynamic equilibrium under various conditions are given in Table 2.

Effect of Shearing Stress on the Molecular Weight of Polymer-like Aggregate. As shown in Fig. 5, the shearing stress (Δp) considerably affects viscosity and the molecular weight of the polymer-like aggregate in dynamic equilibrium.

The dimension of polymer-like aggregate can be estimated from the molecular weight determined by viscometry. If the reduced values η_{sp}/c and $\ln \eta_r/c$ are plotted against c (in grams of SDC per 100 cm^3) and extrapolated to $c=0$, both curves intersect at the same point of ordinate (Fig. 6). The coincidence of intercept of the two curves affords a reasonable value as the limiting viscosity number $[\eta]$.

The correlation of $[\eta]$ with Δp illustrates the fact that decrease in shearing stress tends to increase considerably $[\eta]$ or the dimension of polymer-like aggregate. The plot of $\log \Delta p$ *vs.* $[\eta]$ gives straight lines (Fig. 7). We see that the product $[\eta]\,\Delta p^{\beta}$ is constant, where β is a

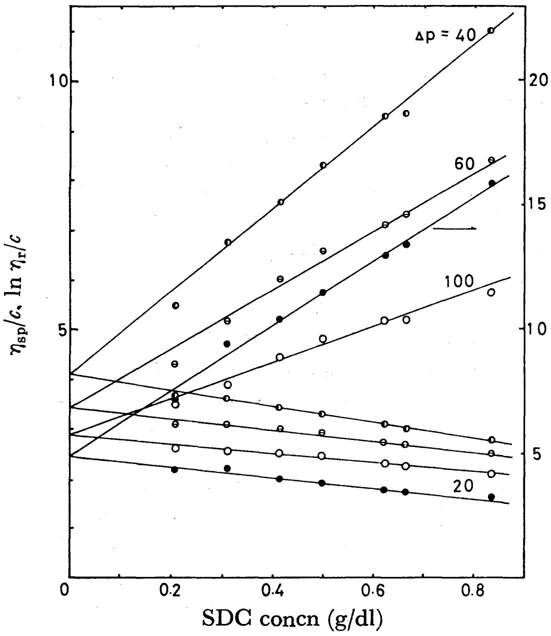

Fig. 6. The plot of $\ln \eta_r/c$ and η_{sp}/c *vs.* the SDC concentration c(g/dl). The reading value extrapolated to the zero concentration affords an apparent intrinsic viscosity $[\eta]$.

** Throughout this paper 1M = 1 mol dm^{-3} and 1 mM = 1 mol m^{-3}.

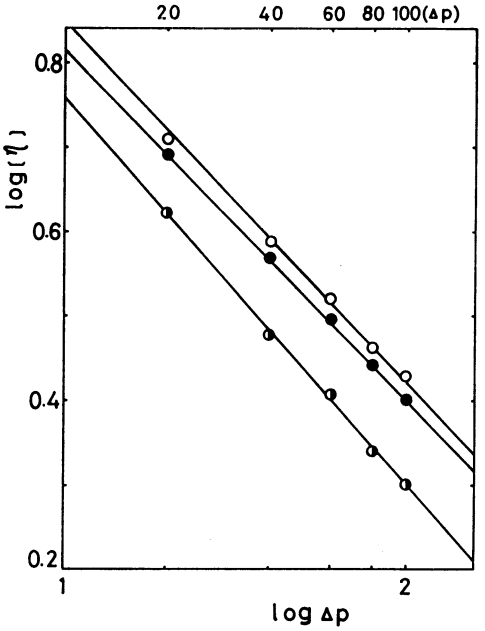

Fig. 7. The relation between log Δp and log [η].
Key ○ pH 6.8, 25 °C, ● pH 7.2, 20 °C, ◑ pH 6.8, 30 °C. Empirical formula is expressed as follows.
at pH 6.8, 20 °C $\log[\eta]=-0.46\log(\Delta p)+1.33$
at pH 6.8, 25 °C $\log[\eta]=-0.46\log(\Delta p)+1.31$
at pH 6.8, 30 °C $\log[\eta]=-0.46\log(\Delta p)+1.22$
at pH 7.0, 20 °C $\log[\eta]=-0.37\log(\Delta p)+1.20$
at pH 7.2, 20 °C $\log[\eta]=-0.42\log(\Delta p)+1.24$

constant.

The intercept of the log (Δp) *vs.* log [η] curve might be utilized as an index of limiting dimension of the aggregate at the pressure difference $\Delta p=1$ cm H_2O. Though the physical meaning of the slope or the power of pressure difference cannot be grasped, the value seems to correspond to pH. Temperature seems to have no effect on the slope. When pH is constant, curves at various temperatures have the same slope.

In the case of linear polymer the molecular weight is estimated by the relation $[\eta]=KM^{\alpha}$. However, this relation is not applicable to the SDC system, since the parameters K and α are unknown. The molecular weight of SDC aggregates can be estimated roughly by use of the computed data for q-value and the data for m obtained by means of light scattering. We assume that q is 5—10 (Fig. 3) and mN or the micellar weight of primary micelle is 2.6×10^4 at 36.5 °C. Thus from Eq. 2, the value of $[M]_{w,t}$ in dynamic equilibrium is estimated to be 2.6×10^5 to 5.2×10^5. The micellar weight of the polymer-like aggregate is calculated to be several millions at 10 °C.10)

pH Change with Flow. The SDC solution undergoes specific hydrolysis when it forms a gel.4,11) This can be explained by a kinetic consideration that the abrupt rise of pH is due to the hydrolysis of SDC. When gel formation takes place, the pH rise is linear against logarithm of the reaction time t in the early stage with the same slope of unity. If we denote the initial concentration of the reactant deoxycholate ion by C, the concentration of the product by x, the rate constant by k, and the ionic product of water by K_W, we obtain the relation

$$pH=\log t+\log(kC/K_W), \qquad (10)$$

where it is assumed that $x \ll C$ in the initial stage of reaction and therefore only the first term of the expansion of $\ln[C/(C-x)]$ is taken. Equation 10 gives the relation $\mathrm{d\,pH}/\mathrm{d}\log t=1$. It was found that the rate constant varies from 1.6×10^{-7} to 20×10^{-7} (min^{-1}) at temperatures 5—35 °C, the activation energy of hydrolysis being calculated to be 14 kcal. We studied the behavior of the counter ions Na^+ and H^+ through the measurement of their activity change with concentration. We have concluded that the adsorption of proton on the micelle surface (or hydrolysis) is necessary for the structure-making of micelle or gel, and that the counter ion Na^+ undergoes exchange with a proton on micelle (gel) formation, and the proton exchanged contributes to the formation of hydrogen bond between deoxycholate molecules.11)

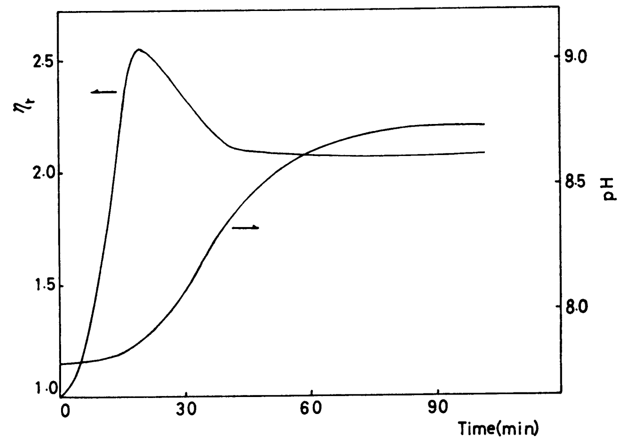

Fig. 8. The time-dependent changes of viscosity and pH which were caused simultaneously in the capillary flow. 1.6×10^{-2} M solution of SDC with addition of 0.5 M NaCl at 15 °C under $\Delta p=80$ cm H_2O.

A special viscometer (Fig. 1) was used in order to determine the pH change accompanied by the change in viscosity. The solution containing SDC of given concentration and 0.5 M NaCl showed a specific rise in pH when viscosity began to rise. However, the rise in pH was gradual, no peak being observed as in viscosity. The dynamic equilibrium in viscosity and pH was reached at the same time. Figure 8 shows an example of both changes in pH and viscosity with time. The measurement of pH shifts was carried out for different concentrations of SDC, shear-rates and temperatures. The results show that the pH shift is independent of concentration and shear-rate, but depends on temperature. On the other hand, viscosity is affected distinctly by shear-rate, concentration and temperature. The characteristic pH behavior indicates that the secondary aggregation or polymerization is not parallel to hydrolysis. The value of pH in equilibrium corresponds to that of the system (Fig. 2, the preceding paper11)) and the range of pH shifts corresponds to the deviation from the straight line with slope 1/2 extrapolated from the singly dispersed region. The mixing of two solutions (SDC and NaCl) indicates that the SDC solution is diluted with NaCl solution, the micelles being broken abruptly due to the shift of equilibrium. After micelles are broken by dilution, new micelles are formed again. In this state the environment of new micelles and singly dispersed species are now rich in sodium ions, almost all the counterions being sodium ions. Micelles of a more stable structure are then formed gradually, the counterions of Na^+ being replaced by protons. This can be considered a relaxation phenomena. Though the exchange reaction is slow, it can be accelerated by mixing or by being carried out in a field of flow. Alternatively, in the early stage of the capillary flow when no rise of viscosity appears, the exchange reaction takes place and stable primary micelles are formed by hydrogen bonding. As soon as sufficient protons are adsorbed on deoxycholate ions in the micelle, the primary micelles begin to associate secondarily forming polymer-like aggregates.

The indispensable role of hydrogen bond in the formation of SDC aggregate was revealed by other studies on pH and pNa behavior of aqueous solution of SDC[11] and the sol-gel transition under high pressure.[12]

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