Aqueous Micro and Nanoreactors Based on Alternating Copolymers of Phenylmaleimide and Vinylpyrrolidone Bearing Pendant L-Proline Stabilized with PEG Grafted Chains

Anselmo del Prado,[1] Mercedes Pintado-Sierra,[2] Marta Juan-y-Seva,[1] Rodrigo Navarro,[2] Helmut Reinecke,[1] Juan Rodríguez-Hernández,[1] Carlos Elvira,[1] Alfonso Fernández-Mayoralas,[2] Alberto Gallardo[1]

[1]Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, Madrid 28006, Spain
[2]Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, Madrid 28006, Spain
Correspondence to: A. Gallardo (E-mail: gallardo@ictp.csic.es)

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ABSTRACT: Proline may work efficiently in water as catalyst of aldol reactions if it is hydrophobically activated. In this work, we have maximized this hydrophobic activation by the preparation of linear alternating copolymers of hydrophobic phenylmaleimide and a vinylpyrrolidone derivative bearing proline. These copolymers were water soluble above pH 5.0 and, unlike the free proline, exhibited efficient catalysis at pH 7.0. Moreover, they catalyzed and presented enantioselectivity in an aggregated form at pH 4.0 (close to the isoelectric point, IEP, of the polymer). This enantioselectivity has been related to the exclusion of water at this IEP. To control the size and stabilize the aggregates, PEG grafted copolymers were prepared by the incorporation of a PEG-macromer (2–10 mol%), which rendered stable nano-aggregates in water at the IEP. At this pH they catalyzed the aldol reaction in a higher rate than the non-grafted polymer, but the enantioselectivity was decreased. © 2017 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2017**, *00*, 000–000

KEYWORDS: catalysis; copolymerization; functionalization of polymers; radical polymerization; stimuli-sensitive polymers

INTRODUCTION Polymer chemistry has different tools that make possible tailoring properties and structure of polymeric materials. Thus, for instance, when two complementary moieties -for example catalyst and co-catalyst- must participate simultaneously in a process, they can be easily combined in a common macromolecular backbone just by copolymerizing two monomeric precursors containing those moieties. Even more, polymer chemistry offers the possibility of obtaining alternating copolymers, which may maximize the interaction between the two required moieties. In this work, this strategy has been combined with the use of polyethylenglycol (PEG)-macromers to obtain aqueous nanoreactors containing L-proline for asymmetric organocatalysis.

Although L-proline is considered as an enzyme mimic of aldolase in polar media,[1–3] its catalytic activity in water is very low.[4,5] Good efficiencies in water may indeed be achieved through "hydrophobic activation,"[6] linking L-proline derivatives to hydrophobic moieties, which actually raises mimicking to a higher level since 3D structure of natural enzymes generally creates a hydrophobic "pocket" at the active centre. This interaction has been achieved by anchoring L-proline to hydrophobic porous solid resins,[7,8] by copolymerizing L-proline based monomers with hydrophobic units such as styrene,[9] or designing more complex macromolecular structures like the described by O'Reilly and coworkers [10–12] and Meijer and coworkers[13,14]

In this work, we propose to optimize the hydrophobic nature of the proline moiety surroundings by preparing alternating copolymers of units containing respectively L-proline and aromatic groups. Specifically, we have synthesized a vinylpyrrolidone derivative bearing protected L-proline, which has been copolymerized with an aromatic maleimide, a 1,2-disubstituted alkene, by radical polymerization. Vinylpyrrolidone (VP) and 1,2-disubstituted vinylics, such as maleic derivatives, have been polymerized in the past to obtain alternating copolymers.[15,16] This alternation is due both to steric and electronic activation reasons: on the one hand, VP belongs to the group of low activated monomers,[17] which

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means that a growing macroradical ended in VP is much more reactive toward a maleic derivative; on the other hand, these 1,2-disubstituted monomers have strong difficulties to homopolymerize because of steric hindrance, which means that a growing macroradical ended in maleic derivative can only propagate by reacting with VP.

This hydrophobic activation may turn the L-proline catalyst efficient in water but, however, may not be enough to achieve enantioselectivity. To do so, it has been reported that water must be excluded from the active centre,[9,13] because water alters the highly organized transition states that are thought to be responsible for the stereoselectivity.[18–20] A strategy described previously in our group to exclude water is to work at the isoelectric point (IEP), where the charge neutralization makes the macromolecules to precipitate and accordingly to exclude water.[21] One disadvantage of this strategy may be the formation of large aggregates at the IEP, which may lead to slow catalyst because of the limited diffusion of reactants. In this work, we have used another tool of polymer chemistry to address this issue by including in the copolymerization PEG macromers, which will lead to graft copolymers with PEG branches able to stabilize nanometer size aggregates in water and thus providing the possibility of reducing diffusion problems.

In summary, we propose the preparation of alternating copolymers of a VP unit bearing pendant L-proline (VPcprol) with phenylmaleimide (PMI) and the grafting to those backbones of PEG chains by including a small amount of PEG-methacrylate (PEGMA) in the reaction. The hypotheses are that the alternation will maximize the hydrophobic interaction making the catalysts highly efficient in water, and that the PEG chains will offer colloidal stabilization at the IEP affording the enantioselective reaction to occur on small entities and therefore allowing a fast reaction.

EXPERIMENTAL

Materials

Vinylpyrrolidone (Sigma-Aldrich) was distilled under reduced pressure before use. 2,2′-Azobis(isobutyronitrile) (AIBN) 98% (Sigma-Aldrich) was recrystallized in ethanol. Polyethylenglycol methyl ether methacrylate average M_n 1.100 (Sigma-Aldrich) was used as received, as well as all other chemicals, which were purchased from commercial suppliers.

Methods

Thin layer chromatography (TLC) was performed on aluminum sheets 60 F254 Merck silica gel and compounds were visualized by irradiation with UV light and/or by treatment with a solution of Ninhydrin in *n*-BuOH/EtOH or H_2SO_4 (5%) in EtOH followed by heating. Flash chromatography was performed using thick walled columns, using silica gel (Merck 60) or deactivated aluminum oxide, Brockmann II (Aldrich).

Monomers ^{1}H and ^{13}C NMR spectra were recorded on a VARIAN NMR system (400 and 500 MHz for ^{1}H, and 100 and 125 MHz for ^{13}C, respectively) using $CDCl_3$ as solvent at room temperature and tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) are reported in parts per million (ppm) relative to TMS. Coupling constants (J values) are reported in Hertz (Hz), and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets), td (triplet of doublets), and m (multiplet or unresolved). The ^{1}H NMR spectra of the polymers were measured on an Inova 300 spectrometer (300 MHz) using $CDCl_3$ or D_2O as solvents at room temperature and TMS as internal standard. ^{1}H NMR spectra of the *in situ* polymerizations were recorded on a VARIAN NMR system (400 MHz) using DMF-d_7 as solvent at 60 °C and hexamethyldisiloxane (HMSO) as reference.

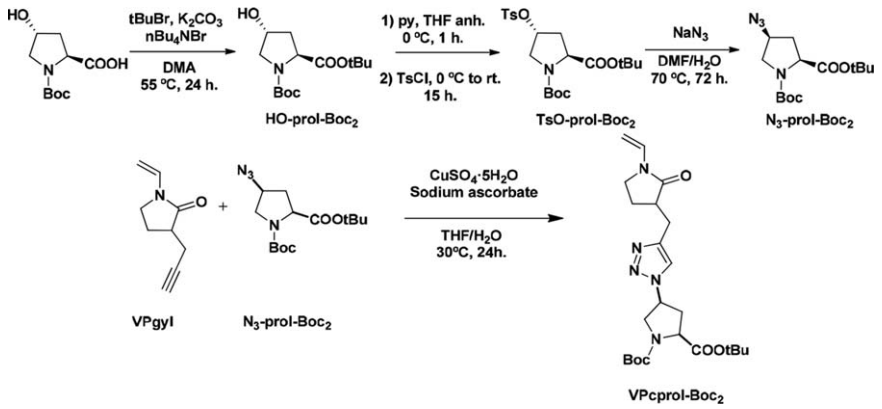
High resolution mass spectra (HRMS) were recorded in an Agilent 1200 Series LC system (equipped with a binary pump, an autosampler, and a column oven) coupled to a 6520 quadrupole-time of flight (QTOF) mass spectrometer. Acetonitrile/water (75:25, v:v) was used as mobile phase at 0.2 mL min^{-1}. The ionization source was an ESI interface working in the positive-ion mode. Fourier Transform Infrared (FTIR) spectra were recorded on a Perkin Elmer RX-1 instrument with a Universal Attenuated Total Reflectance (ATR) device using diamond/ZnSe as internal reflection elements.

Diastereomeric and enantiomeric excess were calculated by HPLC Dionex P680 with DAD detector (lecture at 254 nm), using a chiral chromatographic column Daicel Chiralpak AD-H.

Gel permeation chromatography (GPC) analyses were carried out using a Perkin Elmer chromatographic system equipped with a Waters model 2414 refractive index detector, using Styragel (300 × 7.8 mm, 5 μm nominal particle size) HR3 and HR5 water columns. DMF with 1 wt% LiBr was used as eluent. Measurements were performed at 70 °C at a flow rate of 0.7 mL/min using a polymer concentration of 4 mg/mL. The calibration was performed with monodispersed polystyrene standards in the range of 2.0 and 9000.0 kDa.

DLS experiments were carried out using a Malvern Zetasizer (Zetasizer NS Malvern Instruments, Malvern, UK), working at a scattering angle of 173° relative to the source. This apparatus is equipped with a 4 mW He/Ne laser emitting at 633 nm, a measurement cell, an auto-correlator and a photomultiplier. The measurements were carried out in the fully automatic mode. Intensity autocorrelation functions were analyzed by a General Purpose Algorithm (integrated in the Malvern Zetasizer software) to determine values of zeta potential (in mV) and zeta average diameter (in nm). The measurements were carried out using aqueous solutions of the polymers of 0.5 mg/mL. The influence of the pH on the size was evidenced measuring the polymer samples in pH 7.0 and pH 4.0.

The turbidity change of the aqueous solutions of the polymers (1 mg/mL) as a function of pH was monitored measuring the absorbance at 600 nm in a UV-vis Lambda 35 spectrophotometer (Perkin Elmer Instruments). The initial polymer solution was freshly prepared in an aqueous solution of 0.15 M of NaCl, increasing the pH up to 9.0 with an aqueous solution of



SCHEME 1 Synthesis of VPcprol-Boc_2 via copper(I)-catalyzed azide alkyne cycloaddition (CuAAC).

NaOH 1M to ensure the total deprotonation of the L-proline moieties. A standard aqueous solution 0.1 M of HCl was delivered stepwise. The pH changes were monitored with a SCHOTT Instruments hadylab pH-Meter.

Synthesis of Monomers

The synthetic approach to prepare the protected L-proline vinylpyrrolidone monomer is depicted in Scheme 1. The compounds 3-propargyl-N-vinylpyrrolidone (VPgyl) and phenylmaleimide (PMI) were prepared according to previously reported procedures.[22,23]

(2S,4R)-Di-tert-butyl 4-hydroxypyrrolidine-1,2-dicarboxylate (HO-prol-Boc_2). (2S,4R) *N*-(tert-butoxycarbonyl)-4-hydroxy-L-proline (5.0 g, 21.60 mmol), tetrabutylammonium bromide (3.5 g, 11.00 mmol), K_2CO_3 (59.76 g, 432.40 mmol), and tert-butyl bromide (49.55 mL, 432.4 mmol) were dissolved in *N*,*N*-dimethylacetamide (90 mL). The mixture was stirred at 55 °C during 24 h. Then, distilled water was added to the mixture until a clear solution was obtained. The mixture was extracted with diethylether (3 × 100 mL). The organic phases were collected and dried over anhydrous Na_2SO_4. The solid was removed by simple filtration and the solvent was evaporated under reduced pressure, yielding an oil that was purified silica gel flash chromatography using a mixture of hexane/ethyl acetate (1:1) as eluent. Yield 70%.

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 4.44$ (s, 1H, NC*H*COO), 4.30–4.23 (m, 1H, HOC*H*), 3.60–3.39 (m, 2H, HOCHCH_2N), 2.30–2.20 (m, 1H, HOCHCH*H*CH), 2.06–1.97 (m, 1H, HOCHC*H*HCH), 1.54–1.31 (m, 18H, COO*tBu* ×2) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 172.27$ (CH*C*OOtBu), 154.34 (N*C*OOtBu), 81.26 (CHCOO*C*$(CH_3)_3$), 80.31 (NCOO*C*$(CH_3)_3$), 70.26 and 69.37 (*C*HCOO), 58.63 (HO*C*H), 54.72 (HOCH*C*H_2N), 39.27 and 38.53 (HOCH*C*H_2CH), 28.45 and 28.11 (C(*C*H_3)$_3$ ×2) ppm.

FTIR (cm^{-1}): 3432, 2978, 2935, 2878, 1740, 1702, 1675, 1479, 1457, 1394, 1366, 1256, 1220, 1148, 1128, 1086, 1053, 992, 973, 938, 914, 854, 841, 771.

HRMS (ESI+): calc. *m*/*z*: 310.1625 $(M + Na)^+$, found *m*/*z*: 310.1633.

(2S,4R)-Di-tert-butyl 4-p-toluenesulfonylpyrrolidine-1,2-dicarboxylate (TsO-prol-Boc_2). *p*-Toluenesulfonyl chloride (3.25 g, 16.70 mmol) was added over a solution of HO-pz-Boc_2 (4.0 g, 13.92 mmol) in dry pyridine (17 mL) at 0 °C in an ice bath. The mixture was stirred at 0 °C during 1 h, and then it allowed to warm up to room temperature, keeping the stirring for further 24 h. Afterwards, solvent was removed at low pressure and the residue was purified by silica gel flash chromatography using hexane/ethyl acetate (5:1) as eluent, yielding a yellowish oil. Yield 75%.

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.79$–7.77 (m, 2H, Ar-*H*), 737–7.34 (m, 2H, Ar-*H*), 5.04–4.98 (m, 1H, NC*H*COO), 4.26–4.22 (m, 1H, TsOC*H*), 3.61–3.51 (m, 2H, TsOCHCH_2N), 2.57–2.36 (m, 4H, Ar-CH_3 and TsOCHCH*H*CH), 2.17–2.02 (m, 1H, TsOCHC*H*HCH), 1.44–1.41 (m, 18H, COO*tBu* ×2) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 171.42$ (CH*C*OOtBu), 153.86 and 153.55 (N*C*OOtBu), 145.39 (*C*SO_2), 133.68 and 133.44 (CH3*C*), 130.21 (*C*H—C(CH_3)—CH), 127.90 (*C*H—C(SO_2)—*C*H), 81.76 (CHCOO*C*$(CH_3)_3$), 80.70 (NCOO*C*$(CH_3)_3$), 79.26 and 78.46 (*C*HCOO), 58.09 (TsO*C*H), 52.26 and 51.86 (TsOCH*C*H_2N), 37.42 and 36.10 (TsOCH*C*H_2CH), 28.39 and 28.10 (C(*C*H_3)$_3$ ×2), 21.84 (*C*H_3) ppm.

FTIR (cm^{-1}): 2979, 2934, 2878, 1740, 1702, 1598, 1479, 1457, 1397, 1366, 1257, 1221, 1175, 1151, 1129, 1098, 1052, 1019, 993, 961, 900, 841, 816, 771.

HRMS (ESI+): calc. *m*/*z*: 464.1717 $(M + Na)^+$, found: 464.1725.

(2S,4S)-Di-tert-butyl 4-azidopyrrolidine-1,2-dicarboxylate (N_3-prol-Boc_2). Sodium azide (0.88 g, 13.58 mmol) was added to a solution of TsO-prol-Boc_2 (3 g, 6.79 mmol) in DMF/H_2O (20:14 mL), and left stirring at 60 °C during 48 h. Then, the reaction was quenched adding $CHCl_3$ (20 mL) and saturated NaCl (20 mL). Organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ (2 × 10 mL). The organic phases were collected and dried over anhydrous Na_2SO_4. The solid was removed by simple filtration and the solvent was removed at low pressure and the residue was purified by flash chromatography in silica gel, using hexane/ethyl acetate (7:2) as eluent, yielding an oil. Yield 82%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.32–4.16 (m, 2H, NC*H*COO and N_3C*H*), 3.73–3.63 (m, 1H, N_3CHCH*H*N), 3.50–3.40 (m, 1H, N_3CHC*H*HN), 2.47–2.38 (m, 1H, N_3CHCH*H*CH), 2.16–2.14 (m, 1H, N_3CHC*H*HCH), 1.48–1.43 (m, 18H, COO*tBu* ×2) ppm.

^{13}C RMN ($CDCl_3$, 100 MHz): δ = 170.85 and 170.54 (CH*C*OOtBu), 153.91 and 153.74 (N*C*OOtBu), 81.70 (CHCOO*C*(CH_3)$_3$), 80.41 (NCOO*C*(CH_3)$_3$), 59.34 and 58.40 (*C*HCOO), 58.24 (N_3*C*H), 51.30 and 50.97 (N_3CH*C*H_2N), 36.24 and 35.20 (N_3CH*C*H_2CH), 28.43 and 28.08 (C(*C*H_3)$_3$ ×2) ppm.

FTIR (cm^{-1}):2979, 2934, 2889, 2105, 1746, 1702, 1479, 1457, 1479, 1457, 1395, 1367, 1258, 1220, 1152, 1117, 1055, 1002, 907, 844, 770.

HRMS (ESI+): calc. *m*/*z*: 335.1717 $(M + Na)^+$, found: 335.1725.

(2S,4S)-Di-tert-butyl-4-(4-((2-oxo-1-vinylpyrrolidin-3-yl)methyl)-1H-1,2,3-triazol-1-yl)pyrrolidin-1,2-dicarboxylate (VPcprol-Boc$_2$). On the one hand, VPgyl (2.00 g, 13.4 mmol) and sodium ascorbate (0.53 g, 2.68 mmol) were dissolved in THF (10 mL). On the other hand, $CuSO_4{\cdot}5H_2O$ (1.02 g, 4.02 mmol) were dissolved in 6 mL of distilled water. Both solutions were added at the same time to a third solution of N_3-prol-Boc$_2$ (4.18 g, 13.4 mmol) in 20 mL of THF. The mixture was stirred at 30 °C during 24 h. Then, the reaction was quenched with 5 mL of MeOH. 50 mL of a solution of Na_2CO_3 0.1 M and 50 mL de $CHCl_3$ were added to the mixture. The organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ (2 × 30 mL). All the organic phases were combined and dried over anhydrous Na_2SO_4. The solid residue was removed by simple filtration and the solvent removed at low pressure. The yielding residue was purified by flash chromatography with silica gel, using a gradient solution of hexane/ethyl acetate from 1:1 to 0:1 as eluent. Yield 75%.

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.50 (s, 1H, C-C*H*-N), 7.07 (dd, 1H, N-C*H*═ CH_2, *J* = 16.0 and 9.0 Hz), 5.10–5.02 (m, 1H, N═ N—C*H*—CH_2—N), 4.46–4.10 (m, 4H, N—CH═ CH_2, N—C*H*—COOtBu and N═ N—CH—CH*H*—N), 3.85–3.75 (m, 1H, N═ N—CH—C*H*H—N), 3.41–3.17 (m, 3H, CO—N—CH_2 and CO—CH—CH*H*), 2.98–2.86 (m, 3H, CO—CH—C*H*H, CO—C*H* and CH*H*—CH—COOtBu), 2.59–2.26 (m, 2H, C*H*H—CH—COOtBu and CO—N—CH_2—CH*H*), 3.04–2.89 (m, 1H, CO—N—CH_2—C*H*H), 1.45–1.41 (m, 18H, COO*tBu* ×2) ppm.

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 173.95 (N*C*O), 170.54 (CH*C*OOtBu), 153.66 (N*C*OOtBu), 145.16 (*C*—CH—N), 129.28 (N—*C*H═CH_2), 120.76 (C—*C*H—N), 94.71 (N—CH═ *C*H_2), 81.87 (NCOO*C*(CH_3), 80.77 (CHCOO*C*(CH_3), 58.12 (*C*HCOOC(CH_3), 57.76 and 57.08 (N═ N—*C*H—CH_2—N), 51.36 and 51.09 (N═ N—CH—*C*H_2—N), 42.77 (CO—N—*C*H_2), 42.43 (CO—*C*H), 36.29 and 35.30 (*C*H_2—CH—COOtBu), 28.24 (NCOOC(*C*H_3)$_3$), 27.89 (CHCOOC(*C*H_3)$_3$), 26.55(CO—CH—*C*H_2), 23.35 (N—CH_2—*C*H_2) ppm.

FTIR (cm^{-1}): 3138, 2978, 2933, 2888, 1742, 1698, 1633, 1551, 1479, 1456, 1393, 1327, 1270, 1222, 1153, 1117, 1042, 981, 956, 913, 844, 771.

HRMS (ESI+): calc. *m*/*z*: 462.2711 $(M + H)^+$, found: 462.2699.

Synthesis of Polymers

Alternating Copolymer

Protected copolymer poly(VPcprol-Boc$_2$-alt-PMI) 50:50 was prepared by free radical polymerization in 1,4-dioxane at 60 °C for 24 h using AIBN as initiator. The total concentration of monomer and AIBN was 1 and 1.5 × 10^{-2}, respectively. Reactions were carried out in the absence of oxygen by gently bubbling nitrogen for 20–30 min before sealing the system. After 24 h, the reaction mixture was poured into diethylether, and the resulting precipitate was dried under vacuum overnight. The polymer was obtained as a white solid. To determine the monomer composition, the relative areas of the corresponding monomeric units were used applying the following equations:

$$A_{7.8-6.8} = 1H_{\text{VPcprol-Boc2}} + 5H_{\text{PMI}} \quad (1)$$

$$A_{5.2-3.8} = 3H_{\text{VPcprol-Boc2}} \quad (2)$$

To confirm the tendency to alternation of these monomers, the copolymerization was carried out *in situ* by dynamic ^{1}H NMR ARRAY in DMF-d_7 using VPcprol-Boc$_2$/PMI 4:3 as monomer feed ratio. The experiment was carried out using a Varian 400 NMR equipment. To perform quantitative experiments, the following conditions were used: a pulse sequence of 7 ms equivalent to a 90 tip angle and a 120 s delay time were applied to allow for the total relaxation of the protons and to process the individual data. The spinning rate of the samples was 7 Hz. The sample temperature was maintained at 60 °C using the heat controller of the NMR equipment. To determine the monomer consumption the relative areas of the corresponding monomers were used applying the following equations:

$$H_{\text{VPcprol-Boc2}} = A_{7.25-7.10} \quad (3)$$

$$H_{\text{PMI}} = \frac{A_{7.30-7.25}}{2} \quad (4)$$

$$\text{monomer}\,(\%) = \frac{H_{\text{monomer}}}{\left(H_{\text{PMI}} + H_{\text{VPcprol-Boc2}}\right) t=0} \quad (5)$$

where *A* is the area of the corresponding peak or group of peaks, and monomer is VPcprol-Boc$_2$ or PMI.

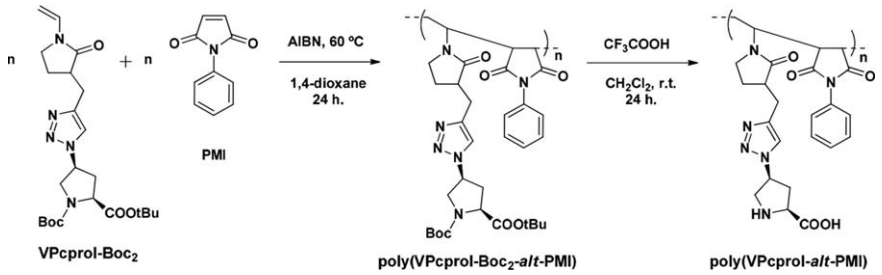
PEG Grafted Terpolymers

Protected PEG grafted terpolymers poly(VPcprol-Boc$_2$-co-PMI-co-PEGMA)-X% (where X is the mol% of PEGMA macromonomer in the feed ratio) were prepared using the same conditions as the alternating copolymer explained above. In this case, X% was 2%, 5%, and 10%. Poly(VPcpro-Boc2-co-PMI-co-PEGMA) polymers were obtained as a white solid. To determine the monomer composition, the following equation was used in addition to eqs 1 and 2:

$$A_{3.8-3.3} = 91H_{\text{PEGMA}} + 2H_{\text{VPcprol-Boc2}} \quad (6)$$

General Deprotection Procedure

The synthesized polymers were dissolved in a mixture of dichloromethane/trifluoroacetic acid (1:1) (2 mL per 100 mg



SCHEME 2 Preparation of the alternating copolymer.

of polymer) and the mixture was then stirred for 24 h. Afterwards, the solvent was removed at low pressure, and the resulting polymers were purified by dialysis in distilled water using membranes of cut-off 1000 Da, followed by freeze drying. All the polymers were obtained as white solids in yields above 95%.

Asymmetric Aldol Reaction

To a solution of polymer (30 mol%, that is amount of L-proline on the polymer relative to *p*-nitrobenzaldehyde reagent) in phosphate buffer (pH 7.0 or 4.0) (it could require sonication for complete solubilization), *p*-nitrobenzaldehyde (x, 1 eq.) and cyclohexanone (y, 5 eq.) were added. The mixture was stirred at room temperature for 24 h. After the specified time elapsed, 0.2 M NaOH (1 mL) was added to the mixture, and was extracted with CH_2Cl_2 (3 $\times$ 1 mL). Then, organic layers were combined, dried over anhydrous Na_2SO_4 and solid was removed by simple filtration. The solvent was removed at low pressure, obtaining a yellowish residue that was redissolved in 1 mL of CH_2Cl_2. From this solution, an aliquot of 100 μL was taken, the solvent was evaporated under reduced pressure and the residue dissolved in a mixture of hexane/isopropanol (4:1) for HPLC analysis.

RESULTS AND DISCUSSION

Synthesis and Catalytic Evaluation of the Alternating Copolymer

VPcprol-Boc_2 was copolymerized with PMI, at an equimolar ratio, by conventional radical reaction (see Scheme 2).

The L-proline derivative was copolymerized in the protected form because of two reasons: (1) free amines may act as transfer agents in the radical process, and (2) the protected monomer exhibited an improved solubility in organic solvents allowing us to find a common solvent for the polymerization reaction. Both units copolymerized properly, being the final copolymer composition 0.50 – calculated from the ^{1}H NMR spectra as indicated in the Experimental section. After deprotection with TFA the copolymer is soluble in water at pH 7.0. Figure 1 shows the spectra of the protected and the free form of the copolymer in $CDCl_3$ and D_2O, respectively. Figure 1 shows the disappearance of the Boc groups at 1.6 ppm. Molecular weights of the copolymers were characterized by GPC in the protected form, using polystyrene standards as reference. Number average molecular weights of polymers were 81 kDa. The dispersity index was 1.7.

To confirm the alternating structure of the copolymers, a copolymerization reaction with a slight excess of the VP derivative was followed using dynamic ^{1}H NMR ARRAY and multiple spectra were collected during the reaction, following procedures previously reported.[24,25] The slight excess of the L-proline derivative was used for the clarity of the graphical presentation, and also to analyze qualitatively the different polymerization rates before and after the consumption of PMI. The consumption of both monomers, VPcprol-Boc_2 and PMI, as a function of their conversion are depicted in Figure 2 using the eqs 3, 4, and 5. An alternating incorporation might occur if both monomers were equally consumed throughout the reaction, regardless of the initial feed molar ratio. For example, a growing chain ending in VPcprol-Boc_2 will preferentially react with activated PMI, providing a new growing radical ended in PMI. This would then only react with VPcprol-Boc_2 because of steric reasons, until one of the monomers was completely consumed. As is shown in Figure 2, the slopes for consumption of both monomers are very similar, indicating that the two monomers are consumed at almost the same rate. The slight deviation between both slopes indicates that the alternation is high but not complete. After the consumption of PMI monomer, the polymerization rate decrees drastically, obtaining only a 5% VPcprol-Boc_2 conversion in a few hours.

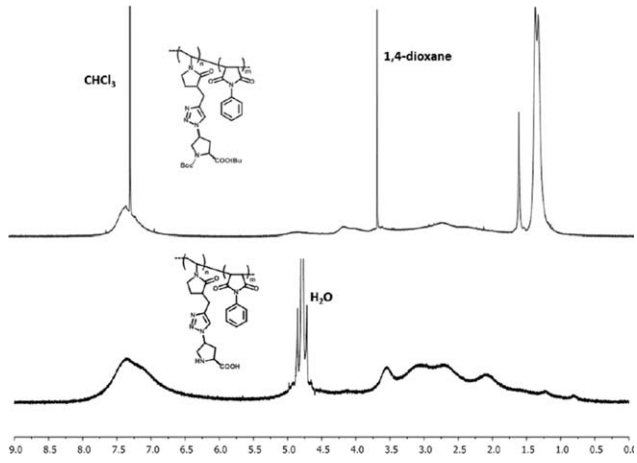


FIGURE 1 ^{1}H NMR spectra of: above, the protected alternating poly(VPcprol-Boc_2-alt-PMI) copolymer and below, the deprotected alternating copolymer, poly(VPcprol-alt-PMI).

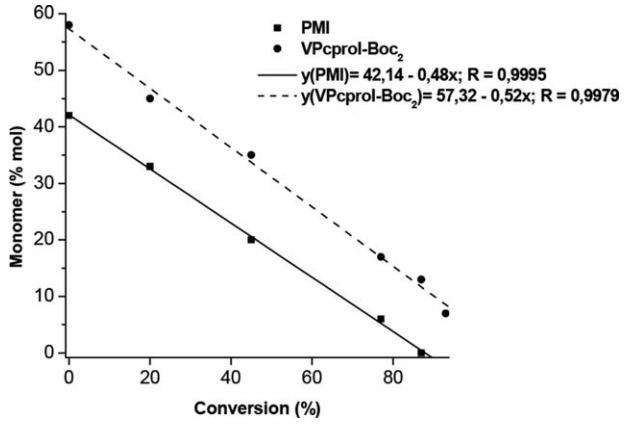


FIGURE 2 Quantity of VPcprol-Boc_2 and PMI in the polymerization reaction versus conversion monitored by dynamic ^{1}H NMR ARRAY.

The solubility of the alternating copolymer was investigated both by Dynamic Light Scattering (DLS) and turbidimetry measurements. According to these results, the copolymer

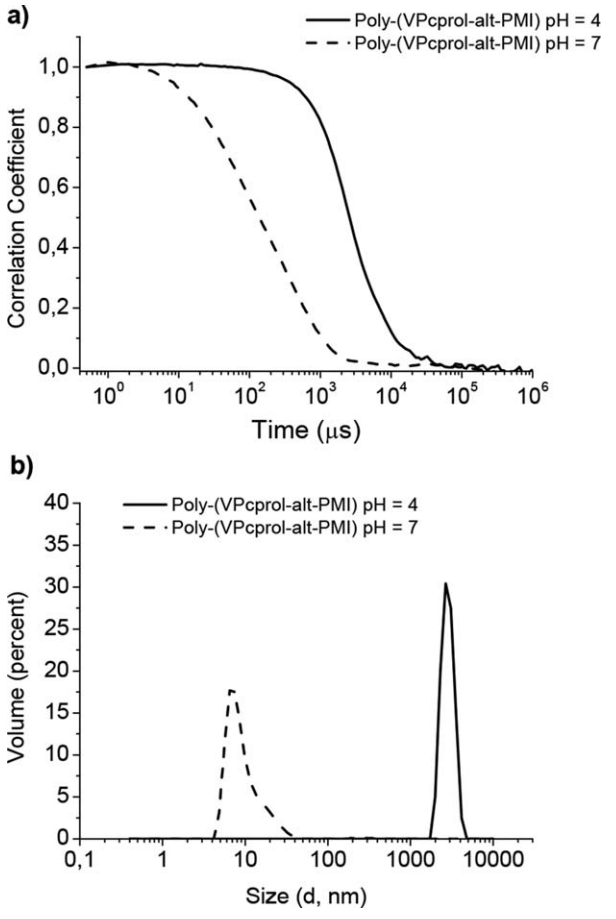
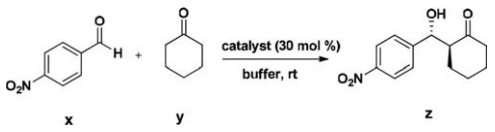


FIGURE 3 Correlation curves (a) and size distribution curves (b) for the poly(VPcprol-alt-PMI) copolymer solutions at two different pH values, that is 4.0 and 7.0. Approaching the isoelectric point (IEP) the solubility of the copolymer decreased while remains soluble at neutral to basic pH values.

TABLE 1 Aldol Reaction Between *x* and *y* Using Copolymer Poly(VPcprol-alt-PMI) as Catalyst (30 mol%) at pH 7.0 and 4.0, and Characteristics of the Entities in Solution (Size and ζ Potential)



		Poly(VPcprol-*alt*-PMI)		L-Proline	
		pH 7	pH 4	pH 7	pH 4
Conversion (%)[a,b]	4 h	n.d.	24	n.d.	n.d.
	24 h	99	91	9	0
Anti/syn[b]	4 h	n.d.	5.5	n.d.	n.d.
	24 h	1.5	4	2.2	-
ee (%)[b]	4 h	n.d.	83	n.d.	n.d.
	24 h	2	81	14	n.d.
Size (nm)[c]		5.2	microns	–	–
ζ potential (mV)[c]		−38	−1	–	–

[a] Determined by ^{1}H NMR.
[b] Determined by HPLC.
[c] Features of polymer entities.
n.d., No determined.

was soluble in aqueous solutions at pH values above 5.0. Below this pH, as evidenced in the turbidimetry curve depicted in Figure 5, the copolymer becomes insoluble because coulombic interactions between opposite charges are maximal at this interval, that is, the range around the isoelectric point (IEP), whose value is in agreement with the previously reported for linear polymers containing pendant L-proline (IEP = 3.8).[9,26] It has to be noted that IEP of free L-proline is 6.3. This remarkable shift of the IEP to lower values in the polymeric form may be related to the differences in accessibility for ionisable groups, the weak amino base and the weak carboxylic acid. Above IEP the net charge is negative since the stoichiometry is lost allowing linear macromolecular chains to expand, solvate and eventually dissolve. In agreement with the turbidimetry measurements, DLS also confirmed the aggregation and precipitation at pH values near to the IEP (see Figure 3 and Table 1). On the one hand, the relaxation time significantly decreased by increasing the pH indicating the formation of smaller entities that upon CONTIN analysis evidenced the formation of smaller objects with sizes in the range of 8–30 nm.

The copolymer was tested as a catalyst in the aldol reaction between *p*-nitrobenzaldehyde (x, 1 eq.) and cyclohexanone (y, 5 eq.) in phosphate buffer pH 7.0 and 4.0. as a model reaction (scheme of Table 1). Table 1 shows the results obtained at these pHs.

These two pHs were chosen to test the catalyst in the soluble and in the non-soluble forms, namely, at pH 7.0 and at

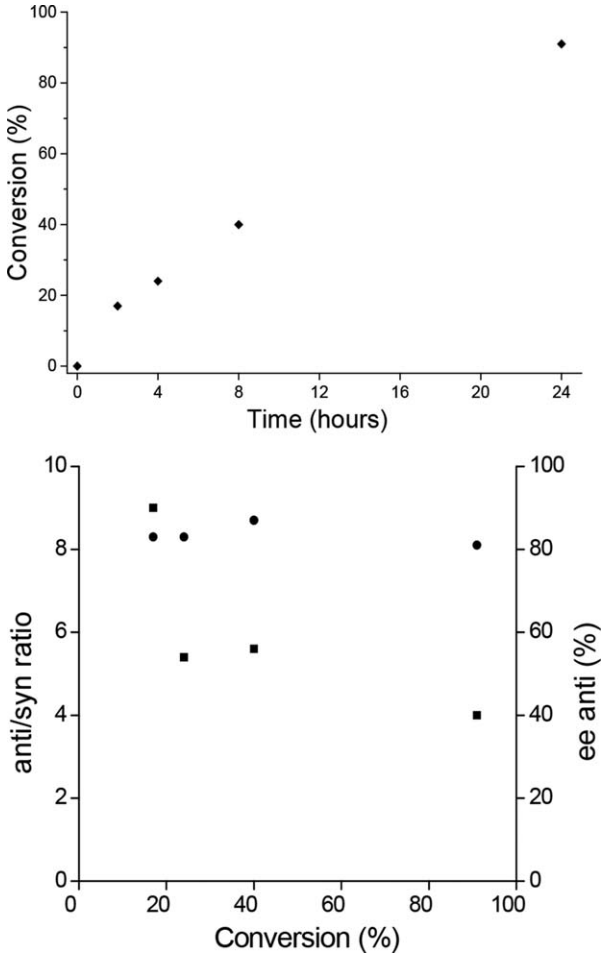


FIGURE 4 Conversion versus time of the aldol reaction of *p*-nitrobenzaldehyde with cyclohexanone at pH 4.0 catalyzed by poly(VPcprol-alt-PMI) (above), and the evolution of the anti/syn ratio (■) and ee (●) with conversion in the same reaction (below).

the pH close to the IEP, respectively. As it has been described before, at pH 7.0 the L-proline moieties are negatively charged and the macromolecules are extended and soluble, being the active catalytic centers solvated by water molecules. At this pH the entities have average sizes of 5.2 nm, which is compatible with unimolecular negatively charged species. At the IEP, on the contrary, the charge cancellation makes the polymer to precipitate and water is expected to be excluded from the aggregates in view of the hydrophobic nature of PMI. Large and non-charged aggregates are found in solution.

Table 1 shows that the copolymer is active at pH 7.0, unlike free L-proline. This result confirms the initial hypothesis related to the hydrophobic interaction of PMI in an alternate sequence distribution. Reactions are non stereoselective, which is in agreement with previous result and it is related to the charge solvation that cause water to be located in the active center, probably influencing the transition states and preventing stereoselectivity.[9] The chains may be seen as "hydrophobic" poly-anions with columbic repulsions between carboxylate units and solvation of the ions with water molecules.

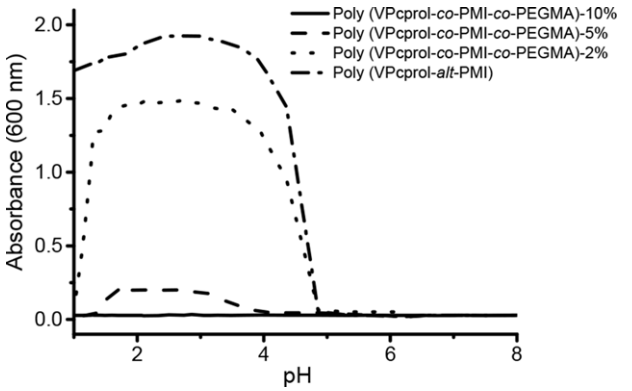


FIGURE 5 Absorbance at 600 nm versus the pH for the copolymer.

At pH 4.0, in despite of the large size of the aggregates, the reaction is quite efficient, exhibiting after 4 and 24 h a 24 and 91% of conversion, respectively. Equally, the reaction rate decreases compared with pH 7.0 due both to the limited diffusion of the reactants and the accessibility to the catalytic

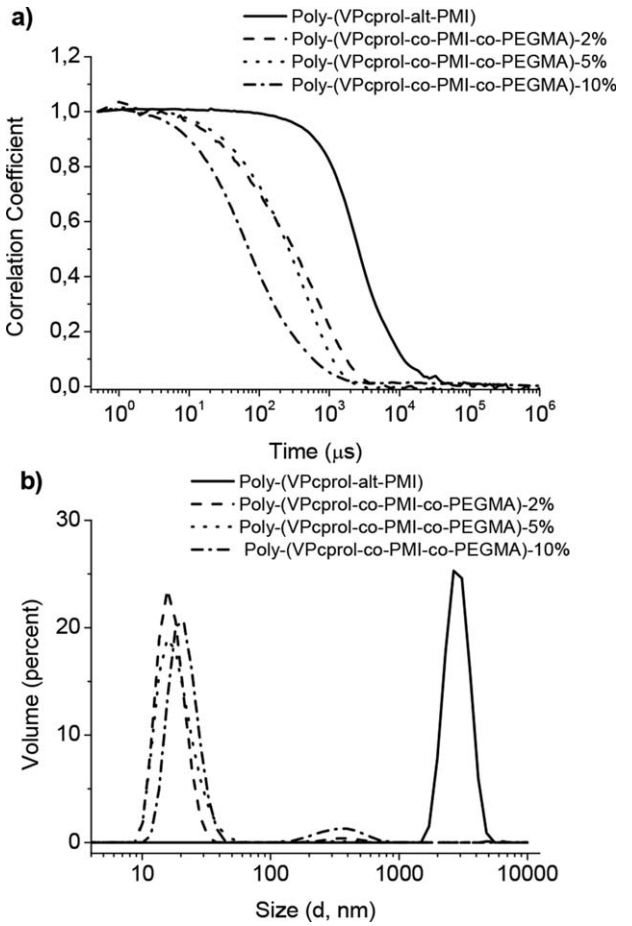


FIGURE 6 Correlation curves (a) and size distribution curves (b) for the copolymer solutions with variable amount of PEGMA. The measurements were carried out at pH 4.0 in a 50mM PBS solution.

TABLE 2 Characteristics of the PEG Grafted Copolymers

	Composition		Molecular Weight		(pH 4.0)	
Polymer	$F/f_{(VPcprol\text{-}Boc2)}$[a]	$F/f_{(PEGMA)}$[a]	$\bar{M}_n$ (kDa)[b]	$Đ$[b]	Size (nm)	ζ Potential (mV)
Poly(VPcprol-co-PMI-co-PEGMA)−2%	0.49/0.47	0.02/0.02	181.8	1.6	15.8	−6
Poly(VPcprol-co-PMI-co-PEGMA)−5%	0.47/0.47	0.05/0.06	170.3	1.7	13.5	−16
Poly(VPcprol-co-PMI-co-PEGMA)−10%	0.45/0.46	0.10/0.09	205.0	1.8	21.2	−17

[a] Determined by ^{1}H NMR using eqs 1, 2, and 6. [b] Determined by GPC.

centers. It is remarkable that at this pH, where the catalytic polymer is not soluble, the reaction is stereoselective: anti/syn diastereoselectivities were 5.5 and 4.0, with enantiomeric excesses of 83% and 81% after 4 and 24 h, respectively. This is in agreement with the proposed exclusion of water from the aggregates and with previous results of literature on linear polymers,[9] as well as on solid supports.[6–8] Figure 4 shows a more detailed study of this reaction at pH 4.0. The reproducibility of the enantioselectivity has been studied carrying out four replicates at 24 h (see data in Table S1, Supporting Information). The results were anti/syn of 4.3 ± 0.4 and ee of 74 ± 6.

Synthesis and Catalytic Evaluation of the Grafted Copolymers

As shown in Table 1 and Figure 3, the size of the aggregates of the alternating copolymer poly(VPcprol-alt-PMI) at pH 4.0 are larger than 1 micron. As it was mentioned in the Introduction, this rather large size may hinder the diffusion of reactants. To obtain smaller aggregates the macromer PEGMA was included in the reaction at molar percentages of 2%, 5%, and 10%. Spectra of the protected and deprotected copolymers can be found at Supporting Information (Fig. S1). The copolymer compositions were calculated from the ^{1}H NMR spectra as indicated in the Experimental section, and are quoted in Table 2. The molar fractions were found to be very close to the feed monomer compositions, which indicate a proper incorporation of the three monomers to the macromolecular chains. Molecular weights of the copolymers were also characterized by GPC in the protected form, using polystyrene standards as reference. Number average molecular weights (between 170 and 205 kDa) of polymers and dispersity indexes (1.6–1.8) can be found in Table 2 as well.

TABLE 3 Aldol Reaction Between *x* and *y* Using Copolymers Poly(VPcprol-co-PMI-co-PEGMA)-X% as Catalyst (30 mol%) at pH 4.0 and 4 h of Reaction

Catalyst	Conv. (%)[a,b]	Anti/syn[b]	ee (%)[b]
Poly-(VPcprol-*alt*-PMI)	24	5.5	83
Poly-(VPcprol-*co*-PMI-*co*-PEGMA)−2%	93	1.8	5
Poly-(VPcprol-*co*-PMI-*co*-PEGMA)−5%	96	2	1
Poly-(VPcprol-*co*-PMI-*co*-PEGMA)−10%	97	1.4	3

[a] Determined by ^{1}H NMR. [b] Determined by HPLC.

The resulting copolymers incorporate grafted PEG chains that may be able to stabilize smaller aggregates at pH 4.0. DLS and turbidimetry analysis shown in Figures 5, 6, and Table 2 confirmed this hypothesis. Turbidimetry curves indicated that the incorporation of an increasing amount of PEGMA within the polymer structure resulted in a lower absorbance of the solution at pH 4.0. As a result, the copolymer with 10 mol% of PEGMA is transparent independently of the solution pH. Similarly, DLS shows that the relaxation times decreased by incorporation of PEGMA.

As a result, the size of the objects formed in solution at pH 4.0 is below than 21 nm incorporating only 2% of PEGMA. It is interesting to note, that the average diameters values observed comprised between 13 and 21 nm are in the range of micelle-type entities.

Thus, most probably the long PEG chains of the PEGMA form the shell of micelles in which proline and VP form the core of the aggregates.

The catalytic studies with these grafted copolymers at pH 4.0 showed a clear higher reaction rate and a decrease on stereoselectivity, when compared with the use of Poly(VPcprol-alt-PMI) (see Table 3). Both effects are related to the smaller size of the aggregates that, on one side increases the reaction rate but on the other permit the presence of water molecules near the catalytic center.

CONCLUSIONS

These studies have confirmed that the catalytic action of supported proline is efficient in water when there is "hydrophobic activation" through the formation of linear and water soluble (above pH 5.0) alternating copolymers of hydrophobic and proline-containing units, in agreement with previous studies in literature. As the alternation optimizes the hydrophobic nature of the proline surroundings, it is proposed that the "hydrophobic activation" is maximized. At pH 4.0, very close to the IEP and to a scenario of charge cancellation, the copolymer precipitates forming micrometric aggregates and it is able to

form enantioselective products, which has been related to the exclusion of water as it has also been indicated previously in literature. The grafting of PEG chains to the linear copolymers has reduced the size of the aggregates to 15–22 nm, which are compatible with micelle-type entities. Grafting increased the reaction rate, which must be related to the reduction of the aggregate size and the easier reactants diffusion. However, enantioselectivity was lost, in agreement with the presence of water molecules in the active center.

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