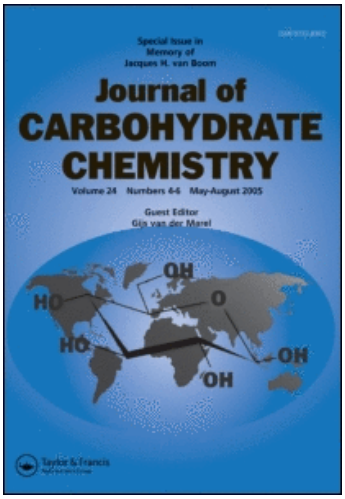

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Hydrogels Produced by Photocrosslinking of Dextran Chain: Characterization and Properties

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The present study describes the synthesis and characterization of dextran hydrogels produced by photocrosslinking of dextran chains. The hydroxyl groups of native dextran were converted to acrylate groups to make hydrogel precursors with different substitution degrees. The hydrogels were photopolymerizated in the presence of a suitable photoinitiator system (thionine/triethylamine) and characterized by ^{13}C,^{1}H NMR, and infrared spectroscopy. The information about microenvironment formed in hydrogel solutions was obtained by fluorescence spectroscopy using pyrene and nabumetone probes. This technique was used also to study the crosslinking process. The results about the solubility and swelling index data of hydrogels showed that their use as models of drug delivery is technically feasible.

Keywords Dextran hydrogel, Photoinitiator system, Photocrosslinking, Drug delivery systems, NMR and infrared spectroscopy

INTRODUCTION

In polymeric systems, hydrogels are materials that when placed in excess water swell rapidly and retain a large volume of water in their swollen three-dimensional structure without dissolution.[1] They are highly biocompatible owing to low surface tension and hydrodynamic properties similar to natural biological gels that can minimize mechanical irritation.

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Chemically, hydrogels are based on hydrophilic polymers, which are crosslinked to prevent dissolution in water and to retain cells, nutrients, drugs, or proteins.[2]

In this work, a synthetic method to produce a dextran hydrogel is described. The hydroxyl groups were converted to acrylate groups to make a hydrogel precursor. The degree of substitution (DS) was controlled by the 3,3-dimethylacryloyl chloride amount. Hydrogels were produced by light-induced polymerization in the presence of thionine and triethylamine. Solubility, swelling index and hydrogel microenvironment in aqueous solution were studied and quantified.

There are few studies that describe the production of hydrogels by photochemical reactions. The present work illustrates the potential future use of these products as models of drug delivery.

EXPERIMENTAL

Chemicals

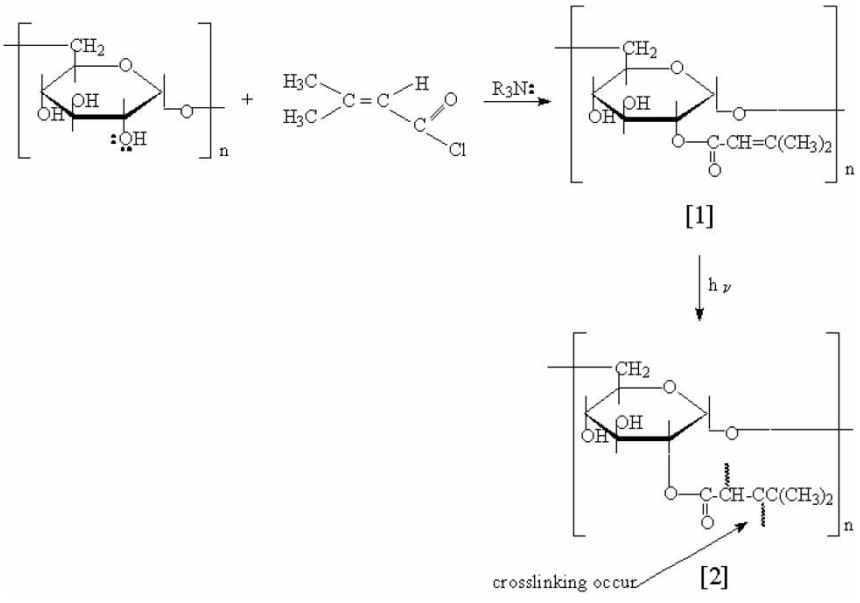
3,3-Dimethylacryloyl chloride (AC, 97%) and triethylamine (TEA, 99%, vacuum-distilled) were purchased from Acros Organics. Dextran was purchased from Pharmacia Uppsala and KBr (99+% FTIR) from Aldrich Chemical. The pyrene and nabumetone photophysical probes were purchased from Fluka and Sigma, respectively. The dye thionine (ThH^+, Aldrich) was used as received. Other chemicals were reagent grade, used without previous purification.

Preparation of Dextran Hydrogel Precursor

Dextran hydrogel precursor was prepared as previously described.[3] Dextran 20% (w/v) was dissolved in aqueous solution under stirring and maintained under constant temperature ($18 \pm 0.01^{\circ}C$). TEA and 3,3-dimethylacryloyl chloride (AC) were added to this solution and remained under continuous stirring for 2 h (Sch. 1). The purification was produced according to literature.[4] The degree of substitution (DS) was controlled by the 3,3-dimethylacryloyl chloride amount. The ratios of dextran (DX), 3,3-dimethylacryloyl chloride (AC), and triethylamine (TEA) are shown in Table 1.

Preparation of Dextran Hydrogel by Photocrosslinking

The light-induced polymerization was performed as described in previous works.[5–7] Dextran hydrogel precursors 3.5% (w/v) were dissolved together with ThH^+ 0.80×10^{-5} mol · L^{-1} and TEA 0.10 mol · L^{-1} in aqueous solution. The resulting solutions were deoxygenated by thoroughly bubbling



Scheme 1: Preparation of dextran hydrogel.

oxygen-free nitrogen and irradiated with a 600 mW Kavo photopolymerizer (Sch. 1). After irradiation the hydrogels were precipitated, washed, and dried in a vacuum oven.

Studies on light-induced polymerization of suitable monomers in the presence of initiators/co-initiators systems are well known.[5–13] In the present work, the photopolymerization method was based on a previous work on the polymerization of methyl methacrylate (MMA) photoinitiated by the dye thionine in the presence of amines as co-initiator.[5–7] Experimental data showed that the cation radicals of the amines, $A^{\bullet+}$, arise from the interaction of the thionine triplet with the amine and deprotonates to amine radical,

Table 1: Molar ratios for the derivatives of dextran with dextran (DX), 3,3-dimethylacryloyl chloride (AC), and TEA amounts.

Acrylate dextran	Molar ratio DX:AC:TEA
DX-AC1	1.0:1.0:6.7
DX-AC2	1.0:0.70:6.7
DX-AC3	1.0:0.33:6.7
DX-AC4	1.0:0.15:6.7
DX-AC5	1.0:0.10:6.7

$^{\bullet}A(-H^{+})$, which is responsible for the chain initiation. Optimum amine concentration yields a more efficient polymerization.

$^{\bullet}A(-H^{+})$ interacts with the monomer (MMA) and initiates a sequence of three events that lead to radical chain polymerization: initiation, propagation, and termination.[14] $R^{\bullet}$, M, and $M^{\bullet}$ denote free radical, monomer molecule, and initiator radical or primary radical, respectively.

$$\text{Initiation}: \quad C + h\nu \xrightarrow{k_d} \rightarrow R^{\bullet} \tag{1}$$

$$R^{\bullet} + M \xrightarrow{k_i} M^{\bullet} \tag{2}$$

$$\text{Propagation}: \quad M_n^{\bullet} + M_k^{\bullet} \xrightarrow{k_{td}} M_x + M_k \tag{3}$$

$$\text{Termination}: \quad M_n^{\bullet} + M_k^{\bullet} \xrightarrow{k_{tc}} M_{x+k} \tag{4}$$

$$M_x^{\bullet} + M \xrightarrow{k_p} M_{x+1}^{\bullet} \tag{5}$$

Characterization of Dextran Derivatives

NMR Spectroscopy

^{13}C and ^{1}H NMR measurements were performed on a DRX500 Bruker NMR spectrometer at 500 MHz. ^{1}H NMR measurements were also used to determine the substitution degree of acrylate groups on dextran (DS), in agreement with previous work.[3]

FT-infrared (IR) Measurement

Infrared spectra were obtained by Fourier transform infrared spectroscopy (FT-IR, Spectrum 2000 Perkin Elmer). The samples were prepared in KBr pellets (5 mg in 200 mg of KBr) and stabilized under controlled relative humidity before acquiring the spectrum.

Solubility and Swelling Test

Dextran acrylate (1 mg) was placed in a tube with each solvent (1 mL). After mixing with an ultrasonicator, the mixture was stored at rt for 7 days and visually observed. Solvents tested were water, dimethyl sulfoxide, dimethyl formamide, methyl pyrrolidone, and acetone.

The swelling-index of dextran hidrogel was calculated by the following equation:

$$Swelling - index, \% = \frac{W_s - W_o}{W_o} \times 100 \tag{6}$$

where W_o is the weight of a dried dextran derivative and W_s is the weight of a swollen derivative after the immersion in water at time t.

Fluorescence Spectroscopy

The fluorescence signals of pyrene (1.0×10^{-6} mol $\cdot$ L^{-1}) and nabumetone (5.0×10^{-5} mol $\cdot$ L^{-1}) were collected by a 600 μm fused silica fiber and coupled into a 1/4 m spectrometer (Oriel Instruments MS257) furnished with a 300 lines/mm grating. An intensified CCD (charge coupled device) with 256 × 1024 pixels was connected at the spectrometer detector port. The specified CCD gating capability was 5 ns, minimum. The CCD gating and time delay was controlled by a model DG535 delay generator, from Stanford Research. The absorption spectra of nabumetone were measured with a Hitachi U-2000 spectrophotometer.

The formation of hydrophobic microdomains can be detected using fluorescent probes. In the Ham effect,[15] the solvent interactions perturb mainly the relative intensities of the vibrational fine structure of the fluorescence spectra of pyrene. Band I of the fluorescence spectrum of pyrene (~372 nm) shows significant intensity enhancement in polar solvents. Thus, the ratio between the fluorescence intensities of peaks I and III (~384 nm) of the emission spectrum of pyrene, I_1/I_3, was used to evaluate the polarity of the local environment.

In addition to the study using pyrene, nabumetone was employed to monitor the solution properties of hydrogels. This molecule is a drug with absorption and emission spectra sensitive to changes in the molecular microvicinity.[16,17]

RESULTS AND DISCUSSION

Preparation of Acrylated Dextran

The synthesis of acrylated dextran (Sch. 1) was based on a previous study of dextran modified by reactions with octanoyl and lauroyl chlorides.[3,18]

The FT-IR spectrum of dextran given in Figure 1A is in agreement with literature[4,19–22] where the absorption bands at 2900 cm^{-1} and 3400 cm^{-1} are attributed to the CH/CH$_2$ vibrations and OH vibrations, respectively. The other absorptions bands are characteristics of polysaccharides in general.

The successful incorporation of the acrylate group into dextran is demonstrated by the presence of an ester band at ~1710 cm^{-1} [19,23] (Fig. 1B). This ester band increased sharply with the increase of the substitution degree of the acrylate group. The presence of pendant vinyl groups in dextran–acrylate was confirmed by the bands at ~3000 cm^{-1} and ~840 cm^{-1}.[21,24] It was difficult to identify the known double-bond absorption band at

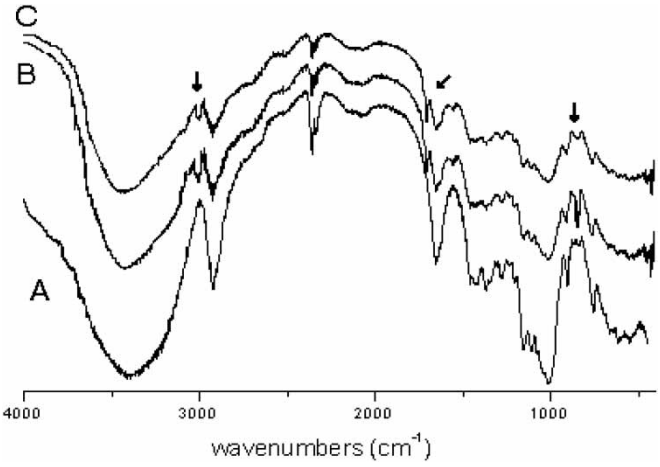


Figure 1: FT-IR spectra of dextran (A), dextran acrylate DX-AC1 (B), and hydrogel based in dextran acrylate DX-AC1 (C).

1656 cm^{-1} due to the existence of small bands of the unmodified dextran in the same region. The broad hydroxyl group band (3000 $cm^{-1} \sim 3800$ cm^{-1}) was not reduced significantly by the incorporation of the acrylate group into dextran, an indication that there might be a number of unreacted free hydroxyl groups in the polysaccharide.

Figure 2 shows the chemical shifts of the ^1H NMR signals for dextran and hydrogel derived from 3,3-dimethylacryloyl chloride. The chemical shifts of the ^1H NMR signals of native dextran (Fig. 2A) were previously evaluated and compared with literature.[21,23,25] New peaks confirm the incorporation of

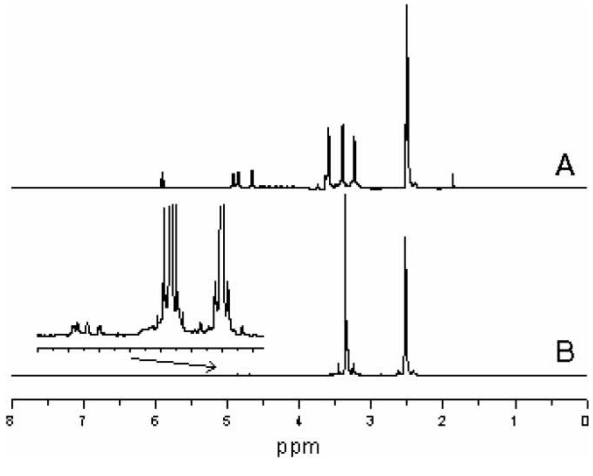


Figure 2: ^1H NMR spectra of dextran (A) and dextran acrylate DX-AC2 (B).

vinyl groups in dextran–acrylate (Fig. 2B): 5.9 ppm, which corresponds with the hydrogen adjacent to double bond (C═C-H); 1.9 ppm, the hydrogen of the methyl substitutes (CH_3) in the acrylate group; and two anomeric proton peaks, one at 4.5 ppm corresponding to dextran and the other at 4.7 ppm, assigned to anomeric protons (C-1) in which the hydroxyl on the neighboring C-2 carbon reacted to form an ester.[21] The peak at 2.5 ppm in both spectra (Fig. 2A,B) are due to solvent DMSO-d_6. After-esterification modifications occur in the spectrum as a consequence of a different electronic environments.[26]

^{1}H NMR measurements were also used to determine the substitution degree of acrylate groups on dextran (DS) (Fig. 5). Previous reports for both dextran[3] and chitosan[18] alkyl derivatives suggest a more favorable reaction with a high concentration of acyl choride. The values reported here indicate the same trend.

New signals appear in ^{13}C NMR spectrum of dextran after the incorporation of an acrylate group (Fig. 3): 170 ppm, carbonyl carbon[21,27], 19.0 ppm, methyl substituent in the acrylate group (CH_3-C═C)[27,28]; and 137 ppm and 127 ppm, carbons in the double bond (C═C).[21,27] Other signals are attributed to the carbonyl carbon of dextran[23,25,29] and the intense signal at $\sim$ 40.0 ppm to the solvent (DMSO-d_6).[27]

Preparation of Dextran Hydrogel by Photocrosslinking

Photocrooslinkg of acrylate groups in acrylated dextran upon light irradiation was conducted within 150 min. The characteristics of the hydrogels produced were measured spectroscopically.

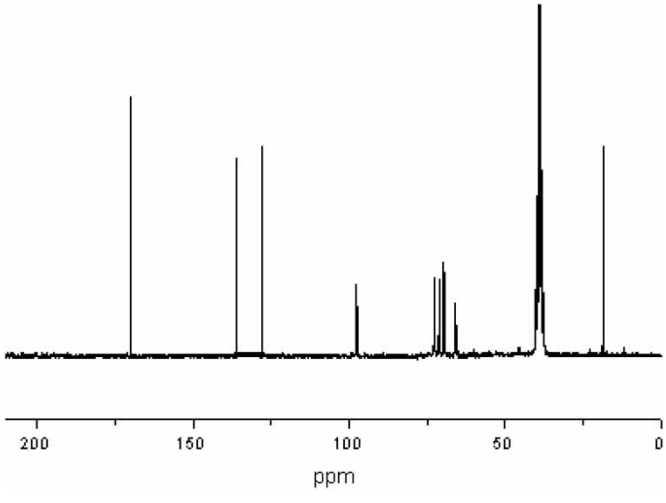


Figure 3: C NMR spectra of dextran acrylate DX-AC2.

In the FT-IR spectrum, the intensities of the peaks at 3000 cm^{-1} and 840 cm^{-1} are decreased after hydrogel formation (Fig. 1C) because of the consumption of double bonds during the photocrosslinking reactions. In the same way, the signals at 137 ppm and 127 ppm in the ^{13}C NMR spectrum of acrylate dextran decrease after light irradiation (results not shown).

Photoinitiators have been used with considerable success in the polymerization of vinyl monomers in homogeneous[5,7,30–32] and microheterogeneous[6,32–34] systems. In previous work, the polymerization of methyl methacrylate (MMA) photoinitiated by dye thionine/amines systems was studied in homogeneous methanol solution[5,7] and sodium dodecyl sulphate micelles.[6] In the current work, the acrylate groups, less soluble in water, inserted in dextran, can be oriented within aggregates formed in solution. These aggregates, similar to those previously described to dextran and alkyl derivatives,[3] increase the effective concentration of double bonds, which leads to an efficient rate of the propagation reaction in free-radical photopolymerization. The radical termination reactions are diffusion controlled and can be retarded by an increase in the viscosity of the medium. Thus, an increase in the propagation rate and a decrease in the termination rate result in a efficient polymerization rate.

Solubility and Swelling Test

The solubility of dextran–acrylate in common organic solvents was enhanced significantly compared with dextran. Native dextran dissolved only in water and dimethyl sulfoxide at rt. Dextran–acrylate, however, dissolved in other solvents as dimethyl formamide and methyl pyrrolidone at rt to form a clear solution. However, dextran–acrylate did not dissolve in acetone at rt.

Swelling kinetics for dextran hydrogel were investigated, as exemplified by DXAC1 and DX-AC3 (Fig. 4). The bulk of the maximum water uptake of dextran hydrogels happened during the initial 100 minutes, except the hydrogels with the highest DS (0.13 and 0.12), 120 minutes. All hydrogels showed a slow swelling process, and this is a necessary property to prevent overhydration that results in the loss of mucoadhesive properties.

To hydrogels with lesser degrees of substitution, the swelling process is faster, achieving a stable state quickly. This occurs because of the lesser hydrophobic content. After establishing equilibrium, all hydrogels showed a good stability for up to 30 days.

Figure 5 relates the behavior of swelling with the DS for dextran hydrogels. In agreement with the literature,[35] when the DS of the dextran hydrogels increased, swelling ratios decreased. Smaller values of swelling index can be attributed to a tighter and more compact structure that limits the water uptake. This inverse relationship between DS and swelling ratio had been

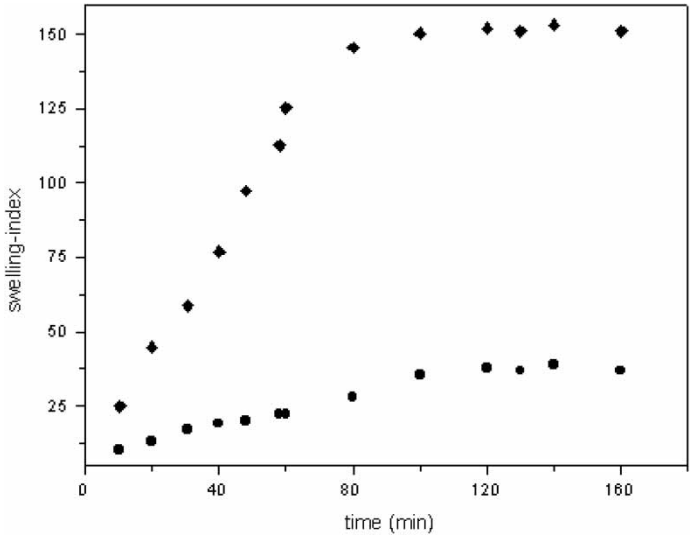


Figure 4: Swelling kinetics of hydrogels (●) DX-AC1 and (◆) DX-AC3.

attributed to the availability of free hydroxyl, groups and to the tightness of the three-dimensional network. A higher DS hydrogel precursor not only would lead to the formation of a tighter three-dimensional network of hydrogel, but also would reduce the availability of free hydroxyl groups, which contribute to the hydrophilicity of the hydrogel.[28]

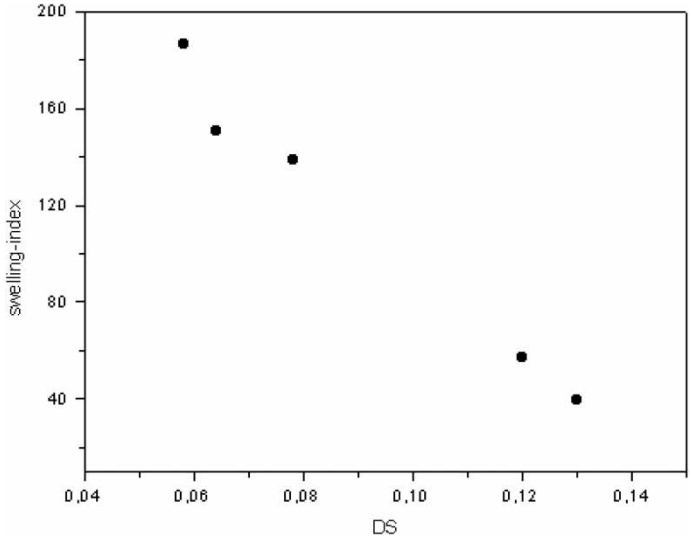


Figure 5: Swelling-index versus degree of substitution (DS) of hydrogel precursor.

Photophysicals Properties of Pyrene and Nabumetone in Hydrogel Solutions

Figure 6 shows the fluorescence of pyrene in water as well as in an aqueous solution of dextran hydrogel. Some observations must be made: first, the overall fluorescence of pyrene intensity increases dramatically in the presence of hydrogel and it remains constant independent of hydrogel concentration. Second, the relative intensity of the third peak (~384 nm) in the vibrational fine structure of pyrene increases with respect to the intensity of the first peak (~372 nm). This behavior indicates that the dextran hydrogel provides a hydrophobic microenvironment capable of solubilizing pyrene. Moreover, the same figure shows that the excimer emission appears (broad band at ~470 nm[15]) when free pyrene molecules are transferred to a hydrogel, microenvironment but decreases with an increase in concentration. When pyrene is incorporated into a hydrogel, it forms a more rigid microenvironment. The fluorescence intensity is increased and the formation of excimer is favored. The excimers are redistributed among the several hydrogels that are formed with the increase in concentration as the emission at 470 nm decreases.

In previous work, the increase of concentration of dextran[3] and chitosan[18] modified (and consequent incorporation of pyrene into the hydrophobic aggregates) caused a variation of fluorescence intensity of pyrene with the increase of the size of the aggregates. Now, the microenvironments are not formed by intra- and/or intermolecular aggregation but by cross-linking. These microenvironment present a pre-established size.

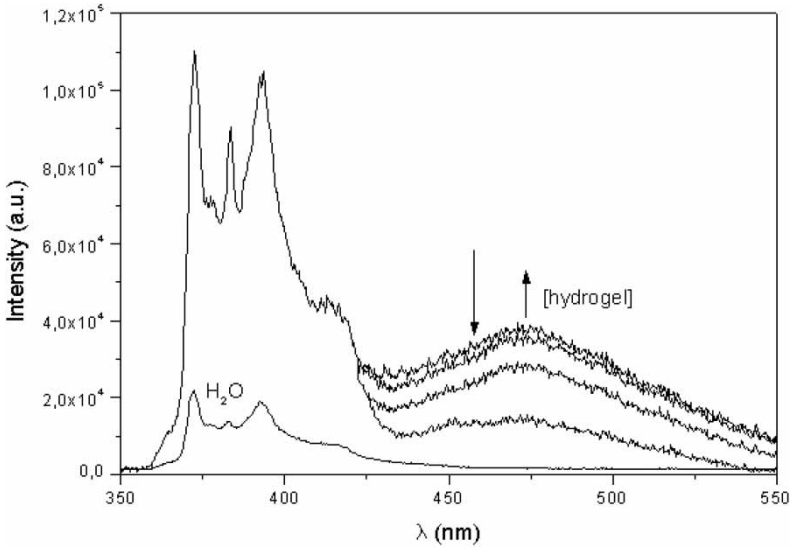


Figure 6: Fluorescence spectra of pyrene as a function of DX-AC2 concentration.

Dextran derivatives solubilized pyrene,[3] but after the crosslinking process, the hydrogel provides an ideal and unchanged environment for the excited pyrene molecules. When the crosslinking process starts, an increase in the fluorescence intensity of pyrene may be expected. No shift was observed in the wavelength of the maximum intensity of pyrene and all samples kept their transparency during the polymerization process.

The emission spectra of pyrene fluorescence were collected during the crosslinking process and in accordance with the irradiation time. Figure 7 shows the behavior observed. The medium polarity, measured by ratios I_1/I_3 of the emission spectra of pyrene fluorescence, decreases due to the formation of hydrophobic environment after the crosslinking of dextran chains. The increase in the number and size of these hydrophobic environments creates a more rigid environment in the pyrene that induces a increase of the local concentration of this probe in the same time that hindered the formation of excimer, and increases the fluorescence intensity. Moreover, the distance between pyrene molecules decreases and the formation of pyrene aggregates in the ground state can occur.

Figures 8 and 9 show similar results obtained with the photophysical probe nabumetone, a molecule that has a weak fluorescence in water and different photophysical behavior in other solutions.[16,17]

The absorption and emission spectra of nabumetone were collected during the crosslinking process for different irradiation times. Figure 8 shows a typical absorption spectra of a naphthalene 2-substituted compound[36] where the longer wavelength band is the most sensitive to changes in the solvent

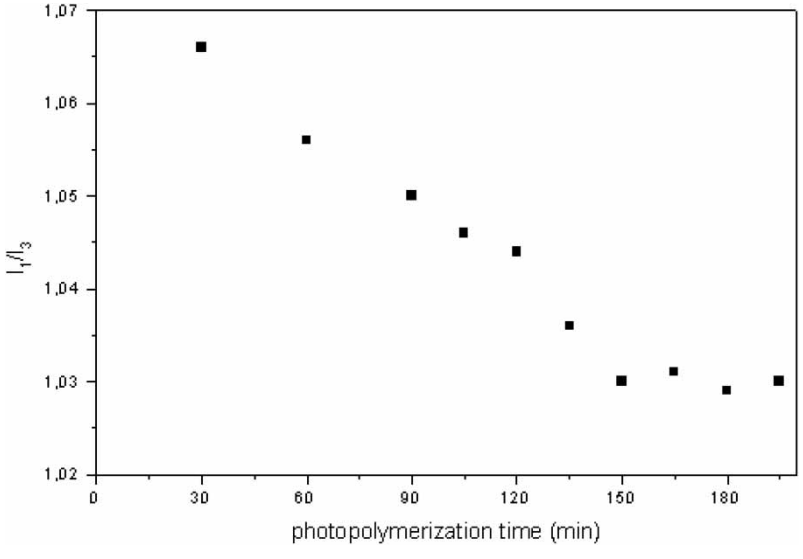


Figure 7: Plot of the fluorescence intensity ratio I_1/I_3 in the presence of acrylate dextran, DX-AC3, as a function of irradiation times.

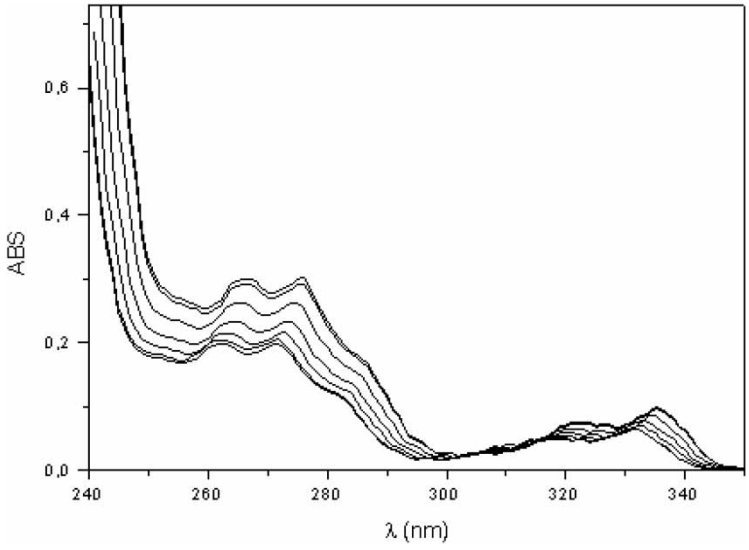


Figure 8: Absorption spectra of nabumetone in hydrogel DX-AC2 solution as a function of irradiation time.

polarity[36] and the maximum absorption can be related to the relative permittivity of the drug environment.[17] Before irradiation and at short times, the spectra remain practically unchanged. At long times of irradiation, the spectral variations are more significant, and after the polymerization, the spectra remain

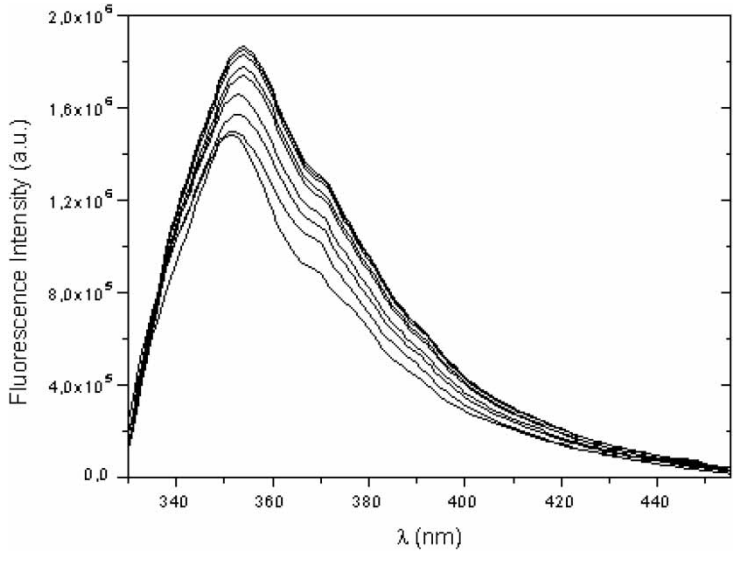


Figure 9: Fluorescence spectra of nabumetone in hydrogel DX-AC2 solution as a function of irradiation time.

not modified. In general, the spectra are shifted to longer wavelengths with the formation of hydrophobic environments during the crosslinking of chain, indicating that nabumetone molecules are transferred from water to a less polar environment inside the hydrogel of dextran. This is a known trend,[16,37] which was observed in all experiments.

The emission spectra of nabumetone show a significant modification when it is dissolved in the dextran derivative during the crosslinking of chains (Fig. 9). The spectra show a behavior similar to the absorption spectra when measured at different irradiation times. The band centered around ~350 nm increases while the probe is being transferred to a more hydrophobic microenvironment where it is protected from water quenching. When the crosslinking is ended, the emission spectra remain practically constant, proving that the nabumetone probe was incorporated into the hydrogel.

CONCLUSION

To generate hydrogels as efficient drug delivery, the crosslinking of a hydrogel precursor based in dextran was promoted by light irradiation. Dextran hydrogels having a wide range of swelling ratios were obtained by the control of the degree of substitution.

The swelling values showed that the hydrogels are expected to be good models of drug delivery systems. Faster photocrosslinking time and enhanced solubility in common organic solvents were additional advantages of these dextran–acrylate hydrogels.

With use of pyrene and nabumetone fluorescent probes, the microenvironment inside of the structure of hydrogels was characterized. The results provide insight into the nature of the hydrophobic environment created by a dextran hydrogel in aqueous media.

ACKNOWLEDGEMENTS

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