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A STUDY OF THE PHOTOSTABILITY OF ERGOCALCIFEROL (VITAMIN D_2) IN ORGANIC SOLVENTS

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ABSTRACT

The photolysis of ergocalciferol (vitamin D_2) in several organic solvents has been studied to determine the influence of solvent dielectric constant and viscosity on the rate of reaction. Ergocalciferol degrades by zero-order kinetics and the rate constants vary from $0.74x10^{-5}$ mol 1^{-1} (methanol) to $1.56x10^{-5}$ mol 1^{-1} (chloroform). The rates appear to be a linear function of the reciprocal of dielectric constant and viscosity of the medium in the range studied.

Introduction

Ergocalciferol (vitamin D_2) is an antirachitic vitamin (Haynes and Murad, 1985) and acts as a regulator of calcium homeostasis and phosphate metabolism (Dollery, 1991). It is sensitive to air, light and heat (Sebrell and Harris, 1971; Lintner, 1973; De-Ritter, 1982; Singh, 1985; Ball, 1992; B.P., 1993) and is rapidly decomposed at 25 and 40° under dry humid conditions (Grady and Thakker, 1980). Under mild acid conditions, the vitamin is isomerised to the inactive 5,6-trans and isotachysterol isomers (DeLuca, 1978). In solution ergocalciferol exhibits reversible thermal isomerisation to its previtamin (Mulder *et al.,* 1971) and is lost upto 38% in total parenteral nutrition (TPN) mixtures within twenty four hours (Allwood, 1984). Exposure of the vitamin to sunlight results in loss of the antirachitic activity (Vanderveen and Vanderveen, 1985). The object of this investigation is to study the influence of ultraviolet light on the stability of ergocalciferol in some organic solvents and to determine the effect of solvent dielectric constant and viscosity on the kinetics of degradation.

Materials And Methods

Ergocalciferol was obtained from Sigma Chemical Company and was found to be chromatographically pure, R_f 0.41 (cyclohexane-ether, 50:50, v/v) /silica gel GF254, activated [lit. (Bolliger and Konig, 1969) R_f 0.40). All solvents were analytical grade or of the purest form available from Merck/BDH and were redistilled before use.

Photolysis

1x10.^{4} M solutions of ergocalciferol (λ_{max} in ethanol, 265 nm, molar absorptivity 18840 M^{-1} cm^{-1}, Moffat, 1986; 20590 M^{-1} cm^{-1}, Sick, 1981) were prepared separately in methanol, ethanol, 2- propanol, 2-methyl propanol, 1-butanol, ethyl acetate, diethyl ether, dichloromethane and chloroform (100 ml). Each solution was placed in a 1 cm quartz cuvette, sealed at the top, and exposed to ultraviolet light using a Philips 30 W mercury discharge ultraviolet tube (88.7% emission at 254 nm and 50.5% emission at 265 nm; Ahmad, 1978) fixed at a distance of 25 cm. The temperature of the solution was maintained at 20 ± 1°C. Absorbance measurements on the solutions were made at suitable intervals with a Shimadzu UV-240 recording spectrophotometer using appropriate controls.

Light intensity measurement

The intensity of the 30W UV tube was determined by potassium ferrioxalate actinometry (Hatchard and Parker, 1956) as 6.42 ± 0.21 x 10^{16} quanta s^{-1}.

Assay

The assay of ergocalciferol in the photolysed solutions was carried out at 265 nm using 20590 M^{-1} cm^{-1} (Sick, 1981) as the value of molar absorptivity. The validity of Beer's Law in each solvent was confirmed at the analytical wavelength prior to the assay. The correlation coefficient values (r= 0.9995-0.9999) indicated a good linear relationship over the concentration range employed in the study. The solvents used do not show appreciable absorbance at the analytical wavelength (Fell, 1986) and hence do not interfere with the assay.

Results and Discussion

Kinetics of photolysis

The photolysis of ergocalciferol in organic solvents results in a loss of absorbance around 265 nm and follows zero-order kinetics. A typical set of absorption spectra measured at different time intervals during uv irradiation in methanol is shown in Fig 1. The concentration versus time plots for the reactions in some organic solvents are shown in Fig. 2 and the zero-order rate constants determined from the slopes of the straight lines are reported in Table 1. A variation in the rate constants with a change in the medium indicates that solvent tends to influence the rate of reaction and is an important factor in the photostability of the vitamin. Since zero-order photolysis reactions often result from oxidations (Carstensen, 1990), the photodegradation of ergocalciferol may

also be considered as an oxidation reaction and the products formed may be the oxidation products of the vitamin (Rosenberg, 1945).

Solvent effect

In order to determine the influence of solvent on the rate of photolysis of ergocalciferol, the zero-order rate constants (log k) were plotted as a function of the reciprocal of solvent dielectric constant (1/e) and a linear relationship with a positive slope was obtained (Fig. 3). This indicates that the reaction is influenced by the solvent polarity and an increase in the dielectric constant leads to a decrease in the rate of reaction. The activation energy of a reaction depends on the type of reaction and does not change rapidly from solvent to solvent. The entropy of activation is always negative and changes with the solvent, becoming more negative as the polarity of the solvent decreases. Reactions leading to the products more polar than the initial substances proceed, as rule, more rapidly in polar media. On the other hand, when the reaction products are less polar than the initial substances, a solvent of low polarity will accelerate the reaction as observed in the photolysis of ergocalciferol. Mathematical equations describing the relationship between log k and 1/ε have been obtained to confirm the above concepts (Martin *et al,* 1983; Laidler, 1987; Racz, 1989) and applied to the study of solvent effects on the reaction rates of pharmaceutical compounds (Garrett, 1967; Ahmad and Tollin, 1981; Ahmad *et al,* 1989; Connors *et al,* 1986; Fasihullah, 1988; Khan *et al,* 1990; Fatmi, 1994).

According to the Hughes-Ingold theory (1935) “an increase in the solvating power of the medium will accelerate the creation and concentration of charges and inhibit the[i]r destruction and diffusion". Thus a reaction in which the charge is on one atom in the reactant but spread over several in the transition state will go slower in a better ion-solvating or polar solvent (Hine, 1962). If the transition state is less polar than the initial state an increase in solvent polarity will increase A G (free energy) and decrease the rate (Connors *et al,* 1986). This may be true for the photolysis behaviour of ergocalciferol for which an increase in solvent polarity leads to a decrease in the rate of reaction and may, therefore, imply the existence of a non-polar or less polar transition state compared to the initial state. Such a transition state will be destabilized by an in-crease in solvent polarity and its rate of reaction will be decelerated. A reaction of this type, as in the present case, will proceed more rapidly with a decrease in solvent polarity as indicated by the rate - reciprocal dielectric constant relationship (Fig. 3).

In addition to altering the activity coefficients of the reactant molecules and the transition state, changes of the solvent system may bring about concomitant changes in surface tension and viscosity thus indirectly affecting the reaction rate (Fung, 1990). Solvent viscosity may influence the diffusion processes and hence the *rate* of reaction (Laidler, 1987). A consideration of the influence of solvent viscosity on the rate of an oxidation reaction may be

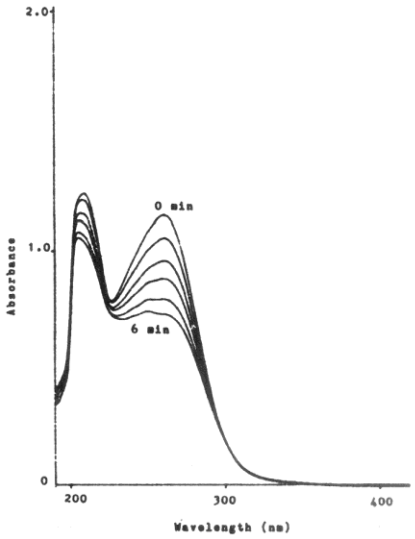
necessary as the viscosity of a liquid affects the rate at which molecules can diffuse through the solution This, in turn, affects the rate at which sensitive compounds can suffer oxidation at the liquid surface (Wallwork and Grant, 1977). A plot of k for the photolysis of ergocalciferol as a function of the reciprocal of solvent viscosity is shown in Fig. 4. The linearity of this relationship indicates the dependence of the rate of photolysis upon the solvent viscosity and may be attributed to the rate of diffusion of the reacting species. The rate increases with a decrease in viscosity as expected for diffusion controlled processes (Laidler, 1987). Thus the photolysis of ergocalciferol is influenced by both the solvent dielectric constant and viscosity. Since the vitamin is soluble only in organic solvents, extreme precaution may be necessary in handling the vitamin in these solvents to minimise the photodegradation reactions.

Table 1

Zero-order rate constants* for the photolysis of ergocalciferol in organic solvents

Solvent	Dielectric constant** ε (25°C)	Viscosity** cp (25°C)	k x 10^5 mol l^{-1} min^{-1}
Methanol	32.63	0.547	0.74
Ethanol	24.30	1.102	0.80
2-Propanol	18.30	2.134	0.84
2-Methyl propanol	17.70	–	0.94
1-Butanol	17.10	2.624	0.86
Ethyl acetate	6.02	0.441	0.85
Diethyl ether	4.33	0.222	1.06
Dichloromethane	9.08	0.425	1.00
Chloroform	4.80	0.542	1.56

*Estimated error is ± 5%.
**Weast (1980).



Photolysis of ergocalciferol in methanol.

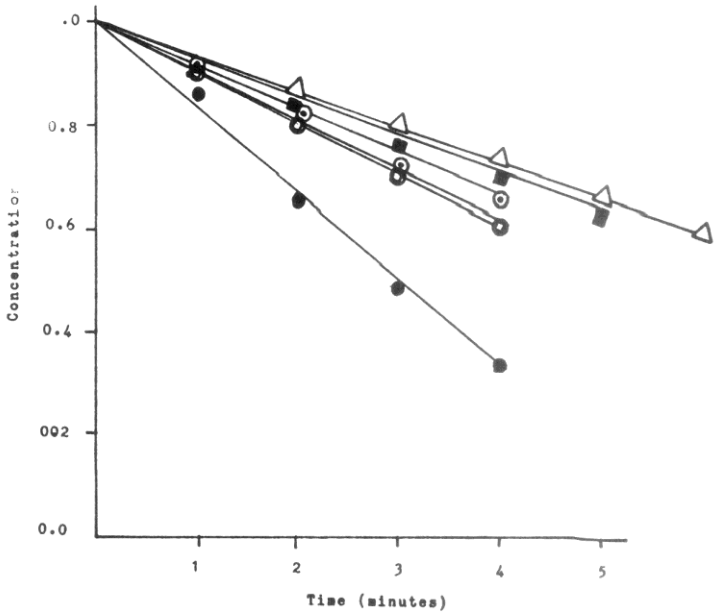


Figure 2: Zero-order plots for the photolysis of ergocalciferol: (A) ethyl acetate; (_) ethnol; (O) 1-buttanol; (O) 2-methyl propanol; (-) diethy1 ether, (O) chloroform.

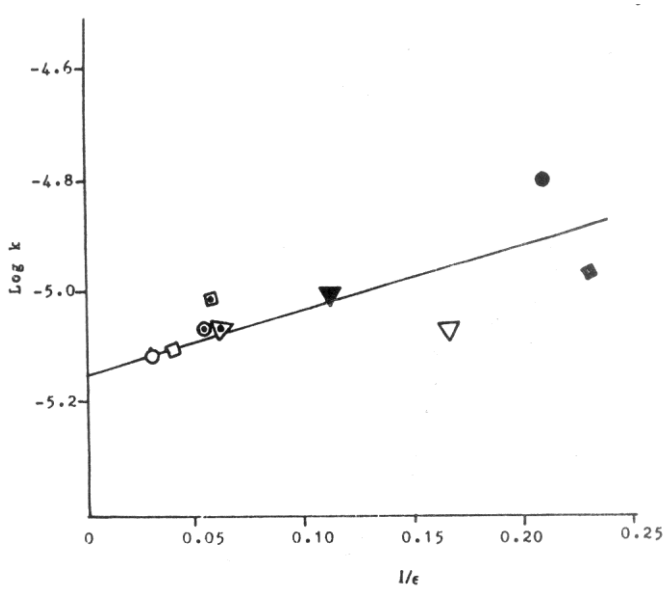


Figure 3. Dependence of the zero-order rate constants for the photolysis of ergocalciferol upon the solvent dielectric constant: (O) methanol; (_) ethanol; (O) 2-propanol; (A) 1- butanol; (-) 2-methyl propanol; (A) dichloromethane; (A) ethyl acetate; (-) diethyl ether; (O) chloroform.

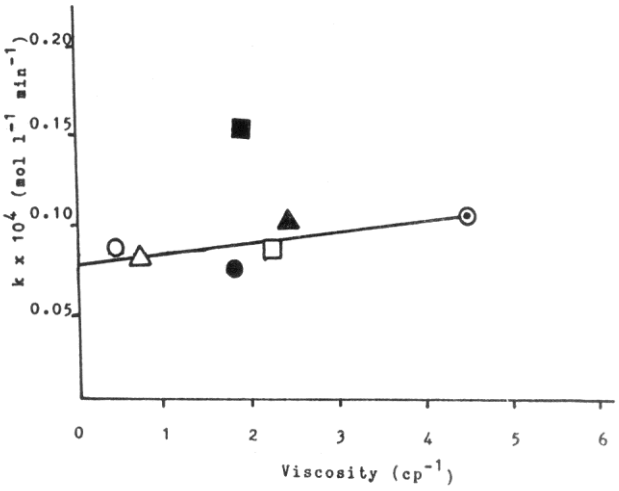


Figure 4. Dependence of zero-order rate constants for the photolysis of ergocalciferol upon the solvent viscosity: (O) 1- butanol; (Δ) ethanol; (O) methanol; (-) chloroform; (-) ethyl acetate; (Δ) dichloromethane; (O) diethyl ether.

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