# Oxyluminescence of Fatty Natural Substances


## Abstract

A number of fatty substances, covering a range from low to high degree of unsaturation, have been observed to emit light when heated. Relative intensities of light emitted at 200C are compared to degree of unsaturation. Activation energies were calculated for those samples giving approximately straight line plots of log $G$ vs. $\frac{1}{T}$, where $G$ is galvanometer deflection and $T$ is the absolute temperature.


## Introduction

OXYLUMINESCENCE is a phenomenon characterized by the emission of light from a material in the presence of oxygen. Ashby (1) and Shard and Russell (2,3) have investigated the oxyluminescence of various polymers and Pittman and Tripp (4) have observed the phenomenon in both unmodified and chemically modified cotton textiles. Zhuravlev (5) has observed the chemiluminescence of oleic acid and olive oil after addition of liver extracts and correlated this phenomenon with the peroxide number. We have investigated fatty substances of animal, vegetable, and marine origin and related products in a preliminary manner.

The apparatus consisted of a cylindrical bar of aluminum 2 in. in diameter and 1¼ in. long recessed at one end to hold a small 60-watt heater. A ¼ in. hole was drilled in the side of the cylinder, about ½ in. from the flat face of the cylinder to which the sample was to be affixed. Two ¼ in. diameter magnets were clamped to diametrically opposite sides of the cylinder with one of their pole faces in the plane of the face of the cylinder which was to hold the sample. This assembly was mounted with the side hole in the cylinder in an upright position. A thermocouple was placed in this hole and thermal contact made by pouring molten Wood's metal into the hole. A 1P21 photomultiplier tube was mounted 7.5 cm from the center of the face of the cylinder that was to hold the sample. This entire assembly was then placed in a light-tight box.

The output from the thermocouple and the amplified 1P21 current were fed into the X and Y axes, respectively, of an X-Y recorder. The heater in the cylinder was supplied with about 15 volts, which served to heat the sample from 50C to 200C in about 25 min.

Isooctane solutions of the materials to be studied were prepared at a concentration of about 16 g/liter. A 2-in. square of fiber glass chromatographic paper was placed in a 3½ in. Petri dish, a 5 ml aliquot of the isooctane solution was pipetted over the chromatographic paper, and the isooctane was allowed to evaporate in a hood. The chromatographic paper was then attached in position over a 1¾ in. diameter hole in the center of a sheet metal disk.

The heater was turned on and when the temperature reached 50C the metal ring holding the chromatographic paper was placed against the face of the cylinder opposite the photomultiplier tube. The magnets held the metal ring firmly against the face of the cylinder, thus holding the chromatographic paper against the face of the cylinder. A recording was then made of the photomultiplier output, in arbitrary units of galvanometer deflection, and the thermocouple temperature from 50C to 200C. An untreated square of chromatographic paper was run as a blank and gave no galvanometer deflection.

Samples of the following laboratory-stored oils, covering a range from a low to a high degree of unsaturation, were studied: (1) a cocoa butter-like fat (6) and a cocoa butter, which contain only relatively small amounts of monoethenoids; (2) sperm oil, a liquid wax, containing a large proportion of monoethenoids; (3) refined cottonseed and sesame oils, which contain from 35 to 50% of diethenoids in addition to monoethenoids and saturates; and (4) tung oil, consisting mainly of conjugated triethenoids. A sample of crude solvent-extracted cottonseed oil was also included.

With the exception of the crude cottonseed oil, which behaved anomalously, these samples gave galvanometer deflections at 200C which increased with the degree of unsaturation.

Cocoa butter-like fats and cocoa butter (iodine values of about 35) gave galvanometer deflections of 15 and 30 units, respectively, at 200C. Sperm oil, sesame oil, and refined cottonseed oil (iodine values ranging from 80 to 110) gave galvanometer deflections of 120, 175, and 205 units, respectively. Tung oil, a drying oil with an iodine value of 163, gave a galvanometer deflection of 230. Crude cottonseed oil, which of course contained considerable nonglyceride constituents, gave a galvanometer deflection of 345 units.

Figure 1 presents two curves typical of those obtained. It will be noticed that curve B exhibits an anomalous behavior at about 180C.

Following previous work (3,4) plots of log $G$ vs $\frac{1}{T}$ were made, where $G$ is the photomultiplier output in arbitrary units and $T$ is the absolute temperature. A number of samples exhibited plots that deviated only slightly from straight lines. Due to the probable complexity of the reactions taking place, straight lines were not expected. Assuming $G$ to be propor-

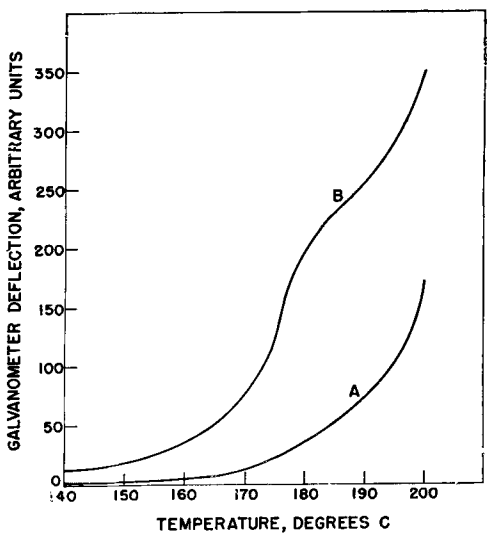


FIG. 1. Typical oxyluminescence-temperature curves: (A) refined cottonseed oil, (B) crude cottonseed oil.

tional to reaction rate, approximate apparent activation energies were calculated for cocoa butter, sperm oil, refined cottonseed oil, and sesame oil using the Arrhenius equation. The range of temperatures used was 140 to 200C. The activation energies calculated ranged from 21 to 31 kcal per mole.

This preliminary investigation indicates that a study of the phenomenon of oxyluminescence in oils and related products may be of value in the elucidation of the mechanism and kinetics of the autoxidation process. The use of oxyluminescence to investigate antioxidants (1,2) in polymers suggests its similar application to oils and related products. In view of the increasing number and diverse nature of the organic materials in which oxyluminescence has been observed, it is suggested that any material susceptible to oxidation under similar conditions to those outlined above might be worthy of investigation by this technique. Further study of this phenomenon is planned at this laboratory. The construction of a more refined apparatus for accurate measurements on selected samples of natural materials and pure model compounds is necessary to determine the role played in this phenomenon by impurities found in natural fatty substances.

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# Fluorodensitometry of Aflatoxin on Thin-Layer Plates

FLUORODENSITOMETRY has been found to be a useful tool for the quantitative estimation of aflatoxin $B_1$ directly from a developed thin-layer chromatographic plate. Standard concentrations of aflatoxin $B_1$ may be easily compared with the unknown samples. This objective procedure has a number of distinct advantages over the visual method of comparison now being used (3,4). Poor quantitative values are obtained by the visual methods unless the thin-layer plates are read by a trained observer. The visual technique requires that the sample be directly compared with a standard spot of approximately the same concentration. Accuracy is dependent on visual discrimination and acumen. Finally, the fluorescent spots may be visually quite faint and the relative intensity very difficult to judge.

The fluorodensitometry technique has been used to quantify the final extracts obtained in the determination of aflatoxin as described by Pons and Goldblatt (4) and Engebrecht, Ayres and Sinnhuber (3). The stationary phases used in these determinations, respectively, are Silica Gel G-HR and Silica Gel G (Brinkman Instruments, Inc.). Comparison of the fluorodensitometric and visual results using these two methods on four cottonseed meal samples is shown on Table I.

The instrument employed in this technique is manufactured by the Photovolt Co. (1115 Broadway, New York 10, N.Y.) and consists of a recorder, a multiplier photometer, an ultraviolet light source, a phototube unit, thin-layer chromatography stage equipment and filters. The ultra violet light passes from a mercury vapor lamp (320–390 mμ emmission), through a primary filter (365 mμ band), through a primary slit (1 mm × 25 mm) and to the stationary silica gel phase on the plate. The aflatoxin $B_1$ absorbs the 365 mμ light and emits fluorescent light at 425 mμ (2). This light passes through the glass plate, the secondary slit (0.1 × 6 mm), the secondary filter (445 mμ band) and into the phototube. The secondary filter permits only the emitted or visible fluorescent light to pass into the phototube; all utlraviolet light from the lamp is screened out.

The developed TLC plate to be read is placed on

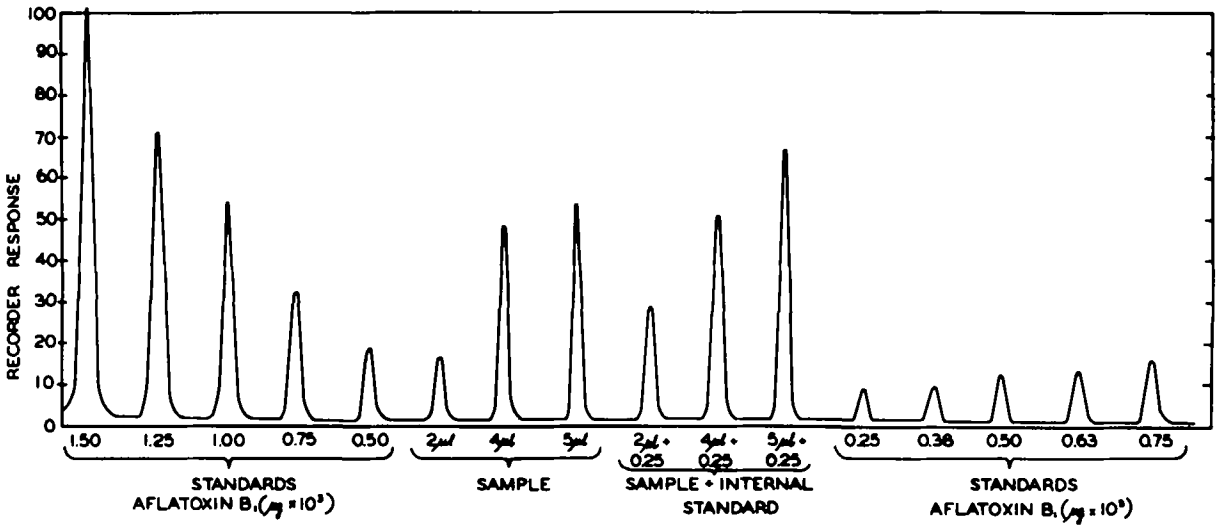


FIG. 1. A typical fluorodensitogram of aflatoxin $B_1$ on a TLC plate.