Bioerodible Hydrogels Based on 2-Hydroxyethyl Methacrylate: Synthesis and Characterization

FEDERICA CHIELLINI,[1] FEDERICA PETRUCCI,[1] ELISABETTA RANUCCI,[2] ROBERTO SOLARO[1]

[1] Department of Chemistry and Industrial Chemistry, University of Pisa, via Risorgimento 35, 56126 Pisa, Italy

[2] Department of Polymer Technology, Royal Institute of Technology, S-100 44 Stockholm, Sweden

Received 6 July 2001; accepted 7 November 2001

ABSTRACT: Biocompatible polymers with specific shape and tailored hydrogel properties were obtained by polymerization of mixtures of 2-hydroxyethyl methacrylate (HEMA) with 1–8 wt % ethylene glycol dimethacrylate (EGDMA) or tetra(ethylene glycol) diacrylate (TEGDA) as crosslinking agents, by using a redox initiator. Introduction of charged positive and negative groups was easily achieved by direct polymerization of appropriate monomer mixtures and by chemical transformation of preformed hydrogels. Investigation of the swelling behavior of the prepared hydrogels evidenced an appreciable dependence on both solvent type and polymer chemical structure. Additionally, the solvation process resulted in being controlled by solvent diffusion, according to a Fickian II mechanism. The presence of several types of water with different melting behavior was observed in fully swollen hydrogels. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 85: 2729–2741, 2002

Key words: biomaterials; 2-hydroxyethyl methacrylate; hydrogels; diffusion; water structure

INTRODUCTION

Among the several classes of biomedical materials, increasing attention is being devoted to polymeric hydrogels, which have the ability to swell in water or in aqueous solutions by forming a swollen gel phase that, in the case of crosslinked systems, will not dissolve regardless of the solvent.[1] An important feature of hydrogels is their biocompatibility, which can be attributed to their ability to simulate living tissue characteristics such as large water content,[2] low interfacial tension with body fluids, and permeability to metabolites, nutrients, and oxygen.[3]

At present, the most investigated hydrogels are those based on 2-hydroxyethyl methacrylate (HEMA), thanks to their ascertained nontoxicity and widespread use in the production of soft contact lenses.[4]

In the present study, we describe the synthesis and characterization of HEMA hydrogels used in the formulation of drug delivery systems and as the basic ingredient for the formulation of scaffolds for tissue engineering applications. Attention was focused on the characterization of samples with different degrees of crosslinking and of swelling in various aqueous solutions. Diffusion coefficients of water were also investigated.

EXPERIMENTAL

Materials

HEMA, 2-(*N*,*N*-dimethylamino)ethyl methacrylate (DAEMA), ethylene glycol dimethacrylate (EGDMA), tetra(ethylene glycol) diacrylate

Correspondence to: R. Solaro (rosola@dcci.unipi.it).
Contract grant sponsor: CNR Progetto Finalizzato.

Journal of Applied Polymer Science, Vol. 85, 2729–2741 (2002)

Table I Feed Compositions Used for the Preparation of HEMA-Based Hydrogels

Run[a]	EGDMA (wt %)	TEGDA (wt %)	Tetrakis (wt %)
E0	0	0	0
E1	1	0	0
E2	2	0	0
E3	3	0	0
E4	4	0	0
E8	8	0	0
ET0	0	0	1
ET1	1	0	1
ET2	2	0	1
ET3	3	0	1
ET4	4	0	1
ET8	8	0	1
T0	0	0	0
T1	0	1	0
T2	0	2	0
T3	0	3	0
T4	0	4	0
T8	0	8	0
TT0	0	0	1
TT1	0	1	1
TT2	0	2	1
TT3	0	3	1
TT4	0	4	1
TT8	0	8	1

[a] 1.0 g of HEMA, 38 μl of a water solution of sodium metabisulfite 0.526*M* and 38 μl of a water solution of ammonium persulfate 0.438*M*, at 37°C for 4 hs. Weight percent is referred to the monomer HEMA content.

(TEGDA), methacrylic acid (MAA), sodium acrylate, and pentaerityrityl tetrakis(3-mercaptopropionate) (Tetrakis), all from Aldrich (USA), were used without any preliminary purification.

Dulbecco's modified Eagle's medium (DMEM), pH 7.4, was purchased from Gibco BRL (USA).

Preparation of HEMA-Based Hydrogels

Poly(HEMA) hydrogels were prepared according to general procedures. Data relevant to the different runs are reported in Table I, whereas some typical examples are described in detail as follows.

Polymerization of HEMA–EGDMA Mixtures in Bulk

One gram (7.7 mmol) HEMA, 10 mg (51 μmol) EGDMA, 38 μL (20 μmol) 0.526*M* sodium metabisulfite water solution, and 38 μL (17 μmol) 0.438*M* ammonium persulfate water solution were mixed in an Eppendorf tube and after few pumping/degassing cycles, the mixture was cured at 37°C for 4 h.

Polymerization of HEMA–EGDMA Mixtures in Water Solution

A solution of 1.0 g (7.7 mmol) HEMA, 10 mg (51 μmol) EGDMA, 38 μL (20 μmol) 0.526*M* sodium metabisulfite water solution, and 38 μL (17 μmol) 0.438*M* ammonium persulfate water solution in 0.5 mL of water was prepared in an Eppendorf tube and after few pumping/degassing cycles, was cured at 37°C for 4 h. The resulting polymeric cylinder was characterized by swelling measurements in water and DMSO.

Polymerization of HEMA–EGDMA Mixtures as Films

Ten grams (77 mmol) HEMA, 100 mg (0.51 mmol) EGDMA, 38 μL (20 μmol) 0.526*M* sodium metabisulfite water solution, and 38 μL (17 μmol) of a 0.438*M* ammonium persulfate water solution were mixed in a glass tube and after few pumping/degassing cycles, the mixture was injected with a syringe between two silanized glasses separated by a 1-mm (or 0.1-mm)-thick silicon spacer. Clamps were applied onto the glasses to ensure a perfect sealing, and the mixture was cured at 37°C for 4 h. The obtained film was characterized by swelling measurements in water, dimethylsulfoxide (DMSO), 0.01*M* pH 7.2 phosphate buffer solution, and DMEM.

Polymerization of HEMA–TEGDA–Tetrakis Mixtures as Films

One gram (7.7 mmol) HEMA, 30 mg (99 μmol) TEGDA, 10 mg (20.5 μmol) Tetrakis, 38 μL (20 μmol) 0.526*M* sodium metabisulfite water solution, and 38 μL (17 μmol) of a 0.438*M* ammonium persulfate water solution were mixed in a glass tube. After a few pumping/degassing cycles, the mixture was cured at 37°C for 2 h, to increase its viscosity, and then was injected with a syringe between two silanized glasses separated by a 100-μm-thick silicon spacer. Clamps were applied onto the glasses to ensure a perfect sealing, and the mixture was cured at 37°C for 2 h.

Preparation of HEMA Hydrogels Containing Ionizable Groups by Direct Polymerization

Preparation of HEMA-based hydrogel films containing MAA or DAEMA units were performed

Table II Compositions of Monomer Mixtures Used for the Preparation of HEMA-Based Hydrogels Containing Ionizable Groups

Run[a]	MAA (wt %)	DAEMA (wt %)
T3C1	1	0
T3C3	3	0
T3C5	5	0
T3C10	10	0
T3A1	0	1
T3A3	0	3
T3A5	0	5
T3A10	0	1

[a] 1.0 g of HEMA, 30 mg of TEGDA, 38 μl of a 0.526*M* water solution of sodium metabisulfite and 38 μl of a 0.438*M* water solution of ammonium persulfate, at 37°C for 4 h.

according to a general procedure. Data relevant to the different runs are summarized in Table II, whereas two typical experiments are described in detail by the following preparations.

Preparation of HEMA Hydrogels Containing MAA Units (Run T3C1)

One gram (7.7 mmol) HEMA, 10 mg (0.12 mmol) MAA, 30 mg (99 μmol) TEGDA, 38 μL (20 μmol) 0.526*M* sodium metabisulfite water solution, and 38 μL (17 μmol) 0.438*M* ammonium persulfate water solution were mixed in an Eppendorf tube and after a few pumping/degassing cycles, the mixture was cured at 37°C for 4 h. The resulting mixture was poured on a silanized glass and covered with another silanized glass, separated by a 100-μm-thick poly(ethylene) spacer. Clamps were applied to the glasses to ensure a perfect sealing, and the mixture was cured at 37°C for 4 h. The hydrogel film was characterized by surface charge analysis.

Preparation of HEMA Hydrogels Containing DAEMA Units (Run T3A1)

One gram (7.7 mmol) HEMA, 10 mg (64 μmol) DAEMA, 30 mg (99 μmol) TEGDA, 38 μL (20 μmol) 0.526*M* sodium metabisulfite water solution, and 38 μL (17 μmol) 0.438*M* ammonium persulfate water solution were mixed in an Eppendorf tube and after a few pumping/degassing cycles, the mixture was cured at 37°C for 4 h. The resulting mixture was poured onto a silanized glass and covered with another silanized glass, separated by a 100-μm-thick poly(ethylene) spacer. Clamps were applied to the glasses to ensure a perfect sealing, and the mixture was cured at 37°C for 4 h. The hydrogel film was characterized by surface charge analysis.

Functionalization of HEMA Hydrogels

Aminolysis of Poly(HEMA) with 1,4-Diaminobutane in Dimethylformamide (DMF) Solution

A mixture of 0.5 g (3.9 mmol of ester groups) poly(HEMA), 0.8 mL (7.9 mmol) 1,4-diaminobutane, and 5 mL anhydrous *N*,*N*-dimethylformamide was heated at 100°C for 24 h. After cooling at room temperature, the reaction product was poured in excess diethyl ether, washed with dichloromethane, and then dried under vacuum to yield 0.5 g of a solid product that was characterized by FTIR spectroscopy and by acid–base titration.

Aminolysis of Poly(HEMA) with 1,4-Diaminobutane in Bulk

A mixture of 0.5 g (3.9 mmol of ester groups) poly(HEMA) and 0.8 mL (7.9 mmol) 1,4-diaminobutane was heated at 100°C for 24 h. After cooling at room temperature, the reaction product was poured in excess diethyl ether and the precipitate was suspended in diethyl ether for 1 week. The solvent was changed every day. The washed polymer was then dried under vacuum to yield 0.5 g of a solid product that was characterized by FTIR spectroscopy and by acid-base titration.

Aminolysis of Poly(HEMA) with 1,4-Diaminobutane in Bulk in the Presence of 1,4-Diaza-2,2,2-Bicyclooctane

A mixture of 0.5 g (3.9 mmol of ester groups) poly(HEMA), 0.8 mL (7.9 mmol) 1,4-diaminobutane, and 47.3 mg (0.42 mmol) 1,4-diaza-2,2,2-bicyclooctane was heated at 100°C for 24 h. After cooling at room temperature, the reaction product was poured in excess diethyl ether, washed with chloroform, and then dried under vacuum to yield 0.5 g of a solid product that was characterized by FTIR spectroscopy and by acid-base titration.

Reaction of Poly(HEMA) with Succinic Anhydride

A mixture of 0.5 g (3.9 mmol of ester groups) poly(HEMA), 86.9 mg (0.87 mmol) succinic anhydride, and 5 mL anhydrous *N*,*N*-dimethylformamide was heated at 50°C for 24 h. After cooling

at room temperature, the reaction product was poured in excess diethyl ether, washed with dichloromethane, and then dried under vacuum to yield 0.5 g of a solid product that was characterized by FTIR spectroscopy and by acid–base titration.

Degradation of HEMA Hydrogels

Hydrolytic Degradation in Phosphate-Buffered Saline

Two grams of powdered hydrogel sample was incubated in 50 mL 0.01*M* pH 7.2 phosphate-buffered saline (PBS) at 37°C for 1 week. The solution was then filtered and its chemical oxygen demand (COD) value was determined. The recovered hydrogel was dried on 96% H_2SO_4 for 1 week and then weighed.

Hydrolytic Degradation in 4% Sodium Carbonate Solution

Two grams of powdered hydrogel sample was incubated in 50 mL of 40 g/L sodium carbonate solution (pH 11.6). The pH of the solution was measured after 1 week and after 3 months. The recovered hydrogel powder was dried on 96% H_2SO_4 for 1 week and then weighed.

Hydrolytic Degradation in Bovine Serum

Two grams of powdered hydrogel sample were incubated in 50 mL of heat-inactivated bovine serum at 37°C for 3 months. The hydrogel powder was filtered, dried on 96% H_2SO_4 for 1 week, and then weighed.

Hydrolytic Degradation in 8 : 2 Ethanol/Water Solution

Two grams of powdered hydrogel sample was incubated in 25 mL 8 : 2 ethanol/water mixture for 1 week at room temperature. The amount of acid groups was evaluated by acid–base titration.

Hydrolytic Degradation in Boiling NaOH

Two grams of hydrogel sample was incubated in 50 mL of boiling 1*N* NaOH solution for 1 h. The surface of the sample was inspected for morphological changes.

Enzymatic Degradation

A sample (2.0 g) of powdered hydrogel was incubated in 100 mL of 0.01*M* pH 7.2 PBS saline containing a known concentration of enzyme [20 mg of papaine (20 UI/mg) from *Carica papaya*, 10 mg of type VII Lipase (30 UI/mg) from *Candida cylindracea*, or 10 mg of Lipase (60 UI/mg) from *Rhizopus delemar*] at 37°C for 24 h. The solution was filtered and the COD value of the filtrate was determined. The recovered hydrogel was dried on 96% H_2SO_4 for 1 week and then weighed.

Hydration of Hydrogels in Controlled Relative Humidity Environments

Circular (3 mm diameter, 15 mg weight) and/or rectangular (4.0 × 3.5 cm, 2.0 g weight) polymeric inserts were placed on a perforated Teflon support inside a glass chamber containing 100 mL solutions of 62, 43, and 17% sulfuric acid, corresponding to 18, 51, and 92% relative humidity, respectively, at 25°C. After 7 days of exposure to the controlled atmosphere, samples were collected and quickly weighed.

Characterization of Materials

DSC determinations were performed on 3-mm-diameter circular inserts (20 mg) obtained from 1-mm-thick hydrogel films, by using a Mettler DSC-30 TA4000 system. Analyses were performed under an 80 mL/min nitrogen flow. Glass transition temperature (T_g) measurements were performed on samples dried for 7 days over 96% H_2SO_4 and then equilibrated in controlled relative humidity atmosphere. The following experimental method was adopted: heating from 25 to 175°C at 10°C/min, cooling from 175 to 25°C at 100°C/min, and then heating from 25 to 175°C at 10°C/min. Measurements of the content of freezing water were performed on samples incubated for 15 days in distilled water. The following experimental method was adopted: cooling from 25 to −50°C at 10°C/min, heating from −50 to −20°C at 10°C/min, from −20 to −10°C at 2°C/min, from −10°C to −20°C at 2°C/min, from −20 to −10°C at 2°C/min, from −10°C to −50°C at 10°C/min, and finally heating from −50 to 25°C at 10°C/min. The total water content in the examined samples was evaluated gravimetrically, whereas the freezing water content was evaluated from the ratio between the heat of fusion (ΔH) of water in the samples and that of pure water.

pH measurements were carried out with a pH meter Metrohm 691 and calibrated with buffer solutions at pH 4.01 and 7.01.

Hydrogel purification was performed by extracting 1.0 g sample of dry hydrogel with 200 mL of boiling ethanol or acetone for 2 days in a Kumagawa extractor. The solvent was removed under vacuum and the residue was weighed. The hydrogel was rinsed with acetone for 2 days, with water for additional 48 h, and finally dried over 96% H_2SO_4 and weighed.

COD analyses were performed according to a standard procedure[5]: 10 mL 0.25*N* potassium bichromate standard solution was mixed with 20 mL of sample solution; then a solution of 0.165 g silver sulfate in 30 mL of 96% sulfuric acid was slowly added. The resulting mixture was refluxed for 2 h and then diluted to about 150 mL. Excess bichromate was titrated with a 0.125*N* solution of $Fe(NH_4)_2(SO_4)_26H_2O$, by using two to three drops of ferroin solution (1.485 g 1,10-phenantrolin monohydrate and 0.695 g $FeSO_47H_2O$ in 100 mL distilled water) as indicator. Solution COD, expressed in mg O_2/L, was evaluated as COD $= 400(b - a)N$, where N is the normality of the Fe(II) solution and a and b are the titrant volumes used for samples and blanks, respectively.

Surface charge measurements were carried out by using the Kelvin probe technique. Polymeric films were placed between two conductor plates, a static one and one vibrating at 200 Hz. The surface charge *SC* was calculated by using the Helmholtz equation:

$$SC = V \times A \times \varepsilon/d$$

where V is the potential difference between the plates in the presence or in the absence of the polymeric material, A is the conductor plate area, ϵ the dielectric constant, and d the distance between the plates. Because the investigated materials are insulators, their dielectric constants were calculated as the square of the refraction index, measured by ellipsometry.

Cell adhesion and proliferation tests were performed by using human umbilical vein endothelial cells (HUVEC) according to a procedure described elsewhere.[6]

RESULTS AND DISCUSSION

Preparation of HEMA Hydrogels

HEMA-based hydrogels were prepared by radical polymerization of HEMA with 1–8 wt % EGDMA or TEGDA as crosslinking agents (Table I). Polymerization runs were carried out at 37°C, either in bulk or in aqueous solution. In the latter case, an amount of water corresponding to 50% of the weight of monomers was used. A water-soluble redox couple, constituted by sodium metabisulfite and ammonium persulfate, was utilized as free-radical initiator. Under these experimental conditions, the time for reaction completion turned out to be 4–5 h.

Polymerization experiments were performed in molds of suitable shape and size. Hydrogels were prepared as cylinders and films because those shapes appeared to be the most suitable for their physical–mechanical characterization and for their potential biomedical applications, respectively. In accordance, the polymerization mixtures were placed within sealed Eppendorf tubes or injected between two silanized glasses separated by 0.1- to 1.0-mm-thick silicone spacers and heated at 37°C. However, 100-μm-thick films obtained by this method were not homogeneous due to the substantial volume contraction occurring during the polymerization process. Therefore, the reaction mixture was heated at 37°C for 2 h before being placed in the mold and then cured at 37°C for a further 2 h.

In all cases, almost quantitative conversions to polymeric products were obtained independent of the shape of the molds. Hydrogels prepared by polymerization in water solution with TEGDA as a crosslinking agent resulted in milky, heterogeneous materials with white opaque inclusions. An increase in the heterogeneity and opacity was observed as the water content in the polymerization feed increased from 25 to 100 wt % with respect to the monomer. This behavior was not observed when using EGDMA as crosslinking agent, probably due to its higher hydrophilicity.

Preparation of Bioerodible HEMA Hydrogels

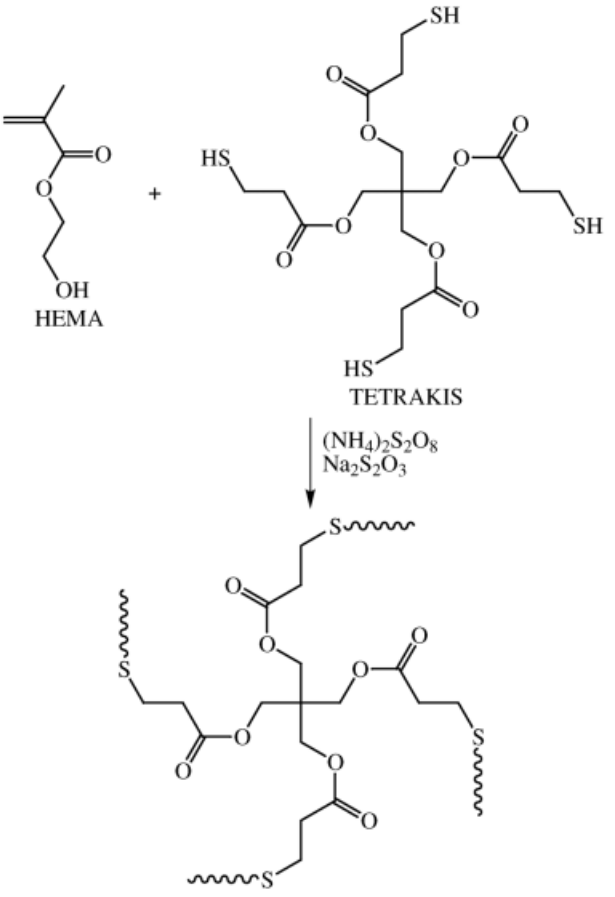
HEMA-based hydrogels exhibit a high degree of chemical stability and mechanical integrity, properties that have been exploited in several applications.[7,8] Still, in many cases, biodegradability is often requested, particularly for biomedical applications.

It is reported that under physiological conditions, poly(HEMA) can be hydrolyzed to poly(MAA). However, high molecular weight PMAA cannot be excreted from the organism and accumulates within the kidneys. To modify the biodegradability of HEMA-based hydrogels, a series of polymerization runs was performed in the pres-

Table III Swelling of HEMA-Based Hydrogels in Water and in DMSO

Sample	Swelling in Water[a] (wt %)	Swelling in DMSO[a] (wt %)
E0	49.0	311
E1	49.4	391
E2	45.9	370
E3	46.8	269
E4	39.6	209
E8	36.4	106
ET0	50.6	n.d.
ET1	53.8	420
ET2	50.0	332
ET3	48.1	265
ET4	42.0	294
ET8	38.8	257
T0	49.1	233
T1	48.0	334
T2	48.8	397
T3	52.3	390
T4	49.7	352
T8	46.5	298
TT0	62.6	n.d.
TT1	70.9	384
TT2	56.7	531
TT3	52.6	510
TT4	49.1	505
TT8	48.5	433

[a] At 37°C for 24 h.



Scheme 1 Formation of star polymers by polymerization of HEMA in the presence of Tetrakis.

ence of 1% Tetrakis, a tetra-functional chain transfer agent (Tables I–IV). These hydrogels should be characterized by a star structure in which four short poly(HEMA) chains are linked by ester bonds to pentaeritrityl (Scheme 1). Due to the presence of EGDMA or TEGDA in the polymerization mixture, poly(HEMA) chains are linked together by bridges containing ester bonds.

Table IV Swelling of 1-mm-Thick HEMA-Based Films in Water, in pH 7.2 PBS, and in DMEM

Sample	Water[a] (wt %)	PBS[a] (wt %)	DMEM[a] (wt %)
E1	51.1	57.8	54.6
E2	45.9	57.5	53.3
E3	44.8	50.8	51.9
T2	48.8	58.1	54.1
T3	51.7	57.0	54.0
T4	50.9	56.0	53.0
TT3	58.3	58.2	57.3
TT4	52.0	65.3	57.3

[a] At 37°C for 24 h.

Polymerization of unsaturated monomers in the presence of multifunctional mercaptans gives star polymers, the branches of which contain an average number of units equal to the monomer/SH groups molar ratio. Accordingly, an average content of 25 HEMA units in each branch was evaluated from the feed composition of the prepared HEMA star polymers. Under physiological conditions, these hydrogels should degrade to low molecular weight poly(MAA), which can be easily excreted by the organism.

Preparation of HEMA Hydrogels Containing Ionizable Groups

A good interaction between biomaterials and tissues is a fundamental requisite for the growth of cells on the biomaterial surface. According to literature, poly(HEMA) hydrogels do not allow for cell adhesion and proliferation.[9–11] A set of hydro-

gels containing ionizable groups was prepared to obtain materials coupling the excellent biocompatibility of poly(HEMA) with the ability to support cell growth.

Experiments were performed by using mixtures containing HEMA, 3 wt % TEGDA, the redox initiator, and 1–10 wt % of either MAA or DAEMA, two monomers containing a carboxyl or a tertiary amine group, respectively (Table II). Also in this case, almost quantitative conversions to polymeric materials were observed.

The same objective could be achieved by the introduction of charged groups onto the hydrogel surface. Accordingly, preformed HEMA hydrogels were reacted for 24 h with succinic anhydride (SA; molar ratio SA/HEMA = 0.2) or 1,4-diaminobutane (DAB; molar ratio DAB/HEMA = 2) in DMF solution, at 50 and 100°C, respectively. Acid–base titration of the resulting hydrogels indicated a content of 3–8% carboxyl monomeric units or 3–15% amine repeating units.

Preliminary surface charge measurements carried out on the resulting films confirmed the presence of positive or negative charges on the surface of hydrogels containing, respectively, amine or carboxyl groups. Accordingly, preliminary *in vitro* tests carried out on poly(HEMA) films containing ionizable groups showed that samples containing amino groups favored cell adhesion. This behavior is in agreement with the larger capacity of interacting with serum proteins and cells shown by polymer with positive surface charge.[12]

Purification of HEMA Hydrogels

Materials for biomedical use must be free from all components including initiator and its decomposition products, unreacted monomers, oligomers, and impurities potentially present in the reagents. These in fact may be released in the body and may be toxic and/or cause serious side effects.

Therefore, at the end of the polymerization, hydrogels were rinsed under continuously flowing water for at least 24 h. After purification, hydrogels still released organic compounds in solution. In particular, 2.0 g of hydrogel film or powder were washed with water for 24 h and with ethanol for 7 days, and finally incubated in water for 7 days. COD analysis of the resulting solution revealed a value of 350 mg O_2/L corresponding to 87.5 mg (CH_2)/L.

As reported by the literature,[13,14] unknown compounds, very likely low molecular weight oligomers and hydrolysis products containing carboxylic groups, are still released in water even after several months of purification.

To reduce the amount of impurities, two specimens of E1 hydrogel were purified according the reported protocol, extracted with boiling ethanol or acetone for 2 days, and then washed for a further 2 days in free-flowing water. A 2.7 and 5.1 wt % decrease was observed by extraction with boiling acetone and ethanol, respectively.

Evaluation of the Interactions of HEMA Hydrogels with Water: Degree of Swelling

The ability of absorbing large amounts of water determines characteristics such as softness, porosity, ease of purification, and permeability of hydrogels. These properties in turn control diffusion and transport of oxygen, essential nutrients, and metabolic waste through the hydrogel network.[15]

Determinations of the swelling degree of the synthesized hydrogels were carried out by placing the samples in the swelling solvent at 37°C for 24 h. The swelling degree R_e was calculated as $R_e = 100(W_e - W_d)/W_d$, where W_e and W_d are the equilibrium weight and dry weight of the sample, respectively. R_e values of the investigated hydrogels ranged between 40 and 70% (Table III). These values are in good agreement with data reported by other authors for HEMA hydrogels.[16–18] The R_e values were not much affected by the type and content of the crosslinking agent or by the presence of Tetrakis in the polymerization feed.

Indeed, hydrogel swelling decreased by 10–20% on increasing the content of crosslinking agent from 1 to 8% and the hydration of samples containing TEGDA was slightly higher than that of samples crosslinked with the less hydrophilic TEGDA. Moreover, other things being equal, hydrogels containing 1% Tetrakis units swelled more than the others. This was the expected behavior, because water absorption must decrease as the degree of crosslinking increases and it is known that the hydrophilicity of the crosslinking agent affects the degree of swelling.[19]

Water is not a good solvent for linear poly(HEMA) and therefore, the swelling degree in water is always very limited, thus leveling off the influence of structural parameters. Indeed, values of the swelling degree in DMSO, a good solvent for linear poly(HEMA), turned out to include between 100 and 500% and were much higher than those recorded in water. However, almost

the same trend was observed in both solvents. It is worth noting that samples containing 2–4% TEGDA swelled more than those containing only 1% crosslinker, indicating that TEGDA large hydrophilicity overcame the negative influence of the increased crosslinking degree.

Measurements of the swelling degree were carried out also on hydrogel films (Table VII). Experiments were performed at 37°C, both in water and in DMEM having pH 7.4, to assess the hydrogel behavior under conditions similar to those found *in vivo*. Moreover, to investigate the effect of pH among the other parameters of DMEM, swelling degrees were evaluated also in pH 7.2 PBS.

The swelling degrees in phosphate buffer turned out to be larger than those measured in water. This behavior, unexpected for hydrogels not containing ionizable groups, may be attributed to the presence of small quantities of MAA present as impurity in the monomer. At the pH of the buffer solution, the carboxyl groups of these units are ionized, thus giving rise to an increase of the hydrophilicity of the sample. The absorption values in DMEM included those collected in water and those collected in PBS.

Hydrogel Hydration Under Controlled Relative Humidity Conditions

When hydrogels are set in humid atmosphere, water molecules tend to pass from the gas phase into the polymer until a thermodynamic equilibrium is eventually reached. Therefore, exposure of polymeric matrices to a controlled relative humidity atmosphere represents a useful method for evaluating the interactions of polymers with water. Accordingly, a set of hydration measurement was carried out on hydrogel samples maintained at prefixed relative humidity atmosphere. Hydration measurements were carried out at 25°C, in sealed glass vessels containing sulfuric acid solutions at concentrations of 62, 43, and 17 wt %, which correspond to 18, 51, and 92% relative humidity, respectively.[20]

As predicted, hydration of all samples increased from less than 1% up to 16–17% as the relative humidity grew from 18 to 92% (Table V). No significant dependence of hydrogel hydration on crosslinking degree and type was detected.

Table V Hydration of HEMA Hydrogels at Different Relative Humidities

	Hydration (wt %) at Different RH		
Run	18	51	92
E1	0.1	4.5	16.8
E8	0.1	4.3	16.4
ET1	0.8	5.1	16.8
ET8	0.6	4.9	16.1

Determination of Water Structure

Water absorbed by a hydrogel can be classified as bound, interfacial, and free water on the basis of the different degrees of interaction of the water molecules with the polar portions of polymeric materials.[21–24]

To evaluate the influence of structural parameters on the interactions of water with HEMA-based hydrogels, the water structure was investigated by DSC. It is generally assumed that on cooling free water freezes at the same temperature as pure water, whereas bound water does not freeze even at very low temperature, due to its strong interactions with the polymer matrix.

HEMA-based hydrogels were incubated in water for 15 days and then analyzed by DSC in the temperature range between −50 and 25°C. On heating, the DSC traces of swollen hydrogels displayed a broad structured endothermic peak around 0°C. The amount of freezing water (W_f) was determined as $W_f = Q_w/\Delta H_w^0$, where Q_w is the melting enthalpy measured in Joules (J), and ΔH_w^0 is the solid–liquid transition enthalpy of hydration water in the hydrogel. The experimental value of ΔH_w^0 turned out to be 392.5 J g^{-1}.

The content of not-freezing water (W_{nf}) in the hydrogel was then evaluated as the total hydration water (W_t) less the amount of freezing water (W_f). The total amount of hydration water, the percentage of freezing water, and the number of moles of not-freezing water per monomeric units diminished with the increase of the degree of crosslinking from 1 to 8% (Table VI). This effect resulted in less importance for hydrogels crosslinked with TEGDA (T1f and T8f samples) because of the higher hydrophilicity of TEGDA as compared to EGDMA (E1f and E8f samples).

The presence of longer crosslinking molecules in the hydrogel makes the network mesh looser, with a relative increase of the amount of not-bound freezing water included within the matrix pores.

No endothermic transition was detected in the DSC of E1f sample, equilibrated at 18, 51, and

Table VI DSC Determination of the Content of Not—Freezing Water in HEMA-Based Hydrogels

Sample	Total Water[a] (wt %)	Freezing Water[b] (wt %)	Not-Freezing Water (wt %)[b]	Not-Freezing Water Moles per m.u.[c]
E1	51.8	24.3	75.7	5.5
E8	37.0	11.7	88.3	3.8
T1	57.1	31.2	68.8	6.6
T8	49.9	29.5	70.5	5.1

[a] Referred to the hydrogel dry weight.
[b] Referred to the total water content.
[c] Moles of not—freezing water per mole of monomeric units.

92% RH atmosphere and containing 0.6, 5.2, and 15.1% of hydration water. This result indicates that at low hydration, all of the water in the hydrogel is present as not-freezing water.

Interestingly, the amount of nonfreezing water present in the investigated hydrogels nicely corresponds to the reported bound water content of poly(HEMA), as determined by NMR measurements.[25]

The mobility of water molecules in HEMA hydrogels was investigated also by measuring spin–lattice (T_1) and spin–spin (T_2) relaxation times of water.[26] Analysis of the relaxation decay pointed to a biexponential trend, in accordance with the presence of two components. The faster and the slower components were attributed to water outside of the polymer network (external water) and inside the network (internal water), respectively. Both components showed T_1 and T_2 values lower than those of pure water, indicating that both types of water, particularly internal water, appreciably interact with the polymer matrix. No clear dependence on hydrogel structure was, however, detected.

DSC Analysis of Hydrogels

DSC analyses were performed between 25 and 175°C on hydrogel samples dried over 96% sulfuric acid as well as on samples hydrated under controlled relative humidity atmosphere. In all cases, the second heating traces showed the presence of a glass transition and the absence of endothermic transitions attributable to melting processes, in agreement with the amorphous structure of the analyzed samples.

Both the chemical composition and the hydration status of the hydrogel affected the T_g (Table VII). Samples crosslinked with EGDMA displayed higher T_g values than TEGDA containing ones having the same degree of crosslinking, in agreement with the higher flexibility of TEGDA. Rather surprisingly, in both cases T_g values slightly decreased with the increase of the crosslinking degree.

The glass transition temperature of hydrogels containing 1% of EGDMA displayed a large decrease with the increase of hydration state of the sample, in accordance with a plasticizing effect of water.[27,28] T_g values evaluated for HEMA/water mixtures by the Fox equation[29] resulted in good agreement with DSC experimental values, pointing out that water–polymer interactions do not cause a significant deviation from the ideal behavior.

Water Diffusion Measurements

Hydrogels' permeability to water and metabolites is of paramount relevance in biomedical and pharmacological applications.[30]

The swelling process due to the absorption of water by the hydrogel can be divided into three steps: diffusion of water molecules into the polymer network, relaxation of the polymer chains, and expansion of the polymer matrix. The swell-

Table VII Glass Transition Temperatures (T_g) of HEMA-Based Hydrogels

Sample	Type	Crosslinker (wt %)	Hydration (wt %)	T_g(°C)	ΔCp (J g^{-1} K^{-1})
E1	EGDMA	1	0	109.4	0.24
E1	EGDMA	1	0.6	99.7	0.24
E1	EGDMA	1	5.2	72.4	0.12
E1	EGDMA	1	15.1	39.9	0.05
E8	EGDMA	8	0	108.7	0.25
T1	TEGDA	1	0	102.4	0.24
T8	TEGDA	8	0	97.8	0.25

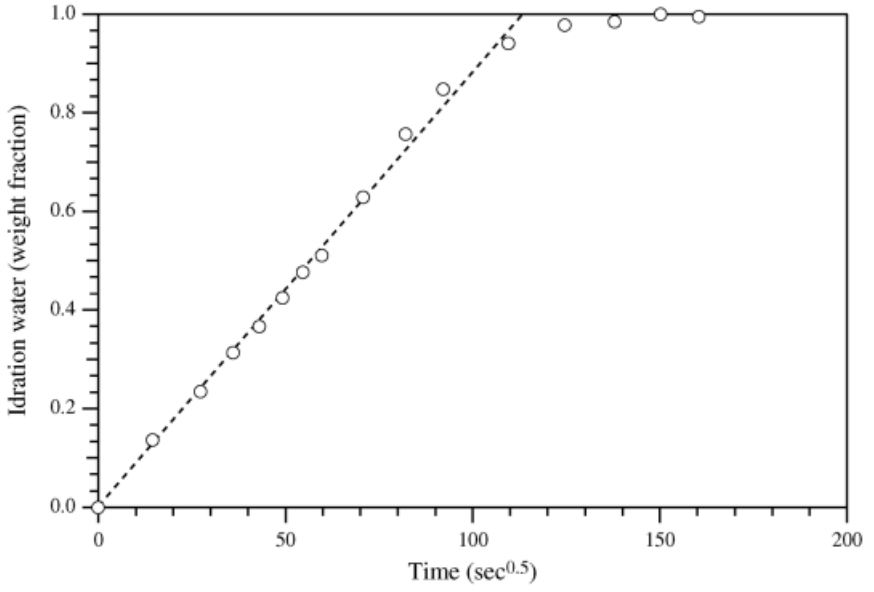


Figure 1 Diffusion plot of water in a HEMA-based hydrogel (ET8 sample).

ing behavior depends on which is the determinant step and the mechanism of solvent penetration may be classified as Fickian diffusion (Type I) or non-Fickian diffusion (Type II).[31]

To measure the swelling kinetics, 1-mm-thick hydrogel films were washed with water for 24 h, dried over 96% sulfuric acid, and then immersed in water at 37°C. The weight of the samples was recorded at different times until a constant weight was eventually reached. Plots of the relative variation of sample weight versus the square root of time (Fig. 1) displayed a linear trend, suggesting that the hydration process is controlled by Fickian diffusion.[18]

By assuming that the thickness of the swollen sample is constant and equal to that of the dry sample,[19] the linear part of the plot of M_t/M_0 versus $t^{1/2}$ may be correlated to D_w, the diffusion coefficient ($cm^2\ s^{-1}$), by the equation:

$$\frac{M_t}{M_\infty} = \frac{4}{\pi^{1/2}} D_w{}^{1/2} t^{1/2} L^{-1} \qquad 0 \le \frac{M_t}{M_\infty} \le 0.6$$

where M_t is the amount of water absorbed at time t, M_0 is the amount of water absorbed at equilibrium, t is the time (s), and L is the thickness of the dry sample (cm).

The values of the diffusion coefficient D_w, that is, the speed of the water front in the different samples, evaluated from the slope of the linear part of the plots, resulted in between 1.4 and 1.8 $\times\ 10^{-7}\ cm^2\ s^{-1}$ (Table VIII), in agreement with the value of about $1.5 \times 10^{-7}\ cm^2\ s^{-1}$ reported in the literature for different poly(HEMA) hydrogels.[4,32]

As expected, D_w values decreased from 1.65 $\times\ 10^7$ to 1.43 $\times\ 10^7\ cm^2\ s^{-1}$ on increasing the EGDMA content from 1 to 8%, because of the increased crosslinking density. On the other hand, hydrogels containing TEGDA and/or Tetrakis showed an opposite trend. This behavior was tentatively attributed to their larger mesh size and hydrophilicity.

Hydrolytic Degradation of HEMA-Based Hydrogels

Biodegradable polymers are materials that undergo relevant structural modifications when placed in appropriate environments. Physical

Table VIII Water Diffusion Coefficient (D_w) in Different HEMA-Based Hydrogels at 37°C

Run	$D_w \times 10^7$ ($cm^2\ s^{-1}$)	Run	$D_w \times 10^7$ ($cm^2\ s^{-1}$)
E1	1.65	T1	1.59
E3	1.55	T3	1.65
E8	1.43	T8	1.75
ET1	1.53	TT1	1.57
ET3	1.51	TT3	1.60
ET8	1.58		1.64

Table IX Hydrolytic Degradation of TT3 Hydrogel Sample in Phosphate Buffered Saline at pH 7.2

Run[a]	COD (mg O_2/L)	Weight Variation[b] (%)
TT3A	914	−18
TT3B	231	−13
TT3C	325	−13
TT3D	346	−12

[a] 2 g of polymer (Sample TT3) in 50 ml of 0.01*M* pH 7.2 phosphate buffer, at 37°C for 7 days.
[b] Referred to polymer dry weight after 24 h washing in water.

agents such as light, heat, and mechanical deformations, and chemical agents, such as oxygen, water, and air pollution, may degrade polymers. Polymeric materials are subjected to a two-step degradation process, one that occurs during processing, such as molding and extrusion, and a second step that takes place with different mechanisms, depending on the application.

Hydrolytic degradation of poly(HEMA) occurs when the ester bonds in the side chains are cleaved; the reaction can be catalyzed by acids, bases, salts, and enzymes.

Hydrolytic degradation of HEMA-based hydrogels was investigated at 37°C in phosphate buffer, either in the presence or in the absence of enzymes, and in serum, to assess the material behavior in solutions that mimic body fluids. Accelerated experiments were also carried out in sodium carbonate at room temperature and in boiling 0.1*N* NaOH, to verify the effective hydrolytic degradability of the investigated hydrogels.

Hydrolysis of poly(HEMA), either linear or crosslinked with diacrylate or dimethacrylate derivatives, affords high molecular weight poly(MAA) that cannot be excreted by the organism. Due to the presence of thioglycolic ester groups, degradation of HEMA-based hydrogels containing star-shaped Tetrakis crosslinks should provide water-soluble oligomeric products that can be easily excreted by the kidneys.

Degradation experiments were carried out in pH 7.2 PBS at 37°C for 1 week on hydrogel specimens previously washed with distilled water for 24 h (Runs TT3fA, TT3fC, TT3fD) or 96 h (Run TT3fB). Specimens used in TT3fC and TT3fD runs were soaked in ethanol for 7 days and washed with water, before being analyzed as powder and film, respectively.

At the end of the experiments, solutions recovered after filtration of the polymer displayed COD values between 231 and 914 mg O_2/L (Table IX). The much higher COD value recorded in TT3fA run may be attributed to the release of impurities from the samples in the phosphate buffer, as indicated by the lower values of COD recorded for thoroughly purified samples.

COD values are related to the presence of organic compounds either formed by a limited hydrolytic process or released by the hydrogels due to the residual presence of impurities. Nevertheless, the recorded 8–13% weight loss corresponds to the hydrolysis of about 20% of ester bonds. This value is very far from the content of acid groups (1% in moles), as determined by acid–base titration of hydrogels recovered at the end of the experiments.

Enzymatic degradation experiments were carried out on a hydrogel sample containing 3% of TEGDA and 1% of Tetrakis (TT3f Sample), in 0.01*M* pH 7.2 phosphate buffer, at 37°C for 24 h. Lipases from *C. cylindracea* and from *R. delemar* and papaine from *C. papaya* were used as hydrolytic enzymes. Both the COD of the solutions and the hydrogel weight loss (Table X) did not show any significant increase of the degradation as compared with data recorded in phosphate buffer. This result indicates that the selected enzymes are not able to catalyze the hydrolytic degradation of the hydrogels.

Degradation in fetal bovine serum was investigated by keeping a dry HEMA hydrogel containing 3% of TEGDA and 1% Tetrakis (TT3f sample) at 37°C for 3 months in heat-inactivated fetal bovine serum. The hydrogel, recovered by filtration, displayed a weight loss of about 10%.

Table X Enzymatic Degradation of HEMA Hydrogels[a]

Enzyme		COD (mg O_2/L)		
Type	(mg)	Enzyme	Enzyme + Polymer	Weight Variation[b] (%)
Lipase C.	10	466	466	−8
Papaine	20	396	698	−12
Lipase R.	10	450	651	−12
None	0	0	310	−9

[a] 2 g of polymer (TT3 sample) in 100 ml of 0.01*M* pH 7.2 phosphate buffer, at 37°C for 24 h.
[b] Referred to the polymer dry weight, after 24 h washing in water.

Polymer degradation was also investigated in sodium carbonate solution. Two specimens of a HEMA hydrogel containing 3% TEGDA and 1% Tetrakis (TT3f sample) were kept at room temperature in 4% sodium carbonate water solution (pH 11.6). The samples, recovered after 7 and 90 days of incubation, displayed a weight loss of 11 and 13%, respectively.

To evaluate the degradation behavior of HEMA hydrogels in a very aggressive environment, samples containing 4% of TEGDA (T4f sample) and 1% of Tetrakis (TT4f) were placed in boiling 0.1*N* NaOH for 1 h. At the end of the experiment, the sample was broken in small strongly swelled fragments. On the contrary, the sample containing Tetrakis completely dissolved in the solution in accordance with a complete hydrolytic degradability. This behavior can be attributed to the presence of Tetrakis residues, which introduce in the macromolecule tetrafunctional structural units that are sites susceptible to hydrolytic degradation.

CONCLUSION

The preparation and structural characterization of new polymeric hydrogels based on HEMA represented the main objective of the present investigation. To this goal, either the bulk composition or the surface characteristics of HEMA-based hydrogels were properly modified to suit their particular pharmacological/biomedical application. The results achieved in the framework of this research activity can be summarized in the following points.

HEMA-based hydrogels with defined shape can be obtained in semiquantitative yields by radical polymerization in proper molds. Hydrogel properties can be fine-tuned by using feed mixtures containing different amounts of various crosslinking agents. In addition to the monomer mixture of Tetrakis, a multifunctional chain transfer agent containing hydrolyzable ester bonds provided the hydrogels of biodegradability character.

DSC investigation showed that the T_g of anhydrous hydrogels is above 95°C, in agreement with the glassy nature of these polymers. T_g values showed a close relationship with hydrogel chemical composition. The even larger dependence of T_g on the extent of hydrogel hydration suggests that water is acting as a plasticizer.

The type and content of crosslinking agent exerted a limited influence on the swelling of HEMA hydrogels in water or phosphate buffer at 37°C. On the contrary, the swelling degree in DMSO, a good solvent of linear poly(HEMA), turned out to be one order of magnitude higher and strictly related to the hydrogel structure. The kinetics of hydrogel swelling in water clearly indicated that the hydration process is mainly controlled by the solvent diffusion.

DSC analysis of the water structure in hydrogels highlighted the presence of at least two types of water structure, characterized by a different melting behavior. The appreciably different results obtained by DSC and NMR analysis indicate that the adopted techniques actually measure different characteristics of water molecules.

The present work was performed with the partial financial support of CNR Progetto Finalizzato "Materiali Speciali per Tecnologie Avanzate II." The authors thank Prof. Emo Chiellini for critical and constructive contribution to the development of this research.

REFERENCES

1. Kim, W. S.; Bae, Y. H.; Okano, T. Pharm Res 1992, 9 (3), 283.
2. Ratner, B. D.; Hoffman, A. S. in Hydrogels for Medical and Related Applications; Andrade, J., Ed.; American Chemical Society: Washington, DC, 1967.
3. Peppas, N. A. in Biomaterials Science, An Introduction to Materials in Medicine; Ratner, B. D., Hoffman, A. S., Schoen, F. J., Lemons, J. E., Eds.; Academic Press: San Diego, CA, 1996.
4. Davis, P. A.; Huang, S. J.; Nicolais, L.; Ambrosio, L. in High Performance Biomaterials; Szycher, M., Ed.; Technomics: Basel, 1991.
5. Skoog, D. A.; West, D. M. Fundamentals of Analytical Chemistry, 6th ed.; Saunder College Publishing: Philadelphia, PA, 1992.
6. Ahluwalia, A.; Basta, G.; Chiellini, F.; Ricci, D.; Vozzi, G. J Mater Sci: Mater Res 2001, 12, 613.
7. Hydrophilic. Polymer Coatings for Medical Devices, 10th ed.; LaPorte, R. J., Ed.; Technomics: Lancaster, PA, 1997.
8. Curtis, A. S. G.; Clark, P. Crit Rev Biocompat 1990, 5, 343.
9. Lydon, M. J. Br Polym J 1986, 18, 22.
10. Horbett, T. A.; Schway, M. B.; Ratner, B. D. J Colloid Interface Sci 1985, 104, 28.
11. Minett, T. W.; Tighe, B. J.; Lydon, M. J.; Rees, D. A. Cell Biol Int Rep 1984, 8, 151.
12. Faris, B.; Mozzicato, P.; Mogayzel, P. J.; Ferrera, R.; Gerstenfeld, L. C.; Glembourtt, M.; Makarski J. S., Jr.; Haudenschild, C. C.; Franzblau, C. Exp Cell Res 1983, 143, 15.

13. Weiss, P. Rev Mod Phys 1959, 31, 449.
14. Brynda, E.; Stol, M.; Chytry, V.; Cifkova, I. J Biomed Mater Res 1985, 19, 1169.
15. Barbieri, R.; Quaglia, M.; Delfini, M.; Brosio, E. Polymer 1998, 39, 1059.
16. Refojo, M. F.; Yasuda, H. J Appl Polym Sci 1965, 9, 2425.
17. Peppas, N. A.; Moynihan, H. J.; Lucht, L. M. J Biomed Mater Res 1985, 19, 397.
18. Migliaresi, C.; Nicodemo, L.; Nicolais, L.; Passerini, P. Polymer 1984, 25, 686.
19. Garcìa, O.; Blanco, M. D.; Gòmez, C.; Teijòn, J. M. Polym Bull 1997, 38, 55.
20. Standard Recommended Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions; ASTM E104-51 (Reapproved 1971), 35, 1091.
21. Jhon, M. S.; Andrade, J. D. J Biomed Mater Res 1973, 7, 509.
22. Stillinger, F. H. ACS Symposium Series 127; American Chemical Society, Washington, DC, 1980; p 11.
23. Quinn, F. X.; McBrierty, V. J.; Wilson, A. C.; Friends, G. D. Macromolecules 1990, 23, 4576.
24. Khare, A. R.; Peppas, N. A. Polymer 1993, 34, 22.
25. Smyth, G.; Quinn, F. X.; McBrierty, W. J. Macromolecules 1988, 21, 3198.
26. Delfini, M.; Chiellini, F.; Solaro, R.; Chiellini, E. Macromol Chem Phys, to appear.
27. Moyninan, H. J.; Honey, M. S.; Peppas, N. A. Polym Eng Sci 1986, 26, 1180.
28. Rault, J.; Gref, R.; Ping, Z. H.; Nguyen, Q. T.; Néel, J. Polymer 1995, 8, 1655.
29. Fox, T. G. Bull Am Phys Soc 1956, 1123.
30. Refojo, M. F. J Appl Polym Sci 1965, 9, 3417.
31. Korsmeyer, R. W. J Polym Sci, Polym Phys Ed 1986, 24, 409.
32. Vázquez, B.; San Roman, J.; Peniche, C.; Cohen, M. E. Macromolecules 1997, 30, 8440.