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The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation

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Summary

Equipment was developed to study the effect of wavelength on vitamin A degradation. Rates of degradation varied with different wavelengths of incident radiation; maximum rates of photolysis were observed over the range 330–350 nm. Degradation was non-exponential, irrespective of wavelength, except at high concentrations of vitamin A. The quatity degraded/unit radiant exposure increased proportionally with concentration up to a threshold of approximately 40–50 I.U./ml, above which quantities degraded became constant with radiant exposure and were unaffected by further increase in concentration. The photolytic reaction did not require oxygen. It is suggested that the photo-degradation of retinol may proceed by two separate reactions, occurring at different rates. The nature of the chemical reaction is unknown. The equipment can be used to assess materials suitable for protecting parenteral infusions containing light sensitive compounds.

Introduction

Vitamin A is extremely light sensitive (Allwood, 1982; DeRitter, 1982). In a previous report it was shown how rapidly degradation occurs when solutions are exposed to ultraviolet light (Allwood and Plane, 1984). The relationship between radiant exposure and degradation rate was found to be non-exponential with evidence of a tailing effect. Experiments were conducted using a fluorescent tube emitting light over the spectral range 254–390 nm.

It is recognized that photochemical reactions depend on the intensity of the light and its wavelength (Lin and Lachman, 1969). In photolytic reactions, the function of the light is to provide activation energy in order to initiate a chemical reaction. The amount of energy taken up by a molecule relies on its ability to absorb light which in turn depends crucially on the wavelength of the radiation. Organic compounds absorb different quanta of energy depending on the wavelength of the light. Therefore the reaction rate will depend on the wavelength of the radiation as well as its intensity. Trissell (1983) has indicated, for example, the most active wavelengths causing photochemical reactions are in the range 290–320 nm.

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In order to study the effect of specific wavelengths of light on vitamin A photosensitivity, equipment was designed which would enable exposure of samples to high intensity monochromatic radiation so that the light energy and photolytic changes could be quantified. The effect of different wavelengths on vitamin A photolysis was measured in order to further elucidate the mechanisms involved. Such information can lead to optimizing methods to protect parenteral solutions from photolytic effects of daylight, by removing the most deleterious wavelengths, using suitable ultraviolet absorbing materials.

Materials and Methods

Vitamin A palmitate was a gift from Roche Products, Welwyn Garden City, Herts. The preparation contained 100,000 I.U./ml in a water miscible vehicle. All samples were prepared by dilution using glass-distilled water in amber glass containers. All analyses were completed within 3–4 hours of ultraviolet exposure.

A calibrated wide-band radiation monitor based on that described by Mountford and Pepper (1981) was used in this study in a similar way to that described by Allwood and Plane (1984). The monitor incorporated a gallium arsenide phosphide (GaAsP) photodiode. The measured sensitivity of the detector to 365 nm radiation was 25.4 ± 1.2 mV/mW/cm^2.

An identical photodiode was connected to a device which integrates ultraviolet irradiance based on a design by Pepper and Mountford (1983). The power supply design was modified to be mains operated. This photodiode was calibrated against the first photodiode using the lamp described below. The irradiance display on the integrator was adjusted using a feedback resistance to give the same voltage as that displayed by the pre-calibrated photodiode. The integrated ultraviolet dose at 365 nm was then measured over a preset time, typically 100 s. A thermopile (model 2M, Alrad Instruments, Newbury) was then connected to the integrator in place of the photodiode. The integrated ultraviolet dose at 365 nm over the preset exposure time was measured and recorded. Relative values of ultraviolet dose at different wavelengths were then measured with respect to the output at 365 nm.

The radiation source used in this study was a 500 W mercury discharge lamp (model Philips SP 500 W1, Rayner Optical Co., Brighton) fitted with a quartz window. An infrared-absorbing water filter comprised distilled water flowing at 4.8 l/min. Care was taken to ensure that cooling water was circulating and free of air bubbles before the lamp was switched on. The cooling water remained circulating for a period of 15 min after the lamp was switched off. These features enabled the working life of the lamp to be extended.

The radiation from the lamp was directed into the input of a monochromator (diffraction grating, Bausch and Lomb, Analytical Systems Division, Rochester, NY 14625). The input slit width was set at 1 cm. The range of wavelengths selected in this study was from 280 nm (UVB) to 400 nm (UVA). The output slit width was set at 1 cm. At this setting the bandwidth was $\pm$ 16 nm (Bausch and Lomb). A sample carriage holder was then placed at the output slit. The detector was then placed at the exit of the sample carriage holder. The sample was contained in a cylinder of borosilicate glass 4 cm long and 1 cm external diameter with each end window comprising 0.2 cm quartz. The sample carriage holder was designed to be centrally placed in the light beam. Two vents, mutually perpendicular, one at each end of the cylinder, allowed the sample that was to be irradiated to be introduced into the cylinder and later removed. In order to calculate the irradiance of the solution at the inner face of the front end window of the cylinder several factors were applied. First the irradiance was measured at the output of the exit slit of the monochromator and related to the irradiance measured at the output of the sample carriage holder. The difference was found to be 3.25%. Secondly the effect of 0.2 cm quartz was estimated to reduce the transmission of ultraviolet radiation by 9.7% (Chemical Rubber Co. Handbook, 1977). Thirdly the calibration factor for the diode and integrator was applied to the integrator reading. A typical measured irradiance value at 365 nm was 16.9 W/m^2. Thus a typical irradiation time for 1000 J/m^2 was 59.2 s.

TABLE 1

RELATIVE OUTPUT FROM MONOCHROMATOR AT DIFFERENT WAVELENGTHS

Wavelength (nm)	Relative output (%)	
280	10	
290	16	UVB
300	20	
310	26	
320	42	
330	56	
340	66	
350	82	
360	94	UVA
365	100 *	
370	104	
380	112	
390	118	
400	124	

* 100% corresponds to 16.9 W/m^2.

Table 1 shows the relative outputs in the sample holder against wavelength selected by the monochromator. 365 nm was chosen as a reference wavelength because this gave the maximum response of the detector (Pepper and Mountford, 1983) and is a major spectral line of mercury.

In order to measure the relationship between exposure and degradation the cell was completely filled to the inlet neck of the vent, placed in the carriage and immediately exposed to a predetermined dose. The whole contents were removed, a sample taken for analysis, the cell completely washed, flushed with vitamin A solution and then refilled for the next irradiation. Background and radiant exposure were determined before and after each batch of 5 consecutive samples.

Since temperature changes of the solution in the cell during irradiation could influence the kinetics of the photochemical reaction, temperature changes were monitored. A hypodermic thermocouple with digital readout (resolution 0.1°C, Digitron Instruments, Hertford, U.K.) was placed in the filled cell. No temperature increase occurred during a 5 min exposure to wavelengths below 370 nm. At 390 nm temperature rose slowly at the rate of approximately 0.1°C/min. During experiments the maximum exposure time employed was 4 min.

Vitamin A was analysed by HPLC (Allwood, 1982). Linearity of response (peak area) over the concentration ranges 0.25–10.0 and 25.0–100 I.U./ml was confirmed ($r > 0.999$; coefficients of variation 2.41% and 0.55%, respectively). Reproducibility of the assay for each experiment was checked by bracketting, standards being injected before and after each batch of five samples.

Results

Influence of wavelength on degradation of vitamin A

The effect of exposure of vitamin A solutions (10 I.U./ml) to various wavelengths over the range 280–400 nm is shown in Fig. 1. The wavelength of the incident light clearly has a substantial in-

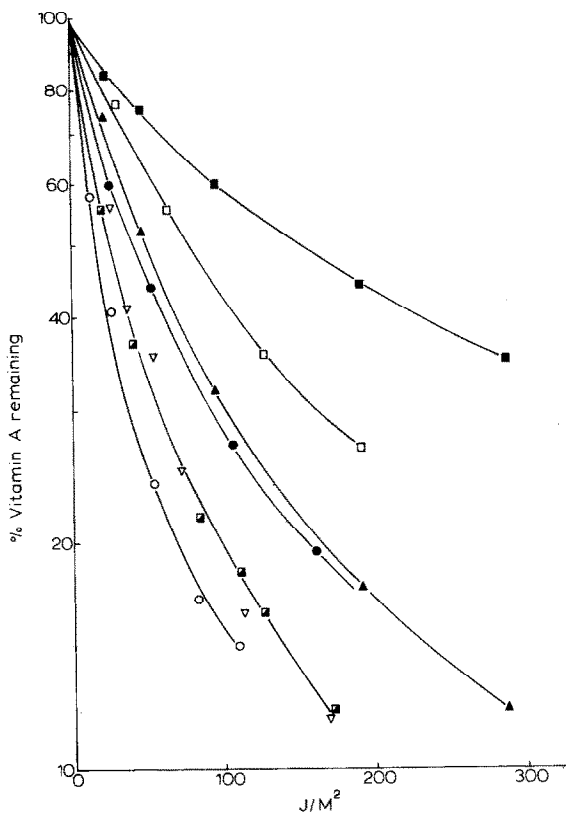

Fig. 1. The degradation of vitamin A (10 I.U./ml) during exposure to different wavelengths. ■, 280 nm; ▲, 300 nm; ▽ 320 nm; ○, 340 nm; ◪, 360 nm; ●, 380 nm; □, 400 nm.

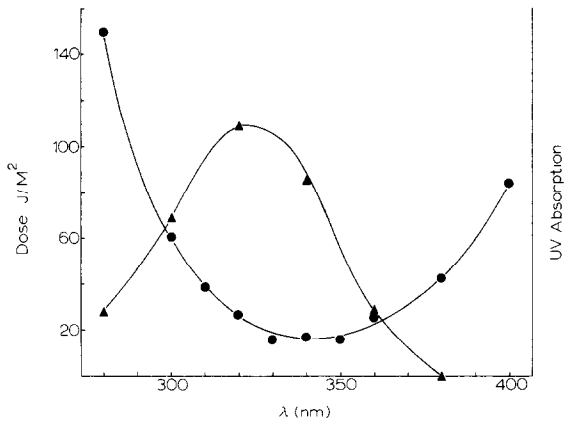

Fig. 2. The relationship between degradation, wavelength and UV absorbing properties of vitamin A. ●, radiant exposure (J/m^2) required to cause 50% degradation; ▲, relative UV absorption of vitamin A.

fluence on the degradation rate of vitamin A. It should also be noted that degradation kinetics are non-exponential, independent of wavelength, the rate of degradation falling as the radiant exposure increases. The relationship between rates of degradation and wavelength is illustrated in Fig. 2. Maximum rates of degradation occur between 330 and 350 nm. Degradation rates are lower at wavelengths above or below this narrow band. This may be compared with the light absorption properties of vitamin A shown in Fig. 2. The λ_{max} is 325 nm and the absorption profile cannot be directly superimposed on the degradation kinetics profile. This suggests that spectroscopic characteristics are not precisely proportional to light energy absorption causing molecular fragmentation. However, the difference between spectral absorption by the molecule and the spectral characteristics of the degradation profile are relatively slight.

Effect of vitamin A concentration on degradation

The degradation of different solutions of vitamin A at 320 nm is illustrated in Fig. 3. The rates of degradation are concentration dependent over the range studied, decreasing as the concentration is raised. It is also apparent that degradation approaches an exponential relationship with radiant exposure. In contrast, the greatest change in degradation rates with increasing radiant exposure occurs at lowest concentrations studied. The interaction between concentration and wavelength is illustrated in Fig. 4. This clearly shows that the influence of wavelength is independent of concentration. Finally, it is possible to calculate the quantity of vitamin A degraded by a constant radiant exposure, irrespective of concentration. This is shown in Fig. 5. At concentrations below approximately 40 I.U./ml, the amount of vitamin A degraded increases almost linearly with concentration. Above a threshold between 40 and 50 I.U./ml, the quantity degraded becomes almost constant, irrespective of concentration.

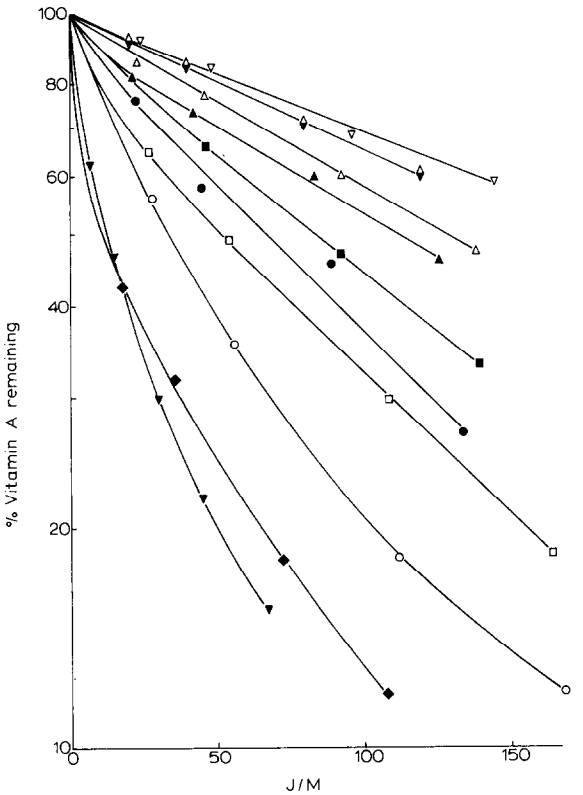

Fig. 3. The degradation of different concentrations of vitamin A during exposure to radiation at 320 nm. ▼, 1 I.U./ml; ◆, 5 I.U./ml; ○, 10 I.U./ml; □, 20 I.U./ml; ●, 30 I.U./ml; ■ 40 I.U./ml; ▲, 50 I.U./ml; △, 60 I.U./ml; ▼, 70 I.U./ml; ▽, 80 I.U./ml.

Influence of other factors on vitamin A degradation

Photolytic reactions often include oxygen as a reactant. The influence of dissolved oxygen was

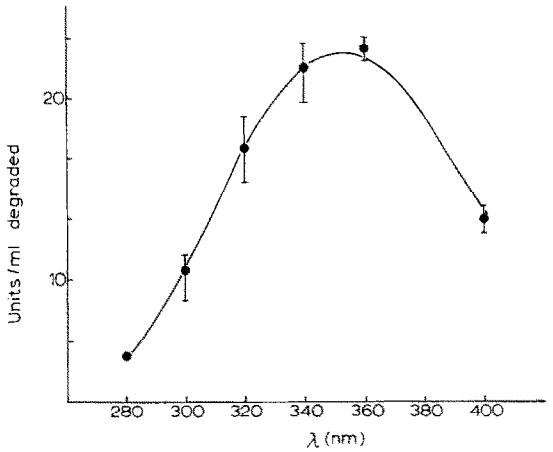

Fig. 4. The effect of wavelength on the photodegradation of vitamin A over the range 40–80 I.U./ml after exposure to a radiant exposure of 50 J/m^2. ●, mean value (bar lines represent S.D. values for each wavelength).

examined since in clinical practice this is not controlled. Solutions of vitamin A (10 I.U./ml) were prepared in closed containers and bubbled with nitrogen for 5 min. Samples were transferred to the cell, previously flushed with nitrogen using a syringe also nitrogen-flushed, and the ports closed using a sealant. After exposure, samples were taken for analysis. During each experiment, nitrogen-flushed and control solutions were alternated. In two experiments representing 20 samples in total, exposed over the range 5–50 J/m^2, at 320 nm the content of the nitrogen bubbled solutions was 103.3% (S.D. 3.4) of the control solutions. It can be concluded therefore that the absence of oxygen has an insignificant effect on vitamin A photolysis (Student's t: $P = 0.05$).

Exposure of any solution to a fixed radiation source may result in a layering effect in which solutions in the segment of the sample closest to the light source may undergo rapid degradation especially if significant proportions of the incident light energy is absorbed by the parent compound and degradation products of the reaction. In order to assess if constant agitation of the vitamin A solution in the cell influenced degradation kinetics, the inlet and outlet ports of the cell were connected by a narrow bore (2 mm diameter)

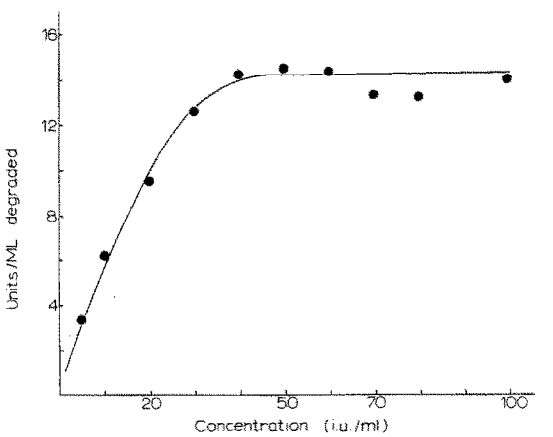

Fig. 5. The effect of concentration on the photodegradation of vitamin A after exposure to a radiant exposure of 50 J/m^2 at 320 nm.

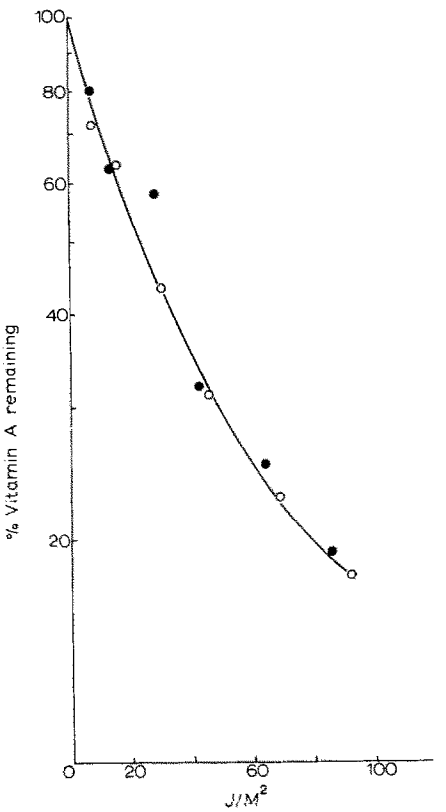

Fig. 6. The degradation of vitamin A during exposure to UV radiation at 320 nm in static (○) or continuously mixing (●) solutions.

rubber tubing, passing through a peristaltic pump. Storage of solutions of vitamin A in the rubber tubing confirmed that the compound was not absorbed by the material. The total volume of the tubing was 2 ml compared with that of the cell which was 1.8 ml. The system provided complete and continuous mixing of vitamin A solution, at a flow rate of 12 ml/min. Exposure to the light source occurred only while the solution passed through the cell. The exposure was proportional to the relative volume of the cell compared with the total volume of the solution in the cell and tubing. Degradation of vitamin A solution containing 10 I.U./ml exposed to 320 nm radiation was measured with the pump running and compared with a static solution in the cell. Results are shown in Fig. 6. This indicates that the mean degradation rate was unaffected by mixing, although results did show that inter-sample variation was greater in the mixed solutions compared with the static solutions.

Discussion

The apparatus described in this paper has been used to study photodegradation of vitamin A. Owing to the compound's sensitivity to UV light the photolytic energy emitted by the lamp was sufficient to allow the measurement of degradation over a wide range of wavelengths. Emitted light energy could be specifically quantified at a particular narrow wavelength band. Exposure periods of between 60 and 240 s were sufficient to achieve 80–90% degradation of vitamin A samples at wavelengths between 290 and 400 nm, except at high concentrations.

Results of the degradation studies indicated that the photochemical reaction kinetics were very dependent on wavelength. Maximum degradation rates were recorded between 330 and 350 nm. This is only slightly greater than the wavelength at which maximum light absorption by the parent compound occurs (325 nm). It is rather higher than the wavelengths considered by Trissell (1983) to be most photoreactive. The photochemical process involves the absorption of sufficient activation energy to enable a chemical reaction to proceed. The ability of UV light to provide appropriate radiant energy depends on the use of a radiation wavelength adequate to supply energy of activation for the chemical process (Lin and Lachman, 1969). Provided a large excess of absorbable light energy is available it would be anticipated that the reaction would be pseudo-first-order and loss of vitamin A would proceed exponentially with radiant exposure. However in a previous study (Allwood and Plane, 1984) it was shown that the reaction kinetics were not exponential with respect to radiant exposure, the rate of degradation falling with increasing radiant exposure. It is possible that photochemical reactions can proceed by more than one pathway. Therefore although the mechanism(s) of photodegradation of vitamin A is unknown, the kinetics data in this study suggest that a likely explanation for the non-exponential degradation is that it proceeds by two separate simultaneous reactions. One reaction may proceed at a slower rate than the other. It has also been observed that the apparent kinetics of vitamin A photolysis change with concentration of the parent compound. At low concentrations the quantity degraded per unit radiant exposure increases with concentration. Above a threshold concentration, the quantity degraded per unit radiant exposure is independent of concentration and becomes dependent on radiant exposure alone. It would appear that the solution becomes saturated with respect to absorbable photon energy delivered by the light source. Light entrapment achieves maximum efficiency at approximately 50 I.U./ml.

As previously stated the chemical changes responsible for photolytic degradation of vitamin A are unknown. It would appear, however, unlike many such reaction mechanisms, that free oxygen or oxygen-generated free radicals can be largely if not entirely excluded since removal of oxygen from the reaction had a negligible influence on reaction kinetics. The chemical reaction(s) depends on direct absorption of energy quanta by the retinol moiety.

From these results it can be concluded that adequate light protection of an infusion containing vitamin A can be achieved provided light within the major absorption range is excluded. At

the present time at least one such system is available (Kirk, personal communication). The wavelengths of light most deleterious to other compounds will depend on the characteristics of the molecule. From the present study it is clear that degradation varies substantially with the spectrum of light and its intensity at a particular wavelength. The equipment described provides a valuable tool to examine this process.

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