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Stability of hydrophilic gels of tretinoin

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Abstract

Tretinoin is the *trans* isomeric form of retinoic acid. Its chemical structure includes a functional acid group and a side chain with conjugated double bonds, both susceptible to redox reactions. The vitamin C redox system comprises L-ascorbic acid, monodehydroascorbic acid and dehydroascorbic acid. Each of these substances has different physicochemical properties but possess antioxidant capacity for both hydrophilic and lipophilic substances. The present work is centred on studying the stability of hydrophilic gels of tretinoin prepared with or without antioxidant. HPLC was used to validate the analytical and extraction techniques to determine the amounts of tretinoin remaining. The results indicate that tretinoin undergoes rapid degradation by oxidation of the conjugated double bonds which is neither retarded nor lessened by the presence of antioxidant.

Key words: Antioxidant; Ascorbic acid; Carbomer® 940; Oxidation; Tretinoin

1. Introduction

Tretinoin is the *trans* isomeric form of retinoic acid (Fig. 1). Its 13-*cis* isomer is denoted as isotretrinoin, from which it differs only in the configuration of unsaturation on the α and β carbon atoms (Boyd and Lubbock, 1989; Underwood, 1989).

Some authors advise its conservation cold and protected from light and air (Bonhomme et al., 1990), and indicate that it is unstable in the presence of strong oxidizing agents (Reynolds, 1993). In contrast, others report tretinoin as stable to oxidation by pure oxygen, as the acid function is unable to undergo subsequent oxidation (Carreras, 1981). On the other hand, it is reduced in alkaline solutions and, with gradual decrease in pH, is recovered in petroleum ether to a maximum of 90% at pH 4, suggesting that its pK_a is 6.0 (Vahlquist, 1982).

A knowledge of the possible degradation pathways of tretinoin is of great help in achieving its ideal pharmaceutical form. The most probable mechanism is oxidation. This reaction is strongly influenced by light, metal ions, oxidizing agents and oxygen, even when in low concentrations

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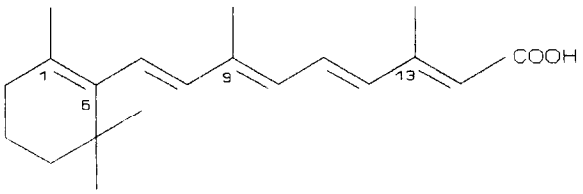

Fig. 1. Chemical structure of tretinoin.

(Franquesa and Alsina, 1985; Ravin and Radebaugh, 1990).

Auto-oxidation is the most common oxidative phenomenon affecting both active principles and pharmaceutical preparations. It can be defined as the reaction of a substance with molecular oxygen (Franquesa and Alsina, 1985; Vadas, 1990; Vigo et al., 1992a,b), with transformation to a peroxide and a free radical. The peroxides increase in content to a maximum at the end of this period, then decompose at a greater rate than that of their formation. Thus, although oxygen absorption continues, the peroxide concentration decreases. At this moment, a great number of hydroxyl free radicals ($OH^{\cdot}$) appear.

All these reactions can continue indefinitely, or terminate due to the action of scavengers or the intraneutralization of the free radicals.

For an auto-oxidation process to begin, the molecules must meet two conditions: first, unsaturation, and second, an ideal spatial configuration in the region of the unsaturated bonds. The point of attack is the hydrogen atoms close to the double bonds and the electronegative groups (Franquesa and Alsina, 1985).

Many oxidations are catalysed by hydrogen ions. Thus, the redox potential of many reactions depends on the pH. This is particularly so for those pharmaceutical products with a weakly acid nature.

Vitamin C is a redox system comprising L-ascorbic acid (AA) (Tappel, 1972; Handbook of Pharmaceutical Excipients, 1986), monodehydroascorbic acid (MDHAA) and dehydroascorbic acid (DHAA). These three substances have different physicochemical properties (Nobile and Woodhill, 1981).

AA is stable when dry, but easily oxidizable in aqueous solution in the presence of air (Vanderveen and Vanderveen, 1990), resulting in its ionization, which is dependent on the pH of the solution. The first constant of dissociation has a pK_1 value of 4.18 and the second, a pK_2 of 11.6 (Nobile and Woodhill, 1981). Under aerobic conditions, the maximum degradation of AA is detected at pH 4 and the minimum at pH 5.6 (Deritter, 1982).

The degradation pathway is oxidation-reduction, where the AA is oxidized to an intermediate free radical, MDHAA. This is relatively unreactive, and when it does react, does so rapidly – either with itself to give AA and DHAA or with other free radicals, thereby terminating these reactions (Seib, 1986; Tolbert, 1986).

DHAA is the hydrophobic component of the vitamin C system. It has a stronger lipid nature than AA and a greater capacity to reduce the interfacial tension. Thus, vitamin C acts as a metabolic detergent and can dissolve, among other substances, fats (Nobile and Woodhill, 1981).

The vitamin C redox system can include compounds able to protect medicaments from oxidative alterations – either natural or resulting from the action of air, light, heat, the presence of metals, etc. With this preventive aim, it can be added to those pharmaceutical preparations exposed to deterioration by oxidative processes (Vanderveen and Vanderveen, 1990).

It is a scavenger of oxygen (Frankel, 1989) and free radicals (Tolbert, 1986; Frankel, 1989; Honegger et al., 1989; Nagy and Degrell, 1989; Vigo et al., 1992a,b), reacting directly with these to form the intermediate stable radical, MDHAA (Seib, 1986). At the same time it can act, among other ways, as a synergist (Frankel, 1989) – potentiating the action of other antioxidants (Bright-See, 1983a,b; Lohmann, 1987; Informacion del Medicamento, 1989; Tannenbaum, 1989; Irache and Vega, 1990).

This work is centred on studying the stability of hydrophilic gels of tretinoin. Two formulations have been designed, one with an antioxidant of proven efficacy: the vitamin C redox system. This was chosen for its possible potentiation of the therapeutic effect of tretinoin (Vigo, 1993).

2. Materials and methods

2.1. Semisolid preparations

Dermatological hydrophilic gels, prepared with the components shown in Table 1, were used as bases (Vigo, 1993).

Carbomer® 940 (Acofarma, Tarrasa, Spain) was dusted onto distilled water (without stirring) and left to settle for 24 h. Then, triethanolamine (TEA) (Acofarma, Tarrasa, Spain) was added, stirring slowly to avoid the inclusion of air (BF Goodrich, 1981). Tretinoin (T) (*trans*-retinoic acid, Merck, Darmstadt, Germany), with or without ascorbic acid (AA) (Merck, Darmstadt, Germany), was dissolved in ethanol and added to the rest of the formula.

Prior to preparation of the gel, the amount of TEA necessary for its neutralization was determined (BF Goodrich, 1981). A pH of around 5.6 was found to be ideal for the stability of both T and AA (BF Goodrich, 1981; Deritter, 1982; Vigo, 1993; Lucero et al., 1994b).

2.2. Analytical and extraction techniques

Tretinoin was detected using a high-performance liquid-liquid chromatograph (HPLC) (Kontron Instrument) consisting of a two-piston pump (Model 420), a variable-wavelength ultraviolet-visible detector (Model 432), and a recorder-integrator (Data Jet Integrator, Konik). The injector was fitted with a loop of 20 μl. The column was 25 cm $\times$ 4.6 mm (Lichrospher® 100, RP18, 5 μm; Merck, Darmstadt, Germany) protected by a precolumn of 4 $\times$ 4 mm (Lichrospher® 100, RP18, 5 μm; Merck, Darmstadt, Germany). The chromatographic conditions were the following: wavelength, 356 nm; flow rate, 1.4 ml/min; mobile phase, methanol/acetonitrile/water/acetic acid in the ratio 80:10:10:0.5. Under these conditions, the limit of detection (5:1, signal-to-noise) was 11 ng/ml. Retinol acetate (Merck, Darmstadt, Germany) was chosen as internal standard (IS) from the bibliography consulted (Fur, 1984; Miller et al., 1984; Milne and Botnen, 1986, De Leenheer et al., 1988).

To validate the analytical method, solutions of semisolid excipient, T, AA and IS were analysed by HPLC under the conditions described above, establishing their retention times and the non-interference between them.

Calibration curves were constructed from solutions of T in absolute ethanol ACS ISO (AEt) (Merck, Darmstadt, Germany), prepared under a nitrogen atmosphere to avoid possible oxidation. The concentrations of T used were the following: 0.341, 0.519, 0.69, 0.853, 1.031, 1.209, 1.55 μg/ml, and 5.625 μg/ml of IS. Ratios of peak areas of T/IS were plotted against the concentration of T.

To verify the variability of the extraction technique, extractions were made of six different samples of each preparation on the same day (within run) and on three successive days (day to day). The results were validated by analysis of variance.

Because of the scarce bibliography on methods to extract T from semisolid preparations (Kundu et al., 1993), different techniques were tested. The following was chosen: 0.1 g of sample was treated with AEt until completely dissolved. This volume was then vacuum filtered (Büschner funnel/Kitasato flask and water jet). Lastly, the volume was made up to 15 ml.

2.3. Degradation kinetics

The amount of T present in the preparations was quantified as a function of time and ambient temperature (22 $\pm$ 1°C). The order of reaction was calculated from the fit of concentration/time pairs to the different general kinetic equations (Franquesa and Alsina, 1985; Carstensen, 1990). The rate constant and the 10 and 50% degradation times (t_{90} and t_{50}, respectively) for each semisolid preparation were also determined.

Table 1
Components of the dermatological bases tested

	Gel T	Gel TAA
Carbomer® 940	1%	1%
Ethanol 96%	15 ml	15 ml
Triethanolamine	3 ml	3 ml
Tretinoin	0.025%	0.025%
Ascorbic acid	–	0.1%
Distilled water	85 ml	85 ml

Table 2
Statistical parameters of the straight line of calibration of T

Coefficient of correlation: 0.9923
Coefficient of determination: 0.9847

Coefficient regression		Estimated constant term
0.4623		0.0073
Standard error	T	Probability
0.0113	40.89	< 0.0001

Source	DF	Sum of squares	Mean square	F	Probability
Regression	1	0.8868	0.8868	1672.3	< 0.0001
Total residuals	26	0.0138	5.3070×10^{-4}		
Error of fit	5	0.0030	6.0400×10^{-4}	1.17	0.3566
Pure error	21	0.0108	5.1270×10^{-4}		
Total	27	0.9006			
Root mean square residuals			2.3028×10^{-2}		
Mean dependent variable			0.4163		
Coefficient variation			5.53%		

3. Results and discussion

The chemical stability of tretinoin formulations was studied because of the possible oxidative decomposition undergone both by this substance (Merck Index, 1976; Franquesa and Alsina, 1985; Ball, 1988; Reynolds, 1993; Vigo, 1993) and by the antioxidant used (Deritter, 1982; Franquesa and Alsina, 1985; Kolar, 1986; Ball, 1988; Reynolds, 1993). Three sections were established: validation of the HPLC analytical technique, validation of the extraction technique, and degradation kinetics of the T in T and TAA gels.

The HPLC analytical technique was validated by performing linear calibration for the T, once the chromatographic conditions had been fixed. In the preliminary studies, good correlation coefficients ($r_{xy} > 0.9000$) were obtained for the different linear calibrations. The statistical study was then carried out by linear regression and analysis of variance of regression.

Table 2 lists the most important parameters of regression. These results indicate the linear regression existing between ratios of peak areas T/IS and concentration of T. At the same time, from the residuals it has been possible to demon-

Table 3
Statistical parameters of the validation of the extraction technique of the gels indicated

Gel	Source	DF	Sum of squares	Mean square	F	Probability
T	Day to Day (A)	2	0.06952	0.03476	2.69	0.1082
	Within run (B)	3	0.04821	0.01007	1.24	0.3368
	AB	6	0.05277	0.00879	0.68	0.6684
	Error	12	0.15495	0.01291		
	Total	23	0.32546			
TAA	Day to Day (A)	2	0.00472	0.00236	0.39	0.6855
	Within run (B)	3	0.01534	0.00511	0.84	0.4959
	AB	6	0.02354	0.00392	0.65	0.6922
	Error	12	0.07275	0.00606		
	Total	23	0.11636			

Table 4
Degradation kinetics of T in gel T

Induction period			Kinetics	r_{xy}	$F_{(1,7)}$	Probability	CV(%)
r_{xy}	$F_{(1,4)}$	Probability	Order 0	0.7803	24.87	0.0020	37.06
0.0128	0.04	0.8560	Order 0.5	0.8615	43.53	< 0.0001	16.48
			Order 1	0.9317	95.51	< 0.0001	11.68
Coefficient regression: 0.0007675			Order 1.5	0.9649	192.18	< 0.0001	9.29
Estimated constant term: 0.42239			Order 2	0.9533	142.99	< 0.0001	21.03
			Coefficient of regression:				
$C_o = 25$ mg/100 ml			$K_d = 0.01022$ [(mg/100 ml)$^{-1/2}$ day^{-1}]				
$t_0 = 21.58$ days			Estimated constant term:				
$t_{90} = 23.71$ days			$2/\sqrt{Co} = 0.2184$ [(mg/100 ml)$^{-1/2}$]				
$t_{50} = 37.79$ days							

strate the fit of the linearity and the accuracy of the analytical method (CV = 5.53%).

Having determined the viability of the analytical technique proposed for quantification of T, the extraction technique of this substance from the formulated gels was validated. This was essential, as no reference was found in the bibliography.

Table 3 shows the data obtained in the analysis of variance. These results indicate that there was no significant statistical difference between the extractions made within run and those day to day. The efficiency of this extraction technique in gel T was 93.75% and in gel TAA 94.58% – thus the presence of ascorbic acid did not affect the extraction of T.

The degradation of T implies the existence of one or more chemical reactions whose rate can be measured by calculating the amount of T remaining with time. To obtain the kinetic data experimentally and later correlate them, it is not necessary to know the degradation mechanism involved (Franquesa and Alsina, 1985; Carstensen, 1990; Ravin and Radebaugh, 1990). It is not simple to define the general rate of this reaction, and in practice is considered at a determinate instant. This parameter varies considerably, not only from the effect of temperature (22 ± 1°C), but also from that of other factors such as pH of the medium, 5.3 and 5.05 for gels T and TAA, respectively (Vigo, 1993; Lucero et al., 1994b).

Table 5
Degradation kinetics of T in gel TAA

Induction period			Kinetics	r_{xy}	$F_{(1,6)}$	Probability	CV(%)
r_{xy}	$F_{(1,4)}$	Probability	Order 0	0.7709	20.19	0.0040	36.96
0.1849	0.91	0.3950	Order 0.5	0.8613	37.26	0.0010	15.62
			Order 1	0.9357	87.28	< 0.0001	10.73
Coefficient regression: 0.0011			Order 1.5	0.9738	223.07	< 0.0001	7.39
Estimated constant term: 0.4188			Order 2	0.9707	198.72	< 0.0001	15.44
			Coefficient of regression:				
$C_o = 25$ mg/100 ml			$K_d = 0.009733$ [(mg/100 ml)$^{-1/2}$ day^{-1}]				
$t_0 = 25.17$ days			Estimated constant term:				
$t_{90} = 27.39$ days			$2/\sqrt{Co} = 0.2015$ [(mg/100 ml)$^{-1/2}$]				
$t_{50} = 42.20$ days							

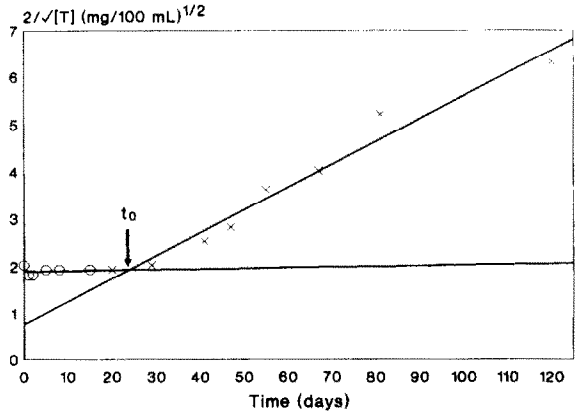

Fig. 2. Degradation of tretinoin in gel T as a function of time after fitting to a kinetic order of 1.5. t_0: time at which the degradation begins and the induction period ends.

It is known that certain degradations are characterized by an induction period during which the process seems to be at rest. This phenomenon has been observed in those involving chain reactions, such as auto-oxidation.

The gels prepared with T (Figs. 2 and 3) showed an induction period. During this time the concentration/time pairs did not fit any kinetic order. After this period, it was observed, for both gel T and TAA, that the degradation of the T best fitted a kinetic order of 1.5 (Tables 4 and 5). The point of intersection of the straight lines defining the induction period and the degradation kinetics of T was considered as t_0, indicating the moment at which this substance commences to degrade. It was 21.58 and 25.17 days for gels T and TAA, respectively (Tables 4 and 5).

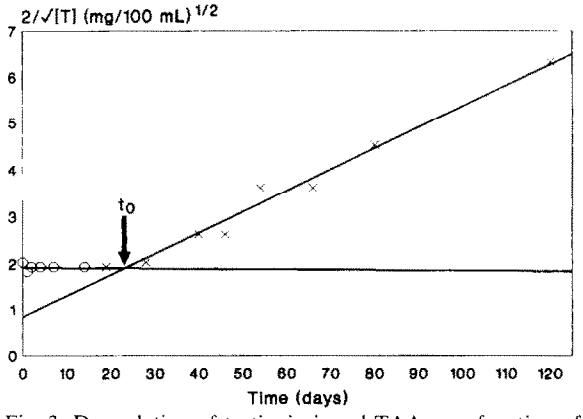

Fig. 3. Degradation of tretinoin in gel TAA as a function of time after fitting to a kinetic order of 1.5. t_0: time at which the degradation begins and the induction period ends.

Once t_0 has been determined, the parameters t_{90} and t_{50} can be calculated. The same tables show the similarity of the results obtained for the two gels. This leads to the idea that T undergoes auto-oxidation, giving rise to unidentified reactive species, typical of this process. Deduction of this kinetic equation is difficult as the degradation products are unstable (and so cannot be isolated), while at the same time the reactions are very fast and consecutive. It is clear that AA scarcely protects T from degradation, as the values of t_{90} obtained in gels T and TAA are very close. If it is accepted that the T is oxidized, it seems that the most probable reaction is by breaking of conjugated double bonds of the side chain, which are more readily attackable by reactive species derived from oxygen (Glas, 1980) than those included within the AA ring.

Another parameter to express the chemical stability of a determinate active principle is t_{50} (Tables 4 and 5). It can be seen clearly that T has a similar t_{50} in gels T and TAA. The same result can be seen in the values of K_d (rate constant), showing that the presence of AA does not reduce degradation of T. The phenomenon shown by these hydrophilic gels demonstrates the chemical instability of T, even when the formulation includes an antioxidant of proven efficacy, as is AA (Lucero et al., 1993, 1994a; Vigo, 1993).

4. Conclusions

Tretinoin, in all the preparations tested, seems to follow a degradation typical of autoxidisable substances, showing an induction period and then fitting a degradation kinetic order of 1.5. At the same time the values of t_{90} and t_{50} demonstrate its chemical instability. This leads to the conclusion that it is better to prepare these hydrophilic gels as extemporary formulations than to add large amounts of antioxidant, which does not ensure their stability with time.

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