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Study of Gelling Behavior of Poly(vinyl alcohol)-Methacrylate for Potential Utilizations in Tissue Replacement and Drug Delivery

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The need of innovative, multifunctional biomaterials for the partial or complete tissue replacement is the driving force for the search of improvements of the performances of the available materials and in the formulation of new ones. Addressing the focus to vitreous substitution, we have explored the possibility of using injectable aqueous solutions of poly(vinyl alcohol), PVA, derivatives able to form hydrogels in the ocular cavity upon UV−vis irradiation with visible light. In particular, we describe the features of hydrogels from methacrylate grafted PVA, PVA-MA, in terms of structural characteristics, degradation processes, release of low- and high- molecular weight molecules, and in vitro gelation kinetics. The mechanical properties, drug delivery tests, and rheology tests suggest that PVA-MA derivatives have the potential to become a useful material for vitreous substitution.

Introduction

Tissue replacement with polymeric materials is a need in reconstructive surgery. The formulation of these materials is a complex task as in many cases the tissue substitute must be multifunctional. Our efforts are addressed to the formulation of a polymeric hydrogel potentially tailored for vitreous substitution. In this case, vitrectomy and vitreoretinal surgery require the partial or total replacement of vitreous with a synthetic material. The suitable material for this task must be transparent, biocompatible with the specific ocular environment, and also possess tamponade properties and possibly drug delivery capabilities. Additionally, they should be storable, sterile, chemically stable, and nonabsorbable. Only a multifunctional biomaterial can supply such vast collection of features.[1]

The currently available materials used as vitreous substitutes range from air to silicon oil or fluorinated liquids to biopolymers aqueous solutions and polymeric hydrogels.[2] More recently, the focus of interest has shifted toward biopolymer solutions such as collagen and hyaluronic acid solutions and mixtures of the two polymers.[3] These systems were tested in terms of their kinetics of degradation in the ocular cavity. Hyaluronic acid and albumin aqueous mixtures were also considered for their nonnewtonian behavior in the replacements of the vitreous.[1] The use of hydrogels based on biopolymers or biocompatible synthetic polymers[4] has been exploited for post-surgical treatments. The main asset offered by polymeric hydrogels is their multifunctionality (i.e., the simultaneous fulfillment of different important functions proper of vitreous as tamponade action, controlled release of drugs, osmotic stability, and transparency features comparable with the natural vitreous). On the other hand, shortcomings of hydrogel materials are their low chemical stability, often associated with the release of cytotoxic contaminants contained in the starting monomers or as degradation byproducts, and the noninjectability of the system. For these limiting reasons, the development of a hydrogel as vitreous substitute has been so far matter of discussion.

Poly(vinyl alcohol), PVA, hydrogels have been studied as a material for biomedical applications[5] including vitreous substitution.[6] PVA hydrogels, produced by γ irradiation of 7% polymer aqueous solution, were implanted in vivo into rabbit eyes, and no inflammatory reaction was observed, although γ ray induced cross-linking of PVA was not practicable for in situ hydrogel formation.[6] In consideration of the desirable features of PVA (chemical versatility,[7] i.e., the possibility to modify PVA chains by means of the reactive hydroxyl moiety contained in the backbone, and biocompatibility[8]), we set out to develop a formulation of a hydrogel that could form directly in the ocular cavity. In recent papers, cross-linking reactions in the absence of photo- or radical initiators have been studied.[9]

In this paper, we report on the potentialities of PVA as a starting material for vitreous substitute, and in particular, on the design of injectable aqueous solutions of methacryloyl derivatized PVA, PVA-MA, containing a photoinitiator, able to form hydrogels in situ when irradiated with the proper wavelength radiation.

Mühlebach et al.[10] showed the possibility of grafting PVA with acrylic and methacrylic acid to obtain acryloyl or methacryloyl derivatized PVA.

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The synthesis of PVA-MA hydrogels can be carried out either by placing the reaction vessel containing the polymer aqueous solution in a photoreactor, or in the case of vitrectomy, by guiding via an optical fiber the radiation necessary to trigger the cross-linking reaction of the PVA-MA aqueous solutions. The latter option minimizes the invasiveness of surgical techniques as the network precursor is injected in the ocular cavity and then cross-linked in situ by irradiation.

In this paper, we describe the synthesis and characterization of aqueous solutions of methacrylated PVA with a degree of substitution ranging from 0.01 to 0.20 according to a method described by Hennink et al. for the grafting of methacryloyl dextran.[11,12] Photoinitiated radical polymerization was employed to cross-link methacrylated PVA to obtain macroscopic polymer hydrogels. The resulting networks were studied in terms of equilibrium swelling and elastic properties. The use of such hydrogels as drug delivery devices is a key point for the formulation of a multifunctional biomaterial for vitreous substitution, as it is often required in vitrectomy replacement a simultaneous drug releasing action. To understand how the diffusion process of the drugs is influenced by the structure of the polymer network, kinetics of release of both low and high molecular weight solutes in the hydrogels was determined according to the free volume theory. In this study, the performances of the PVA hydrogels in vitreous substitute application were evaluated by means of in vitro rheology tests for the assessments of the sol−gel transition. In particular, the influence of important parameters for in situ vitreous replacement such as polymer and initiator concentrations, irradiation power, and time exposure to UV fixed radiation were investigated.

Materials and Methods

Materials. 4-(*N*,*N*-Dimethylamino)pyridine, DMAP, was a Fluka product. Poly(vinyl alcohol) with nominal weight average molecular weight of 70 000 g/mol (degree of deacetylation: 99%) and 13 000 g/mol (degree of deacetylation: 98%), bovine serum albumine, BSA, and methylene blue were Sigma products.

Glycidyl methacrylate, GMA, was purchased from Fluka. The photoinitiator, 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one was supplied by Ciba. Dimethyl sulfoxide, DMSO, acetone, HCl, and NaOH were purchased from Carlo Erba. All chemicals were reagent grade and used without further purification.

Water was Milli-Q purity grade (18.2 MΩ cm), produced with a deionization apparatus (PureLab) from USF.

Dialysis membranes (cut off 12 000 g/mol) were purchased from Medicell International Ltd and prepared according to standard procedure.

Synthesis of Poly(vinyl alcohol)-methacrylate, PVA-MA. PVA was grafted with a methacrylate moiety according to the procedure described by Hennink[11,12] and Anseth.[13] Typically, 10 g of PVA with weight average molecular weight of 70 000 g/mol was dissolved in 250 mL of DMSO added with 5 g of DMAP. After displacement of oxygen by purging N_2 for 1 h, GMA was added in the proper molar ratio with the polymer repeating units and left for 48 h under stirring in the darkness. DMAP was neutralized by the addition of concentrated hydrochloric acid to prevent alkaline hydrolysis of the methacrylic ester. Samples with stoichiometric degrees of substitution, D.S., of 1, 2, 5, 10, and 20% were prepared.

Table 1. Theoretical and Experimental Degree of Substitution, D.S., of PVA-MA[a]

$D.S._{th}$ (%)	$D.S._{exp}$ (%)
1	1.0
2	1.7
5	3.3
10	7.2
20	16.8

[a] Errors within 10%.

After completion, the reaction mixture was dialyzed against water to purify the derivative of PVA and to exchange DMSO with water. PVA-MA samples with a content of methacrylic ester up to 2% were soluble in water, whereas samples with higher D.S. values precipitated during dialysis. Water-soluble samples were precipitated very slowly by adding acetone up to a volume ratio of 50:50 (v/v). The precipitate was collected, finely broken up, and finally dried.

For the water-insoluble samples, after dialysis the precipitate was collected, finely dispersed, and dried.

Experimental D.S. values were determined by ^{1}H NMR as the ratio of the peak areas of the vinyl protons at 5.50 and 6.20 ppm and the unsubstituted methine moiety at 4.07−4.2 ppm of the PVA backbone. As a check, the peak areas of grafted methine were compared with the unsubstituted CH of the backbone confirming, within the experimental errors, the same results obtained with the analysis based on the vinyl protons. For NMR analysis, a Bruker 400 MHz was used. Spectra were registered using acetone as reference.

The stoichiometric and experimentally determined D.S. are given in Table 1.

Synthesis of the Hydrogel Based on PVA-MA. Typically a 9% (w/v) aqueous or DMSO solution of PVA-MA was added with the photoinitiator at a concentration of 3 g/L. The mixture was irradiated in a photoreactor with a power of 12 W at 365 nm (Photochemical Multirays Reactor, Helios Italquarz) for 5 min. The system was left for 12 h at 25 °C. After this equilibration period, the gel was set, and extensive washings were carried out to remove the excess of reactants.

Each gel was prepared in three replicas to average out possible differences and heterogeneities occurring in the gels. Polymer hydrogels at a working concentration of 9% (w/v) were transparent.

Rheological Characterization. The mechanical properties of the networks measured in compression mode were determined by dynamic mechanical analysis (DMA) as described by Meyvis et al.[14] The DMA measurements were carried out with a DMA-7 dynamic mechanical analyzer (Perkin-Elmer) equipped with a parallel plate accessory (D = 20 mm). Disks measuring 20 mm in diameter and 2−4 mm in thickness were cut from the slabs and completely immersed in a water bath. The experiments were performed at room temperature by applying a different static stress between 30 and 300 mN, to ensure a good contact

between hydrogel and plates, and dynamic strain of 0.2% at frequencies of 1 Hz. Three replicas of each sample were measured.

Kinetics of gelation was followed by DSR200 rotational rheometer (Rheometric Scientific) equipped with a thermostated transparent measuring cell with plate-plate geometry, illuminated with a UV radiation at 365 nm by an optical fiber with a diamater of 8 mm. Storage modulus (G') and loss modulus (G'') were measured during the gelation process (sol−gel transition) by applying a stress less than 15 Pa (always in the linear viscoelastic region) at a fixed frequency of 1 Hz (6.28 rad/s) with a plate distance of 2−3 mm. All runs were performed at 37 °C.

In view of a potential use in vitrectomy, a screening of the parameters important for the gelation kinetics was made by studying the process with aqueous solutions of 13 000 g/mol PVA-MA with concentrations ranging from 2 to 9% (w/v) in the presence of the initiator with concentrations ranging from 0.1 to 0.5% (w/v), respectively. Incident radiation at 365 nm was used at 1, 10, 50, and 100% of the maximum output power (40 W) for an irradiation time of 60, 300, 600, and 1200 s.

Loading and Release of Diffusible Molecules and Hydrogel Degradative Studies. Release of low molecular weight molecules was tested by loading methylene blu, MB, in PVA-MA hydrogels. Samples with a volume of 5 cm^3, prepared in cylindrical shape, were equilibrated for 24 h in a 100 mL MB aqueous solution at a concentration of 1×10^{-3} M. The loaded hydrogels were placed in a volume of water 20 times larger than the hydrogel volumes, thermostated at 25 °C, and the kinetics of release was followed spectrophotometrically at 665 nm. The concentration of released MB was determined using a molar extinction coefficient of 81 600 M^{-1} cm^{-1}.[15]

In another set of experiments, the release of high molecular weight molecules was studied using bovine serum albumin, BSA, as probe. The loading was carried out by exposing preformed PVA-MA hydrogels to an 85 mM BSA aqueous solution at pH 7.0 for 24 h. The release of BSA from hydrogels into 100 mL of milliQ quality water was followed spectrophotometrically at 280 nm using a molar extinction coefficient of 50 M^{-1} cm^{-1}.

The degradation and swelling behavior of the photopolymerized and dialyzed hydrogels were investigated by measuring periodically the weight of swollen and dried networks after swelling.

Results and Discussion

The general synthetic route for coupling a vinyl moiety with a hydroxylated polymer was first described by Hennink in his papers on the grafting of the polysaccharide dextran with a methacrylic moiety.[11,12] The introduction of this group can in principle occur either by the attachment of a methacryloylglyceryl or of a methacryloyl side chain to the hydroxyl function of the polymer backbone. In the latter case, the derivatization of the polymer produces a glycidol molecule. The presence of this molecule during the grafting reaction was observed by gas chromatography−mass spectrometry determination,[12] leading to the conclusion that the effective pathway to the vinyl derivatization of dextran proceeds through a trans-esterification with the production of a glycidol molecule as leaving group.

This reaction scheme is consistent with the findings we have collected in the case of the grafting reaction of a methacrylic moiety on PVA. The proton NMR spectra of a PVA-MA sample with D.S. = 1.7% in deuterated water (Figure 1a) and with D.S. = 16.8% in deuterated DMSO (Figure 1b) show the resonances assigned on the basis of the peak integrals and by comparison with the NMR spectrum of PVA before grafting. For all the investigated degrees of substitution, the presence of a glyceryl spacer between the hydroxyl moiety of the backbone and the methacryloyl side chain was not detected in the spectra, confirming the conclusions drawn in the reported studies carried out on dextran.[11,12] In Table 1, the comparison of the stoichiometric degree of substitution and of the actual values obtained by integrating the resonance peaks of the vinyl moiety and of the backbone methine groups is shown.

Aiming to complete the in situ formation of a chemical hydrogel from injectable aqueous solutions of PVA-MA, the cross-linking reaction was initiated by irradiating at 365 nm the polymer solution contained in a 10 mL beaker in the presence of the photoinitiator.

This procedure yielded macroscopic hydrogels obtained with 4 mL of aqueous solution at a polymer concentration of 9% (w/v). Mold-shaped, homogeneously transparent hydrogels were obtained with all the D.S. values investigated. Hydrogel volumes were equal to those of the starting solutions, with the exception of the hydrogel with the highest cross-linking density (D.S. 16.8) where some shrinking accompanied by solvent rejection was observed. In analogy with the hypothesis suggested by Hennink for methacrylated grafted dextran,[16] we propose a PVA-MA networking model where a wire of polymethacrylate is sandwiched with two PVA chains, holding them together (see Chart 1).

The networking is assured when the polymethacrylate wire switches from one PVA chain to another one, and intermolecular photocross-linking of PVA macromers occurs. This structural feature is essential in preserving the overall shape of the gel even when some of the ester side chains undergo hydrolytic degradation, as will be seen when degradation processes of PVA-MA hydrogels are described.

The grafting reaction was carried out at different glycydyl methacrylate contents to obtain PVA chains at different substitution degrees. The hydrophobic character of the grafted PVA chain increased with D.S., and for this reason, the cross-linking reactions with polymer chains with a D.S. higher than 0.05 were carried out in DMSO subsequently replaced by water. Although not significant for biomedical applications, we have included in this work the study of highly substituted PVA chains (up to 20%) for a better understanding of the swelling properties of highly cross-linked hydrogels.

Structural Characterization of PVA-MA Hydrogels. The structural features of PVA-MA hydrogels were investigated by dynamic−mechanical analysis and equilibrium swelling measurements, two well-established meth-

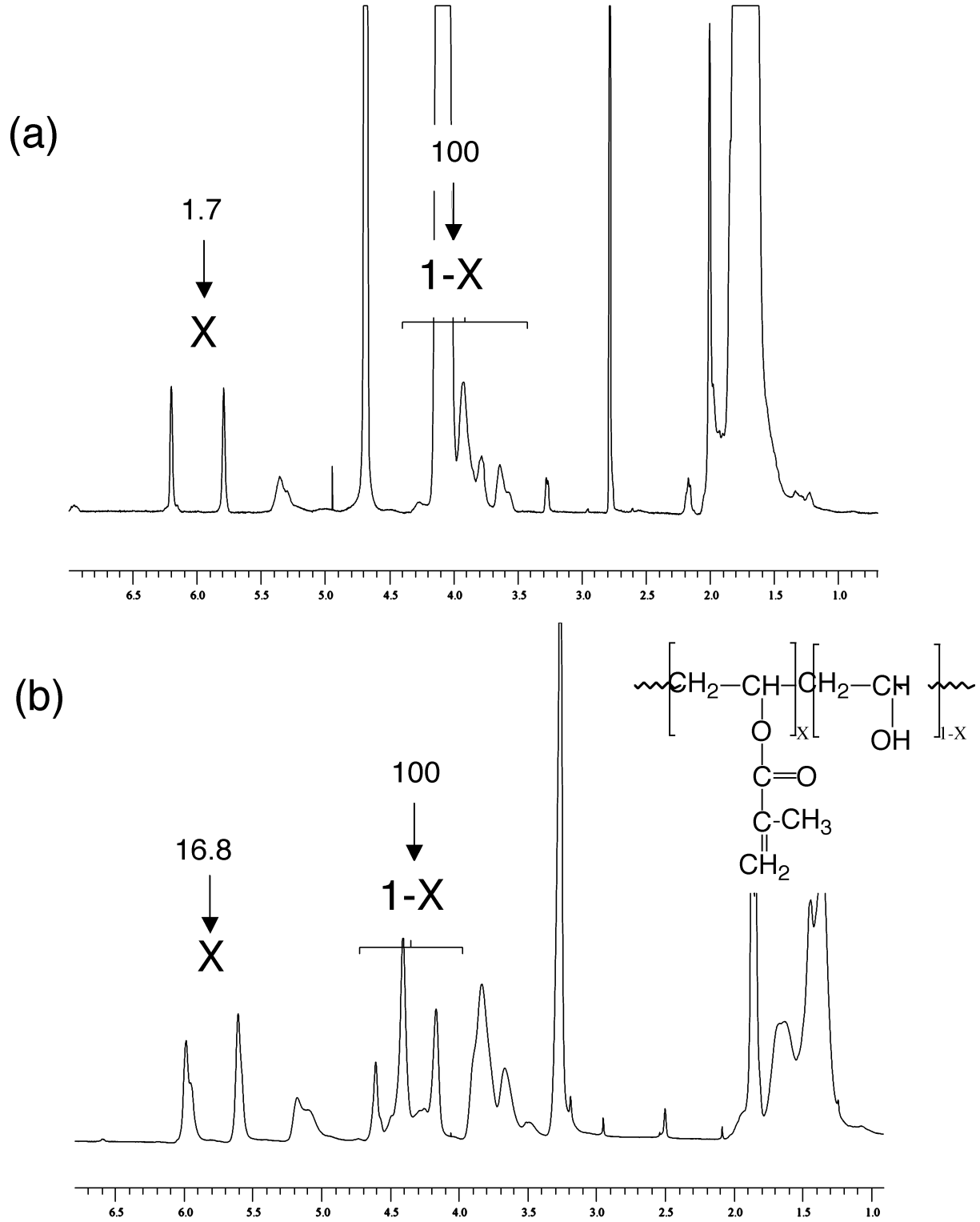


Figure 1. 400 MHz proton NMR of PVA-MA derivatives with (a) D.S. 1.7% in D_2O and (b) D.S. 16.8% in deuterated DMSO.

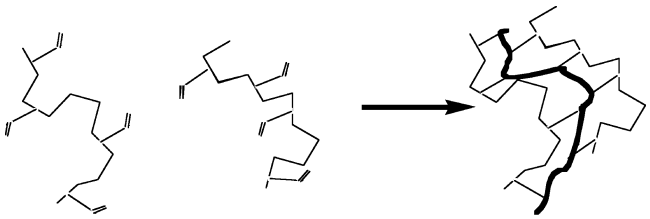
ods in polymer network studies. In our case, the attention was addressed particularly toward the determination of the Flory−Huggins interaction parameter, χ, as the presence of an increasing content of methacrylic moiety in the hydrogels imparts an overall hydrophobic character to the systems.

The Flory swelling theory[17] successively modified by Peppas[18] for the case of network formation in solution relates the average molecular weight between cross-links, M_c, with the swelling behavior and χ assuming tetra-functional junctions

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{(\rho_2^{-1}/v_1)[\ln(1-\phi_2) + \phi_2 + \chi\phi_2^2]}{\phi_0\left[\left(\frac{\phi_2}{\phi_0}\right)^{1/3} - \frac{1}{2}\left(\frac{\phi_2}{\phi_0}\right)\right]} \quad (1)$$

where ϕ_0 and ϕ_2 are the polymer volume fraction in the hydrogel in the relaxed and swollen state, respectively,

Chart 1. Scheme of Crosslinking Reaction with Formation of Polymerized Methacyrlate Moiety (Bold Line) According to Hypothesis Formulated by Hennink et al.[16]



Partially substituted PVA-MA polymer

Table 2. Results of the Equilibrium Swelling and Dynamic−Mechanical Measurements on PVA-MA Hydrogels[a]

D.S. (%)	$\phi_0 \times 10^2$	$\phi_2 \times 10^2$	$E_c \times 10^{-4}$ (Pa)	M_c (g/mol)[b]	χ[b]	ξ (Å)[b]
1.0	4.2	1.4	0.03	18 000	0.50	380
1.7	5.1	2.4	0.58	14 400	0.50	290
3.3	6.7	8.4	9.6	5000	0.50	100
7.2	6.7	12	12	4500	0.57	90
16.8	6.7	18	21	3400	0.56	70

[a] PVA molecular weight 70 000 g/mol. [b] Average values over five samples; errors within 10%.

obtained by weight analysis. ρ_2 is the density of the dry polymer (1.269 g/cm^3 for atactic PVA), and υ_1 is the molar volume of the solvent (i.e., 18 cm^3 mol^{-1} for water).

If equilibrium swelling measurements are coupled with dynamomechanic analysis, the simultaneous determination of M_c and χ is possible, yielding the simultaneous determination of both structural and interaction parameters of the investigated hydrogels.

In a DMA experiment at constant frequency of the applied force and for relative elongation up to 0.2%, a linear behavior between the stress and the strain is observed. Elastic modulus, E_c, is related to the effective chain density ν_e/V_0 taking into account an affine-like deformation behavior of the network, where V_0 is the dry hydrogel volume

$$\frac{\nu_e}{V_0} = \frac{E_c}{3RT}[\phi_0^{-2/3}\phi_2^{-1/3}] \quad (2)$$

and to M_c by means of

$$M_c = \frac{\rho_2}{\frac{\nu_e}{V_0} + \frac{2\rho_2}{M_n}} \quad (3)$$

The results of this study are summarized in Table 2.

The M_c values at different degrees of cross-linking obtained by eq 3 can be converted in an average mesh size, ξ, of the hydrogel[19] considering an equivalent chain

$$\xi = \phi_2^{-1/3}(C_\infty n)^{1/2} l \quad (4)$$

where n is the number of bonds between cross-links, and l and C_∞ are the carbon−carbon bond length and the characteristic ratio (i.e., 1.54 Å and 8.8 for PVA, respectively).

These results indicate a good correlation between the molecular weight between cross-links, M_c, and the degree of substitution of the PVA-MA samples. From Table 2, it can be noted that the hydrophobicity of the network increases, reflected by an increase in the value of χ and promoted by the increased grafting with the vinyl moiety. This effect occurring at high D.S. values is at the basis of the observed network shrinkage accompanied by solvent rejection and indicating a possible phase separation of the network or aggregation of methacrylate moiety. The increase in χ values in the hydrogels determined by combining dynamomechanical and equilibrium swelling measurements is confirmed by static light scattering measurements on aqueous solution in PVA-MA for D.S. ranging from 0.5 to 2% (not shown). Moreover, data from Table 2 show that PVA-MA with higher D.S. values form more elastic networks with increasing values of E_c. However, at the highest D.S. values, M_c levels off because of an increase in the amount of network imperfections such as loops that originate from intramolecular cross-linking. For low D.S. (1.0 and 1.8%) values, there is a loss of polymer material of about 30% due to uncompleted conversion during the gel formation. Consequently, after the setting of the polymer network at the equilibrium swelling conditions, the entangled but not chemically linked chains are eluted in the external solvent.

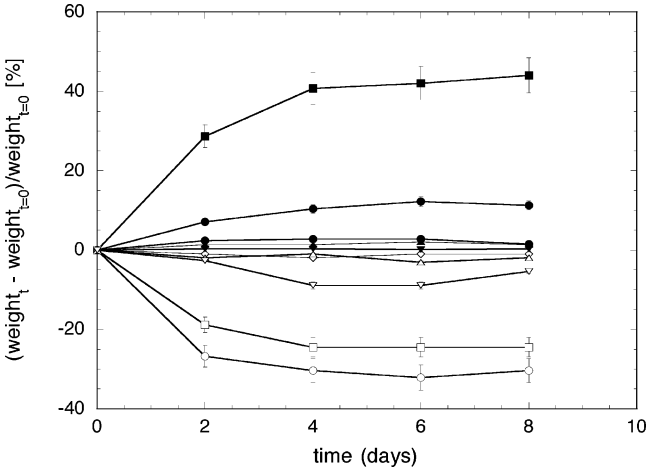


Figure 2. Study of the partial degradation of PVA-MA hydrogels in terms of the relative (%) weight change with respect to the initial state of hydrogels based on PVA-MA with different D.S. Full and empty symbols refer to swelled and corresponding dried networks after swelling, respectively: (●, ○: D.S. 1%; ■, □: D.S. 1.8%; ◆, ◇: D.S. 3.3%; ▲, △: D.S. 7.2%; and ▼,▽: D.S. 16.8%).

These results are necessary in the interpretation of the release behavior of the gels described in the next section.

Network Partial Degradation after One Week of Dialysis. In general, for all hydrogels, the transport properties are strongly dependent on the cross-linking density of the network. In our case, partial degradation occurs by ester linkage cleavage in the body of the hydrogels, thus decreasing the cross-linking density of the overall network with the loss of segments of polymer chains. Measurements of degradation were carried out on hydrogels equilibrated for several days at pH 7 at 25 °C.

The degradation process was monitored by weighing out the swollen and dried networks after the swelling process at different times and referring the weight changes to the initial weights. Figure 2 shows that a partial degradation process occurs mainly in the PVA-MA hydrogels with the lowest degree of cross-linking, leading to a decrease of the cross-

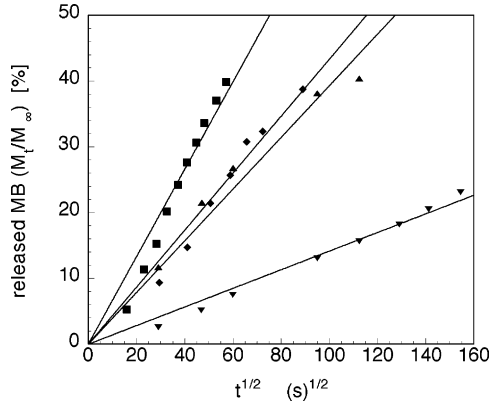


Figure 3. Cumulative release (%) of methylene blue in PVA-MA hydrogels with different D.S. (%): ■ D.S. 1.7; ◆ D.S. 3.3; ▲ D.S. 7.2; and ▼ D.S. 16.8.

linking density after 4 days. Positive values of the *y*-axis in Figure 2 refer to measurements on swollen samples, corresponding to an increase of absorbed water due to a decrease of the cross-linking density, attributed mainly to the occurrence of an hydrolysis process, although we cannot exclude some loss of material due to disentanglements of noncross-linked chains. Negative values refer to measurements on dried samples after swelling, denoting a mass loss due to both network degradation (hydrolysis) and elution of free PVA chains. After this time, the degradation process levels off with the conservation of the overall shape and integrity of the network. This can be explained taking into account the structure of the junction zones. Assuming valid the network model formulated by Hennink et al. for radical cross-linking of vinyl containing side chains,[16,20] the PVA chains zipped by the polymethacrylic wires (Chart 1) will not move upon hydrolysis of some ester links, maintaining the original macroscopic features of the hydrogel. This process is very limited, if any, for hydrogels with higher cross-linking density. The more compact structure of these hydrogels caused by the reduced solvation capacity of water as attested by a χ value larger than 0.5, shown in Table 2, slows down the hydrolysis and the partial degradation of these hydrogels.

Kinetics of Release. To evaluate a bioactive function (namely, a drug release) of these hydrogels, we have tested the release of a low molecular weight probe molecule (i.e., methylene blue, MB) and of large molecule such as BSA by monitoring the release of solutes from the hydrogel into the outside aqueous medium.

The release behavior is different for the two types of probes and depends on the average mesh size of the matrix and on the hydrodynamic diameter of the probe molecule.

When the PVA-MA hydrogel was loaded with MB, the release was governed, after an initial burst, by a Fickian behavior. In Figure 3, the trends of the cumulative release versus the square root of time are reported.

The diffusion coefficient of the solute was calculated according to the formula[21]

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{D_m t}{\pi r^2}} \qquad (5)$$

which is valid for monolithic devices with a cylindrical geometry and for values of fractional amount of released material, M_t/M_∞, up to 0.4, where M_t is the cumulative amount of released MB at time t, $M\infty$ is the amount of MB released as time approaches infinity, and r is the radius of the hydrogel cylinder at the equilibrium state. Figure 3 shows that it is possible to control the diffusion coefficient by varying the structural feartures (i.e., the M_c, of PVA-MA hydrogels).

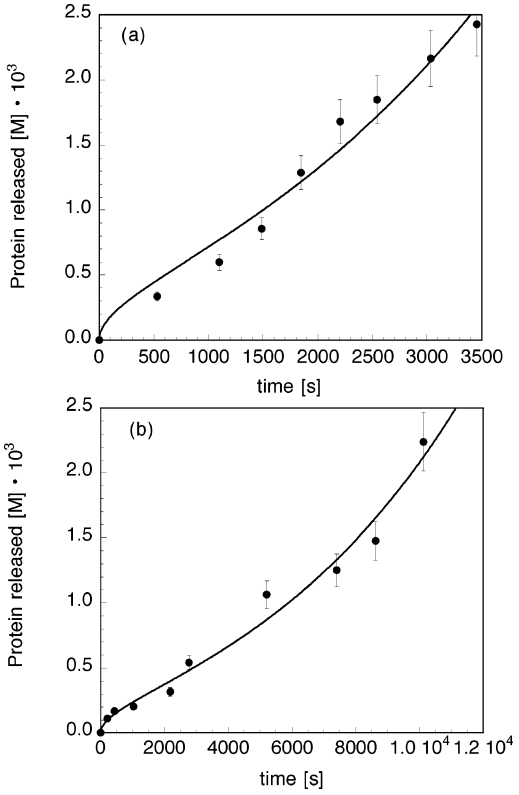


Figure 4. Concentration of released protein as a function of time: (a) D.S. = 1.0%, (b) D.S. = 3.3%. Lines are the nonlinear best fit according to eq 6 (see text).

We have also tested these hydrogel systems in view of their possible use as protein delivery devices. In this case, a qualitatively different mode of release occurs when molecules characterized by large hydrodynamic volumes as BSA are loaded. The delivery of the protein is more sensitive to pore size transformation occurring during the release. This effect is expected to be modulated by the overall kinetics of the high molecular weight drug. The observed trend for the hydrogels based on PVA-MA with a degree of substitution up to 7.2 is consistent with a release coupled with a gel degradation (see Figure 4).

The release curve in the presence of hydrogel partial degradation described by[22]

$$M_t = A(2P_0 e^{kt} C_0 t)^{1/2} \qquad (6)$$

allows by a nonlinear fit the evaluation of the preexponential factor (i.e., $A(2P_0C_0)^{1/2}$) and of the time constant k. The knowledge of the surface of the cylindrical hydrogel slab, A, and of the loaded concentration of protein in the gel volume, C_0, permits the determination of the permeability of the protein in the hydrogel and its diffusion coefficient, D_{BSA}, by means of $D_{BSA} = P_0/C_0$, where P_0 is the perme-

Table 3. Diffusion Coefficients for Methylene Blue and BSA in PVA-MA Hydrogels at Different Molecular Weights between Cross-Links

M_c (g/mol)[a]	$D_{MB} \times 10^5$ (cm²/s)	$D_{BSA} \times 10^7$ (cm²/s)
18 000	1.0	3.02
14 400	0.84	
11 500		2.59
10 600		1.96
5000	0.49	
4500	0.40	
3900		0.65
3400	0.09	

[a] Averaged values over four samples; errors within 10%.

ability of the drug before occurrence of matrix degradation. The release behavior of PVA-MA hydrogel with the highest cross-linking density cannot be framed in this interpretation as the hydrogel pore size is comparable or smaller than the protein average dimensions in solution (i.e., 72 Å).[23] In this case, the release is due to superficially adsorbed protein not influenced by a simultaneous limited degradation of the matrix.

A summary of the results obtained for the release of MB and of BSA is given in Table 3.

Diffusional properties of matrixes depends on the relation between the average size of the percolating solute and the size of the openings in the gel. The origin of such openings can be considered as the combination of structural features (i.e., degree of cross-linking, chain flexibility, and dynamic factors, i.e., chain conformational fluctuations, and thermal fluctuations). We have used the free volume theory,[23] a model for diffusion in hydrogels that takes into account the aforementioned sieving action of the network, for connecting the experimental diffusion coefficient of methylene blue and BSA with the structural features of PVA-MA hydrogels. Literature values for the diffusion coefficient in water, D_0, for MB and BSA are 1.01×10^{-5} and 0.59×10^{-6} cm^2 s^{-1}, respectively. According to this theory, there is a linear dependence of $\ln(D_m/D_0)$, where D_m is the diffusion coefficient of the solute inside the hydrogel and the ratio of the polymer volume fraction over the water volume fraction, $\phi_2/(1-\phi_2)$. This parameter is directly related to the state of hydration of the gel

$$\ln\left(\frac{D_m}{D_0}\right) = \ln(\Psi) - k r_s^2 \left(\frac{\phi_2}{1-\phi_2}\right) \qquad (7)$$

where Ψ is a factor accounting for the sieving effect of the matrix, k is a dimensional constant, and r_s is the hydrodynamic radius of the solute. This equation is strictly valid only when the gel pore size is larger than the overall dimension of the percolating molecule.[24] Use of eq 7 as fit equation of the experimental data shows in Figure 5a that the intercept, $\ln(\Psi)$, is about zero, indicating that the hydrodynamic size of the solute, MB, is much smaller than the sieving characteristic dimension of the matrix. On the contrary, in the diffusion of BSA, Figure 5b, the intercept is finite and negative indicating that the sieving effect of the network is present.

Kinetics of Gelation. This study was carried out by measuring the storage and loss moduli during the sol−gel

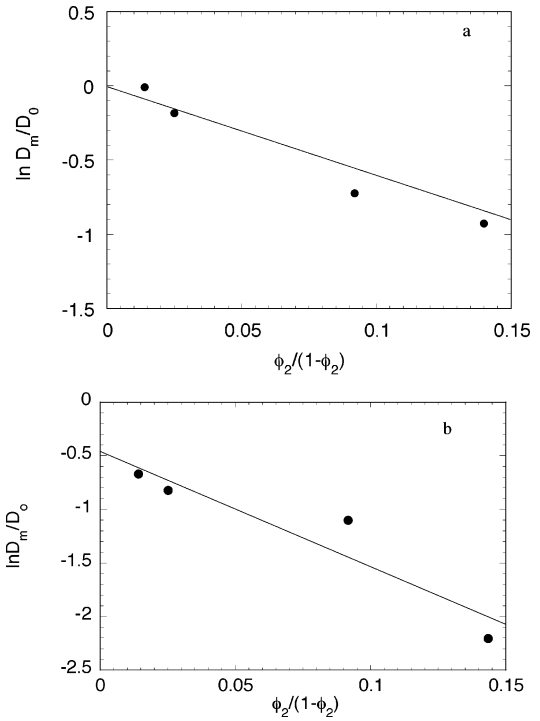


Figure 5. Logarithm of the normalized diffusion coefficient of (a) methylene blue and (b) BSA as a function of the inverse of hydrogel hydration.

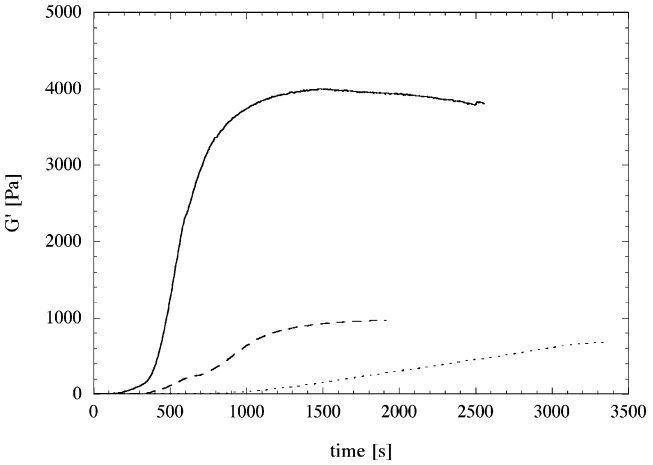


Figure 6. Storage modulus, G', as a function of time at 37 °C. Polymer concentration: 9% (w/v). UV output power conditions: continuous line, 40 W; dashed line, 20 W; and dotted line, 4 W.

transition occurring in aqueous solutions of PVA-MA with a molecular weight of 13 000 Da. The choice of this molecular weight was made considering the convenience to perform a vitrectomy using a less viscous solution.

The polymer aqueous solution of PVA-MA in the presence of the photoinitiator was irradiated with a UV radiation at 365 nm.

Figure 6 shows that the gelation of a 9% (w/v) PVA-MA aqueous solution is accomplished within 1 h by choosing an initiator concentration of 0.05 g/L with a radiation power from 0.4 to 40 W exposing the sample for an irradiation time of 300 s.

This approach to vitreous replacement offers the possibility to tune the gelation process in a systematic way. In fact, gelation time can be regulated by intervening on one or more of the parameters influencing the process (i.e., polymer and

initiator concentrations, irradiation power, and exposure time). As a result of the screening study, the polymer concentration cannot be lower than 4% (w/v), as this parameter intervenes also in the elastic properties of the gel. In fact, a polymer solution at 9% (w/v) can be considered a good compromise in terms of transparency and mechanical properties. On the other hand, photoinitiator concentration cannot be too high as it may cause some metabolic or tissue damage. In our tests, an initiator concentration of 0.02 g/L was sufficient to trigger the photoreticulation process.

However, in future applications of the outlined method, other considerations, besides the timing of the surgical operation, can pose limits in the choice of the conditions for obtaining the gel. The fine-tuning of the method can only be obtained with a more detailed study of the influence of the different parameters on the properties of this gelling system.

Conclusion

We have shown that injectable aqueous solutions of PVA-MA can be photocrosslinked, yielding transparent hydrogels able to maintain the shape of the mold after irradiation at 365 nm in the presence of the photoinitiator. This approach can be envisaged as potentially useful for in situ formation of a hydrogel for tissutal replacement as required, for example, in vitrectomy where an aqueous solution containing PVA-MA as polymeric network precursor can be injected in the ocular cavity and cross-linked via local irradiation by means of an optical guide. The hydrogels studied in this work have been prepared in a photoreactor for the evaluation of their structural characteristics, showing that network pore size can be easily tailored by controlling the degree of substitution of PVA-MA. In this way, several hydrogel properties such as diffusion of low and high molecular weight molecules, mimicking the delivery of conventional drugs or genes, can be fine-tuned for multifunctional use of the hydrogels. Small hydrodynamic solute molecules were found to diffuse with a Fickian behavior, whereas, for high molecular weight, solute diffusion is coupled with partial degradation of the network.

In vitro gelling kinetics was studied to gain knowledge on the various combinations of the main parameters influencing the process.

Promising preliminary in vitro cytotoxycological tests have been carried out on human neuroblastoma cells. Although more stringent in vitro and in vivo tests are needed, the picture gathered so far indicates that PVA based hydrogels are promising materials requiring more in depth investigations aimed at assessing the potentiality of this soft condensed matter system as a biomaterial suitable for vitreous replacement.

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