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Photostability of Solid-State Ubidecarenone at Ordinary and Elevated Temperatures under Exaggerated UV Irradiation

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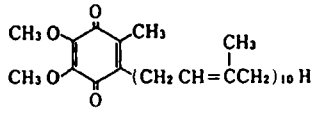
Abstract □ The photostability of ubidecarenone was investigated. Two irradiation apparatus, a grating monochromator and a high-pressure mercury vapor lamp, were employed at ordinary and elevated temperatures. Both physicochemical and chemical stabilities were significantly affected by irradiation wavelength, with UV light causing the greatest changes. The degree of degradation was a function of the light absorption properties of the substrate and markedly increased when the absorption became >30%. The photolytic degradation followed apparent first-order kinetics at all wavelengths and was promoted with temperature elevation. The Arrhenius plot gave an activation energy in the solid state different from that in the liquid state. These activation energies linearly decreased with increasing intensity of UV light.

Keyphrases □ Ubidecarenone—photostability, ordinary and elevated temperatures, exaggerated UV irradiation, activation energies □ Photostability—solid-state ubidecarenone, ordinary and elevated temperatures, exaggerated UV irradiation, activation energies □ Degradation—ubidecarenone photostability, ordinary and elevated temperatures, exaggerated UV irradiation

Preformulation study is of prime importance in the rational development of dosage forms for drug substances labile against various environmental factors. In designing a solid dosage form, it is necessary to know the inherent stability of the drug substance. There have been many reports concerning the behavior of organic compounds when subjected to heat or moisture. Photochemical mechanisms of solid-state reactions also have been reviewed (1), but not from the viewpoint of stabilization. Because of the complexity of photochemical reactions, there has been very little reported on the photostability of solid dosage forms (2–9).

Ubidecarenone [2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone (I)], a lipid-soluble benzoquinone derivative with a melting point of ~48° (10), is widely used in Japan for the treatment of angina. It is a yellow or orange crystalline powder; on exposure to light, I gradually decomposes and the color changes to dark yellow (10). The dosage forms commercially available are tablets, granules, and hard or soft gelatin capsules; these are photo-protected with a package system using light-resistant films.

The objective of the present investigation was to obtain useful informations on the behavior of I in the presence of light and heat under ordinary and accelerated storage conditions as the first step toward photostabilization.



I

Emphasis was placed on the photostability of the drug itself.

EXPERIMENTAL

Samples—Ubidecarenone, 170 mg, was accurately weighed and compressed into a flat-faced tablet 15 mm in diameter, using a compression-tension testing machine[1]. To keep the surface condition constant, a fixed compression force of 200 kg was used. Tablets were used for the quantification of appearance change by light irradiation. For the kinetic study 50 mg of ubidecarenone was dissolved in 50 ml of *n*-hexane–ether (1:1). Sixty microliters was placed on a quartz-glass plate (26 × 38 mm) and evaporated at room temperature. The oily sample was then cooled to 0–4° for 24 hr and allowed to crystallize. A 60-μg sample (<70 μm in diameter) was dispersed over the plate to illuminate all molecules as uniformly as possible. Samples were stored over silica gel in a desiccator in the dark until the irradiation test.

UV Irradiation—Two irradiation apparatus were employed. To investigate the effect of irradiation wavelength on the appearance change or photolytic degradation, a grating monochromator[2] with a 5-kW xenon lamp adjusted for 290–500 nm, a 5- to 21-nm intervals, was used (8). A band width of 5 nm was employed at all wavelengths. The amount of energy irradiated to each sample was calculated from the counts of the integrating photometer attached to the monochromator. Tablets or crystalline samples were attached to the front of the sample holder of the monochromator and exposed to UV rays. Elevation of temperature in the monochromator was prevented by a cooling water jacket surrounding the light source; the surface of samples was maintained at 25°.

In the accelerated irradiation test at ordinary and elevated temperature, the samples were placed in a thermostated jacket (Fig. 1). Water at the prescribed temperature was allowed to circulate through the jacket, and the temperature in the sample chamber was monitored with a thermocouple sensor. The jacket was placed in a fading tester[3] equipped with a 400-W mercury vapor lamp for color fading, as reported previously (11), and exposed to UV rays. The distance between the light source and the sample was 30 cm. The temperature in the fading tester was maintained below 27° by a constant-operating fan. To control the UV intensity irradiated to the sample, several optical filters[4] having various light transmission properties were attached to the front of the sample chamber.

[1] Autograph model IS-5000; Shimadzu Co., Kyoto, Japan.
[2] Model CRM-50; Japan Spectroscopic Co., Tokyo, Japan.
[3] Model MH-1; Mitsubishi Electric Co., Tokyo, Japan.
[4] Toshiba Kasei Kogyo Co., Tokyo, Japan.

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Table I—Irradiation Energies after 1 hr of Irradiation Through Various Glass Optical Filters

Filter	Irradiation Energy (300–400 nm), erg/cm^2
None	1.21×10^8
UV-25	1.02×10^8
UV-D25	9.97×10^7
UV-35	7.60×10^7
VY-42	2.09×10^5

The irradiation energies (300–400 nm) through these filters at the surface of a sample were measured with a UV intensity meter[5] (Table I). Samples were withdrawn from the monochromator or fading tester at designated time intervals for tristimulus colorimetry or high-performance liquid chromatographic (HPLC) analysis.

Colorimetric Measurements—To follow a quantitative change in surface color of the sample, a color and color difference meter[6] equipped with a halogen lamp as the light source was employed, and the surface color of the tablets in the L,a,b coordinate system was measured as reported previously (11). The Hunter's color difference ΔE (12) before and after irradiation was calculated to evaluate color darkening. After the measurement, samples were returned to the monochromator and irradiation was continued.

Absorption Measurements—At every irradiation, UV absorption spectra [semi-integral attenuance spectra (13)] of the sample powders in the gas phase were measured by a multipurpose recording spectrophotometer[7] with an end-on type photomultiplier. Air was used as the absorption control in accordance with the method described previously (5).

HPLC Analysis—A liquid chromatograph[8] equipped with a fixed-wavelength UV detector[9] was used for HPLC analysis. Ubidecarenone was quantified at 254 nm. The prepacked column[10] (30 cm × 3.9-mm i.d.) was operated at ambient temperature at a flow rate of 1.4 ml/min. The mobile phase consisted of a solvent system of n-hexane–ether (85:15). The internal standard solution of benzophenone was prepared at a concentration of 100 μg/ml. All solvents and reagents were HPLC and analytical grade, respectively, and were used as received.

After irradiation, samples on the plate were washed several times with ether, 30 μl of the internal standard solution was added, and the mixed solution was evaporated to dryness under vacuum. The residue was then dissolved in 300 μl of n-hexane–ether (1:1); 2 μl of this solution was injected onto the chromatograph with a 10-μl syringe by the on-flow technique. A typical chromatogram of the sample obtained after 15-min irradiation is shown in Fig. 2. The amount of ubidecarenone was the mean of three determinations.

RESULTS AND DISCUSSION

Color Darkening of the Tablet Surface—The coloration reaction of solid pharmaceutical preparations by light may be localized on the surface, differing from that of liquid preparations in which all drug molecules take part in the reaction. It is nevertheless possible to qualitatively estimate the photostability of the drug by the extent of coloration or color fading.

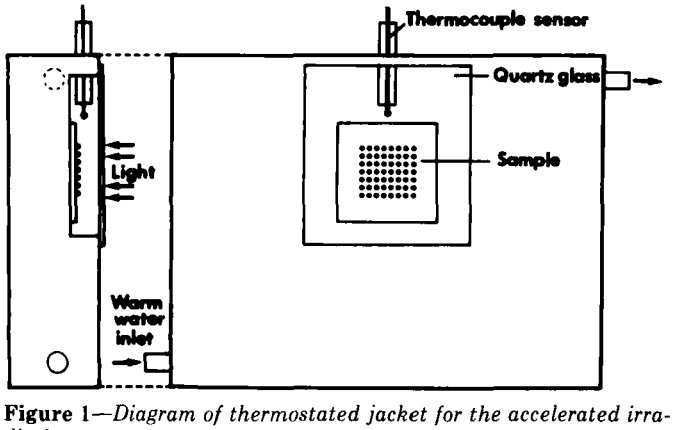

Figure 1—*Diagram of thermostated jacket for the accelerated irradiation test.*

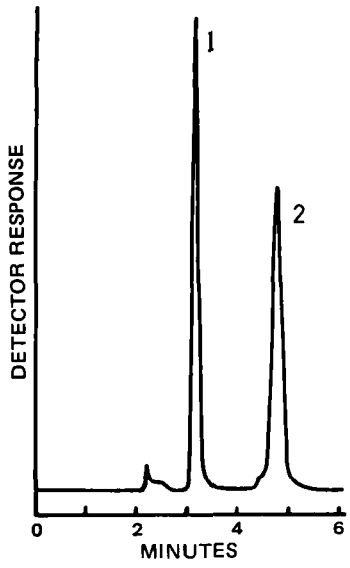

Figure 2—*Typical chromatogram of photodegraded ubidecarenone. Key: (1) benzophenone (internal standard); (2) ubidecarenone.*

Figure 3 shows the action spectrum for color darkening of a tablet surface irradiated under an intensity of 3.49×10^8 erg/cm^2. The color difference (ΔE) increased with decreasing irradiation wavelength and reached its maximum at ~350 nm. The color darkening was observed also in the visible region over 400 nm and resulted in a significant color difference even at ~450 nm. Above 480 nm, ΔE remained <2 NBS units, indicating that apparent color darkening did not occur in this wavelength region in view of the relationship between visual perception and color difference (14). Such behavior in coloration was strikingly different from the results obtained for sulfisomidine (8) and indomethacin (15), which were photostable against visible light. The photostability of ubidecarenone over a wide range of irradiation wavelength suggests that the selection of a desirable light source and the optimization of illumination conditions are necessary for studying the photoreaction of pharmaceuticals.

The process of these color changes was examined under irradiation with a mercury vapor lamp, and the change in ΔE against time was followed up to the maximum irradiation energy of 2.62×10^8 erg/cm^2 (after 120-min irradiation). For the kinetic interpretation of this process, the increase in ΔE against time may be expressed as (9):

$$\frac{d\Delta E}{dt} = k(\Delta E)^n \qquad \text{(Eq. 1)}$$

where t and k are irradiation time and the color-darkening rate constant, respectively, and n is constant. Integrating Eq. 1 under the initial condition ($\Delta E = 0$ at $t = 0$), we obtain:

$$\log \Delta E = \frac{1}{1-n} \log t + \frac{1}{1-n} \log [(1-n)k] \quad (n \neq 1) \qquad \text{(Eq. 2)}$$

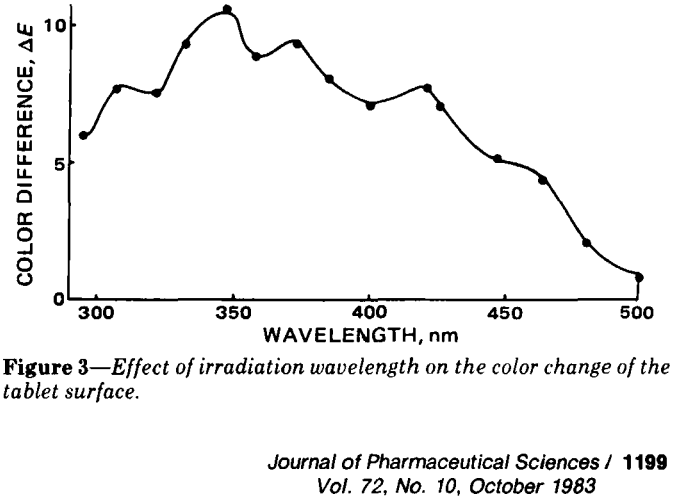

Figure 3—*Effect of irradiation wavelength on the color change of the tablet surface.*

[5] Model UVR-365, Tokyo Optical Instruments Co., Tokyo, Japan.
[6] Model ND-101, Nippon Denshoku Co., Tokyo, Japan.
[7] Model MPS-50L, Shimadzu Co., Kyoto, Japan.
[8] Model LC-3A, Shimadzu Co., Kyoto, Japan.
[9] Model SPD-2A, Shimadzu Co., Kyoto, Japan.
[10] μPorasil, Waters Associates, Milford, MA 01757.

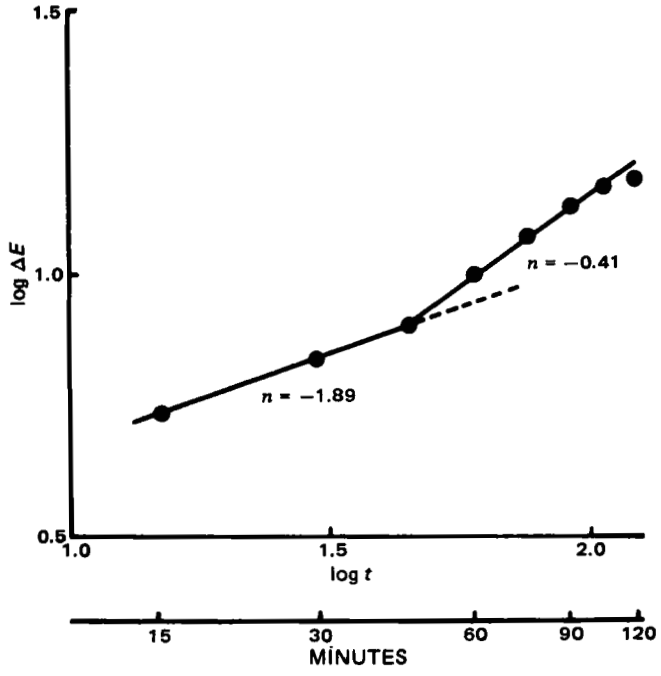

Figure 4—*Double-logarithmic plot for the color change process (cf. Eq. 2).*

Equation 2 indicates that a linear relationship with a slope of $1/(1 - n)$ should hold between ΔE and t on the double-logarithmic scale. The double-logarithmic plot of the color darkening process gave two excellent straight lines with different slopes (Fig. 4); the color darkening rate in the latter period was much higher than that in the earlier period of irradiation.

Physicochemical Change of Crystals—The results shown in Fig. 4 suggest that the mechanism of color darkening may undergo a change during the process of irradiation. The color darkening may be attributable to change in the surface condition of the tablet in addition to structural change of the drug molecules. Figure 5 shows photomicrographs of ubidecarenone crystals irradiated for various times. No change in appearance of the wrinkled surface was observed up to 30 min of irradiation, but after 50 min liquefaction of the surface layer of crystals began and gradually extended inward. After 90 min all crystals were liquefied to oily droplets. This may be due to the formation of a photoproduct with a lower melting point than the original substance. These physicochemical changes must affect the UV absorption properties of solid-state samples. Figure 6 shows the absorption spectra obtained under the same irradiation condition as Fig. 5. The spectrum of the original state before irradiation gave an absorption maximum and minimum at 278 and 236 nm, respectively. As

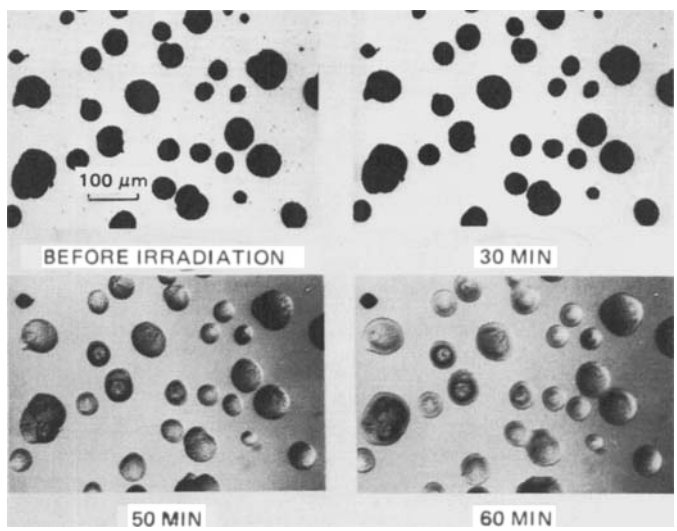

Figure 5—*Liquefaction of ubidecarenone crystals irradiated using a mercury vapor lamp.*

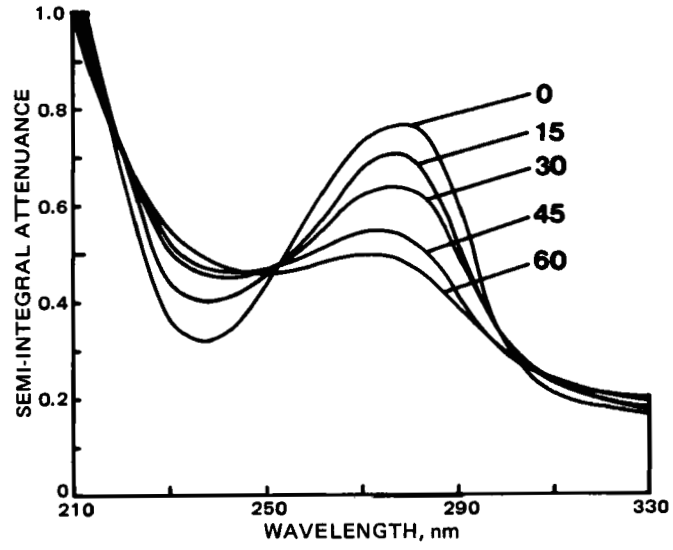

Figure 6—*Effect of irradiation time on the absorption spectrum of solid-state ubidecarenone. Numbers on the graph refer to irradiation time in minutes.*

the irradiation time proceeded, the absorption curve showed a flattening tendency, increase in the minimum and decrease in the maximum. The isosbestic points were confirmed at 254 and 300 nm up to 30 min of irradiation. After 45 min, however, these points were not obtained, and the absorption maximum and minimum shifted to shorter and longer wavelengths, respectively. The disappearance of isosbestic points after 45 min was closely related to the alteration in color-darkening rate (Fig. 4) and the beginning of liquefaction (Fig. 5). The presence and absence of isosbestic points suggest occurrence of a photochemical reaction on the crystal surface.

Photolytic Degradation at Various Irradiation Wavelengths—The photolytic degradation is also believed to be affected by irradiation wavelength. Figure 7 shows the semilogarithmic plot of percent remaining ubidecarenone against irradiation energy at several representative wavelengths. Good linear relationships existed between both parameters at every wavelength, indicating that the photolytic degradation followed apparent first-order kinetics. The irradiation energy was directly proportional to time. Even under the same irradiation energy, the degree

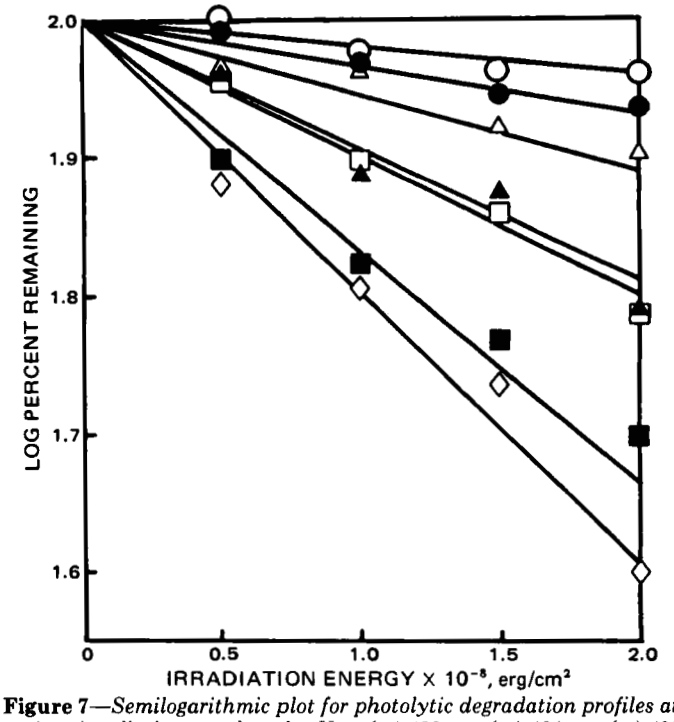

Figure 7—*Semilogarithmic plot for photolytic degradation profiles at various irradiation wavelengths. Key: (○) 480 nm; (●) 464 nm; (△) 426 nm; (▲) 400 nm; (□) 373 nm; (■) 347 nm; (◇) 290 nm.*

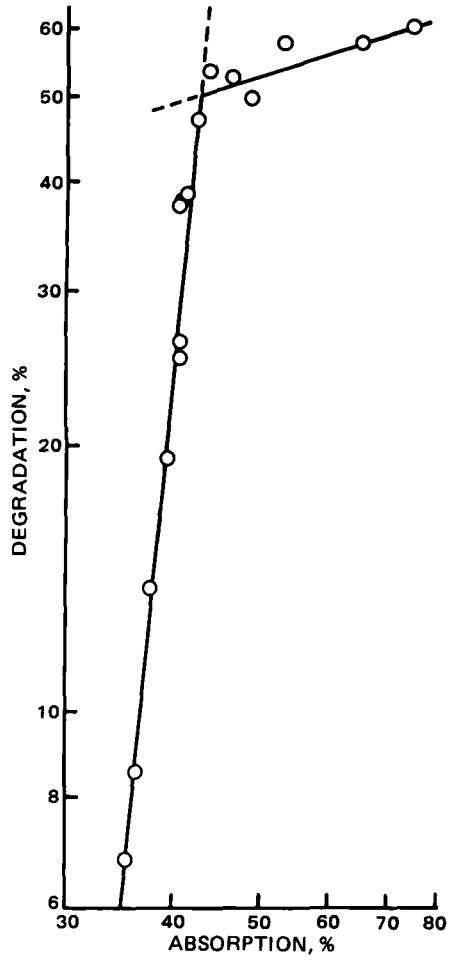

Figure 8—*Relationship between percent degradation and percent absorption of irradiated light energy.*

of degradation varied largely depending on the wavelength: it increased with decreasing irradiation wavelength. According to Grottus–Draper's law, only the light absorbed by a substrate can subject it to chemical change. Therefore, the results obtained in Fig. 7 may be attributed to the fact that the semi-integral attenuance increased with decreasing wavelength above 290 nm, as recognized in Fig. 6.

Supposing that the intensity of reflected light at the surface of substrate is negligibly low compared with that of light either absorbed by or transmitted through the substrate, the absorbance may be replaced

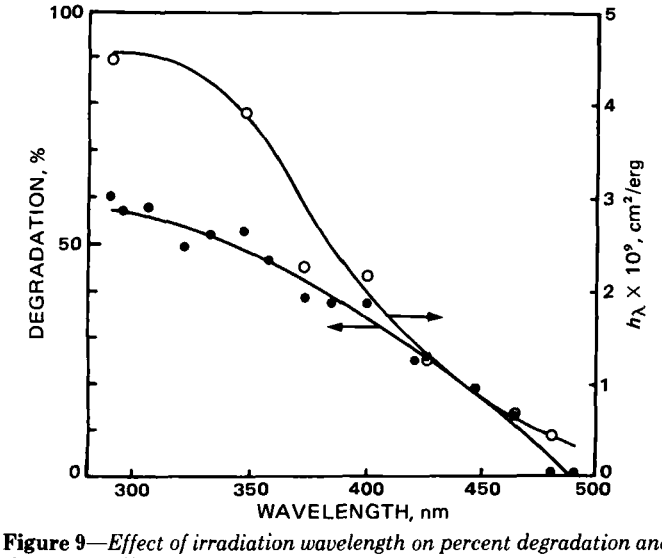

Figure 9—*Effect of irradiation wavelength on percent degradation and the energy efficiency constant,* h_λ.

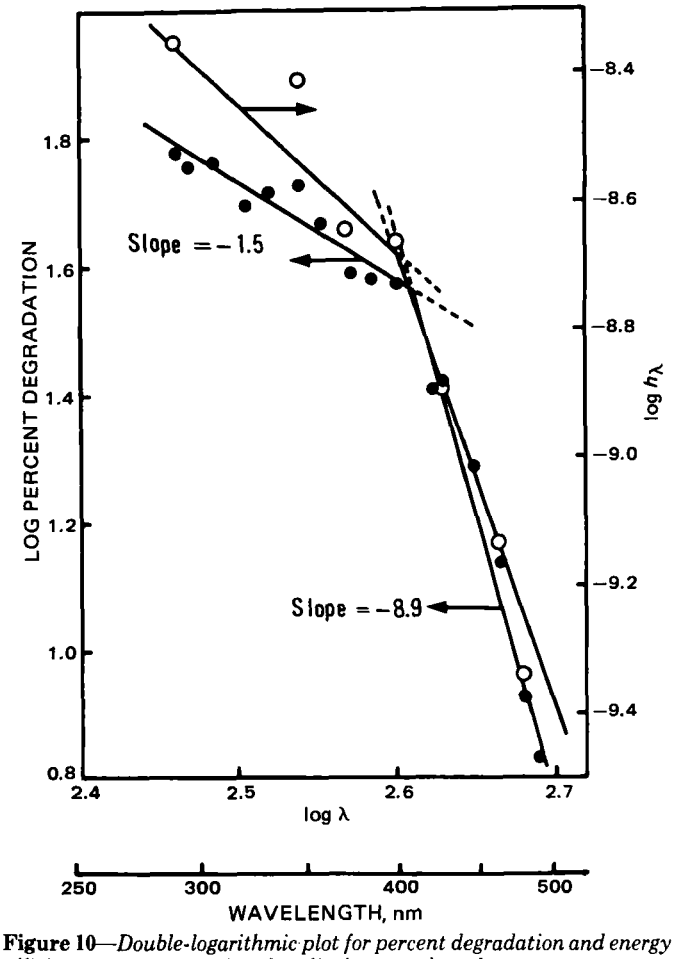

Figure 10—*Double-logarithmic plot for percent degradation and energy efficiency constant against irradiation wavelength.*

with semi-integral attenuance, ${}_pE_t$. The percent absorption, A, is then given by:

$$A\ (\%) = 100 - 10^{\log T} \qquad \text{(Eq. 3)}$$

where, $\log T = 2 - {}_pE_t$. Thus, the quantitative relationship between the degree of degradation and light absorption can be given as in Fig. 8; the percent degradation under the same irradiation energy of 2.0×10^8 erg/cm^2 in the range of 290–500 nm is plotted in this graph against percent absorption at that irradiation wavelength, using ${}_pE_t$ values of the original curve before irradiation in Fig. 6. It is clear that the percent degradation is quite sensitive to light absorption in the region of lower percent absorption, whereas it is less sensitive for higher percent absorption: the critical point existed at ~43% absorption. Photolytic degradation could no longer take place below 30% absorption. This result indicates that the absorption of light by ubidecarenone is not necessarily effective in producing degradation. In such a situation after a molecule had absorbed a quantum of light energy, it might collide with other molecules to raise their kinetic energy, resulting in only an elevation of the system temperature.

The slope of each line in Fig. 7 is equivalent to a degradation rate constant with a unit of min^{-1} at one irradiation wavelength. However, due to the spectral irradiation energy distribution of the light source, the required irradiation time differs depending on the irradiation wavelength even though under the same irradiation energy. It is not, therefore, reasonable to regard the slope as the degradation rate constant. In this case, an energy efficiency constant, h_λ, with a unit of cm^2/erg is used for convenience. Figure 9 shows the dependencies of the percent degradation and the energy efficiency constant obtained from Fig. 7 on the irradiation wavelength under the irradiation energy of 2.0×10^8 erg/cm^2. The percent degradation decreased with increasing wavelength, and no degradation occurred >480 nm. This pattern was in agreement with that of appearance change in Fig. 3. The energy efficiency constant was also reduced significantly with increasing irradiation wavelength. These parameters were replotted on the double-logarithmic scale (Fig. 10). For all parameters, good regression lines with an intersection at ~410 nm were estab-

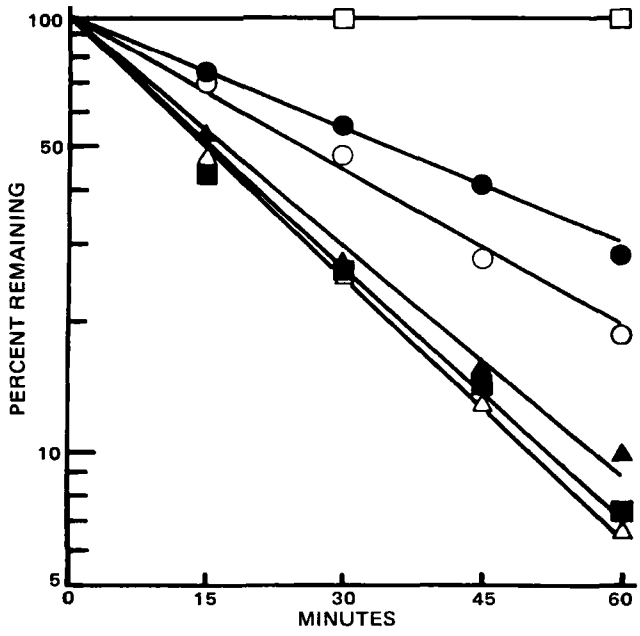

Figure 11—*Semilogarithmic plot for photolytic degradation profiles at various temperatures. Key: (□) 60° in the dark; (●) 25°; (○) 35°; (▲) 45°; (■) 55°; (△) 60°.*

lished over the whole range of wavelengths, but the slopes in two wavelength regions differed markedly. On the basis of this fact, we can deduce that though ubidecarenone is highly degraded by UV light, the effect of irradiation wavelength is much greater in the visible region than in the UV region.

Effect of Temperature on Photolytic Degradation—In examining the photostability of solid drugs with low melting point, it is of value to know how the photostability will be affected by temperature, as drugs stored at higher than melting point may degrade. Very little has been reported concerning the effect of temperature in photochemical reactions. In such cases the contributions of elevated temperature were promotive (16) or repressive (17–19) to the reaction.

Figure 11 shows the semilogarithmic plot of percent residual ubidecarenone against irradiation time at various temperatures. The time

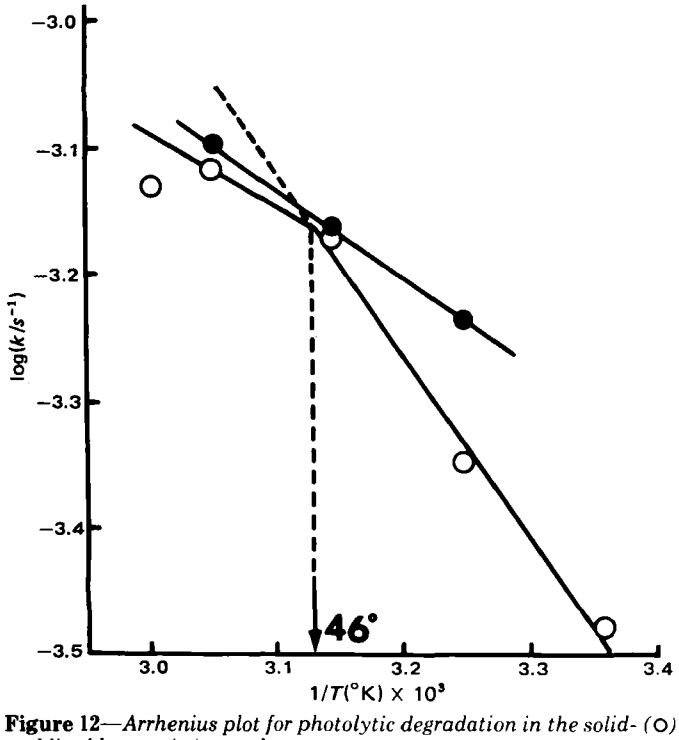

Figure 12—*Arrhenius plot for photolytic degradation in the solid- (○) and liquid-state (●) samples.*

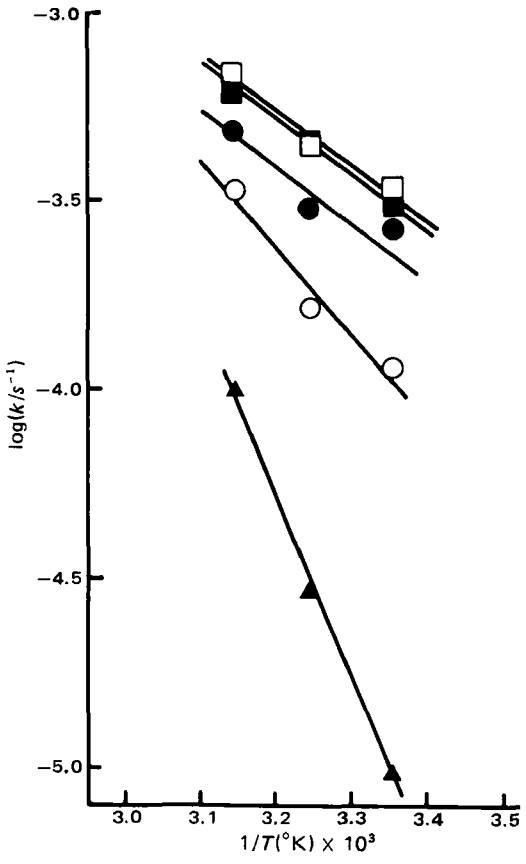

Figure 13—*Arrhenius plot for the photolytic degradation of ubidecarenone covered with various optical filters (see Table I). Key: (□) without filter; (■) UV-25; (●) UV-D25; (○) UV-35; (▲) VY-42.*

dependency of degradation was confirmed to follow apparent first-order kinetics at every temperature, as expected from Fig. 7. The degradation was significantly accelerated by elevation of temperature. It was evident that even at 60°, no degradation occurred at all in the dark, which suggests that the thermal reaction by itself cannot participate in the chemical degradation.

These degradation rate constants are given as an Arrhenius plot in Fig. 12. In the case of a liquid-state sample which was not allowed to recrystallize, the plots gave only one satisfactory line over the whole range of temperatures. In contrast, the plots for a solid sample gave two straight lines with different slopes; a critical temperature of 46° obtained as an intersection of two lines agreed with the initiating temperature for melting on the differential thermal analysis (DTA) curve of the sample. The slopes of these lines, therefore, indicate the activation energies for photodegradation in the solid and liquid states. The line in the range of temperatures higher than the melting point was adjacent and parallel to that of the liquid-state sample, giving an activation energy of 3.14 kcal/mole, which was much lower than that of 6.67 kcal/mole in the solid state. The liquid-state sample was far more prone to photodegradation than the solid sample. The difference in the activation energy depending on the existing states of sample seems to rely on the mobility of molecules subjected to the reaction in addition to the light absorption properties. In any case, these activation energies were much lower than the reported activation energies for typical drug decomposition in the liquid phase (20). It is worthy to note that reactions with such low activation energy take place so readily that photostable pharmaceutical preparations may not be able to be formulated without extreme care.

Under the various irradiation energies using several optical filters (Table I), the time dependency of degradation was examined at 25°, 35°, and 45°. The results are summarized as an Arrhenius plot in Fig. 13. This graph reveals a thermodynamic relationship between the tendency of degradation and UV intensity. As expected from the foregoing discussions, the higher the UV intensity, the more rapid the degradation. In contrast, the effect of temperature on the degradation rate constant was more significant with decreasing UV intensity. The ratios of the degradation rate constant for the sample without a filter to that for the sample covered with a VY-42 filter at 25° and 45° were 34.5 and 8.6, respectively.

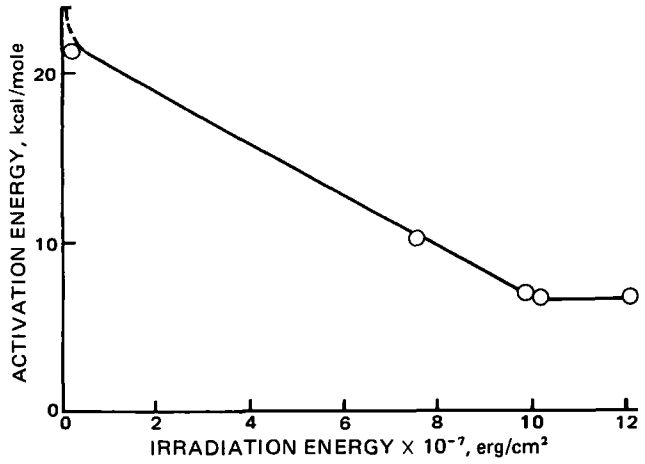

Figure 14—*Effect of UV intensity on the activation energy.*

These values were smaller by one or two orders of magnitude in comparison with the UV intensity ratio of 579, irradiated on each sample. In this respect, the elevation of temperature could be verified again to have a synergistic effect on the chemical degradation of ubidecarenone. The effect of UV irradiation energy on the activation energy calculated from the slope of each regression line is shown in Fig. 14. The activation energy linearly decreased up to $\sim 1.0 \times 10^8$ erg/cm^2; with values larger than this it was no longer dependent on the UV intensity. It should be pointed out that even under very low intensity (in the case of filter VY-42), the activation energy approached a finite value of 21.3 kcal/mole, which is within the usual range of activation energies for hydrolysis or oxidation (21).

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Determination of Busulfan in Plasma by GC–MS with Selected-Ion Monitoring

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Abstract □ A GC–MS technique with selected-ion monitoring is described for the determination of busulfan in plasma. Busulfan is extracted from plasma with methylene chloride and converted to 1,4-diiodobutane. Analysis by GC–MS with selected-ion monitoring (m/z 183) gave a relative standard deviation of ±4.3% (n = 5) at the 10-ng/ml level.

Keyphrases □ Busulfan—determination in human plasma, GC–MS with selected-ion monitoring of 1,4-diiodobutane □ GC–MS analysis—selected-ion monitoring, determination of busulfan in human plasma

The alkylating agent, busulfan, 1,4-butanediol dimethanesulfonate, is the drug of choice in the treatment of chronic myeologenous leukemia. The drug has been in clinical use since the 1950's, but the fate of the drug in humans has only been studied by administering radioactively labeled compound and measuring total radioactivity in plasma and urine (1, 2). This paper describes the conversion of busulfan to 1,4-diiodobutane, and the subsequent quantitation of this material by GC–MS with selected-ion monitoring.

EXPERIMENTAL

Synthesis of 1,5-Pentanediol Dimethanesulfonate (Internal Standard)—Methanesulfonic anhydride (9 g) was added carefully to a stirred mixture of 1,5-pentanediol (5.5 g) in pyridine–methylene chloride (40 ml, 1:1). After stirring overnight at 25° the mixture was filtered, and the organic phase was washed with water. The organic phase was evaporated to ~5 ml and left at 4° for 48 hr. The crystals which formed were separated, washed with ice-cold water, and dried (yield: 9%); mp 35° [lit. (3) 34–35°]. The compound was identified by GC–MS[1] after conversion to the corresponding 1,5-diiodo derivative according to the procedure given below. There were prominent peaks at m/z 324 (M^+, 8%), 199 (5), 197 (53), 169 (5), 155 (26), 70 (8), and 69 (100).

Conversion of Busulfan to 1,4-Diiodobutane—Busulfan was con-

[1] LKB 2091; the ionizing energy was 70 eV.

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