

- (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  $12 \times 10^{-3}$  wt. % for polysorbate 20,  $9 \times 10^{-3}$  wt. % for polysorbate 60, and  $6.2 \times 10^{-3}$  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<sup>1</sup>. 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<sup>2</sup> [polyoxyethylene (20) sorbitan monolaurate], polysorbate 60<sup>3</sup> [polyoxyethylene (20) sorbitan monostearate], and polysorbate 80<sup>4</sup> [polyoxyethylene (20) sorbitan monooleate]. These surfactants

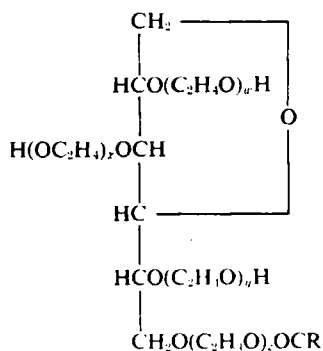
<sup>1</sup> Model DMA 02/C, Anton Paar Kg., A-8054, Graz, Austria.

<sup>2</sup> Lot 1337, Tween 20, Atlas Chemical Industries Inc., Wilmington, Del.

<sup>3</sup> Lot 854, Tween 60, Atlas Chemical Industries, Inc.

<sup>4</sup> Lot 1147, Tween 80, Atlas Chemical Industries, Inc.

have the following general chemical formula:



where the RCO-- group represents the fatty acid moiety and  $n = w + x + y + z$  is the number of ethylene oxide units, which is equal to 20 for the surfactants considered (9). In this study, samples from these commercially available lots were analyzed as received. Further refining was not attempted because methods available (10) can effect only partial removal of the impurities. All solutions were prepared in distilled water on a weight-to-weight basis.

**Density Measuring Apparatus**—The system consisted of a digital precision density meter<sup>1</sup>, circulator<sup>2</sup>, and a digital thermistor thermometer<sup>3</sup>. The precision density meter consisted of a readout unit and a sample compartment. The sample compartment contained a hollow, V-shaped oscillator made of glass. The oscillator had a fixed volume ( $V$ ), and it was filled and emptied through two ports located on the outside of the sample compartment. The oscillator could be excited to an undamped oscillation by a magnetic rod which was activated electronically. The readout unit measured the frequency of the oscillation ( $f$ ) and displayed it as the oscillation period ( $T$ ), where  $T = 1/f$ . The circulator was connected with short rubber tubing to the sample compartment. When in operation, it could maintain the temperature of the sample compartment to within  $\pm 0.02^\circ$ . The temperature of the sample compartment was monitored during each run with the digital thermistor thermometer.

**Instrument Theory** The digital precision density meter can be represented by an equivalent system consisting of a hollow body of mass ( $M$ ) and of volume ( $V$ ) suspended from a spring with a spring constant ( $C$ ). When the hollow body is filled with a liquid of density  $d$  and is set into motion, the oscillation period for the system is given by the following expression (11):

$$T = 1/f = 2\pi\sqrt{(dV + M)/C} \quad (\text{Eq. 1})$$

According to this relationship, which is general for any liquid, the oscillation period is only a function of the density of the liquid used. Expansion of Eq. 1 and further transformation yield:

$$T^2 = Ad + B \quad (\text{Eq. 2})$$

where  $A$  and  $B$  are the instrument's constants and are defined by ( $A = 4\pi^2V/C$ ) and ( $B = 4\pi^2M/C$ ).

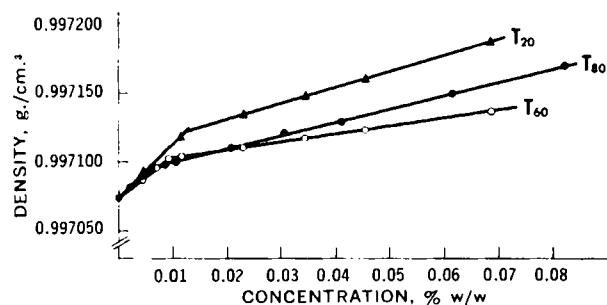


Figure 1—Solution density versus concentration of polysorbate 20 ( $T_{20}$ ), polysorbate 60 ( $T_{60}$ ), and polysorbate 80 ( $T_{80}$ ) in water at  $24.88^\circ$ .

<sup>1</sup> Lauda/Brinkmann, model K-21R, Brinkmann Instrument Inc., Westbury, NY 11590

<sup>2</sup> Model 1501, United System Corp., Dayton, OH 45403

Table I—Densities of Aqueous Solutions of Polysorbates 20, 60, and 80 at  $24.88^\circ$

Concentration, wt. %	Density, g./cm. <sup>3a</sup>		
	Polysorbate 20	Polysorbate 60	Polysorbate 80
0.0000	0.997074	0.997074	0.997074
0.0020	—	—	0.997081
0.0041	—	—	0.997089
0.0046	0.997093	0.997088	—
0.0062	—	—	0.997096
0.0069	0.997101	0.997096	—
0.0082	—	—	0.997098
0.0091	0.997110	0.997102	—
0.0103	—	—	0.997100
0.0114	0.997118	0.997103	—
0.0205	—	—	0.997110
0.0229	0.997134	0.997110	—
0.0308	—	—	0.997121
0.0344	0.997149	0.997117	—
0.0410	—	—	0.997129
0.0455	0.997162	0.997124	—
0.0615	—	—	0.997150
0.0687	0.997187	0.997138	—
0.0820	—	—	0.997171

<sup>a</sup> Average of four determinations.

For two liquids of differing densities, it readily follows that the difference in their densities is given by the equation:

$$(d_1 - d_2) = \frac{1}{A}(T_1^2 - T_2^2) \quad (\text{Eq. 3})$$

In this expression,  $A$  has the same definition as before and therefore represents the calibration constant for the instrument;  $T_1$  and  $T_2$  are the oscillation periods when the oscillator is filled with liquids of densities  $d_1$  and  $d_2$ , respectively.

**Density Measurement Procedure**—Generally, the instrument was started 1 hr. prior to any density measurements to assure temperature equilibration. The hollow oscillator was washed by injecting distilled water through the injection port and emptying it by forced air several times. Next, the instrument was calibrated by obtaining the oscillation period for distilled water ( $T_1$ ) and for anhydrous ethanol ( $T_2$ ). These two liquids were used because their respective densities at various temperatures are reported in the literature (12). The densities of the aqueous solutions of the polysorbates were measured against distilled water as the reference.

All measurements were done at  $24.88 \pm 0.02^\circ$ . It was necessary to wait for 15 min. between each sample injection for the temperature to reequilibrate. The accuracy of the measurements was investigated with distilled water. It was determined to be  $\pm 3.0 \times 10^{-6}$  g./cm.<sup>3</sup> of the reported value (12). Accuracy of this order of magnitude is easily obtainable since the instrument determines the density through precise measurement of frequency rather than determination of weight and volume.

## RESULTS AND DISCUSSION

The densities of aqueous solutions of the three surfactants used in this study are reported in Table I. Each reported density is the mean of four separate determinations. In Fig. 1, the densities of the aqueous solutions of each surfactant are plotted against their re-

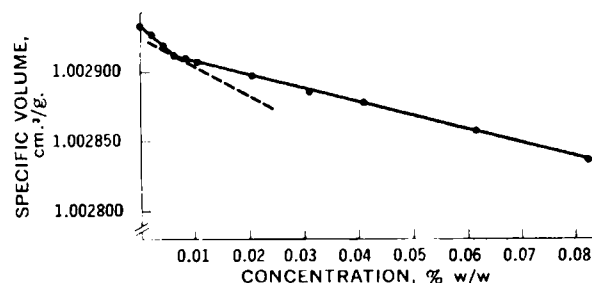


Figure 2—Specific volume versus concentration of polysorbate 80 in water.

**Table II**—Partial Specific Volume of Water in Aqueous Solutions of Polysorbates 20, 60, and 80 at 24.88°

Concentration, wt. %	Partial Specific Volume of Water, cm. <sup>3</sup> /g.		
	Polysorbate 20	Polysorbate 60	Polysorbate 80
0.0020	1.002934	1.002934	1.002934
0.0040	1.002934	1.002934	1.002934
0.0050	1.002934	1.002934	1.002934
0.0055	1.002934	1.002934	1.002929
0.0060	1.002934	1.002934	1.002924
0.0065	1.002934	1.002934	1.002919
0.0070	1.002934	1.002934	1.002918
0.0075	1.002934	1.002932	1.002918
0.0080	1.002934	1.002929	1.002918
0.0085	1.002934	1.002925	1.002918
0.0090	1.002934	1.002921	1.002918
0.0095	1.002934	1.002915	1.002918
0.0100	1.002929	1.002911	1.002918
0.0110	1.002921	1.002911	1.002918
0.0120	1.002915	1.002911	1.002918
0.0140	1.002907	1.002911	1.002918
0.0160	1.002902	1.002911	1.002918
0.0180	1.002901	1.002911	1.002918
0.0200	1.002901	1.002911	1.002918
0.0300	1.002901	1.002911	1.002918
0.0400	1.002901	1.002911	1.002918
0.0600	1.002901	1.002911	1.002918

spective concentration. Each curve consists of two linear segments of different slopes. The abrupt change in slope occurs at the CMC of the surfactant (1-8). The CMC values for polysorbate 80, polysorbate 60, and polysorbate 20 were  $6.2 \times 10^{-3}$ ,  $9.0 \times 10^{-3}$ , and  $12 \times 10^{-3}$  g./100 g., respectively. Bjaastad *et al.* (10) obtained CMC's of  $6.0 \times 10^{-3}$  and  $4.5 \times 10^{-3}$  g./100 ml. for partially purified polysorbate 80 and polysorbate 60, respectively, at room temperature. The higher value obtained for polysorbate 60 by the method employed in this investigation is probably due to impurities in the commercial sample used.

The volume ( $V$ ) of a solution composed of  $n_1$  moles of constituent  $x_1$  and  $n_2$  moles of constituent  $x_2$  is related at constant temperature and pressure to  $n_1$  and  $n_2$  by the relationship (13):

$$V = n_1 \bar{V}_1 + n_2 \bar{V}_2 \quad (\text{Eq. 4})$$

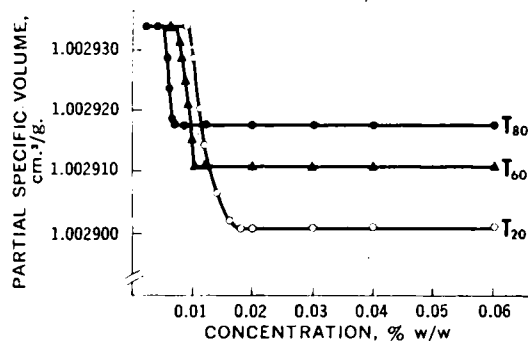
where  $\bar{V}_1$  and  $\bar{V}_2$  are the partial molal volumes of the constituents  $x_1$  and  $x_2$ , respectively, and are defined by the following expressions:

$$\bar{V}_1 = (\partial V / \partial n_1)_{T,P,n_2} \quad (\text{Eq. 5})$$

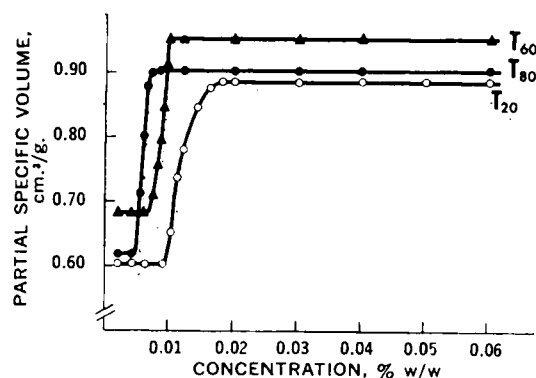
$$\bar{V}_2 = (\partial V / \partial n_2)_{T,P,n_1} \quad (\text{Eq. 6})$$

The subscripts  $T$ ,  $P$ ,  $n_1$ , and  $n_2$  refer to the constant temperature and pressure conditions at constant amount of component one or two, respectively. If grams is chosen instead of moles as the concentration unit, Eqs. 4-6 are valid for partial specific volumes: These multiplied by their respective molal weights give the corresponding partial molal volumes.

The commercial polysorbates used in this study are impure and their exact molal weights cannot be used for such conversions without introducing some errors in the values of their partial molal



**Figure 3**—Partial specific volume of water in aqueous solutions of polysorbate 20 ( $T_{20}$ ), polysorbate 60 ( $T_{60}$ ), and polysorbate 80 ( $T_{80}$ ).



**Figure 4**—Partial specific volume of polysorbate 20 ( $T_{20}$ ), polysorbate 60 ( $T_{60}$ ), and polysorbate 80 ( $T_{80}$ ) in aqueous solutions.

volumes. Thus, the densities of each polysorbate solution were used to calculate the partial specific volumes of the surfactants ( $\bar{v}_2$ ) and that of the water ( $\bar{v}_1$ ) rather than their respective partial molal volumes. The method of intercepts (13) was used in these calculations. In this method, the specific volume (1/density) of each of the solutions was plotted *versus* the surfactant concentration (weight percent). A smooth curve was fitted through the points. If a tangent is drawn to the curve of such a plot at any one point, the intercept of this tangent upon the ordinate representing 100% water is equal to  $\bar{v}_1$ . Similarly, the intercept upon the 100% surfactant ordinate is equal to  $\bar{v}_2$ . Figure 2 is such a plot for polysorbate 80 solutions. The broken line is a tangent drawn to the point on the curve corresponding to a solution containing 0.0065 g. of surfactant/100 g. of solution. The partial specific volumes of the water in aqueous solutions of each surfactant were determined graphically. For the surfactants, however, graphical extrapolation of the tangents to the 100% surfactant ordinate was not practical. Therefore, all values for  $\bar{v}_2$  were obtained by determining the straight-line equations fitting the tangent lines. From these equations, the intercepts upon the ordinate representing 100% surfactant were calculated.

The values of  $\bar{v}_1$  and  $\bar{v}_2$  at several concentrations were obtained for the three surfactant solutions (Tables II and III) and are plotted *versus* concentration of the polysorbates in Figs. 3 and 4, respectively. An inspection of Figs. 3 and 4 shows that below the CMC the values of  $\bar{v}_1$  and  $\bar{v}_2$  are constant, indicating that the surfactants behave as normal undissociable solutes at these concentrations. Depending on the magnitude of change in the slope of specific volume *versus* concentration plot, the values of  $\bar{v}_1$  and  $\bar{v}_2$

**Table III**—Partial Specific Volumes of Polysorbates 20, 60, and 80 in Aqueous Solutions at 24.88°

Concentration, wt. %	Partial Specific Volume of Surfactants, cm. <sup>3</sup> /g.		
	Polysorbate 20	Polysorbate 60	Polysorbate 80
0.0020	0.602934	0.683490	0.621355
0.0040	0.602934	0.683490	0.621355
0.0050	0.602934	0.683490	0.621355
0.0055	0.602934	0.683490	0.712929
0.0060	0.602934	0.683490	0.802924
0.0065	0.602934	0.683490	0.877919
0.0070	0.602934	0.683490	0.902918
0.0075	0.602934	0.709182	0.902918
0.0080	0.602934	0.759339	0.902918
0.0085	0.602934	0.797043	0.902918
0.0090	0.602934	0.845027	0.902918
0.0095	0.602934	0.910607	0.902918
0.0100	0.655471	0.954131	0.902918
0.0110	0.735479	0.954131	0.902918
0.0120	0.779511	0.954131	0.902918
0.0140	0.844574	0.954131	0.902918
0.0160	0.876396	0.954131	0.902918
0.0180	0.885254	0.954131	0.902918
0.0200	0.885254	0.954131	0.902918
0.0300	0.885254	0.954131	0.902918
0.0400	0.885254	0.954131	0.902918
0.0600	0.885254	0.954131	0.902918

undergo a sharp change near or at the CMC of each surfactant, which continues over a narrow concentration range beyond the CMC. This type of behavior has been observed for potassium *n*-octoate (5) and for potassium laurate and lauryl sulfonic acid (8). The concentration dependency of  $\bar{v}_1$  and  $\bar{v}_2$  is due to the changing ratio of monomeric to micellar surfactant (14, 15). After this transition region,  $\bar{v}_1$  and  $\bar{v}_2$  begin to become constant again due to the diminishing ratio of the monomeric surfactant to the micellar surfactant.

It also has been suggested (16) that the decrease in the partial specific volume of water with a concomitant rise in that of the surfactant is due to change of water structure upon micelle formation. Below the CMC where the surfactant molecules exist in their monomeric form, the hydrophobic portions of these molecules are tightly surrounded by water molecules (17, 18). Thus, these monomers are subjected to high compression by the strong cohesive field of water molecules. At the same time, by their interposition between water molecules, these monomers effectively reduce the attraction of water molecules for each other which results in less compression on water. Upon aggregation, the area of contact between water and the hydrophobic portions of the surfactant is greatly reduced on account of the large size of the micelle as compared to single molecules. This reduction of the contact area releases most of the water molecules that were originally bound to the surfactant molecules and were the cause of their compression. For these reasons, micelle formation is accompanied by a reduced compression of the surfactant molecules (larger partial specific volume) and by an increased compression of the water molecules (smaller partial specific volume).

#### REFERENCES

- (1) A. W. Ralston and C. W. Hoerr, *J. Amer. Chem. Soc.*, **64**, 772(1942).
- (2) P. Debye and E. E. Anacker, *J. Phys. Colloid Chem.*, **55**, 644 (1951).
- (3) B. Farhadieh, N. A. Hall, and E. R. Hammarlund, *J.*

*Pharm. Sci.*, **56**, 18(1967).

- (4) J. Grindley and C. R. Bury, *J. Chem. Soc.*, **1929**, 679.
- (5) D. G. Davies and C. R. Bury, *ibid.*, **1930**, 2263.
- (6) R. G. Paquette, E. C. Lingafelter, and H. V. Tartar, *J. Amer. Chem. Soc.*, **65**, 686(1943).
- (7) A. T. Florence, *J. Pharm. Pharmacol.*, **18**, 384(1966).
- (8) H. Lall, *J. Colloid Sci.*, **8**, 414(1953).
- (9) "Guide to the Use of Atlas Surfactants and Sorbitol in Cosmetic and Pharmaceutical Products," LD-104, Atlas Chemical Industries, Inc., Wilmington, Del., 1965, p. 25.
- (10) S. G. Bjaastad, N. A. Hall, and A. L. Thakkar, *J. Pharm. Sci.*, **54**, 1529(1965).
- (11) Precision Density Meter, Model DMA 02/C, "Instruction Manual," Anton Paar Kg. Graz, Austria, 1970, p. 2.
- (12) "Handbook of Chemistry and Physics," 50th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1970, F-4.
- (13) G. N. Lewis and M. Randall, *J. Amer. Chem. Soc.*, **43**, 233 (1921).
- (14) P. Ekwall, T. Rosendahl, and N. Lofman, *Acta Chem. Scand.*, **11**, 590(1957).
- (15) S. H. Yalkowsky and G. Zografi, *J. Pharm. Sci.*, **59**, 798 (1970).
- (16) P. A. Winsor, "Solvent Properties of Amphiphilic Compounds," Butterworths Scientific Publication, London, England, 1954, p. 48.
- (17) H. V. Tartar, *J. Colloid Sci.*, **14**, 115(1959).
- (18) P. White and G. C. Benson, *J. Phys. Chem.*, **64**, 559(1960).

#### ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1972, from *Pharmaceutical Product Division, Abbott Laboratories, North Chicago, IL 60064*

Accepted for publication June 13, 1973.

Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Houston meeting, April 1972.

The author thanks Mr. G. H. Barlow for providing the digital precision density meter and familiarization with its use.

## Effect of Wavelength on Production of Previtamin D<sub>2</sub>

ELIANE ABILLON<sup>▲</sup> and RENÉ MERMET-BOUVIER

**Abstract** □ On the basis of a previously established reaction scheme, the formation rate of various isomers produced by UV irradiation of ergosterol at low temperature was calculated for wavelengths between 240 and 320 nm. Particular attention was given to previtamin D<sub>2</sub>, the precursor of vitamin D<sub>2</sub>; the production optimum and the time required to obtain it were investigated in relation to the irradiation wavelength.

**Keyphrases** □ Previtamin D<sub>2</sub> photochemical formation from ergosterol—effect of irradiation wavelengths □ Ergosterol photochemical conversion to previtamin D<sub>2</sub>—effect of irradiation wavelength □ UV irradiation of ergosterol—conversion to previtamin D<sub>2</sub>, effect of various wavelengths

Much work has been done on the photoisomerization of ergosterol (1–3), and various reaction schemes have been proposed (4–6). Only the thermal transformation of previtamin D<sub>2</sub> to vitamin D<sub>2</sub> was reported (2) and is insignificant if the temperature of the irradiated medium is under 10°.

The production of vitamin D<sub>2</sub> is achieved in two steps:

1. UV irradiation of ergosterol (I) at a low temperature, which results in the formation of the following isomers: previtamin D<sub>2</sub> (II), tachysterol<sub>2</sub> (III), lumisterol<sub>2</sub> (IV), and toxisterols.

2. Separation of previtamin D<sub>2</sub>, which becomes vitamin D<sub>2</sub> (V) through thermal transformation.

The purpose of this work was to determine the effect of the irradiation wavelength on the percentage of the previtamin formed. The absorption properties were investigated as a function of wavelength for the isomers concerned in accordance with a recently determined reaction scheme representing the different photochemical transformations.

#### PHOTOCHEMICAL REACTION SCHEME OF ERGOSTEROL AT LOW TEMPERATURE: CORRESPONDING KINETICS

The reaction scheme (7, 8) used in this study is shown in Scheme I. The quanta transformation yield values are indicated and are ex-