Physicochemical Properties of Prostaglandin $F_{2\alpha}$ (Tromethamine Salt): Solubility Behavior, Surface Properties, and Ionization Constants

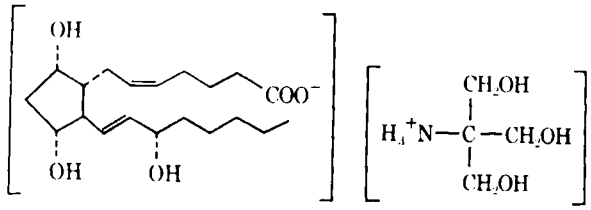
T. J. ROSEMAN▲ and S. H. YALKOWSKY

Abstract □ The pH-solubility profile of the tromethamine salt of $PGF_{2\alpha}$ was determined. The intrinsic solubility of the free acid is 0.0042 *M* at 25° and, as expected for a carboxylic acid, the solubility increased as a function of pH. However, at a pH value just above 5, there is a marked increase in solubility, which is significantly greater than would be expected from the ionization constant of the acid. This type of behavior is attributed to the formation of micelles that solubilize the drug once a critical concentration and pH are reached. Independent determination of the critical micellar concentration (CMC) by drop volume or potentiometric techniques demonstrated that $PGF_{2\alpha}$ tromethamine does form micelles. The CMC increased with the degree of ionization and, therefore, is a function of pH. The pKa of $PGF_{2\alpha}$ was determined by titration in aqueous media to be 4.90 ± 0.02. The pKa increased above the CMC as a function of concentration to a maximum value of 5.6. When a plot of concentration *versus* pKa is made, the point of deviation from the true value of 4.90 is in good agreement with the CMC reported by the drop volume technique. The interrelationship between the potentiometric, solubility, and surface properties of $PGF_{2\alpha}$ tromethamine are presented. Interestingly, these properties are qualitatively similar to those reported for certain bile acids that have similar functional groups present on the parent cholanic acid nucleus.

Keyphrases □ Prostaglandin $F_{2\alpha}$, tromethamine salt—solubility behavior, surface properties, and ionization constants □ pH-solubility profiles—prostaglandin $F_{2\alpha}$ tromethamine salt, micelle formation □ Micelle formation—factor in solubilization of prostaglandin $F_{2\alpha}$ tromethamine salt

Prostaglandins are a class of biologically active lipids found in various animal and human tissues. In recent years, the scientific literature of many disciplines has virtually exploded with publications on prostaglandin research. Several reviews (1–3) summarized prostaglandin research in the areas of chemical synthesis, biosynthesis and metabolism, analytical methods, biology, and clinical applications. From a clinical point of view, $PGF_{2\alpha}$ and PGE_2 have been the most widely tested prostaglandins; their use in induction of labor and in therapeutic abortion is well documented (4).

Prostaglandins have been administered to patients as parenteral and vaginal solutions, vaginal tablets, and suppositories. Yet, studies on their physicochemical properties have been limited. Although Johnson and Saunders (5) reported that $PGF_{2\beta}$ and PGE_1 do not form micelles, their studies did not cover a sufficiently wide concentration range. Data are presented which demonstrate that $PGF_{2\alpha}$ (as the tromethamine salt) does indeed form micelles. The present article describes an in-depth study on certain physicochemical properties of $PGF_{2\alpha}$. These properties are characterized with regard to solubility behavior, surface properties, and ionization constants, and the interrelationships among them are demonstrated. In this respect, these findings are consistent with the studies of Ekwall *et al.* (6) using bile salts. They showed that



I

micelle formation affects other physicochemical properties such as ionization and solubility behavior. A knowledge of these properties will provide not only a rational basis for the design of formulations but also an insight into other areas of prostaglandin research. For example, structure–activity relationships based upon partition coefficient data can be influenced by micelle formation since the presence of micelles may alter partition values. Micelle formation and ionization behavior can also influence the absorption pattern of the drug molecule.

EXPERIMENTAL

Prostaglandin $F_{2\alpha}$ was used as its tromethamine salt (1). This salt was selected because it is crystalline and could be obtained in high purity. The free acid exists as a wax-like material and is not only difficult to purify but also is subject to weighing errors when small quantities are needed. As the tromethamine salt, $PGF_{2\alpha}$ was 99.2% pure by GLC and showed one major spot using TLC. The melting point was 100.3–101.3°. All solutions were prepared with compression distilled water, and reagent grade chemicals were used.

Solubility Determinations—Appropriate quantities of $PGF_{2\alpha}$ (tromethamine) were placed into 20-ml. glass screw-capped vials containing distilled water. The solution was then adjusted to the desired pH with 0.1 *N* HCl. The final solutions were examined visually to be sure that excess drug was present. The vials were then sealed with a wax film[1] and rotated end-over-end in a thermostated water bath at 25 ± 0.1°. After equilibration, the pH was again measured and this equilibrium value was used in reporting the data. Samples were rotated for at least 48 hr., which was previously shown to be sufficient for establishing equilibrium. At pH values ≤3, some drug decomposition was noted at 48 hr.

After equilibration, samples were centrifuged for approximately 15 min. and then filtered through a 13-mm., 0.22-μ filter[2]. The resulting clear solutions were analyzed for their $PGF_{2\alpha}$ content by GLC. One milliliter was extracted with 3 × 10 ml. of chloroform after the addition of 2 ml. of 0.2 *M* pH 3 citrate buffer. Previous studies demonstrated that this procedure yields quantitative recovery of $PGF_{2\alpha}$. The chloroform was evaporated to dryness at 37° with a stream of nitrogen. The residue was then dissolved in 0.1 ml. of pyridine and silylated with a 4:1 mixture of *N,O*-bis(trimethylsilyl)acetamide[3]–trimethylchlorosilane[3] containing 3 mg./ml. of cholesteryl acetate as the internal standard. The volume of silylating reagent varied depending upon the amount of $PGF_{2\alpha}$ present. The reagent was swirled in the flask to ensure that all $PGF_{2\alpha}$ was silylated. After al-

[1] Parafilm, American Can Co., Neenah, Wis.
[2] Millipore Corp., Bedford, Mass.
[3] Specially purified grade, Pierce Chemical Co., Rockford, Ill.

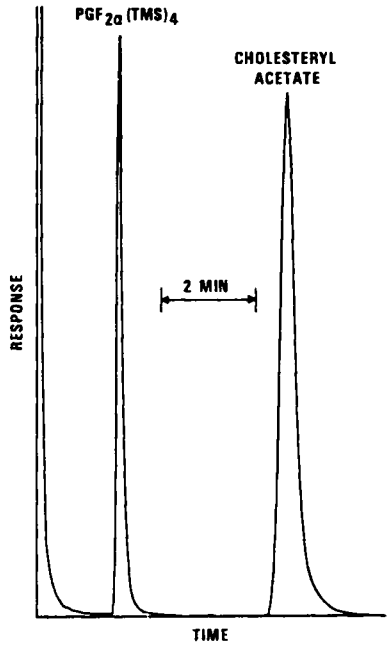


Figure 1—*GLC chromatogram of silylated $PGF_{2\alpha}$ and internal standard (cholesteryl acetate).*

lowing the solution to stand 15 min., a 1-μl. sample was injected into a chromatograph[4] under the following conditions: glass column, 1.2 m. × 0.63 cm. (4 ft. × 0.25 in.) 1% SE-52 on Gas Chrom Q (80–100 mesh); column temperature, 215°; detector temperature, 265°; and flash heater temperature, off. The helium gas flow was 60 ml./min.; air and hydrogen were adjusted to give maximum response.

Two references were prepared at each assay time. Exactly 1 mg. of $PGF_{2\alpha}$ tromethamine was weighed on an electrobalance[5] and dissolved in 0.1 ml. pyridine. It was then silylated in an identical manner as the samples. A typical chromatogram is shown in Fig. 1. The $PGF_{2\alpha}$ concentration, C, in milligrams per milliliter was calculated from the following expression:

$$C = \frac{R}{R_1} C_1 \frac{V_1}{V_{12}} \qquad \text{(Eq. 1)}$$

where:

R = ratio of sample peak area to internal standard peak area
R_1 = ratio of standard peak area to internal standard peak area
C_1 = concentration of standard
V_1 = volume of sample reagent
V_{12} = volume of solution extracted

CMC Determinations—Solutions of $PGF_{2\alpha}$ tromethamine were prepared by dissolving the drug in distilled water and adjusting the pH with 0.001–0.1 *N* HCl or 0.001–0.1 *N* NaOH. Solutions were then transferred to a 2- or 10-ml. syringe[6] equipped with a Teflon plunger. The syringe was mounted on an infusion pump[7] adjusted to deliver 0.08 ml./min. The surface tension (γ) of the solution was calculated from the average volume of 10 drops at ambient temperatures (25 ± 2°). The apparatus and calculations were reported previously (7).

pKa Determinations—The pKa values were determined by titrating aqueous solutions of different concentrations of $PGF_{2\alpha}$ tromethamine with 1.0 *N* HCl. The hydrochloric acid normality was chosen to minimize volume changes during the titration. The solutions were decarbonated by bubbling with purified nitrogen for 15 min. Titrations were carried out in a nitrogen atmosphere. A digital pH meter[8] equipped with a combination electrode[9] was used to measure pH.

[4] Hewlett Packard, model 402, Skokie, Ill.
[5] Cahn Corp., Paramount, Calif.
[6] Hamilton, Whittier, Calif.
[7] Sage, White Plains, N. Y.
[8] Sargent-Welch model NX, Skokie, Ill.
[9] Sargent-Welch model S-30072-15, Skokie, Ill.

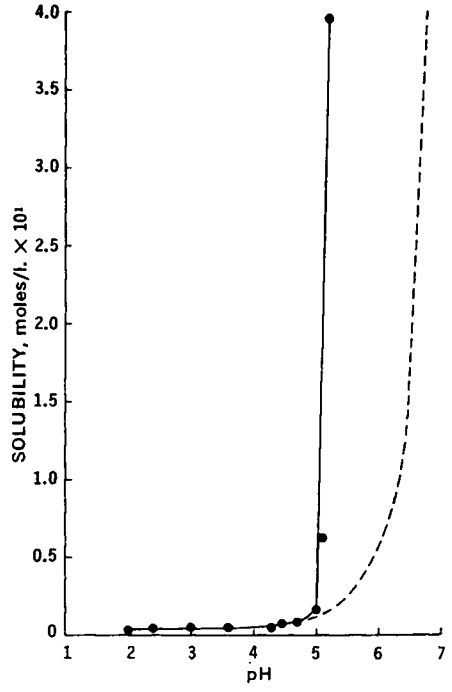


Figure 2—*pH-solubility profile of $PGF_{2\alpha}$ tromethamine. Key: ●, experimental value; and – –, predicted based upon pKa = 4.9.*

The hydrochloric acid was added using a syringe[6] calibrated to deliver 0.01 ml. Titrations of small volumes (2–4 ml.) were accomplished by using a cone-shaped container and a microstirring bar. After the addition of each increment of hydrochloric acid, the solution was allowed to equilibrate to a constant pH before the pH was recorded. The pKa at different percents of drug neutralized was calculated from the titration curve according to the method of Albert and Serjeant (8), and the pKa values were calculated from the following expression:

$$\text{pKa} = \text{pH} + \log \frac{C_T(\beta) - H_3O^+}{C_T(1-\beta) + H_3O^+} + \frac{0.505\sqrt{I}}{1 + 1.6\sqrt{I}} \qquad \text{(Eq. 2)}$$

where C_T is the total concentration of prostaglandin present (P^- + PH) and β is the degree of neutralization, *i.e.*, PH/(P^- + PH). The ionic strength contribution to the pKa was negligible below the CMC. At concentrations below the CMC, the reported pKa is an average of nine values from the titration curve.

RESULTS AND DISCUSSION

Solubility—The intrinsic solubility of the free acid of $PGF_{2\alpha}$ is 1.5 mg./ml. (0.0042 mole/l.). As shown in Fig. 2, the solubility of $PGF_{2\alpha}$ as the tromethamine salt increases with pH. The sharp (35-fold) increase in solubility with pH between 5.0 and 5.2 is far greater than would be expected on the basis of normal ionization

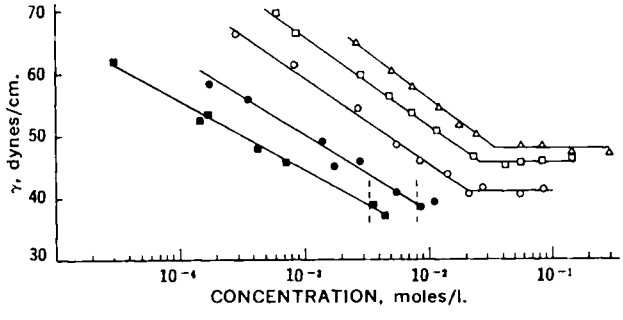


Figure 3—*Effect of pH on the surface tension plots of $PGF_{2\alpha}$ tromethamine in water. Solutions to the right of the vertical lines were colloidal for pH 3.0 and 5.0. Key: ■, pH 3.0; ●, pH 5.0; ○, pH 6.0; □, pH 8.0; and △, pH 10.0.*

Table I—CMC (Moles per Liter) of $PGF_{2\alpha}$ Tromethamine

pH	Medium	CMC Method—Titration	CMC Method—Surface Tension
10.0	Water	—	0.033
8.0	Water	—	0.026
6.0	Water	0.022	0.021
5.8	Water	0.022	—
5.5	Water	0.014	—
5.0	Water	—[a]	—[a]
3.0	Water	—[a]	—[a]
6.0	0.2 *M* Tromethamine	—	0.018
6.0	0.2 *M* NaCl	—	0.022
3.0	0.2 *M* Tromethamine	—	—[a]

[a] Since micelle formation could not be distinguished from precipitation, no value is reported.

of the acid. For purposes of comparison, the solubility that would be predicted on the basis of the following equation (9) is also shown:

$$S = S_0\left(1 + \frac{K_a}{H_3O^+}\right) \qquad \text{(Eq. 3)}$$

where S_0 is the intrinsic solubility of the acid and $K_a = 1.26 \times 10^{-5}$. This theoretical plot assumes that the solubility of the salt is much greater than the free acid. Although the exact solubility of the salt could not be determined, it is at least 500 mg./ml.

Micelle Formation—The unusual solubility behavior suggested that some type of molecular association, such as micelle formation, occurs above pH 5. Therefore, surface tension experiments were initiated to determine whether or not micelle formation occurs and to evaluate the CMC as a function of pH. Figure 3 shows the surface tension *versus* concentration plots for $PGF_{2\alpha}$ tromethamine at several pH values. At pH 3 and 5, surface tension lowering is evident, but the limited solubility of the drug precluded the distinction between micelle formation and precipitation. At the higher pH values, a well-defined plateau region is present, and the CMC is readily determined from the point of intersection of the two straight lines. These values are tabulated in Table I along with values calculated from the titration data. It was reported previously that, based on surface tension data, prostaglandins $F_{1\beta}$ and E_1 (5) do not form micelles. The discrepancy between that report and the present data could lie in the fact that the earlier study was not extended to sufficiently high concentrations to detect micelles.

The dependence of the CMC on pH is shown in Fig. 4. The points at the lower pH values represent the total solubility of the monomeric material, and it was not possible to measure a true CMC. From the data in Fig. 4, it can be seen that in the neutral pH range the micelles formed are likely to be mixed micelles composed of unionized and ionized $PGF_{2\alpha}$. This type of behavior is in agreement with results of similar studies using other surfactants (10, 11). Figure 5 shows the effect of salt on the surface tension–concentration plots at pH 3 and 6. Neither the slopes (discussed later) nor the CMC values (Table I) appear to be very dependent upon the presence of salt. Independent determination of exact CMC values utilizing the dye

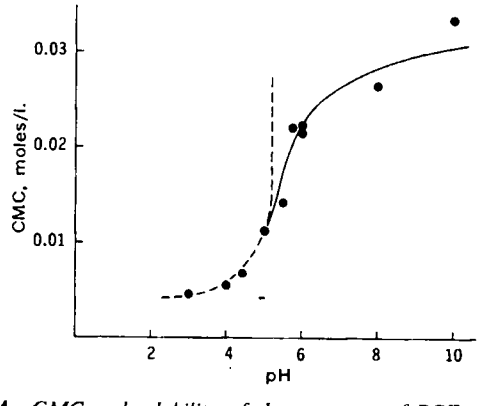


Figure 4—*CMC and solubility of the monomer of $PGF_{2\alpha}$ tromethamine as a function of pH. Key: - - -, solubility of the monomer; and —, CMC.*

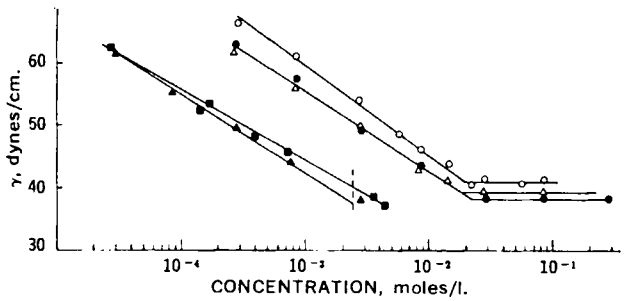


Figure 5—*Effect of salt on surface tension plots of $PGF_{2\alpha}$ tromethamine at pH 3.0 and 6.0. Solutions to the right of the vertical line were colloidal for pH 3.0. Key: ▲, pH 3.0, 0.2 M tromethamine; ■, pH 3.0, water; △, pH 6.0, 0.2 M tromethamine; ●, pH 6.0, 0.2 M NaCl; and ○, pH 6.0, water.*

solubilization method was unsuccessful due to assay interference by $PGF_{2\alpha}$. However, an increase in solubility of the dye (anthracene) was noted at approximately the CMC value. The CMC value calculated from titration data was in agreement with the value from surface tension measurements.

Micelle formation describes an association of several molecules showing a change in slope of a physical property at a critical concentration. Certain molecules may show this type of behavior and yet aggregate according to a stacking-type arrangement (12). This type of association can be characterized for rigid molecules where cooperative interaction of hydrophobic portions of the molecules is limited. Estimates of thermodynamic constants from CMC data may be misleading when this occurs (12). In the case of $PGF_{2\alpha}$, sufficient data are not available to determine the specific nature of the association process. However, space-filling molecular models show that $PGF_{2\alpha}$ has flexible hydrophobic chains (upper and lower) which could interact in a cooperative manner and probably form micelles in the classical sense.

Titration Curves—The titration curves in Fig. 6 are plots of pH *versus* fraction of total titrant added, β, obtained at various concen-

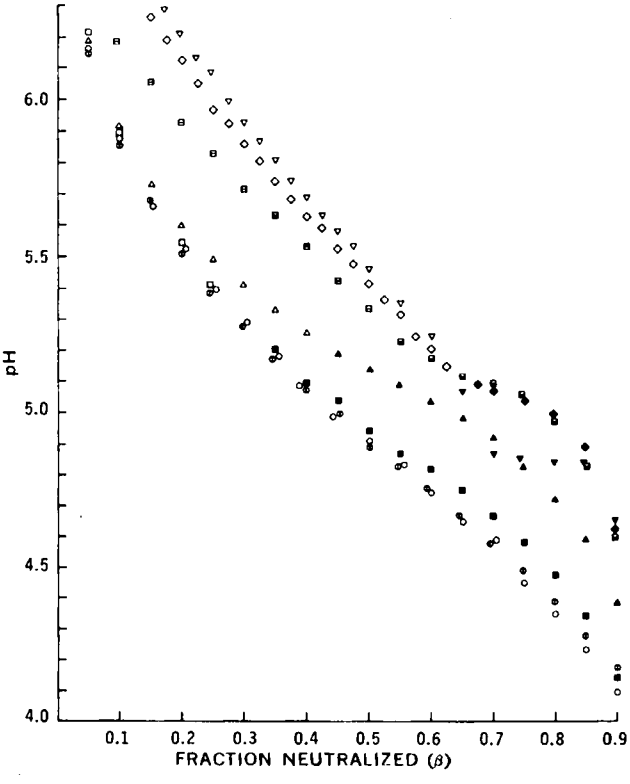


Figure 6—*Representative titration curves for $PGF_{2\alpha}$ tromethamine for various concentrations. Shaded symbols indicate precipitation. Key: ⊕, 0.001 M; ○, 0.002 M; □, 0.01 M; △, 0.02 M; ⊟, 0.05 M; ◇, 0.10 M; and ▽, 0.50 M.*

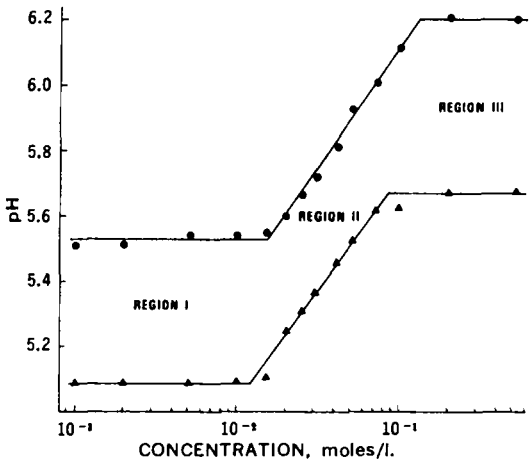


Figure 7—*pH-concentration profile for $PGF_{2\alpha}$ tromethamine. Key: ●, $\beta = 0.2$; and ▲, $\beta = 0.4$.*

trations. The fraction of total titrant is also referred to as the degree of neutralization of the system. The curves are independent of drug concentration at low concentrations, but they are displaced toward higher pH values as the concentration is increased and again become nearly superimposable at high drug concentrations. The shaded symbols indicate the regions where the system is colloidal, and these occur at $PGF_{2\alpha}$ tromethamine concentrations equal to or greater than 0.01 *M* and below a pH of about 5.1. These points were found to be less reproducible and more time dependent than those obtained for the true solutions, because equilibrium within the oil droplets could not be reached rapidly and with certainty. These points are indicated only to show the behavior of the titration curves in the situation where oiling out occurs. The slow dissolution of the oil droplets would explain why precipitation occurs in some cases at higher pH values than noted in the solubility profile. Only the values obtained for true solutions were used in calculating the data for the next section.

When pH is plotted against drug concentration at a constant β value, three distinct regions are noted, as in Fig. 7 for $\beta = 0.2$ and 0.4. At concentrations within the first region (I), only the monomeric species is present and a constant value for pH results. Above the CMC, the curves deviate from ideality. In this region (II), the pH changes with concentration due to the contribution of two species, monomer and micelle. Above 0.1 *M* (Region III), the pH again becomes constant since the micellar species predominates and the fraction of monomers becomes negligible.

The titration curves in Fig. 6 were also used to determine the CMC of $PGF_{2\alpha}$ as a function of pH. By plotting the drug concentration at which each curve crosses a particular pH, two curves are obtained which intersect at the CMC. When only monomers are present, as in Fig. 7, β is independent of concentration; however, it begins to deviate from the monomer value at the CMC (Fig. 8). This procedure for determining the CMC is equivalent to the pH-stat method described by Wood *et al.* (13). The CMC at pH 6.0 was in good agreement with the value determined from surface tension measurements (Table I and Fig. 3).

Ionization Constants—The pKa of $PGF_{2\alpha}$ tromethamine was

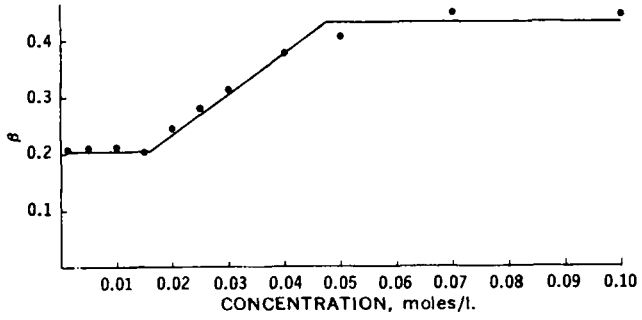


Figure 8—*Degree of neutralization (β) of $PGF_{2\alpha}$ tromethamine as a function of concentration at pH 5.5.*

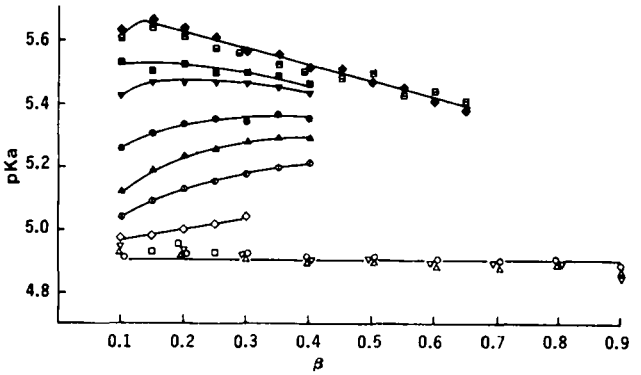


Figure 9—*pKa of $PGF_{2\alpha}$ tromethamine as a function of β at different concentrations. Key: △, 0.001 M; ○, 0.002 M; ▽, 0.005 M; □, 0.01 M; ◇, 0.02 M; ⊕, 0.03 M; ▲, 0.04 M; ●, 0.05 M; ▼, 0.07 M; ■, 0.1 M; ◆, 0.2 M; and ⊟, 0.5 M.*

calculated for each point in the raw titration curves (Fig. 6) as described in the *Experimental* section. The plots in Fig. 6 consider three variables (solute concentration, pH, and β), which are interrelated as shown in Eq. 2. The resultant pKa values as a function of β for different drug concentrations are shown in Fig. 9. At concentrations below 0.01 *M*, the pKa is equal to 4.90, as would be expected for an aliphatic carboxylic acid, and is independent of β and prostaglandin concentration. As the drug concentration is increased above the CMC, the pKa becomes dependent upon both β and prostaglandin concentration. In all cases, the apparent pKa increases as a function of the fraction of micelles present. This is shown graphically in Fig. 10A, where the plots are made for constant β values. Again, three regions are present which correspond to those described in Fig. 7. This type of behavior has been noted for other surfactant systems (14–16).

The higher pKa of the micellar $PGF_{2\alpha}$ compared to the monomer results from the fact that the negative carboxyl groups in the micelle are closer to one another than they are in free solution. In this respect, they resemble titratable groups of a polyelectrolyte (17–21). The accumulation of negative charges on the micellar surface places each individual carboxylate in a negatively charged environment. As a result, there are more protons in the vicinity of each carboxyl

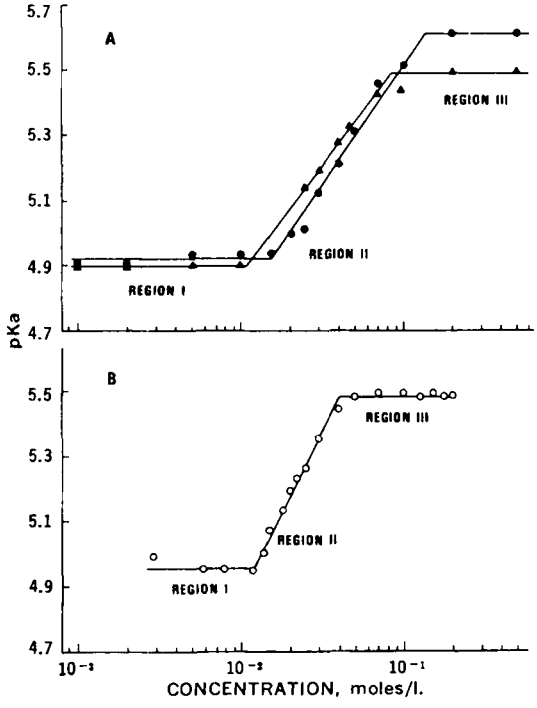


Figure 10—*(A) pKa as a function of concentration at different values of β for $PGF_{2\alpha}$ tromethamine. Key: ▲, $\beta = 0.4$; and ●, $\beta = 0.2$. (B) pKa as a function of concentration for cholic acid at 20° (14).*

group. Thus, deprotonation of each group is inhibited and the observed pKa increases. The magnitude of the increase in pKa is related to the number and closeness of the negative charges on the surface of the micelle. Therefore, the pKa of the ionized micelle ($\beta \rightarrow 0$) is greater than that of the more neutral micelle ($\beta \rightarrow 1.0$), and a negative slope would be expected for a pKa *versus* β plot. Ideally, the pKa of micelles at $\beta = 1.0$, where all of the carboxyl groups are in their neutral protonated form, should be equal to about 4.9 (the intrinsic value of a free aliphatic carboxyl group). When this is the case, the surface potential of the micelle at any value of β can be determined from the difference between the pK observed at that value of β and the intrinsic pK of the titratable group (17–21). The surface potential could be used to calculate the size of the micelle (21). However, other factors such as hydrogen bonding and changes in micelle size and shape tend to complicate the situation, especially for polyfunctional structures such as the prostaglandins.

The maximum surface potential of the micelle was calculated to be −41 mv. This value is less than the values reported for dimethyldodecylamine oxide (22) and long-chain acylcarnitines (21) and suggests that $PGF_{2\alpha}$ forms a loosely packed micelle.

At concentrations slightly above the CMC, the pK *versus* β plots (Fig. 9) have a positive slope. As mentioned earlier, a negative slope would be expected for micelles or a fixed mixture of monomers and micelles. This apparent discrepancy is explained by the fact that as β increases, the CMC decreases and, therefore, the fraction of micelles increases. This causes an increase in the observed pKa, which overcompensates for the decrease due to the neutralization of the surface charge as β increases. The following equation expresses this dependence in mathematical terms (11):

$$\text{pKa(observed)} = \text{pH} + \log\left[\frac{\beta - (C_0\beta_m/C_T)}{(1 - C_0/C_T) - (\beta - C_0\beta_m/C_T)}\right] \quad \text{(Eq. 4)}$$

where β_m is the degree of neutralization of the monomers, C_T is the total concentration of prostaglandin, and C_0 is the CMC.

The dependence of the CMC upon β is evidenced by the difference in concentration at which the breaks occur between Regions I and II (Fig. 10A). At 0.015 *M*, the curve at $\beta = 0.2$ represents only monomer. At higher values of β, the systems are a mixture of monomers and micelles.

Influence of Micelles on Solubility Profile—Returning now to the solubility–pH behavior of $PGF_{2\alpha}$ tromethamine, it is apparent that the rapid increase in solubility just above pH 5 is related to micelle formation. Figure 4 shows that the solubility of the monomeric acid and salt differs by less than a factor of 8. This does not, then, account for the observed solubility profile. Based upon Fig. 3 (see pH 3 and 5 runs), either the unionized acid of $PGF_{2\alpha}$ does not form micelles or the micelles are not very soluble. Hence, the observed solubility behavior (pH > 5) is due to the contribution of ionized drug, because it forms micelles that are extremely soluble. In the pH range of 3–8, the micelles are mixed due to the presence of both ionized and unionized species. The exact ratio of salt to acid is governed by the pH of the system, as described by the Henderson–Hasselbalch equation (23). There appears to be a critical ratio that is necessary for the rapid increase in solubility. Above pH 5.10, the ratio[10] of salt to acid is greater than 2:1; below this pH, there are less than two salt molecules for each acid molecule. Not surprisingly, as β approaches a value of 0.6 (Figs. 6 and 9), precipitation is noted for concentrations well above the CMC. This observation is consistent with the requirement that a critical ratio of salt to acid of near 2.0 be exceeded.

It is not clear why at least two molecules of salt are required to solubilize one molecule of acid. However, this type of behavior is not unusual in pharmaceutical systems. If the acid and salt are thought of as two distinct species whose relative concentrations are controlled by the pH of the solution, these observations are strikingly similar to those of Higuchi and Lach (24–26) for complexes of definite stoichiometry such as benzocaine–caffeine (1:1) and barbital–caffeine (2:1). Furthermore, some bile salts and their corresponding acids were shown by Ekwall *et al.* (6) and Ekwall (27) to exhibit a marked increase in solubility when the pH, and thus the acid–salt ratio, reaches a critical value.

[10] This ratio is based upon the pKa of the micelle being 5.38 at pH 5.10.

Table II—Area (Å²) per Molecule of $PGF_{2\alpha}$ Tromethamine at the Air–Water Interface

pH	Medium	Area per Molecule
3.0	Water	85.9
5.0	Water	75.5
6.0	Water	67.5
8.0	Water	64.1
10.0	Water	63.9
6.0	0.2 *M* Tromethamine	74.0
6.0	0.2 *M* NaCl	72.5
3.0	0.2 *M* Tromethamine	74.2

The similarity between $PGF_{2\alpha}$ and bile salts, *e.g.*, cholic acid, goes beyond the solubility–pH behavior mentioned previously. The CMC values of certain bile salts (28) fall in the same high concentration range as those of $PGF_{2\alpha}$, and their ionization constants (14) show a similar dependence on concentration (Fig. 10B). There is also an obvious similarity between the functional groups on cholic acid and $PGF_{2\alpha}$ (one carboxyl and three hydroxyl groups in each case). Moreover, in spite of its greater molecular weight, the molecular volume of cholic acid (28) is close to the value calculated for $PGF_{2\alpha}$ from its group contributions (29).

Area per Molecule—The area per molecule of $PGF_{2\alpha}$ was calculated from Eq. 5, the Gibbs adsorption equation (30):

$$\Gamma = \left(\frac{1}{kT}\right)\left(\frac{d\gamma}{d \ln C}\right) \quad \text{(Eq. 5)}$$

where Γ is surface excess of material adsorbed (moles/cm.²), k is the Boltzmann constant, T is absolute temperature, and $d\gamma/d \ln C$ is 1/2.303 times the slope of surface tension–log concentration curves in Figs. 3 and 5. Wherever applicable, activity coefficient corrections were made for variation in ionic strength. In the case of the $PGF_{2\alpha}$ tromethamine salt at low ionic strength, no correction was made for the adsorption of counterions at the interface (30, 31). The apparent lack of need for the full correction factor of 2.0 is evident in Table II. The areas calculated for pH 8 and 10 in the absence of added electrolyte differ by 17% from those obtained when a correction factor is not appropriate, *i.e.*, in swamping electrolyte or when only free acid is present. This might indicate that $PGF_{2\alpha}$ is adsorbed as an ion-pair rather than as free ions. The areas per molecule of the free acid or of the anion in swamping electrolyte (for which Eq. 5 is valid) average 77 Å². It is possible to construct space-filling molecular models, oriented with all of the polar groups on one side of the molecule, that have areas of about 85 Å². This orientation is reasonable since it would be expected that the polar groups would orient toward the polar aqueous phase. In this respect, prostaglandin $F_{2\alpha}$ again resembles the cholic acid molecule which contains three α-hydroxyl groups and a carboxylic acid moiety which are oriented toward the water at an air–water interface (28).

CONCLUSION

The pKa of $PGF_{2\alpha}$ was found to depend upon the concentration of drug titrated. The pKa increased from 4.9 for the monomer to 5.6 for the micellar state. This increase in pKa is consistent with the presence of a net negative charge at the surface of the micelle. The presence of these negatively charged micelles have been shown to account for the unusual solubility behavior observed for $PGF_{2\alpha}$ tromethamine salt. The existence of molecular aggregates was confirmed by surface tension measurements as well as the titration behavior.

REFERENCES

(1) J. W. Hinman, *Annu. Rev. Biochem.*, **41**, 161(1972).

(2) J. R. Weeks, *Annu. Rev. Pharmacol.*, **12**, 317(1972).

(3) T. O. Oesterling, W. Morozowich, and T. J. Roseman, *J. Pharm. Sci.*, **61**, 1861(1972).

(4) S. M. M. Karim, "The Prostaglandins, Progress in Research," Medical Technical Publishing Co., Ltd., Oxford and Lancaster, England, 1972.

(5) M. C. R. Johnson and L. Saunders, *Biochim. Biophys. Acta*, **218**, 543(1970).

(6) P. Ekwall, K. Fontell, and A. Sten, "Second International Congress of Surface Activity," vol. I, Academic, New York, N. Y., 1957, p. 357.
(7) E. L. Rowe, *J. Pharm. Sci.*, **61**, 781(1972).
(8) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N. Y., 1962.
(9) A. N. Martin, J. Swarbrick, and A. Cammarata, "Physical Pharmacy," Lea & Febiger, Philadelphia, Pa., 1969, pp. 310–312.
(10) K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants," Academic, New York, N. Y., 1963, p. 76.
(11) F. Tokiwa and K. Ohki, *J. Phys. Chem.*, **70**, 3437(1966).
(12) P. Mukerjee, "Abstracts, APhA Academy of Pharmaceutical Sciences," vol. 2, no. 2, 1972, p. 158.
(13) J. H. Wood, B. M. Richard, and H. L. Smith, *ibid.*, vol. 2, no. 1, 1972, p. 68.
(14) P. Ekwall, T. Rosendahl, and N. Löfman, *Acta Chem. Scand.*, **11**, 590(1957).
(15) A. Veis and C. W. Hoerr, *J. Colloid Sci.*, **15**, 427(1960).
(16) S. H. Yalkowsky and G. Zografi, *J. Pharm. Sci.*, **59**, 798 (1970).
(17) F. Tokiwa and K. Ohki, *J. Phys. Chem.*, **71**, 1824(1967).
(18) F. Tokiwa and K. Ohki, *J. Colloid Interface Sci.*, **24**, 219 (1967).
(19) C. Tanford, "The Physical Chemistry of Macromolecules," Wiley, New York, N. Y., 1961, pp. 475–481.
(20) W. Shankland, *J. Colloid Interface Sci.*, **34**, 9(1970).
(21) S. H. Yalkowsky and G. Zografi, *ibid.*, **34**, 525(1970).
(22) F. Tokiwa and K. Ohki, *ibid.*, **27**, 247(1968).
(23) A. N. Martin, J. Swarbrick, and A. Cammarata, "Physical Pharmacy," Lea & Febiger, Philadelphia, Pa., 1969, p. 237.
(24) T. Higuchi and J. L. Lach, *J. Amer. Pharm. Ass., Sci. Ed.*, **43**, 524(1954).
(25) *Ibid.*, **43**, 527(1954).
(26) *Ibid.*, **43**, 349(1954).
(27) P. Ekwall, "Proceedings on the International Conference on Biochemical Problems," vol. 1, 1953, p. 103.
(28) P. P. Nair and D. Kritchevsky, "The Bile Acids," Plenum, New York, N. Y., 1971, chap. 8.
(29) S. H. Yalkowsky and G. Zografi, *J. Pharm. Sci.*, **61**, 793 (1972).
(30) L. I. Osipow, "Surface Chemistry," Reinhold, New York, N. Y., chap. 7.
(31) B. A. Pethica, *Trans. Faraday Soc.*, **50**, 413(1954).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 8, 1973, from *Pharmacy Research, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication May 14, 1973.

Presented to the 13th National Meeting, Academy of Pharmaceutical Sciences, Chicago, Ill., November 1972.

The laboratory assistance of Mrs. R. Butler is greatly appreciated.

▲ To whom inquiries should be directed.

Determination of CMC and Partial Specific Volume of Polysorbates 20, 60, and 80 from Densities of Their Aqueous Solutions

BAHRAM FARHADIEH

Abstract □ The CMC of the surfactants was determined by measuring the density of their aqueous solutions at 24.88° with a new instrument which can measure accurately the change in natural frequency of a hollow oscillator of constant volume when it is filled with a solution of unknown density. The frequency change is transformed into density by calibrating the instrument with liquids of known densities. The CMC values obtained were 12×10^{-3} wt.% for polysorbate 20, 9×10^{-3} wt.% for polysorbate 60, and 6.2×10^{-3} wt.% for polysorbate 80. By applying the intercept method, partial specific volumes of water and the surfactant were obtained from the density of each surfactant solution. These partial quantities indicated that the formation of micelles is associated with an increase in the partial specific volume of the surfactant and a concomitant decrease in that of water.

Keyphrases □ Polysorbates 20, 60, and 80—determination of CMC's and partial specific volumes from densities of their aqueous solutions □ CMC and partial specific volume, polysorbates 20, 60, and 80—determination from density of aqueous solution □ Density measurements, polysorbates 20, 60, and 80—used to determine CMC and partial specific volume

Various methods have been used in the determination of the critical micelle concentration (CMC) of ionic and nonionic surfactants. In general, these methods rely on the abrupt change that occurs in a number of physicochemical properties of the surfactant solutions as the CMC is approached (1–3). Partial volume quantities are a direct measure of the changes occurring in the body of the solutions but have rarely been used in the study of aggregation (4–8). The limited use of this method has been due mainly to difficulties involved in obtaining accurate measurements at constant temperatures and at low concentrations of the associating solute (6, 7). The availability of new instrumentation has greatly reduced such measurement difficulties.

The study reported here deals with the determination of densities of the aqueous solutions of polysorbates 20, 60, and 80 using a digital precision density meter[1]. The CMC for each surfactant and the partial specific volumes of the components of each surfactant solution are obtained from the corresponding density data. The results are used to describe changes that occurred in these solutions during aggregation.

EXPERIMENTAL

Chemicals—The nonionic surfactants examined were a group of three commercial products of pharmaceutical interest: polysorbate 20[2] [polyoxyethylene (20) sorbitan monolaurate], polysorbate 60[3] [polyoxyethylene (20) sorbitan monostearate], and polysorbate 80[4] [polyoxyethylene (20) sorbitan monooleate]. These surfactants

[1] Model DMA 02/C, Anton Paar Kg., A-8054, Graz, Austria.
[2] Lot 1337, Tween 20, Atlas Chemical Industries Inc., Wilmington, Del.
[3] Lot 854, Tween 60, Atlas Chemical Industries, Inc.
[4] Lot 1147, Tween 80, Atlas Chemical Industries, Inc.