BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 39 105—112 (1966)

Studies on Long Chain Alkyl Amides of Water-soluble Poly-α-amino Acids. III. The Association of Poly-DL-alanine Dodecylamides in Aqueous Solution

By Shoichi IKEDA, Masakazu MAEKAWA* and Toshizo ISEMURA
Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka

(Received May 15, 1965)

Poly-DL-alanine dodecylamide, prepared by the polymerization of *N*-carboxy-DL-alanine anhydride with dodecylamine initiation at different anhydride-to-initiator ratios, ([A]/[I]), is soluble in aqueous hydrochloric acid solutions to form micelles. Its micellar properties at pH 2.0 have been investigated by means of hydrodynamic methods. The material prepared at [A]/[I] 5 has a critical micelle concentration around 0.2—0.3 g. dl^{-1}, which can be detected by Archibald sedimentation measurements. The micelle has been determined by the Archibald method to consist of about 13 monomer molecules. The molecules in the micelle are hydrogen bonded intermolecularly to form a structure similar to the β-conformation. Hydrogen bonding

---

* Present address: Mitsubishi Paper Mills, Inc., Takasago, Hyogo.

between peptide groups is more important for the micelle formation than hydrophobic bonds between dodecyl groups. The materials prepared at [A]/[I] 15 and 20 associate into large micelles, consisting of about 320 and 240 monomers respectively, as deduced from the sedimentation and viscosity measurements. The micelles exhibit even a flow birefringence. The structure of micelles seems to be a disordered one composed of randomly coiled monomer molecules; intermolecular hydrogen bonding and hydrophobic bonding are cooperative in its stabilization.

A series of amphipathic polypeptides, poly-DL-alanine dodecylamide (PADA) with degrees of polymerization from 5 to 30, has been synthesized by the polymerization of *N*-carboxy-DL-alanine anhydride with dodecylamine initiation, and their physical chemical properties have been investigated in relation to the degree of polymerization.[1,2] PADA is soluble in aqueous hydrochloric acid solution of pH below 5.5, and the surface tension of its aqueous solution decreases considerably with the concentration. The surface activity of PADA decreases with increasing the degree of polymerization, but the lowest molecular weight PADA behaves like a typical amphipathic substance or a surface active agent. It is then expected that PADA molecules will be associated in aqueous solution to form micelles.

It has been accepted that intermolecular hydrophobic bonds, which should be strong between long chain alkyl groups, are important as a cohesive force for micelle formation. In the case of PADA, an additional cohesion, i. e., intermolecular hydrogen bonds between peptide groups, will also contribute to the stability of its micelle. Therefore, the effect of hydrogen bonding should be reflected in the associated states of PADA molecules such as the size of micelle. While PADA has a constitution somewhat similar to that of polyoxyethylene dodecyl ether, the latter being unable to form intermolecular hydrogen bond, it would be of interest to examine the micellar properties of PADA in comparison with those of polyoxyethylene dodecyl ether.

In the present work, the micellar properties of PADA in aqueous hydrochloric acid solutions have been investigated by means of measurements of viscosity, sedimentation and flow birefringence. It was found that the association of PADA is specifically dependent on its degree of polymerization. Since it has been confirmed[3] that poly-DL-alanine is molecularly dispersed in an aqueous solution, the hydrophobic force between dodecyl groups is operative and important in the association of PADA as in the other amphipathic substances; here evidence was also obtained that intermolecular hydrogen bonding between peptide groups also exerts a profound effect on the micellar association. The size and structure of the micelles were discussed in the light of the intermolecular forces and the molecular conformation.

Experimental

Materials.—PADA was synthesized by the polymerization of *N*-carboxy-DL-alanine anhydride by dodecylamine initiation in dioxane.[1] The anhydride-to-initiator molar ratio, [A]/[I], was varied from 5 to 20, to obtain PADA samples with different degrees of polymerization.

Titration of Terminal Amino Groups.—To obtain the number-average degree of polymerization of PADA, the anhydrous titration of terminal amino groups were carried out.[4] About 3 mg. of PADA were dissolved in 1 ml. of a formic acid - acetic acid (1 : 9 v/v) mixture, and the solution was titrated to a blue point of crystal violet with 0.05 N perchloric acid dissolved in acetic acid. The blank titer was then subtracted from the solution titer. The mixed solvent was used, because the indicator color was blue in formic acid even without any titer, and also because PADA was insoluble in acetic acid alone.

Preparation of Aqueous Solutions.—Almost all the experiments were made with PADA solutions in aqueous hydrochloric acid at pH 2.0. The pH's of the solutions were adjusted by the addition of hydrochloric acid a day after solutions were made. Solutions of different concentrations were prepared by diluting a ca. 1 g. dl^{-1} stock solution with water and by readjusting the pH's with hydrochloric acid. The pH values were measured by a Horiba Model H pH-meter. Each solution was filtered through a No. 4 glass filter before use.

Electric Conductivity.—The specific conductances were measured at 25°C with a Yanagimoto MT-7 Conductivity Bridge. The electrodes of a conductivity cell were made of white platinum, since platinized platinum electrodes blocked the electric current completely because of the adsorption of PADA on their surfaces. The cell constant was 0.450, as calibrated by 0.01 M potassium chloride.

Density.—The densities of the solutions were determined at 25°C by an Ostwald type pycnometer with a volume of 3 ml. The partial specific volume of PADA, $\bar{v}$ (ml. g^{-1}), was calculated from the equation

$$\rho=\rho_0+(1-\bar{v}\rho_0)c$$

where ρ (g. ml^{-1}) is the density of the solution at a given concentration, c (g. ml^{-1}), and ρ_0 (g. ml^{-1}) is the density of the solvent, i. e., of 0.01 N hydrochloric acid.

Viscosity.—An Ubbelohde type viscometer with a flow time 140 sec. for water was used for the viscosity measurements. The relative viscosities were referred to 0.01 N hydrochloric acid at 25°C.

---

[1] T. Isemura, S. Ikeda, F. Tokiwa and J. Noguchi, This Bulletin, **34**, 1236 (1961).

[2] S. Ikeda and T. Isemura, ibid., **35**, 1523 (1962).

[3] S. Ikeda, unpublished.

[4] M. Sela and A. Berger, *J. Am. Chem. Soc.*, **77**, 1893 (1955).

Sedimentation. — *The Archibald Method.* — The Archibald method[5-7] was adapted to the determination of the micellar molecular weight of PADA [A]/[I] 5. The measurements were performed in a Hitachi UCA-1 Analytical Ultracentrifuge using a schlieren optical system. A piece of stretched human hair was drawn in place of a schlieren diaphragm. The rotor speed read by an odometer was 41000 r. p. m., and the temperature measured by a radiation thermocouple was 30±1°C. The solutions were centrifuged in a standard cell, putting a thin layer of Dow-Corning Silicone 555 at the cell bottom.[8] Photographic films were enlarged tenfold on an Olympus Model CP-20 Precision Contour Projector and traced on graphic paper. For the calibration of the schlieren pattern to the concentration scale, a synthetic boundary cell of the Pickels type was utilized.

Sedimentation Velocity.—The sedimentation coefficients of PADA [A]/[I] 15 and 20 were determined in a Spinco Model E Analytical Ultracentrifuge using a schlieren optical system. The rotor speed was 44770 r.p.m. The sedimentation coefficients were reduced to those at 25°C by the correction of the solvent viscosity coefficients.

Flow Birefringence.—Both the extinction angles and the birefringences were measured in a Rao Flow Birefringence Instrument, Model 7-B. The birefringence was determined at 517 mμ of a zirconium lamp. The rotor speed was read by counting the speed of the driving motor with a tachometer.

Refractive Index Increment.—The refractive index increments at 547 mμ were measured in a Shimadzu Differential Refractometer of the Debye type against 0.01 M hydrochloric acid at 25°C.

Results

Characterization.—PADA is soluble in formic acid, dichloroacetic acid, phenol and *m*-cresol, in which it will be molecularly dispersed without association. The determination of the molecular weight of PADA in these solvents, however, involves numerous difficulties.* Therefore, the number of terminal amino groups was determined by the anhydrous titration to estimate an approximate number-average degree of polymerization. As is shown in Table I, the degree of polymerization, x_{NH_2}, derived from the number of amino groups is almost equal to the [A]/[I] value, indicating that the synthetic procedure can be well controlled in obtaining PADA of desired molecular weight. The number-average degree of polymerization, x_n, was thus assumed to be equal to the [A]/[I] value. The weight-average degree of polymerization, x_w, was estimated from the number-average one, assuming that the molecular weight distribution follows the Poisson distribution.[12] The results are summarized in Table I.

TABLE I. DEGREE OF POLYMERIZATION OF PADA

Sample number	[A]/[I] $=x_n$	x_{NH_2}	x_w	M_n	M_w	[η] at 25°C in 85% HCOOH
B4201	5	6.4	5.8	539	596	0.036
B4202	10	10.9	10.9	959	1023	0.043
B4203	15	17.5	15.9	1427	1493	0.053
B4204	20	22.8	21.0	1804	1872	0.063
A808 NaOCH$_3$	49					0.086

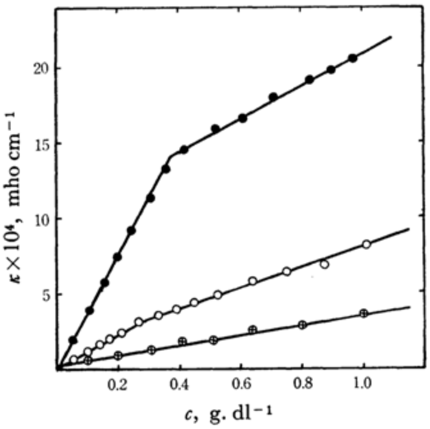

Fig. 1. Specific conductances of PADA solutions at pH 5.0.
○, [A]/[I] 5; ⊕, [A]/[I] 15; ●, dodecylammonium chloride in water

The specific conductances of PADA solutions at pH 5.0 are shown in Fig. 1, together with those of dodecylammonium chloride in water. The latter data are in accord with the published ones.[13] On the conductance curve of PADA [A]/[I] 5, a break similar to the critical micelle concentration (CMC) for dodecylammonium chloride appeared. The CMC of PADA [A]/[I] 5 was found to be 0.295 g. dl^{-1} or 5.4×10^{-3} M. A similar break was observed in the surface tension curve.[1] The CMC was somewhat lower as the pH was lowered.

The [A]/[I] 10 preparation was unstable in an aqueous solution. Its relative viscosity markedly increased with the time elapsed after the solution

---

[5] W. J. Archibald, *J. Phys. Chem.*, **51**, 1204 (1947).

[6] S. M. Klainer and G. Kegeles, ibid., **59**, 952 (1955).

[7] H. K. Schachman "Ultracentrifugation in Biochemistry," Academic Press, New York (1959), p. 181.

[8] A. Ginsburg, P. Appel and H. K. Schachman, *Arch. Biochem. Biophys.*, **65**, 545 (1956).

* The application of the Archibald method was limited by such factors as the negative value of the $1-\bar{v}\rho_0$ term in organic acids, as has been observed elsewhere,[9] the corrosive nature of these acids against the available cell materials and the high viscosity of *m*-cresol. The freezing point depression in formic acid or phenol was much larger than that to be expected from the [A]/[I] value and it was also strongly dependent on the concentration in a dilute region, probably because PADA is ionized at its terminal amino groups or at each peptide group, as has been noted in polyamides.[10,11]

[9] M. Goodman, E. S. Schmitt and D. A. Yphantis, *J. Am. Chem. Soc.*, **84**, 1283 (1962).

[10] J. R. Schaefgen and C. F. Trivisonno, ibid., **73**, 4580 (1951).

[11] H. G. Fendler and H. A. Stuart, *Makromol. Chem.*, **25**, 159 (1957).

[12] S. G. Waley and J. Waston, *Proc. Roy. Soc.*, **A199**, 499 (1949).

[13] A. W. Ralston, C. W. Hoerr and F. J. Hoffman, *J. Am. Chem. Soc.*, **64**, 97 (1942).

was made up, and it did not settle within several days. The sedimentation pattern of the same solution consisted of many discrete peaks. Thus this preparation was not investigated further.

The reduced viscosities of PADA of solutions at pH 2.0 are shown in Fig. 2. The values of the intrinsic viscosities, $[\eta]$, are given in Table II together with those of the partial specific volumes. The latter was also calculated from the partial molar volumes of the constituent atoms.[14] Assuming the micelles to be unhydrated ellipsoids of revolution, the axial ratios of the PADA micelles were estimated from the intrinsic viscosities and partial specific volumes by means of the Simha's equation.[15]

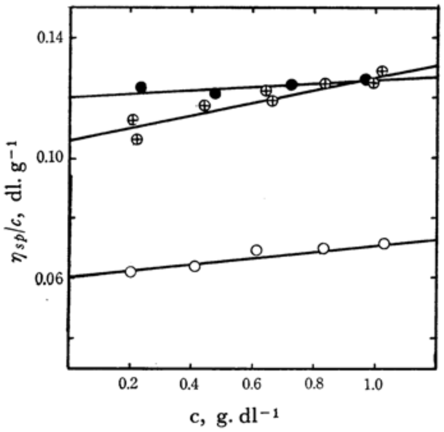

Fig. 2. Reduced viscosities of PADA solutions at pH 2.0.
○, [A]/[I] 5; ⊕, [A]/[I] 15; ●, [A]/[I] 20

TABLE II. INTRINSIC VISCOSITY AND PARTIAL SPECIFIC VOLUME OF PADA AT pH 2.0

Sample number	$[\eta]$ dl. g^{-1}	$\bar{v}$* ml. g^{-1}	Axial ratio, a/b	
			Prolate	Oblate
B4201	0.060	0.834 (0.861)	6.1	1/ 8.7
B4203	0.106	0.793 (0.811)	9.90	1/17.9
B4204	0.120	0.782 (0.770)	10.9	1/20.8
A808**	0.079	0.726 (0.735)		

* Values in parentheses were calculated from molar volumes of constituent atoms.[14]

** Data in distilled water.

The electrostatic effect of ionized terminal groups has not been taken into account in all cases, since it seems likely that a good amount of hydrochloric acid exists to suppress the effect, at least, at pH 2.0.

The Association of PADA [A]/[I] 5.—The Archibald method of sedimentation is based on the fact that the equation for sedimentation equilibrium is formally satisfied at both the meniscus and the bottom of a solution in a sedimentation cell, irrespective of the time of centrifugation. If a solution is centrifuged in a cell with an angular velocity, ω (sec^{-1}), and if the concentration at a distance, r (cm.), from the rotor axis is given by c (g. ml^{-1}) at time t, the apparent molecular weight at the meniscus or the bottom can be obtained by the equation[5-7,16,17]:

$$M_{app}(t)=\frac{\boldsymbol{R}T}{\omega^2(1-\bar{v}\rho)}\left(\frac{\mathrm{d}c/\mathrm{d}r}{rc}\right)_{m\text{ or }b}$$

where T is the temperature and $\boldsymbol{R}$ is the gas constant. The suffix m or b denotes the meniscus or the bottom. For a given initial concentration, c_0 (g. ml^{-1}), $M_{app}(t)$ can be evaluated from the schlieren pattern at any time, t, and then extrapolated to zero time to obtain $M_{app}(0)$ for c_0. The extrapolation is usually performed by postulating a linear relation of $M_{app}(t)$ with t. The apparent molecular weight, $M_{app}(0)$, obtained in this way should be equal at both the meniscus and the bottom; it can be related to the weight-average molecular weight, M_w, of the solute by

$$M_{app}{}^{-1}(0)=M_w{}^{-1}+2Bc_0+\cdots$$

where B is the second virial coefficient.

The determination of the micellar molecular weight of PADA [A]/[I] 5 is somewhat complicated, since it has the CMC. The validity of the Archibald method has been demonstrated even for association-dissociation systems, whether the conversion velocity is fast or slow.[18] However, as far as monomers and micelles coexist in a solution, the usual procedure of the Archibald method, as described above, can not be applied; an extrapolation must be made to the CMC rather than that to an infinite dilution. A similar procedure has been widely adopted in the case of light scattering in micellar solutions.[19-21] In the Archibald method the values of CMC and $\{(\mathrm{d}c/\mathrm{d}r)/r\}_{\mathrm{CMC}}$ must be first determined from the plots such as those of $\{(\mathrm{d}c/\mathrm{d}r)/r\}_{m\text{ or }b}$ against c_m or c_b, in order to obtain the micellar molecular weight. However, these values could not be determined very accurately for the present system, probably because the rates of the mutual conversion between monomers and micelles were not sufficiently fast as compared with the sedimentation velocities, so that the values $M_{app}(t)$ were not independent of the initial concentration.

Consequently, the usual procedure was first adopted for the determination of the molecular weight. Nevertheless, it gave an approximate

---

[14] E. Friedman, T. J. Gill, III, and P. Doty, ibid., **83**, 4050 (1961).

[15] R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

[16] G. Kegeles, S. M. Klainer and W. J. Salem, ibid., **61**, 1286 (1957).

[17] H. Fujita, H. Inagaki, T. Kotaka and H. Utiyama, ibid., **66**, 4 (1962).

[18] G. Kegeles and M. S. N. Rao, *J. Am. Chem. Soc.*, **80**, 5721 (1958).

[19] P. Debye, *J. Phys. Colloid. Chem.*, **53**, 1 (1949).

[20] P. Debye and E. W. Anacker, ibid., **55**, 644 (1951).

[21] L. M. Kushner and W. D. Hubbard, *J. Phys. Chem.*, **58**, 1163 (1954).

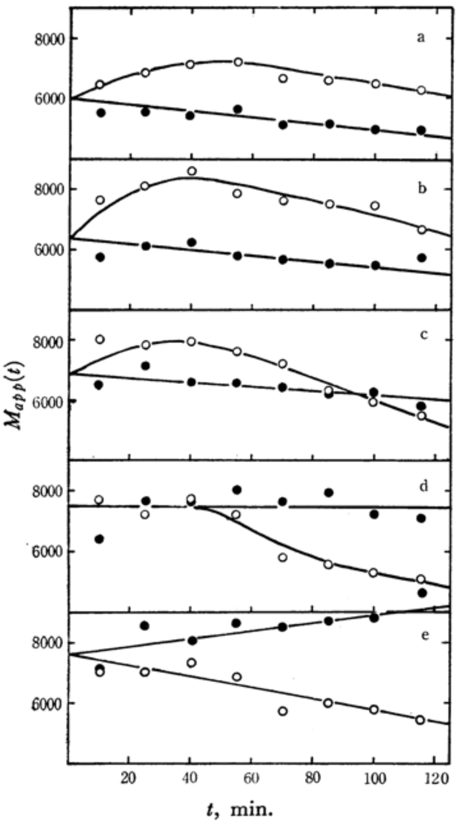

Fig. 3. The $M_{app}(t)$ versus t curves of PADA [A]/[I] 5 solutions at pH 2.0.
○, meniscus; ●, bottom
Rotor speed, 41000 r. p. m.; temp.; 30°C
Concn. (g. dl^{-1}): a, 1.02; b, 0.82; c, 0.61; d, 0.41; e, 0.20.

value for the CMC. The plots of $M_{app}(t)$ against t were drawn for different initial concentrations and the curves were extrapolated to zero time of centrifugation to obtain $M_{app}(0)$. They are shown in Fig. 3. Linear extrapolation was always possible at the cell bottoms, but the slopes of the straight lines varied from negative to positive as the inital concentrations were lowered. On the other hand, some of the plots at the menisci were not linear but upward convex: they initially rise up and then fall gradually with time. Then the intercept at zero time at the meniscus can be made coincident with that at the bottom, thus giving an identical value of $M_{app}(0)$ for a given initial concentration. Such anomalous behavior may be qualitatively explained in the following way.

In general, the fractionation effect of a polydisperse system under centrifugal field results in a decrease in $M_{app}(t)$ with time at the meniscus and in its increase at the bottom. On the contrary, the non-ideality of the system has an opposite effect on the change in $M_{app}(t)$ with time.[16,17] In the present system, however, the micelle formation at the bottom or the dissociation of micelles into monomers at the meniscus seems to be more important than the fractionation effect. At the bottoms for high initial concentrations, the decrease in $M_{app}(t)$ with time can be ascribed to the non-ideality of the micellar solutions. For the lowest initial concentration investigated, the concentration at the bottom increases with time, reaching and then exceeding the CMC after some time of centrifugation; micelles are formed and accumulate there to increase the $M_{app}(t)$ value with time. At the intermediate initial concentration, the micelle-forming effect compensates for the non-ideality of the micellar solution, and $M_{app}(t)$ becomes practically independent of the time.

The initial rise in $M_{app}(t)$ at the meniscus can also be attributed to the non-ideality of the micellar solution. The decrease in $M_{app}(t)$ after some time reflects the dissociation of micelle into monomers through the CMC region. At the lowest initial concentration, the curve monotonously decreases with time, suggesting the dissociation of micelle into monomers. Near the CMC or even above it, micelles leaves the meniscus faster than monomers, and the dilution at the meniscus brings about the dissociation of the remaining micelles; thus $M_{app}(t)$ decreases with time. These results indicate the presence of rather a broad concentration region for micelle formation.

After such a justification for the non-linear relation of $M_{app}(t)$ with t, the same intercept, $M_{app}(0)$, can be derived from both the meniscus and the bottom.* Then the intercept, $M_{app}(0)$, was plotted against c_0, as is shown in Fig. 4. The curve clearly indicates[22] that the CMC locates around 0.3 g. dl^{-1}. The linear part of the curve is extrapolated to the CMC The intercept gives

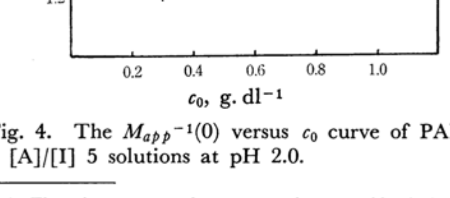

Fig. 4. The $M_{app}{}^{-1}(0)$ versus c_0 curve of PADA [A]/[I] 5 solutions at pH 2.0.

---

* There is no reason, however, to have an identical value for $M_{app}(0)$ at the meniscus and at the bottom, epecially for the present system. Solute molecules adsorbed at the meniscus, i. e., at the air-water interface, need not necessarily have an identical apparent molecular weight with that in solution or at the bottom, for they may have different conformations or different associated states.

22) E. Hutchinson, *J. Colloid Sci.*, **9**, 191 (1954).

TABLE III. MICELLAR PROPERTIES OF PADA [A]/[I] 5 (B4201)

CMC g. dl^{-1}	M_w	m (Number of association)	B mol. ml. g^{-1}
0.3	7700	13	2.5×10^{-3}

the weight-average molecular weight of the micelle, while the slope gives its second virial coefficient, omitting some contribution of the monomers present. The micellar molecular weight and the second virial coefficient of PADA [A]/[I] 5 obtained in this way are listed in Table III. The molecular weight, 7700, or the number of association, 13, is very small as compared with that of the corresponding derivatives of polyoxyethylene.[23-25]

A method similar to that described above was applied to a solution of 1.0 g. dl^{-1} at pH 5.0; it gave a value of $M_{app}(0)$ 3300. The micellar molecular weight would be lower than that at pH 2.0, even if the second virial coefficient is taken into account. Hence, lowering the pH serves merely to promote the association of PADA; free hydrochloric acid would act to suppress the electrostatic interaction.

The Association of PADA [A]/[I] 15 and 20. —The sedimentation velocities of PADA [A]/[I] 15 and 20 in solution were both sufficiently high, even at low rotor speeds. The sedimentation pattern of PADA [A]/[I] 15 consisted of two components, one amounting to about 95% and the other appearing as a faster shoulder. The shoulder is probably a remnant of discretely heterogeneous aggregates, as was observed in the [A]/[I] 10 preparation. PADA [A]/[I] 20 gave a symmetrical peak. The sedimentation coefficients of the main peaks are plotted against the concentration in Fig. 5. From the extrapolation to infinite dilution, the sedimentation constants, $s°$, were obtained; they are given in Table IV.

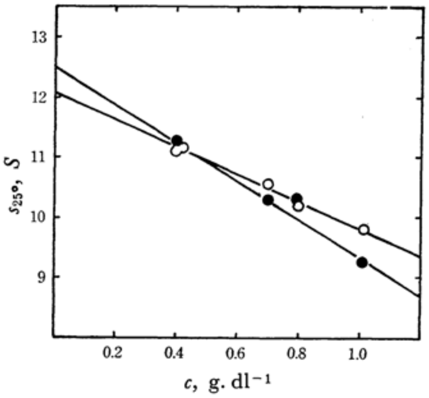

Fig. 5. Sedimentation coefficients of PADA solutions at pH 2.0.
●, [A]/[I] 15 (main component); ○, [A]/[I] 20

TABLE IV. MICELLAR PROPERTIES OF PADA [A]/[I] 15 AND 20

[A]/[I]	$s^0_{25°}$ S	Simha-Perrin			Scheraga-Mandelkern	
			M	m	M	m
15 (B4203)	12.5	Prolate Oblate	509000 599000	340 400	477000	320
20 (B4204)	12.1	Prolate Oblate	468000 576000	250 310	447000	240

Assuming the micelles to be unhydrated ellipsoids of revolution, and omitting the contribution of minor components in the [A]/[I] 15 preparation, the micellar molecular weights were estimated by means of the Simha-Perrin tretament.[15,26,27] The values for both prolate and oblate ellipsoids are listed in Table IV. They may be limits of the micellar molecular weights. Alternatively, assuming the micelles to have a structure similar to random coils, the Scheraga-Mandelkern equation[28]

$$M^{2/3}=\frac{Ns^0[\eta]^{1/3}\eta_0}{\beta(1-\bar{v}\rho_0)}$$

can give another estimate of the micellar molecular weights, as is also given in Table IV. Here η_0 is the viscosity coefficient of the solvent, N the Avogadro number and β is a shape factor having a value of 2.5×10^6 for random coils.

The micellar molecular weights obtained by the Scheraga-Mandelkern treatment are rather in better agreement with those obtained by the Simha-Perrin treatment for the prolate ellipsoidal model, although the latters are extreme cases without hydration. PADA might have the micellar molecular weights of 480000 and 450000 for [A]/[I] 15 and 20, respectively. Their numbers of association are 320 and 240, respectively. Therefore, the PADA micelles are much larger than those of the corresponding polyoxyethylene derivatives.[23-25] Since poly-DL-alanine does not associate in an aqueous solution,[3] it seems likely that the number of association of PADA micelle decreases with increasing the degree of polymerization, as far as the latter exceeds about ten.

At pH 5.0 the sedimentation experiments were performed on solutions of about 1 g. dl^{-1}. The sedimentation coefficients were 5.6 S and 5.4 S for [A]/[I] 15 and 20, respectively. They are lower than those at pH 2.0, This again indicates that the micelles at higher pH's consist of a smaller number of molecules than those at lower pH's, and that the excess hydrochloric acid merely suppresses the electrostatic effect.

---

[23] J. Stauff and J. Rasper, *Kolloid-Z.*, **151**, 148 (1957).

[24] T. Nakagawa, H. Inoue, K. Kuriyama and T. Oyama, *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zassi*), **79**, 345 (1958).

[25] F. Tokiwa and T. Isemura, This Bulletin, **35**, 1737 (1962).

[26] F. Perrin, *J. phys. radium*, [7] **7**, 1 (1936).

[27] J. W. Mehl, J. L. Oncley and R. Simha, *Science*, **92**, 132 (1940).

[28] H. A. Scheraga and L. Mandelkern, *J. Am. Chem. Soc.*, **75** 179 (1953).

Discussion

It may be supposed that the instability of the solution of the [A]/[I] 10 preparation that there are two kinds of PADA micelles, depending on the degree of polymerization. In fact, the low molecular weight PADA forms a micelle consisting of only 13 monomer molecules, while the higher molecular weight PADA forms a micelle the number of association of which amounts to a few hundred for [A]/[I] 15 and 20.

Structure of the Small Micelle.—It has been shown from infrared spectral studies(1,2) that PADA [A]/[I] 5 assumes the β-conformation in the solid state. In the β-conformation the polypeptide chains are extended and arranged in an antiparallel way, and hydrogen bonds are formed between peptide groups of different chains. A similar structure may be expected to exist in the micelle. In solutions of acid pH's, however, the intermolecular hydrogen bonds would compete with the electric repulsion between ionized amino groups. If the solution is not dilute enough, ionized amino groups would be shielded electrically by other ionized molecules and the PADA molecules would associate into a micelle through the intermolecular hydrogen bonds: PADA has a structure similar to the β-conformation. If the solution is so dilute that the electric repulsion is not sufficiently suppressed, the intermolecular hydrogen bonds would be disrupted and the PADA molecules would assume a random coil conformation without associating with one another. Therefore, a sharp change in solution properties should occur at some intermediate concentration. The CMC would correspond to this concentration, where the association-dissociation of molecules and the coil-β transition of conformation take place simultaneously.

If the micelle has a structure similar to the β-conformation in the solid, dodecyl groups are directed outwards within the plane of polypeptide chains alternately. The micellar dimension can be roughly estimated for this model. This axial ratio of an oblate ellipsoid approximating this model was about 9, which is comparable with that derived from the intrinsic viscosity. Alternatively, by converting the model into an equivalent disk, the second virial coefficient may be evaluated by means of an excluded volume equation.(29) It was found to be 1—2×10^{-3} c. g. s., which was of the same order of magnitude as the observed value, 2.5×10^{-3} c. g. s. The correction for polydispersity and intermolecular interaction may explain the discrepancy between the calculated and observed values.

From these considerations regarding the micellar size and shape in relation to the conformation of PADA in solid, it may be concluded that the micelle of PADA [A]/[I] 5 has a structure similar to the β-conformation.

Structure of the Large Micelles.—The micelles of high molecular weight PADA are different from the small micelle of the [A]/[I] 5 preparation. The micellar size decreases with the degree of polymerization, and poly-DL-alanine does not associate in an aqueous solution. Such a dependence of the micellar size on the monomer size is similar to that of polyoxyethylene derivatives.(25) Hydrophobic bonds between dodecyl groups are certainly an important factor in the cohesion of the micelles. However, intermolecular hydrogen bonding between peptide groups is also effective in the association, since the micellar size of PADA is much larger than that of polyoxyethylene derivatives. In connection with the conformation in the solid state and the hydrodynamic behavior of the micelles, it can be concluded that the large micelles of PADA [A]/[I] 15 and 20 have a structure similar to that of random coils; the conformation of each molecule is randomly coiled in these micelles.

It may be expected that the large PADA molecule can be oriented by shear flow to exhibit flow birefringence. Preliminary experiments on flow birefringence were made with the two preparations, [A]/[I] 15 and 20. The results concerning PADA [A]/[I] 20 are shown in Fig. 6. A slight dependence of the extinction angle, χ, and of the birefrigence increment, $\Delta n/c$ on the concentration was observed. Since the micellar structure and its rigidness are not known accurately, no definite conclusion on the micellar size and shape can be derived from these results. If the micellar shape is assumed to be a hydrodynamically

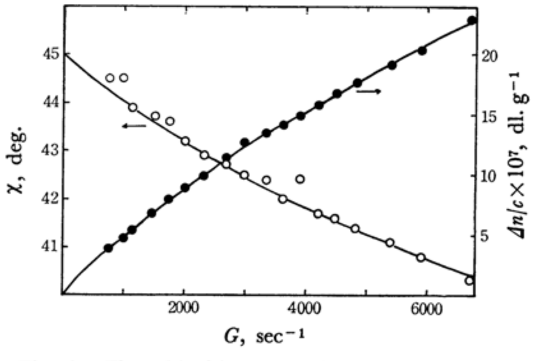

Fig. 6. Flow birefringence of PADA [A]/[I] 20 solution at pH 2.0.
Concn., 0.94 g. dl^{-1}; temp., 16°C
○, Extinction angle; ●, Birefringence increment

TABLE V. OPTICAL PROPERTIES OF PADA MICELLES

[A]/[I]	Θ sec^{-1}	g_1-g_2 $\times10^{-3}$	dn/dc ml.g^{-1}	Ellipsoid	n_1	n_2
15 (B4203)	1000	0.6	0.185	Prolate	1.55	1.59
20 (B4204)	5000	1.0	0.187	Oblate	1.64	1.56

---

29) A. Isihara, *J. Chem. Phys.*, **18**, 1446 (1950).

equivalent ellipsoid derived by the Simha treatment, the rotary diffusion coefficient, Θ, and the optical anisotropy factor, g_1-g_2, can be obtained by the use of the equations of Peterlin and Stuart.[30] These values are listed in Table V. The rotary diffusion coefficients, however, lead to too large micellar dimensions when they are compared with those derived from the sedimentation experiments. This may be due to the polydispersity of the micelles and to the concentration dependence of the extinction angle.

If the data on birefringence increment, $\Delta n/c$, and refractive index increment, $\mathrm{d}n/\mathrm{d}c$, are combined, the principal refractive indices, n_1 and n_2, for the ellipsoids of revolution can be obtained; these values are also given in Table V. It was found that the principal refractive indices of the micelles are equal to each other for the two preparations, [A]/[I] 15 and 20, whether a prolate or an oblate ellipsoid is postulated. This suggests that the micelles formed from these two preparations have similar structures.

---

30) A. Peterlin and H. A. Stuart, *Z. Physik,* **112**, 1 (1939).