International Journal of Pharmaceutics, 93 (1993) 85–90

IJP 03026

Stabilization-oriented preformulation study of photolabile menatetrenone (vitamin K_2)

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(Received 10 May 1992)
(Modified version received 31 July 1992)
(Accepted 27 August 1992)

Key words: Menatetrenone; Photostability; Illuminance; Wavelength dependency; Prediction; Preformulation

Summary

The photostability of menatetrenone in rape seed oil as a solvent was investigated under irradiation with a fluorescent lamp to obtain basic information on encapsulation of the drug. The drug was susceptible to degradation by visible light below 480 nm and was most strongly degraded at around 430 nm, showing wavelength dependency. The degradation followed apparently first-order kinetics for a given initial concentration and exhibited a concentration dependency. The greater the initial concentration, the smaller was the degradation rate constant. Semilogarithmic plots of the apparent degradation rate constant against the reciprocal of illuminance demonstrated a linear relationship similar to that of Arrhenius-type behavior, and prediction of photostability under ordinary illumination conditions was suggested to be possible from the data obtained under conditions of accelerated illumination. Geometrical scale factors relating to the dosage form were also investigated, and it was found that round capsules were the most favorable shape from the viewpoint of stability.

Introduction

Menatetrenone (vitamin K_2; 2-methyl-3-all-*trans*-tetraprenyl-1,4-naphthoquinone) was originally developed in Japan and has been used as injections for the treatment of hypoprothrombinemia due to a lack of vitamin K in newborns. Because the drug is very photolabile, it is necessary to protect the commercial dosage form from light. The photostabilities of the drug in several kinds of injections or infusions have been investigated from the viewpoint of quality assurance (Yamaji et al., 1978, 1979; Yamano et al., 1978; Mori et al., 1979, 1980; Ishibashi et al., 1980). Recently, it has been reported that accidental deaths were caused by anaphylactic shock owing to a solubilizing agent added to the drug in injections, and therefore the development of oral dosage forms is desirable in place of such injections.

Soft gelatin capsules may be recommended as the most suitable dosage form, since the drug has a low melting point (34–38°C) and is fat-soluble. However, there exist neither extensive studies on the kinetic evaluation of the stability of photolabile drug in this dosage form nor a detailed

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examination of conditions that might affect the photodegradation kinetics.

The purposes of the present preformulation study were to obtain basic information to facilitate the photostabilization of encapsulated menatetrenone under irradiation with a fluorescent lamp and to investigate the possibility of predicting the degree of photostability through data obtained under conditions of accelerated irradiation. Consequently, an interpretation of the photodegradation mechanism is beyond the scope of the present investigation. A solution to this problem may indicate ways of improving photolabile solid dosage forms.

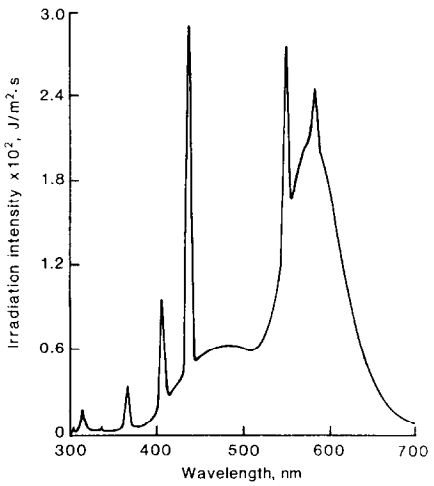

Fig. 1. Spectral irradiation intensity curve of the fluorescent lamp at an illuminance of 2000 lx.

Materials and Methods

Materials

Menatetrenone (lot no. KK6102CB, Nisshin Chemicals Co., Japan) and rape seed oil (Nacalai Tesque Co., Japan) as a solvent were purchased from the manufacturers. The commercial solvents for HPLC analysis and tocopherol acetate as an internal standard were used without further purification.

Methods

Accurately weighed menatetrenone was dissolved in rape seed oil and the solution was thoroughly stirred by a magnetic stirrer in a 200 ml flask to prepare eight kinds of solutions ranging in drug concentrations from 0.125 to 12 mg/ml. Unless otherwise specified, only one concentration (1 mg/ml) was used for irradiation tests. A fixed amount of each solution was placed in a plastic dish (35 mm i.d., 10 mm in depth) with a quartz glass cover so that a constant depth (7 mm) could be maintained.

Irradiation tests

To study the effect of specific wavelength of light on the photostability of menatetrenone, the same grating monochromator as described in our previous paper (Matsuda et al., 1989) was used within the wavelength range of 290–537 nm. Samples were exposed to light at each wavelength until the intensity level of the irradiation reached the same value (1.1×10^5 J/m^2). In the ordinary stability tests a white fluorescent lamp (type FL20SW, Toshiba Co., Japan) was used as a light source. The illuminance and irradiation intensity on the surface of the sample solution were measured with a portable spectroradiometer (model LI-1800, LI-COR Inc., NE, U.S.A.), and these parameters were varied by adjusting the distance between the light source and sample. Fig. 1 shows the spectral irradiation intensity curve of this lamp, as measured on the surface of sample solution under irradiation conditions.

To investigate the effects of volume and exposed area of solution on the photostability, sample solutions (0.5 mg/ml) were placed in cylindrical plastic bottles with flat-faced bottoms of 2.4, 3.3, 4.0, 4.8 and 5.4 cm in diameter up to depths of 0.4, 0.8, 1.2, and 3.2 cm. The outer walls of these bottles were covered with aluminum foil to shield the incidence of scattered light so that the solution could be exposed only to a parallel beam of incident irradiation with a lamp. Unless otherwise specified, the irradiation tests were carried out at 25°C. As for irradiation tests with the fluorescent lamp at different temperatures, plastic dishes with a transparent quartz glass cover, in which a constant amount of sample solution was placed, were fixed in a thermostated jacket main-

tained at 25, 35, 45 or 55°C and were exposed to light (2000 lx). Each sample solution was stirred with a magnetic stirrer to ensure homogeneity of photodegradation.

HPLC analysis

Residual menatetrenone after irradiation was determined by on a HPLC system (Waters Co.) equipped with a UV detection (270 nm); the prepacked column (Lichrospher Si60, 125 mm × 4.0 mm i.d., Merck) was operated at 27°C at a flow rate of 1.4 ml/min. The mobile phase consisted of a solvent system of *n*-hexane-ether (95:5). A chloroformic solution of tocopherol acetate (2 mg/ml) was used as an internal standard. After irradiation, an accurately weighed sample solution (approx. 30 mg) was mixed with chloroform in a flask, 40 μg of the internal standard solution was added, and the mixture was evaporated to dryness under vacuum. The residue was then dissolved in 1 ml of a mixed solvent of *n*-hexane-ether (4:1) and the concentration of unchanged menatetrenone was determined chromatographically by using 6 μl of this solution. The mean of values determined in triplicate was used. The calibration curve for menatetrenone showed good linearity ($r > 0.999$; $n = 5$), and the reproducibility of the data was invariably good. All procedures were carried out in the dark.

Results and Discussion

Effect of irradiation wavelength on photostability

For satisfactory stabilization of any photolabile drug in soft gelatin capsules it is essential to examine its photosensitivity to light over a wide range of wavelengths. Fig. 2 shows the effect of the wavelength of light on photodegradation in rape seed oil under irradiation of constant intensity (1.1×10^5 J/m^2). No degradation occurred at wavelengths above 480 nm. However, a significant decrease in the percentage remaining was observed below 480 nm, showing a minimum at around 430 nm followed by a further increase at lower wavelengths. This result suggests that, due to menatetrenone being so readily decomposed even by visible light, the drug must be stored carefully if a fluorescent lamp is used in a stock room.

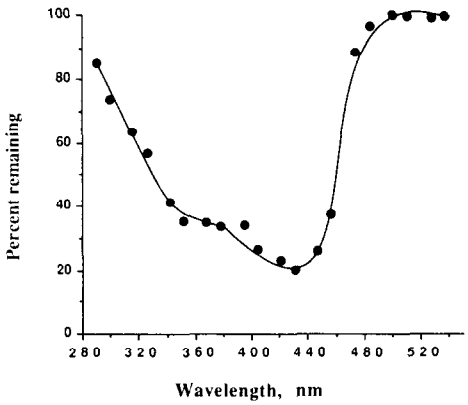

Fig. 2. Effect of wavelength on the photodegradation of menatetrenone after irradiation at a light intensity of 1.1×10^5 J/m^2.

Photostability under fluorescent lamp

Fig. 3 shows typical semilogarithmic plots of the percentage remaining of menatetrenone vs time at different initial concentrations under irradiation with a fluorescent lamp (2000 lx). The plots show good linearity ($r < -0.995$; $n = 8$) over a wide range of concentrations investigated, suggesting that the photodegradation followed apparently first-order kinetics for a given concentration. However, as was evident from the difference of slope of each degradation curve, a clear dependency of degradation rate on the initial con-

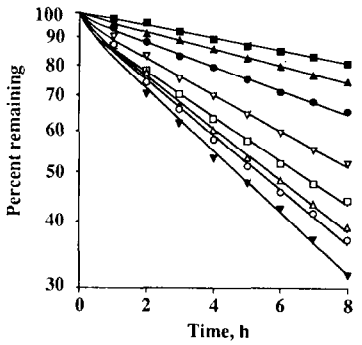

Fig. 3. Semilogarithmic plot for degradation of menatetrenone at different concentrations under irradiation by the fluorescent lamp. Concentration (mg/ml): (▼) 0.125, (○) 0.25; (△) 0.5, (□) 1.0, (▽) 2.0; (●) 4.9, (▲) 8.0 (■) 12.0.

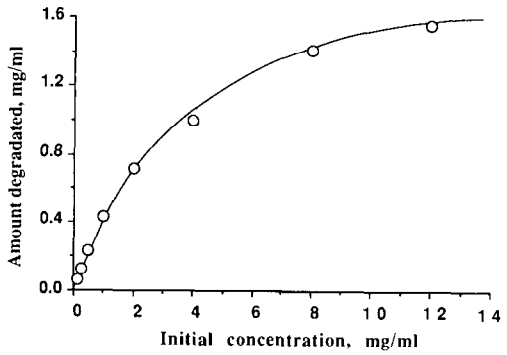

Fig. 4. Effect of initial concentration on the amount of menatetrenone degraded after cumulative irradiation at 10000 lx h.

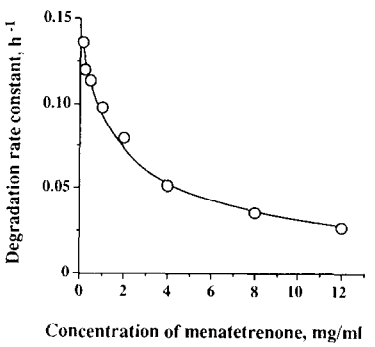

Fig. 5. Effect of concentration on the apparent degradation rate constant of menatetrenone irradiated with the fluorescent lamp.

centration, where photodegradation was apparently much more strongly inhibited with an increase in initial concentration, was observed in contrast to the general rule of first-order kinetics; the percentage remaining after 8 h irradiation was 81% at the highest concentration (12 mg/ml), whereas it decreased to 34% at the lowest concentration (0.125 mg/ml). Similar dependencies have been reported for photodegradation of Irgasan DP300 (Matsumoto et al., 1981) and vitamin A (Allwood and Plane, 1986). However, no quantitative interpretation, including our investigation, has been made since the apparent kinetics of those degradation processes changed with concentration of parent drug. The effect of concentration on photodegradation by constant irradiation energy (5 h irradiation, 10000 lx h) is illustrated in Fig. 4. At lower concentrations, the amount of menatetrenone undergoing degradation increased considerably with increase in concentration, however, at higher concentrations, the amount degraded levelled off and was likely to be constant irrespective of the concentration. From the results shown in Figs 3 and 4, the concentration-dependent degradation in this study might be due to the fact that although the total amount of photolytic energy emitted by the lamp remained unchanged, the energy absorbed by a molecule present in the solution decreased relative to an increase in the initial concentration.

The degradation rate constants calculated from the slopes of the regression lines in Fig. 3 are plotted in Fig. 5 vs initial concentration. The higher the initial concentration, the lower was the value of the degradation rate constant.

As mentioned in Materials and Methods, it is possible to carry out accelerated irradiation tests with the same light source by varying the distance between the light source and sample. In this case, the relationship between illuminance and irradiation intensity on the surface of a sample solution must be confirmed beforehand. Fig. 6 shows the effect of illuminance on the total irradiation intensity in the wavelength range (300–480 nm) which strongly affected the degradation. A clear proportional relationship was established between both parameters, suggesting that illuminance could be used as a measure of irradiation intensity. Semilogarithmic plots of the percentage remaining vs time at different illuminances resulted in linear traces resembling those in Fig. 3, their slopes becoming steeper with increasing illuminance. Thus the effect of illuminance on the

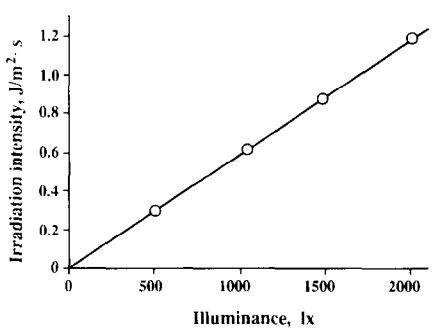

Fig. 6. Relationship between illuminance and irradiation intensity (300–480 nm) of the fluorescent lamp.

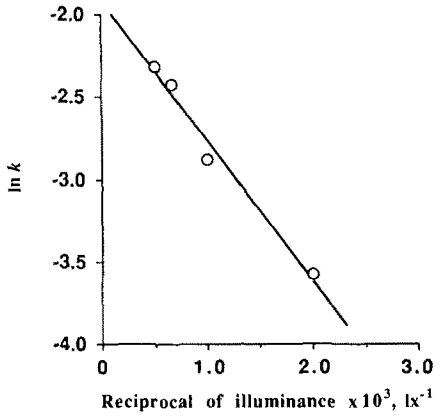

Fig. 7. Semilogarithmic plots of apparent degradation rate constant vs reciprocal of illuminance.

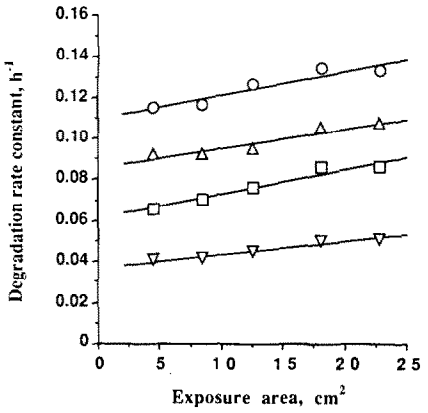

Fig. 8. Effects of exposure area and depth of sample solution on the apparent degradation rate constant under irradiation by the fluorescent lamp. Depth (cm): (○) 0.4, (△) 0.8, (□) 1.2, (▽) 3.2.

degradation rate constant is illustrated in Fig. 7. Semilogarithmic plots of the apparent degradation rate constant against the reciprocal of illuminance conformed closely to a linear relationship, as well as the results obtained by Mendenhall (1984) and Tokunaga et al. (1985), similar to that of Arrhenius-type behavior. This result suggests that photostability under ordinary illumination condition can readily be predicted from the data obtained under conditions of accelerated illumination. Although the physical meaning of the apparent activation energy for photodegradation calculated from the slope of the line is unclear from the results of these investigations, it should be useful as an important measure for evaluating the photosensitivity of a drug.

Table 1 summarizes the temperature dependency of the degradation rate constant determined under irradiation with a fluorescent lamp. A slight increase in degradation rate constant was found as the temperature rose. The value of the activation energy (2.46 kJ/mol) obtained from the Arrhenius plots was very small. This suggests that the activation energy was not correlated with the degradation kinetics, in contrast to the data of Matsumoto et al. (1981).

TABLE 1

Apparent first-order degradation rate constants at various temperatures obtained under the fluorescent lamp

Temperature (°C)	Degradation rate constant (h^{-1})
25	9.20×10^{-2}
35	10.0×10^{-2}
45	9.68×10^{-2}
55	10.3×10^{-2}

Effect of geometric scale factor of dosage form on the photostability

In designing soft gelatin capsules of menatetrenone, it is also important to investigate the scale effect of this dosage form on photostability. Therefore, the degradation rate constants obtained by varying both the inner diameter of the bottle and the depth of the sample solution at an illuminance of 2000 lx are summarized in Fig. 8. Although the degradation rate constant increased with either increasing exposure area or decreasing depth of solution it was more strongly affected by the depth of the solution than by the exposure area. If all light beams radiating from the fluorescent lamp are parallel, the degradation rate constant should be constant, irrespective of the exposure area. However, a significant effect of exposure area was observed, in contrast to expectations. This phenomenon might be due to scattered light straying into the sample solution, the contribution of which may be significant. In cases where the intensity of incident light was kept constant, the reason why the drug was apparently stabilized with increasing depth of solu-

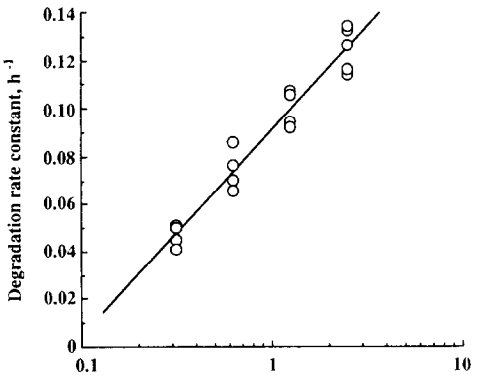

Fig. 9. Relationship between apparent degradation rate constant and exposure area per unit volume of sample solution.

tion was attributable to a lowering of the relative absorption efficiency of light energy per molecule with increasing number of drug molecules, in addition to the supply of light energy to the lower parts of the solution being insufficient.

It is possible to envisage various volumes of soft gelatin capsules by arbitrarily combining the data for both exposure area and depth of solution in Fig. 8. Fig. 9 shows the relationship between the degradation rate constant and exposure area per unit volume of sample solution on a semilogarithmic scale. Irrespective of the exposure area and depth of solution, a good linear relationship was established between both parameters, suggesting that the larger the exposure area per unit volume, the lower was the photostability. It is conceivable from this result that the photostabilizing design of encapsulated menatetrenone should be more satisfactorily achieved by using round capsules rather than oval, oblong, tube-shape forms, since the solid having the minimum surface area per unit volume is a sphere.

In conclusion, the dosage form design of photolabile drugs should carefully be carried out by considering the irradiation conditions and the effects of both geometric and formulation factors.

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