

Auto-oxidation of Linoleic Acid in Micellar Solution

By J. SWARBRICK and C. T. RHODES

The rates of auto-oxidation of different concentrations of linoleic acid, solubilized in various aqueous solutions of Brij 35, a nonionic surfactant, at 39.6° have been determined as a function of the changes in the spectrophotometric absorbance at 233 m μ which arises from the formation of conjugated diene as the process of auto-oxidation proceeds. For any one surfactant concentration over the range of linoleic acid concentrations studied, the maximum rate of conjugated diene formation (equivalent to the rate of oxidation in the initial stages of the reaction) was found to be directly proportional to the initial micellar concentration of linoleic acid, expressed as the weight ratio of linoleic acid to surfactant. As the concentration of surfactant was increased, the change in the maximum rate, as a function of the weight ratio of linoleic acid to surfactant, decreased. These results are discussed in the light of previous work dealing with the auto-oxidation of solubilized oils.

SURFACE-ACTIVE AGENTS are widely used in the preparation of pharmaceutical products; increased attention is now being focussed on the stability of such formulations toward oxidation (1-4) and hydrolysis (5-9). Studies concerned with the auto-oxidation of aliphatic and aromatic aldehydes and methyl linoleate dispersed in various surfactant solutions have been undertaken recently by a number of workers (1-4) using a manometric technique in which the uptake of gaseous oxygen was determined directly. In the present paper the auto-oxidation of a solubilized polyunsaturated essential fatty acid, linoleic acid, has been investigated by following the change in ultraviolet absorption of the systems. Tukamoto (10), who used a similar approach to evaluate the antioxidant activity of several compounds, was able to correlate the increase in absorption, due to diene conjugation, of linseed oil undergoing auto-oxidation with changes in chemical peroxide value and, to a certain extent, viscosity. Change in diene conjugation has been employed also by Carless and Nixon (1) in studies concerned with the auto-oxidation of methyl linoleate solubilized and emulsified in aqueous solutions of potassium laurate and a nonionic surfactant. It was found that the height of the peak did not parallel the oxygen uptake after the initial stages of oxidation.

Although a complete account of the mechanism of auto-oxidation of polyunsaturated fatty acids is not yet available, it is believed that the initial free radical process involves the early formation of some conjugated diene which reacts with oxygen to form conjugated hydroperoxide (11, 12). Decomposition of the peroxide leads to the formation of other free radicals which serve to initiate further oxidation chains, *i.e.*, the process is autocatalytic (13). Throughout the

early stages of auto-oxidation, the increase in diene conjugation, measured spectrophotometrically at 230-236 m μ , increases parallel with oxygen uptake and peroxide formation (14). Consequently, during this early stage of the reaction, the increase in ultraviolet absorption affords a convenient method of following the auto-oxidation of initially nonconjugated unsaturated fatty acids. This method has the advantage that secondary reactions, leading to the destruction of double bonds, will not complicate the primary rate determination, whereas it might be difficult, if not impossible, when using a manometric technique alone to separate the oxygen uptake arising from the primary conjugation and the secondary scission reactions. Consequently, the kinetics of the primary auto-oxidation of linoleic acid will be better represented by the data obtained from conjugation rather than manometric studies.

EXPERIMENTAL

Linoleic acid¹ was packed in 5-ml. glass vials and stored under nitrogen in the dark until required. The solubility curve of linoleic acid in aqueous solutions of Brij 35,² a polyoxyethylene lauryl alcohol consisting mainly of C₁₂H₂₆(CH₂CH₂O)₂OH, was determined at 39.6° by observing the onset of turbidity (Fig. 1). Aqueous solutions containing 1.74, 2.50, 4.00, and 6.00% w/v Brij 35 were prepared and 40-ml. samples placed in 200-ml. amber glass bottles in order to standardize the effect of light and ensure an adequate supply of oxygen between sampling. On the basis of the solubility curve (Fig. 1), various concentrations of linoleic acid were added so as to give solutions containing from 20 to 90% of the maximum possible weight solubilized at each surfactant concentration studied. The bottles were sealed with waterproof tape and placed on a tray submerged in a water bath maintained at 39.6°. The tray and bottles were moved through a horizontal sweep of 4.5 cm. at the rate of 36 sweeps per minute. At suitable time intervals aliquots were removed, diluted in alcohol (60% v/v), and the

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¹ Purified grade, Fisher Scientific Co.

² Atlas Powder Co., Inc.

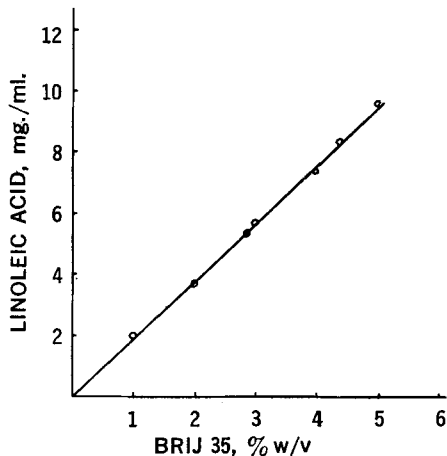


Fig. 1.—Solubility of linoleic acid in Brij 35 solutions at 39.6°.

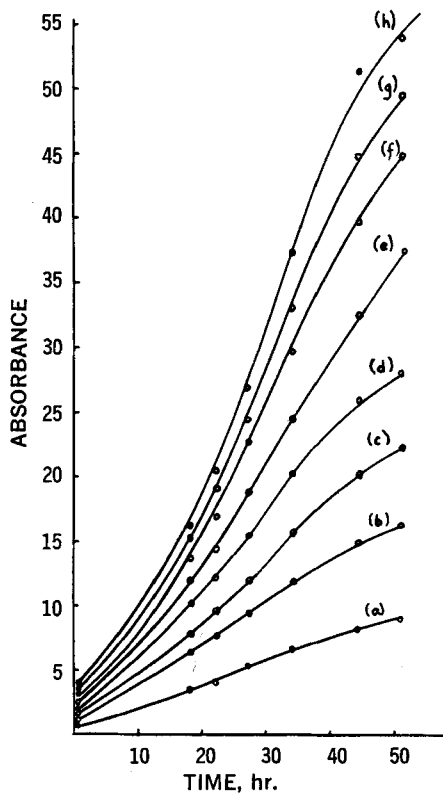


Fig. 2.—Change in absorbance at 233 $m\mu$ of linoleic acid in Brij 35 solutions (1.74%) at 39.6° as a function of time. Key: Initial linoleic acid concentration in mg./ml.: (a) 0.59, (b) 1.02, (c) 1.36, (d) 1.61, (e) 2.08, (f) 2.41, (g) 2.74, (h) 3.08.

absorbance measured at 233 $m\mu$ on a Beckman DU spectrophotometer using 1-cm. cells. All absorbance values were calculated on the basis of an undiluted 1-ml. sample of solution. The pipets used to take the samples were calibrated gravimetrically at 39.6°. The ethanolic solutions of linoleic acid obeyed Beer-Lambert's law over the range of concentrations studied.

RESULTS AND DISCUSSION

The absorbances at 233 $m\mu$ for increasing concentrations of linoleic acid solubilized in 1.74% Brij 35 solutions are plotted in Fig. 2 as a function of time. It can be seen that R_v , the rate of absorbance change per ml., equivalent to (dA/dt) , where A is the absorbance per ml. of original solution and t is the time in hours, reaches a maximum equivalent to the maximum rate of oxidation of the linoleic acid molecule, and then declines. This type of plot was typical of the changes from the other three surfactant concentrations studied. Figure 3 shows the maximum rates, $(dA/dt)_{max.}$, for all the systems investigated plotted against the initial concentration of linoleic acid.

The authors have plotted $(dA/dt)_{max.}$ against the initial linoleic acid concentration rather than the concentration of linoleic acid present at the time ($t_{max.}$) when the maximum rate is operative because it is not possible to calculate accurately the concentration of unreacted acid remaining at any one time. This introduces no serious error since the change in concentration is virtually constant, on a per cent basis, for all the systems examined. Thus, when using a value for the molar extinction coefficient of 22,700 for the hydroperoxide (12), calculations show that the percentage oxidation of linoleic acid, in all systems studied, lies within the range 8.8 ± 1.5 to 10.7 ± 1.9 at $t_{max.}$, where $t_{max.}$ in all instances lay between 25 and 30 hr. This means that the slopes of the curves in Figs. 3 and 4 will be virtually equivalent, relative to one another, regardless of whether the initial linoleic acid concentration or the estimated linoleic acid concentration is plotted as abscissa. Accordingly, all reaction constants are quoted as *apparent* rather than absolute constants.

Previous work (3) has established the micelle as the site of oxidation in solubilized systems. The rate of oxidation should therefore be related to the

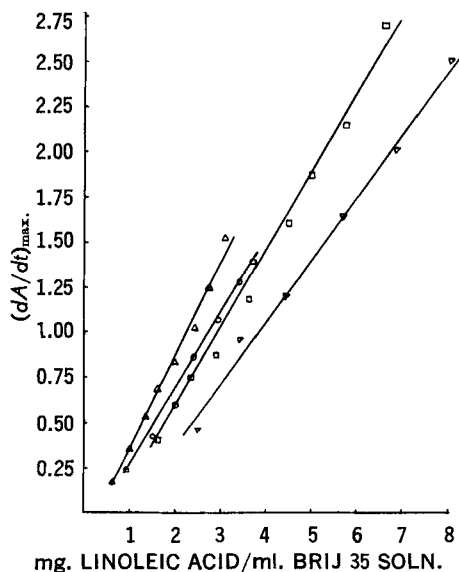


Fig. 3.—Plot of maximum rate of absorbance change against initial over-all linoleic acid concentration. Key: Brij 35 concentration: Δ , 1.74%; \circ , 2.50%; \square , 4.00%; ∇ , 6.00%.

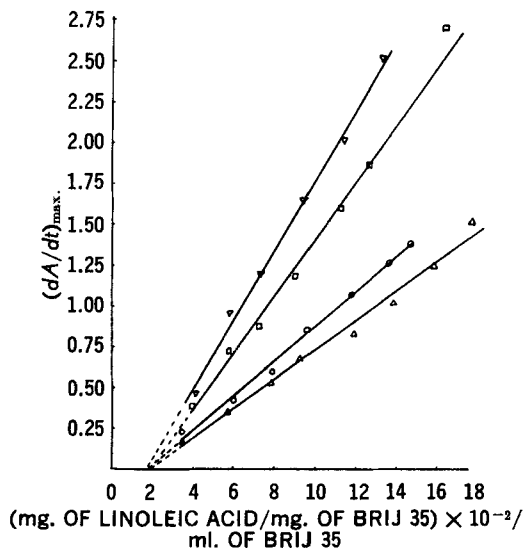


Fig. 4.—Plot of maximum rate of absorbance change against micellar ratio, *i.e.*, effective *initial* micellar linoleic acid concentration. Key: Brij 35 concentration: Δ , 1.74%; \circ , 2.50%; \square , 4.00%; ∇ , 6.00%.

TABLE I.—VARIATION OF MAXIMUM OXIDATION RATE PER mg. INITIAL LINOLEIC ACID, R_w , WITH SURFACTANT CONCENTRATION

Brij, mg./ml.	k'	R_w
17.4	9.12	0.525
25.0	10.65	0.426
40.0	17.81	0.445
60.0	21.39	0.356

micellar concentration of linoleic acid rather than the total concentration added. Accordingly, it is necessary to treat the solution as a two-phase system consisting of an aqueous continuous phase and a micellar pseudo-phase and estimate the distribution of the components between them. The concentration of surfactant in the continuous phase will approximate to the critical micelle concentration (CMC) of the ternary system (3); the micellar surfactant concentration will therefore equal the total concentration minus the CMC. In the present study the total concentration of surfactant is so large in comparison to the likely CMC that no appreciable error is introduced if the micellar concentration is taken as being the same as the total amount of surfactant present. While it may be difficult to estimate the distribution of slightly water-soluble additives between the micellar and aqueous continuous phase, especially as the concentration of additive is varied, it is reasonable to assume, in the case of water-insoluble materials such as linoleic acid (15), that they are located exclusively in the micellar pseudo-phase. Consequently, the micellar linoleic acid is equal to the total amount added.

The rates given in Fig. 3 have therefore been replotted against the ratio of the micellar weights of *initial* linoleic acid to Brij 35 per unit volume of

Brij 35 solution (Fig. 4). This concentration term is used in preference to the micellar weight of linoleic acid per unit volume of surfactant solution since it automatically corrects for the several surfactant concentrations used. As a result, all the rates are compared under identical conditions, in so far as relative micellar amounts of additive and surfactant are concerned. Figure 4 shows that the maximum rate of oxidation varies linearly with the micellar ratio when the latter exceeds a value of 1.85×10^{-2} , or

$$(dA/dt)_{\max.} = k'(\text{micellar ratio} - 1.85 \times 10^{-2}) \quad (\text{Eq. 1})$$

where k' is an *apparent* reaction constant for the formation of conjugated diene in each of the various surfactant systems examined and (micellar ratio) is expressed as the micellar weight ratio of *initial* linoleic acid to surfactant per unit volume of Brij 35 solution.

Since the molecular weights are in the ratio of approximately 280:1200 or 1:4.3, the weight ratio of 1.85×10^{-2} is equivalent to a micellar *molar* ratio of 7 to 8×10^{-2} , *i.e.*, at this concentration, which is common for all four surfactant concentrations studied, there are seven or eight linoleic acid molecules associated with every 100 surfactant molecules in the micellar pseudo-phase. Linoleic acid, because of its long hydrocarbon chain and weakly polar head group, is predominantly lipophilic. The site of solubilization within the micelle will therefore be the hydrocarbon interior, although the hydrated carboxylic acid group is probably like that of other solubilized acids, in that it is anchored at the junction of the palisade layer and the hydrocarbon nucleus (16). The reactions at the unsaturated sites along the chain will thus take place in the hydrocarbon interior of the micelle. If, as seems likely from available data on aggregation numbers (17), we assume that each micelle contains about 50 surfactant monomers, then the extrapolated intercept in Fig. 4 implies that approximately four linoleic acid molecules have to be present in the micelle interior before the reaction becomes first order with respect to concentration. One possible explanation is that when less than this number of additive molecules are present the position of the head group restricts movement of the hydrocarbon chain sufficiently to limit the rate of oxidation probably by making the propagation step difficult. However, this restriction becomes insignificant when more than three or four molecules are present because the number of linoleic hydrocarbon chains is now sufficient to allow ready propagation of the reaction. A somewhat similar explanation has been advanced by Swarbrick and Carless (3) to account for the auto-oxidation of benzaldehyde solubilized in a series of ampholytic surfactants, although in this case the oxidation site on the molecule was the weakly polar aldehyde group located in the palisade layer of the micelle.

Figure 4 compares R_w , the rate per ml. solution, under identical conditions of micellar saturation, it takes no account of the rate per mg. micellar linoleic acid (R_w) as a function of micellar saturation. From Fig. 4

$$R_w = \frac{(dA/dt)_{\max.}}{(\text{Brij})(\text{micellar ratio} - 1.85 \times 10^{-2})} \quad (\text{Eq. 2})$$

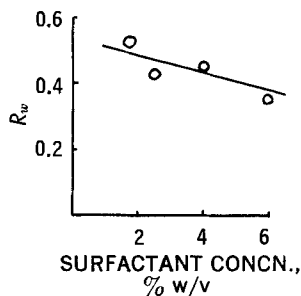


Fig. 5.—Variation of maximum rate of oxidation per mg. initial linoleic acid, R_w , with surfactant concentration.

where (Brij) is the surfactant concentration in mg. per ml. solution.

Thus from Eqs. 1 and 2

$$R_w = \frac{k'}{(\text{Brij})} \quad (\text{Eq. 3})$$

The calculated values for R_w and k' derived from Fig. 4 are shown in Table I.

The quantity R_w , which represents the *apparent* reaction constant calculated with respect to unit surfactant concentration, would, if all the surfactant systems were behaving identically, be constant in value. It is however obvious that R_w , although constant for any one surfactant concentration, decreases as higher surfactant concentrations are employed (Fig. 5). This is somewhat surprising since the only difference in the four sets of systems studied, assuming the aggregation number to remain constant, is that the number of micelles per unit volume increases directly with the surfactant concentration. These systems were not deliberately catalyzed with metal ions as those studied previously using the manometric technique (1-4). Consequently, no direct comparisons can be drawn. It may be however that, under the conditions used

in the present work, the number of initiating free radicals produced by photo- and thermal catalysis is relatively low and independent of linoleic acid and surfactant concentration. As the surfactant concentration is raised, the number of initiators may fall below the number of micelles; this could lead to the observed fall in R_w . Studies involving the addition of a metal catalyst are in progress to test this hypothesis. Nevertheless, the present results are of immediate practical importance since no manufacturer would deliberately contaminate his product with a metallic catalyst. Thus, the relationships established in this study indicate that pharmaceutical formulations of solubilized linoleic acid and possibly other unsaturated molecules should contain as much surfactant as possible in order to reduce the concentration of additive in the micelles.

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