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Degradation of Retinyl Acetate in Simple Solvent Systems

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ABSTRACT

The degradation of vitamin A acetate was studied by u.v. spectrophotometry in three solvent systems: absolute ethanol, ethanol:water (95:5) and hexanes. Three potential degradative factors were investigated: exposure to light, presence of air and temperature. In all systems studied, exposure to light was the major factor. In the ethanol systems, the rate constants calculated for the degradation in the presence or in the absence of oxygen are not significantly different. Analysis of the data by first order kinetic models yielded the best fit in all cases. The activation energy for the degradation reaction in absolute ethanol was found to be 56 ± 8 kJ/mol. The presence of water in ethanol did accelerate the reaction, but the dependence of the rate on the water content is not a simple one.

INTRODUCTION

Fortification of foods with respect to their vitamin A content is usually accomplished using retinyl esters as acetate and palmitate (Bauernfiend & Cort, 1975), since they are believed to be more stable than retinol itself (Bondi & Sklan, 1984). Over the years a number of studies dealing with the stability of retinol and its esters have been published (for example, see reviews by Morton, 1970; Wolf, 1981 and Isler & Kienzle, 1984). It has been reported that these compounds are sensitive to heat (Hopkins, 1920; Finkel'shtein *et al.*, 1970; Wilkinson *et al.*, 1981), air (Mattill, 1927;

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Bolomey, 1947; Budowski & Bondi, 1960; Kozlov *et al.*, 1969; Evteeva & Gagarina, 1983) and light (Chevalier & Dubouloz, 1936; Smith *et al.*, 1939; Mousseron-Canet *et al.*, 1966; Sattar *et al.*, 1977; de Man, 1981), as well as to the presence of catalysts and pro-oxidants (Powick, 1925; Marcus, 1931; Smith, 1939; Holman, 1950; Henbest *et al.*, 1957; Budowski & Bondi, 1960; Blain & Shearer, 1963; Ogata *et al.*, 1970), and also to the nature of the solvent medium used (Dann, 1932; Chilcote *et al.*, 1949; Forlano & Harris, 1960*a*, *b*; Dobrucki, 1976). More specifically, the kinetics of degradation of vitamin A compounds have been investigated in a number of solvents and/or matrixes and reports have been conflicting: for example, both zero (Garrett, 1956; Sattar *et al.*, 1977) and first (Higuchi & Reinstein, 1959) order kinetics have been suggested, along with autocatalytic chain reactions showing an induction period (Budowski & Bondi, 1960; Kozlov *et al.*, 1969; Gagarina *et al.*, 1983; Suyama *et al.*, 1983), etc.

In an attempt to identify more clearly some of the factors responsible for the degradation of retinyl acetate, a kinetic study was undertaken, testing four factors: exposure to light, presence of oxygen, temperature and nature or composition of the solvent.

MATERIALS AND METHODS

Reagents and equipment

Retinol (type X) and retinyl acetate, type I, were obtained from Sigma Chemicals, St. Louis, Missouri, and were used without further purification.

Both absolute and 95% ethanol were purchased from Commercial Alcohols, St. Laurent, Québec; they were filtered before use. Hexanes, reagent, were from Caledon Laboratories, Georgetown, Ontario.

Nitrogen (O_2-free) and oxygen (extra-dry) were from Union Carbide Canada, Montréal, Québec.

Low actinic glassware (Pyrex) had the following specifications, according to the manufacturer (Corning Glass Works, Corning, NY): transmittance of 0%, 1% and 4% at wavelengths of 300, 400 and 500 nm, respectively. Clear glassware (Pyrex, 2 mm thickness) by comparison, has transmittances of 70%, 80% and 92% at wavelengths of 320, 330 nm and for visible light, respectively. U.v.–visible spectra were recorded on a double beam spectrophotometer, Perkin-Elmer model Lambda 3 (Perkin-Elmer, Norwalk, Connecticut).

Light intensities were measured with a Weston Illumination Light meter, model 756 (Weston Instruments, New Jersey).

Experimental method

All experiments were run in duplicate.

In order to assess the relative importance of the four individual factors tested (exposure to light, presence of oxygen, temperature and nature of the solvent), four series of experiments were initially set up as follows.

Series 1: exposure to light

Retinyl acetate was dissolved in absolute ethanol and the solutions were kept in glass-stoppered flasks, at ambient laboratory temperature, which ranged from 15 to 28 °C (average 22–23 °C) over the time of the experiments. One duplicate set was kept in low actinic glass-stoppered flask while the other set, in clear glassware, was exposed to the laboratory fluorescent lights. The lights were kept continuously on and the flasks were kept in the same positions throughout, in order to ensure a constant light exposure (intensity: 650 lux, for Series 1 to 4). Samples were withdrawn at various time intervals, and their u.v.–visible spectra were recorded.

Series 2: presence of oxygen and composition of the solvent

Solutions of retinyl acetate were made up in 95 % ethanol and kept at ambient temperature, under the same conditions of light exposure as in Series 1. One set of solutions was made up using air-saturated 95 % ethanol (air was bubbled through the solvent for 20 min prior to making the solutions, and for 10 min after it was made). Another set used O_2-free 95 % ethanol (nitrogen was bubbled through the solvent and solution, in the same manner as for the air-saturated solutions). In this latter case, a nitrogen headspace was kept above the solutions at all times during the experiments. A control set of solutions was made up with O_2-free 95 % ethanol and kept in low actinic glassware.

In addition to testing for the effect of the presence of oxygen, the air-saturated set of this series is used for comparison with Series 1 in terms of solvent composition, i.e. water content of ethanol.

Series 3: temperature

Solutions of retinyl acetate were made up using either O_2-free absolute ethanol or O_2-saturated absolute ethanol (oxygen was bubbled through, as in Series 1). These solutions were kept under an inert atmosphere (N_2) and under an oxygen atmosphere, respectively, for the duration of the experiment.

Experiments were run at 30 °, 40 ° and 50 °C; the solutions were kept at constant temperature in a water bath linked to a Thermocirculator (Perkin-Elmer). Although this series was performed under the same lighting conditions, the fact that the flasks were immersed in water definitely modified the light intensity, and possibly the composition of the light reaching the solutions.

Activation energies were calculated using Arrhenius' equation.

Series 4: nature of the solvent

Solutions of retinyl acetate were made up in air-saturated hexanes, which is a mixture of hexane isomers, mainly *n*-hexane and methylcyclopentane. They were kept in glass-stoppered flasks at ambient laboratory temperature, and under the same light exposure as for Series 1 and 2. A set of control solutions was kept in low actinic glassware.

Kinetic method

Reactions were followed by monitoring the absorbance (A) of the main band showing a maximum at 325 nm; this band is attributed to the all-*trans*-retinyl acetate (Planta *et al.*, 1962). The degradation of all-*trans*-retinyl acetate leads to a decrease of the band intensity at 325 nm, and to the appearance of new absorption maxima at other wavelengths, as shown in Fig. 1.

Kinetic data were analyzed by the various integrated rate laws, and the equation yielding the best fit was used to both assign the order of the reaction and to calculate the rate constant: in all cases studied, the first order integrated rate equation yielded the best fit.

Depending on the solvent composition, two different first order rate laws had to be used. This is because reactions performed in either absolute or 95 % ethanol showed a non-zero value of absorbance at 325 nm at the end of the reaction, indicating an equilibrated reaction, i.e. one in which a portion of the initial reactant is still present at infinite time. On the other hand, reactions done in hexanes did go to completion in terms of the

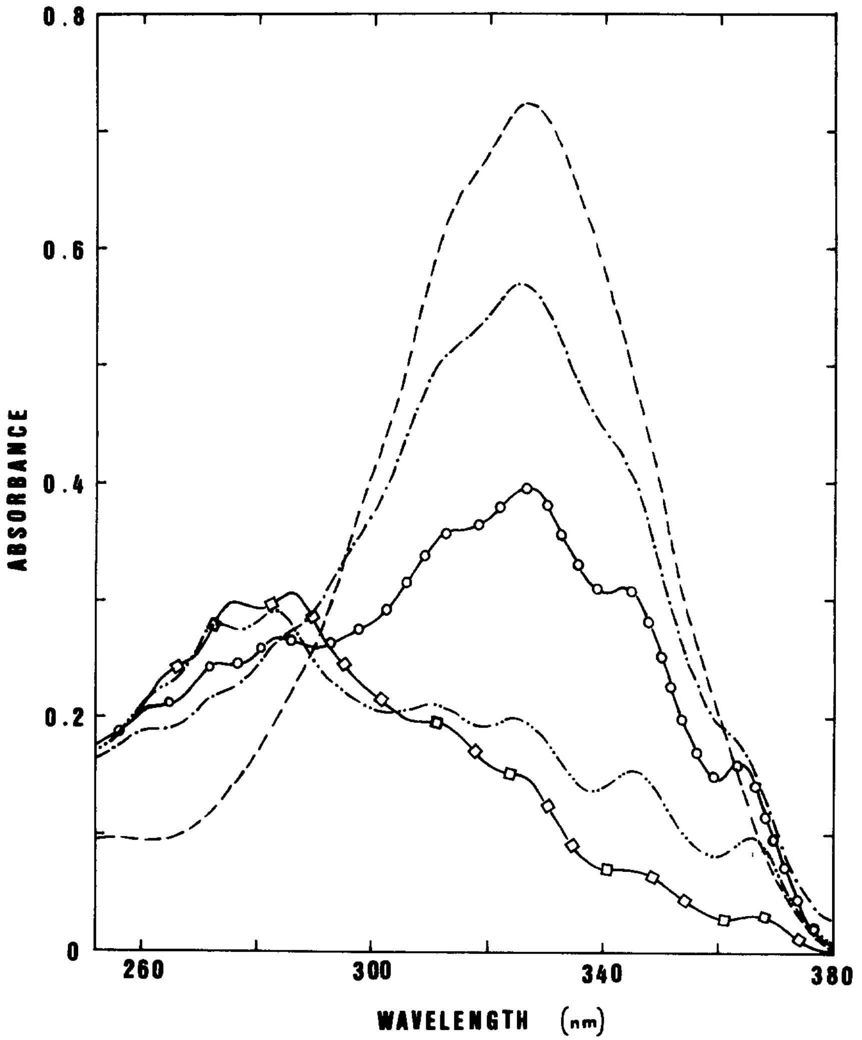

Fig. 1. Spectral changes of retinyl acetate in clear glassware; absolute ethanol, light intensity: 650 lux. Time 0 h, ------; 405 h, —●—●—; 842 h, —○—○—; 1700 h, —●●—●●—; 2372 h, —□—□—.

disappearance of the initial reactant; they were thus analyzed by the appropriate model.

Kinetic models

Equilibrated reaction

The reaction may be summarized by the following equation:

$$\text{All-}\textit{trans}\text{-retinyl acetate} \underset{k_{-1}}{\overset{k}{\rightleftharpoons}} \text{Products} \tag{1}$$

Defining x as the concentration of products formed and a, $a - x$ and $a - x_e$ as the concentrations of all-*trans*-retinyl acetate at times $t = 0$, $t = t$ and $t =$ infinity, respectively, the first order reaction may be described as follows

$$\mathrm{d}[\mathit{Products}]/\mathrm{d}t = k_1(a - x) - k_{-1}(x) \tag{2}$$

At equilibrium, i.e. after infinite time, it may be stated that

$$k_1(a - x_e) - k_{-1}(x_e) = 0 \tag{3}$$

By using the integrated rate law for equilibrated reaction, one obtains

$$\ln [(x_e - x)/x_e] = -(k_1 + k_{-1})t \tag{4}$$

In order to use the absorbance values obtained experimentally, one must transform eqn (4) accordingly. Thus if it is stated that A_0, A_t and A_{infinite} represent the absorbances recorded at times $t = 0$, $t = t$ and $t =$ infinity, respectively, then it may be stated that

$$A_0 = e_1 aL \tag{5}$$

$$A_t = e_1(a - x)L \tag{6}$$

$$A_{\text{infinite}} = e_1(a - x_e)L \tag{7}$$

where e_1 is the absorptivity of reactant at 325 nm, and L is the cell pathlength.

After substitution into the proper equation and rearrangements, eqn (4) becomes

$$\ln [(A_t - A_{\text{infinite}})/(A_0 - A_{\text{infinite}})] = -(k_1 + k_{-1})t \tag{8}$$

or

$$\ln (A_t - A_{\text{infinite}}) = -(k_1 + k_{-1})t + \ln (A_0 - A_{\text{infinite}}) \tag{9}$$

Thus a plot of $\ln(A_t - A_{\text{infinite}})$ vs. time yields $-(k_1 + k_{-1})$ as the slope. It is to be noted that the value obtained is not the sole value of k_1 but the sum of $k_1 + k_{-1}$; it will be seen later on that k_1 may still be evaluated individually in the system studied.

Reaction going to completion

The reaction may be summarized by the equation:

$$\text{Reactant} \xrightarrow{k_2} \text{Products} \tag{10}$$

Defining a, $a - x$ and 0 as the concentration of reactant at times $t = 0$, $t = t$ and $t =$ infinity, respectively, and x as the concentration of products at time $t = t$, it may be written that

$$\mathrm{d}[Products]/\mathrm{d}t = k_2(a - x) \tag{11}$$

The integrated rate law is

$$\ln[a/(a - x)] = k_2 t \tag{12}$$

Using A_0, A_t as previously described (eqns 5 and 6), and $A_{\text{infinite}} = 0$, eqn (12) rearranges into

$$\ln[A_0/A_t] = k_2 t \tag{13}$$

or

$$\ln(A_t) = -k_2 t + \text{constant} \tag{14}$$

Thus a plot of $\ln(A_t)$ vs. t yields $-k_2$ as the slope.

RESULTS AND DISCUSSION

Series 1: Effect of exposure to light (650 lux) at ambient temperature (average 23 °C) in absolute ethanol

Figure 1 shows the spectral changes occurring during the reaction time. Figure 2 shows the change of absorbance at 325 nm with time for both the reaction run in clear glassware and for the control run in low actinic glassware. It indicates that the reaction is equilibrated, i.e. the value of A_{325} does not reach zero at the end of the reaction. It also clearly indicates that exposure to light causes a very important acceleration of the degradation reaction, since all other parameters are identical in the two runs (concentration, presence of air, temperature and solvent composition).

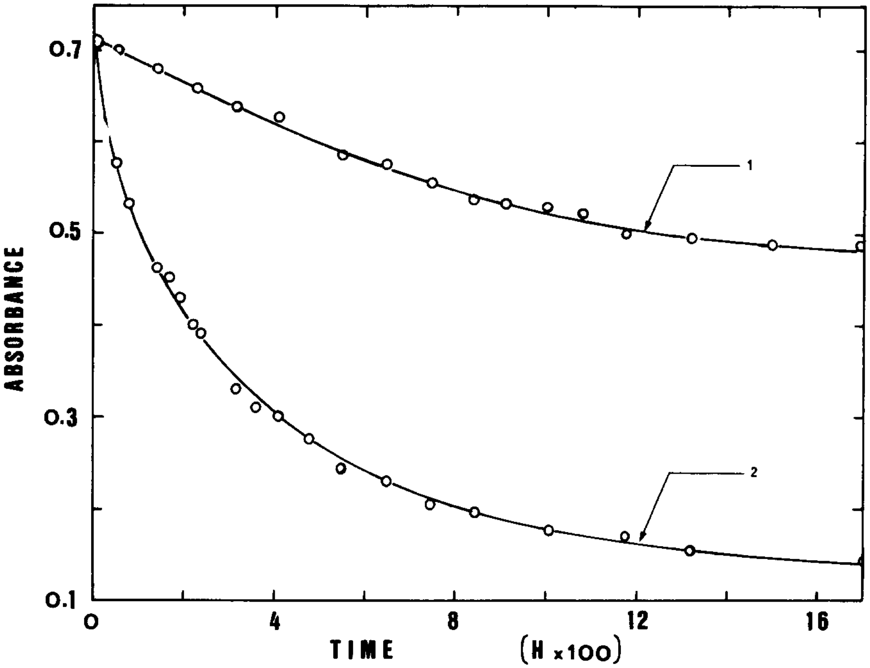

Fig. 2. Changes in A_{325} with time for retinyl acetate in absolute ethanol; (1) low actinic and (2) clear glassware.

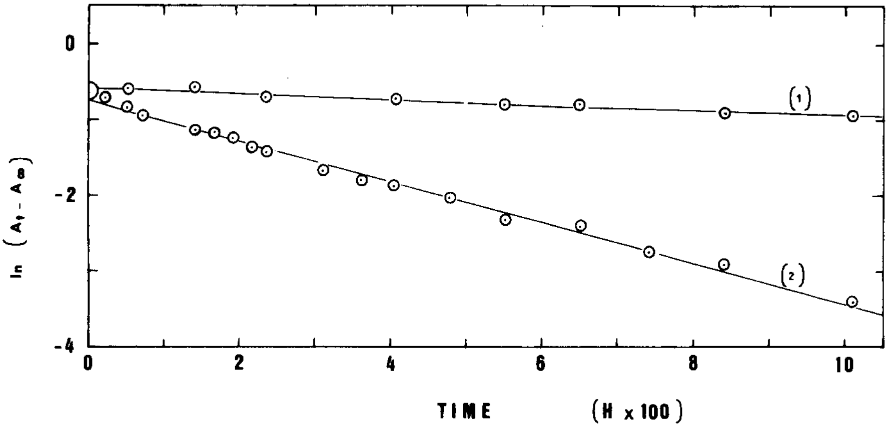

Fig. 3. Kinetic plot, first order equilibrated reaction, retinyl acetate in absolute ethanol, light intensity; 650 lux; (1) low actinic and (2) clear glassware.

Kinetic analysis of the data showed that a best fit was obtained when using a first order integrated rate law. For runs that had been exposed to light, the data were analyzed over at least 3 half-lives (Fig. 3). The control runs (low actinic glassware) were also analyzed as first order reactions, but only tentatively, due to the fact that eqn (8) requires the value of A_{infinite} to proceed; since it was estimated that it would have required at least 25 000 h to reach equilibrium, the A_{infinite} values used for the calculations were the ones obtained from the reactions run in the clear glassware.

It was mentioned previously that the slope of the plot of ln $[A_t - A_{\text{infinite}}]$

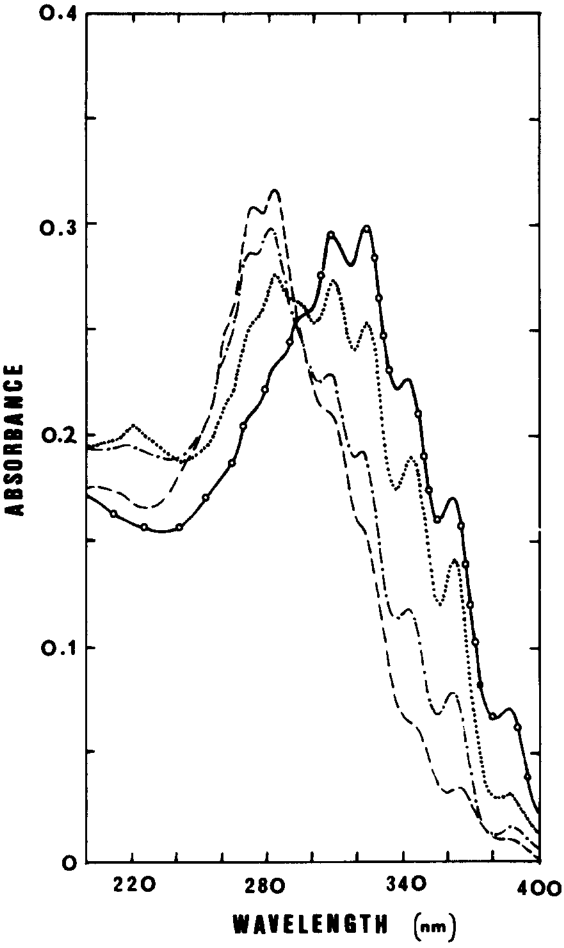

Fig. 4. Regeneration of degraded retinyl acetate; stored in the dark after exposure to light for 2372 h, -----; 1105 h, —·—·—; 2856 h, ······; 10 344 h, —O—O—.

vs. time is $-(k_1 + k_{-1})$. In order to estimate the values of the individual rate constants, a simple experiment was tried following mention made a while ago (Smith *et al.*, 1939) that vitamin A solutions which had degraded showed some regeneration of the band at 325 nm when taken from a lighted environment and stored back in the dark; the result is shown in Fig. 4. From this last experiment, the value of k_{-1} may be evaluated individually if it is assumed that the backward reaction is not affected by light. This assumption is justified by the fact that exposure to light is definitely the major factor for the forward reaction (Fig. 2) and by the fact that the corresponding experiment run in actinic glassware shows a slow, continuous decrease in absorbance at 325 nm till it reaches equilibrium at infinite time. The value of k_{-1} being so small (it is estimated to be about $5 \times 10^{-5}\,h^{-1}$) in comparison to the sum $(k_1 + k_{-1})$, $2{\cdot}6 \times 10^{-3}\,h^{-1}$, it may be neglected for all practical purposes; i.e. the value of the slope obtained from Fig. 3 may be taken as the value of k_1.

The fact that the reaction was found to be an overall first order one is in agreement with some previous reports (Higuchi & Reinstein, 1959; Forlano & Harris, 1960*a*; Slater *et al.*, 1979) which state first order kinetics. Comparison of the numerical values of the rate constants are

TABLE 1
Rate Constants of Retinyl Acetate Degradation

Solvent	*Glassware*	*Conc.* (M) × *10*^{5}	$k(h^{-1}) \times 10^3$
Abs. Ethanol	Clear	1·5	2·71
	Actinic	1·5	0·40
	Clear	3·1	2·46
	Actinic	3·1	0·35
95% Ethanol	Clear (O_2-free)	2·96	10·0
	(air-saturated)	2·96	10·7
	Actinic	2·96	0·30
	Clear (O_2-free)	1·49	15·3
	(air-saturated)	1·49	15·4
	Actinic	1·49	0·53
	Clear (O_2-free)	0·75	21·5
	(air-saturated)	0·75	21·7
	Actinic	0·75	0·63
Hexanes	Clear	3·05	7·63
		1·52	9·88
		0·77	9·30

difficult to make since Higuchi & Reinstein (1959) do not report lighting conditions, while Forlano & Harris (1960*a*) did their work with solutions kept in 'amber bottles'. And finally, as shown in Table 1, the rate constants calculated vary depending on the initial concentration of retinyl acetate; this type of behaviour has been reported before (Higuchi & Reinstein, 1959) and is currently under investigation; it will be reported upon in a separate paper.

Spectral changes shown in Fig. 1 are very similar to those reported by Dalvi & Morton (1951) for irradiated retinyl acetate or neo-vitamin A esters in ethanol; new maxima at wavelengths higher than the original one (325 nm) may be attributed to isoanhydrovitamin A (Sobotka *et al.*, 1944) or to the ethyl ether of isoanhydrovitamin A (Oroshnik, 1954), or to anhydrovitamin A (Takashima *et al.*, 1979; Blattna & Parizkova, 1982). New peaks or shoulders at wavelengths < 325 nm could be due to geometric isomers of the initial all-*trans*-vitamin A since some of these show secondary peaks below 325 nm (Robeson *et al.*, 1955; Hubbard, 1956; Planta *et al.*, 1962), or to so-called oxidation products or 'rehydro structure' (Forlano & Harris, 1960*a*).

Series 2: effect of the presence of air, at ambient temperature and in 95 % ethanol

The spectral changes occurring over the reaction time are shown in Fig. 5. The major degradation factor is still exposure to light, as is shown in Figs 6 and 7. The presence of oxygen leads to no significant difference in the degradation rates measured. As in the case of Series 1, the data was analyzed with the various integrated rate laws, and the best fit was obtained using the first order equation for equilibrated reactions. The rate constants (Table 1) show no significant difference between the O_2-free and the air-saturated samples. On the other hand, in comparison with the results of Series 1, an important observation is to be noted: the water content of the solvent (ethanol) does influence the degradation rate of retinyl acetate. The difference is significant for both the light exposed runs ($k = 1{\cdot}5 \times 10^{-2}\,h^{-1}$ for 95 % ethanol, $k = 2{\cdot}7 \times 10^{-3}\,h^{-1}$ for absolute ethanol), and for the non-exposed runs ($5{\cdot}3 \times 10^{-4}\,h^{-1}$ for 95 % ethanol, $4{\cdot}0 \times 10^{-4}\,h^{-1}$ for absolute ethanol). This is in agreement with the results of Forlano & Harris (1960*a*) obtained in isopropanol, and with those of Takashima *et al.* (1979) obtained in ethanol. These authors found close to a five-fold increase in the retinyl acetate degradation rate constant

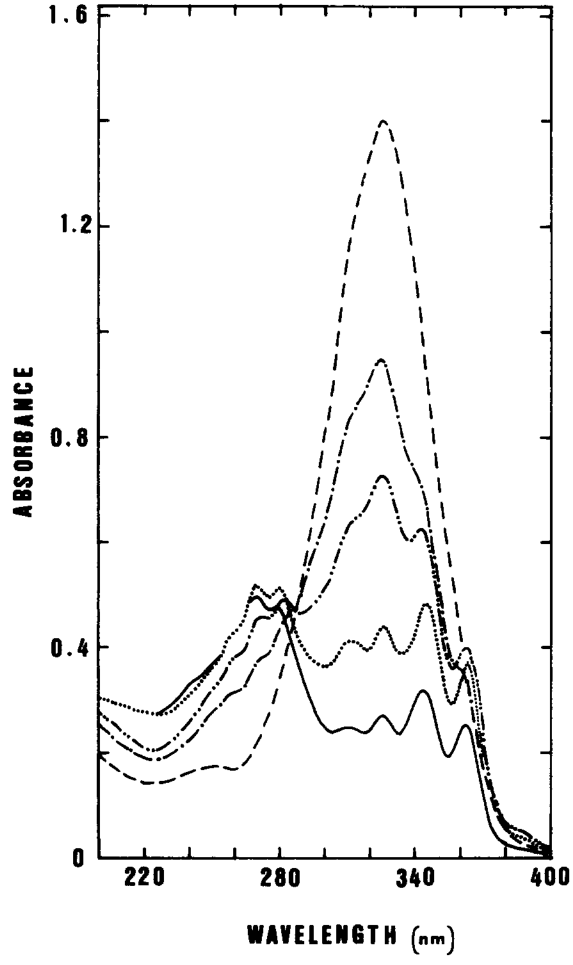

Fig. 5. Spectral changes of retinyl acetate in 95% ethanol; clear glassware, light intensity: 650 lux; time 0 h, – – – – –; 18 h, —●—●—; 45 h, —●●—●●—; 134 h, ● ● ● ● ●; 360 h, ————.

when going from absolute to 90% ethanol; this study found a four- to six-fold increase of the rate constant, when going from absolute to 95% ethanol (Table 1).

Although some authors still report a sensitivity of retinyl acetate to oxygen (Sattar *et al.*, 1977; Fung & Rahwan, 1978; Evteeva & Gagarina, 1983), our finding that oxygen plays a very small role, if any, in the degradation of these compounds has been reported in the literature a

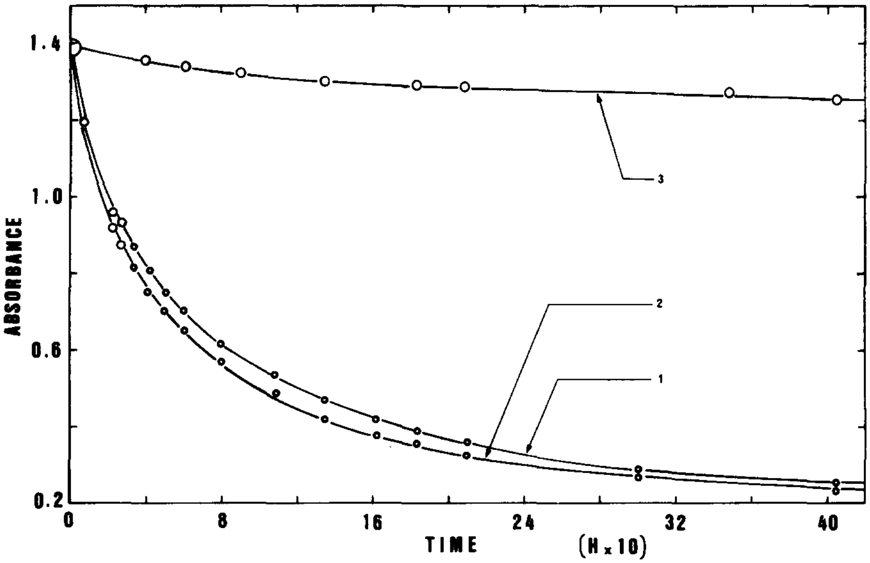

Fig. 6. Changes in A_{325} with time for retinyl acetate in 95 % ethanol; (1) clear glassware, O_2-free; (2) clear glassware, air-saturated and (3) low actinic glassware, O_2-free.

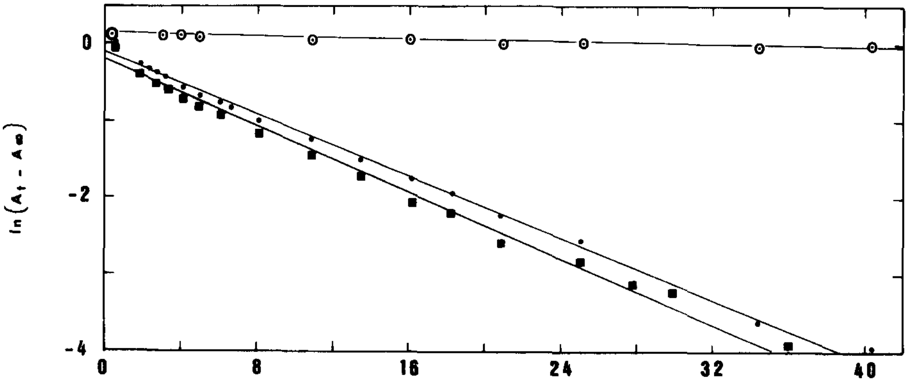

Fig. 7. Kinetic plot, first order equilibrated reaction, for retinyl acetate in 95 % ethanol, light intensity: 650 lux; —⊙—⊙—, O_2-free, low actinic glassware; —●—●—, O_2-free, clear glassware; —■—■—, air-saturated, clear glassware.

number of times (Dann, 1932; Embree, 1941; Krukovsky *et al.*, 1943, 1944; Bieri, 1951; Nakai *et al.*, 1983).

Series 3: effect of temperature, in absolute ethanol saturated with either N_2 or O_2, and kept under the corresponding atmosphere during the run

These results are shown in Table 2 and in Fig. 8. Once again the presence of oxygen (O_2-saturated solvent, under a blanket of O_2) leads to no significant difference in both the rate constants and the activation energies calculated, in comparison to runs performed in N_2-saturated ethanol and kept under a blanket of N_2.

TABLE 2

Rate Constants and Activation Energies in O_2-free and O_2-saturated Ethanol

Solvent	*O_2-free ethanol*			*O_2-saturated ethanol*		
Temp. (°C)	*30*	*40*	*50*	*30*	*40*	*50*
$k_{exp}(h^{-1})$	$6{\cdot}7\times10^{-3}$	$1{\cdot}2\times10^{-2}$	$2{\cdot}9\times10^{-2}$	$7{\cdot}0\times10^{-3}$	$1{\cdot}3\times10^{-2}$	$2{\cdot}6\times10^{-2}$
r	0·9960	0·9997	0·9983	0·9989	0·9976	0·9988
E_a (kJ/mol)		$58{\cdot}9\pm10{\cdot}0$			$53{\cdot}5\pm5{\cdot}8$	

The activation energies found are in agreement with previously reported values (Garrett, 1956: in amber bottles, analyzed as zero order initially, then first order; Kozlov *et al.*, 1969: lighting conditions not reported, analyzed as a chain reaction, activation energy calculated for the first order of the reaction but differ significantly from others (Higuchi & Reinstein, 1959; Forlano & Harris, 1960*a*; Wilkinson *et al.*, 1981). It is somewhat difficult to evaluate and/or compare results of this nature since, in some cases, lighting conditions are not specified and also sometimes the order of the reaction studied was reported to be different from one: thus the rate constants used to calculate the activation energy would differ substantially from the ones that a first order analysis would yield.

Series 4: effect of the nature of the solvent; air-saturated hexanes

Kinetic analysis of the data showed a best fit using a first order integrated rate law (eqn (12)), for a reaction going to completion in terms of the

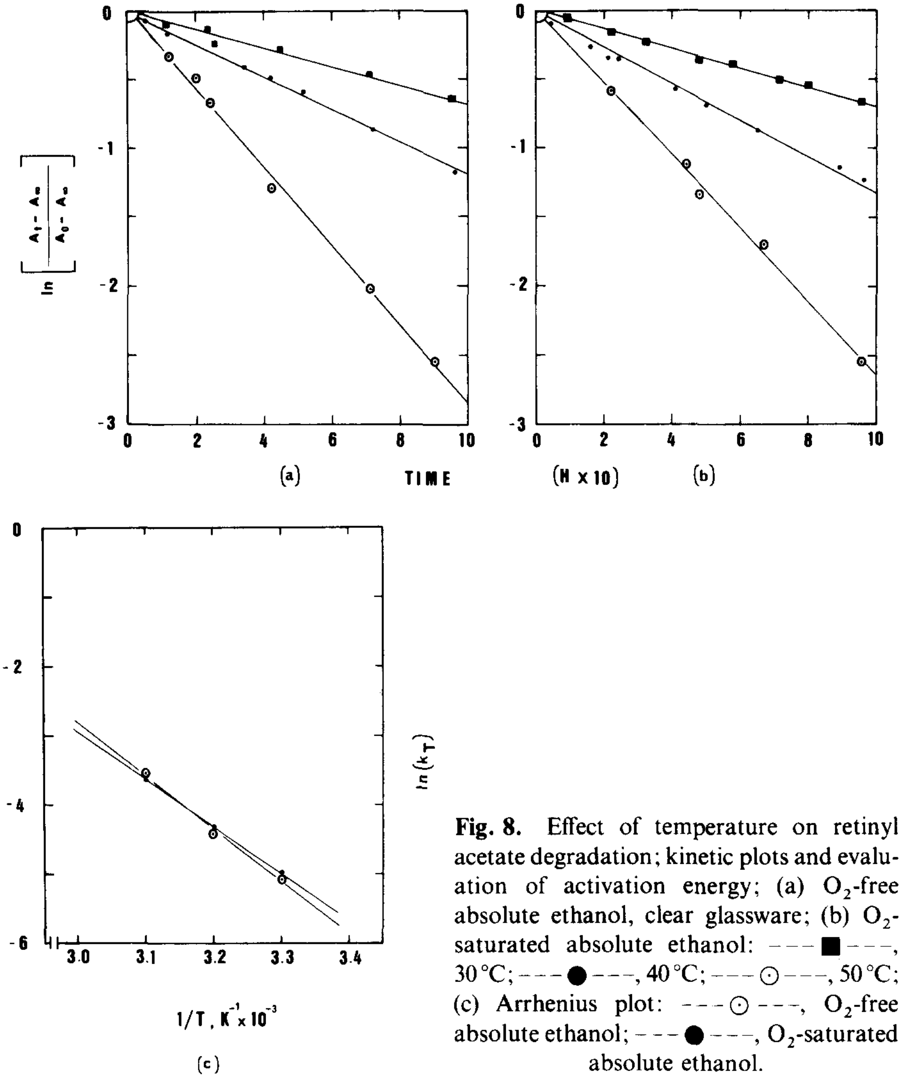

Fig. 8. Effect of temperature on retinyl acetate degradation; kinetic plots and evaluation of activation energy; (a) O_2-free absolute ethanol, clear glassware; (b) O_2-saturated absolute ethanol: ---■---, 30 °C; ---●---, 40 °C; ---⊙---, 50 °C; (c) Arrhenius plot: ---⊙---, O_2-free absolute ethanol; ---●---, O_2-saturated absolute ethanol.

initial reactants (Figs 9 and 10). The rate constants computed are intermediate between the ones obtained for the reaction in absolute ethanol and the ones in 95 % ethanol, under the same conditions of temperature, light exposure and contact with air.

Analysis of the data leads to assigning a first order to this reaction. This is in disagreement with the results of Budowski & Bondi (1960) who used retinyl palmitate in paraffin oil and with those of Kozlov *et al.* (1969) who

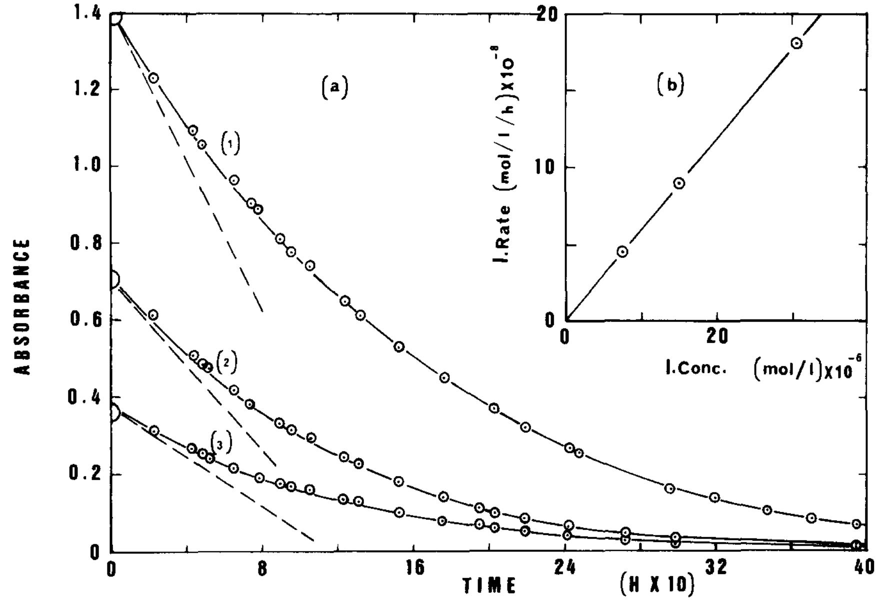

Fig. 9. Changes in A_{325} with time for retinyl acetate in hexanes, clear glassware, light intensity; 650 lux; (1) $3{\cdot}05 \times 10^{-5}$ M; (2) $1{\cdot}52 \times 10^{-5}$ M; (3) $0{\cdot}77 \times 10^{-5}$ M.

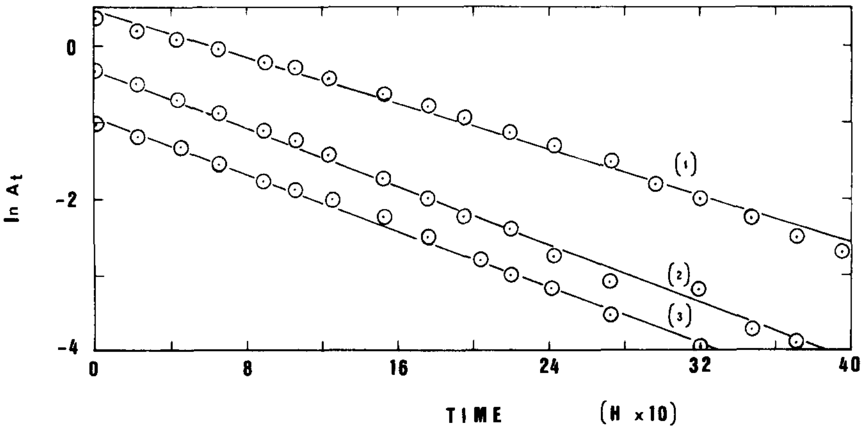

Fig. 10. Kinetic plot, first order non-equilibrated reaction, retinyl acetate in hexanes, clear glassware; (1) $3{\cdot}05 \times 10^{-5}$ M; (2) $1{\cdot}52 \times 10^{-5}$ M; (3) $0{\cdot}77 \times 10^{-5}$ M.

used retinyl acetate in decane: they both reported an autocatalytic chain reaction with an induction period. As with the series run in ethanol (95 % and absolute), the rate constants show a dependence on the initial concentrations of retinyl acetate, which is not explained at this time.

Spectral changes occurring over the reaction time in this solvent are shown in Fig. 11. It is to be noted that these changes differ from those occurring both in absolute and in 95 % ethanol (Figs 1 and 5). First the reaction goes to completion in terms of the disappearance of the initial reactant, as monitored by the absorbance at 325 nm; secondly, no new maxima at wavelengths higher than 325 are noticeable, eliminating iso-anhydrovitamin A as one of the products of the reaction (Oroshnik, 1954). This indicates that the degradation reactions occurring are

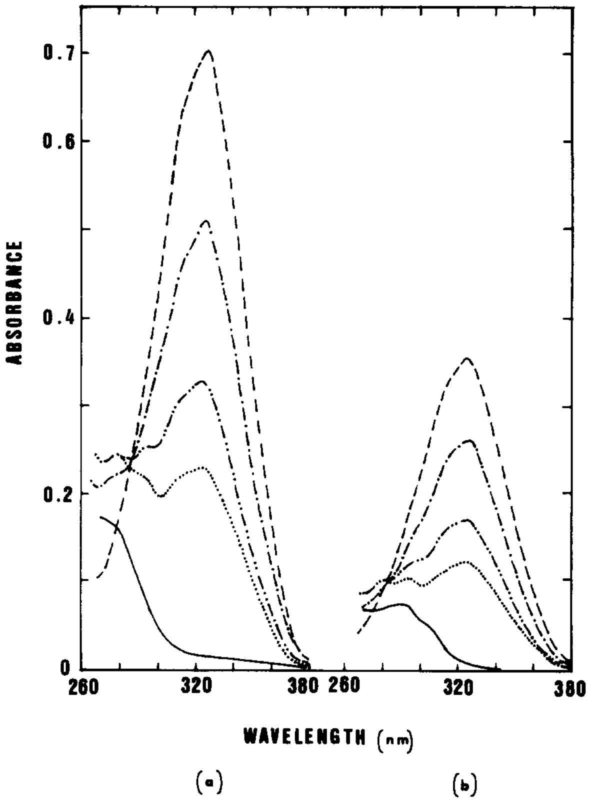

Fig. 11. Spectral changes of retinyl acetate in hexanes, light intensity: 650 lux; (a) initial concentration: $1\cdot52 \times 10^{-5}$ M; (b) initial concentration: $0\cdot77 \times 10^{-5}$ M; time 0, -----: 43 h; —●—●—; 98 h, —●●—●●—; 131 h, ······; 395 h, ——.

following different pathways; a change in solvent nature (mainly polarity in this case) causes certain pathways to be eliminated. It has to be remembered that what is in fact measured by the kinetic analysis is the sum of all the reactions taking place.

CONCLUSIONS

In the three systems studied, exposure to fluorescent light was a far more important factor in the degradation rate of retinyl acetate than the other factors: namely, presence of oxygen, temperature and nature of the solvent medium.

In all cases studied, the presence of oxygen leads to non-significant differences in the rate constants computed when compared to runs performed in the absence of O_2.

The presence of water in ethanol does accelerate the degradation reaction but the dependence of the rate on the water content is not a simple one. The activation energy found is 56 ± 8 kJ/mol.

The spectral changes occurring during the reaction time indicate that more than one degradation mechanism is operating in the systems studied.

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