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Composition of Stearic Acid Monolayers from Calcium-Containing Substrates

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Monomolecular layers were formed from stearic acid on substrates 10^{-4} *M* in calcium chloride containing sodium phosphate, sodium bicarbonate, or ammonium chloride as a buffer. Analyses of skimmed or plated films were performed by the evaluation of their infrared spectra. On all buffers no calcium stearate was observed at pH <4 and complete conversion was observed at pH >9. In the pH range 5–7, less calcium stearate was formed in the monolayer on the sodium phosphate buffer than on the other buffers. These results were found to be in satisfactory agreement with the force-area characteristics of stearic acid monolayers spread on calcium-containing substrates observed by earlier workers. When the monolayer was removed by plating to build up multilayers, the calcium stearate was shown to be present as the monohydrate and not as the anhydrous salt. Heating a multilayer to 50° caused the loss of water and also of a substantial proportion of stearic acid.

Introduction

A knowledge of the composition of monolayers is essential for the understanding of their properties, particularly their interaction with ions in the substrate.

Several workers have analyzed the metallic content of stearic acid films spread on substrates containing calcium ions at varying pH values. Langmuir and Schaefer¹ determined the metal ion content of skimmed stearic acid monolayers formed on bicarbonate buffers containing calcium salts and found that increasing amounts of calcium stearate were found with increasing pH. Havinga² performed similar experiments and found a smaller fraction of calcium stearate than Langmuir and Schaefer did at the same pH. It should be noted that Havinga mentions the possibility that his samples were contaminated with copper ions from the trough.

A different method of analysis was adopted by Sobotka, Demeny, and Chanley,³ who plated calcium stearate monolayers onto chromium-plated slides from 10^{-4} *M* calcium chloride solutions labeled with Ca^{45} . A comparison of the β -particle count of the plated sample with a standard containing a known amount of Ca^{45} made it possible to determine the extent of calcium stearate formation, which was found to occur to a lower degree at pH <8 than observed by Langmuir and Schaefer; at higher pH levels, essentially complete conversion to the calcium salt was observed.

Other, less extensive studies have also been reported. The infrared spectra of multilayers of calcium and barium stearate were measured by Francis and Ellison,⁴ who observed a disappearance of the absorption peak

at 1710 cm^{-1} when the acid was converted to the alkaline earth salt. Kimizuka and Koketsu⁵ using Ca^{45} measured the calcium ion content of a stearic acid monolayer on its solution substrate. The calcium:stearate molar ratio in the monolayer at pH 8.2 and a calcium ion concentration of 10^{-5} *M* was 1:2.

This paper reports the composition, as determined by infrared absorption spectra and chemical analyses, of stearic acid films removed by plating or skimming from calcium-containing substrates containing different buffer ions. Some observations have been made which may explain some of the discrepancies between the results of earlier workers.

Experimental

Stearic acid of 99.4% purity⁶ was used. Its purity was established by vapor phase chromatography, the impurity being almost entirely the C_{16} acid, and no acids higher than C_{18} were present. The acid was dissolved in Fisher's "Spectranalyzed" grade hexane (20 mg./100 cc.) for application. Substrates were 10^{-4} *M* calcium chloride solutions containing bicarbonate, phosphate, or ammonium chloride as a buffer. All reagents were A.R. grade and dissolved in water which was deionized and then distilled carefully in an all-glass still. The bicarbonate buffer contained 2.5×10^{-4} *M* sodium carbonate and 2.5×10^{-4} *M* sodium bicarbonate, with the pH adjusted by the addition of hydrochloric acid. The phosphate buffer consisted of different proportions of disodium hydrogen phosphate and potassium dihydrogen phosphate, the total molar concentration being 2.5×10^{-4} . The ammonium chloride solution was 10^{-3} *M* and its pH adjusted with ammonium hydroxide or hydrochloric acid.

Anhydrous calcium stearate was prepared by mixing equimolar solutions of calcium chloride and stearic acid in hot methanol, adjusting the pH to 10 with concentrated ammonium hydroxide, filtering the precipitate, rinsing several times with hot methanol, and drying *in vacuo* at 50° for 48 hr.

(1) I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **58**, 284 (1936).

(2) E. Havinga, *Rec. trav. chim.*, **71**, 72 (1952).

(3) H. Sobotka, M. Demeny, and J. D. Chanley, *J. Colloid Sci.*, **13**, 565 (1958).

(4) S. A. Francis and A. H. Ellison, *J. Opt. Soc. Am.*, **49**, 131 (1959).

(5) H. Kimizuka and K. Koketsu, *Nature*, **196**, 995 (1962).

(6) Supplied through the courtesy of R. J. Grabenstetter of Procter and Gamble Co. Research Laboratories.

Calcium stearate monohydrate was prepared by the method of Kawano.⁷ Hot aqueous solutions of sodium stearate and calcium chloride were mixed, the precipitate filtered and then washed several times with water. The sample was allowed to dry in air at room temperature. When this sample was then dried to constant weight at 50° *in vacuo*, its weight loss corresponded to the monohydrate.

The substrate was contained in a Teflon-coated (S-122 fluorocarbon, Miller Stephens Chemical Co., Inc.) glass trough (14 × 64 × 1 cm.) or a Cenco Hydrophil balance trough. The hexane solution of stearic acid was spread on the surface between two Teflon-coated barriers until a visible lens was just formed. The film was swept from the surface and the procedure repeated three or four times to ensure a clean surface for the final spreading.

The film was allowed to remain on the surface for 10 min. after spreading before removal for analysis. No significant difference was found between films which were removed 10 min. and 60 min. after spreading. The film was removed from the surface by either a skimming or plating technique. With skimming, the barrier was advanced until the film visibly collapsed; then a strip of silver chloride (2.5 × 1.2 × 0.05 cm.) was dipped below the surface of the substrate and moved across the trough repeatedly while the barrier was further advanced. In this way almost all the collapsed film was collected on the silver chloride. The silver chloride strip had been previously cleaned by washing with methanol, then with 0.01 *M* sodium thiosulfate solution, and finally with water.

The Langmuir-Blodgett plating technique was used to transfer the monolayer to the silver chloride strip. Three strips of silver chloride, cleaned as before, were attached to a hand-operated plating device which allowed the strips to be raised and lowered in the solution. The stroke length was 1.5 cm. and the plating rate 1–3 cm. sec.⁻¹. After five complete plating cycles at 25 ± 5 dynes cm.⁻¹, the barrier was advanced 3 cm. to maintain this pressure; this sequence was repeated. Normally, a total of 250–300 layers was deposited on each side of the silver chloride strip. Although it was possible to plate over the entire pH range, the ease of plating was pH dependent and appeared to be the most satisfactory at pH 7. The multilayer was of the Y-type with a hydrophobic outer surface; drying was not necessary before analysis.

The composition of the samples was determined by infrared analysis. The infrared spectra of both stearic acid⁸ and calcium stearate⁷ show sharp peaks at 2920–2940, 2860–2870, 1465–1475, and 720 cm.⁻¹, all attributed to C–H vibrations; absorption at 2920–2940 cm.⁻¹ was the strongest. Stearic acid has a sharp peak at 1710 cm.⁻¹ attributed to the C=O group and not shown by calcium stearate. Anhydrous calcium stearate shows a peak for COO⁻ at 1583 cm.⁻¹ and calcium stearate monohydrate a split carboxyl peak at 1580 and 1540 cm.⁻¹; none of these peaks is shown by stearic acid. Silver chloride shows no significant absorption throughout the region 3000–1000 cm.⁻¹. The above frequencies were confirmed directly in this laboratory by pressing small samples of stearic acid or calcium stearate prepared as described earlier onto silver chloride strips of the same dimensions used for removing the monolayers. The spectra were obtained using a Perkin-Elmer Model 21 spectrophotometer with a sodium chloride prism and a beam condenser (No. 127–1271) with a potassium bromide prism. The plated silver chloride strips were placed in series so that the beam passed through a total of about 2000 monomolecular layers.

In some cases chemical analyses of skimmed monolayers were performed. A larger trough (90 × 28 cm.) was used and eight skimmings yielded about 3 mg. of material. The material was dried at 50° for 48 hr., weighed on a microbalance, and then wet-ashed first with concentrated nitric acid and then with concentrated hydrochloric acid to convert the material to the chloride salt. The residue was dissolved and then titrated in the usual way using 0.01 *M* sodium EDTA with murexide (Fisher M273) as indicator. Under these conditions the calcium determination was accurate to ±3%. Attempts to determine sodium by flame photometry showed that its mole fraction was less than 1% of that of calcium.

Results

The infrared spectra showed only the presence of stearic acid and calcium stearate for both skimmed and

plated samples over the entire pH range. No evidence could be found for the presence of bicarbonate, phosphate, or ammonium ions, or of significant quantities of free water.

Multilayers plated at pH <5 showed only C–H and C=O peaks, showing the presence of pure stearic acid. Multilayers plated between pH 5 and 9 showed C–H, C=O, and split carboxylate peaks, indicative of a mixture of stearic acid and calcium stearate monohydrate. At pH >9, only C–H and split carboxylate peaks were observed, showing the absence of stearic acid. Identical results (within experimental error) were obtained by the skimming and plating techniques.

When a sample plated at a pH >5 was dried *in vacuo* at 50° for 24 hr., the split carboxylate peak observed before drying was removed and replaced by the single peak at 1583 cm.⁻¹ for anhydrous calcium stearate. Clearly, drying *in vacuo* dehydrated the plated sample and the change in spectra was confirmation that the calcium stearate was plated originally as the monohydrate. One other very important change took place on heating at 50° *in vacuo*. In addition to the removal of water, heating also removed some of the stearic acid, shown by a decrease in COOH absorption at 1710 cm.⁻¹. This effect was examined further and found to be observed for both skimmed and plated samples heated in air or *in vacuo* at 50° for 24 hr. Heating *in vacuo* was more efficient than heating in air in removing stearic acid, but the latter procedure still removed a substantial fraction (about 54%) of the stearic acid.

The quantitative analysis of the samples was carried out as follows. For any given sample

$$K_1 A_{\text{CH}} = N_{\text{CH}}$$

$$K_2 A_{\text{COO}^-} = N_{\text{COO}^-}$$

$$K_3 A_{\text{COOH}} = N_{\text{COOH}}$$

where the *A* values are the absorbances of CH at 2940, COO⁻ at 1580, and COOH at 1710 cm.⁻¹, and where *N* refers to the corresponding number of moles of the species. The *K* values are assumed to be constant. It should be noted that since calcium stearate is present as the monohydrate, we are always referring to the hydrated stearate (COO⁻ · 0.5H₂O) but for convenience will write COO⁻. The mole fraction of stearate ion is then

$$X_{\text{COO}^-} = N_{\text{COO}^-} / (N_{\text{COO}^-} + N_{\text{COOH}})$$

and substituting from above

$$X_{\text{COO}^-} = [K_2 / K_1 A_{\text{COO}^-}] / [K_2 / K_1 A_{\text{COO}^-} + K_3 / K_1 A_{\text{COOH}}]$$

For pure calcium stearate monohydrate, $N_{\text{CH}} = N_{\text{COO}^-}$ and $K_2 / K_1 = A_{\text{CH}} / A_{\text{COO}^-}$ for the pure salt. The value of K_2 / K_1 was obtained from the spectrum of a few milligrams of calcium stearate monohydrate pressed onto a silver chloride strip or from the multilayer plated at pH 9, when no stearic acid was found to be present; in both cases $K_2 / K_1 = 1.06$.

Similarly, for pure stearic acid $K_3 / K_1 = A_{\text{CH}} / A_{\text{COOH}}$. The value of $K_3 / K_1 = 2.00$ was obtained from spectra of multilayers plated at pH 2 (when no calcium stearate could be observed).

Assuming that the values of K_2 / K_1 and K_3 / K_1 did not vary with composition, it was possible to calculate the

(7) M. Kawano, *J. Japan. Chem. Soc.*, **81**, 1806 (1960).

(8) N. Trzebowski and H. Dunken, *Wiss. Z. Friedrich-Schiller-Univ. Jena Math. Naturwiss. Reihe*, **10**, 45 (1961).

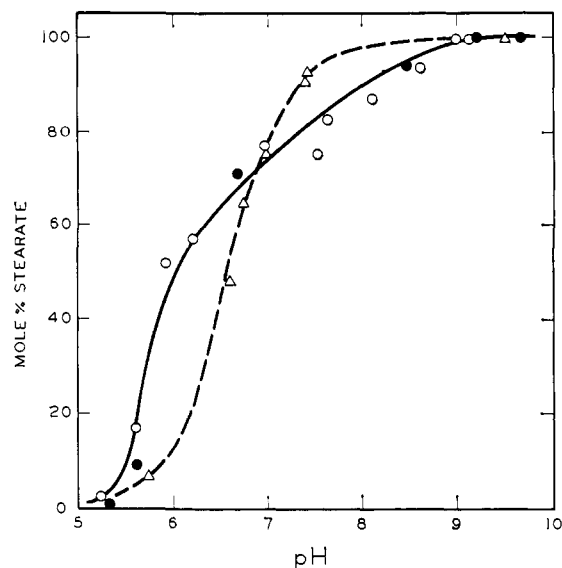


Fig. 1.—Composition of stearic acid-calcium stearate mono-hydrate films plated (and skimmed) from 10^{-3} M bicarbonate (O), ammonium (●), and phosphate (Δ) substrates, as determined by infrared analysis.

mole fraction of stearate ion for the different samples. The results showing mole fraction of the stearate ion *vs.* pH for three different buffers are shown in Fig. 1. The composition of the monolayers on bicarbonate and ammonium chloride buffers was, within experimental error, identical. However, the mole fraction of stearate ion on a phosphate buffer had a different pH dependency, as shown also in Fig. 1. For example, at pH 6.0 on a bicarbonate or ammonium chloride buffer, the mole fraction of stearate was 0.5; on a phosphate buffer it was but 0.1.

Samples which had been dried at 50° *in vacuo* were analyzed by infrared techniques and also by chemical means; agreement between the two methods was within 5%. This comparison provides a valuable corroboration of the infrared method. The results of these comparisons are not reported here because samples so heated had lost appreciable amounts of stearic acid.

Discussion

The results reported in this paper on the analyses of monolayers spread on substrates containing bicarbonate as a buffer are in satisfactory agreement with those of Havinga² and Sobotka³ but do not agree with those of Langmuir and Schaefer,¹ particularly at pH < 7 (see Fig. 2). All these workers also used bicarbonate buffers. Langmuir suggested that his samples may have contained additional sodium salts from drops of the solution which may have adhered during skimming. The method of analysis used did not allow calcium to be differentiated from sodium contamination. This suggestion was supported by the fact that at pH > 9 when complete conversion to calcium stearate occurred, Langmuir's method led to an apparent conversion of 119%.

There was one important difference in the technique used by Langmuir and that of the other workers.^{3,4} Langmuir's skimmed samples were dried at 100° before analysis but Havinga and Sobotka allowed their samples to dry at room temperature. We have shown that stearic acid can be sublimed readily from skimmed or plated layers by heating in air at 50° . The desorp-

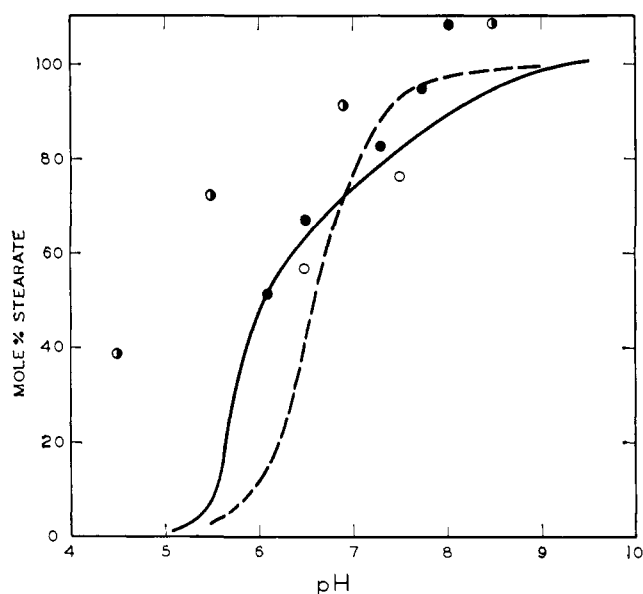


Fig. 2.—Comparison of results reported here on bicarbonate and ammonia buffers (—) and on a phosphate buffer (---) with those (on bicarbonate buffers) of Langmuir and Schaefer¹ (●) and of Havinga² (○), both of whom used chemical analyses, and of Sobotka, Demeny, and Chanley³ (●), who employed a radiometric technique.

tion of stearic acid has also been reported by Gregg and Widdowson.⁹ Multilayers were plated from substrates containing barium ions at different pH values and then heated *in vacuo*. The desorption of stearic acid was measured by observing the change in thickness of the multilayer. Recently, the desorption *in vacuo* of stearic acid monolayers plated onto a quartz slide has been observed even at room temperature.¹⁰

It is possible that Langmuir's samples lost stearic acid by subliming at 100° and so that an apparently higher percentage of calcium would have been found. However, the extent of this loss is unknown and may be small since Langmuir reported a recovery rate of at least 90%, based upon the amount of stearic acid spread upon the surface.

Studies by Thomas and Schulman¹¹ of the force-area characteristics of monolayers of myristic and stearic acids spread on substrates containing calcium ions and saturated with carbon dioxide showed that the films were more rigid at relatively low pH levels than at high pH levels. Clearly, these changes must be related to the formation of calcium stearate. The force-area data of several workers¹²⁻¹⁴ may be compared with our analytical data on the following basis (see Fig. 3). The area per molecule at zero compression on a substrate at pH 2 is taken as the area per molecule of stearic acid (a_{HSt}) and at pH 9 as the area per stearate ion (a_{St-}). If we assume that at intermediate pH levels the areas of the two species are additive, then the observed area (a_{obsd}) for a monolayer where the degree of dissociation is α is given by

$$a_{obsd} = \alpha a_{St-} + (1 - \alpha) a_{HSt}$$

(9) S. J. Gregg and E. E. Widdowson, *Nature*, **144**, 666 (1939).

(10) G. L. Gaines, Jr., and R. W. Roberts, General Electric Research Laboratory Report No. 62-RL-3135 C., Oct., 1962.

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(12) T. Sasaki and R. Matuura, *Bull. Chem. Soc. Japan*, **24**, 274 (1951).

(13) G. Di Bari, M.S. Thesis, Polytechnic Institute of Brooklyn, 1963.

(14) J. A. Spink and J. V. Sanders, *Trans. Faraday Soc.*, **51**, 1154 (1955).

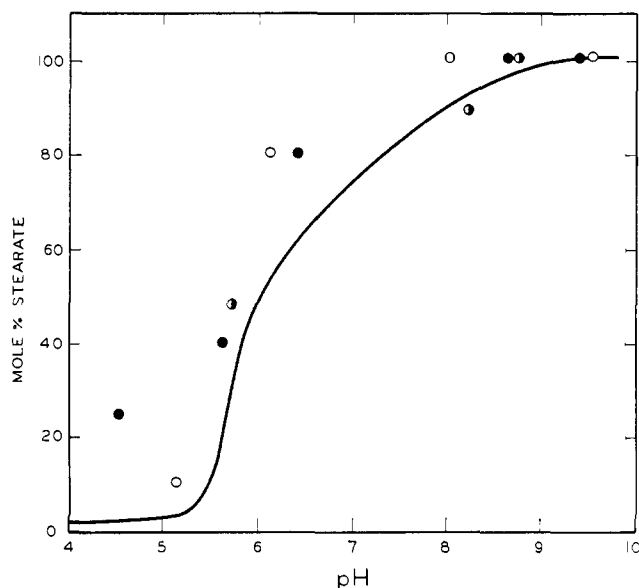


Fig. 3.—Comparison of results reported here on sodium bicarbonate or ammonium chloride buffers (—) with those derived from the force-area data of Spink and Sanders¹⁴ (●), Sasaki and Matuura¹² (○), and Di Bari¹³ (⊙).

The values of α shown in Fig. 3 were derived in this way from the force-area data. With the exception of one point at pH 4.5, the agreement between the force-area data and the analytical data is quite satisfactory. It is worth noting that the point at pH 4.5 was obtained on a substrate containing $5 \times 10^{-4} M$ calcium chloride compared to $10^{-4} M$ calcium chloride used in the analytical work. The influence of the alkaline earth cation concentration in the substrate at pH 5.7 has been studied for stearic acid monolayers spread on strontium chloride solutions.¹⁵ This work showed that an increase of strontium chloride in the substrate from 10^{-4} to $10^{-3} M$ increased the amount of strontium in the monolayer by a factor of two. The difference in calcium chloride concentration would appear to be a reasonable explanation for the deviation of the force-area data from the analytical data at this point. The agreement between the two sets of data is a strong indication that the removal of the monolayer for analysis has not altered its composition appreciably.

The difference between the composition of monolayers spread on a sodium bicarbonate or ammonium chloride solution and those on sodium phosphate solutions at pH < 7 may be due to the formation of complexes between calcium and phosphate ions. From the reported stability constants¹⁶ for calcium phosphates it appears that most of the calcium ions are bound at pH > 5 and the free calcium ion concentration will thereby be much lower than in sodium bicarbonate or ammonium chloride solutions. From the work mentioned above¹⁵ it follows that the degree of conversion should, therefore, be lower. At the present time, however, there is no reasonable explanation for the small but significant increase in the mole fraction of stearate formed in monolayers on sodium phosphate solution at pH > 7 above the fraction on sodium bicarbonate or ammonium chloride solutions. Although the influence of calcium ion concentration would be

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(16) T. D. Farr, "Phosphorus," Chemical Engineering Report No. 8, Tennessee Valley Authority, Wilson Dam, Ala., 1950.

expected to be less at almost complete ionization, the phosphate curve would never be expected to be above the bicarbonate or ammonium chloride curve.

The sodium content of the monolayer was less than 1% and illustrates the very marked specificity for calcium over sodium. This specificity had been observed previously for stearic acid² and for palmitic acid¹⁷ monolayers spread on mixed solutions of calcium and sodium ions. This specificity has been attributed to binding of a covalent character rather than ionic attraction.^{2,18} Davies and Rideal¹⁹ have applied the Stern theory to the interaction of sodium and calcium ions with stearic acid monolayers and derived a value of λ_p , the energy of polarization for these ions. The results were in agreement with the conclusions above and showed that the calcium binding had a much more covalent character than the sodium.

Danielli¹⁸ has estimated that the apparent pK_a for a carboxylic acid in a close-packed monolayer is about 3 units higher than in aqueous solution. The explanation for the increase was that a potential ψ is set up by the ionization of the monolayer and this potential increases the hydrogen ion concentration just below the surface. The relationship between the hydrogen ion concentration just below the surface and in the bulk is given by²⁰

$$pH_s = pH_b + e\psi_0/KT$$

where ψ_0 is the potential at the monolayer-solution interface. Surface potential measurements of stearic acid monolayers on substrates containing sodium ions²¹ and infrared analysis of skimmed samples from similar substrates²² are consistent with a pK_a increase of 2-3 units.

However, in this study on calcium-containing substrates, pK_a is 6.0, or an increase of but 1 unit above the value in aqueous solution. This result would indicate that ψ_0 is much smaller on a calcium-containing substrate than on a sodium-containing substrate at the same degree of stearate formation. If the calcium is bound (at least partially) in a covalent manner, then the surface charge density will be lower than in the case of completely ionic bond formation and, therefore, ψ_0 is expected to be smaller. Surface potential measurements have been made of stearic acid monolayers spread on $5 \times 10^{-4} M$ calcium chloride solutions.²³ The surface potential falls sharply from 300 mv. at pH 6.5 to zero at pH 9. The surface potential is given by

$$S.P. = 4\pi n(\alpha\mu_{St^-} + (1 - \alpha)\mu_{HSt}) + \psi_0$$

where μ_{HSt} is the vertical component of the stearic acid dipole, μ_{St^-} the vertical component of the stearate dipole, and n the number of molecules cm^{-2} . The value of μ_{HSt} is generally accepted to be 210 mD.²⁴ If our assumption that ψ_0 is small is correct, then most of the change in the surface potential must be attributed

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(18) J. F. Danielli, "Surface Chemistry," Butterworths, London, 1959, pp. 87-93.

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to a decrease in the vertical dipole moment. The dipole moment of calcium stearate in the monolayer is not known and so this decrease cannot be calculated. It is worth noting, however, that since the monolayer plates as calcium stearate monohydrate, it is likely that some water molecules must be strongly bound by the monolayer. The contribution of the dipole moment of these bound water molecules might play an important role in reducing the over-all vertical dipole moment and thus the surface potential.

The finding that calcium stearate monohydrate is present in the plated multilayer is of great interest in accounting for some aspects of the behavior of multilayer electrodes. Calcium stearate multilayers have been used as electrodes which are specific for calcium ions under certain conditions.²⁵ These multilayers have a specific conductance of 10^{-4} ohm $^{-1}$ cm. $^{-1}$ at 25°, one which is much higher than would be antici-

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pated. Sodium stearate,²⁶ for example, has a specific conductance of 7.2×10^{-11} ohm $^{-1}$ cm. $^{-1}$ at 170°. However, the presence of 1% water in sodium stearate²⁷ increases the specific conductance by a factor 10^8 . It seems very likely, therefore, that the presence of water of hydration in the calcium stearate multilayer is in part responsible for the specific conductance observed.

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[CONTRIBUTION NO. 132 FROM CHARLES F. KETTERING RESEARCH LABORATORY, YELLOW SPRINGS, OHIO]

Photoreduction of Ethyl Chlorophyllide *a* by Ascorbic Acid in Ethanol-Pyridine Solutions

BY G. R. SEELY AND A. FOLKMANIS

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Quantum yields for the photochemical reduction of ethyl chlorophyllide *a* by ascorbic acid in ethanol-pyridine solutions have been determined. According to a proposed mechanism, photoreaction occurs principally between the pyridinium ascorbate ion pair and the chlorophyllide solvates Chl(eth)₂ and Chl(eth)(py). Pyridine acts not only to neutralize ascorbic acid, but also, in the form of pyridinium ion, to stabilize the immediate products of photoreaction by transferring hydrogen. The photoreaction is inefficient, the maximum quantum yield of about 0.02 being attained only when the ascorbic acid concentration approaches 0.1 *M*. When ethanol is the solvent component in excess, the reduced chlorophyllide first formed is converted by acid into a mixture of two reduced pheophorbides in equilibrium, one absorbing maximally near 525, the other near 620 m μ . The rate of regeneration of chlorophyllide or pheophorbide in the dark is apparently limited by the availability of an active form of oxidized ascorbic acid. Structures are proposed for the reduced pheophorbides and chlorophyllide. As aids to understanding the reaction the distribution of chlorophyllide among the three possible solvate species was investigated by spectral methods, and the extent of ionization and ion pair formation in ascorbic acid solutions was investigated by conductivity and indicator methods.

Introduction

The reversible photoreduction of chlorophyll and its derivatives by ascorbic acid has attracted much interest because of its possible relationship to the primary process of photosynthesis.^{1,2} It is known from qualitative work that for efficient reduction to occur, an organic base such as pyridine and an "activator" such as ethanol or water must be present,³⁻⁵ but their precise roles have not been established. Chlorophyll or pheophytin is regenerated in the dark, the latter if the reduced chlorophyll loses magnesium.⁴

We wish to present quantum yields for photoreduction at various ascorbic acid concentrations in solvents of composition varying from pure ethanol to pure pyridine and to propose a mechanism to account for the necessity of both ethanol and pyridine. Most previous work has employed water-pyridine mixtures; ethanol-pyridine mixtures have the advantages that the entire solvent composition range may be employed,

and magnesium loss from reduced chlorophyll is slower. For incidental reasons ethyl chlorophyllide *a* was used instead of chlorophyll; replacement of the phytyl group of chlorophyll by an ethyl group is not expected to affect the solution photochemistry significantly, and it should be possible to make valid comparison with chlorophyll reduction as studied by others.

Experimental

Materials.—Ethyl chlorophyllide *a* was prepared from *Datura stramonium* by the method of Holt and Jacobs⁶ and purified chromatographically. Ethyl pheophorbide *a* was prepared in solution as needed by treating ethyl chlorophyllide in ethanol with 0.01 ml. of concentrated HCl, excess acid being neutralized on addition of pyridine.

Ascorbic acid (Eastman) was at first used directly but later recrystallized from 1:1 ethanol-water. Some runs with unrecrystallized ascorbic acid gave evidence of the presence of a retarder; others did not, but gave the same yields as with recrystallized acid. Other organic reagents were usually recrystallized, but dehydroascorbic acid (Mann) and β -carotene (Nutritional Biochemicals) were used as received. Absolute ethanol was further dried by distillation from Mg(OC₂H₅)₂; pyridine was dried over BaO and distilled.

Air was removed from the reaction cells by bubbling through them prepurified nitrogen (Matheson) which had passed through

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