Hydrogels Based on Water-Soluble Poly(ether urethanes) Derived from L-Lysine and Poly(ethylene glycol)

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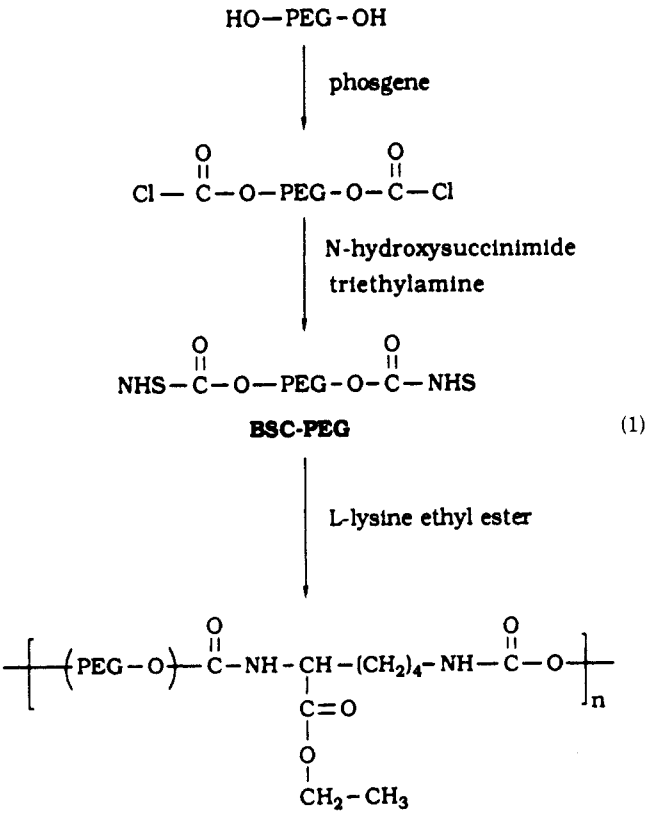
ABSTRACT: Water-soluble poly(ether urethanes) (M_w up to 170 000) have been prepared by the copolymerization of bis(succinimidyl) carbonate derivatives of poly(ethylene glycol) (PEG) with L-lysine (Lys) in a strictly alternating fashion. The resulting copolymers have physical properties similar to those of PEG while the carboxylic acid groups of L-lysine provide multiple pendent groups along the polymer backbone for further functionalization. By using PEG chains of different molecular weights, a homologous series of PEG–Lys copolymers was prepared containing one lysine unit every 1000, 2000, 4000, and 8000 Da. The pendent carboxylic acid groups were utilized in cross-linking reactions leading to amide cross-links, new acyl semicarbazide cross-links, and hydroxyethyl acrylate and hydroxyethyl methacrylate derived cross-links. The resulting hydrogels formed transparent, highly swollen films. At an equilibrium water content of about 80%, these films exhibited a relatively high degree of tensile strength ranging from 0.26 to 1.09 MPa. Due to their favorable physicomechanical properties and the nontoxicity of the components, PEG–Lys-derived hydrogels may find applications as biomaterials.

Introduction

Due to their physiological acceptability,[1] relatively good biocompatibility,[2,3] stability over long implant periods,[4-6] and excellent physical and mechanical properties,[1,3] polyurethanes are widely used in medical applications. Common features of the medically used polyurethanes are their water insolubility and their lack of reactive, functional groups. As a result, these polymers have not been used as injectable drug carriers or as backbones in cross-linked hydrogels. To improve the applicability of polyurethanes as biomaterials, we developed a reaction scheme (eq 1) in which individual chains of poly(ethylene glycol) (PEG) are copolymerized with derivatives of the natural amino acid L-lysine (Lys) via urethane linkages to yield strictly alternating poly(ether urethanes) of high molecular weight.

In the past, PEG chains were copolymerized with a variety of difunctional comonomers. For example, copolymers of poly(oxyethylene)–dicarboxylic acids with aliphatic and aromatic amines have been reported.[7,8] Block copolymers of PEG with polyesters,[9] poly(L-proline),[10] and poly(γ-benzyl L-glutamate)[11] are also known and have been suggested as biomaterials. Poly(ethylene glycols) cross-linked by copolymerization with triols and diisocyanates have been used to prepare hydrogels and hydrogel membranes.[12-14] Pretula and co-workers have reported the preparation of PEG ionomers with phosphate diester linkages.[15]

In contrast, we employed the α- and ϵ-amino groups of L-lysine to link PEG chains of variable length via urethane bonds. The resulting poly(ether urethane) copolymers provided multiple pendent groups (represented by "ethyl ester" in eq 1) at strictly controlled, predetermined intervals. After further derivatization, a family of new, structurally related copolymers was prepared, carrying pendent chains with different types of functional groups (Figure 1). This feature facilitated the attachment of drugs to the polymer backbone (to be reported in a separate manuscript) and the design of cross-linking reactions for the preparation of degradable and nondegradable hydrogels.



(1)

(1) Poly(PEG-Lys-OEt)

Experimental Section

Materials and Methods. The following chemicals were used without further purification: PEG1000, PEG2000, PEG4000, 20% phosgene solution in toluene, L-lysine ethyl ester dihydrochloride (Fluka), PEG8000, triethylamine, *tert*-butyl carbazate, 4 M HCl/dioxane, 1,6-diisocyanatohexane, 2-hydroxyethyl acrylate (HEA), 2-hydroxyethyl methacrylate (HEMA), benzoin methyl ether (BME) (Aldrich), *N*-hydroxysuccinimide (Schweizerhall); 1,6-diaminohexane, dicyclohexylcabodiimide (DCC), and benzylamine (Kodak). HPLC-grade solvents were used for synthesis and for GPC. ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-200 spectrometer. FT-IR spectra were obtained on a Mattson Cygnus 100 spectrophotometer. In organic solvents

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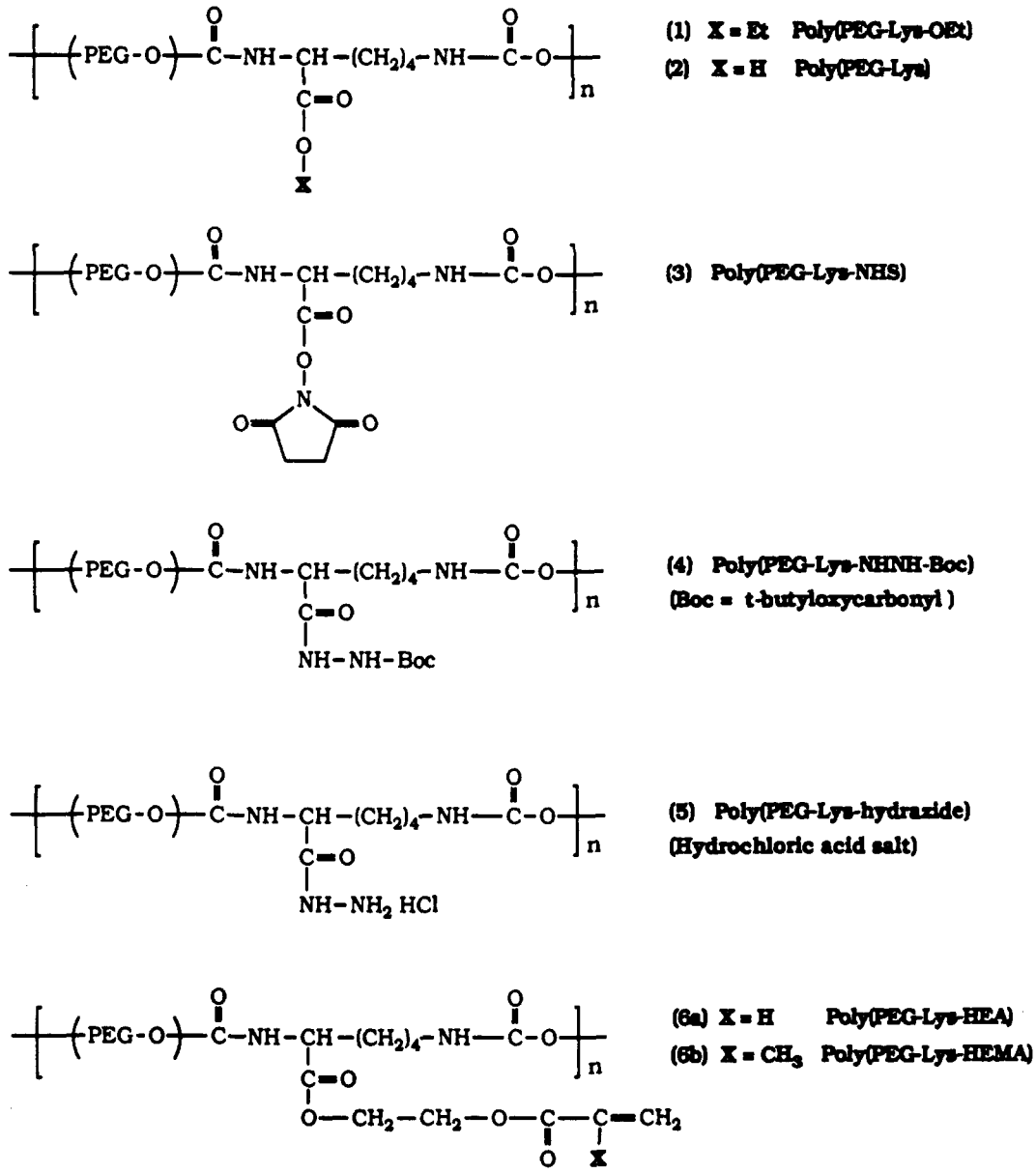


Figure 1. Structure of poly(PEG–Lys) and its derivatives.

molecular weights were determined by GPC as reported previously,[16] relative to PEG standards. For aqueous GPC, in 0.1 M acetate buffer (pH 5.4) as the mobile phase, two TSK gel columns (TSK G-2000 and TSK G-4000) were used in series. The mechanical strength of 10 cm × 0.5 cm films was determined on an Instron Tensile Tester Model 1122 at a strain rate of 1000%/min for dry films and 100%/min for swollen films. Thermal properties were determined on a Du Pont 910 differential scanning calorimeter (DSC).

The amount of active carbonate in bis(succinimidyl) carbonate derivatives of PEG (BSC-PEG) was determined by a titrimetric procedure with a known excess of benzylamine.[17] The amount of free carboxylic acid groups in poly(PEG–Lys) (2) was determined by nonaqueous titration with sodium methoxide in methanol–toluene using thymol blue as the indicator. *N*-Hydroxysuccinimide (NHS) was determined spectrophotometrically by treating solutions with 1 M ammonium hydroxide and measuring the absorbance at 260 nm as described by Miron and Wilchek.[18] The amount of available acrylate or methacrylate groups in poly(PEG–Lys–HEA) and poly(PEG–Lys–HEMA) was determined by reaction with morpholine followed by titration of the tertiary amine formed.[19] BSC-PEG and poly(PEG–Lys) derivatives were prepared using the illustrative procedures for PEG2000 given below. For other PEG units (PEG1000, PEG4000, and PEG8000) the molar ratio of the reagents was adjusted according to the amount of terminal hydroxyl groups present.

Preparation of BSC-PEG. *Warning*! This procedure uses phosgene, a highly toxic reagent.

Previously published procedures[20,21] for the preparation of mono(succinnimidyl) carbonate derivatives of methoxy-PEG were adopted for the activation of PEG by doubling the amount of phosgene and NHS used in the reaction. BSC-PEG was purified by recrystallization from 2-propanol followed by washings with cold 2-propanol and hexane.

Preparation of Poly(PEG–Lys–OEt) (1) from PEG2000 in an Interfacial Reaction System. Lys-OEt·2HCl (1.1 g, 4.5 mmol) was dissolved in water (150 mL) in a 500-mL three-necked round-bottomed flask fitted with an overhead stirrer. Sodium bicarbonate (1.7 g, 21 mmol) was added followed by BSC-PEG2000 (10 g, 4.4 mmol) in methylene chloride (150 mL). The mixture was stirred vigorously for 2 h and then acidified to pH 2 with 0.1 N HCl. The methylene chloride phase was separated from the aqueous phase, washed with a saturated solution of NaCl, and dried over $MgSO_4$. The solution was concentrated to 25 mL by evaporation and added into 100 mL of precooled ether (4 °C). Poly(PEG–Lys–OEt) precipitated and was collected by filtration. After drying, a white crystalline solid (7.4 g, 75%) was obtained. FT-IR (film on NaCl, cm^{-1}): ν characteristic bands at 2883 (CH), 1720 (C=O of urethane), shoulder at 1740 (C=O of ester), 1112 (CO). ^1H NMR ($CDCl_3$): δ 5.38 (1 H, d, α-NH of Lys), 4.90 (1 H, m, ϵ-NH of Lys), 4.16–4.23 (6 H, terminal CH_2 of PEG + CH_2CH_3), 3.62 (173 H, PEG overlapping with α-CHNH), 3.13 (2 H, m, ϵ-CH_2NH), 1.31–1.91 (6 H, br m, CH_2 of Lys), 1.26 (3 H, t, CH_3). ^{13}C NMR ($CDCl_3$): δ 172.2 (C=O of ester), 156.3 (C=O of urethane from α-NH), 155.8 (C=O of urethane from

ε-NH), 63.8–70.4 (CH_2 of PEG), 61.3 ($-OCH_2CH_3$), 53.6 (α-CH of Lys), 40.5, 32.2, 29.3 and 22.2 (CH_2 of Lys), 14.1 ($-OCH_2CH_3$).

Hydrolysis of Poly(PEG–Lys–OEt). Poly(PEG–Lys–OEt) (5 g) was dissolved in H_2O (50 mL). The pH of this solution was adjusted to 11.5 and was maintained at this value for 5 h by the continuous addition of 0.01 N NaOH. After 5 h the reaction mixture was acidified with 0.1 N HCl to pH 4, and the polymer was extracted into methylene chloride (200 mL). The methylene chloride extract was washed with a saturated soluction of NaCl, dried over $MgSO_4$, and concentrated to 15 mL. The polymer was precipitated by adding the solution to anhydrous ether (100 mL). After cooling to 4 °C for several hours the product was collected by filtration, washed with cold ether, and dried under vacuum. Yield: 3.5 g (71%). FT-IR (film on NaCl, cm^{-1}): ν characteristic bands at 2868 (CH), 1721 (C=O of urethane), shoulder at 1740 (C=O of acid), 1110 (CO). ^1H NMR ($CDCl_3$): similar to 1 except that the absorption at 1.26 ppm (CH_3 of the ethyl ester) was not observed. ^{13}C NMR ($CDCl_3$): 173.4 (C=O of acid), 156.5 (C=O of urethane from α-NH) and 155.9 (C=O of urethane from ε-NH), 63.7–70.4 (CH_2 of PEG), 53.4 (α-CH of Lys), 40.3, 31.9, 29.1, and 21.9 (CH_2 of Lys).

Preparation of Poly(PEG–Lys–NHS) (3). Poly(PEG–Lys) (1.0 g, 0.46 mmol based on the molecular weight of the polymer repeat unit) was dissolved in methylene chloride (10 mL). To this solution was added dry and finely powdered NHS (0.26 g, 2.3 mmol). The flask was cooled in an ice–water bath, and DCC (0.10 g, 0.50 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 24 h. The precipitated 1,3-dicyclohexylurea (DCU) was removed by filtration, and the filtrate was added into ether (25 mL). After cooling to 4 °C the crude material was collected by filtration and purified by two recrystallizations from 2-propanol. Yield: 0.72 g (71%). FT-IR (film on NaCl, cm^{-1}): ν 2873 (CH), 1815 and 1788 (C=O of succinimide), 1740 (C=O of ester) and 1110 (CO). ^1H NMR ($CDCl_3$): similar to **2** except that an additional absorption at 2.81 ppm (4 H, s, NHS protons) was observed.

Preparation of Poly(PEG–Lys–NHNH–Boc) (4). Poly(PEG–Lys) (2.2 g, 1.0 mmol based on the molecular weight of the polymer repeat unit) was dissolved in methylene chloride (20 mL). To this solution was added *tert*-butyl carbazate (260 mg, 2.0 mmol). The flask was cooled in an ice–water bath, and DCC (410 mg, 2.0 mmol) was added. The reaction mixture was then stirred at 0 °C for 1 h and at room temperature for 24 h. The precipitated DCU was removed by filtration, and the filtrate was added into ether (100 mL). After cooling to 4 °C the crude material was collected by filtration and purified by recrystallization from 2-propanol. Yield: 1.7 g (80%). ^1H NMR ($CDCl_3$): δ 8.55 (1 H, m, N*H*NHBoc), 6.92 (1 H, m, NHN*H*Boc), 5.75 (1 H, d, α-NH of Lys), 5.35 (1 H, br s, ε-NH of Lys), 1.35 (9 H, s, *t*-Bu). Other peaks were similar to those found in **2**.

Preparation of Poly(PEG–Lys–hydrazide) (5). Poly(PEG–Lys–NHNH–Boc) (5.5 g, 2.1 mmol based on the molecular weight of the polymer repeat unit) was added to a solution of 4.0 M HCl–dioxane (75 mL) and stirred for 2 h at room temperature. The polymer settled at the bottom of the flask as an oil. The HCl–dioxane layer was decanted, and the polymer layer was added to ether (100 mL). The polymer that precipitated was isolated, washed with ether, and dried under vacuum. It was further purified by recrystallization from 2-propanol (50 mL). Yield: 4.1 g (84%). The complete removal of the Boc group was confirmed by ^1H NMR (disappearance of the *t*-Bu peak at 1.35 ppm).

Preparation of Poly(PEG–Lys–HEA) (6a) and Poly(PEG–Lys–HEMA) (6b). Poly(PEG–Lys) (2.2 g, 1.0 mmol based on the molecular weight of the polymer repeat unit) was dissolved in methylene chloride (70 mL). HEMA (0.26 g, 2 mmol) was added to the solution followed by 4-(dimethylamino)pyridine (0.02 g, 1 wt %). The reaction flask was cooled in an ice bath, and DCC (0.206 g, 1 mmol) was added in small portions. The reaction was stirred for 5 h at room temperature and then cooled again in an ice bath to maximize the precipitation of dicyclohexylurea which was removed by filtration. The filtrate was concentrated to about 10 mL and then dropped into cold ether. Poly(PEG–Lys–hydroxyethyl methacrylate) precipitated. After 2 h, the solvent was decanted and the product was washed with cold ether. It was further purified by recrystallization from 2-propanol (30 mL) and dried under high vacuum. Yield: 1.98 g (90%). FT-IR (film on NaCl, cm^{-1}): ν characteristic bands at 2900 (CH), 1720 (C=O of urethane), shoulder at 1740 (C=O of ester), 1637 (C=O of unsaturated ester), 1110 (CO). ^1H NMR ($CDCl_3$): δ 6.08 (1 H, s, =C*H*H), 5.57 (1 H, s, =CH*H*), 5.39 (1 H, d, α-NH of Lys), 4.95 (1 H, m, ε-NH of Lys), 4.33 (4 H, m, terminal CH_2 of PEG), 4.16 (4 H, m, $-OCH_2CH_2O-$), 3.70 (173 H, PEG overlapping with α-C*H*NH), 3.13 (2 H, m, ε-CH_2NH), 1.90 (3 H, s, $=CCH_3$), 1.31–1.81 (6 H, br m, CH_2 of Lys). The same procedure was used for the preparation of poly(PEG–Lys–HEA).

Preparation of "Amide" Hydrogels. 1,6-Diaminohexane (0.024 g, 0.20 mmol) in methylene chloride (3 mL) was added with stirring to a solution of poly(PEG–Lys–NHS) (**3**; 0.92 g, 0.40 mmol based on the molecular weight of the polymer repeat unit) in methylene chloride (15 mL). After 5 min, the mixture was poured into the cavity of a trimethylsilane-coated glass casting mold. The mold was covered with filter paper to facilitate the slow and uniform evaporation of the solvent. The film was allowed to dry in a glovebox for 48 h and then peeled from the mold. The thickness of the membrane in the dry state was approximately 0.1 mm. To determine the amount of residual, free amino groups in the cross-linked film, a specimen of the membrane weighing approximately 0.1 g was swollen in methylene chloride (5 mL) and the amount of free amines was determined by titration with standard perchloric acid using methyl red as indicator. The end point was reached when a stable pink color was obtained.

Preparation of "Semicarbazide" Hydrogels. Finely powdered sodium bicarbonate (1.5 g) was added to a solution of poly(PEG–Lys–hydrazide·HCl) (1.5 g, 0.67 mmol based on the molecular weight of the polymer repeat unit) in methylene chloride (20 mL). The suspension was stirred at room temperature for 30 min and filtered. To the clear filtrate was added with vigorous stirring 1,6-diisocyanatohexane (56.3 mg, 0.33 mmol) in methylene chloride (20 mL). After 5 min, the solution was poured into a glass mold and a film was cast and analyzed for residual hydrazide groups as described above for "amide" hydrogels.

Preparation of Photo-Cross-Linked "Methacrylate" and "Acrylate" Hydrogels. Poly(PEG–Lys–HEMA) (1 g) was dissolved in dioxane (10 mL). Benzoin methyl ether (2 mg, 0.2 wt %) was added as the photoinitiator. The solution was flushed with nitrogen and poured into a glass mold. A medium-pressure mercury lamp (long wave, 100 W) was placed 18 cm above the mold. The mold was irradiated for 2 h. Then the residual solvent was removed in vacuo. After 24 h the film was peeled off from the mold. Cross-linked membranes of poly(PEG–Lys–HEA) were prepared in the same way.

Swelling Measurements. Swelling was measured according to the procedure published by Peppas.[22] The weight of a sample of the dry membrane in air and in hexane was determined. The sample was then allowed to swell in water for 1 h, and its weight in air and in hexane was determined. From these four weights the swelling ratio and the equilibrium water content (EWC) were calculated. Alternatively, the volume change upon swelling was determined by the actual measurement of the dimensions of a rectangular specimen.

Weight Loss Measurements of Hydrogels. A specimen of the dry hydrogel membrane was accurately weighed and placed inside a scintillation vial. To the vial was added 5 mL of phosphate buffer (0.1 M, pH 7.4). The vial was kept closed at constant temperature (37 °C). After predetermined time intervals, the hydrogel was removed from the buffer, gently rinsed with three portions of distilled water and transferred to a preweighed sample tube. The membrane was dried to constant weight under high vacuum. From the difference between the initial and final weights, the percent weight loss was determined. Measurements were made in triplicate, using different sets of samples for each data point.

Results and Discussion

Copolymer Synthesis. Recently, the mono(succinimidyl) carbonate derivative of poly(ethylene glycol) has been employed for the modification and cross-linking of proteins.[20,21] We adopted this approach for the prepa-

Table I
Comparison of ^{1}H NMR Spectra of Poly(PEG–Lys–OEt) Derived from PEG Units with Different Molecular Weights

polymer	ratio of PEG protons to CH_3 protons[a] calcd	exptl
poly(PEG1000–Lys–OEt)	29.0:1	29.6:1
poly(PEG2000–Lys–OEt)	59.3:1	60.9:1
poly(PEG4000–Lys–OEt)	119.9:1	102.2:1
poly(PEG8000–Lys–OEt)	241.0:1	not determined[b]

[a] CH_3 group of the ethyl ester side chain. [b] The signal of the CH_3 protons was too weak to be determined accurately relative to the intensity of the PEG protons.

ration of PEG-based, linear poly(ether urethanes) by activating PEG with phosgene and *N*-hydroxysuccinimide to yield the corresponding bis(succinimidyl) carbonate derivative (BSC–PEG). This activated "prepolymer" was subsequently reacted with L-lysine ethyl ester to give a poly(ether urethane) consisting of strictly alternating units of PEG and L-lysine ethyl ester 1; eq 1.

By slightly modifying a previously published procedure,[20,21] BSC–PEG could be prepared in essentially quantitative yields and with up to 99.5% purity after two recrystallizations. The amount of available "active" carbonate was determined by nonaqueous titration with benzylamine.[17] Only those preparations having more than 97% of the theoretical amount of reactive carbonate groups were used in the subsequent polymerization reactions.

The interfacial polymerization of L-lysine ethyl ester with BSC–PEG in methylene chloride–water at pH 8 yielded high polymers. The degree of polymerization could be controlled by varying reaction time, concentration of the monomers, and stirring speed. Under optimized conditions, poly(ether urethanes) with a degree of polymerization of up to 50 were obtained. This corresponded to a final *weight-average* molecular weight of about 170 000 when PEG of molecular weight 2000 was used. Since the maximum obtainable degree of polymerization was largely independent of the length of the PEG chains, the molecular weight of the copolymers tended to increase with the increasing length of the PEG unit. The polymerization was complete within about 2 h.

The structure of poly(PEG–Lys–OEt) was confirmed by IR and ^{1}H and ^{13}C NMR (see the Experimental Section for spectral data). IR showed formation of the urethane bond at 1720 cm^{-1}. The ethyl ester peak was seen as a shoulder of the urethane peak. In the ^{1}H NMR spectrum, two distinct peaks at 5.41 and 4.92 ppm were assigned to the urethane NH protons derived from the α- and ϵ-amino groups of L-lysine, respectively. An identical observation was made in the ^{13}C NMR spectrum which showed two peaks at 156.4 and 156.0 ppm. This is in agreement with the molecular structure of the copolymer.

By using BSC–PEG of molecular weights 1000, 2000, 4000, and 8000, a homologous series of copolymers was obtained, differing only in the length of the PEG chain in between lysine ethyl ester residues. The ^{1}H NMR spectra of these polymers were compared (Table I). The ratio of the integrations of the PEG protons to the protons of the CH_3 group of the ethyl ester side chains was found to be very close to the expected values, except for the polymer derived from PEG8000 where the intensity of the PEG protons was too high to permit the accurate determination of the integration ratio.

A major advantage of these copolymers is the presence of reactive pendent groups at predetermined intervals along the polymer backbone. The spacing of these groups depends on the length and molecular weight distribution of the PEG chains used in the synthesis of the copolymer. Ideally, when monodisperse PEG is employed, the distance between the pendent groups will be constant. In our syntheses, purified PEG preparations of low polydispersity were used. For example, PEG2000 had an exact number-average molecular weight of 2034 (±5%) and a polydispersity of 1.034 (as determined by GPC relative to monodisperse standards of PEG). Similar polydispersities were determined for the other PEG preparations.

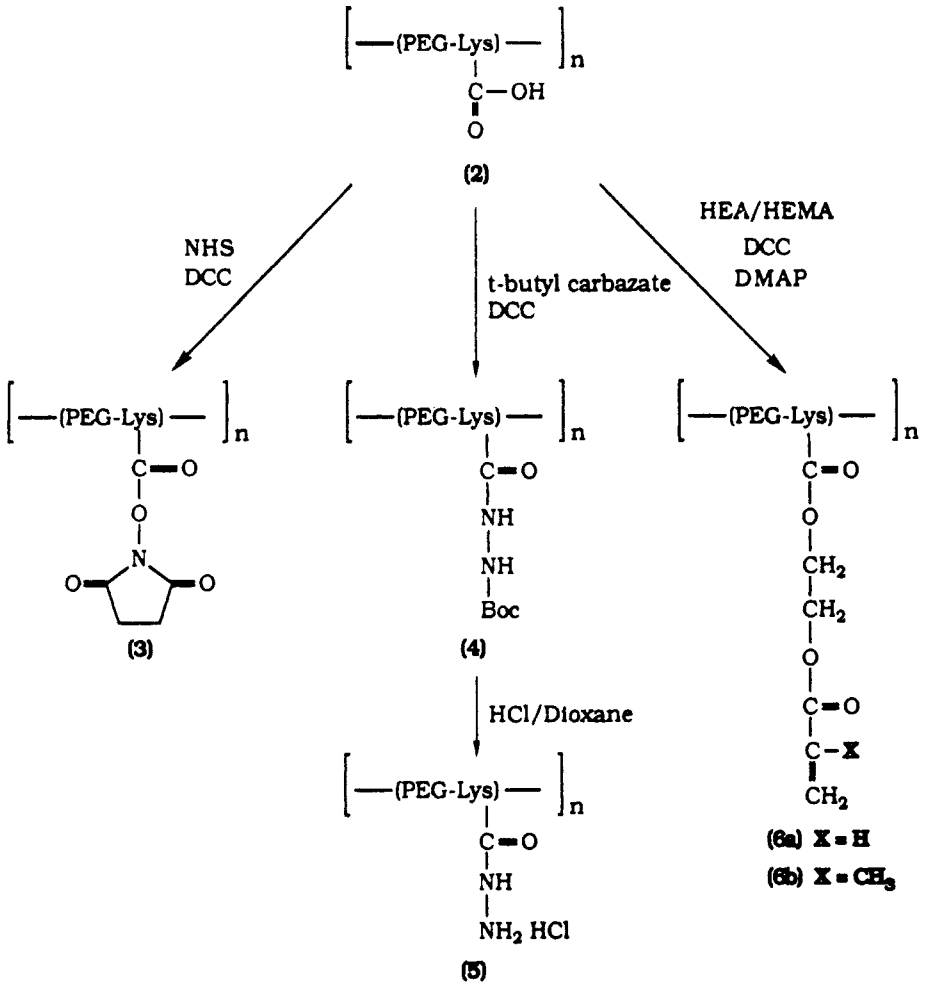
Derivatization Reactions. The possibility of preparing functionalized polymers carrying a variety of different pendent groups such as the *N*-hydroxysuccinimide active ester (**3**), the hydrazide (**5**), or the hydroxyethyl acrylate (or methacrylate) derived spacers (**6a,b**) (Figure 1) is an important requirement for the successful use of PEG–Lys copolymers in the design of cross-linking reactions. To facilitate the necessary derivatization reactions, the ethyl ester side chains of poly(PEG–Lys–OEt) (**1**) must first be hydrolyzed to free carboxylic acid groups.

Since the urethane backbone linkages are susceptible to cleavage by strong base, the ethyl ester side chains were hydrolyzed at room temperature under mild conditions at pH 11.5. The hydrolysis was followed by monitoring the disappearance of the CH_3 signal of the ethyl ester side chain at 1.26 ppm in the ^{1}H NMR spectrum. The reaction was complete after about 5 h. The quantitative formation of free carboxylic acid side chains was further confirmed by nonaqueous titration with sodium methoxide. Size-exclusion chromatographic analysis of poly(PEG–Lys) (**2**) and poly(PEG–Lys–OEt) (**1**) in aqueous buffer on TSK gel columns indicated the absence of detectable backbone degradation during side-chain hydrolysis.

The free acid copolymer (**2**) was first converted to the *N*-hydroxysuccinimide active ester (**3**) (eq 2). The degree of side-chain substitution was ascertained by ^{1}H NMR spectroscopy, using the integration ratio of the NHS and PEG protons. **3** was further analyzed by reaction with a known excess of benzylamine, followed by nonaqueous titration of the residual, unreacted benzylamine. The results obtained by the titrimetric assay corresponded to data obtained by NMR spectroscopic analysis, indicating that **3** contained about 95% of the theoretically expected amount of active ester groups. The polymeric, water-soluble, active ester derivative will be a very convenient intermediate in drug attachment or cross-linking schemes.

Next **2** was used for the preparation of poly(PEG–Lys–hydrazide) (**5**). Polymers having pendent hydrazide groups represent a useful addition to poly(PEG–Lys) (**2**) since carboxylic acid groups and hydrazide groups complement each other in their ability to serve as attachment points for cross-linkers. Surprisingly, our attempt to prepare **5** by hydrazinolysis of poly(PEG–Lys–OEt) with hydrazine in methanol (using well-established procedures in peptide chemistry[23]) resulted in considerable backbone degradation. We therefore reacted **2** with *tert*-butyl carbazate and obtained the protected polymer poly(PEG–Lys–NHNH–Boc) (**4**). The free hydrazide hydrochloride (**5**) was obtained by treatment of **4** with 4.0 N HCl in dioxane. The complete removal of the Boc group was confirmed by ^{1}H NMR and nonaqueous titration. Overall, more than 90% of all carboxylic acid side chains had been converted to free hydrazide groups.

In another reaction sequence, HEA or HEMA groups were attached to the pendent carboxylic acid side chains of **2** via hydrolytically cleavable ester bonds (eq 2). The amount of acrylate–methacrylate groups on the polymer was ascertained by a nonaqueous titration method.[19] Under optimized conditions, HEA or HEMA groups were linked to about 95% of all available carboxylic acid side chains.

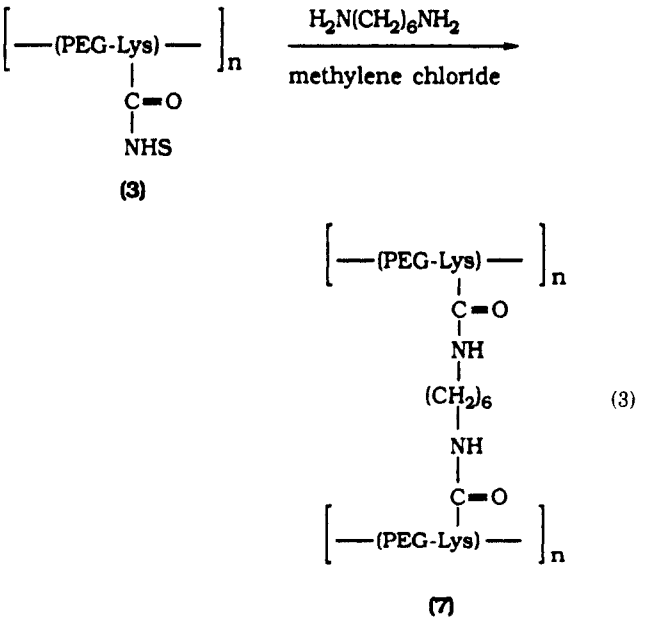


In this way photo-cross-linkable formulations were obtained. However, contrary to most other polymers carrying HEA or HEMA, the pendent chains described here were intentionally designed with cleavable linkages. Thus, hydrogels derived from cross-linking of poly(PEG–Lys–HEA) or poly(PEG–Lys–HEMA) can be expected to slowly solubilize under physiological conditions and can be considered, for example, in the formulation of degradable, implantable drug delivery devices.

Synthesis of Cross-Linked Hydrogels. Reaction of the active pendent groups of poly(PEG–Lys) derivatives with suitable bifunctional reagents led to the formation of cross-linked networks. In these networks, the constant spacing of the reactive pendent groups along the polymer backbone has the potential of leading to the formation of uniformly spaced cross-links at predetermined intervals. This represents an important difference to the well-known radiation-cross-linked poly(ethylene oxide) hydrogels[24] and hydrogels derived from PEG, triol, and diisocynates[12] in which the cross-links are formed in a random fashion.

In a particularly convenient reaction scheme, poly(PEG–Lys–NHS) (3) was cross-linked using hexamethylenediamine (eq 3) to obtain amide cross-links (referred to as "amide" hydrogels (7)). Upon addition of hexamethylenediamine to a solution of 3, the reaction mixture gelled after 5 min. After slow removal (48 h) of the solvent by evaporation in an atmosphere of nitrogen, transparent, water-insoluble, and highly swellable films were obtained. In preliminary stability tests, "amide" hydrogels appeared to be nondegradable under physiological conditions (phosphate buffer, pH 7.4, 37 °C).

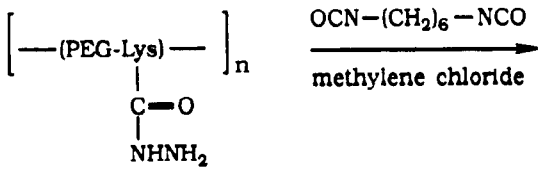
The cross-linking reaction led to the release of *N*-hydroxysuccinimide (NHS) which remained embedded within



(3)

the membrane and had to be removed by washing with water. To ensure the complete removal of NHS, the films were successively washed with several portions of water and the washings were analyzed for free NHS spectrophotometrically as described by Miron and Wilcheck.[18] Since "amide" hydrogels swell considerably, about 80% of the total amount of NHS was released in the first washing and no more NHS was detectable in the supernatant after four washings of 5 min each.

In an alternative approach, the reaction of poly(PEG–Lys–hydrazide) (5) with 1,6-diisocyanatohexane was ex-



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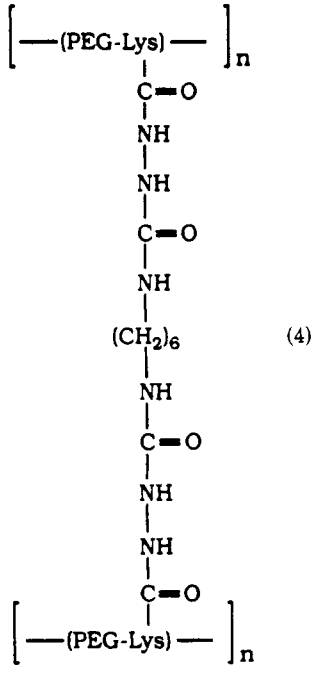

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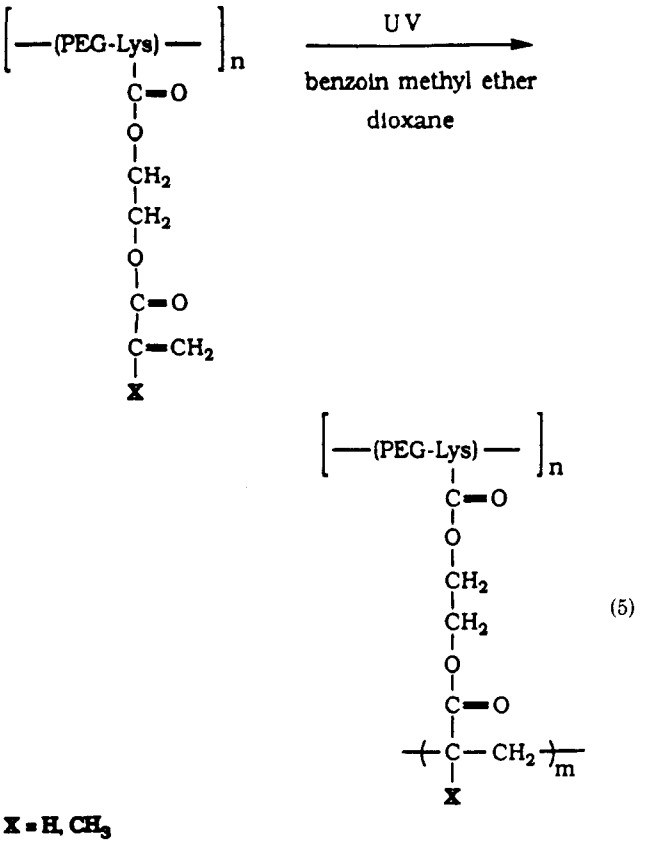
plored (eq 4). In analogy to the known reaction of monomeric acyl hydrazides with isocyanates,[25] this cross-linking scheme gave rise to acyl semicarbazide cross-links which had heretofore not been used for the preparation of hydrogels. Since the hydrazide groups of poly(PEG–Lys–hydrazide·HCl) (5) did not react with the diisocyanate, the polymer salt was neutralized with triethylamine (TEA) or by the addition of finely ground, solid sodium bicarbonate to the methylene chloride solution of the polymer. After neutralization, cross-linking occurred rapidly and water-insoluble, transparent films ("semicarbazide" hydrogels (8)) were obtained.

For PEG units of molecular weight 2000, hydrogels prepared under optimized conditions contained only 5% of the initial amount of free hydrazide groups, indicating that almost all side chains had reacted with the cross-linking agent. Since "semicarbazide" hydrogels prepared from PEG4000 and PEG8000 contained 10 and 18% of the initial amount of free hydrazide groups after cross-linking, the cross-linking reactions became less efficient as the distance between cross-links increased. The "semicarbazide" hydrogels were free of residual 1,6-diisocyanatohexane and did not contain other detectable impurities (as determined by FT-IR).

The photo-cross-linking reaction of poly(PEG–Lys–HEA) and poly(PEG–Lys–HEMA) led to the formation of poly(HEA) or poly(HEMA) chains covalently attached to and physically intertwined with the strands of poly(PEG–Lys) (eq 5). The resulting cross-linked hydrogel thus consists of a network of two interconnected polymers and should have physicomechanical properties that differ from the properties of the chemically cross-linked "semicarbazide" or "amide" hydrogels.

Probably due to the low density of acrylate or methacrylate groups in the reaction mixture, initial attempts



X = H, CH_3 (5)

to photo-cross-link poly(PEG–Lys–HEA) or poly(PEG–Lys–HEMA) failed. To optimize the reaction, different conditions were explored. In one set of experiments, the amount of UV irradiation was kept constant, while solvent effects and the influence of different initiators on the mechanical strength of the resulting cross-linked membranes were studied (Table II).

Without initiator, the reaction mixture remained water soluble, indicative of the absence of significant cross-linking. Among the tested solvents no correlation between solvent polarity and the success of the cross-linking reaction was observed. Overall, the type of initiator added appeared to be the most important reaction parameter. Benzoin methyl ether gave consistently stronger membranes than either benzophenone or acetophenone. Decreasing the initiator concentration from 0.1 to 0.004 wt % led to very weak and partly water-soluble membranes, while increasing the initiator concentration above 0.1 wt % did not improve membrane strength. Thus, in all subsequent experiments, a concentration of 0.1 wt % of benzoin methyl ether in dioxane was used for the preparation of photo-cross-linked membranes.

Since it is known that PEG-based polymers are readily cross-linked by γ radiation,[12] we checked whether the backbone of poly(PEG–Lys) was involved in the observed cross-linking reaction under UV light. In one control experiment, poly(PEG2000–Lys) was subjected to optimized cross-linking conditions. In a second control experiment, a *mixture* of poly(PEG2000–Lys) and HEA was subjected to the same conditions. In both the cases, the reaction products remained completely water soluble. These experiments confirmed that the formation of swellable hydrogels during the irradiation of poly(PEG–Lys–HEA) was due to the reaction of covalently linked pendent acrylate double bonds.

Physicomechanical and Chemical Properties. Melting Temperatures in the Dry State. In the low molecular weight range (200–8000), the melting temperature (T_m) of PEG increases with increasing molecular

Table II
Effect of Solvent and Initiator on the Photo-Cross-Linking Reaction of "Acrylate" Hydrogels Derived from Poly(PEG2000–Lys–HEA)

initiator (0.1 wt %)	solvent	result[a]
none	toluene	–
	water	–
	dioxane	+
benzophenone	toluene	+
	methylene chloride	+
acetophenone	toluene	–
benzoin methyl ether	toluene	+ +
	dimethyl sulfoxide	+ +
	ethyl acetate	+ +
	chloroform	+ +
	dioxane	+ + +

[a] Qualitative examination: (–) no membrane was obtained, (+) very weak highly swellable membrane was obtained, (+ +) membrane with reasonable strength was obtained, (+ + +) strong membrane was obtained.

weight from –65 to +63 °C, while for PEG chains above about 8000, T_m is no longer a sensitive function of the molecular weight.[26] A comparison of T_m for PEG chains of molecular weight 2000, 4000, and 8000 and poly(PEG–Lys–OEt) copolymers (1) having PEG units of the same molecular weight indicated that the melting temperature of poly(PEG–Lys–OEt) copolymers depends on the chain length of the PEG units from which they are derived and not on the molecular weight of the copolymer itself. Furthermore, the linear copolymers and the cross-lined hydrogels had almost identical melting temperatures. Thus, by changing the length of the PEG unit, the melting temperature of cross-linked hydrogels can be varied from about 30 to 53 °C (Table III).

Water Uptake and Swelling. Since both radiation-cross-linked poly(ethylene oxide) (PEO) hydrogels[24] and PEO gels cross-linked with 1,2,6-hexanetriol and dicyclohexylmethane-4,4′-diisocyanate[12] have an equilibrium water content (EWC) greater than 80%, we expected poly(PEG–Lys)-derived hydrogels to swell significantly in water.

Indeed, as determined by gravimetric[22] and volume measurements, all poly(PEG–Lys)-derived hydrogels swelled extensively and took up water rapidly. Thin films (0.1–0.2 mm) reached equilibrium within about 1 h. The EWC of the chemically cross-linked "semicarbazide" hydrogels was 81–91% and was higher than the EWC of the photo-cross-linked "acrylate" and "methacrylate" hydrogels (Table IV). Superimposed on this was the trend of the EWC to increase with decreasing cross-link density. Overall, among the few hydrogels tested so far, the EWC could be varied from about 64 to 91%.

Mechanical Strength in the Dry and Swollen State. In the dry state, the chemically cross-linked hydrogels formed extremely strong films whose tensile strength surpassed 60 MPa. The films were also very ductile and elongated from 700% (for hydrogels derived from PEG2000) to 1200% (for hydrogels derived from PEG8000). The photo-cross-linked hydrogels were significantly weaker and had a tensile strength of 10–20 MPa and an elongation at break of 160–470%. When stretched hydrogel films were heated above their melting point, they collapsed back to their original unstretched state, similar to the behavior of heat shrinking wrapping materials.

As expected, the mechanical strength decreased significantly in the swollen state (Table IV). While all swollen hydrogels behaved like perfect elastomers, there were no generally valid correlations between EWC, tensile strength, elongation, and modulus. Particularly striking was the exceptionally high strength of the "methacrylate" hydrogels. When PEG4000 chains were used, these hydrogels reached a tensile strength of 1.09 MPa at an EWC of 78% and rank among the mechanically strongest, highly swollen hydrogels reported in the literature so far. For comparison, "acrylate" and "semicarbazide" hydrogels of similar EWC had significantly lower tensile strength values, and for hydrogels derived from copolymers of methacrylate esters and *N,N′*-dimethylacrylamide, a tensile strength of only 0.21 MPa at an EWC of 80% had been reported.[27]

Hydrolytic Stability. The hydrolytic stability of hydrogels was studied in two different test systems. All hydrogels were subjected to physiological conditions (phosphate buffer, pH 7.4, 37 °C), and their stability was determined quantitatively by monitoring the weight loss of immersed membranes as a function of time. In the second test system, the stability of selected hydrogels was determined in a qualitative fashion at 25 °C over a wide range of pH values.

Stability under Physiological Conditions. "Amide" hydrogels lost about 40% of their initial weight within the first day. Thereafter no further weight loss occurred over the next 20 days. Since the release of NHS (formed during the cross-linking reaction) can account for only about 5% of the total weight of the hydrogel, the high initial weight loss indicated that a considerable fraction of the PEG–Lys copolymer chains remained unbound to the cross-linked network. On the other hand, 90% of all added amino groups were consumed by the cross-linking reaction, as determined by nonaqueous titration of the cross-linked gel. Thus, a large proportion of the cross-links formed must involve intramolecular rather than intermolecular linkages. In any event, the lack of further weight loss over the subsequent period of 20 days is clearly a reflection of the hydrolytic stability of the amide cross-links in physiological phosphate buffer.

"Semicarbazide" hydrogels lost about 11% of their initial weight within the first day and continued to lose weight at the rate of about 1% per day thereafter. The hydrogels disintegrated completely after about 40 days when the total cumulative weight loss reached about 50%. The continuous weight loss shown by "semicarbazide" hydrogels is an indication of the potential of the semicarbazide cross-links to degrade under physiological conditions.

"Acrylate" and "methacrylate" hydrogels showed no initial weight loss during the first day, indicative of the more complete entanglement of all polymer chains. Thereafter, "acrylate" and "methacrylate" hydrogels lost weight at a rate of 1.7 and 1.1% per day, respectively. The faster weight loss of the "acrylate" hydrogels could be a reflection of the more hydrophilic nature of the acrylate cross-links as compared to methacrylate. As the degradation process advanced, the daily rate of weight loss accelerated. Although the membranes became progressively weaker, they maintained their structural integrity up to a cumulative weight loss of about 60% and rapidly dissolved thereafter.

Stability as a Function of pH. "Photo-cross-linked" hydrogels and "semicarbazide" hydrogels were exposed to aqueous solutions of varying pH at room temperature, and the time required for the complete dissolution of the films was noted (Table V). Both types of hydrogels were found to be most stable in weakly acidic to neutral media and extremely unstable in strongly basic media.

Stability of the Polymer Backbone. The observed degradation of the hydrogels could in principle result from the degradation of backbone urethane linkages. To test this, samples of poly(PEG–Lys–OEt) (1), representing the

Table III
Melting Temperatures of PEG, Poly(PEG–Lys–OEt), and Cross-Linked Hydrogels as a Function of the Length of the PEG Chain

mol wt of PEG unit used in synthesis	melting temp[a] (°C) for				
	PEG	poly(PEG–Lys–OEt)[b]	"semicarbazide"hydrogel	"acrylate" hydrogel	"methacrylate" hydrogel
2000	52	38 (88 000)	38	33	26, 29[c]
4000	59	50 (132 000)	48	45	47
8000	62	53 (201 000)	53		

[a] Determined by DSC at a heating rate of 1 °C/min. [b] Numbers in parentheses are the weight-average molecular weights of the copolymers used in these experiments. [c] Two endothermic transitions were observed.

Table IV
Mechanical Properties of Swollen, Degradable Hydrogels

hydrogel	PEG chain length	equilibrium water content (%)	elongation at break (%)	tensile strength (MPa)	Young's modulus (MPa)
"semicarbazide"	2000	81	50	0.26	0.46
	4000	87	70	0.14	0.27
	8000	91	111	0.19	0.17
"acrylate"	2000	68	62	0.64	0.91
	4000	80	29	0.31	1.08
"methacrylate"	2000	64	35	0.97	2.97
	4000	78	52	1.09	2.18

Table V
Short-Term Stability of Hydrogels at 25 °C in Various Aqueous Media[a]

conditions	time required for dissolution (days)	
	"semicarbazide"	"acrylate"/"methacrylate"
1 N HCl	5–8	5–7
0.1 N HCl	>8[b]	>8[b]
0.01 N HCl	>8[b]	>8[b]
deionized water (pH = 6)	>8[b]	>8[b]
borate buffer (pH = 9)	5–8	8–12
0.01 N NaOH	5 h	1–2 h
0.1 N NaOH	3–5 h	5–10 min
1 N NaOH	1 h	5 min

[a] Thin films of hydrogels were kept for up to 8 days in the indicated media, and the time required for complete dissolution of the films was noted. [b] No physical changes were noted.

polymer backbone, were dissolved in phosphate buffer (pH 7.4) and kept at 60 °C. GPC analysis of the solutions over a period of 30 days showed that the molecular weight of poly(PEG–Lys) remained constant. Hence, the degradation of "semicarbazide" hydrogels must involve the cleavage of the semicarbazide cross-links, while the degradation of the "acrylate" and "methacrylate" hydrogels must involve the cleavage of the ester bonds connecting the acrylate or methacrylate spacers to the poly(PEG–Lys) backbone.

Conclusions

Strictly alternating copolymers of the new bis(succinimidyl) carbonate derivatives of PEG (BSC–PEG) and the natural α-amino acid L-lysine were prepared by the formation of urethane linkages between the α- and ε-amino groups of L-lysine and the terminal hydroxyl groups of PEG. The interfacial polymerization with L-lysine ethyl ester proceeded under mild conditions and yielded copolymers with degrees of polymerization of up to 50. Since these copolymers were exclusively derived from structural units having an extensive record of nontoxicity and biocompatibility, we expect the PEG–Lys copolymers to be useful biomaterials, and a detailed investigation of their biological properties is currently in progress.

When monodisperse preparations of PEG are used in the synthesis, the resulting copolymer has pendent groups at readily controllable and uniformly spaced intervals along the polymer backbone. Derivatization reactions, used to transform the pendent carboxylic acids to a variety of functionalities proceeded in close to quantitative yield. The ease with which the derivatization reactions could be brought to completion can probably be attributed to the high degree of solvation of the PEG chains and their intrinsic flexibility. The facile derivatization of poly(PEG–Lys) is in contrast to the general observation that it is often difficult to perform quantitative derivatizations of polymers.

The availability of a family of structurally related copolymers carrying different functionalized pendent chains facilitated the design of cross-linking reactions. The controllable distance between individual cross-links represents a major advantage of poly(PEG–Lys)-derived materials. Hydrogels with amide-, semicarbazide-, and acrylate-based cross-links were prepared. The semicarbazide cross-links hydrolyzed under mild conditions, resulting in the dissolution of "semicarbazide" hydrogels within about 40 days under simulated physiological conditions. The "amide" hydrogels appeared to be significantly more stable in short-term stability tests under physiological conditions. The "acrylate" and "methacrylate" hydrogels were cross-linked by strands of poly(HEA) or poly(HEMA) and therefore represented a network of two interconnected polymers. The exceptional strength of these hydrogels in the swollen state can probably be attributed to this structural feature.

In summary, poly(PEG–Lys) and its polymeric derivatives represent a new and versatile family of PEG-based poly(ether urethanes) that may find a variety of industrial and medical applications.

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