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Degradation kinetics of α-tocopherol in hydrophilic gels

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Abstract

The present work studies the degradation kinetics of α-tocopherol in hydrophilic gels incorporating vitamin C as antioxidant. HPLC was used to detect the amounts of α-tocopherol remaining and to validate the analytical and extraction techniques. The results indicate that α-tocopherol undergoes degradation by oxidation of the hydroxyl group, and that this is reduced by the presence of the antioxidant.

Keywords: α-tocopherol; Antioxidant; Ascorbic acid; Carbomer® 940; Oxidation

α-tocopherol (α-T) is the most potent and generally most predominant form of vitamin E (Burton et al., 1983; US Pharmacopeia, 1995; Hoffman-La Roche). Its structure includes a saturated side chain whose non-esterified component is highly susceptible to oxidation. The non-esterified α-T is less stable due to the free phenol group; its oxidation leads successively to the formation of α-tocopherylquinone, α-tocopherol red, *p*-quinone, α-tocopherol purple and small amounts of dimers and trimers (Tappel, 1972; Deritter, 1982). Auto-oxidation is the most common oxidative phenomenon affecting both active principles and pharmaceutical preparations (Franquesa and Alsina, 1985; Vigo et al., 1992a,b). This may in turn be reduced in the presence of certain substances such as vitamin C (Rodilla et al., 1989). Vitamin C is a redox system comprising L-ascorbic acid (Tappel, 1972; Handbook of Pharmaceutical Excipients, 1986), monodehydroascorbic acid and dehydroascorbic acid. These three substances have different physicochemical properties (Nobile and Woodhill, 1981). The degradation pathway is oxidation, in which ascorbic acid is oxidized to an intermediate free radical, monodehydroascorbic acid, which is relatively unreactive but when it does react it does so rapidly, either with itself to give ascorbic acid and dehydroascorbic acid, or with other free radicals, so terminating the reaction (Seib, 1986; Tolbert, 1986; Honegger et al., 1989).

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Table 1
Statistical parameters of the regression of calibration of α-T

Coefficient of correlation: 0.9929					
Coefficient of determination: 0.9859					
Coefficient of regression 0.0546			Estimated constant term 0.0509		
S.E. 0.0013		T 42.72	$P<0.0001$		
Source	DF	Sums of squares	Mean square	F	P
Regression	1	1.7195	1.7195	1825.1	<0.0001
Total residuals	26	0.0245	9.4215×10^{-4}		
Error of fit	5	0.0028	5.6300×10^{-4}	0.5455	0.7399
Pure error	21	0.0217	1.0320×10^{-3}		
Total	27	1.7440			
Root mean square error			3.0694×10^{-2}		
Mean of dependent variable			0.7848		
Coefficient of variation			3.91		

This work studies the chemical stability of hydrophilic gels of α-T. Two formulations have been designed, one with the vitamin C redox system and the other without this antioxidant. The choice was based on the potentiation of the in vivo scavenger action of α-T (Doba et al., 1985; Furuse, 1987; Sies, 1989; Reynolds, 1993; Vigo, 1993). Hydrophilic gels were prepared (Vigo, 1993). The gel base was prepared with Carbomer® 940 (1% w/w, Acofarma, E-Tarrasa) by the usual procedure. α-T and α-TAA gels were prepared by dissolving ascorbic acid (AA) (0.1% w/w, Merck,

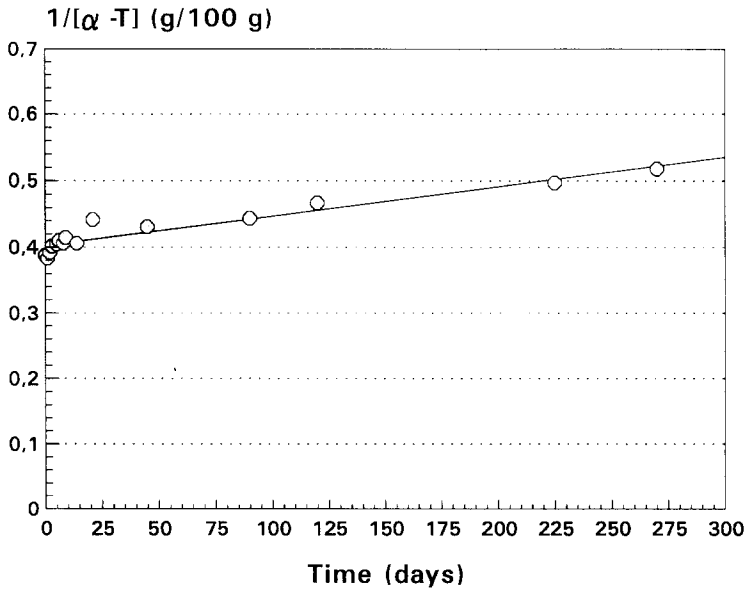

Fig. 1. Degradation of α-tocopherol in gel α-T as a function of storage time after fitting to a 2nd order kinetics.

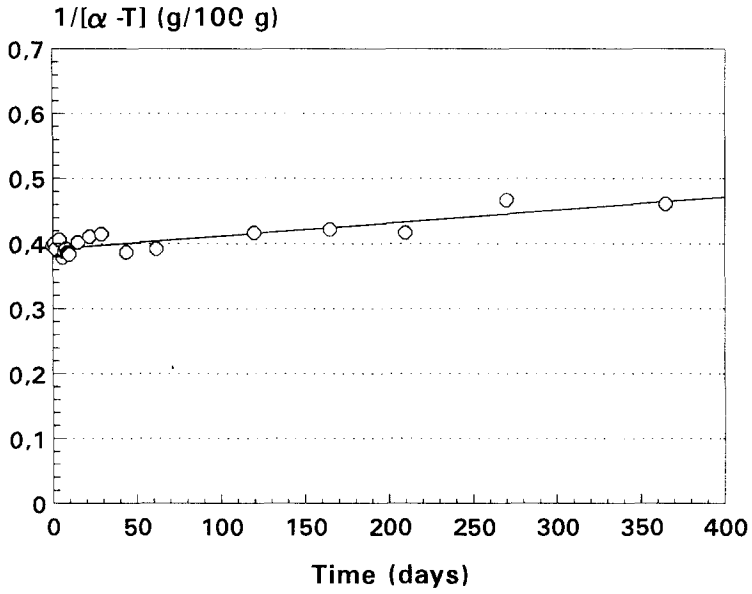

Fig. 2. Degradation of α-tocopherol in gel α-TAA as a function of storage time after fitting to a 2nd order kinetics.

G-Darmstadt), α-tocopherol (α-T) (2.5% w/w, DL-α-tocopherol, Merck, G-Darmstadt) or both substances in the ethanol 96% (15% w/w), followed by incorporation to the rest of the formula. The formulations were left in repose at ambient temperature for 24 h before carrying out the assays (BF Goodrich, 1981; Deritter, 1982; Lucero et al., 1994a,b,c).

α-T was detected by high performance liquid–liquid chromatography (HPLC), using a chromatograph (Kontron Instrument) comprising a two-piston pump (Mod. 420), a variable-wavelength ultraviolet-visible detector (Mod. 432) and a recorder-integrator (Data Jet Integrator, Konik). Injection was with a loop of 20 μl. The column was 25 × 4.6 cm (Lichrospher® 100, RP18, 5 μm, Merck, G-Darmstadt) protected by a precolumn of 4 × 4 mm (Lichrospher® 100, RP18, 5 μm, Merck, G-Darmstadt). The chromatographic conditions were the following: wavelength 292 nm, flow rate 1.4 ml/min, and mobile phase methanol:water (93:7). Under these conditions the detection limit (signal:noise 5:1) was 15 ng/ml. In our work, retinol acetate (Merck, G-Darmstadt) was chosen as internal standard (IS) (Miller et al., 1984; Milne and Botnen, 1986; De Leenheer et al., 1988) to avoid interference with the degradation products of the active principle. Calibration curves were then made from solutions of α-T in absolute ethanol ACS ISO (Merck, G-Darmstadt), obtained under nitrogen atmosphere to prevent possible oxidation. The concentrations used were the following: 5.925, 7.90, 9.875, 11.85, 13.825, 15.80 μg/ml of α-T and 17.775 μg/ml of IS. Quotients of peak area α-T/IS were plotted against concentration of α-T. The extration technique used for α-T was that proposed by ourselves (Vigo et al., 1992c).

The amount of α-T present in the gels was quantified as a function of storage time and at ambient temperature. Other parameters determined were the rate constant and the times of 10 and 50% degradation (t_{90} and t_{50}, respectively) for each hydrophilic gel (Franquesa and Alsina, 1985; Carstensen, 1990).

For validation of the analytical technique by HPLC, calibration curves were made for α-T when the chromatographic conditions had been fixed. Preliminary studies yielded good correlation coefficients ($r_{xy} > 0.9000$) for the different calibration curves. A statistical study was then carried out on linear regression and analysis of variance

Table 2
Degradation kinetics of α-T in gels α-T and α-TAA

Gel α-T					Gel α-TAA			
Kinetics	r_{xy}	$F_{(1,13)}$	P	CV	r_{xy}	$F_{(1,13)}$	P	CV
Order 0	0.8632	82.06	<0.0001	3.32	0.7528	51.76	<0.0001	2.82
Order 0.5	0.8761	91.88	<0.0001	1.62	0.7585	53.41	<0.0001	1.42
Order 1	0.8881	103.22	<0.0001	3.67	0.7638	54.98	<0.0001	3.16
Order 1.5	0.8995	116.31	<0.0001	1.51	0.7686	56.47	<0.0001	1.44
Order 2	0.9100	131.40	<0.0001	2.93	0.7729	57.87	<0.0001	2.90
Coefficient of regression: $K_d = 0.0004444$ $((g/100\ g)^{-1}\ d^{-1})$					$K_d = 0.000199$ $((g/100\ g)^{-1}\ d^{-1})$			
Estimated constant term: $1/C_0 = 0.4030$ $((g/100\ g)^{-1})$					$1/C_0 = 0.3919$ $(g/100\ g)^{-1}$			
$C_0 = 2.5/100$ g					$C_0 = 2.5$ g/100 g			
$t_{90} = 100.1$ d					$t_{90} = 221.1$ d			
$t_{50} = 901.1$ d					$t_{50} = 2010.0$ d			

of the regression. Table 1 shows the most important parameters of regression. The results indicate the linear regression existing between quotients of peak area α-T/IS and concentration of α-T. At the same time, from the residuals it has been possible to demonstrate the fit of the linearity and the accuracy of the analytical method (CV = 3.91%). Having determined the validity of the analytical technique for quantifying α-T, the technique of its extraction from the formulated gels was validated. Table 1 shows the data obtained from the analysis of variance. These results indicate that there was no statistically significant difference between extractions made on the same day ($P = 0.7895$ and 0.1377, α-T and α-TAA, respectively) and on successive days ($P = 0.7895$ and 0.1222, α-T and α-TAA, respectively). The yield of this extraction technique from gel α-T was 95.32% and from gel α-TAA was 99.36%, so that the presence of AA favourably affects the extraction of α-T.

The preparations made with α-T (Figs. 1 and 2) undergo degradation with time. In the case of gel α-T, this best fits 2nd order kinetics (Table 2), leading to the conclusion of a chain reaction characteristic of auto-oxidation (Franquesa and Alsina, 1985). The same kinetics are shown by gel α-TAA. The values of parameters t_{90} and t_{50}, are much higher for gel α-TAA than for gel α-T. Thus, the addition of antioxidant (AA) slows the oxidation of α-T, greatly increasing the chemical stability of the active principle in the hydrophilic gel (gel α-TAA) by more than 100% with respect to that without antioxidant. This is explained by the AA in solution having a higher degradation rate than that of α-T (Willson, 1983), thereby creating a reducing environment so that the α-T is not oxidized. These results corroborate the proven efficacy of the vitamin C redox system (Lucero et al., 1993, 1994a,b,c), and at the same time demonstrate the increase in chemical stability of α-T in hydrophilic gels.

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