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Synthesis, characterization and properties of polyacrylic systems derived from vitamin E

Carmen Ortiz, Blanca Vázquez and Julio San Román*
Instituto de Ciencia y Tecnología de Polímeros, Juan de la Cierva, 3, 28006-Madrid, Spain
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A methacrylic derivative of the α-tocopherol (vitamin E), V, has been synthesized and characterized by ^{1}H and ^{13}C n.m.r. spectroscopy. The homopolymerization of the new monomer was studied in solution in dimethylformamide at 50°C using AIBN as initiator. The kinetics of polymerization were followed gravimetrically and compared with those obtained for 2-hydroxyethyl methacrylate polymerized in the same conditions. Copolymers of the novel methacrylate with 2-hydroxyethyl methacrylate, H, were obtained in a range of feed composition between 5 and 20 mol%. The reactivity ratios were calculated according to the general copolymerization equation from composition data of copolymer samples determined by ^{1}H n.m.r. spectroscopy and by using both the linearization method and non-linear least squares analysis. The values obtained indicate that this system seems to fit a typical statistical copolymerization. Molecular weight and glass transition temperature of the copolymers increased with increasing vitamin E methacrylate content. It was observed that the values of the glass transition temperature were influenced by the sequential distribution of the comonomeric units. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: polymeric drugs; antioxidants; vitamin E hydrogels)

INTRODUCTION

Many laboratories have been involved in the investigation of oxygen-induced injury and oxidative process at the cellular level. Conclusive results have demonstrated that oxygen exposure, particularly in lung injury, is believed to be mediated through various reactive intermediates formed during the reduction of oxygen or oxidative compounds, including superoxides radicals, hydroxyl radicals and hydroperoxides[1,2]. For protection from the ravages or effects of these reactive molecular species it is necessary for the cells either to prevent the formation of these reactive intermediates or to minimize their damaging influences once they are formed. Several defence mechanisms have now been identified which undoubtedly participate to varying degrees towards controlling these reactive intermediates in the environment of the cell. Superoxide dismutase, catalase, the glutathione system and vitamin E have been the most frequently investigated of protective systems. Superoxide dismutasa (SOD) found in mitochondria (manganese form) and cytosol (cupro-zinc form), catalyses the reaction

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2.$$

Catalase is believed to function intramedullarly to remove hydrogen peroxide formed in the dismutation reaction. The glutathione system consists of a battery of enzymes, including selenium-dependent glutathione peroxidase which is believed to function intracellularly to remove inorganic and organic peroxides and possibly by inhibition of peroxidative attack on membrane lipids[3]. Vitamin E has long been regarded as membrane-protecting and has individually been the subject of many reviews and symposia[4].

There have been a number of animal experiments dealing with vitamin E effects on oxygen-induced pulmonary damage. Vitamin E deficiency markedly enhances the pulmonary toxicity of oxygen[5].The mechanisms for the protection and the relationship of vitamin E to polyunsaturated lipids has been a subject of several review articles and symposia[6]. Vitamin E is decreased in the low birth weight infant and this deficiency has been associated with RBC haemolysis[7], and retrolental fibroplasias (RLF) which is believed to represent oxygen toxicity[8]. Vitamin E supplementation has been shown to protect the vitamin E deficient experimental animal from oxygen-induced lung damage, and preliminary studies in idiopathic respiratory distress syndrome (IRDS) affected infants have suggested improvement[9].

To date, there has been no attempt to synthesize a monomeric acrylic or vinylic compound containing the vitamin E residue, which would be of great interest owing to the promising characteristics of the vitamin E molecule. In this sense, it should also be considered that the vitamin E being linked to a methacrylic back-bone through an aromatic ester of limited stability could be hydrolysed in the biological environment in mild conditions.

This paper reports on the preparation and characterization of the methacrylic derivative of vitamin E (α-tocopherol). The kinetics of solution homopolymerization of this monomer, together with those of 2-hydroxyethyl methacrylate, have been investigated. In addition, copolymers of the vitamin E acrylic derivative with 2-hydroxyethyl methacrylate were obtained from a range of feed compositions. Reactivity ratios were determined by using both linearization and non-linearization methods. Also, average molecular weights and glass transition temperatures of the copolymers are reported.

EXPERIMENTAL

Materials

Vitamin E (α-tocopherol) (Merck) was used as-received

* To whom correspondence should be addressed

without further purification. Methacryloyl chloride (Fluka A.G.) and triethylamine (Scharlau) were purified by distillation under reduced pressure. Azobisisobutironitrile (AIBN) (Merck) was recrystallized from methanol (melting point 104°C). HEMA was purified according to the literature[10]. The solvents diethyl ether (Quimicen), isopropanol (Quimicen) and dimethylformamide (Scharlau) were purified by standard procedures.

Synthesis of α-tocopheryl methacrylate

α-Tocopherol (20 mmol), triethylamine (20 mmol) and diethyl ether (50 ml) were introduced to a three-necked flask and the methacryloyl chloride (20 mmol) was added dropwise with constant stirring at room temperature under nitrogen atmosphere. The reaction mixture was then stirred for 24 h at room temperature. The reaction medium was filtered to remove the triethylamine chlorhydrate and the unreacted reagents were removed by successive extraction with 5% NaOH solution. After drying over $MgSO_4$, the solvent was removed by flash distillation and then under reduced pressure until constant weight.

Polymerization kinetics

The kinetics of solution polymerization for α-tocopheryl methacrylate or 2-hydroxyethyl methacrylate were determined gravimetrically. The corresponding monomer was polymerized using AIBN ([AIBN] = 1.5×10^{-2} mol l^{-1}) as free radical initiator, and dimethylformamide ([M] = 1 mol l^{-1}) as solvent. The reactions were carried out in Pyrex standard ampoules sealed at high vacuum. The ampoules were placed in a thermostatic bath at 50°C and kept for the time of interest. The polymer was precipitated in excess methanol, filtered off, washed and finally dried in vacuum.

Copolymerization reactions

The pair of monomers α-tocopheryl methacrylate, V, and 2-hydroxyethyl methacrylate, H, were copolymerized in a solution of dimethylformamide using the same experimental conditions as for the polymerizations (T^a = 50°C, [AIBN] = 1.5×10^{-2} mol l^{-1} and [M] = 1 mol l^{-1}) using a range of monomer feeds between 5 and 20 mol% of the vitamin E derivative. The reactions were allowed to proceed for the required time to obtain copolymers at conversions of < 5%. The reaction mixture was precipitated in a large excess of diethyl ether, filtered off, washed, and finally dried in vacuum until constant weight.

Characterization

N.m.r. spectra of monomer and polymers were recorded using a Varian XLR-300 spectrophotometer operating at 300 MHz for ^1H n.m.r. experiments and 75.5 MHz for ^{13}C n.m.r. experiments. The ^1H n.m.r. and ^{13}C n.m.r. spectra of the monomer were recorded at 80°C in 5% (w/v) and 25% (w/v) solutions respectively in deuterated chloroform. The ^1H n.m.r. and ^{13}C n.m.r. spectra of the polymer were recorded at 25°C in the same conditions using a pulse width of 13 μs and a relaxation delay of 4 s. These conditions ensured the complete relaxation of all ^{13}C nuclei analysed. Relative peak intensities were measured from peak areas calculated by means of an electronic integrator or by triangulation and planimetry.

Weight- and number-average molecular weights of the polymers and copolymers were determined by gel-permeation chromatography (g.p.c.) (Waters-510) with an Injector 7125 and a Pye Unicam UV Detector, PU-4025. The signal at λ_{max} = 273 nm was used as reference. Three columns packed with PL-styragel of pore sizes 10^3, 10^4 and 10^5 Å (Polymer Laboratories) were used. The elution solvent was *N,N*-dimethylformamide with 1% LiBr and the flow rate was maintained at 1 ml min^{-1}. Calibration was carried out with standard poly(methyl methacrylate) samples obtained from Polymer Laboratories with average molecular weights in the range 2990–1.4×10^6 and a polydispersity of 1.1.

The glass transition temperature T_g was determined with a Perkin Elmer DSC-7 differential scanning calorimeter. The samples were placed in aluminium pans, heated at 500 K for 30 min and then cooled at constant rate to −50°C prior to the measurement. Measurements and calibration were carried out at heating rates of 20°C min^{-1}. Calibration of the calorimeter was performed by determining the melting point and melting enthalpy of fusion for indium. T_g was taken as the midpoint of the heat capacity transition.

RESULTS AND DISCUSSION

Synthesis of the methacrylate derivative of vitamin E, V

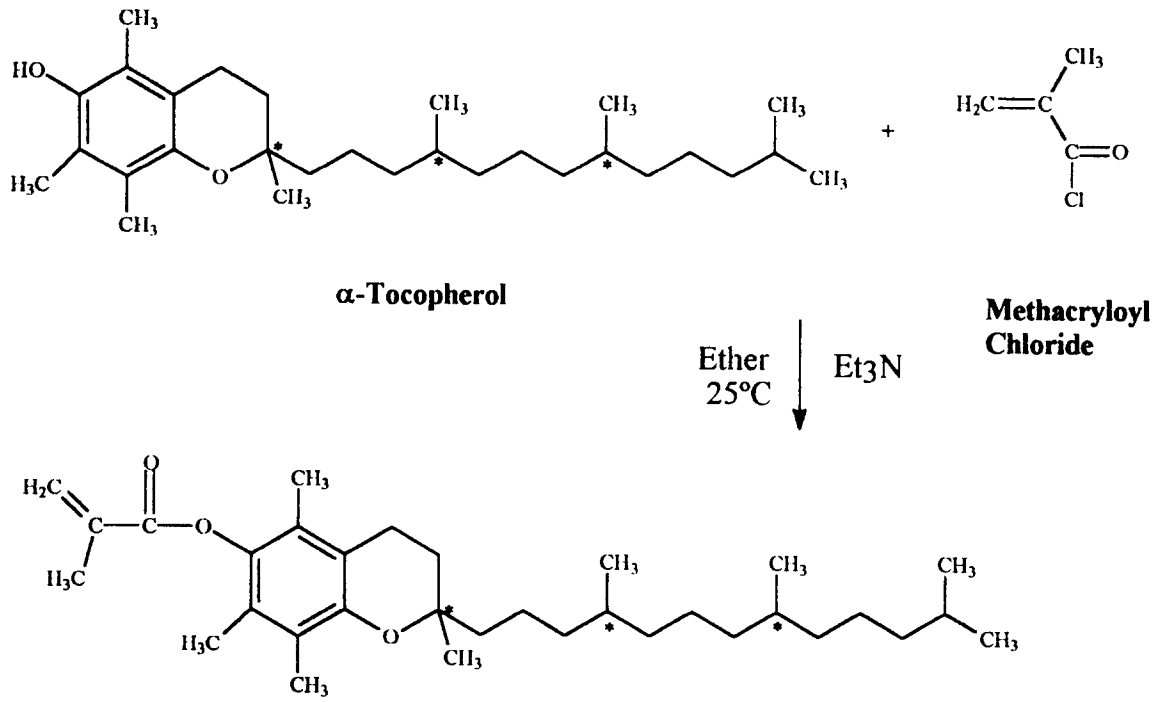
The methacrylic derivative of the vitamin E, V, was prepared by the reaction of α-tocopherol with methacryloyl chloride in the presence of triethylamine as catalyst with a yield of 74%. The chemical reactions are shown in *Scheme 1*. *Figure 1* shows the ^1H n.m.r. spectrum of α-tocopheryl methacrylate recorded at 80°C, where the signals due to the vinyl protons of the methacrylic residue can be clearly observed. The rest of the signals belonging to the α-tocopheryl residue were assigned by comparison with the spectrum of the α-tocopherol. *Figure 2* shows the ^{13}C n.m.r. spectrum of this monomer with the assignment of signals that were made by using the DEPT-135 (distortionless enhancement by polarization transfer) technique. The signals pertaining to the methacrylic residue can be clearly observed. The signals of the vinylic carbons appear at 137 and 126 ppm, the signal of the carboxylic group appears at 165 ppm and the signal corresponding to the $(CH_3)_\alpha$ appears at 19 ppm.

Kinetics of polymerization

The kinetics of the polymerization reaction of vitamin E methacrylate were studied in dimethylformamide at 50°C, and compared with that of 2-hydroxyethyl methacrylate. Conversions were determined gravimetrically. *Figure 3* shows the time–conversion relationship for both polymerizations, where straight lines with no induction time can be seen. The rate of polymerization can be computed from this slope. Considering the steady state, the rate equation can be expressed by[11]

$$R_p = \frac{K_p}{K_t^{1/2}} (2fK_d[I])^{1/2}[M] \quad (1)$$

where K_d, K_p and K_t are the rate constants for decomposition of AIBN, propagation and termination respectively, and f is the efficiency of the initiator (I). It is established that equation (1) holds for alkyl methacrylates whenever the conversion is below 10%[11], so that we can assume that this equation also holds for the vitamin E methacrylate. Regarding the hydroxyethyl methacrylate, it has been reported that when the polymerization reaction is carried out in thermodynamically good solvents, the reaction order with respect to the monomer has values similar to those found in the polymerization of analogous alkyl methacrylates[12]. In this study the polymerization reaction of hydroxyethyl methacrylate has been carried out in



Scheme 1

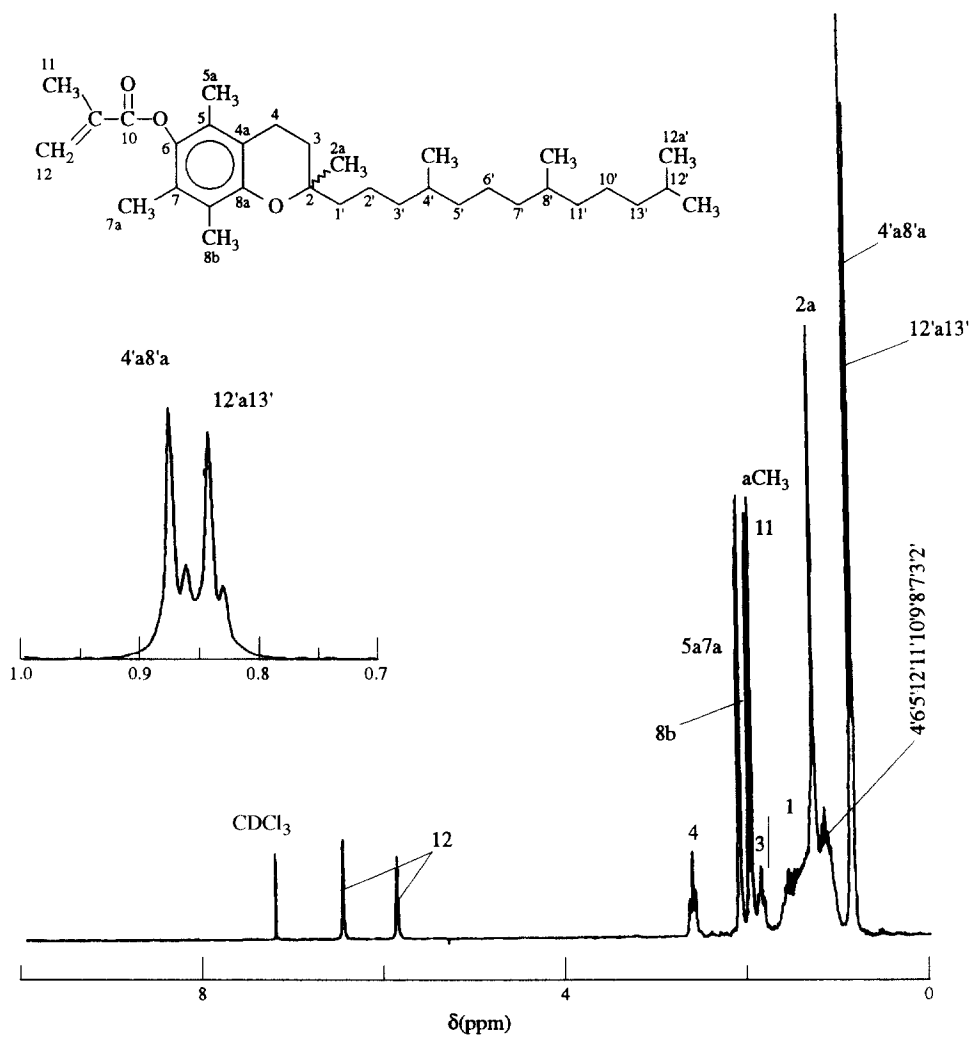


Figure 1 ^1H n.m.r. spectrum of α-tocopheryl methacrylate in deuterated chloroform at 80°C

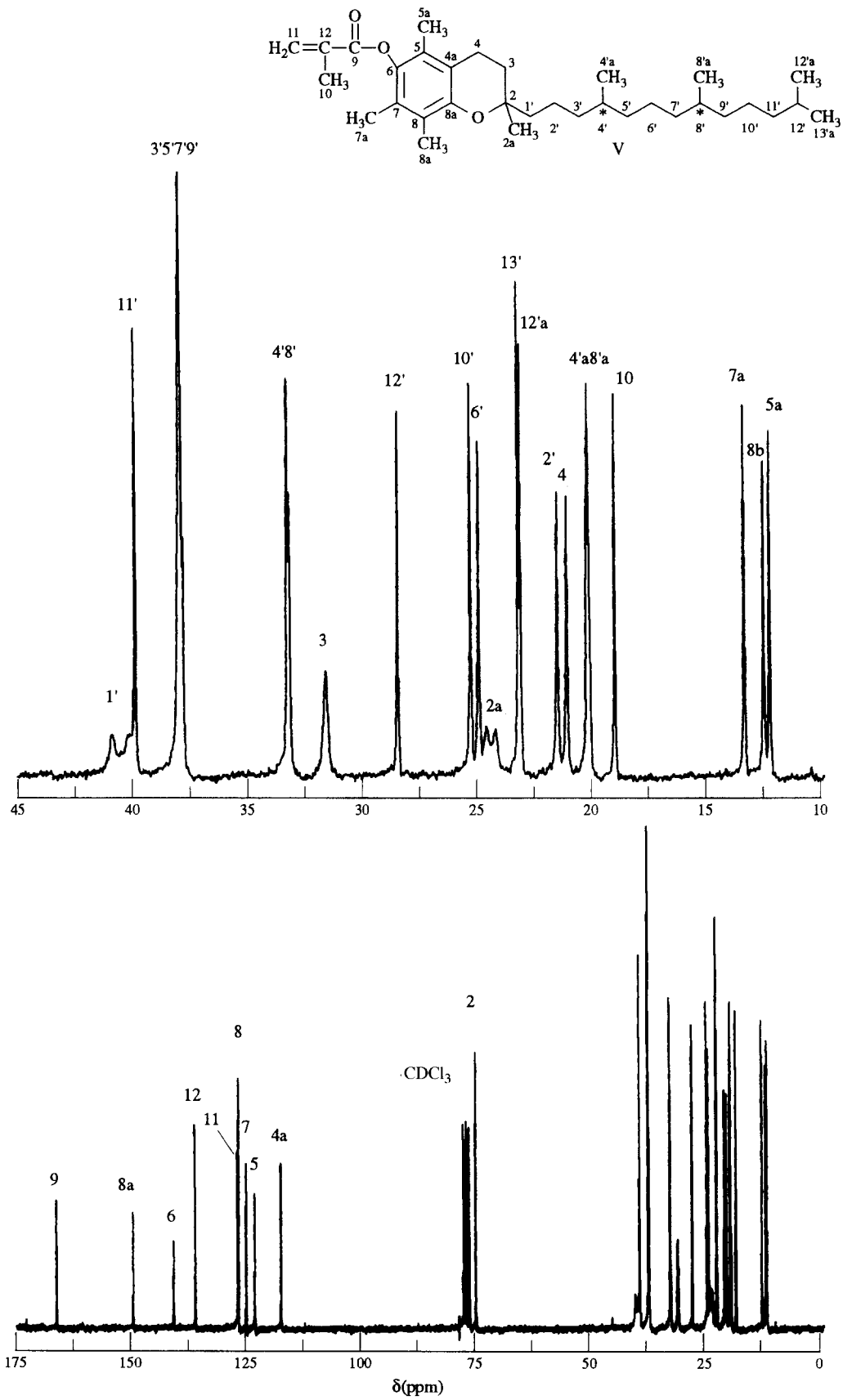


Figure 2 ^{13}C n.m.r. spectrum of α-tocopheryl methacrylate in deuterated chloroform at 80°C

dimethylformamide, in which a homogeneous medium is obtained. Thus, assuming equation (1) to be valid and using values of $K_d = 2.14 \times 10^{-6}\ s^{-1}$ [13] and $f = 0.6$, a value of $K_p/K_t^{1/2} = 0.15\ mol^{1/2}\ l^{-1/2}\ s^{-1/2}$ was calculated for the polymerization of α-tocopheryl methacrylate and a value of $0.27\ mol^{1/2}\ l^{-1/2}\ s^{-1/2}$ for the radical polymerization of 2-hydroxyethyl methacrylate. It is clear that the kinetic ratio for the system based on polyV is lower than that of the system based on polyH. This result could be explained by the fact that the K_t for the radicals ending in V is lower than that of the polyH radicals owing to the steric effect of the side group of α-tocopheryl. Then we can assume that both

K_p and K_t for polyH are higher than those for polyV but the increase in K_t is inferior to that of K_p.

2-Hydroxyethyl methacrylate/vitamin E methacrylate (H/V) copolymers

Copolymers of V and H were prepared in a composition interval between 5 and 20 mol% of the vitamin E derivative.

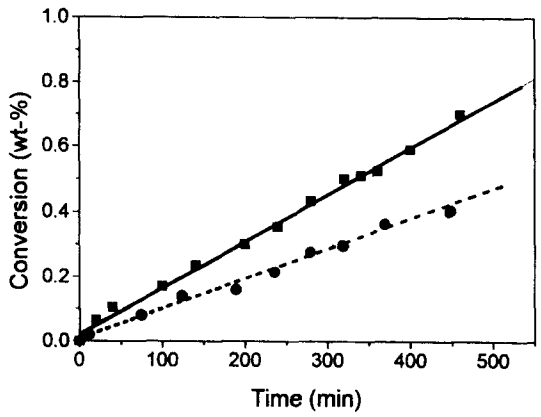


Figure 3 Time–conversion relationship for the radical polymerizations of V (●) and H (■) in dimethylformamide at 50°C: [AIBN] = 1.5 × 10^{-2} mol l^{-1}, [M] = 1 mol l^{-1}

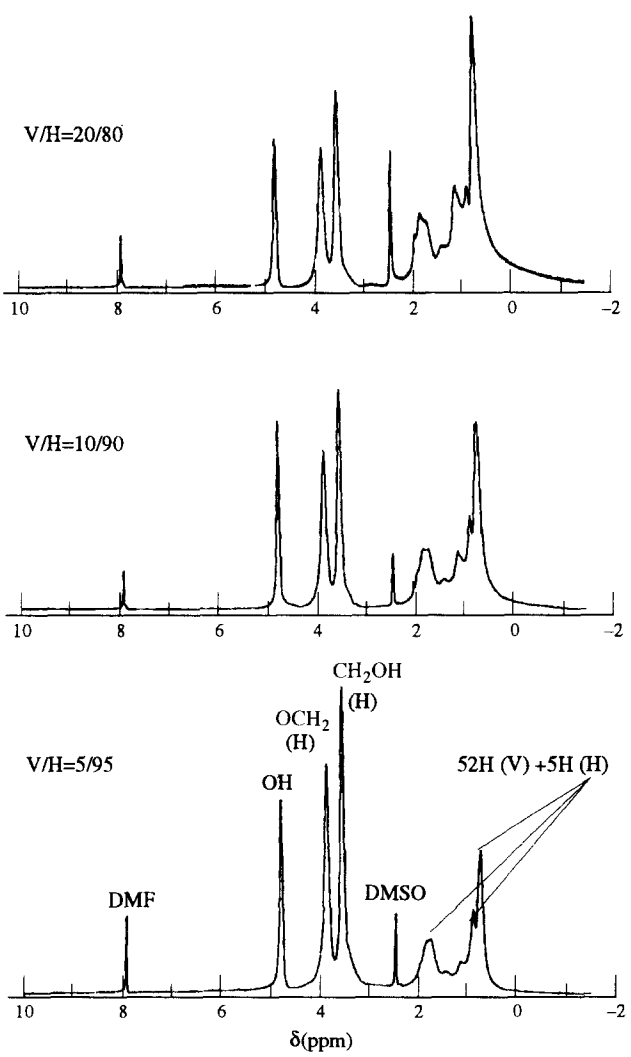


Figure 4 ^1H n.m.r. spectra of V/H copolymers of different composition (V/H molar ratio) in deuterated chloroform at 25°C

For compositions higher than 20 mol% of V monomer, a negligible amount of copolymer was obtained after 24 h of reaction time. This is consistent with the known antioxidant properties of the α-tocopheryl group. *Figure 4* shows the ^1H n.m.r. spectra of three copolymer samples with different compositions. The mole fraction of the corresponding monomers in the copolymer chains was calculated by considering the signals at 3.58 and 3.90 ppm assigned to the methylene protons of the $-OCH_2-$ and $-CH_2OH$ groups of the H monomer and the integration of the signals between 0.8 and 1.8 ppm corresponding to the rest of the protons at high field. The values of copolymer composition are reported in *Table 1*. The content of V monomer in the copolymer was superior to that in the feed in all cases.

The reactivity ratios r_V and r_H were calculated by using Fineman–Ross[14] and Kelen–Tüdõs[15] linearization methods as well as the application of the non-linear least squares analysis suggested by Tidwell and Mortimer[16]. *Figure 5* shows the diagrams obtained by the application of both linearization methods, where straight lines with good correlations can be observed. Also, the mathematical treatment suggested by Behnken[17] and Tidwell and

Table 1 Copolymer composition for the system V/H

f(V), feed	*f*(V), copolymer	Conversion (wt%)
0.030	0.05	5.42
0.060	0.10	4.82
0.075	0.12	4.73
0.100	0.16	4.62
0.125	0.19	4.30
0.150	0.22	3.80
0.175	0.25	3.75
0.200	0.27	3.90

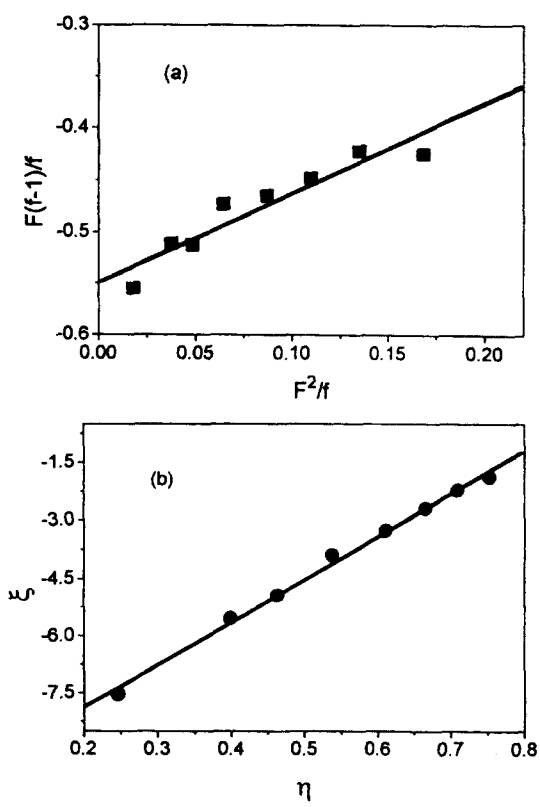


Figure 5 Diagrams of Fineman–Ross[14] (*a*) and Kelen–Tüdõs[15] (*b*) for the radical copolymerization of vitamin E methacrylate and 2-hydroxyethyl methacrylate in solution at 50°C initiated by AIBN

Mortimer[16] was applied to our system. This treatment provides the 95% confidence limit which gives an idea of the experimental error and the goodness of the experimental conditions used to calculate the composition data. The limits defined by the area of the elliptical diagram drawn in *Figure 6* show that all the reactivity ratios determined by the linearization methods and the non-linear approach are located in the central sector of the ellipse, and those that are approximately nearest the centre are the values determined by the non-linear least squares method by Tidwell and Mortimer. The values of r_V and r_H determined by the three methods are quoted in *Table 2*. The product $r_V \times r_H$ remains less than unity, indicating that the copolymers present a predominantly statistical distribution of monomeric units. Thus, the relative reactivity of growing chains ending in V or H are 1.40 and 1.87 respectively; so, both growing radicals present more reactivity towards the other monomer than towards its own monomer, this tendency being higher for the H-ending radical.

The values of number- and weight-average molecular weights, polydispersity and glass transition temperature of copolymers are shown in *Table 3*. All the copolymer systems analysed present high molecular weight and a molecular weight distribution near 2, according to a normal radical polymerization process. The change of the molecular weight with the average composition is insufficient to be able to detect a clear effect of the composition on the length of the macromolecular chains. Probably, the large size and low polarity of the α-tocopheryl side group present in the V molecules and propagating radicals could contribute to the steric hindrance for the termination reaction. However, the differences are in the limits of the experimental error associated with the SEC technique and the universal calibration applied.

The values of the glass transition temperatures T_g for poly(α-tocopheryl methacrylate) and poly(2-hydroxyethyl methacrylate) were 430 K and 358 K respectively. The higher value obtained for polyV is consistent with the steric effect of the large volume of the α-tocopheryl side group, which can prevent the free rotation and decrease the flexibility of the main chain. The values of T_g of the copolymers ranged between those of the corresponding homopolymers; however, it was observed that the incorporation of very low contents of V monomer in the copolymer caused an unexpected increase in the T_g of the copolymer with respect to that of polyH, if we take into consideration that the average T_g of the homopolymers is 394 K. This fact indicates the influence of the

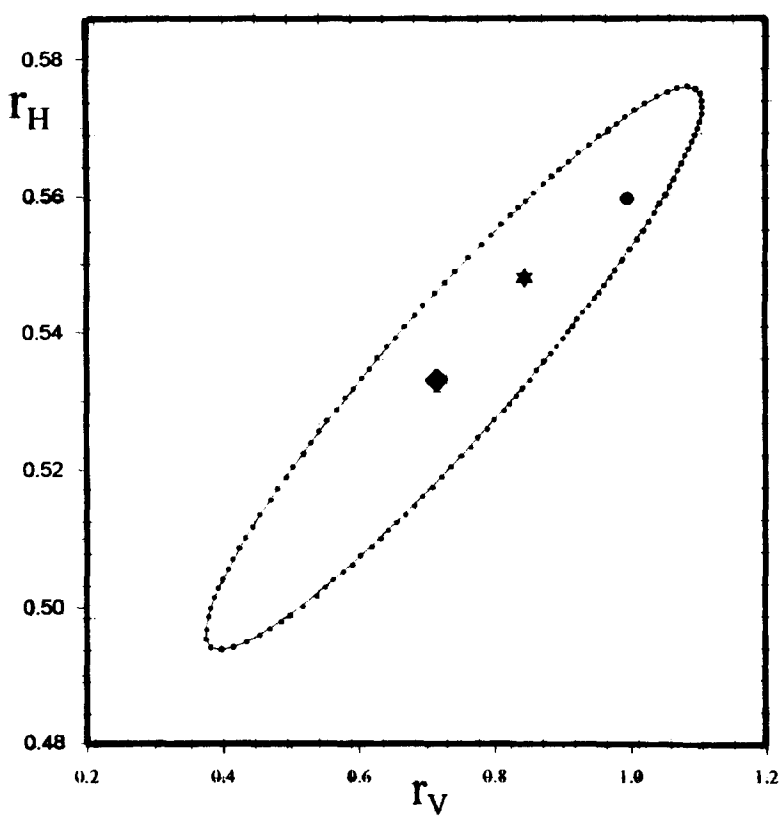


Figure 6 95%-confidence diagram for the reactivity ratios of V and H monomers, determined by the non-linear least squares method[16,17]. Values of reactivity ratios: (♦) Tidwell–Mortimer[16]; (★) Fineman–Ross[14]; (●) Kelen–Tüdõs[15]

Table 2 Values of reactivity ratios for the free radical copolymerization of vitamin E methacrylate and 2-hydroxyethyl methacrylate in solution at 50°C using AIBN as initiator: [AIBN] = 1.5×10^{-2} mol l^{-1}

Method	r_V	r_H	$1/r_V$	$1/r_H$	$r_V \times r_H$
Fineman–Ross[14]	0.85 ± 0.12	0.55 ± 0.01	1.18	1.81	0.46
Kelen–Tüdõs[15]	0.98 ± 0.10	0.55 ± 0.02	1.00	1.82	0.55
Tidwell–Mortimer[16]	0.72	0.53	1.4	1.87	0.37

Table 3 Values of number- and weight-average molecular weights, polydispersity and glass transition temperature for V/H copolymers prepared in a solution of dimethylformamide at 50°C using AIBN as initiator: [AIBN] = 1.5×10^{-2} mol l^{-1}

f(V), copolymer	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n	T_g (K)
0.05	105	218	2.06	365
0.1	125	230	1.84	374
0.18	140	263	1.87	378
0.27	158	350	2.21	394

sequential distribution of the copolymer on the glass transition temperature of the copolymer. In this respect different studies have been published. The effects of the monomer arrangement or sequence distribution on T_g have successfully been considered independently by Barton[18] and by Johnston[19]. The treatment proposed by Johnston is based on the 'free volume theory' and can be considered an extension of the pioneer work by Fox[20]. The linear expression due to Johnston can be expressed as

$$(1/T_g) - (\omega_1 P_{11}/T_{g11}) - (\omega_2 P_{22}/T_{g22}) = (1/T_{g12})(\omega_1 P_{12} + \omega_2 P_{21})$$

where T_g is the copolymer glass transition temperature, ω_1 and ω_2 are the average weight fractions of monomeric units 1 and 2 in the copolymer chains, T_{g11} and T_{g22} are the glass transition temperatures of the homopolymers and T_{g12} that of the alternating copolymer, and P_{11}, P_{12}, P_{21} and P_{22} refer to the probabilities of having various linkages.

In contrast, Barton has proposed an equation based on the 'entropic theory' by extension of the pioneering suggestion of DiMarzio and Gibbs[21] taking into consideration the T_g of both homopolymers and that of the alternating copolymer, T_{g12}, together with the average composition and sequence distribution specified by the mole fractions of diads 11, 12 or 21 and 22.

Figure 7a and *b* shows the linear diagrams obtained from the application of experimental data to both Johnston's (*a*) and Barton's (*b*) treatments. From the slopes of the straight lines the values of $T_{g12} = 417$ K and 421 K were obtained respectively, showing a good agreement between themselves. It is clear from the T_{g12} values that the alternating diad 12 or 21 presents a glass transition temperature higher than the arithmetical average of the T_g of both homopolymers (*Table 4*). The physical significance of these results is that the alternating diad shows a lower flexibility than that expected from the combination of both kinds of monomeric unit. This indicates that the flexibility of the alternating diad is strongly influenced by the bulky tocopheryl side group present in the methacrylic monomer derived from the vitamin E. The stiffness of polymeric segments is a property of great importance in determining the interactions of the active functional groups with the surrounding medium. This is of particular importance in biological applications because of the selectivity of macromolecular compounds, like proteins, enzymes, polysaccharides, etc., in biochemical reactions produced in the physiological medium. Moreover, it is believed that the oxidative protection of vitamin E, and, therefore, its anti-ageing effect, is associated with the deposition and interaction of vitamin E derivatives into the cytoplasmatic membrane of cells. This is probably the reason for the administration of vitamin E as alkyl esters like acetate.

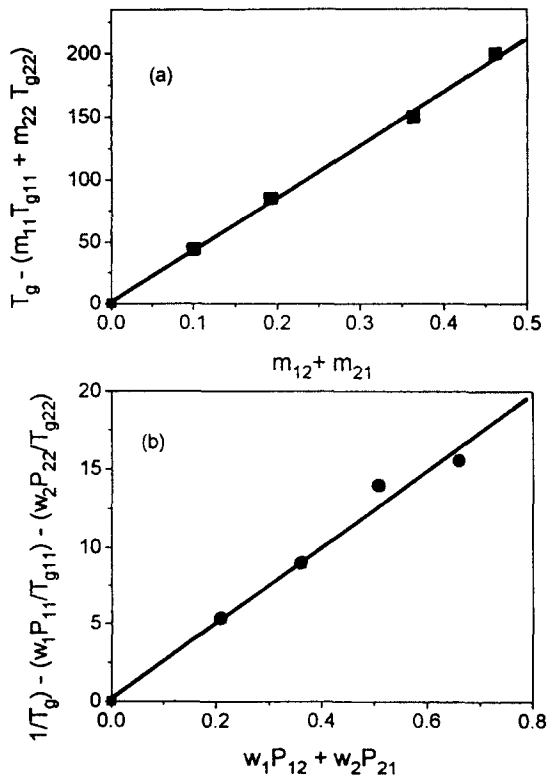


Figure 7 Application of the Barton[18] (*a*) and Johnston[19] (*b*) treatments to V/H copolymers

Table 4 Values of glass transition temperature of the poly(vitamin E methacrylate) T_g(V), poly(2-hydroxyethyl methacrylate) T_g(H) and those of the alternating diad T_g(HV) obtained by the Barton[18] and Johnston[19] treatments

T_g(H)	358
T_g(V)	430
$[T_g(H) + T_g(V)]/2$	394
T_g(HV)	
Barton's treatment[18]	417
Johnston's treatment[19]	421

As is clearly observed in *Table 4*, the introduction of H units in macromolecular polyacrylic derivatives of vitamin E not only provides molecular systems of relatively high stability. It also provides a support with hydrophilic character, which means better transport through the extracellular medium and higher flexibility than the pure poly(vitamin E methacrylate). This improves the capability of copolymer systems proposed with the appropriate composition of V and H units to interact with the cytoplasmatic wall of cellular systems. We are studying this point by the application of 20:80 V/E copolymer systems in the regeneration of the Achilles tendon in rabbits. The preliminary results obtained indicate a clear activation of the healing process of the tendon as a consequence of the cellular stability promoted by the vitamin E system with excellent biocompatibility in comparison with control animals.

ACKNOWLEDGEMENTS

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REFERENCES

1. Roberts, R. J., in *Advances in Pharmacology and Therapeutics, Vol. 8, Drug-Action Modifications Comparative Pharmacology*, ed. G. Olive. Pergamon Press, Oxford, UK, 1979, p. 53.
2. Fridovich, I., in *Free radicals in Biology*, Vol. I, ed. W. A. Pryor. Academic Press, New York, 1976, p. 239.
3. McCay, P. B., Gibson, D., Fong, K. and Hornbrook, K. R., *Biochim. Biophys. Acta*, 1976, **431**, 459.
4. Diplock, A. T. and Lucy, J. A., *FEBS Lett.*, 1973, **29**, 205.
5. Tierney, D. F., Ayers, L. and Kasuyama, R. S., *Annu. Rev. Resp. Dis.*, 1977, **115**, 59.
6. Whitting, L. A., *Am. J. Clin. Nutr.*, 1974, **27**, 952.
7. Graeber, J. E., Williams, M. L. and Oski, F. A., *J. Peds.*, 1977, **90**, 282.
8. Johnson, L., Schaffer, D. and Boggs, T. R., *Am. J. Clin. Nutr.*, 1974, **27**, 1158.
9. Ehrenkranz, R. A., Bonta, B. W., Ablow, R. C. and Warshaw, J. B., *Ped. Res.*, 1980, **11**, 569.
10. Fort, R. J. and Polyzoidis, T. M., *Eur. Polym. J.*, 1976, **12**, 685.
11. Odian, G., *Principles of Polymerization*, 3rd edn. Wiley Interscience, New York, 1992.
12. Wichterle, O., in *Encyclopedia of Polymer Science and Engineering*, Vol. 15, ed. H. F. Mark, N. G. Gaylord. Interscience, New York, 1971, p. 273.
13. Tulig, T. J. and Tirrel, M., *Macromolecules*, 1982, **15**, 459.
14. Fineman, M. and Ross, S. D., *J. Polym. Sci.*, 1950, **5**, 259.
15. Kelen, T. and Tüdõs, F., *J. Macromol. Sci. A*, 1975, **9**, 1.
16. Tidwell, P. W. and Mortimer, G. G. A., *J. Polym. Sci. A*, 1965, **3**, 369.
17. Behnken, D. W., *J. Polym. Sci. Part A: Polym. Chem. Ed.*, 1964, **2**, 645.
18. Barton, J. M., *J. Polym. Sci. Polym. Symp.*, 1970, **30**, 530.
19. Johnston, N. W., *J. Macromol. Sci. Rev. Macromol. Chem. C*, 1976, **14**, 215.
20. Fox, T. G., *Bull. Am. Phys. Soc.*, 1956, **1**, 123.
21. DiMarzio, E. A. and Gibbs, J. H., *J. Polym. Sci.*, 1959, **40**, 121.