

Photocuring of cinnamic carbohydrate esters: Controlling the reaction by monocinnamoylated derivatives

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Abstract

A monofunctional and a polyfunctional monomer were studied for use as potential co-photopolymers. The monomers were the totally cinnamoylated derivatives of *p*-nitrophenol (PNPCN) and D-manitol (MNTCN), and their structures were characterized by several spectroscopic techniques. The derivatives obtained were cross-linked by irradiation in the ultraviolet region, where the photosensitive monomers showed maximum sensitivity. The thermal properties of these photosensitive monomers and the mixtures prepared with different MNTCN:PNPCN ratios were investigated by differential scanning calorimetry under nitrogen atmosphere, and their photoreactivity was investigated in thin films. Photoreaction products were analyzed by gel permeation chromatography. The effect of NPCN as a chain blocker in the MNTCN photocrosslinking reaction was evaluated by UV–visible spectroscopy and by ¹H NMR.

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1. Introduction

The crosslinking and subsequent insolubilization of photosensitive polymers is one of the oldest photochemical reactions (Delzenne, 1972; Delzenne, 1974). The first synthetic photopolymer used, which solved the problems associated with natural photopolymers (Delzenne, 1974; Egerton et al., 1981; Minsk, Smith, Van Deusen, & Wright, 1959; Tsuda, 1964), was polyvinyl cinnamate (PVCN) (Minsk et al., 1959), which, in turn, was replaced by a broad class of photopolymers based on cycloadditions (Delzenne, 1972; Williams, Laakso, & Borden, 1971). The polymers (olefins) are insolubilized by cyclodimerization of two cinnamoyl groups (excited chromophores) belonging to two different chains by means of a “four-centre” polymerization step (Wendell, 1983), involving the absorption of light energy (Ranby, 1998). This photoreaction

takes place in crystalline state, providing polyesters that contain units of cyclobutane. The carbonyl group provides the double reactive bond with the necessary polarization and the phenyl group increases the polarisability, thus increasing the chromophore’s power to absorb light (Williams, 1974). The presence of truxylic rather than truxinic acid in the crosslinked PVCN requires stereoregularity between chains (Reiser, 1980). The light-induced photoreaction and molecular reorientation of thin polymer films containing cinnamoyl groups depend on the regioisomerism of the these groups (Chae, Lee, Kim, Lee, & Ree, 2003a; Chae, Lee, Ree, Jung, & Kim, 2003b; Ichimura, Akita, Akiyama, Kudo, & Hayashi, 1997; Lee et al., 2003; Lin, Chu, Huang, & Reiser, 1992). In recent times, the synthesis of monomers and polymers that contain photoreactive groups has received fresh attention because of their potential use in emerging fields of microelectronics, liquid crystal screens, holographic head-up-displays, nonlinear-optical materials, integrated circuits, etc. (Balaji, Grande, & Nanjundan, 2004; Chae et al., 2003a, 2003b; Lee et al., 2003). Monomers and polymers with

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α,β -unsaturated carbonyl groups, such as cinnamoyl groups, possess a high degree of photosensitivity, good pre-irradiation solubility, the potential to form thin films, resistance to solvents, plasmas and strong acids after crosslinking, and good thermal stability; they undergo crosslinking as a result of UV radiation and can be used as negative-type photoresists.

In previous studies we described the use of crosslinked cinnamic carbohydrate esters for immobilising different enzymes (García-Ruiz, 2000; Marín-Zamora, Rojas-Melgarejo, García-Cánovas, & García-Ruiz, 2005; Rojas-Melgarejo, Marín-Iniesta, Rodríguez-López, García-Cánovas, & García-Ruiz, 2006; Rojas-Melgarejo, Rodríguez-López, García-Cánovas, & García-Ruiz, 2004a, Rojas-Melgarejo, Rodríguez-López, García-Cánovas, & García-Ruiz, 2004b), and the dependence of the immobilized enzymatic activity on the crosslinking density (ρ) reached by the polymer during its irradiation (Rojas-Melgarejo, Rodríguez-López, García-Cánovas, & García-Ruiz, 2004c). After irradiation, the synthesized polymers that act as carriers of reactive groups (Fréchet, 1981) are insoluble in the aqueous medium used for enzyme reaction and/or storage.

Small variations in irradiation time provoke substantial changes in the crosslinking density and immobilized enzymatic activity attained, so that a method is needed to control the process and to provide more reproducible results. This problem can be solved by preparing mixtures of the immobilization support and an agent to limit the crosslinking density, regardless of the irradiation time and conditions used, thus controlling any increase in molecular weight and support branching. In this way, the physical-chemical characteristics (pore size and hydrophobic–hydrophilic ratio) of the final polymer obtained (Rojas-Melgarejo et al., 2004a) can be suited to the properties of the enzyme to be immobilized and to the specific application for which it is destined, and it will be possible to limit the cinnamoyl groups available to react in an uncontrolled way and thus modify the physical-chemical characteristics of the initial support obtained. For this reason, the principal aim of this study was to control the photocrosslinking process that takes place in monomers containing photoreactive cinnamoyl groups in an attempt to produce polymers for a specific application. In the present work, we used the totally cinnamoylated derivative of D-manitol (MNTCN) as monomer for crosslinking through the action of UV light, and *p*-nitrophenol (PNPCN) cinnamate as a chain blocker of the photocrosslinking reaction.

2. Materials and methods

2.1. Materials

D(–)-Manitol was supplied by Merck (Germany), while pure *p*-nitrophenol (PNP) and *trans*-cinnamic acid chloride were purchased from Sigma Chemical Co. (Spain). All other chemicals were of analytical grade and were supplied by Panreac Chemical, S.A. (Spain). Ultrapure water from a

Milli-Q system (Millipore Corp.) was used throughout this research.

2.2. Synthesis

2.2.1. Preparation of the totally cinnamoylated derivative of D-manitol (MNTCN)

The preparation followed a modified version of the method proposed by Van Cleve (Van Cleve, 1963), in which 0.02 mol (3.6 g) of D-manitol was dissolved in 100 ml of pyridine. The mixture was heated at 60 °C for 1 h to ensure complete dissolution. After cooling to room temperature, 0.15 mol (24.0 g) of cinnamic acid chloride was added. The reaction was allowed to proceed at room temperature for 4 h, after which the resulting mixture was poured into vigorously stirred cool water. The precipitate obtained, after decanting and filtering this mixture, was dissolved in chloroform and purified by adding, one drop at a time, to vigorously shaken hexane. The solid obtained was redissolved and reprecipitated before being dried on P₂O₅ at reduced pressure (yield: 76.4); mp = 98–99 °C (Fig. 1).

FT-IR (KBr, cm^{–1}): 1719 (C=O ester in the cinnamoyl groups); 1632 (vinylene C=C stretching vibration in the cinnamoyl groups); 1578, 1508, 1491 and 1449 (C=C stretching vibration of the benzene ring in the cinnamoyl groups); 1382; 1328; 1312; 1286; 1267; 1252; 1203, 1149 and 1069 (C–O ester stretching in the cinnamoyl groups); 1026; 984 (*trans*-vinylene C–H deformation vibration in the cinnamoyl groups), 870; 766 and 706 (=C–H out-of-plane bending of the benzene ring), 676. ¹H NMR (CDCl₃, δ in ppm): 4.39–4.73 (m, 2+2H, >CH–CH₂–O), 5.60–5.62 (m, 2H, >CH–CH–CH₂–), 5.91–5.93 (m, 2H, >CH–CH–CH<), 6.40–6.57 (m, 6H, Ar–CH=CH–), 7.24–7.59 (m, 30H, ArH), 7.67–7.88 (m, 6H, Ar–CH=). The integration signals corresponded to totally esterified derivatives. The absence of hydroxyl groups was confirmed by analysing samples to which deuterated water had been added; ¹³C NMR (CDCl₃, δ in ppm): 62.16 (>CH–CH₂–OOC–), 68.63 (>CH–CH₂–), 69.35 (>CH–CH–CH<), 116.67

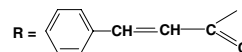
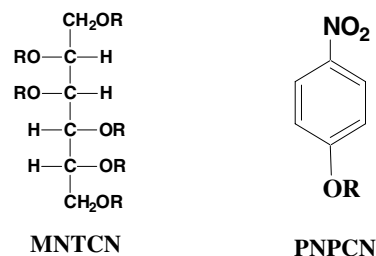


Fig. 1. Schematic representation of the structure of the photocrosslinkable monomers, MNTCN and PNPCN.

($-\text{CH}=\text{CH}-\text{COO}-$), 117.00 ($-\text{CH}=\text{CH}-\text{COO}-$), 117.21 ($-\text{CH}=\text{CH}-\text{COO}-$), 128.32 (ArH), 129.02 (ArH), 131.23 (ArH), 133.65 ($\text{Ar}-\text{CH}=\text{CH}$), 145.62 ($\text{Ar}-\text{CH}=\text{CH}$), 146.08 ($\text{Ar}-\text{CH}=\text{CH}$), 146.65 ($\text{Ar}-\text{CH}=\text{CH}$), 165.43 ($-\text{COO}-\text{CH}_2-$), 165.56 ($-\text{COO}-\text{CH}<$), 166.30 ($-\text{COO}-\text{CH}<$).

2.2.2. Preparation of the cinnamoylated derivative of *p*-nitrophenol (PNPCN)

To prepare this photoreactive monomer, 0.02 mol (2.8 g) of *p*-nitrophenol was reacted with 0.03 mol (4.0 g) of cinnamic acid chloride. The reaction and purification conditions were similar to those described in the previous section for MNTCN (yield: 78.2); mp = 145–146 °C (Fig. 1).

FT-IR (KBr, cm^{-1}): 1739 ($\text{C}=\text{O}$ ester in the cinnamoyl groups); 1634 (vinylene $\text{C}=\text{C}$ stretching vibration in the cinnamoyl groups); 1597 ($\text{C}=\text{C}$ stretching vibration of the benzene ring in the cinnamoyl groups); 1523 (NO_2 asym. stretching); 1494; 1352 (NO_2 sym. stretching); 1311; 1221 and 1147 ($\text{C}-\text{O}$ ester stretching in the cinnamoyl groups); 980 (*trans*-vinylene $\text{C}-\text{H}$ deformation vibration in the cinnamoyl groups); 871; 853; 769 and 707 ($=\text{C}-\text{H}$ out-of-plane bending of the benzene ring); 678. ^1H NMR (CDCl_3 , δ in ppm): 6.60–6.66 (d, 1H, $\text{Ar}-\text{CH}=\text{CH}-$), 7.38–7.40 (d, 2H, $\text{H}_m\text{Ar}-\text{NO}_2$), 7.44–7.46 (m, 3H, ArH), 7.59–7.62 (m, 2H, ArH), 7.89–7.95 (d, 1H, $\text{Ar}-\text{CH}=\text{CH}-$), 8.27–8.32 (d, 2H, $\text{H}_o\text{Ar}-\text{NO}_2$). The integration signals corresponded to totally esterified derivatives. The absence of hydroxyl groups was confirmed by analysing samples to which deuterated water had been added; ^{13}C NMR (CDCl_3 , δ in ppm): 116.2 ($-\text{CH}=\text{CH}-\text{COO}-$), 122.3 ($\text{H}_m\text{Ar}-\text{NO}_2$), 125 ($\text{H}_o\text{Ar}-\text{NO}_2$), 128.3 (ArH), 129 (ArH), 131 (ArH), 133.8 ($\text{Ar}-\text{CH}=\text{CH}$), 147.8 ($\text{Ar}-\text{CH}=\text{CH}-$ and $\text{Ar}-\text{NO}_2$), 155.6 ($-\text{COO}-\text{Ar}-\text{NO}_2$), 164.1 ($-\text{CH}=\text{CH}-\text{COO}-$); e.m.; m/z (%): 270 (M^+ , 0.3), 132 (8.9), 131 (100), 103 (45.7), 101 (10.6), 77 (27.7), 63 (8.0), 51 (8.1).

2.3. Instruments

The UV–visible spectra were obtained with a UV/visible UNICAM 8625 spectrophotometer. Analyses and measurements were made in quartz cuvettes. Infrared spectra were obtained in a Nicolet Impact 400 spectrophotometer. The cinnamoyl ester derivatives were prepared as thin films on KBr plates. The ^1H NMR spectra were obtained in a Bruker 400 MHz UltraShield™ spectrophotometer provided with broad band probe at room temperature, with tetramethylsilane (TMS) as internal standard and using CDCl_3 as solvent. The proton decoupled ^{13}C NMR spectra were obtained in the same instrument and probe, operating at 100.82 MHz. The GC–MS analysis was made in a Hewlett Packard 5980-A gas chromatograph coupled to a Hewlett Packard 5710-A mass spectrometer. The samples were introduced by means of solid mass accessories, and the electron impact technique used an ionization energy of 70 eV. The data obtained were expressed as units of mass (m/e) and the relative abundance of each fragment is given

as a percentage of the spectrum base peak. The weight-average molecular weights (\overline{M}_w) of the irradiated samples were measured by using a Waters gel permeation chromatography (GPC) apparatus equipped with a set of two columns (Waters Styragel® HR1 and HR3 columns: effective molecular-weight range of 100–5000 and 500–30,000, respectively) and calibrated with poly(methyl-methacrylate) standards of 2580–31,600 \overline{M}_w . In these measurements, a flow rate of 1.0 ml min^{-1} was employed and THF was used as the eluent. Differential thermal analysis was performed on a differential scanning calorimeter (model TGA-DSC 2920). The measurements were carried out over the temperature range of 10–300 °C at a heating rate of 10 °C min^{-1} under a flow (5 ml min^{-1}) of dry N_2 .

2.4. Films preparation

An emulsion was prepared from a solution (25 g l^{-1}) of each of the MNTCN:PNPCN (mol:mol) mixtures being studied in chloroform (Table 1), by adding dropwise over a transparent quartz cuvette at room temperature. The desired solid film was formed as the solvent evaporated (Rojas-Melgarejo et al., 2004c).

2.5. Photoreactivity studies

All the assays were carried out under the same light intensity, using an Osram HOL-125W mercury vapour lamp without its external glass in order to obtain maximum intensity in the ultraviolet region, which coincides with that of the maximum sensitivity of the monomers obtained. The lamp was placed $\approx 17 \text{ cm}$ from the film being irradiated, which is equivalent to providing a power of 1.6 mW cm^{-2} , as determined by a Nova-Laser power/energy monitor (OPHIR Optronics Ltd.). The monomer samples were irradiated for different times (0–110 min) at room temperature. Immediately after irradiation, the UV–visible spectrum of the crosslinked polymer film obtained was recorded in the 240–340 nm range. In the work conditions used

Table 1
Composition data for the mixtures prepared with MNTCN and PNPCN

Ratio ^a	m_1^b	m_2^b	$m_{>\text{C}=\text{C}}^c$
[1:0]	0.0260	–	0.1558
[3:1]	0.0238	0.0079	0.1507
[2:1]	0.0228	0.0114	0.1482
[1:1]	0.0203	0.0203	0.1421
[1:2]	0.0167	0.0333	0.1335
[1:3]	0.0141	0.0426	0.1272
[1:4]	0.0123	0.0490	0.1228
[1:5]	0.0108	0.0541	0.1189
[1:6]	0.0098	0.0577	0.1167

Final concentration of 25 g l^{-1} .

^a MNTCN:PNPCN molar ratios.

^b m_1 , m_2 : MNTCN and PNPCN mol, respectively, in the mixtures.

^c Photoreactive double bonds mol in the films.

(constant irradiation intensity and transparent support), the crosslinking density (ρ , so much per unity of crosslinked monomer units) is independent of the initial concentration of sample (Reiser, 1980), which made the supports easier to prepare and guaranteed that the results would be comparable and reproducible. In these conditions, the crosslinking density and irradiation time (t) fulfil the equation:

$$\rho/t = 2.303 \times I \times \phi \times \varepsilon \times M_0 \quad (1)$$

where I is the intensity of illumination, ϕ is the quantum yield, ε is the gram extinction coefficient and M_0 is the initial molecular mass of the polymer (Egerton, Pitts, & Reiser, 1981; Lin et al., 1992). The fraction of photocrosslinked bonds was monitored using the following equation (Balaji et al., 2004):

$$\rho = (A_0 - A_t)/(A_0 - A_\infty) \quad (2)$$

where A_0 , A_t and A_∞ are the absorbance intensities at 280 nm due to the double bond of the photosensitive group after irradiation times $t = 0$ (non-irradiated sample), $t = t$ and $t = \infty$, respectively, obtained from the UV–visible spectra recorded. Similarly, the photochemical reaction can be followed by ^1H NMR spectroscopy, observing the disappearance of peaks at $\delta = 6.40\text{--}6.66$ ppm and $7.67\text{--}7.95$ ppm of the corresponding spectra in samples exposed to different irradiation times. In all cases, the results were obtained as mean value of three different samples. The reaction rate (v) was calculated using the first derivative ($\text{d}\rho/\text{d}t$) of the equation obtained after fitting the representation of crosslinking density (ρ) vs irradiation time ($t_{\text{irrad.}}$, min). To quantify the reaction rate, one unit of photocrosslinking (U) was defined as the fraction of monomer units crosslinked per minute in the above mentioned irradiation conditions and at 25°C .

3. Results and discussion

3.1. Thermal properties

Differential scanning calorimetry analysis (DSC) of the totally cinnamoylated derivatives corroborated the melting point data obtained for both non-irradiated monomers. In the case of PNPCN, peaks corresponding to melting and solidification can be seen in successive heating ramps. In the case of MNTCN, thermal crosslinking led to no melting point being observed in the second heating cycle. For samples prepared with different MNTCN:PNPCN ratios, peaks of heat absorption were observed in all cases at short irradiation times, although these were not observed in subsequent cycles. Such a peak appeared at a slightly higher temperature (131°C) in the [1:4] preparation than in those prepared at [1:1] (111°C) or [1:3] (130°C) or [1:6] (127°C). At long irradiation times small peaks were observed at $168\text{--}169^\circ\text{C}$, particularly in the [1:4] sample. The results described in this section reflect the formation of a greater or lesser quantity of impeded residues (see Section 3.2.2).

3.2. Photochemical properties

3.2.1. Variations in the ultraviolet and ^1H NMR spectra

For this analysis, three samples of each of the MNTCN:PNPCN (mol:mol) mixtures being studied were prepared (Table 1). The influence of irradiation on the photoresponse of the monomers was examined by measuring changes in the absorption intensity UV ($\lambda = 280$ nm) at several irradiation times. Spectra were obtained (Fig. 2) in which isobestic points were observed at 205 and 327 nm during the initial stages of UV irradiation due to the *trans*–*cis* photoisomerization of the double bonds (Chae et al., 2003b; Ichimura et al., 1997). In addition, the absorption intensity at 280 nm decreased rapidly and almost totally disappeared after 10 min of irradiation. However, with increasing concentrations of PNPCN, the time required for the reaction to finish also increased. For example, while 30 min were necessary for completion with MNTCN films, this time rose to 50 and 110 min for the [1:1] and [1:6] MNTCN:PNPCN ratios, respectively. For an irradiation time of 0.5 min, MNTCN showed a conversion degree of 35%, while, in the presence of PNPCN ([1:1] ratio), conversion was only 14% and as low as 5% for the [1:6] mixture. Such behaviour was similar for all the radiation times assayed. For an irradiation time of 25 min (final stages of the photocrosslinking process) the reaction was almost totally completed in the case of MNTCN films (99%), while in the case of the [1:1] mixture, conversion was 88% and only 71% at [1:6]. The decrease in absorbance intensity at 280 nm following irradiation reflected the disappearance of double bonds due to the formation of cyclobutane rings by cycloaddition [$2\pi + 2\pi$] of

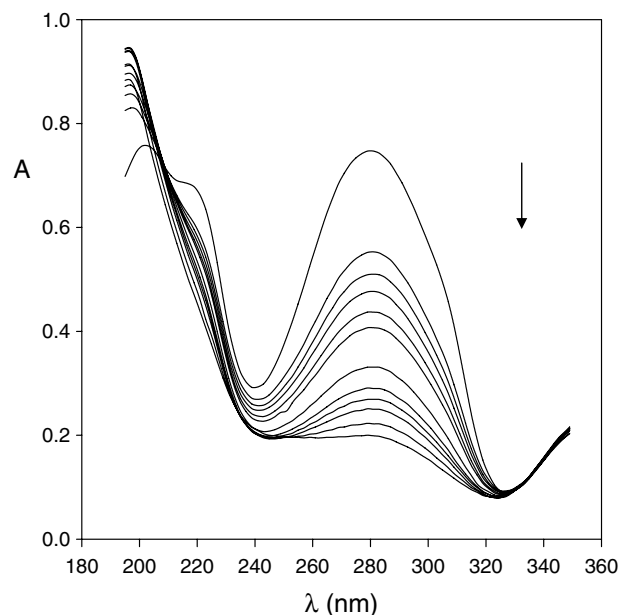


Fig. 2. Changes in the UV spectral patterns of MNTCN in thin film upon irradiation. Top to bottom, after irradiation time $t = 0, 10, 20, 30, 45, 60, 120, 180, 240, 300, 450$ and 600 s.

the cinnamoyl groups present in adjacent positions. These destroy the conjugation in the π -electron system, provoking the final formation of the polymer. Both monomers react photochemically in a similar way to that observed for cinnamic acid and its derivatives (Balaji et al., 2004; Oriol, Piñol, Serrano, & Tejedor, 2003). The value of maximum absorption ($\lambda = 280$ nm) was used to calculate the crosslinking density (ρ) according to Eq. (2), or the fraction of crosslinked monomers. The representation of ρ versus irradiation time shows that crosslinking density behaves logarithmically with respect to this time (Fig. 3), which agrees with the results of other authors (Reiser, 1980; Reiser, 1989). Note the greater variation in the ρ values calculated for the shorter irradiation times, while any variation was much less at longer times. However, when the concentration of PNPCN was increased, variations in the initial stages of the process were less pronounced, revealing how low concentrations of this monocinnamoylated derivative slow down the photocrosslinking reaction, probably by preventing the MNTCN chains from coming together. The slopes of the straight lines traced tangentially to the curve for a given irradiation time provide the photocrosslinking reaction rates at different times of the process. The representation of the residual reaction rate (v_R , %) versus irradiation time illustrated the differences between the various photopolymer films prepared (Fig. 4). In all cases, the reaction rate tended to zero for irradiation times in excess of 10 min (Fig. 4; inset). The beginning of the photocrosslinking reaction and the rate depended on the concentration of PNPCN in the prepared sample. For example,

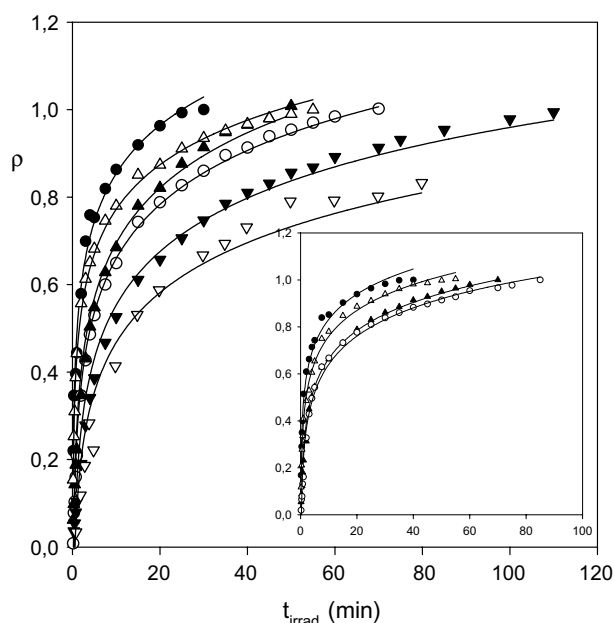


Fig. 3. Effect of irradiation time on crosslinking density in samples prepared with different proportions of MNTCN:PNPCN: (●) [1:0], (△) [2:1], (▲) [1:1], (○) [1:5], (▼) [1:6] and (▽) [0:1]. Inset: (●) [3:1], (▲) [1:2], (○) [1:3] and (△) [1:4]. Representation of crosslinking density, ρ , vs irradiation time, $t_{\text{irrad.}}$ (min). Data obtained by UV–visible spectroscopy.

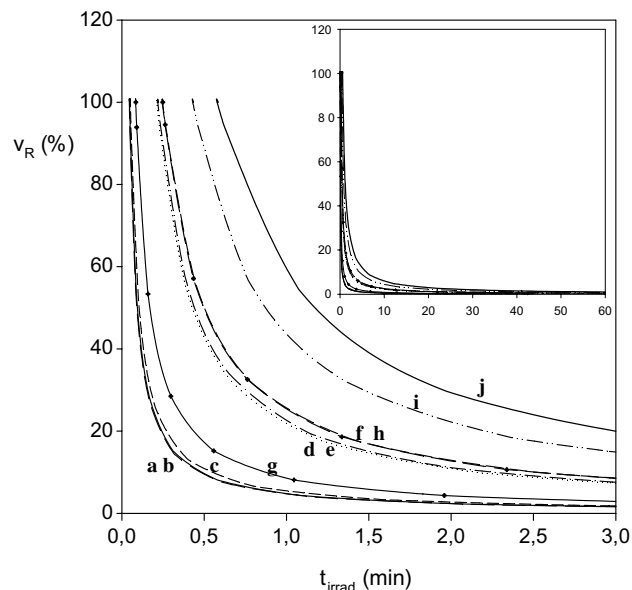


Fig. 4. Effect of irradiation time on residual photocrosslinking rate in samples prepared with different proportions of MNTCN:PNPCN: (a) [1:0], (b) [3:1], (c) [2:1], (d) [1:1], (e) [1:2], (f) [1:3], (g) [1:4], (h) [1:5], (i) [1:6] and (j) [0:1]. Representation of residual photocrosslinking rate, v_R (%), vs irradiation time, $t_{\text{irrad.}}$ (min). The residual reaction rate (v_R , %) was calculated taking the initial reaction rate (v_0 , 100%) to be that calculated for an irradiation time ($t_{\text{irrad.}}$, min) required to obtain a ρ value of zero in the corresponding fitted equation. Data obtained by UV–visible spectroscopy.

the films prepared with [3:1] and [2:1] ratios showed similar behaviour to the sample containing no blocking PNPCN groups ([1:0]), while those prepared with a higher proportion of PNPCN ([1:1], [1:2] and [1:3]) all showed similar behaviour to each other, but lower reaction rates and a lag period at the beginning of the reaction five times greater than that observed in the above mixtures with a lower proportion of PNPCN. With the addition of increasing quantities of PNPCN ([1:4] and [1:5] ratios), mixed photocrosslinking became the main reaction and the polymerization degree (DP) diminished, while the photopolymerization rate increased, although the number of photoreactive units in the reaction mixture was lower (Table 1), since the greater mobility of the PNPCN molecules probably facilitated their orientation in the mixture and permitted a more effective arrangement of the double photoreactive bonds. Thus, the film formed with the [1:4] ratio tended to behave like the mixtures prepared with the lowest proportion of PNPCN although with slightly lower reaction rates and a lag at the beginning of the photoreaction that was double the length. Similarly, the film formed with the [1:5] ratio tended to behave like the mixtures prepared with intermediate quantities of PNPCN, the graph obtained superimposing that obtained with the [1:3] proportion. For very high concentrations of PNPCN ([1:6] ratio), the crosslinking process is governed by this photopolymer and the PNPCN dimerization reaction becomes quantitatively important (its photodimerization

rate was only 10% of that measured in MNTCN films: Figs. 3 and 4), the accumulation of these dimers among the MNTCN chains leading to very low crosslinking rates. This sample showed the lowest reaction rates and longest lag at the beginning of the photoreaction.

In order to evaluate the ^1H NMR spectroscopy technique for use in monitoring the reactivity of the photoreaction, several samples (25 g l^{-1}) were prepared at different irradiation times (0–7 min) using pure MNTCN and a [1:2] mixture with PNPCN. The signals at $\delta = 7.24$ – 7.62 ppm (due to the aromatic protons) were used as reference because they are not involved in the photochemical reaction. However, the intensity of the signals at $\delta = 6.40$ – 6.66 ppm and 7.67 – 7.95 ppm (due to the vinylic protons) decreased with UV irradiation as a result of photodimerization and the formation of cyclobutane rings. The data referring to the integrated areas of the signals obtained permitted us to calculate the relation between the number of vinylic and aromatic hydrogens in the sample analyzed. In this case, the crosslinking density, ρ , was defined by the following equation:

$$\rho = 1 - (\text{H}_{\text{vinylic}}/\text{H}_{\text{aromatic}})_t / (\text{H}_{\text{vinylic}}/\text{H}_{\text{aromatic}})_0 \quad (3)$$

where $(\text{H}_{\text{vinylic}}/\text{H}_{\text{aromatic}})_t$ corresponds to the relative intensity of the double photoreactive bond after irradiation, and $(\text{H}_{\text{vinylic}}/\text{H}_{\text{aromatic}})_0$ is the relative intensity before irradiation. As the irradiation time increased, we observed a diminution in the intensity of the bands due to the double bond compared with the intensity of those due to the aromatic ring; the appearance of signals at $\delta = 3.22$ ppm was accompanied by an increase in the signals at $\delta = 4.39$ – 4.73 ppm and 5.60 – 5.93 ppm due to the formation of the β -truxynic cyclobutane ring during photodimerization (Oriol et al., 2003) and its overlapping with the signals originated by the aliphatic chain of MNTCN. The values of ρ obtained for different irradiation times in samples of MNTCN:PNPCN prepared in different proportions are shown in Fig. 5 (inset). As in the ultraviolet studies, the crosslinking density in all cases behaved logarithmically with respect to the irradiation time and the slopes of the straight lines traced tangentially to the curve for a given irradiation time enabled the corresponding photocrosslinking rates to be calculated (Fig. 5). The presence of PNPCN led to longer irradiation times being necessary to reach a given value of ρ and a decrease in the corresponding reaction rate. Furthermore, as the irradiation time increased the resulting solid became more insoluble in deuterated chloroform so that deuterated methanol (CD_3OD) was added to increase solubilization. For very long exposure times, the resulting polymer could not be solubilized, making ^1H NMR analysis impossible. Therefore, although this aspect would be a limiting factor in the application of ^1H NMR spectroscopy to the analysis of photoreactive monomers that give rise to polymers of very high molecular weight, it could well be advantageous for analysing the initial and intermediate stages of the photoreaction, which are

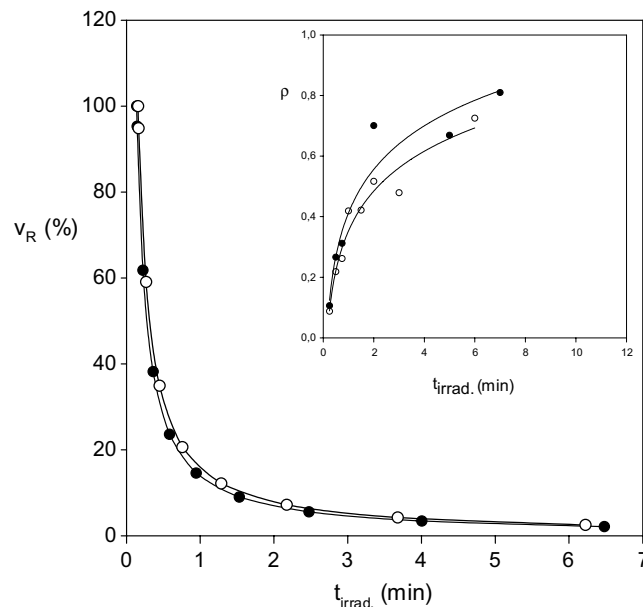


Fig. 5. Effect of irradiation time on residual photocrosslinking rate in samples prepared with different proportions of MNTCN:PNPCN: (●) [1:0] and (○) [1:2]. Representation of residual photocrosslinking rate, v_R (%), vs irradiation time, t_{irrad} (min). Inset: Effect of irradiation time on crosslinking density. Representation of crosslinking density, ρ , vs irradiation time, t_{irrad} (min). Data obtained by ^1H NMR spectroscopy.

the most influential stages when characterising and differentiating photoresists.

Although both UV–visible and ^1H NMR spectroscopy can be used to monitor the photoreaction, comparison of both techniques shows that (i) photodimerization can only be monitored by the former if the absorbance value of the monomer film to be irradiated is adjusted to the measuring range of the spectrometer used, while ^1H NMR spectroscopy permits assays at widely differing concentrations of monomer, (ii) ^1H NMR spectroscopy provides more structural information concerning the photoprocess taking place, indicating both the disappearance of the photoreactive double bond and the appearance of cyclobutane rings, (iii) the use of the ^1H NMR technique depends on the possibility of dissolving the polymer formed during irradiation, while this is not a problem for following the process by UV–visible spectroscopy, and (iv) in the initial and intermediate stages of the photoreaction studied both UV–visible and ^1H NMR spectroscopy can be used since the conclusions reached are similar. However, the results obtained in both cases are not identical due to the lower sensitivity of ^1H NMR spectroscopy (Lee et al., 2003) for monitoring the photoprocess studied and due to the greater degree of processing of the irradiated samples necessary to obtain the corresponding ^1H NMR recording. Such processing may introduce errors into the measurements which are difficult to evaluate and which will depend on the chemical nature of the monomers used, their ratio

in the mixture and the irradiation time. This explains why the differences observed in both samples by ^1H NMR are less than those observed by the UV–visible technique and why the former technique is more suitable for mixtures that require long irradiation times for insolubilization.

3.2.1.1. Effect of nature of the cinnamate groups. From films prepared with different MNTCN concentrations it was seen experimentally that crosslinking density was independent of the initial concentration of the sample (Eq. (1); data not shown), so that the differences in the reaction rates measured cannot be due to the different concentrations of the photoreactive groups present in the prepared mixtures but to the different reactivities of the two monomers used (MNTCN and PNPCN: Figs. 3 and 4) as a result of the different chemical nature of the molecule to which the cinnamoyl groups in each monomer are bound (Balaji et al., 2004) and the contribution each makes to the overall rate of the process (Fig. 6). Hence, for the samples prepared at [1:1]–[1:6] ratios, the overall rate of the process was governed by the lower photoreactivity of PNPCN, while for the mixtures prepared with higher concentrations of MNTCN ([1:0]–[2:1]) the rate was determined by the latter monomer. Moreover, the maximum reactivity reached suggests the existence of an optimal molar relation ([MNTCN]:[PNPCN] = [1:4]), possibly due to the mixed photocrosslinking of double photoreactive bonds belonging to both types of monomers. This optimal ratio would depend on the chemical nature of the photoreactive monomers used and must be determined experimentally in each case.

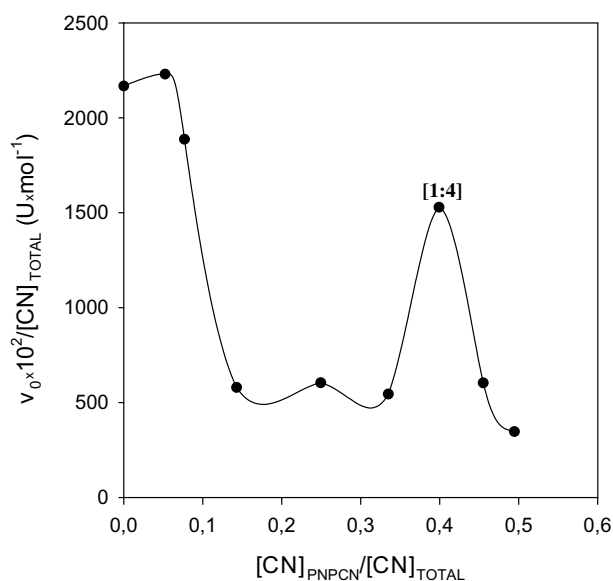


Fig. 6. Effect of cinnamate group nature on photocrosslinking initial rate. Representation of initial photocrosslinking rate per mol of total cinnamate groups, $v_0 \times 10^2 / [\text{CN}]_{\text{TOTAL}}$ (U mol^{-1}), vs PNPCN cinnamate group concentration per mol of total cinnamate groups, $[\text{CN}]_{\text{PNPCN}} / [\text{CN}]_{\text{TOTAL}}$.

3.2.2. Photoreaction monitoring by GPC data analysis

The retention times corresponding to PNPCN and MNTCN monomers were determined by injecting non-irradiated samples (25 g l^{-1} in chloroform) prepared with the corresponding monomers. Injection of irradiated samples (40–36,000 s) confirmed the position of the MNTCN dimers, trimers and oligomers, and the PNPCN dimers from the previously obtained calibration curve. The correlation between the molecular weight of the species studied and the retention time at which they eluted showed that the width of the integrated peaks corresponded to species of MNTCN monomer (alone or with one or two PNPCN groups), MNTCN dimers (alone or with a PNPCN group), or MNTCN trimers alone, while the rest of the chromatographic peaks were assumed to correspond to oligomers of the reacting species. Furthermore, the existence of MNTCN species with PNPCN groups was evidently due to the presence of shoulders at lower retention times partially overlapping the corresponding MNTCN monomers, dimers, trimers and oligomers, thus breaking the symmetry of these peaks.

The signals recorded in the chromatograms were integrated in a range that included the weight-average molecular weights of the species obtained alone or with one or several blocking PNPCN groups (Fig. 7). For samples prepared in similar conditions and exposed to the same irradiation times, it can be seen that the [1:4] mixture showed higher values (corresponding to MNTCN monomers and dimers alone or with blocking PNPCN groups), while the values corresponding to trimers and, especially, oligomers with or without blocking groups, were lower than those obtained with the [1:1], [1:3] and [1:6] ratios. This clearly illustrates the blocking effect of the PNPCN groups on MNTCN photodimerization, since products of lower molecular weight were accumulated, while those with a higher degree of crosslinking were obtained in greater quantities with mixtures containing a lower or higher proportion of blocking groups than the mixture prepared with the [1:4] ratio.

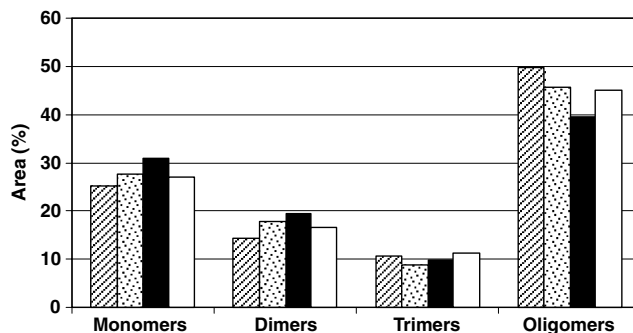


Fig. 7. Analysis by GPC of samples prepared with different MNTCN:PNPCN ratios and irradiated with UV light. Area values (%) calculated for each photoreaction product obtained after irradiation. MNTCN:PNPCN ratios: ▨ [1:1], ▤ [1:3], ■ [1:4] and □ [1:6].

4. Conclusions

MNTCN and PNPCN were synthesized and their structures were confirmed by spectral studies, applying UV–visible, FT-IR, ^1H NMR and ^{13}C NMR spectroscopic, and gc-ms spectrometry techniques. The DSC data obtained for MNTCN and PNPCN confirmed the melting point values obtained for these monomers. The DSC recordings revealed heat absorption peaks at temperatures slightly higher (low irradiation times) or of greater intensity (high irradiation times) in the case of the [1:4] samples, probably due to the presence of highly impeded photocrosslinking products with a greater number of chain blockers in their structure.

Photoreactivity studies of monomers in thin films suggest that these monomers are highly photosensitive, which is a prerequisite for negative photoresists and other applications. The rate of photocrosslinking depended on the proportion of PNPCN present in the reaction mixture since it acts as a chain blocker of the process. The reaction rate of the film prepared depended on whether it was mainly governed by the photocrosslinking process of the MNTCN (highest rate for [1:0], [3:1] and [2:1] ratios), the PNPCN (lowest rate for [1:6] ratio), the mixture of both MNTCN and PNPCN ([1:1], [1:2], [1:3] and [1:5] ratios), or of the MNTCN chains containing chain blockers (for the [1:4] ratio, with a higher rate than that expected for this composition), in all cases regardless of the time at which it was measured. The higher quantity of photoreaction products with low weight-average molecular weights obtained after irradiation of the [1:4] sample was confirmed by GPC analysis. Although both UV–visible and ^1H NMR spectroscopy can be used to monitor the photoreaction, the results obtained by each method are not identical due to the lower sensitivity of ^1H NMR spectroscopy compared with UV–visible spectroscopy and due to the greater degree of processing of the irradiated samples necessary before obtaining the corresponding ^1H NMR recording.

This study opens up the possibility of testing the effect of other monocinnamoylated compounds for preparing immobilization supports with photocrosslinkable cinnamate groups and for adapting their physical–chemical characteristics (molecular size, branching, pore size, hydrophobic-hydrophilic ratio and effect of the chemical nature of the monocinnamoylated compound added) to the requirements necessary for the immobilization of cells, enzymes and/or biomolecules of industrial interest.

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References

- Balaji, R., Grande, D., & Nanjundan, S. (2004). Photoresponsive polymers having pendant chlorocinnamoyl moieties: synthesis, reactivity ratios and photochemical properties. *Polymer*, 45, 1089–1099.
- Chae, B., Lee, S. W., Kim, S. B., Lee, B., & Ree, M. (2003a). Photoreaction and molecular reorientation in films of novel photo-sensitive polyesters containing *n*-alkyl side groups and 1,4-phenylene-diacyloyl units in the backbone. *Langmuir*, 19, 6039–6049.
- Chae, B., Lee, S. W., Ree, M., Jung, Y. M., & Kim, S. B. (2003b). Photoreaction and molecular reorientation in a nanoscaled film of poly(methyl 4-(methacryloyloxy)cinnamate) studied by two-dimensional FTIR and UV correlation spectroscopy. *Langmuir*, 19, 687–695.
- Delzenne, G. A. (1972). Recent advances in photo-cross-linkable polymers. In I. Skeist (Ed.), *Reviews in polymer technology* (Vol. 1, pp. 185–215). New York: M. Dekker.
- Delzenne, G. A. (1974). Photographic applications of photopolymers. Photophysical and photochemical aspects. *Industrie Chimique Belge*, 39, 249–265.
- Egerton, P. L., Hyde, E. M., Trigg, J., Payne, A., Beynon, P., Mijovic, M. V., et al. (1981). Photocycloaddition in liquid ethyl cinnamate and in ethyl cinnamate glasses. The photoreaction as a probe into the micromorphology of the solid. *Journal of the American Chemical Society*, 103, 3859–3863.
- Egerton, P. L., Pitts, E., & Reiser, A. (1981). Photocycloaddition in solid poly(vinyl cinnamate). The photoreactive polymer matrix as an ensemble of chromophore sites. *Macromolecules*, 14, 95–100.
- Fréchet, J. M. J. (1981). Synthesis and applications of organic polymers as supports and protecting groups. *Tetrahedron*, 37, 663–683.
- García-Ruiz, P. A., (2000). Patent P2000002457.
- Ichimura, K., Akita, Y., Akiyama, H., Kudo, K., & Hayashi, Y. (1997). Photoreactivity of polymers with regioisomeric cinnamate side chains and their ability to regulate liquid crystal alignment. *Macromolecules*, 30, 903–911.
- Lee, S. W., Kim, S. I., Lee, B., Choi, W., Chae, B., Kim, S. B., et al. (2003). Photoreactions and photoinduced molecular orientations of films of a photoreactive polyimide and their alignment of liquid crystals. *Macromolecules*, 36, 6527–6536.
- Lin, A. A., Chu, C.-F., Huang, W.-Y., & Reiser, A. (1992). Reactant preordering solid photopolymers. *Pure and Applied Chemistry*, 64, 1299–1303.
- Marín-Zamora, M. E., Rojas-Melgarejo, F., García-Cánovas, F., & García-Ruiz, P. A. (2005). Cinnamic ester of D-sorbitol for immobilization of mushroom tyrosinase. *Journal of Chemical Technology and Biotechnology*, 80, 1356–1364.
- Minsk, L. M., Smith, J. G., Van Deusen, W. P., & Wright, J. F. (1959). Photosensitive polymers. I. Cinnamate esters of poly(vinyl alcohol) and cellulose. *Journal of Applied Polymer Science*, 2, 302–307.
- Oriol, L., Piñol, M., Serrano, J. L., & Tejedor, R. M. (2003). Synthesis, characterization and photoreactivity of liquid crystalline cinnamates. *Journal of Photochemistry and Photobiology A: Chemistry*, 155, 37–45.
- Ranby, B. (1998). Photochemical modification of polymers-photocross-linking, surface photografting, and lamination. *Polymer Engineering and Science*, 38, 1229–1243.
- Reiser, A. (1980). The physical chemistry of crosslinking photopolymers. *Journal de Chimie Physique*, 77, 469–481.
- Reiser, A. (1989). In *Photoreactive polymers: The science and technology of resists*. John Wiley, New York, p. 47.
- Rojas-Melgarejo, F., Marín-Iniesta, F., Rodríguez-López, J. N., García-Cánovas, F., & García-Ruiz, P. A. (2006). Cinnamic carbohydrate esters show great versatility as supports for the immobilization of different enzymes. *Enzyme and Microbial Technology*, 38, 748–755.

- Rojas-Melgarejo, F., Rodríguez-López, J. N., García-Cánovas, F., & García-Ruiz, P. A. (2004a). Immobilization of horseradish peroxidase on cinnamic carbohydrate esters. *Process Biochemistry*, 39, 1455–1464.
- Rojas-Melgarejo, F., Rodríguez-López, J. N., García-Cánovas, F., & García-Ruiz, P. A. (2004b). Stability of horseradish peroxidase immobilized on different cinnamic carbohydrate esters. *Journal of Chemical Technology and Biotechnology*, 79, 1148–1154.
- Rojas-Melgarejo, F., Rodríguez-López, J. N., García-Cánovas, F., & García-Ruiz, P. A. (2004c). Cinnamic carbohydrate esters: new polymeric supports for the immobilization of horseradish peroxidase. *Carbohydrate Polymers*, 58, 79–88.
- Tsuda, M. (1964). Some aspects of the photosensitivity of poly(vinyl cinnamate). *Journal of Polymer Science Part A*, 2, 2907–2916.
- Van Cleve, J. W. (1963). Direct benzylation of D-fructose. In R. L. Whistler & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (Vol. 2, pp. 237–238). London: Academic Press.
- Wendell, L. D. (1983). Polymerization of unsaturated compounds by photocycloaddition reactions. *Chemical Reviews*, 83, 1–47.
- Williams, J. L. R. (1974). Photoreactive polymers. In E. Sélégny (Ed.), *Polyelectrolytes* (pp. 507–528). Dordrecht, Netherlands: Reidel.
- Williams, J. L. R., Laakso, T. M., & Borden, D. G. (1971). Patent FR2058289.