Photochemical and Oxidative Degradation of the Solid-State Tretinoin Tocoferil

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The photostability of tretinoin tocoferil was investigated under irradiation with three kinds of lamps, *i.e.*, a cool white fluorescent lamp, a UV-A fluorescent lamp and a D65 fluorescent lamp. A combination of the cool white fluorescent lamp and the UV-A fluorescent lamp, and the D65 lamp having relative spectral power distribution similar to that of direct daylight, correspond to options 2 and 1 in ICH Guidelines, respectively. The photodegradation apparently followed second-order kinetics under these light sources and the degradation rate constant under exposure by the D65 lamp was larger than that by the cool white fluorescent lamp. The drug was susceptible to degradation by visible and UV light below 480 nm and was degraded most remarkably at around 420 nm, showing a wavelength-dependency. The semi-logarithmic plots of apparent degradation rate constant against the reciprocal of illuminance showed a good linear relationship in the Arrhenius-type fashion, and the photostability under ordinary illumination conditions could be predicted from the data obtained under the accelerated illumination conditions. The rate of oxidative degradation was slightly accelerated with the rise of temperature. Thermodynamic parameter was calculated from the Arrhenius plot. The degradation rate constant rapidly increased in proportion to partial pressure of oxygen below 20 kPa.

Key words tretinoin tocoferil; photostability; illuminance effect; oxidation; preformulation study

Tretinoin tocoferil ((±)-3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-tridecyl)-2*H*-1-benzopyran-6-yl (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate) is an α-tocopherol ester of all-*trans* retinoic acid (RA) and has been safely used in the treatment of decubitus and skin ulcer to stimulate the proliferation of human skin fibroblasts.[1] Toxicity tests in animal models have shown that tretinoin tocoferil is at least 150 times less toxic than all-*trans* RA and does not induce teratogenesis. 13-*cis* RA and all-*trans* RA are known to undergo *Z–E* isomerization (due to the existence of four unsaturated double bonds) and oxidation when exposed to light and air.[2] RA underwent autooxidation in 90% ethanol at 25—85.5 °C to give epoxides, dioxetanes, an endoperoxide and double-bond cleavage products.[3] Therefore tretinoin tocoferil is also likely to be susceptible to photodegradation and oxidation.

The information obtained from the stability test of the drug is useful for development and stabilization of the dosage forms, but there are no reports concerning the photostability and the oxidation of tretinoin tocoferil.

The purpose of the present investigation was to evaluate photostability and oxidation profiles of tretinoin tocoferil under various environmental conditions to establish a reasonable stabilization design against light and oxygen. Elucidation of the degradation mechanism was therefore beyond the aim of this study.

Experimental

Materials Bulk tretinoin tocoferil was obtained from Nisshin Flour Milling Co., Japan. The commercial solvents for HPLC analysis and Sudan II as an internal standard were used without further purification.

Methods Tretinoin tocoferil (50 mg) was dissolved in 50 ml of dichloromethane. An aliquot of the solution (50 μl) was placed in a glass tube and evaporated to dryness at room temperature. The sample was further dried under vacuum at room temperature.

Irradiation Tests Two irradiation apparatuses were employed: To study the effect of specific wavelength of light on the photostability of tretinoin tocoferil, the same grating monochromator (model CRM-FA, Japan Spectroscopic Co., Tokyo, Japan) as described in our previous paper,[4] equipped with a 5-kW xenon lamp, was used. Samples were exposed until the intensity level of the irradiation reached a definite energy(1.8×10^5 J/m^2). To study the effect of photon sources on the photostability, a cool white fluorescent lamp (type FL20SW/M), a D65 fluorescent lamp (FLR20S·D-EDL-D65/M), or a UV-A fluorescent lamp (FLR20S·BL/M, all three lamps from Nagano Science Equipment MFG. Co., Japan) were equipped in a light-irradiation tester (LIGHTTRON LT-120(D3CJ), Nagano Science Equipment MFG. Co., Japan). The spectral irradiation intensity curves of these lamps were measured with a portable spectroradiometer (model LI-1800, LI-COR Inc., Neb., U.S.A.). Figure 1 shows the relative spectral power distribution of the lamps as measured on the surface of a sample under an irradiation condition.

Unless otherwise specified, the irradiation tests were carried out at 3500 lx and 25 °C for the cool white and D65 fluorescent lamps. The irradiation intensity was set at 10 J/m^2·s for the UV-A fluorescent lamp at 25 °C. Total irradiation intensity per second (J/m^2·s) in the wavelength range 300—500 nm was calculated from the data obtained by the spectroradiometer and corrected according to the experimental illuminance or UV intensity.

To investigate the effect of oxygen on the photostability, the samples were placed in a jacket which had a quartz glass window on the front side, and oxygen, air or argon gases were passed through the jacket at a flow rate of 500 ml/min.

Oxidative Stability To estimate the oxidative stability of tretinoin tocoferil, the degradation data was measured as follows: The glass tubes described above were stored in a thermostat maintained at various temperatures. The jacket put in the thermostat and a mixture of gases of nitrogen and oxygen, which were adjusted to various partial pressures of oxygen with gas blender equipment (GM-2A, Kofloc Co., Japan), were passed through it at a flow rate of 500 ml/min.

Assay Procedures for Tretinoin Tocoferil The assay for tretinoin tocoferil content of all samples after storage was carried out by a HPLC system (Waters Co.) equipped with a photodiode array detector (358 nm, model 996, Waters Co.) ; the prepacked column (NUCLEOSIL 50-5, 150 mm×4.6 mm i.d., Chemco Co.) was operated at room temperature at a flow rate of 1.2 ml/min. The mobile phase was composed of *n*-hexane–ethyl acetate (100 : 1). A solution of Sudan II in *n*-hexane (16 mg/100 ml) was used as an internal standard. After irradiation, 1 ml of internal standard solution was added to the glass tube and stirred until the sample completely dissolved. The concentration of tretinoin tocoferil unchanged was determined chromatographically by using 50 μl of this solution. The experimental results shown are the average of three measurements. A standard calibration curve for tretinoin tocoferil gave good linearity ($r>0.999$; $n=5$), and the reproducibility of the data was invariably good. All procedures were carried out in the dark.

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Results and Discussion

Estimation of Chemical Structure of Photoproducts Figure 2 shows the chromatograms resulting from the HPLC analysis of the sample after 1 h-irradiation under the D65 lamp at 3500 lx. The sample after irradiation showed many peaks, and 3—7 formed as a result of photochemical reaction in addition to tretinoin tocoferil, 1.

Tretinoin tocoferil is a α-tocopherol ester of all-*trans* RA, and retinoids are generally unstable to heat, oxygen, and light.[5,6] Bempong *et al*.[2] investigated the degradation products formed after prolonged exposure of solution and solid samples of all-*trans* RA to light. Many degradation products such as 13-*cis* RA, the other isomer, all-*trans* 5,6-epoxy RA and isomers of 5,6-epoxy RA were obtained from the RA solution after exposure to air and light. Therefore, many isomers of tretinoin tocoferil would likely be formed by light irradiation. The UV spectra corresponding to these peaks were obtained by diode array technique (Fig. 2) and exhibited the similar spectrum and absorption maximum as those of the intact spectra, suggesting that these products were geometrical isomers. The photoisomers of RA exhibit λ_{max} of the UV spectra at around 350 nm.[7] In addition, 5,6-epoxy might be formed by the irradiation of tretinoin tocoferil as well as RA.[2] However, the photodegradation mechanism was not elucidated since it was beyond the scope of the present study.

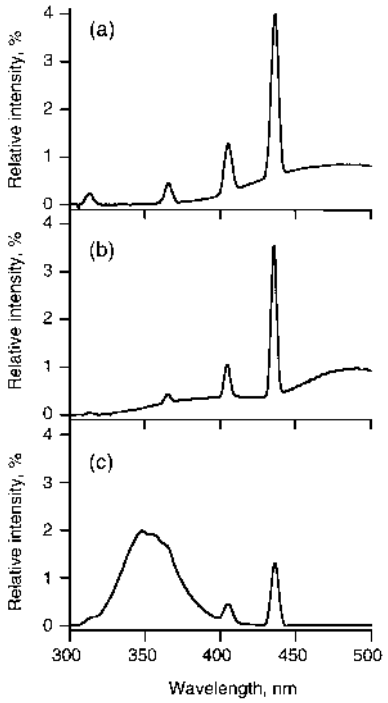

Fig. 1. Relative Spectral Power Distribution of Various Lamps

a, Cool white fluorescent lamp (2009 lx); b, D65 fluorescent lamp (1952 lx); c, UV-A fluorescent lamp.

Irradiation Wavelength Dependency on Photostability Since the photolabile drugs are sensitive to light within a particular wavelength range, photodegradation rate is effected by the kind of light sources with different spectral power distribution. Therefore, to reasonably stabilize any photolabile drug it is important to examine its photosensitivity over a wide range of wavelengths. Figure 3 shows the effect of wavelength of light on the photodegradation of tretinoin tocoferil under the constant irradiation intensity (1.8×10^8 J/m^2). No degradation was observed at a wavelength over 500 nm. However, a significant decrease of the percent remaining occurred $<$500 nm, showing the minimum at around 420 nm followed by subsequent increase at further short wavelengths. This suggests that the drug must be stored carefully when exposed to light from a fluorescent lamp and daylight, because tretinoin tocoferil is so easily decomposed by visible and UV light.

Photostability under Various Light Sources The relative spectral power distribution measured by a portable spectroradiometer for three light sources is shown in Fig. 1. The D65 fluorescent lamp corresponds to option 1 of the photon source in the photostability test of the ICH Guidelines[8] and

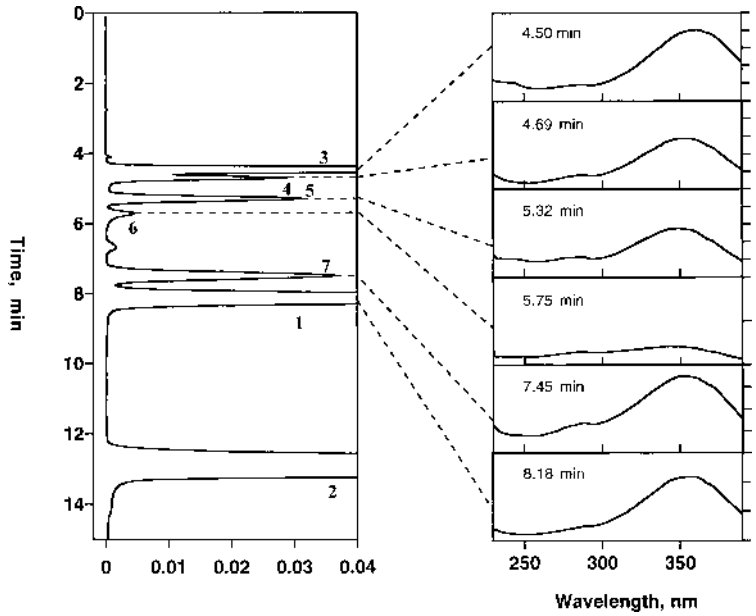

Fig. 2. HPLC Chromatograms of Tretinoin Tocoferil Exposed to D65 Fluorescent Lamp for 1 h , and UV Spectra of Tretinoin Tocoferil and Its Degradation Products

1, Tretinoin tocoferil; 2, internal standard; 3—7, photoproducts.

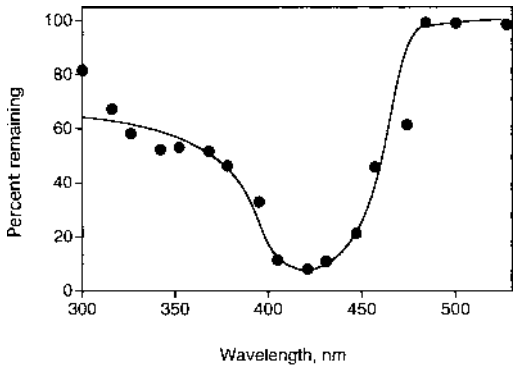

Fig. 3. Effect of Irradiation Wavelength on the Photodegradation of Tretinoin Tocoferil (Irradiation Intensity: 1.8×10^5 J/m^2)

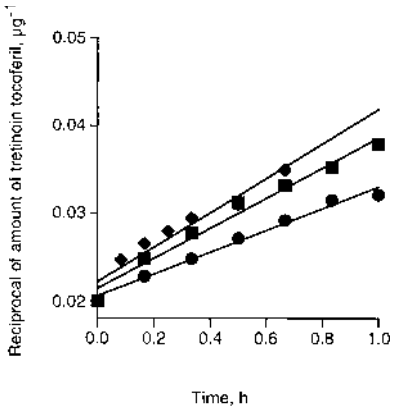

Fig. 4. Time-Courses of Photodegradation of Tretinoin Tocoferil under Various Light Sources

Light source: ●, cool white fluorescent lamp (3500 lx); ■, D65 fluorescent lamp (3500 lx); ◆, UV-A fluorescent lamp (10 J/m^2·s).

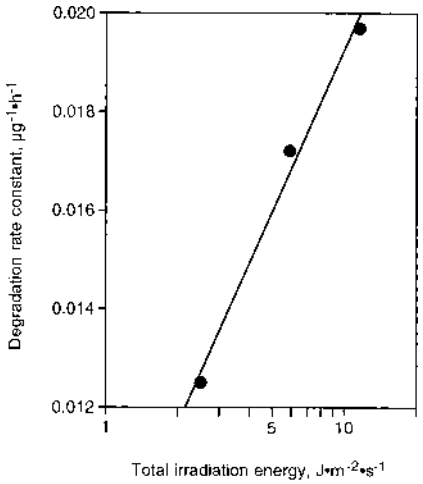

Fig. 5. Relationship between Degradation Rate Constant of Tretinoin Tocoferil and Total Irradiation Energy per Second (300—500 nm) of Various Light Sources

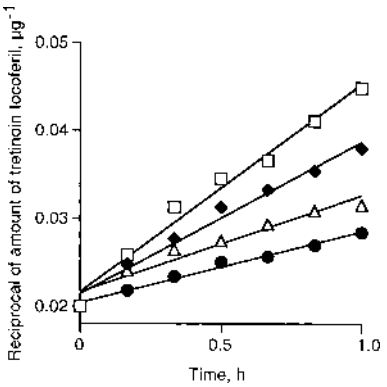

Fig. 6. Time-Courses of Photostability of Tretinoin Tocoferil at Different Illuminances by D65 Fluorescent Lamp

Illuminances (lx): ●, 1000; △, 2000; ◆, 3500; □, 5000.

has continuous light intensity in the wavelength range 300 to 700 nm, similar to direct daylight. On the other hand, a cool white fluorescent lamp, which is used as indoor illumination, had lower light in the UV region below 400 nm compared with the D65 lamp. The UV-A fluorescent lamp emits light in the wavelength range 300 to 400 nm. A combination of the cool white and UV-A fluorescent lamps was applied as option 2 in the guideline.

The photodecomposition of tretinoin tocoferil was studied under the three light sources. The percent remaining values of tretinoin tocoferil were fitted to a first-order model, however the first-order equation did not seemed to provide a good fit ($r<0.986$). Figure 4 shows the plots of the reciprocal amount of tretinoin tocoferil against time when the drug was exposed to light from these three sources. The plots showed good linearity ($r\approx0.990$) in all light sources, suggesting that the photodegradation followed the apparent second-order kinetics. Kurotori *et al.*[9] reported that the photodecomposition reaction of nafamstat mesilate followed the pseudo-second order reaction, but there are no reports that the photodegradation reaction followed the apparent second-order kinetics.

Thus the degradation rate constants obtained from the slope of straight lines for cool white, D65, and UV-A fluorescent lamps were 0.0125, 0.0172, 0.0197 $\mu g^{-1}\cdot h^{-1}$, respectively. This result suggested that tretinoin tocoferil was more unstable under exposure by the D65 lamp irradiating light below 400 nm than that by the cool white fluorescent lamp in spite of their having the same illuminances. These differences in degradation rates are attributable to the spectral power distribution and light intensity of these lamps (Fig. 1). Therefore, the cumulative irradiation intensity of light in the wavelength range of 300—500 nm under experimental conditions was calculated according to the method reported by Matsuda *et al.*[4] Figure 5 shows the relationship between the degradation rate constant of the drug and total irradiation intensity per second (300—500 nm) of various light sources. Irrespective of the sources, a good relationship was observed between the two parameters. This indicated that the total irradiation intensity per second could be a parameter by which to estimate the photostability of tretinoin tocoferil and that the stability under the irradiation by a different light source or daylight was predictable if the total irradiation intensity per second could be the measured under that condition.

Effect of Illuminance on the Photostability of Tretinoin Tocoferil It is possible to carry out an accelerated irradiation test with the same light source using the light irradiation tester described above, which can control illuminance, ultraviolet energy and temperature. Figure 6 shows the plot of reciprocal amount of tretinoin tocoferil remaining against irradiation time at several levels of illuminance. Good linear relationships existed between the two parameters at all illu-

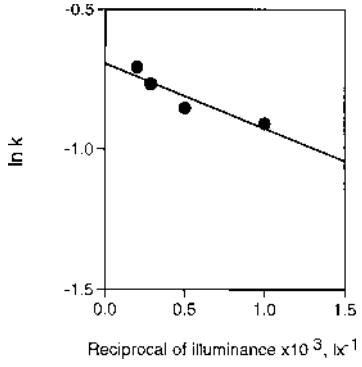

Fig. 7. Semilogarithmic Plots of Apparent Degradation Rate Constant against the Reciprocal of Illuminance

Table 1. Effects of Temperature on the Photodegradation Rate Constant of Tretinoin Tocoferil

Temperature (°C)	Degradation rate constant, $\mu g^{-1} \cdot h^{-1}$
15	1.94×10^{-2}
25	1.72×10^{-2}
35	2.73×10^{-2}
45	2.73×10^{-2}

Table 2. Effects of Oxygen, Air and Argon on the Photodegradation Rate Constant of Tretinoin Tocoferil

Atmospheric gas	Degradation rate constant, $\mu g^{-1} \cdot h^{-1}$
Oxygen	2.43×10^{-2}
Air	2.36×10^{-2}
Argon	1.95×10^{-2}

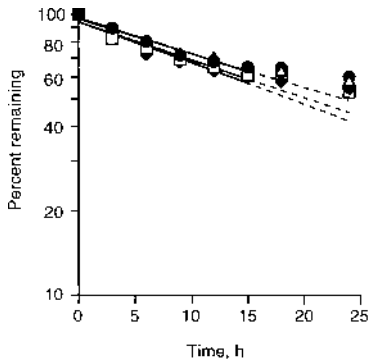

Fig. 8. Semilogarithmic Plots for the Thermal Degradation of Tretinoin Tocoferil at Various Temperatures

Temperatures (°C): ●, 40; △, 25; ◆, 35; □, 45.

minances, indicating that the photolytic degradation followed apparent second-order kinetics and the degradation rate constant increased with increasing illuminance. Semilogarithmic plots of the apparent degradation rate constant against the reciprocal of illuminance are shown in Fig. 7. A good linear relationship ($r>0.930$, $n=4$) was established between the parameters, suggesting that the stability of the drug under ordinary illuminance in a hospital pharmacy or home could be estimated from the data obtained under accelerated illuminance conditions. There have been several reports dealing with plots similar to the Arrhenius-type fashion relating to drug photostability.[10—13] This plot must therefore be useful to evaluate the photosensitivity of a drug.

Effect of Temperature on the Photostability of Tretinoin Tocoferil In spite of variation of reaction mechanism and kinetic order, drug degradation generally will be affected by storage temperature. There have been few reports concerning the effect of temperature on the photochemical reaction.[14,15] The effect of temperature on photodegradation of tretinoin tocoferil under irradiation by the D65 fluorescent lamp is summarized in Table 1. The degradation was accelerated by a rise in temperature. The calculated activation energy by Arrhenius equation for the photodegradation was 11.2 kJ/mol, which was less than that of menatetrenone[10] or almost the same as that of ubidecarenone.[14] Matsumoto *et al.*[15] observed the following temperature dependency on photodegradation: a) Activation energy is required in the formation process of products. b) Vibrational level participates in photodegradation. c) An aggregated form is generated and is subjected to photodegradation. d) The reaction is influenced by viscosity of the reactant. The reason why a slight temperature-dependency was observed in the photodegradation might have been due to the generation of reaction relating to temperature during the degradation process after exiting of the drug. However, since these reaction mechanisms are complicated, it would be difficult at this point to speculate which reaction is affected by temperature. Furthermore, sample viscosity would decrease with a rise in temperature and consequently the reaction rate would be increased, because tretinoin tocoferil is a resinous substance.

Effect of Oxygen on the Photostability Table 2 summarizes the effect of oxygen on the photodegradation rate constant obtained under irradiation by the D65 fluorescent lamp. The degradation rate constant under argon atmosphere became less than that under air or oxygen atmosphere. Photodegradation of cianidanol[16] was inhibited by lowering the concentration of oxygen surrounding the reactant, so that oxygen was assumed to participate in the photolytic degradation of cianidanol. 5,6-Epoxy RA forms by irradiation of RA with a fluorescent lamp[2] and this epoxide is known to be an autoxidation product of RA. It is shown below that tretinoin tocoferil was subjected to oxidation; therefore, oxygen would be slightly related to photodegradation of this drug.

Effect of Temperature on Oxidation Process It is also important to investigate the effect of temperature on oxidative stability of this drug. Tretinoin tocoferil was stored at various temperatures, and the percent of the drug remaining was followed at designated time intervals. RA and retinoids (*e.g.*, retinal, retinol) are known to undergo autoxidation, and the resulting products of RA were epoxide, dioxetanes, endoperoxide, and double-bond cleavage products.[3] The peaks of degradation products were scarcely observed in the HPLC chromatogram after 24-h storage at 358 nm, suggesting that the conjugated double bonds were broken by autoxidation of tretinoin tocoferil. Figure 8 shows a semilogarithmic plot of tretinoin tocoferil degradation profiles at various temperatures. Straight lines were obtained at each temperature except for the latter period of reaction, indicating that the degradation of the drug followed the first-order kinetics at the initial stage. This phenomenon might be due to the resinous state of the drug. Consequently, only the surface of sample might be subjected to degradation. The degradation rate constants

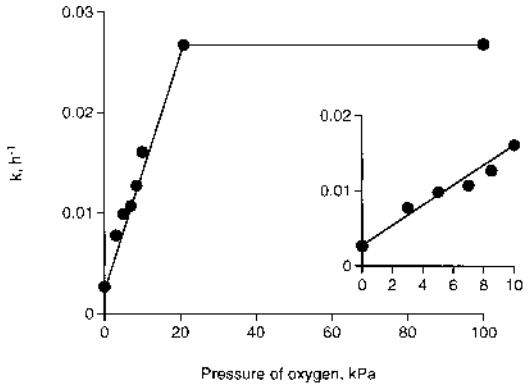

Fig. 9. Effect of Partial Pressure of Oxygen on the Degradation Rate Constant at 70 °C

were estimated from the data of a linear part of the degradation curve. There was a slight difference among these degradation rate constants.

The activation energy obtained from the Arrhenius plot was 3.0 kJ/mol and very small. Activation energy of the oxidative reaction for ascorbic acid and morphine were reported to be 32.6[17] and 95.3 kJ/mol,[18] respectively. Compared with these values, tretinoin tocoferil is likely to be easily oxidized even at ordinary or lower temperature. The shelf life (T_{90}) at 25 °C estimated by extrapolation was approximately 3.8 h, while T_{90} at 70 °C was 3.4 h.

Effect on Oxygen Pressure on the Thermal Degradation The partial pressure of oxygen is thought to be a particularly important factor in determining the autoxidation of pharmaceutical products. Figure 9 shows the effect of the pressure of oxygen on the oxidative degradation rate constants of tretinoin tocoferil. The degradation rate constant rapidly increased in proportion to the partial pressure of oxygen below 20 kPa and was kept constant at a pressure of 20 kPa or above. In general, the radicals derived from a substrate generate in the initial step of autoxidation, then peroxide formation by the reaction of that radical with oxygen occurs in the second step. Therefore, autoxidation of tretinoin tocoferil might be inhibited under low oxygen pressure. The degradation might still be observed even under nitrogen atmosphere because of the oxygen remaining in the sample.

It is concluded from these results that tretinoin tocoferil easily decomposes by light of ordinary illuminance and the wavelength of light significantly affects the degradation. In addition, it has become apparent that the oxidation of a drug depends on the pressure of oxygen.

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