Purification of oleic acid and linoleic acid

Ravindra L. Arudi, Mark W. Sutherland, and
Benon H. J. Bielski

*Department of Chemistry, Brookhaven National
Laboratory, Upton, NY 11973*

Summary To permit kinetic studies of the reactivity of unsaturated fatty acids towards oxygen radicals, it is essential to remove traces of hydroperoxides and other conjugated lipid impurities commonly present in commercial samples. Removal of these impurities has been satisfactorily achieved for oleic and linoleic acids by anaerobic low temperature recrystallization from acetonitrile. The UV spectra of commercial and purified samples are compared.—**Arudi, R. L., M. W. Sutherland, and B. H. J. Bielski.** Purification of oleic acid and linoleic acid. *J. Lipid Res.* 1983. **24:** 485–488.

Supplementary key words hydroperoxides • low temperature crystallization

The elucidation of the basic mechanism of the autoxidation of polyunsaturated fatty acids (PUFA) has been one of the great challenges to chemists (1). As is well known, the lability of these compounds toward dioxygen and light is linked to the degree of unsaturation and is directly responsible for the presence of lipid hydroperoxides and conjugated impurities in even the purest of commercial samples.

Recent studies of the rate of reaction of superoxide radical (O_2^-) with linoleic acid (LH) (2)[1] and its hydroperoxide (LOOH) (3) suggest that the ratio of $k_1(LOOH)(O_2^-)/k_2(LH)(O_2^-)$ (where k_1 and k_2 are the respective second order rate constants) is at least 10^4. Such a high ratio makes it apparent that rate studies of HO_2/O_2^- with PUFAs would be significantly affected by the presence of even 0.1% of hydroperoxide impurities.

Therefore, in order to carry out meaningful kinetic studies, we have found it necessary to prepare PUFA samples of the highest purity possible. While over the years other researchers have developed purification methods (4) with varying degrees of success, we have found most of these procedures unsatisfactory for our needs. By recrystallization from acetonitrile under strict anaerobic conditions at low temperatures, we have succeeded in preparing PUFA samples of a purity level satisfactory for kinetic studies with HO_2/O_2^-.

Abbreviations: PUFA, polyunsaturated fatty acids; LH, linoleic acid; LOOH, linoleic hydroperoxide.

[1] Bielski, B. H. J., R. L. Arudi, and M. W. Sutherland. Unpublished results.

MATERIALS

Oleic acid and linoleic acid were purchased in the highest available purity (>99.0%) from Sigma Chemical Co. and Nu-Chek Preparations Inc. For comparison, linoleic acid was also obtained from Supelco, Inc. Acetonitrile and methanol (glass distilled) were purchased from Burdick and Jackson Laboratories, Inc., and were used without further purification. Propionitrile from Aldrich Chemical Co., was distilled once before use. Ultra high purity nitrogen (Matheson Co.) was used throughout this project. Spectral analyses were carried out using a Cary 210 spectrophotometer.

METHODS

Conventional and commercially available low temperature recrystallization devices (such as the one shown on p. 198, Ace Glass catalog 800) are not suitable for purification of PUFAs by this procedure since they do not allow continuous purging of solutions with N_2 or the easy removal of mother liquor, by forcing it through the filter (using a positive pressure of N_2). The basic design of the apparatus used for recrystallization of PUFAs is shown in **Fig. 1.** Several such vessels of varying capacity were used, depending upon the size of the sample to be purified. In a typical run, a solution of PUFA in acetonitrile is filtered (coarse glass frit) into the recrystallization vessel at room temperature and purged with ultra high purity nitrogen for 15 min. While N_2 is passing through the solution via D (set A for flow from I to II; close B; open C) the vessel is slowly lowered into the acetone–dry ice cooling mixture. The temperature of this cooling mixture is controlled by periodic addition of pulverized dry ice. PUFA crystals begin to appear within a few minutes and the crystallization goes to completion within 15–20 min. The mother liquor is removed from the crystals by changing the direction of nitrogen flow (set A for flow from I to III; close C; open B and increase the N_2 pressure to 4–5 psi). The PUFA crystals are washed once with a minimum amount of precooled solvent (added under nitrogen through the top of the flask) by thoroughly wetting the crystals (add solvent and bubble vigorously with N_2 for several minutes) and removing the filtrate as before. The yields of crystallized unsaturated fatty acids are subject to several factors such as: *1)* the quantity of starting material per unit volume of solvent; *2)* the temperature at which the PUFA is recovered; and *3)* the length of time allowed for crystallization. The overall yield of linoleic acid after 18 recrystallizations was typically 35–40%; oleic acid was recovered in 70–75% yield after 6 recrystallizations.
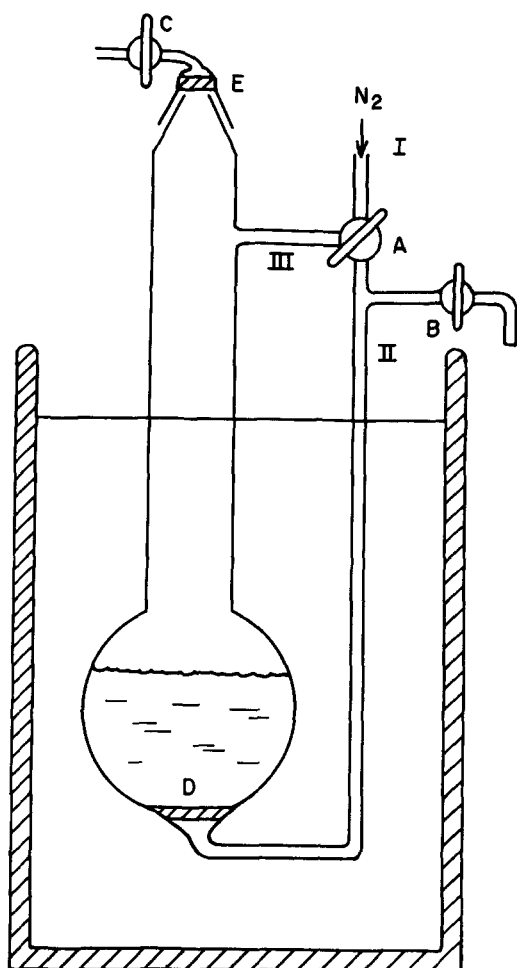
Fig. 1. Low temperature recrystallization apparatus. Stopcocks A, B, and C (Teflon) control the direction of nitrogen flow. Glass frit D (coarse, 1-in diameter) allows for nitrogen purging and filtering, while frit E (coarse, 3/4-in diameter) keeps atmospheric dust out of the system. I, II, and III indicate direction of nitrogen flow.

Fig. 2. Absorption spectra of 2.5 μl of linoleic acid from various sources added to 3 ml of methanol at 22°C. a, Sigma Chemical Co.; b, Nu-Chek Preparations, Inc.; c, Supelco, Inc.; and d, Sigma sample recrystallized 18 times.

While the qualitative monitoring of the progress of purification is readily achieved by recording the UV spectra of the filtrates, the quantitative evaluation of such spectra requires the isolation of the purified fatty acid. After removal of all traces of solvent on a high vacuum line, the final product is stored under N_2 at −70°C in the dark. Based on spectral characteristics these samples are stable for several months.

RESULTS AND DISCUSSION

The composition and nature of the impurities observed in commercial PUFA samples are very complex. A major source of these impurities is the cross contamination of various PUFAs as they are structurally so very similar and difficult to separate. For example, the conjugated impurities in linoleic acid are most likely derived
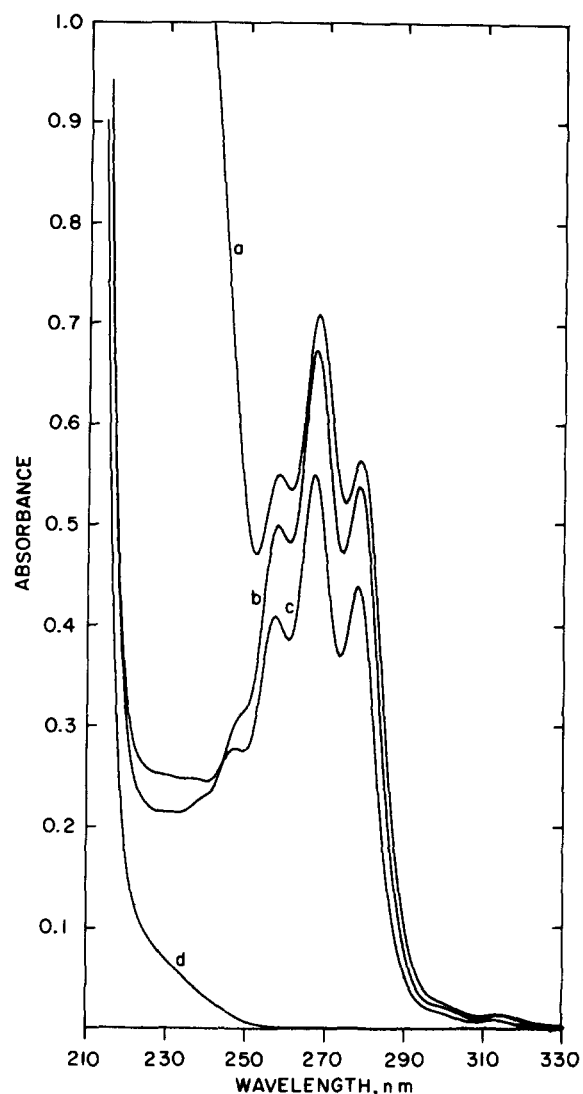
TABLE 1. Optical absorbance of recrystallized linoleic acid[a] (5 μl in 3 ml of CH_3OH) as a function of wavelength and number of recrystallizations (n)

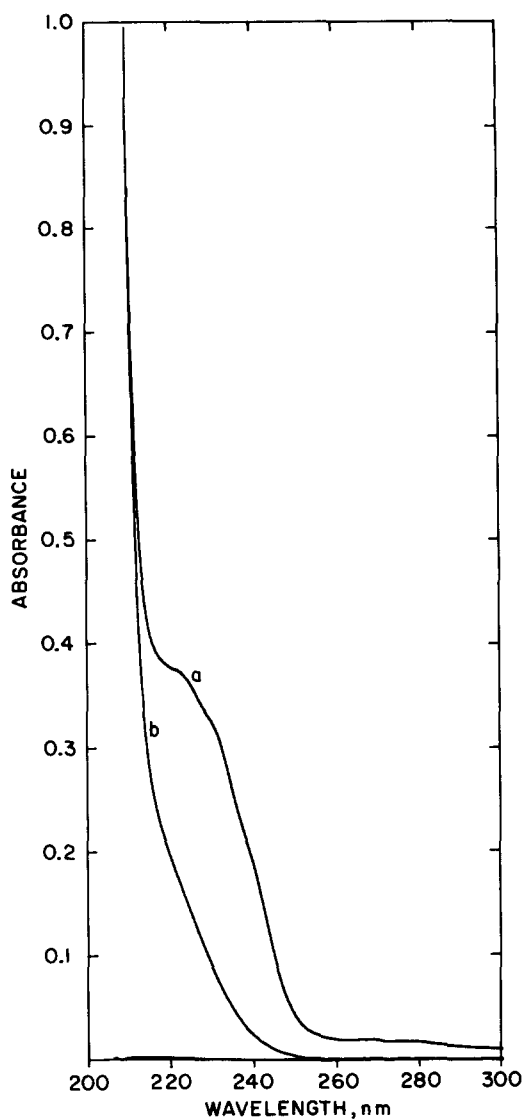| n	230 nm	250 nm	270 nm	290 nm
0	1.496	0.648	0.832	0.140
2	0.762	0.276	0.361	0.050
4	0.490	0.146	0.187	0.023
6	0.304	0.084	0.078	0.014
8	0.250	0.048	0.036	0.005
10	0.140	0.027	0.021	0.004
12	0.138	0.022	0.008	0.002
14	0.130	0.016	0.006	0.001
16	0.130	0.009	0.002	0.000
18	0.130	0.007	0.000	0.000
20	0.130	0.007	0.000	0.000

[a] Sigma Chemical Co. (99% purity).

Fig. 3. Absorption spectra of 2.5 μl of Sigma oleic acid (a, before and b, after six recrystallizations) added to 3 ml of methanol at 22°C.

from trace amounts of linolenic and arachidonic acid. While the bulk of these higher unsaturated fatty acids and their decomposition products can be removed by known purification methods, removal of trace amounts has proven very difficult. Although an increase in absorbance near 230 nm is often associated with the formation of conjugated hydroperoxides in linoleic acid, earlier studies on the alkaline isomerization of PUFAs (5, 6) suggest that trace amounts of the more labile linolenic and arachidonic acids could give rise to impurities absorbing in the 230 to 330 nm range.

The UV spectra for various commercial samples of linoleic acid (purity 99%) are shown in **Fig. 2.** While absorption due to impurities in the 250–290 nm region is similar irrespective of source, the absorption at 230 nm can vary dramatically. As the purity of samples from

a given supplier differs for different batches, the spectra shown in Fig. 2 reflect a random selection.

By screening a number of common organic solvents and their mixtures, acetonitrile was found to be most suitable for the low temperature recrystallization of linoleic acid. The best results were obtained when 1 g of linoleic acid was recrystallized from 8–9 ml of acetonitrile at −30 to −35°C under strict anaerobic conditions. While about 90% of all the conjugated impurities could be removed by eight recrystallizations, the removal of the remaining impurities was found to proceed asymptotically as illustrated in **Table 1.** Starting with a sample of linoleic acid from Sigma, the total removal of the impurities required 18 recrystallizations. Further purification did not lower the absorbance at 230 and 250 nm (See Table 1). The purification progress was monitored by recording the UV spectrum of the treated sample after every two recrystallizations. The samples for the spectral analyses were prepared by removing the solvent under high vacuum. The spectrum of the 18X sample is shown in Fig. 2. Assuming the density of linoleic acid is $d^{22°} = 0.901$ gram/cm^3 (7), the corresponding $E_{1\,cm}^{1\%}$ at 230 nm in methanol is 0.87.

Commercial oleic acid samples contain conjugated dienoic impurities resulting from oxidation and/or isomerization of linoleic acid impurities and possibly oleic acid hydroperoxides. As acetonitrile was shown to be effective in the removal of such impurities in the case of linoleic acid, it was also used in the purification of oleic acid. It was found that oleic acid could be rendered free from impurities upon six recrystallizations at −20°C. Corresponding UV spectra before and after purification are shown in **Fig. 3.** The $E_{1\,cm}^{1\%}$ at 230 nm in methanol of this sample assuming $d^{25°} = 0.895$ g/cm^3 is 1.22 (7).

Preliminary purification of linolenic and arachidonic acid show the presence of significant amounts of tetraenoic and higher conjugated impurities. As both these unsaturated fatty acids have lower melting points, attempts to recrystallize them from acetonitrile were unsuccessful. Partial success has been achieved by using a mixture of acetonitrile and propionitrile (1:2) at −60°C. The results of this work will be reported upon completion.▩

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