Stability of unsaturated methyl esters of fatty acids on surfaces

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Summary The stability of unsaturated methyl esters is greater when they are adsorbed on silica gel than when a glass surface is used. Storage of small samples adsorbed on silica gel may be a convenient addition to conventional methods of protecting labile fats against autoxidation.

Supplementary key words autoxidation · silica gel storage

PRESERVATION of samples before analysis is important to biochemists handling materials containing polyunsaturated fats. When storage is necessary, it should be at low temperature, under an inert gas, possibly with added antioxidant. These measures decrease but do not always prevent autoxidative and enzymatic changes.

In the course of investigating the increased stability to autoxidation found for unsaturated methyl esters chromatographed on silver nitrate–silicic acid (1), the effects of adsorption of these esters on different surfaces was studied. The type of surface can be important in several ways. It may contain atoms of transition metals, causing ordinary metal catalysis (2). Very small amounts of certain metals can have profound effects on autoxidation rates (3–5). The orientation of molecules on the surface can also influence oxidations. When cottonseed oil is spread on a gelatin surface there is less oxidation than on a glass surface (6). Since the π bonding of unsaturated esters to silver ions is strong enough to permit chromatographic separation of compounds with different degrees of unsaturation, we hoped that the specific binding of the unsaturated bonds of methyl esters from biological materials to AgNO₃-impregnated silica gel might be strong enough to afford significant protection against autoxidation. Hopefully, this would be a useful adjunct to methods commonly used to protect small quantities of unsaturated fats.

Materials. Pentane, hexane, cyclohexane, diethyl ether, and silver nitrate were reagent grade and were used as received. Cyclohexene, bp 83°C, was distilled to remove the *p*-methoxyphenol (bp 243°C) added as a stabilizer by the manufacturer. It was then passed through a silica gel column and stored in the dark at 4°C. Silica gel (Baker 3405, lot 34110) was used as received for one part of the experiment. It was impregnated with AgNO₃ (9% by weight) by addition of an aceto-

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Abbreviations: GLC, gas–liquid chromatography.

nitrile solution of the reagent to an acetonitrile slurry of the adsorbent, followed by removal of the solvent with vacuum and heat in a rotating evaporator. A mixture of methyl esters with 0–6 methylene-interrupted double bonds was made from commercial esters of >90% purity. The ester mixture dissolved in cyclohexane was stored at −20°C until purified by chromatography. A 2 × 14 cm column of silica gel (Baker 3405) was washed with 50 ml of pentane. A cyclohexane solution of the esters was poured on the top of the column. 50 ml of pentane eluted 0.0024 g (I). 100 ml of 4% diethyl ether in pentane eluted 0.2375 g (II). 75 ml diethyl ether eluted 0.0176 g with typical autoxidation odor (III). I and III were discarded. II was diluted with 50 ml of hexane to give a concentration of 0.00475 g/ml. The flask was stoppered and kept at −20°C except when in use. GLC composition of the mixture was palmitate, 3.29%; oleate, 9.4%; linoleate, 12.1%; linolenate, 15.3%; arachidonate, 15.4%; eicosapentaenoate, 18.9%; and docosahexaenoate, 25.5%.

Procedure. A 1-ml aliquot of hexane containing 0.00475 g of mixed esters was pipetted into an empty 125-ml Erlenmeyer flask to serve as a control. 1-ml aliquots were also pipetted into similar flasks containing 0.1 g of AgNO₃–silica gel, 0.5 g of AgNO₃–silica gel, 0.1 g of silica gel, or 0.5 g of silica gel. The flasks were swirled to mix the contents. They were loosely covered with beakers to exclude dust but not air, and then placed in a 4°C refrigerator. The refrigerator was dark except for occasional openings to remove and return contents. Sampling initially consisted of the addition of cyclohexene–diethyl ether 1:1, mixing, removal of an appropriate volume with a microsyringe, and direct injection on a polar GLC column. The flask was immediately replaced in the refrigerator for storage until the next sampling. Because the broad solvent peaks interfered with accurate measurement of early peaks, the sampling technique was modified. A larger amount of cyclohexene was added and the flask was swirled. A portion was withdrawn and filtered through a small plug of Pyrex wool in a disposable Pasteur pipette. The cyclohexene was evaporated with nitrogen at room temperature. The sample was then dissolved in pentane and injected immediately onto the GLC column.

Gas–liquid chromatography. A Barber-Colman model 10 with a flame ionization detector was used for analyses. The column was 12.2% (w/w) ethylene glycol succinate on acid-washed, siliconized Chromosorb G, 6 mm × 41 inches, 80–100 mesh. At 185–200°C and 15–20 psig of argon, methyl palmitate was eluted in 2–3 min. Accuracy was within ±5% of major components.

Calculation. Methyl palmitate was presumed to be unchanged under conditions of the experiment. The area ratios of the unsaturated esters to methyl palmitate were

calculated, divided by the initial area ratios for the individual esters, and multiplied by 100 to give percentages of the original esters remaining.

Results. There was a considerable difference between the stability of the unsaturated esters on glass and on the two adsorbents. After 16 days at 4°C there were no esters with more than three double bonds found on the glass surfaces (three trials). After 42 days at 4°C there were varying amounts of all the original compounds on the adsorbents. The differences between the two adsorbents are shown in Table 1. Although the silver nitrate-treated silica gel preserved the unsaturated esters longer than the glass surface, the unaltered silica gel was even better. It is possible that crystallization of silver nitrate in the pores of the silica gel reduced the surface area. Oxygen uptake by soybean oil on silica gel was an inverse function of the surface area when the amount of oil was less than required for a monomolecular layer (7). It is equally possible that Ag+ promotes some oxidation.

Comparison of oxidation on different surfaces. A very crude estimate of the difference in stability on a glass surface with that on the silica gel may be obtained by comparing the time required by a given ester to reach about 50% of its original value. Fortuitously, the values for the 63-day silica gel sample and the 5-day glass control sample are similar for most of the esters. This is shown in Table 2. The esters stored on silica gel might be considered 63/5, or 12.6, times more stable than the control. A more satisfying approach is to assume that methyl oleate does not autoxidize until the majority of the polyunsaturated materials are gone. Inspection of the GLC analyses for samples containing three or more double bonds indicated that the value for 18:1 was as likely to be above 100% as below (7 vs. 5), and the 18:1/16:0 ratios did not decrease. Chromatograms in which methyl oleate deviates from 100% by more than 5% are presumed to err by inaccuracy in the palmitate peak. They are corrected by multiplying each component by the factor necessary to adjust oleate to 100%. From the original percentage composition and percentage remaining at a given time, the actual amount of each ester oxidized is calculated. A plot of μmoles of each ester oxidized vs. the number of double bonds in the ester is roughly linear. Fig. 1 shows typical plots. The data from Table 2 have been included. The slope of a line gives the average μmoles of ester oxidized per double bond. Division by the days of storage gives the average oxidation rate in μmoles per bond per day. The comparison made from the data in Table 2 can also be made from Fig. 1. The slope of the 5-day glass sample is 0.283; the oxidation rate is 0.057 μmoles/bond/day. The slope of the 63-day, 0.5-g silica gel sample is 0.231; the oxidation rate is 0.0037 μmoles/bond/day. The ratio of the oxidation rates shows that the esters are 15 times less stable on glass

TABLE 1. Variation of stability of unsaturated methyl esters with surface after 42 days[a]

Methyl Ester	AgNO₃–SiO₂	SiO₂
	% remaining[b]	
18:1[c]	97.6	103.1
18:2	41.7	106.2
18:3	24.7	79.6
20:4	10.4	83.5
20:5	8.7	76.3
22:6	7.9	59.9

[a] 0.00475 g of ester mixture on 0.1 g adsorbent, 4°C, dark.
[b] Relative to 16:0.
[c] Number of carbon atoms:number of double bonds.

TABLE 2. Comparison on silica gel and glass surface[a]

Methyl Ester	63 Days Silica Gel[b]	5 Days Glass
	% remaining[c]	
18:1[d]	104.8	101.5
18:2	85.2	84.0
18:3	75.9	74.2
20:4	75.0	65.7
20:5	67.7	57.1
22:6	64.7	60.2

[a] 0.00475 g of mixed methyl esters, 4°C, dark.
[b] 0.5 g.
[c] Relative to 16:0.
[d] Number of carbon atoms:number of double bonds.

than on silica gel. For practical purposes it is sufficient to say that storage on silica gel has a significant protective effect.

Discussion. There is considerable evidence that polyunsaturated esters of fatty acids are much more susceptible to autoxidation than monounsaturated esters (8–10). The linear correlation of susceptibility to autoxidation with number of double bonds is not unreasonable, since there has been no oxidation of methyl oleate. However, this does not coincide with the relative reactivities of 18:2 and 18:3 shown by a recent literature survey (11). It is possible that our results have been influenced by the unequal composition of the starting mixture, or that decomposition products of the highly unsaturated esters have changed the rates of 18:2 and 18:3. Determination of the oxidation rates of single esters would answer these questions.

There are several possible explanations, not mutually exclusive, for the effect of silica gel. A primary factor may be differences in trace metal content of this silica gel and glass. The importance of surface geometry of the adsorbent to adsorption (12, 13) and to chromatographic separation has been mentioned (14). Optimum pore size and surface area for chromatographic separations have been related to the size of the molecules separated (15, 16). It is possible that moisture content of the silica gel
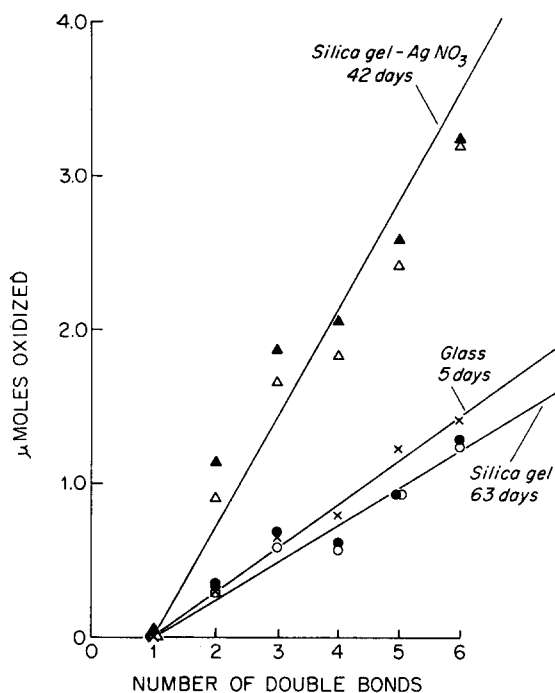
Fig. 1. Relation of oxidation to amount of unsaturation (15.2 μmoles (0.00475 g) of mixed esters, 4°C, dark). ×—×, Glass, 5 days, uncorrected (Table 2); ●—●, silica gel, 0.1 g, 63 days, corrected; ○—○, silica gel, 0.5 g, 63 days, uncorrected (Table 2); ▲—▲, AgNO₃–silica gel, 0.1 g, 42 days, uncorrected; △—△, AgNO₃–silica gel, 0.5 g, 42 days, corrected.

as well as its surface had an effect on the stability of the esters. This was demonstrated with methyl linoleate on cellulose (17, 18), where oxidation was markedly reduced by humidification. Water has clearly been shown to increase the binding of some compounds to silica gel (13). There was a substantial increase in the heat of adsorption of benzene and hexane after hydration of the silica gel. A logical presumption is that with a stronger bond to silica gel, an unsaturated molecule becomes less reactive with atmospheric oxygen.

In view of the general observations that oxidation rates for unsaturated materials are connected with surface area and moisture content, it is not difficult to accept our results. Determining basic reasons for the observed differences in oxidation rates would be of practical value because a better understanding of the most relevant factors would facilitate choice of a better support.

Conclusion. Highly unsaturated fatty acid esters when stored on silica gel were 12 to 15 times more stable than when stored on a glass surface. Therefore, in addition to usual precautions of low temperature and protection from oxygen, the use of silica gel to provide extra stability is recommended. Deposition of fatty acid esters on to silica gel by evaporation of solvent and subsequent removal with a polar solvent are simple and rapid pro-

cedures. Since the unsaturated methyl esters are stable on silica gel for days, the collection and temporary storage of fractions from GLC separations would be facilitated by use of silica gel. If the technique is used for other lipid compounds, caution should be exercised, since undesirable changes may be accelerated by silica gel (19, 20).

REFERENCES

1. Slawson, V., and R. Stein. 1970. Comparative autoxidative susceptibility of fatty esters with 0–6 methylene-interrupted double bonds. *Lipids.* **5:** 713–717.
2. Holman, R. T. 1954. Autoxidation of fats and related substances. *Progr. Chem. Fats Other Lipids.* **2:** 51–98.
3. Ingold, K. U. 1968. Principles of metal catalyzed lipid oxidation. *In* SIK Symposium on Metal Catalyzed Lipid Oxidation. R. Marcuse, editor. SIK-Rapport no. 240. Kalleback, Göteborg, Sweden. 11–41.
4. Uri, N. 1956. Metal ion catalysis and polarity of environment in the aerobic oxidation of unsaturated fatty acids. *Nature* (London). **177:** 1177–1178.
5. Heaton, F. W., and N. Uri. 1961. The aerobic oxidation of unsaturated fatty acids and their esters: cobalt stearate-catalyzed oxidation of linoleic acid. *J. Lipid Res.* **2:** 152–160.
6. Togashi, H. J., A. S. Henick, and R. B. Koch. 1961. The oxidation of lipids in thin films. *J. Food Sci.* **26:** 186–191.
7. Honn, F. J., I. I. Bezman, and B. F. Daubert. 1951. Autoxidation of drying oils adsorbed on porous solids. *J. Amer. Oil Chem. Soc.* **28:** 129–133.
8. Holman, R. T. 1966. General introduction to polyunsaturated acids. *Progr. Chem. Fats Other Lipids.* **9:** 3–12.
9. Holman, R. T., and O. C. Elmer. 1947. The rates of oxidation of unsaturated fatty acids and esters. *J. Amer. Oil Chem. Soc.* **24:** 127–129.
10. Gunstone, F. D. 1958. An Introduction to the Chemistry of Fats and Fatty Acids. John Wiley and Sons, New York. 114–115.
11. Ackman, R. G., J. C. Sipos, and P. M. Jangaard. 1967. A quantitation problem in the open tubular gas chromatography of fatty acid esters from cod liver lipids. *Lipids.* **2:** 251–257.
12. Adamson, A. W. 1967. Physical Chemistry of Surfaces. 2nd ed. Interscience, New York, London, and Sydney. 406.
13. Kiselev, A. V. 1961. Surface chemistry, adsorption energy and adsorption equilibria. *Quart. Rev. Chem. Soc.* **15:** 99–124.
14. Sporer, A. H., and K. N. Trueblood. 1959. A quantitative study of the relation between molecular structure and adsorption affinity on silicic acid–Celite columns. *J. Chromatogr.* **2:** 499–512.
15. Klein, P. D. 1961. Silica gel structure and the chromatographic process. Effect of pore diameter on the adsorption and differential migration of sterol acetates. *Anal. Chem.* **33:** 1737–1741.

16. Waksmundzki, I. A., J. Oscik, J. Matusewicz, R. Nasuto, and J. Rozylo. 1961. The structure of silica gels adsorbing specifically pyridine, quinoline, and acridine. *Przem. Chem.* **40:** 387–390. *Chem. Abstr.* **56:** 2018i (1962).
17. Labuza, T. P., H. Tsuyuki, and M. Karel. 1969. Kinetics of linoleate oxidation in model systems. *J. Amer. Oil Chem. Soc.* **46:** 409–416.
18. Labuza, T. P., N. D. Heidelbaugh, M. Silver, and M. Karel. 1971. Oxidation at intermediate moisture contents. *J. Amer. Oil Chem. Soc.* **48:** 86–90.
19. Smith, L. L., and T. Foell. 1962. Thin-layer chromatography of steroids on starch-bound silica gel chromatoplates. *J. Chromatogr.* **9:** 339–344.
20. Marinetti, G. V., J. Erbland, and E. Stotz. 1959. The structure of beef heart plasmalogens. *J. Amer. Chem. Soc.* **81:** 861–864.