information on the mechanism of tannin–protein interactions and changes associated with various processes.

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Photoactivatable Time–Temperature Indicators for Low-Temperature Applications[1]

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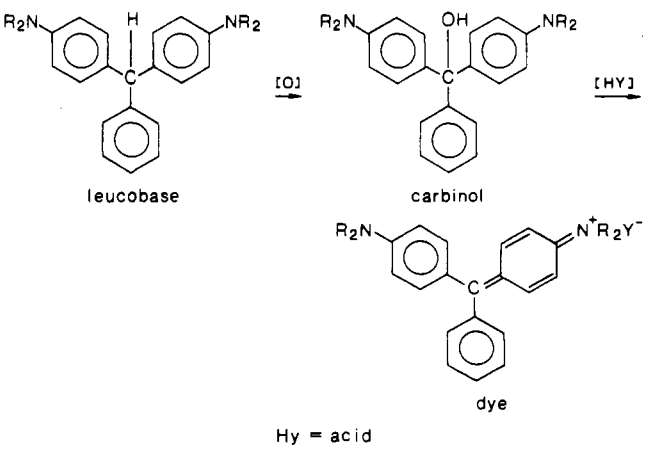
This paper describes a photoactivatable time–temperature indicator based on a leucobase system. A leucobase is mixed in a polymeric matrix with a material that generates acid upon exposure to light. Photoexcitation causes the formation of a thermally sensitive, color-forming product. Following this activation step, a progressive color development occurs at a rate that increases with temperature. The indicator is useful for monitoring the freshness of perishable products, particularly those stored at subambient temperatures.

Many articles of commerce—both food and nonfood—are perishable. Particularly when the perishable is enclosed in packaging, it may not be readily apparent when the article has exceeded its useful lifetime. It is even more difficult to determine precisely where an article is positioned on an imaginary graph that plots its deterioration as a function of time. Since the rate at which a perishable deteriorates is generally a function of its integrated time–temperature exposure—at least within a restricted range of time–temperature—a time–temperature indicator is a useful tool for those who are concerned with the freshness of perishable products. Principal applications seen for these indicators involve monitoring the freshness of perishable products, particularly those stored at subambient temperatures. This paper discusses the development and application of a shelf life monitoring indicator for temperature-sensitive products. The indicator uses color-changing, time–temperature integrating materials that remain inactive until activated by shining actinic radiation.

In the literature, there are several devices available that can respond to temperature in much the same way as perishable commodities in which loss of quality is directly related to the combined effects of the degree and the duration of the storage temperature (Hu, 1972; Blixt and Tiru, 1977; Labuza, 1982; Farquhar, 1982; Fields and Prusik, 1985; Zall and Fields, 1986). The indicator devices described are all visual in nature and feature systems using polymeric materials, enzymes, etc., to measure temperature abuse of the products. However, most are not readily adaptable for use at refrigeration temperatures, primarily because ambient temperatures for storage of the test materials are, in effect, *high* temperatures relative to test conditions. For this reason the "clock" for the indicating materials must be started when low-temperature storage of the monitored sample is begun. The activation mechanism, brief exposure to ultraviolet light of a preselected wavelength, described in this paper is especially convenient to do the same. The photoactivation procedures has potential advantages such as (i) activation of color change at a specified point in time and (ii) totally nonintrusive nature of activation. We have discovered that a suitable photoactivatable time–temperature indicator can be based on color development from a leucobase. Leucobases are the colorless forms that are the precursors to diphenylmethane and triarylmethane dyes (Venkataraman, 1952). Examples of triarylmethane leuco bases include those of Malachite Green dye, Brilliant Green dye, Crystal Violet dye, etc. By selecting from among these and other suitable leucobases, as well as mixtures of two or more of them, a wide variety of desired colors can be obtained. Color selection entirely depends on types of monitoring devices employed to record the growth of color development. Thus, a He–Ne laser, which emits at 632 nm, is a convenient light source for monitoring the reflectivity of the indicators derived from the leucobases.

It is known that colorless leucobase is converted to dye in a two-step process:



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It should be noted that the leucobase and the carbinol are colorless forms, while the dye form is colored. Thus, when the reaction is conducted in a controlled oxygen atmosphere, acidification becomes the critical step in the activation of color development.

It was realized that the use of a photoinducible acid is an elegant way to conduct such acidification because in that case the process of photoactivation can also provide control over the precise moment of system activation. A number of chemical compounds are known to generate acids upon excitation with actinic radiation. Among these photoacids are *o*-nitrobenzaldehyde and substituted *o*-nitrobenzaldehydes (Pashayan et al., 1976, 1977; George and Scaiano, 1980), trihalo alcohols (Maslowski, 1974), and compounds of the form $[Ph_n(I, S)]^+ [(P, Sb, As)F_m]^-$, where n is 2 or 3 and m is 4 for P and 6 for Sb or As (Crivello and Lam, 1979a,b).

In selecting the particular photoacid generator, the following aspects were taken into consideration:

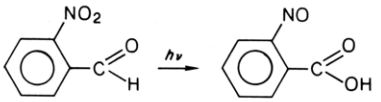
(a) Since the response to time–temperature exposure generally depends on the extent of photoactivation, it is important either that the photoacid generator not respond to ambient light exposure or that the indicator be protected from such exposures (e.g., by incorporating an opaque cover sheet or by keeping the indicator in the dark).

(b) The photoactivation step must be reproducible. That can be accomplished conveniently and easily by, for example, using a reproducible light source or monitoring the photoactivation exposure.

(c) Wavelengths in the visible range are convenient to use for activation, but they can also cause problems. If time–temperature exposure is monitored by changes in reflection density, then it is important that the light whose reflection is being measured not be capable of causing additional activation. Since it is convenient to use reflection of visible light, preferably there should be no activation at wavelengths longer than 400 nm. Thus, a material activatable at wavelengths in the range between about 200 and 400 nm is ideal for this system.

(d) The photoacid should be thermally stable in the application environment; i.e., it should not be thermally activatable.

Upon taking into consideration all the above requirements, we found that for our system *o*-nitrobenzaldehyde is the ideal photoacid generator. Ultraviolet light (approximately 350 nm) converts *o*-nitrobenzaldehyde to *o*-nitrosobenzoic acid:



EXPERIMENTAL SECTION

Preliminary experiment was done by placing 60 mg of colorless Malachite Green leuco [bis[*p*-(dimethylamino)-phenyl]phenylmethane] purchased from Fisher Scientific Co. in 1 mL of an organic solvent (alcohol, acetone, and chloroform were each used in separate tests, with similar results). The solution was then mixed with 30 mg of *o*-nitrobenzaldehyde (also purchased from Fisher Scientific) crystals. Pieces of filter paper were dipped in the resulting solution and dried inside a hood. The coated pieces appeared faint gray and remained so for days when stored in the dark. However, when individual strips were irradiated with UV light (Model 250A, Xenon Corp.) for 5 s each, the strips were "activated" and developed a very faint green color. The photoactivated samples, when left at different temperatures (such as room temperature, refrigeration temperature, etc.), developed more green color at different rates.

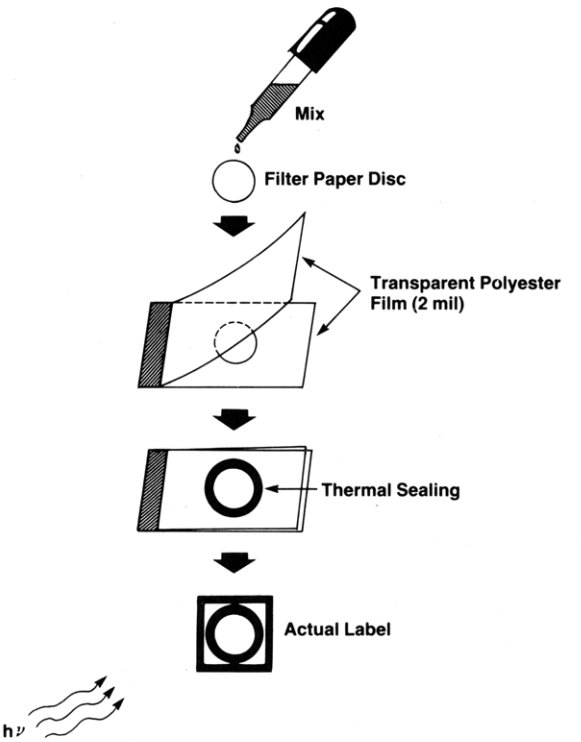


Figure 1. Steps involved in the indicator labels preparation.

Although the experimental demonstration was very clear and unambiguous in the above case, several practical disadvantages became apparent in making these indicators useful for commercial applications. For increasing the shelf life of the unactivated strips and to promote easy handling of the indicator labels, we realized the necessity of making the indicator labels by coating and by laminating the coated substrates.

The following steps were carefully carried out to develop such laminated indicator labels. Our experimental work included systematic investigations in the following areas: (1) selection of proper polymeric materials to coat the leucobase and photoacid on various substrates and subsequent lamination of the coated labels; (2) establishing an appropriate photoactivation procedure; (3) systematic spectrophotometric investigation of color change of the photoactivated labels, held at various temperatures, with time.

Initially, an aqueous medium (gel) was prepared by heating a mixture of 4 g of poly(vinyl alcohol) (PVA) in 100 mL of water at 80 °C for a few minutes. The PVA (from Aldrich) had a molecular weight of 115 000 and was 99–100% hydrolyzed. A 1-mL sample of the PVA gel and 60 mg of Malachite Green leuco were mixed well by grinding together. Strips of filter paper (12.5 mm × 100 mm each) were coated with the above mix either by using a Mayer rod or through a silk screen and were then dried at room temperature inside a dark hood. Fifteen milligrams of *o*-nitrobenzaldehyde (ONB) was dissolved in 1 mL of reagent ethanol. Dried coated strips, 12.5 × 6.5 mm each, were briefly soaked with the ONB solution and were laminated as follows: A coated strip was thermally sealed between two sheets of transparent, heat-sealable polyester film (100-μm thickness, obtained from Kapak Corp.) to form a "label". The steps involved in the actual lamination procedure are shown in Figure 1. Some of these labels were stored in the dark at room temperature, and some were stored at refrigeration temperature for time–tem-

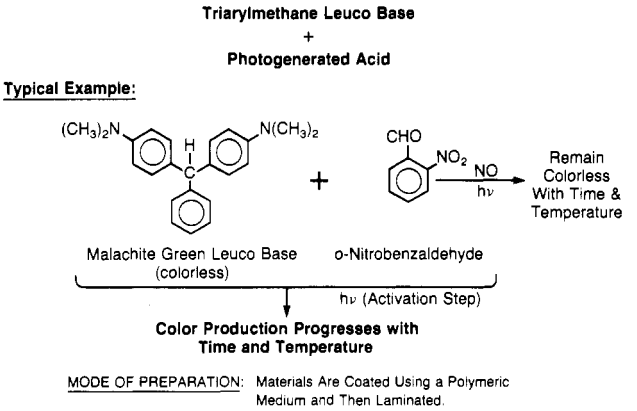


Figure 2. Overall schematic of this photoactivatable time–temperature indicator system.

perature monitoring of the unactivated labels. Photoactivation of the other labels was accomplished by irradiating individual labels either with a 100-W mercury arc lamp (obtained from Oriel Corp.) or with a UV flash lamp (Model 250A, Xenon Corp.) for a specific period of time (from 5 to 20 s). The polyester film filtered out light having a wavelength shorter than 300 nm. After photoactivation, the optical reflectivities (R) ranged from 90 to 95% (still virtually colorless).

The decrease in reflectance with time (gradual green color development) at a constant temperature was monitored with an optical scanning wand (Fields and Prusik, 1985) that employs 632-nm light to measure the reflectivity of the label relative to a standard (reference) bar code. In practice, a rectangular hole was cut in a bar code label, and the indicator label was placed under the hole. The wand scanned both the bar code label and indicator label, as the two labels were held together. All reflectivity values are an average of at least 10 scans. Over a period of time, the reflectivity of the indicator label decreased at a temperature-dependent rate. For comparison, the reflectivity was also measured by a Perkin-Elmer UV–visible spectrophotometer, Model 553, with an integrated sphere attachment. The measurements correlated well with those obtained by the optical scanner. The unactivated labels were also monitored for a period of more than 1 month, and no color development was observed either at refrigeration temperature (6 °C) or at room temperature. Using Crystal Violet leuco instead, in the same system, the final color developed was violet. Similarly, blue color appeared when a mixture of leucobases of Malachite Green and Crystal Violet was used in the proportion of 5:1, respectively.

The above procedure has demonstrated that coatings on the filter paper from an aqueous dispersion of a leucobase and a photoacid generator can be used as a time–temperature indicator. However, to develop a more convenient one-step coating procedure, e.g., applying both leucobase and the photoacid simultaneously, we used a polymeric binder that is primarily soluble in organic solvents. Since both the leucobase and ONB are also soluble in organic solvents, it was found that one-step coating could be accomplished using poly(vinyl acetate) in solutions containing different mixtures of ethanol and water.

Accordingly, polymeric gels were prepared from 10% poly(vinyl acetate) (PVAc, MW 120000, Aldrich) solutions in different mixtures of ethanol and water, the alcohol content ranging from 60% to 90%. Mixtures of 300 mg of leucobase (Malachite Green) and 25 mg of ONB in 10 mL of each of these polymeric gels were coated in Whatman No. 41 filter papers with use of a Paasche airbrush.

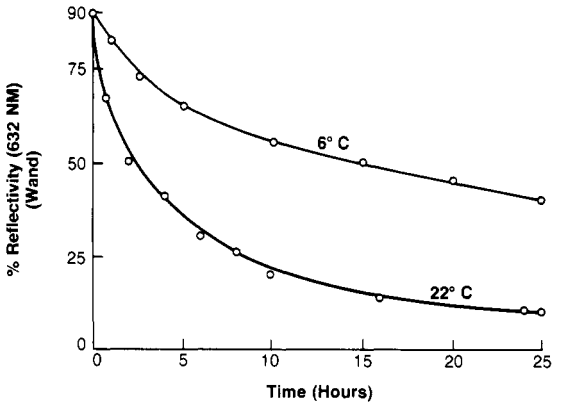


Figure 3. Changes in reflectance with time (in % R scale) for photoactivated labels prepared in poly(vinyl alcohol) and held at 22 and at 6 °C.

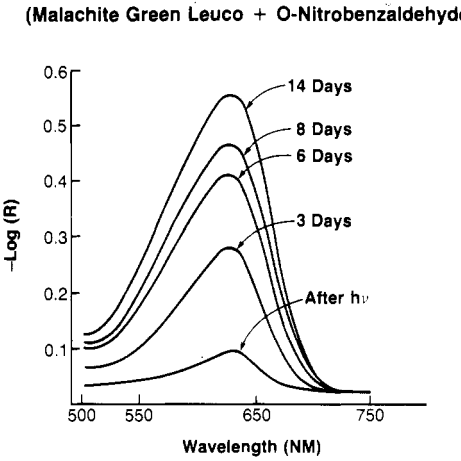


Figure 4. Changes in reflectance (in $-\log R$ scale) of such samples held at 6 °C.

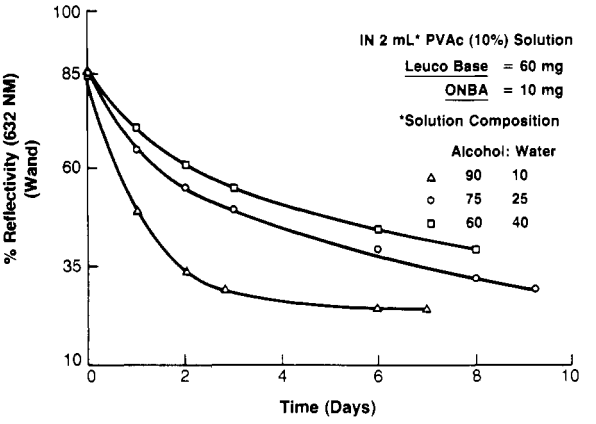


Figure 5. Effect of solvents on time–temperature color development at 6 °C.

The coated samples were dried in a dark hood for 4 h and then laminated and photoactivated by the methods described earlier. The rates of color development of the activated labels at room temperature and at 6 °C were also measured by the same procedures already described.

In another set of experiments, we established the effects of coating thickness as well as the effect of extended photoactivation. Thus, a coating mix was prepared by blending together leucobase (Malachite Green), ONB, and PVAc gel (previously made in an alcohol/water mixture). Pieces of filter paper were coated by spraying the coating mixture with a Paasche airbrush. Several samples were prepared with two or more coatings of the same materials. The coated strips were dried and laminated. After pho-

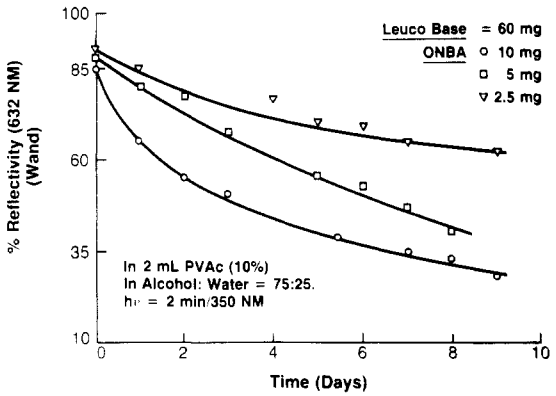


Figure 6. Comparison of changes in reflectance with various concentrations of photoacid at 6 °C.

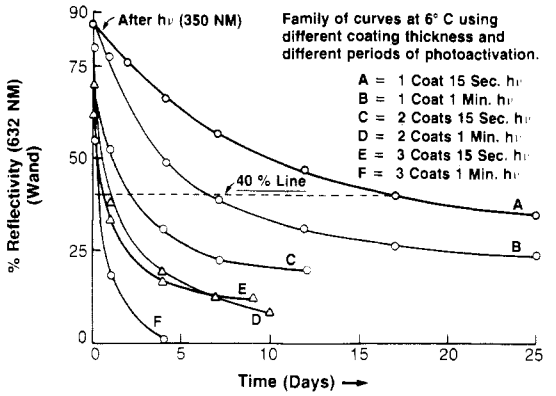


Figure 7. Family of curves at 6 °C using different coating thickness and different periods of photoactivation.

toactivation, the reflectivity at 632 nm was monitored while the samples were stored at refrigeration temperature (6 °C). Different periods of photoactivation were also attempted on samples having different coating thicknesses.

RESULTS AND DISCUSSION

Figure 2 represents the overall schematic of this time–temperature indicator system. Figure 3 shows the typical plots at room temperature (22 °C) and refrigeration temperature (6 °C) for the samples prepared in poly(vinyl alcohol). Changes in reflectance of such samples at 6 °C in $-\log R$, which is roughly equivalent to the absorbance as measured by the integrated sphere technique, are shown in Figure 4.

As mentioned in the Experimental Section, poly(vinyl acetate) is the preferred coating medium because it is more soluble than poly(vinyl alcohol) in organic solvents, such as alcohol or alcohol/water mixtures. For the coated labels in poly(vinyl acetate), the greater the alcohol concentration in an alcohol/water mixture of solvents, the more rapid was the development of color. The effects of such solvents on the rate of color development at 6 °C are shown in Figure 5.

Another parameter that can influence the rate of color development is the amount of photoacid used during the preparation of coating mixture. Figure 6 shows typical results. A faster rate is obtained as the concentration of the photoacid (ONB) is progressively increased up to the limit imposed by the requirement of making uniform coatings.

In this photoactivatable time–temperature indicator system, faster color change can also be achieved by using thicker coatings. These are prepared (see the Experimental Section), for example, by making multiple coatings on a single area. The combined influence on rate of color

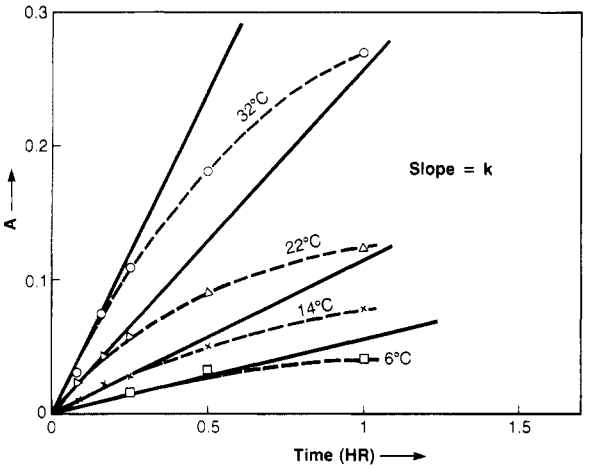


Figure 8. Absorbance at 632 nm as a function of time for photoactivated systems held at 6, 14, 22 and 32 °C.

change of coating thickness and degree of photoactivation permits a single indicator composition to monitor the freshness of products whose shelf lives differ from each other by a factor of at least 30. Figure 7 shows such results at 6 °C where, depending on the coating thicknesses and degree of photoactivation, reflectivity equivalent to 40% is achieved within times ranging from approximately 12 h to 17 days. Samples A and B each had one coat of coating mixture, but they had different periods of photoactivation (350-nm light). More activation resulted in a faster rate of change in reflectivity. Samples C and D each had two coats; again rates varied according to period of photoactivation. Samples E and F were prepared by applying a third coat on top of two coats. Again, the rates varied with extent of photoactivation. Immediately after photoactivation, the reflectivities (R) of the samples ranged from 85 to 90%; however, for simplicity, all starting points are considered to be 87% in Figure 7.

The results of Figures 6 and 7 suggest that the thermal activation rate for these indicators was clearly dependent upon the amount of photoacid production. Optimization of this step appears to be critical both to generate a high contrast in color response as well as to maintain reproducibility. A specific amount of change in reflectance can be used as a guide to control the extent of photoactivation. Accurate monitoring of the intensity of the UV light source is rather essential to accomplish this goal.

It is rather surprising that when a mixture of leucobase and photoacid is exposed to actinic radiation, the rate at which color develops depends on the temperature; i.e., the color development is an indication of time–temperature exposures. Although at this point we do not understand the basis of this effect, we have established the fact that after photoacidification of the leucobases efficient color development with time and temperature can occur only in the presence of oxygen in air. Thus, in actual experimentation, two pieces of coated labels were placed separately inside two UV-transmitting cells. The coated sides were positioned upward facing the clear windows of the absorption cells. The open end of one of the cells was connected to a source of oxygen-free nitrogen to keep the label free from oxygen. The other cell was left open to the laboratory environment. The labels inside the cells were irradiated by a UV flash lamp (Model 250A, Xenon Corp.) for the same time. After photoactivation, the labels were allowed to stay overnight under their respective environments. After 24 h, it was observed that the label under nitrogen remained almost colorless ($R \approx 90\%$), whereas

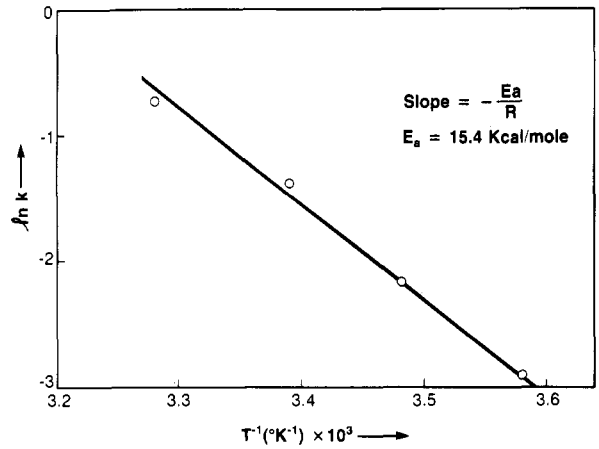


Figure 9. Natural logarithm of rate constant (in units of absorbance/hour) plotted against T^{-1} for this photoactivated system. Slope gives an activation energy of 15 kcal/mol.

the one exposed under environmental conditions developed a beautiful green color ($R \approx 40\%$). Ideally, calibration of the amount of color development can be achieved by controlling the access of oxygen to the sample. The cover sheet (lamination) used for preventing unwanted photoactivation thus provides a second function as a semipermeable barrier to oxygen (more efficient at lower temperatures). It also protects the coating from physical damage during handling.

We determined the activation energy of this photoactivatable system for a representative batch of labels by measuring the relative reaction rate obtained from a plot of absorbance (at 630 nm) as a function of time. Figure 8 shows plots of absorbance vs time at different temperatures. The initial slopes of the plots in Figure 8 (units of absorbance/hour) gave relative rate constants, k, plotted on a traditional ln k vs T^{-1} plot to obtain an activation energy of 15 kcal/mol, as shown in Figure 9. The loss of quality in many food products also obeys the Arrhenius relationship, where the rate of deterioration (determined by sensory tests and other chemical tests at periodic intervals of storage) varies logarithmically with the reciprocal of absolute temperature of storage. If deterioration of the sensitive material has an "effective activation energy" comparable to that of the indicator, development of a specified depth of color indicates quantitatively the end of the safe lifetime for use of the stored substance. Not all perishable materials have the same response (effective activation energy) to changing temperature. The most recommended compositions of indicator preparations are chosen to correspond to the temperature sensitivity of common foodstuffs. However, as shown in Figures 6 and 7, the activation energy of the present indicator may be adjusted, e.g. by variation of the nature and concentration of the photoacid, by variation of intensity or duration of the photoactivating exposure, or by varying the concentration of the leuco dye, to match very closely the temperature response of any sensitive material. Thus, this indicator system may be tailored to monitor the critical thermal history of materials having widely variable spoilage characteristics.

CONCLUSIONS

We have presented a photoactivatable time–temperature indicator system unique in the convenience of preparation, activation, and interrogation of the samples used for true temperature monitoring. Activatable indicators have several definite advantages over those where the color development progresses as soon as labels are fabricated. Some disadvantages or concerns that may inhibit the performances of this system, unless carefully utilized, may include (i) the possibility of activation by ambient light exposure and (ii) the potential difficulties in developing a reproducible activation dose.

We have demonstrated the system's applicability covering a wide range of time and temperatures, including refrigeration and frozen temperatures. Thus, these indicators should be capable of monitoring a wide range of products from food to pharmaceuticals, as well as various nonfood items such as photographic film, paints and resins, blood and blood plasma, vaccines, and other biological products. We have demonstrated rather simple and inexpensive ways for fabrication of the indicator labels using commercially available dyes and dye precursors. With the color development data of these indicator labels, decisions can be made for rotating and discarding the inventories so that consumers can always get uniform and high-quality products.

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Registry No. ONB, 552-89-6; Malachite Green Leuco Base, 129-73-7; Crystal Violet, 548-62-9; poly(vinyl acetate), 9003-20-7.

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