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# PHOTO-STABILITY OF CIANIDANOL IN AQUEOUS SOLUTION AND IN THE SOLID STATE

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## ABSTRACT

Cianidanol was exposed to light in aqueous solution and in the solid state for stability studies. Photo-decomposition of cianidanol occured by exposure to UV light below approximately 340 nm in aqueous solution and below approximately 310 nm in the solid state. In aqueous solution, photo-induced oxidation, which was liable to be remarkable with increasing pH value, and other photo-decomposition without participation of oxygen occured simultaneously. In the solid state, the photo-decomposition was inhibited by lowering the concentration of surrounding oxygen to below 0.1 % and cianidanol monohydrate was more stable to light than cianidanol tetrahydrate. Further,

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solid-state cianidanol exhibited remarkable coloration by exposure to light when mixed with magnesium alumina silicate and this coloration was inhibited by drying magnesium alumina silicate and removing water-soluble impurities contained in commercially available magnesium alumina silicate.

## INTRODUCTION

There are many pharmaceutical medicaments which undergo light-induced color change when exposed to natural or artificial light. However, little investigations dealing with effect of light on stability of medicaments in the solid state have been published compared to effects of temperature and humidity because photochemistry in the solid state is still not well established. Most of these investigations in pharmaceutical field have been related to color change of the surface of solid-state medicaments measured with color difference meter, and hence reports dealing with quantitative investigations of photolytic degradation in the solid state have scarcely been published. Recently, Matsuda *et al.* reported the investigations about kinetics of photolytic degradation of ubidecarenone[2)] by measuring residual ubidecarenone in irradiated solid-state sample by HPLC. In addition, one of authors reported the investigation about the effect of wavelength on the photolytic degradation of nifedipine3) under exposure to light by quantitative measurement of the residual percent of intact nifedipine in tablets.

Cianidanol is one of the flavans obtained from wood and leaves of Uncaria gambir, and its therapeutic effect on oral administration has recently been noted.4) We have investigated its stability in aqueous solution5) to obtain informations which must be grasped for the manufacture in liquid dosage forms. Similarly investigations on the influences of humidity and light on the stability of cianidanol in the solid state are very important for determination of optimum formulations or optimum package forms in solid dosage forms. Preliminary studies revealed that cianidanol was very stable to heat and humidity, but labile to light in the solid state as well as in an aqueous solution. In this study, we quantitatively investigated the influence of pH of aqueous solution and wavelength of light on photo-stability of cianidanol in an aqueous solution and then effects of wavelength of light and surrounding oxygen on the photo-stability of cianidanol in the solid state. In addition, the difference of photo-stability between two crystal forms of cianidanol and the influence of excipients mixed with cianidanol on photo-stability were investigated.

## EXPERIMENTAL

Materials —— Cianidanol was obtained from Zyma S. A. and recrystallized from water, then dried over phosphorus pentoxide. Corn starch (JP X), crystalline cellulose (JP X), lactose (JP X) and magnesium alumina silicate (Fuji Chemical Industry Co., Toyama, Japan; NEUSILIN ® $US_2$) were used as excipients. Other chemicals were of reagent grade quality. Deionized then distilled water was used in all experiments.

Photo-Stability Studies in Aqueous Solution —— Four hundred milligrams of cianidanol was dissolved in 200 ml of each buffer solution of pH 8.5, pH 8.0, pH 6.9, pH 5.9, pH 4.0 and pH 3.0. On the other hand, 400 mg of cianidanol was dissolved in 200 ml of each buffer solution previously flushed with nitrogen for 1 hour at 60 ml/min. Six milliliters of each solution was placed in a pyrex glass tube (100 mm x 13 mm i.d., thickness 0.7 mm), then sealed. Each pyrex glass tube was mounted on the turn table rotating at approximately 45 r.p.m. beside a light source to receive a same amount of light. High pressure mercury lamp (300 W) was used as a light source and placed 14 cm apart from sample. When the effect of wavelength was investigated, a series of color glass filters (Toshiba Kasei Kogyo Co., Tokyo, Japan; color glass filter UV-29, UV-31 and UV-35) were placed between the light source and the turn table at a distance of approximately 3 cm from the light source.

<u>Photo-Stability Studies of Cianidanol in the Solid State</u> —— Twenty-five microliters of cianidanol aqueous solution in concenration of 2 W/V % prepared at 60° and 25 $\mu$l of water were mixed in a hole of a slide galss (diameter of hole : 15 mm), then allowed to stand to give white crystals, which was converted to two crystal forms according to the procedure reported by Dermanci *et al.*6) as follows. The white crystals of cianidanol on a slide galss was air-dried and then stored for three days at 20° over $P_2O_5$ to afford cianidanol monohydrate. The sample of cianidanol monohydrate prepared in this manner was further stored for two days at 20° and 75 % R.H. to afford cianidanol tetrahydrate. The sample of solid-state cianidanol on a slide glass was mounted on the turn table and irradiated with high pressure mercury lamp in the same manner as in the photo-stability studies in aqueous solution.

When the effect of surrounding oxygen on the photo-stability of solid-state cianidanol was investigated, the sample on a slide glass was mounted into a pyrex glass tube (200 mm x 22.5 mm i.d., thickness 1.2 mm) where oxygen absorbent (Mitsubishi Gas Kagaku Co., Tokyo, Japan; AGELESS ® F-50) was admitted at the bottom. Then the pyrex glass tube was stoppered with a silicone rubber stopper. The concentration of oxygen in the pyrex tube was confirmed to be less than 0.1 % with oxygen detecting agent (Mitsubishi Gas Kagaku Co., Tokyo, Japan; AGELESSEYE®) which was admitted into the pyrex glass tube together with oxygen absorbent. Then the pyrex glass tube containing the sample of solid-state cianidanol was mounted on the turn table and irradiated with high pressure mercury lamp.

Measurement of Color Difference of Cianidanol-Excipient Tablet —— Powder of cianidanol monohydrate and tetrahydrate was prepared according to the procedure reported by Dermanci *et al*.6) Each powder (below 60 mesh) was mixed with twice an excipient by weight, and each mixture was directly compressed under 100 kg/cm$^2$ into 0.3 g flat faced tablet, 13 mm in diameter and approximately 3 mm thickness using a potassium bromide tablet die. When magnesium alumina silicate was used as excipient, it was treated previously in the following manner. A part of magnesium alumina silicate was dried under reduced pressure at 60° for 6 hours to make its water content to be 8.6 % (water content before drying was 16.0 %). Another part of magnesium alumina silicate was stored for two days at 20° and 75 % R.H. to make its water content to be 22.8 %. Furthermore, 5 g of magnesium alumina silicate was washed with seven 500 ml portions of water and then dried under reduced pressure at 60° for 6 hours to make its water content to be 8.6 %. Three kinds of magnesium alumina silicate thus prepared were mixed with a powder of cianidanol, respectively. One side of the tablets was irradiated with high pressure mercury lamp, then the surface color on the irradiated side was measured with color difference meter (Nippon Denshoku Co., Tokyo, Japan; Model ND-101) at appropriate intervals. Hunter's color difference formula employed to evaluate the degree of coloration is expressed by the following equation.

$$\Delta E\ (Lab) = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

where, $\Delta E$ (Lab) : color difference in the Lab system; $\Delta L$, $\Delta a$, $\Delta b$ : difference between two lightnesses, L, and those between chromaticity coordinates, a and b in the Lab systems.

<u>Assay Method of Intact Cianidanol by Gas-Liquid Chromatography</u> —— The concentration of intact cianidanol in the aqueous solution was assayed with gas-liquid chromatograph by the method proposed previously.5) The residual cianidanol in a solid-state sample on a slide glass was assayed by the same method as that of aquous solution after recovery of intact solid-state cianidanol with methanol.

<u>DTA and TG of Solid-State Cianidanol</u> —— Crystals of cianidanol monohydrate or tetrahydrate on a slide glass

prepared for photo-stability test were scrapped from a slide glass, then each 5 mg of the crystals was subjected to DTA and TG (Shimadzu Co., Kyoto, Japan; model DT-20B). No change in DTA and TG curves of the samples during photo-stability test was confirmed by comparison with those of the samples subjected to dark reaction. Measurement of the Absorption Spectra of Solid-State Cianidanol —— In order to measure the absorption spectra of solid-state cianidanol in the gas phase, a drop of 2 % aqueous solution of cianidanol prepared at 60° was placed on the quartz glass plate. The sample was allowed to stand for 2 hours for crystallization on it, then stored for 3 days at 20° over $P_2O_5$. The sample of cianidanol monohydrate was placed in the sample holder of the spectrophotometer (Shimadzu Co., Kyoto, Japan; model UV-210).

## RESULTS AND DISCUSSION

### Effect of Wavelength of Light on Photo-Stability of Cianidanol in Aqueous Solution

Cianidanol was irradiated with light of high pressure mercury lamp passing through color glass filter (UV-29 or UV-33 or UV-35) in an aqueous solution at pH 8.5. Then the concentration change of residual cianidanol with time was measured compared with that of light-intercepted sample. Compared to that of light-intercepted sample, a remarkable lowering and a slight lowering of the concentration of cianidanol were observed when irradiated with light passing through UV-29 and UV-33 color glass filter, respectively, as shown in Fig. 1. In an aqueous solution at pH 8.5, cianidanol was not stable without exposure to light because epimerization of cianidanol to epicatechin and base-catalized oxidation of them occured as described previously.5) In this case, it can be assumed that the reactivity of epicatechin for photo-decomposition is the same as that of cianidanol. Therefore, the concentration change of the sum of cianidanol and epicatechin against time was plotted in Fig. 1. On the other hand, the concentration change of cianidanol was identical with that of a light-intercepted sample when irradiated with light passing through UV-35 color glass filter, that is, any contributin of light to the degradation of cianidanol was not observed. From the light-transmission curve of UV-35 color glass filter shown in Fig. 2, the wavelength passing through UV-35 was found to be above approximately 340 nm. In preliminary experiments, benzenoid $\pi-\pi^*$ absorption band relating to phenol skelton of cianidanol was observed ($\lambda_{max}$ 280 nm) in an aqueous solution. From these results, the $\pi-\pi^*$ excited state of cianidanol generated by irradiation with UV-light between 340 nm and 250 nm

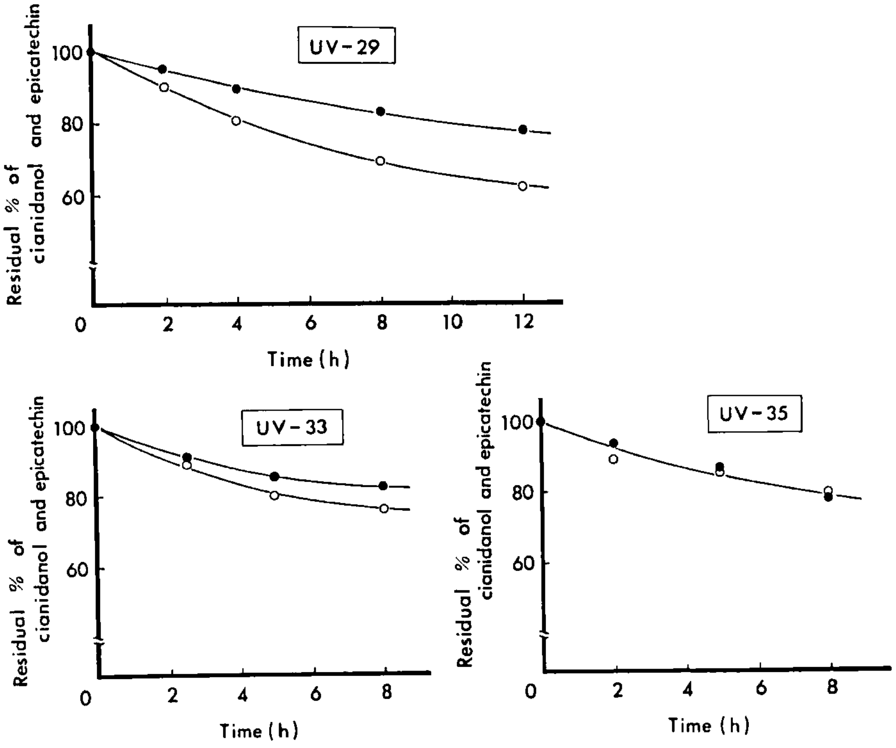

FIGURE 1

Photo-Stability of 0.2 % cianidanol Aqueious Solution at pH 8.5 under Irradiation through Various Color Glass Filters with High Pressure Mercury Lamp
-●-, light-intercepted sample;
-○-, irradiated sample.

which was lower limit of light-transmission of pyrex glass was assumed to participate in photolytic degradation of cianidanol in an aqueous solution.

## Effect of pH on Photo-Stability of Cianidanol in Aqueous Solution

Residual percent of cianidanol in aqueous solution at pH 3.0-8.0 against exposure time was plotted in Fig.

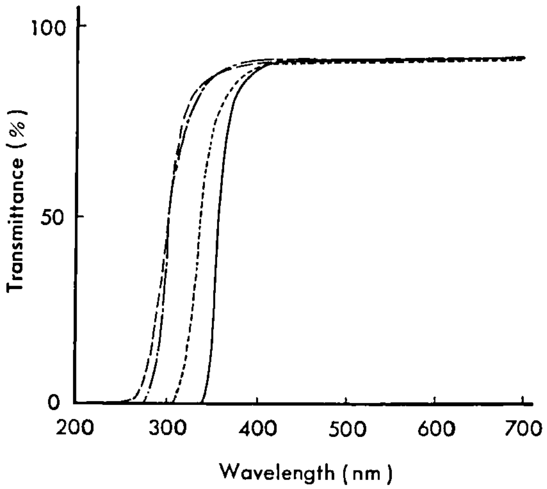

FIGURE 2

Light Transmission Curves of Various Color Glass Filters and Pyrex Glass
— — — —, UV-29; ---------, UV-33;
————, UV-35; —·—·—, pyrex glass.

3. Irradiated solutin exhibited lowering of the concentration of cianidanol at all pH while all light-intercepting samples were very stable at this pH range. On the other hand, the lowering of the concentration of cianidanol was also observed in aqueous solution free from oxygen at all pH as shown in Fig. 4. Consequently, the photolytic degradation without participation of oxygen other than photo-induced oxidation was supposed to occur simultaniously. The difference between the residual percents of cianidanol in Fig. 3 and Fig. 4 at the same exposure time over all pH solutions can be assumed to indicate the degradation by only photo-induced

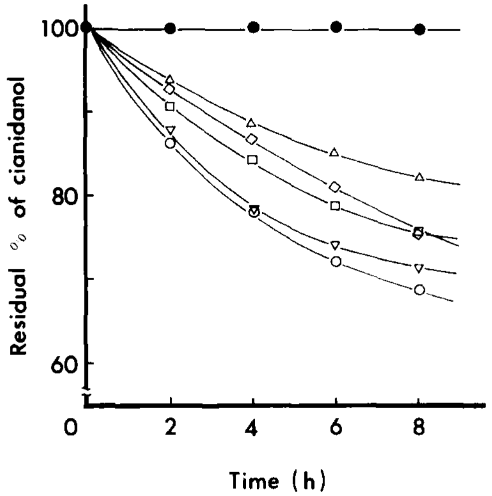

FIGURE 3

Effect of pH on the Photo-Stability of Cianidanol in Aqueous Solution under Irradiation with High Pressure Mercury Lamp
Initial concentration of cianidanol, 0.2 % W/V %.
-▽-, pH 3.0; -◇-, pH 4.0; -△-, pH 5.9;
-□-, pH 6.9; -○-, pH 8.0;
-●-, light-intercepted sample at each pH.

oxidation. From the results shown in Fig. 3 and Fig. 4, this difference was plotted against exposure time over all pH solutions as shown in Fig. 5. It was estimated from the results shown in Fig. 5, that the degradation by photo-induced oxidation was remarkable with increasing pH value of the solution.

### Effect of Wavelength of Light on Photo-Stability of Cianidanol in the Solid State

Cianidanol monohydrate and tetrahydrate on a slide glass were irradiated through a color glass filter with high pressure mercury lamp and residual percent of

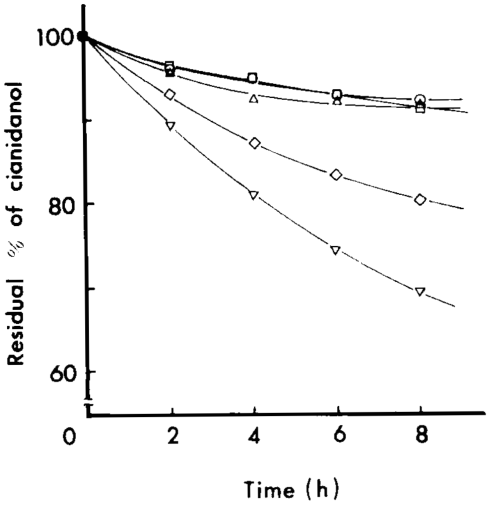

FIGURE 4

Effect of pH on the Photo-Stability of Cianidanol in Aqueous Solution Flushed with Nitrogen under Irradiation with High Pressure Mercury Lamp
Initianl concentration of cianidanol, 0.2 W/V %.
-▽-, pH 3.0; -◇-, pH 4.0; -△-, pH 5.9;
-□-, pH 6.9; -○-, pH 8.0.

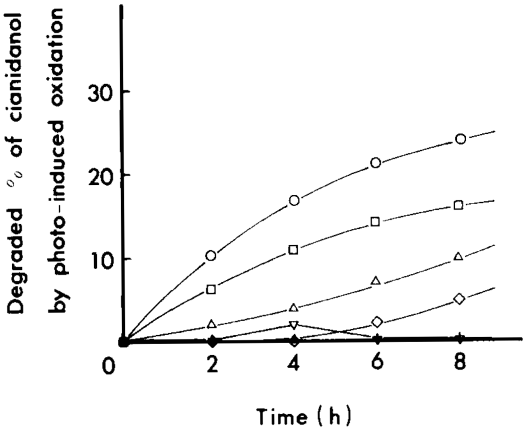

FIGURE 5

Effect of pH on the Percent of Cianidanol Degraded by Photo-Induced Oxidation under Irradiation with High Pressure Mercury Lamp.
-▽-, pH 3.0; -◇-, pH 4.0; -△-, pH 5.9;
-□-, pH 6.9; -○-, pH 8.0.

cianidanol on a slide glass was assayed at periodic time. As shown in Fig. 6, both crystal forms of cianidanol were very stable to the light passing through UV-33 color glass filter. On the other hand, both crystal forms were labile to the light passing through UV-29 color glass filter to exhibit lowering of the residual percent of cianidanol. From the light-transmission curves of UV-29 and UV-33 color glass filters shown in Fig. 2, the wavelength of light passing through UV-29 filter was found to be above approximately 240 nm and that through UV-33 filter was found to be above approximately 310 nm. As shown in Fig. 7, benzenoid $\pi-\pi^*$ absorption band relating to phenol skelton was observed ( $\lambda_{max}$ 280 nm) in the ultraviolet absorption spectra of cianidanol in the solid state. From these results, the $\pi-\pi^*$ excited state of cianidanol generated under irradiation of UV light below 310 nm was assumed to participate in photolytic degradation in the solid state.

## Effect of Surrounding Oxygen on Photo-Stability of Cianidanol in the Solid State

Cianidanol tetrahydrate on a slide glass mounted in a pyrex glass tube, in which the conentration of oxygen was made to be below 0.1 % was irradiated with high pressure mercury lamp. As shown in Fig. 8, cianidanol tatrahydrate was found to be very stable in

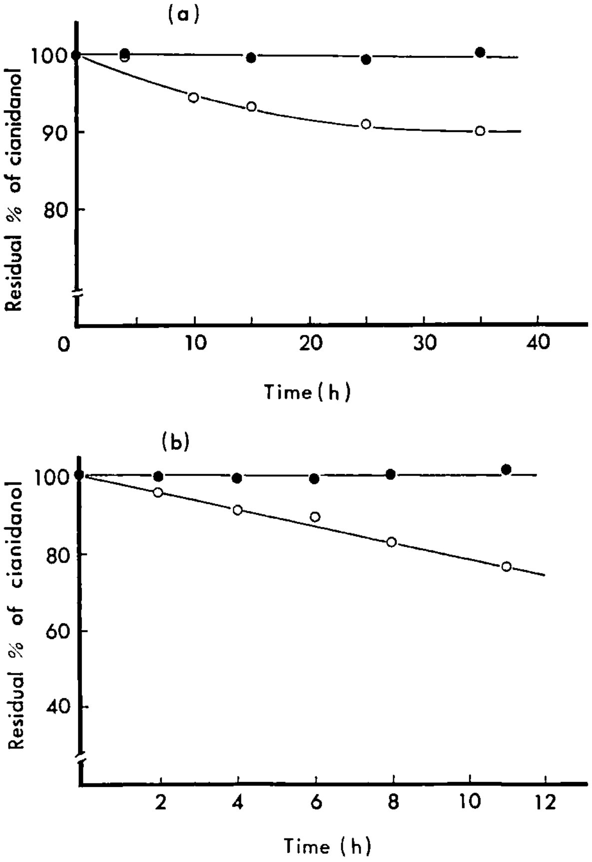

FIGURE 6

Photo-Stability of Cianidanol in the Solid State under Irradiation through UV-29 or UV-33 Color Glass Filter with High Pressure Mercury Lamp
(a) cianidanol monohydrate, (b) cianidanol tetrahydrate.
-○-, UV-29; -●-, UV-33.

an atomospher free from oxygen even if it was exposed to UV light. On the other hand, solid-state cianidanol in a pyrex glass tube without oxygen absorbent was degraded under irradiation of light. From these results, surrounding oxygen was assumed to participate in the photolytic degradation of cianidanol in the

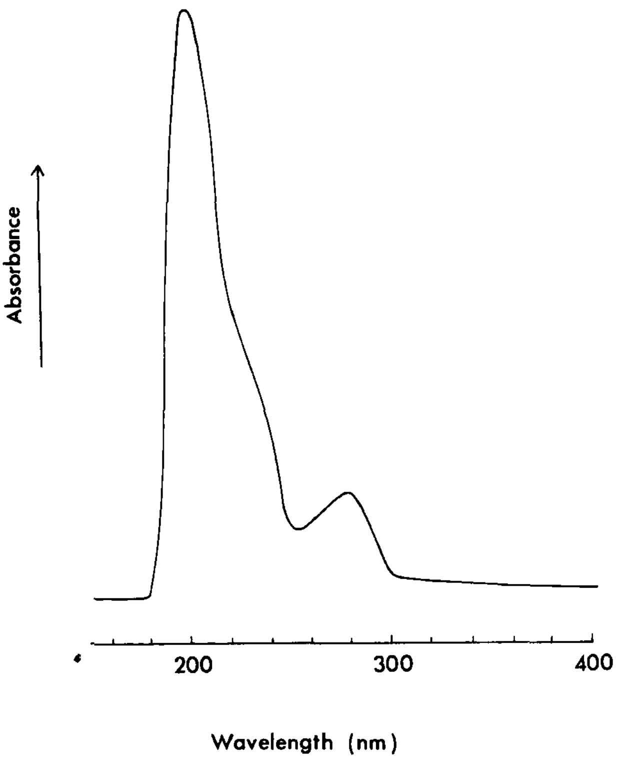

FIGURE 7

UV-Absorption Spectra of Cianidanol (Monohydrate) in the Solid State

solid state. It has been known that cianidanol was liable to be degraded by autoxidation in an aqueous solution without exposure to light.[7)] Consequently, these effects of surrounding oxygen and wavelength of light on photo-stability of cianidanol in the solid state were supposed to suggest that photolytic degradation of cianidanol in the solid state was photo-induced autoxidation via $\pi$-$\pi^*$ excited state of cianidanol.

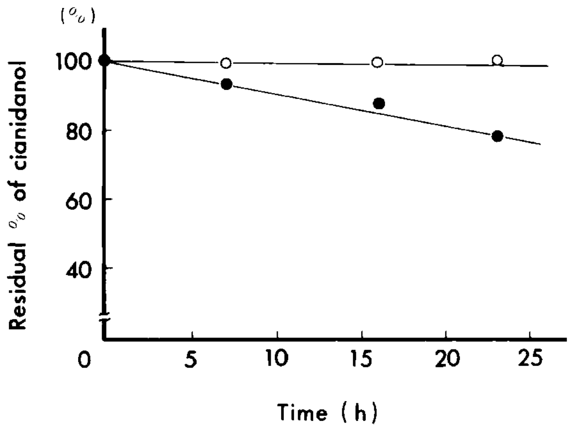

FIGURE 8

Photo-Stability of Solid-State Cianidanol (Tetrahydrate) Admitted into Pyrex Glass Tube with Oxygen Absorbent under Irradiation with High Pressure Mercury Lamp
-○-, with oxygen absorbent;
-●-, without oxygen absorbent.

## Qualitative Analysis of Photolytic Degradation Product of Cianidanol in the Solid State

The degraded sample was scrapped off after an irradiation of cianidanol tetrahydrate on a slide glass with high pressure mercury lamp for 24 hours, then approximately 15 mg of the collected sample was dissolved in 10 ml of water containing 10 mg of o-phenylenediamine. The mixture was refluxed for 1 hour to precipitate as an brown solid after cooling. On the other hand, approximately 15 mg of a light-intercepted sample was dissolved in a 10 ml of water containing 10 mg of o-phenylenediamine, then similarly refluxed. However, any precipitates were not observed. It has been known that 1,2-diketone and o-phenylenediamine undergo condensation to afford quinoxizaline which is generally water-insoluble.8) In this investigation, the isolation and the identification of a photolytic degradation product was not able to be carried out because of its low yield. However, the results obtained from the qualitative analysis can be assumed to suggest that the photolytic degradation product has o-quinone structure which is generated by oxidation of catechol moiety of cianidanol. Hathway et al.7) reported that cianidanol was oxidized in an aqueous solution to afford a polymer via o-quinone structure of cianidanol. Further studies on the chemical structure of the photolytic degradation product in the solid state are in progress.

## Photo-Stability of Cianidanol Monohydrate and Tetrahydrate

Cianidanol monohydrate and tetrahydrate on a slide glass were exposed to a same amount of light with high pressure mercury lamp. Consequently, cianidanol monohyrate found to be more stable to light than cianidanol tetrahydrate as shown in Fig. 9 and color change of monohyrate appeared to be less remarkable than that of tetrahydrate. From the DTA and TG curves of these crystal forms shown in Fig. 10, a mode of dehydration found to be very different between

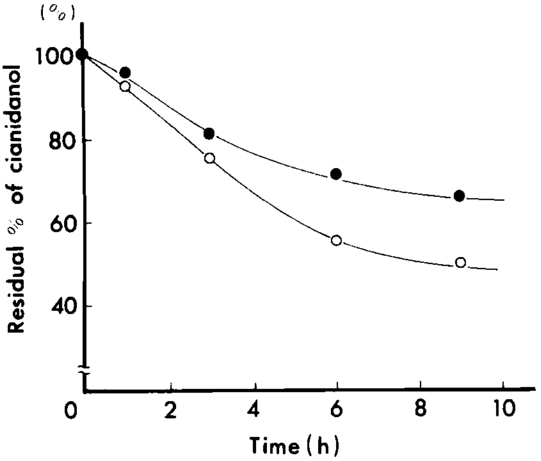

FIGURE 9

Photo-Stability of Cianidanol Monohydrate and Cianidanol Tetrahydrate in the Solid State under Irradiation with High Pressure Mercury Lamp
-●-, cianidanol monohydrate;
-○-, cianidanol tetrahydrate.

monohydrate and tetrahydrate. Namely, three moles of water of crystallization contained in tetrahydrate were eliminated below approximately 100° while water of crystallization contained in monohydrate was scarcely eliminated below 100°. It has been known about the photo-induced oxidation of phenols that phenoxy radical is initially generated from $\pi$-$\pi^*$ excited state of phenol <u>via</u> phenoxy radical cation which is stabilized by solvation with water.9) Therefore, a part of water of crystallization in cianidanol tatrahydrate which was easily eliminated at low temperature was supposed to participate in the stabilization of phenoxy radical cation of cianidanol to accelerate oxidation of

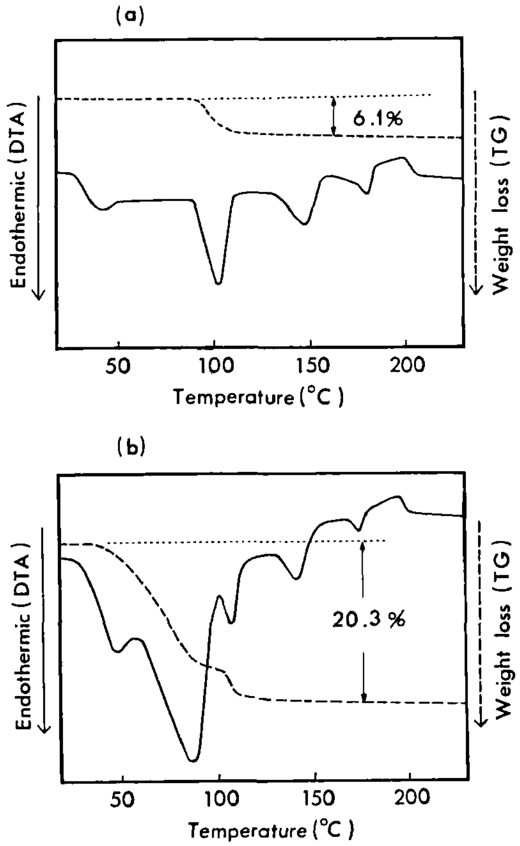

FIGURE 10

DTA and TG Curves of Cianidanol Monohydrate and Cianidanol Tetrahydrate
(a) cianidanol monohydrate, (b) cianidanol tetrahydrate.

cianidanol in the solid state. Further work is required to determine the actual reason for the difference of photo-stability between these ctystal forms. Including this investigation, further studies on preparation of a more photo-stable crystal forms which have no water of crystallization or have one eliminated at higher temperature than that of monohydrate are in progress.

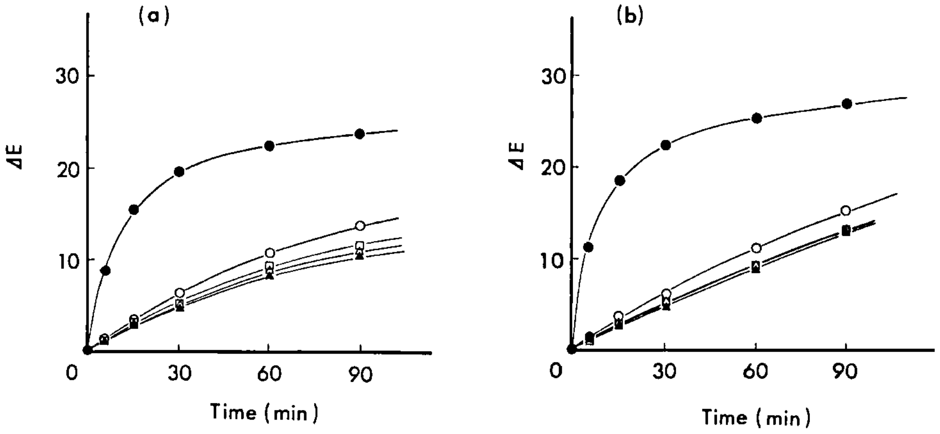

FIGURE 11

Degree of Coloration of Tablet Surface Containing Cianidanol and Excipient under Irradiation with High Pressure Mercury Lamp
(a) cianidanol monohydrate, (b) cianidanol tetrahydrate.
-○-, without excipient; -●-, Mg Al silicate;
-□-, corn starch; -△-, lactose;
-▲-, crystalline cellulose.
1 : 2 mixture of cianidanol and excipient was used for preparation of tablets.

## Photo-Stability of Solid-State Cianidanol in the Presence of Excipients

The 1 : 2 (W/W) mixture of cianidanol powder (tetrahydrate or monohydrate) and an excipient (corn starch, lactose, crystalline cellulose and magnesium alumina silicate) was compressed into a tablet followed by exposure to light with high pressure mercury lamp. Then the coloration of tablet surfaces was measured with color difference meter. As shown in Fig. 11, tablets containing magnesium alumina silicate as excipient exhibited remarkable coloration, namely

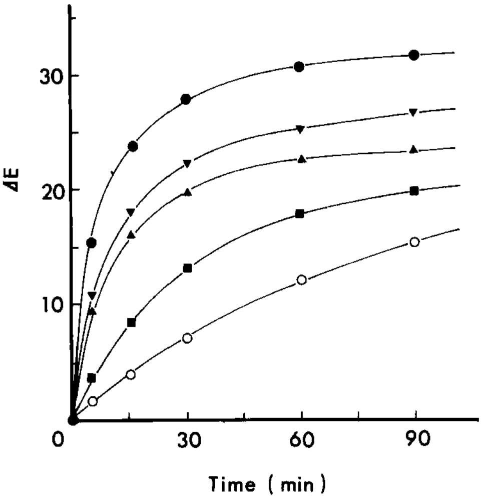

FIGURE 12

Effect of Water Contents in Mg Al Silicate on Coloration of Surface of Tablet Containing Cianidanol-Mg Al Silicate
water content
-●-, 22.8 %; -▼-, 16.0 %; -▲-, 8.6 %;
-■-, washed, then dried one (water content; 8.6 %).
-○-, without excipient.

high ΔE value, under exposure to light compared to tablets containing other excipients. In addition, this remarkable coloration was inhibited by lowering the water content of magnesium alumina silicate and washing it with water as shown in Fig. 12. This commercially available magnesium alumina silicate has been prepared from magnesium chloride, aluminum hydroxide and sodium silicate and hence a trace amount of metal ions such as $Mg^{2+}$ are estimated to be contained as impurities. When cianidanol was mixed with magnesium alumina silicate, these metal ions which was water-soluble were assumed to accelerate the photo-induced oxidation similarly to metal ion catalyzed oxidation of sulpyrine reported by Yoshioka et al.[10)] On the other hand, it was assumed to suggest from the results relating to the influence of water content of magnesium alumina silicate that photo-induced autoxidation of cianidanol was accelerated by stabilization of phenoxy radical cation of cianidanol with surrounding water as mentioned above.

## CONCLUSIONS

Cianidanol was exposed to light in aqueous solution and in the solid state for photo-stability studies, and the following conclusions were drawn

1) Cianidanol was found to be stable to light approximately above 340 nm in aqueous solution and in the solid state.
2) In aqueous solution, photo-induced oxidation and other photo-decomposition without participation of oxygen were found to occur simultaneously, and the former was liable to be remarkable with increasing pH value.
3) In the solid state, it was found that the photo-decomposition was inhibited by lowering the concentration of surrounding oxygen to below 0.1 % and cianidanol monohydrate was more stable to light than cianidanol tetrahydrate. Consequently,

photo-induced oxidation via $\pi-\pi^*$ excited state of cianidanol was suggested as main photolytic degradation reaction in the solid state.

4) When mixed with magnesium alumina silicate, solid-state cianidanol was found to exhibit remarkable coloration under exposure to light and the coloration was found to be inhibited by drying magnesium alumina silicate and removing water-soluble impurities contained in magnesium alumina silicate.

## ACKNOWLEDGEMENT

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