

# Autoxidation of Polyunsaturated Fatty Esters on Silica

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## ABSTRACT

The stability of unsaturated methyl esters of fatty acid adsorbed on silica gel and silicic acid was studied by gas liquid chromatographic estimation of disappearance relative to a saturated internal standard. Variables included silica-ester ratio, agitation, adsorbent particle size, and degree of unsaturation. Under the conditions of the experiment, destruction of substrate unsaturated ester at 80 C was more a function of time than of unsaturation or initial purity.

## INTRODUCTION

It was recently shown (1,2) that stability of unsaturated oil and methyl esters of polyunsaturated fatty acids is greatly increased when adsorbed on silica gel. This finding was considered significant, not only from a practical viewpoint but also because of its possible implication of a mechanism of autoxidation different from that elucidated in solutions or in neat oils (3-6). It may also have a bearing on the mechanism of autoxidation of lipids in some ordered arrangement as in layers or membranes (7,8), a reaction that has been implicated in oxidative tissue damage as in the

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aging process (9).

In a further extension of this research, a comparison has been made of the rates of oxidation of methyl esters with different degrees of unsaturation adsorbed on silica gel. The results appear to confirm the different mechanism of autoxidation of adsorbed polyunsaturates.

## MATERIALS AND METHODS

Pentane, cyclohexane, diethyl ether, silica gel (J.T. Baker 3405, lot 34110) and silicic acid (J.T. Baker 0324, lot 32080, 200-325 mesh) were reagent grade and were used without further purification. The adsorbents contained some metal impurities, as determined by emission spectroscopy. The ppm of Fe, Co, Ni, Cu, Ca and Mg were 100, not detected (ND), 0.8, 0.1, 45 and 120, respectively, for the silica gel, and 5, ND, 0.5, 0.2, 1 and 0.1, respectively, for the silicic acid. Traces of Ti, Al and Na were observed; no detectable amounts of Cr, Sr, Ba or Ag were found. Methyl linolenate, methyl eicosapentaenoate and methyl docosahexaenoate were of variable purity, which was checked, in part, by UV spectrum and gas liquid chromatographic (GLC) purity.

A rotating shaker was altered in such a way that vigorous agitation of the solid adsorbent could be achieved. The shaker was maintained at 80 C.

TABLE I

Oxidation of Unsaturated Esters on Silica Gel at 80 C

Methyl esters	Time, days	Per cent oxidized		
		Experiment 1 <sup>a</sup>	Experiment 2 <sup>b</sup>	Experiment 3 <sup>b</sup>
18:3	2	67	---	63
20:5	2	70	---	---
22:6	2	---	---	69,64
18:3	4	89	87	82
20:5	4	91	---	---
22:6	4	---	83	90

<sup>a</sup>Disappearance of 0.5 mg unsaturated ester on 0.5 silica gel was measured relative to 0.1 mg Me 18:0. The unsaturated esters contained significant conjugation. Gas liquid chromatography on EGS.

<sup>b</sup>Disappearance of 0.5-0.6 mg unsaturated esters on 0.5 g silica gel was measured relative to 0.05 mg Me 16:0 and 0.1 mg Me 20:0. Gas liquid chromatography on OV-1.

TABLE II  
Effect of Gel-Unsaturated Ester Ratio

Methyl ester	Gel Unsaturated ester, g/mg	Per cent of original oxidized <sup>a</sup>	
		2 Days	4 Days
18:3	0.20	63	88
	1.0	67	89
	2.0	62	90
20:5	0.19	62	88
	0.92	70	91
	1.8	68	87

<sup>a</sup>Disappearance of 0.5 mg of unsaturated ester was measured relative to 0.1 mg Me 18:0. Gas liquid chromatography on EGS. (Experiment 1 oxidation values for gel-ester ratio = 1 also appear in Table I.)

### Gas Liquid Chromatography

Analyses for the first experiments were performed with polar columns. A Barber-Colman Model 20 apparatus was equipped with a 6 ft x 1/8 in. 12% DEGS column. A Barber-Colman Model 10, with a flame-ionization detector was equipped with a 40 in. x 6 mm 12% EGS column. Because the retention time for 22:6 at the maximum recommended temperature (195 C) was ca. 45 min, later analyses were performed using a nonpolar column. A 40 in. x 6 mm 3% OV-1 column was used in the Barber-Colman Model 10. The retention time of Me. 22:6 on the nonpolar column was 22 min at 185 C and 20 psig A.

### Calculations

The progress of autoxidation was measured on GLC by the disappearance of the unsaturated methyl ester relative to that of an added saturated ester. The saturated ester was presumed unchanged, although it is recognized that saturated esters may autoxidize (10). From relative autoxidation rates of 1:100:1000 for Me 18:0, Me 18:1 and Me 18:2 (11), it was presumed that the saturated ester would be substantially unchanged as long as a trace of unsaturated ester remained.

The per cent of the original unsaturated ester remaining was estimated from the ratio unsat.-sat. after oxidation and extraction. This ratio was divided by the initial unsat.-sat. ratio and multiplied by 100. Weight ratios were used for calculation of the initial mixtures unless GLC indicated gross deviation. In these instances, GLC initial ratios were used. Single values in tables represent single chromatograms.

### Procedure

The disappearance of unsaturated methyl esters relative to saturated internal standards was measured by GLC. Methyl esters were

deposited on a silica support by pipetting appropriate volumes of unsaturated and saturated ester solutions into a flask containing a cyclohexane slurry of silica gel or silicic acid. Cyclohexane was used to prepare the standard solutions since they could be stored at -20 C in the solid state. It was hoped that immobilization in this manner might decrease autoxidation during storage and, indeed the poly-unsat.-sat. ratios of freshly mixed stock solutions showed no significant decrease during 6 months of storage. Concentrations of unsaturated methyl esters in the solutions were ca. 0.5 mg/ml cyclohexane.

The flasks, containing cyclohexane, silica and ester, were swirled to mix the contents and the solvent was removed under vacuum on a rotating evaporator, below 50 C. The dry powder was transferred to a clean 125 ml Erlenmeyer flask to avoid an ester film on the inside surface of the original flask.

The flasks were heated at 80 C open to air. Portions (80-200 mg) were removed at intervals with a small porcelain spoon for GLC analysis. The silica was transferred to a 9 in. glass disposable Pasteur pipette containing a small plug of pyrex wool. Esters were extracted from this small column with two or three 1 ml portions of diethyl ether. The solvent from each extraction was evaporated in a stream of nitrogen before the next extraction. The 1 ml vials containing the solvent were held in a heating block at 35 C until the solvent had just evaporated, and the resulting methyl ester mixture was subjected to GLC analysis as rapidly as possible.

### RESULTS

Several types of experiments were carried out, all at 80 C. This temperature was conveniently accessible, yet high enough so that reasonably fast oxidation rates prevailed. It was first confirmed that thin films of unsaturated esters, that is, films not adsorbed on silica gel or on silicic acid but simply spread on the bottom of a 125 ml Erlenmeyer flask, underwent the normal rapid autoxidation. Samples of 0.25-0.5 mg Me 18:3 were 21% oxidized after 30 min and 63% oxidized after 1 hr at 80 C. Similar samples of Me 22:6 were 66% oxidized after 30 min and 96% oxidized after 1 hr. Esters with different degrees of unsaturation oxidize at different rates, as is usually expected. The rate is considerably more rapid than when adsorbed, since, as seen below, the same amount of either ester required 4 days for 80-90% oxidation. Thus the earlier observation of decreased surface oxidation (2) is confirmed.

Most of the experiments were carried out with the use of silica gel as the adsorbent. A first set of results is given in Table I, under run 1. We were concerned, however, that although freshly opened vials of material were used, there was evidence of contamination by oxidation products. The 18:3/18:0 ratio was 4.49 by GLC for a mixture made up to have a ratio of 4.80 w/w. Worse, the 20:5/18:0 ratio was 2.95 by GLC as compared to 5.23 w/w. Also, the UV spectra indicated the presence of some conjugated diene in the 18:3 material and of both conjugated diene and triene in the 20:5 material. It is known, of course, that in the usual autoxidation studies, impurities can have significant effects (3,6,12-14).

New samples of esters were therefore used for runs 2 and 3 of Table I. No conjugated dienes were seen in either sample. Moreover the 22:6/20:0 ratio was 5.44 by GLC and 5.52 w/w, or the same within experimental error; even better agreement was found between the 18:3/20:0 ratios. The results of the three runs are essentially the same, however, and it thus appears that the presence of oxidation impurities does not materially affect the oxidation rates of the adsorbed ester. As a precaution, however, all subsequent runs were made with the second set of samples.

A second series of experiments was planned to determine the effect of varying the ratio of ester to silica gel. The results of a 10-fold variation are summarized in Table II. No significant effect is present.

A point of interest was whether our uniformly slow oxidation rates for the adsorbed esters could possibly be a reflection of oxygen diffusion to the surface as a rate limiting step. To check this possibility, Me 18:3 and Me 22:6 adsorbed on silica gel were allowed to oxidize under conditions of vigorous shaking such that the particles were continuously tumbled. The results of the dynamic (shaken) and static (unshaken) runs are given in Table III. There appears to be no effect of greatly increasing the ease of access of oxygen to the silica gel particles.

The 2-4 day periods used for most of the runs allowed sufficient oxidation to occur for any differences in rates between the various esters to be magnified. It was desirable, however, to investigate the oxidation rate law more closely by obtaining values for the degrees of oxidation at shorter times. A set of such runs gave results summarized in Table IV. These results, being reported as per cent of original ester oxidized, necessarily exhibit more scatter than do the data for the longer times. The point is that our actual measurement is of the relative

TABLE III  
Comparison of Dynamic and Static Oxidation of Unsaturated Esters at 80 C

Methyl ester	Time, days	Per cent of original oxidized <sup>a</sup>	
		Dynamic	Static
18:3	2	58 (60) <sup>b</sup>	50 (54) <sup>b</sup>
22:6	2	63	63
18:3	4	88	83
22:6	4	91	88 (87) <sup>b</sup>

<sup>a</sup>Disappearance of 0.5-0.6 mg unsaturated ester on 0.5 g silica gel was measured relative to 0.05 mg Me 16:0 and 0.1 mg Me 20:0. Gas liquid chromatography on OV-1.

<sup>b</sup>Duplicate gas liquid chromatography value.

amount of ester not oxidized, and the per cent oxidation is obtained by comparing this measurement with that for an untreated or reference sample. Each measurement separately is subject to ca. 5% uncertainty in absolute value, although the precision is actually somewhat better. The effect is that the smaller the degree of reaction, the more uncertain is its actual value. A reported 10% degree of reaction is, for example, probably subject to 50% relative error. With this aspect in mind, there appears to be no significant difference in oxidation rate between the various esters, with the possible exception of the 3.5 hr results.

Finally, we wished to test for sensitivity to at least minor changes in the nature of the adsorbent. This was done by carrying out a set of runs using silicic acid. The results are given in Table V. Silicic acid appears to be a somewhat more inert supporting material than silica gel, although the effect is not large. It should be noted that the apparent ester-adsorbent ratios may not be strictly comparable between the two adsorbents. The silica gel was 60-200 mesh, while the silicic acid was 200-325 mesh; the average particle sizes and probably also the specific surface areas were thus different. In addition, the loss in weight on ignition was 8.2% for the silica gel and 23.8% for the silicic acid, according to the manufacturer's specifications. This means that the number of surface sites per gram of material was probably different for the two adsorbents, even for the same mesh size particles. Moreover the nature of such sites may have been somewhat different because of the different water content.

DISCUSSION

The above results appear to support the following immediate conclusions: (a) Oxidation rates for adsorbed unsaturated esters are one-

TABLE IV

Early Stages of Oxidation<sup>a</sup>

Methyl ester	Hours 80 C	Per cent oxidized	Average per cent oxidized
18:3	3.5	0, 6.8	3.4
22:6	3.5	16.6 (17.2), <sup>b</sup> 20.2	18.4
18:3	5.75 <sup>c</sup>	16.5, 20.1	18.3
22:6	5.75 <sup>c</sup>	28.8 (29.0), <sup>b</sup> 21.9	25.3
18:3	22 <sup>c</sup>	48.2, 47.3	47.8
22:6	22 <sup>c</sup>	56.5, 53.4	55.0

<sup>a</sup>Disappearance of 0.5-0.6 mg unsaturated ester on 0.5 g silica gel was measured relative to 0.05 mg Me 16:0 and 0.1 mg Me 20:0. Gas liquid chromatography on OV-1.

<sup>b</sup>Duplicate gas liquid chromatography value was not used in average to prevent weighting.

<sup>c</sup>Samples were removed and stored at -20 C and extracted just before analysis.

twentieth to one-thirtieth as fast as those for esters in bulk. (b) The oxidation rates of adsorbed esters are not sensitive to (c) the ester-adsorbent ratio at the level of 1 mg/g, (d) variation in the ease of access of oxygen to the surface of the particles of adsorbent, or (e) to the molecular weight or the degree of unsaturation of the ester. Finally, (f) the rate may be sensitive to the type of silicious adsorbent used.

The data were tested with various rate laws, using average values for the degrees of oxidation of each ester at each of the various times, as obtained from the individual observations reported in Tables I-IV. The results agree fairly well with the first order rate law,  $A/A_0 = e^{-kt}$ , where  $A/A_0$  denotes the fraction of ester remaining at time  $t$ , and  $k$  is the apparent first order rate constant. The corresponding semi-logarithmic plot is shown in Figure 1. With the exception of the 3.5 hr point for Me 18:3, all of the data lie sufficiently close to a straight line for distinctions between the esters to seem unwarranted, in confirmation of point (e) above. (Somewhat better agreement does result if separate straight lines of slightly different slope are fitted to the 18:3 and the 22:6 points, but it is doubtful that the precision of the data justifies this level of detail.) The data do not fit a simple second order plot ( $A_0/A$  vs.  $t$ ) at all, nor do they give a straight line when plotted as  $A/A_0$  vs.  $t^{1/2}$ , or as  $(1-A/A_0)^2$  vs.  $t$ . These last are forms sometimes followed when diffusion through a deepening layer of material is involved (15a).

Returning to the first order plot, the line in Figure 1 is drawn so as to intercept  $A/A_0 = 90\%$  at  $t = 0$ ; this gives a better fit to the points than does a line drawn to  $A/A_0 = 100\%$ . Such an intercept suggests the presence of a fast component to the rate law. This component could be bulk phase oxidation occurring during the preparation of the system, but we have no reason to believe that our procedures could have caused an acceleration of this ordinarily

slow process. Another explanation is suggested below. It should be noted that the 3.5 hr point for Me 18:3 would, if given full weight, suggest a 1-2 hr induction period. A more detailed investigation would be needed to establish such a feature, however.

The results for silicic acid are included in Figure 1. If first order behavior is assumed, the oxidation rate is about two-thirds of that on silica gel. The data, while limited, are fit best by a line drawn to 100%  $A/A_0$  at  $t = 0$ , suggesting that no fast component is present in the rate law.

An aspect of possible importance to any discussion of mechanism is the following. The specific surface area of our samples is ca. 300  $m^2g^{-1}$  (16,17). If we take the radius of 325 mesh material to be ca.  $2 \times 10^{-4}$  cm and the density of silica gel to be ca. 2.5, then for spherical particles the specific surface area is ca. 0.5  $m^2g^{-1}$ . Thus most of the "surface" of our adsorbents must be interior surface; the materials are highly porous. The adsorbed esters are probably distributed fairly uniformly throughout the interior of each particle. A possibility that must be considered, therefore, is that a bulk-type reaction is occurring in our systems but that it is slow because of being limited in rate by that of diffusional access of the oxygen. The lack of dependence of the rate on the degree of agitation is not diagnostic, since diffusion of oxygen into and through individual particles could be the slow step. There are, however, some counterindications. Oxygen diffusion into particles should not obey a first order rate law; also qualitative observations on the temperature dependence of the oxidation indicate that it is much larger than would be expected for a gas diffusion process. For example, the earlier results (2) indicated ca. 30% oxidation of esters with three or more bonds on silica gel after 63 days at 4 C while the present results show that only ca. 1 day is required at 80 C. The comparison generates an

TABLE V

Oxidation of Unsaturated Esters on Silicic Acid at 80 C<sup>a</sup>

Methyl ester	Time, days	Per cent oxidized	
		Experiment 2	Experiment 3
18:3	2	—	52,45
22:6	2	—	43
18:3	4	77	75
22:6	4	73	78,76

<sup>a</sup>Disappearance of 0.5-0.6 mg unsaturated esters was measured relative to 0.05 mg Me 16:0 and 0.1 mg Me 20:0 on 0.5 g silicic acid. Gas liquid chromatography on OV-1.

apparent activation energy of 11 kcal mol<sup>-1</sup>. Gas diffusion coefficients are proportional to the average molecular velocity and hence to T<sup>1/2</sup> (18). Another point is that the more unsaturated esters should provide a deeper oxygen "sink"; more oxygen would have to diffuse into a particle for a given degree of loss of original ester and a slower rate of disappearance would be expected, contrary to our observation. Finally, the absolute rates are rather small to be explained in terms of gas diffusion. The reaction itself does not appear to be of the bulk type, since we observe little if any induction period.

Our tentative conclusion is that the rate limiting step is a direct chemical reaction between oxygen and adsorbed ester, uncomplicated by the free radical chains involved in the bulk reaction (4). A calculation is instructive at this point. A loading of 1 mg ester per gram of adsorbent of surface area 300 m<sup>2</sup>g<sup>-1</sup> corresponds to an apparent film thickness of ca. 0.03 Å. The actual thickness of a hydrocarbon chain is ca. 3 Å so that only ca. 1% of the surface is actually occupied. Adsorbed ester molecules are thus far apart on the average and it is very unlikely that they would be able to participate in a cooperative or chain process. A first order rate law should be obeyed since oxygen diffusion is now not considered to be rate controlling. There should be no induction period.

We next consider the possible nature of the oxidation step itself. One possibility is that the adsorbent inhibits oxidation by isolating the ester molecules from each other so that no chain reaction is possible. The observed rate might then be similar to that of the *initiation* step of the bulk reaction. The difficulty with this picture is that the rate of oxidation should be at least approximately proportional to the number of double bonds in the ester molecule, contrary to observation.

The above difficulty is resolved if active sites are present, capable of catalyzing oxidation at a

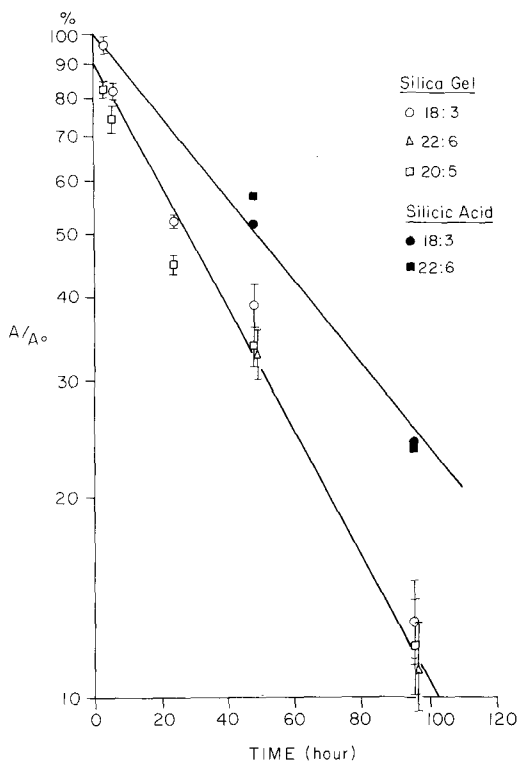


Fig. 1

double bond, with only one double bond activated per molecule of ester. The rate of oxidation would now conform to observation (3). The restriction to one activated double bond per molecule can be accounted for in at least two ways. One possibility is that the spacing between activating adsorption sites does not match the distance between double bonds, so that only one bond in a given molecule can be optimally adsorbed.

An alternative possibility is that the active sites are those of metal impurities. Metal oxides are catalysts for the oxidation of various organic molecules, and of course metal ions are known to be catalysts in the homogeneous autoxidation of unsaturated esters (14,20). Although the free radical aspect of autoxidation is usually emphasized, the possibility of nonfree radical initiation in the presence of trace metal impurities has been considered (21,22). Transition metals such as cobalt, manganese, nickel, iron and copper are especially active. This possibility could take one of two forms. Ester adsorption might be localized on metal oxide sites, but with the sites far enough apart that only one double bond per molecule is activated. This would be a stoichiometric

mechanism. Less than a stoichiometric number of sites would be needed, however, if surface diffusion allowed ester molecules to migrate to a catalytic site for reaction. For first order kinetics to be observed, it would be necessary for the site reaction to be the slow step. Not much is known about surface diffusion rates on solids, but indications are that they can be quite appreciable (15b), so this dynamic mechanism may not be unreasonable.

The 100 ppm of iron in the silica gel corresponds, on a mole basis, to ca. 0.5 mg of unsaturated ester. If the iron were entirely on the surface, this amount would barely suffice to provide the adsorption sites necessary for the stoichiometric mechanism; the amount would probably be sufficient to account for the possible fast component in the oxidation, however. Neither iron nor the other likely candidates, nickel and cobalt, are present in sufficient amount in either adsorbent to hold the quantities of ester used. While the stoichiometric mechanism thus seems to be ruled out, the dynamic one could still apply. It is of course suggestive that the silicic acid was less active than the silica gel and also contained smaller amounts of transition metal impurities.

We believe that the present results establish a potentially important phenomenon, namely the reduced rate and nonautocatalytic nature of the oxidation of adsorbed unsaturated esters. A number of additional parameters remain to be studied. These include the dependence of the oxidation rate on the partial pressure of the oxygen, on temperature, on the degree of hydration (or of heat treatment) of the adsorbent, and on the surface concentration of metal impurities. Our present data may reflect this last parameter: The disappearance of the fast component to the rate law on going from silica gel to silicic acid could be due to there being a smaller concentration of some active impurity in the latter adsorbent.

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