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Synthesis and polymerization of novel methacrylate having glycerophosphorylcholine

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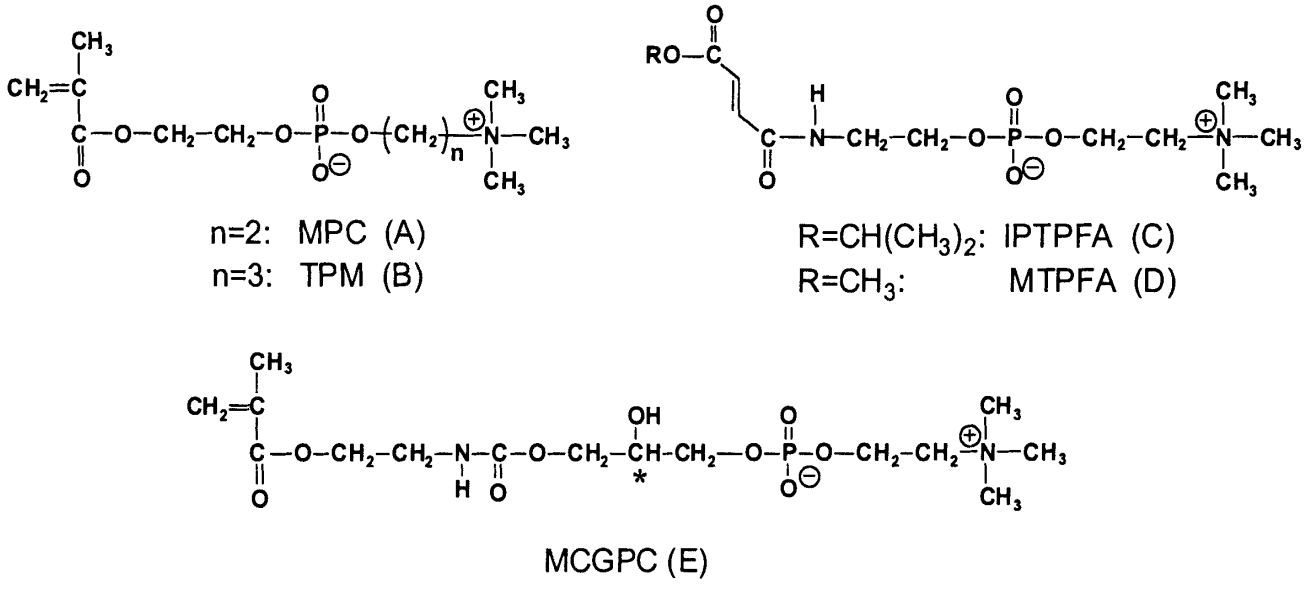
Summary

A novel vinyl monomer, 1-(2-methacryloyloxyethyl) carbamoyl-*sn*-glycero-3-phosphorylcholine (MCGPC), was synthesized from *sn*-3-glycero-phosphorylcholine (GPC) and 2-methacryloyloxyethyl isocyanate (MOI) in the presence of di-*n*-butyltin dilaurate (BTL) as a catalyst, and its critical micelle concentration (CMC) was determined to be 2.7×10^{-3} mol/l. MCGPC was copolymerized with *n*-butyl methacrylate (BMA) in the presence of a radical initiator in ethanol for 24 hr and 48 hr at 60 C. Bovine serum albumin (BSA) was adsorbed onto membranes prepared from copolymers of MCGPC with BMA to estimate the blood compatibility of those copolymers. The BSA adsorption was reduced with an increase of the composition of MCGPC in copolymers.

Introduction

A vinyl monomer bearing a phosphorylcholine group, i.e., 2-methacryloyloxyethyl phosphorylcholine (MPC) [Scheme 1, A] was synthesized and investigated to apply to useful medical materials, excellent blood compatibility and depression of protein adsorption and platelet adhesion [1-7]. We reported synthesis and brief study on biocompatibility of copolymers obtained from relative compounds such as 2-[3'-(trimethyl-ammonium)propylphosphoryl]ethyl methadrylate (TPM) [Scheme 1, B], which was added a methylene to an ethylene unit in the phosphorylcholine analogous group [8], alkyl-2-[2'-(trimethylammonium)ethyl phosphoryl]ethyl fumaramate [RTPFA: alkyl = isopropyl (IPTPFA) [Scheme 1, C] and methyl (MTPFA) [Scheme 1, D]] [9]. These results suggest that the introduction of phosphorylcholine groups into a polymer surface is useful for improvement of protein adsorption-resistance properties.

The present paper describes a novel vinyl monomer 1-(2-methacryloyloxyethyl) carbamoyl-*sn*-glycero-3-phosphorylcholine (MCGPC) [Scheme 1, E] was synthesized as a derivative having a phospholipid polar group, and its critical micelle concentration (CMC) was determined. MCGPC was radically copolymerized with *n*-butyl methacrylate (BMA) to obtain the corresponding copolymers. The copolymer membranes were characterized by water fraction (*H*) and adsorption test for bovine serum albumin (BSA).



Scheme 1. Structure of phospholipid derivatives

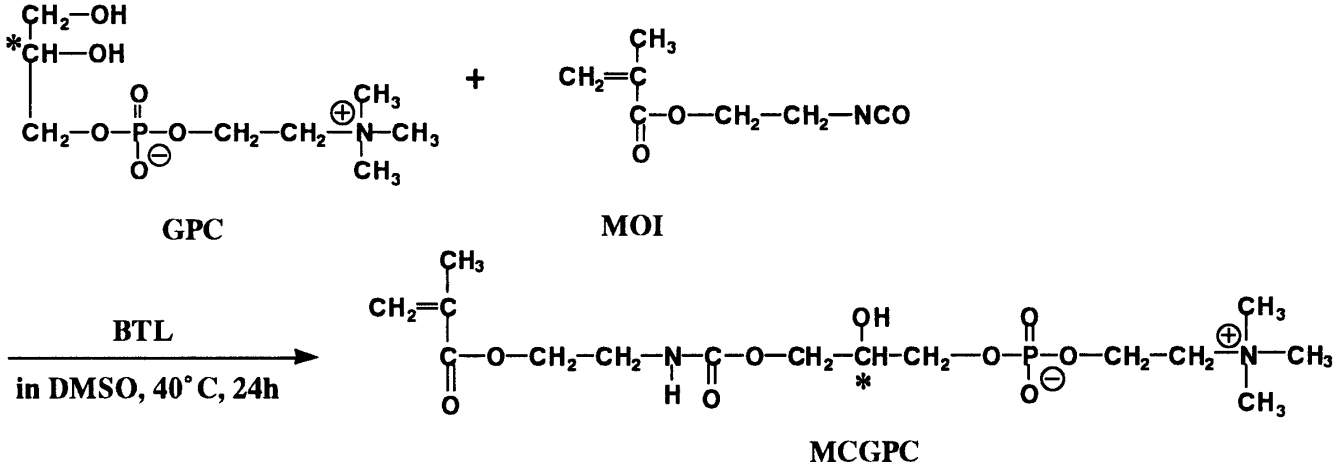
Experimental

Materials

sn-3-Glycero-phosphorylcholine (GPC) was supplied by Nippon Oil & Fats Co. Ltd., and lyophilized for a day before use. 2-Methacryloyloxyethyl isocyanate (MOI) was supplied by SHOWA DENKO K.K. and used without further purification. BMA and dimethyl sulfoxide (DMSO) were purified by distillation under reduced pressure. Methanol (MeOH) was purified according to the conventional method. 2,2'-Azobisisobutyronitrile (AIBN) was purified by recrystallization from MeOH. BSA was purchased from Sigma Chemical Co. Ltd., used without further purification. Other special grade reagents and solvents were used without further purification. Distilled water was used throughout the experimental.

Synthesis of MCGPC

MCGPC was synthesized by the reaction of hydroxyl groups in GPC and an isocyanate group in MOI in the presence of di-*n*-butyltin dilaurate (BTL) as a catalyst, as shown in Scheme 2. A typical reaction was as follows. GPC (2.1g, 8.0×10^{-2} mol) and BTL (10mg) were stirred in DMSO (65ml) at 50 °C for 2 h to prepare suspension after degassed with dry nitrogen gas. MOI (3.6g, 2.3×10^{-2} mol) was added into the suspension and kept at 40 °C for 24 h with stirring. The reaction mixture was cooled until r.t. and was separated by column chromatography ($CHCl_3$ / MeOH / H_2O = 65 / 25 / 4 (vol / vol / vol)) on silica gel to purify. The isolated product was distilled under reduced pressure to obtain pure MCGPC: white viscous solid, yield 42.5% (1.40g). ^{1}H NMR (δ in ppm from TMS in D_2O): 6.06 (s, 1H, CH_2=, cis), 5.66 (s, 1H, CH_2=, trans), 4.18 (m, 2H, $COOCH_2$-), 4.00 (m, 2H, $POCH_2$-), 3.82-3.92 (m, 3H, -CHOH, -CH_2N), 3.59-3.68 (m, 4H, $NHCOOCH_2$-, $CHCH_2O$-), 3.40 (m, 2H, CH_2NH-), 3.16 (s, 9H, $-N^+(CH_3)_3$), 1.85 (s, 3H, $CH_3C=CH_2$); ^{13}C NMR (δ in ppm from TMS in D_2O): 170.58 ($C=CH_2C=OO$-), 159.27 (NHC=OO-), 136.76 ($CH_3C=CH_2$-), 127.95 ($CH_3C=CH_2$-), 69.39 (-CHOH), 67.27 ($C=OOCH_2CH_2$-), 67.20 (CH_2OP), 66.36 ($C=OOCH_2CH_2$-), 64.97 ($POCH_2CH_2$-), 60.37 ($POCH_2CH_2$-), 54.93 ($-N^+(CH_3)_3$), 40.39 (CH_2CHOH), 18.34 ($CH_3C=CH_2$); IR analysis (KBr method, cm^{-1}): 3400 (N-H), 1730 (C=O), 1230 and 1100 (PO-O-).



Scheme 2. Synthesis of MCGPC

Radical solution copolymerization procedure

Radical solution copolymerization of MCGPC (M_1) with BMA (M_2) was performed with AIBN as an initiator in MeOH at 60 °C for 2 h in a polymerization tube. The polymerized solution was poured into excess *n*-hexane / ethanol (EtOH) = 9 / 1 (vol / vol) to precipitate polymers. The obtained copolymer was dried *in vacuo* at r.t. for 2 days. The composition of the copolymer was determined by ^{1}H NMR measurement.

Determination of critical micelle concentration (CMC)

Solutions of several concentrations (1.0×10^{-6} ~ 1.0×10^{-2} mol/l) of MCGPC were prepared in distilled water saturated with pyrene. Fluorescence spectra from 352 to 500 nm were measured with a JASCO FP-777 spectrofluorometer using a quartz cell ($1 \times 1 \times 4.0$ cm^3) and excitation wavelength at 342 nm, scanning rate at 50 nm/min. The CMC was determined from variation of the ratios (I_3/I_1) of the first signal (I_1) at 372-373 nm and the third signal (I_3) at 383-385 nm, according to the method by Thomas [10].

Measurement of water content (H)

A polymer membrane was prepared from a casting method, as follows. The obtained polymer (0.5g) was dissolved in 5 ml of EtOH. The polymer solution was spread on Teflon plate to allow the solvent to evaporate at r.t. The obtained membrane was dried *in vacuo* at 40 °C over night to eliminate residual solvent, and used for measurement of equilibrium water content *(H)*. The polymer membrane was immersed in water to equilibrate at 30 °C. The saturated polymer membrane, removed excess water by wiping with a dry filter paper, was weighted every one hour until the weight kept constant. Water content *(H)* was calculated from following equation:

H = ((weight of hydrated membrane) – (weight of dry membrane)) / (weight of hydrated membrane)

Adsorption procedure of BSA

The sample polymer was coated on a disposable cell, made of poly(methyl methacrylate, (MMA)) for spectrometry (semi-micro type, 10mm × 20mm × 4mm), by a solvent evaporation method as follows. 2.0 ml of EtOH solution contained 0.5 wt% of the test polymer was poured into a disposable cell and kept for 30 min. The polymer solution was removed from the disposable cell, and the cell was dried at r.t. for 6 h and then dried *in vacuo* at 40 °C over night. The amount of BAS adsorbed on polymer membrane was determined by the micro BCA method [11]. The amount of adsorbed-BSA was calculated from the content of BSA solution eluted from the surface of the disposable cell in sodium dodecyl sulfate (SDS) aq. solution. In order to calculate the amount of BSA in the SDS solution, the protein assay kit (micro BCA protein assay regent kit, #23235, Pierce, Rockford, IL, USA) was used. The amount of adsorption of BSA onto polymer surface was calculated by measuring the absorbance at 562 nm based on BAS by u.v.-v.i.s. measurement with a Shimadzu UV-2200 spectrophotometer. The calibration curve was made from the values and the BSA concentration.

Other measurements

Chemical structures of the synthesized monomer and the polymers were determined by measurements of ^{1}H and ^{13}C NMR spectra with a JEOL NMR EX-270 instrument using $CDCl_3$ and D_2O as solvents. FT IR spectra were obtained using Shimadzu FT IR-8100A. The reduced viscosity (η_{SP}/C) was measured using an Ostwald viscometer (No.3) in ethanol at 30 °C.

Results and discussion

Synthesis of MCGPC

Optically active MCGPC was synthesized according to Scheme 2, and identified from ^{1}H, ^{13}C NMR and FT IR spectra, as described in experimental section. Purified MCGPC of white viscous solid showed remarkable deliquescence as well as raw GPC.

The GPC has both the primary and the secondary hydroxyl groups in a molecule, and the reactivity of the primary hydroxyl group may be higher than that of the secondary one. In order to selectively obtain MCGPC by the reaction of the primary hydroxyl group of GPC with isocyanate group of MOI, the reaction of GPC with MOI carried out in various molar ratios (GPC / MOI = 1 / 1, 1 / 1.5, 1 / 2 (mol/mol)). However the yield was very low under these reaction conditions. In this case, hydrolysis reaction of MOI preferentially took place with a little water in the reaction system. Excessive amount of MOI was necessary in monomer feed to obtain MCGPC by the reaction of GPC with MOI. Therefore the molar ratio of 1 / 3 (mol/mol) for GPC / MOI was performed.

Table 1. Radical Copolymerizations of MCGPC (M_1) and MPC (M_1) with BMA (M_2) at 60 °C[a]

Run	M_1 in feed (mol%)	Solvent (ml)	Polym.time (h)	M_1 in copolymer[b] (mol%)	Yield (%)	h_{sp}/C[c]	*(H)*[d]
1	MCGPC (30.0)	MeOH (2.0)	24	37.8	68.7	0.18	0.43
2	MCGPC (20.0)	MeOH (2.0)	48	16.0	81.2	0.18	0.28
3	MCGPC (10.0)	MeOH (2.0)	24	11.7	22.1	0.14	0.22
4	MPC (31.1)	EtOH (2.0)	12	29.6	55.0	0.35	0.67

[a] AIBN : 2,2'-azobisisobutyronitrile, [AIBN] / [total monomer] = 0.01, M_1+M_2 = 0.5g

[b] Measured by ^{1}H NMR

[c] Measured in EtOH

[d] Equilibrium water fraction

H = ((weight of hydrated membrane) – (weight of dry membrane)) / weight of hydrated membrane

Radical copolymerization

MCGPC (M_1) was radically copolymerized with BMA (M_2) in MeOH (2ml) at 60 °C for 24 and 48 h. M_1 of the concentrations of 10, 20 and 30 (mol%) was used in monomer feed. Table 1 summarizes the result of these radical copolymerizations together with result of used 30 mol% of MPC as M_1. All copolymerizations proceeded homogeneously throughout. ^{1}H NMR spectrum of poly(MCGPC-*co*-BMA) (run 1 in table 1) is shown in Figure 1. All peaks of the copolymer were assigned to MCGPC and BMA units. The compositions of M_1 in the copolymers were calculated from integrated values of ^{1}H NMR peaks corresponding to a $-N^+(CH_3)_3$ group (9H, 1 in Figure 1) for a MCGPC unit and a $-CH_3$ group (3H, b in Figure 1) in the main chain.

Polymer yield and the content of M_1 increased with M_1 content in feed. Reduced viscosity for poly(MPC-*co*-BMA) was higher than that for poly(MCGPC-*co*-BMA), indicating that the molecular weight of poly(MCGPC-*co*-BMA) was lower because of steric hindrance due to long side-chains in MCGPC, compared with MPC.

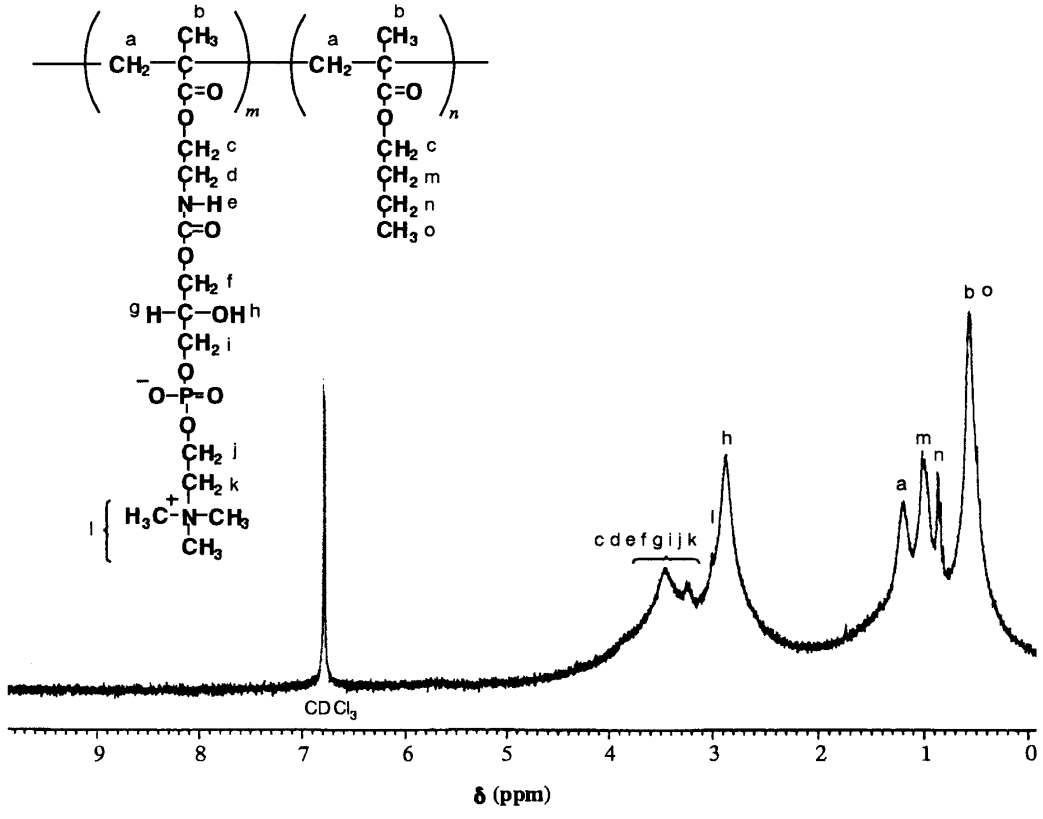


Figure 1. ^{1}H NMR spectrum for poly(MCGPC-*co*-BMA) in $CDCl_3$

Molecular Aggregation of MCGPC in Water

Molecular aggregation of MCGPC was investigated according to the fluorescence probe method by Thomas *et al.* [10,12]. Pyrene using as a fluorescence probe in the measurement of CMC depicts high hydrophobic nature, and its solubility in water is very low. Fluorescence spectrum of pyrene was measured in distilled water at excitation wavelength of 342 nm. Fluorescence spectra of pyrene in various concentrations of MCGPC aqueous solution were measured, and fluorescence intensity (I_3/I_1) were calculated. In the relationship between fluorescence intensity ratio (I_3/I_1) and MCGPC concentration in distilled water signal intensity (I_3/I_1) remarkably changed at 2.7×10^{-3} mol/l in water, indicating that the CMC of MCGPC was 2.7×10^{-3} mol/l. That is, MCGPC gathered in aqueous solution over CMC, and then formed micelles. The CMC of MPC was measured in our laboratory as 1.3×10^{-3} mol/l. Balance between hydrophilic and hydrophobic groups in a molecule became poor because a glicero skeleton in MCGPC is longer, compared with MPC. MCGPC easily formed micelles with comonomers having hydrophobic nature such as BMA in water over the CMC, as well as MPC, to obtain corresponding copolymers. The micelle formation ability of MCGPC was lower than that of MPC in this copolymerization system. Thus, the reduced viscosity for poly(MCGPC-*co*-BMA) became low as shown in table 1.

When the concentration of BMA with hydrophobic nature was high in the feed, MCGPC cannot form micelle in polar solvent. Thus, the obtained polymers became soluble in the mixture of *n*-hexane / MeOH = 9 / 1 (vol

/ vol) because the yield and molecular weights of soluble polymers were low. In additions, when the concentration of MCGPC was 10 mol% in monomer feed, the yield was low (run 3 in table 1).

The water content of the copolymer

The result of water content measurements for copolymer membranes prepared by the solvent evaporated method is shown in table 1. The value of the water content (*H*) of poly(MCGPC-*co*-BMA) increased with increasing of the MCGPC content in copolymer, however, it was lower than that of poly(MPC-*co*-BMA). The reason for lower water content may result from relatively rigid urethane groups in the side-chain.

Assay of BSA absorbed amount using micro BCA method

BSA adsorption test for poly (MCGPC-*co*-BMA)s was carried out using micro BCA method [11]. The results are shown in Figure 2, compared with poly(MPC-*co*-BMA), poly(BMA) and poly(MMA). The BSA adsorption was reduced with an increase of the composition of MCGPC in copolymers, and poly(MCGPC-*co*-BMA)s more strongly depressed adsorption of BSA than poly(BMA) and poly(MMA).

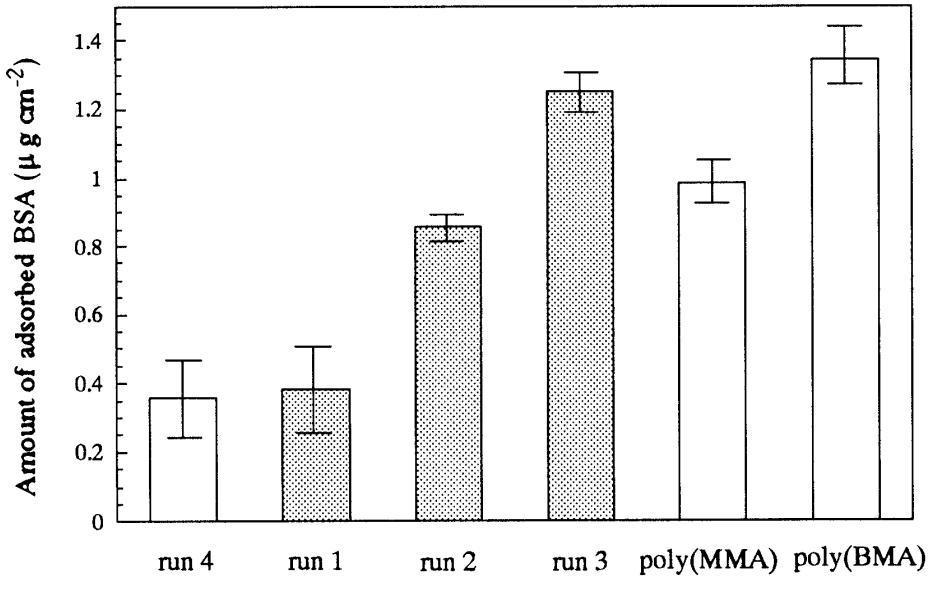


Figure 2. Amount of BSA adsorbed onto poly(MMA) cell coated with various polymer

Even though the water contents (*H*) of poly(MCGPC-*co*-BMA) were low, the BSA adsorption onto the polymer membrane was effectively depressed. This may result from the fact that the structure having a long side-chain greatly influenced the water content (*H*) in poly(MCGPC-*co*-BMA). The copolymer, introduced MCGPC having both a phosphorylcholine unit and a glycero skeleton, reduced BSA adsorption, as well as poly(MPC-*co*-BMA) with excellent anti-protein adsorption.

Conclusions

1. A novel vinyl monomer bearing glycerophosphorylcholine, MCGPC, was synthesized, and copolymerized with BMA in the presence of radical initiator in MeOH.
2. CMC of MCGPC was determined to be 2.7×10^{-3} mol/l by the fluorescent probe method.
3. The BSA adsorption of poly(MCGPC-*co*-BMA) was reduced with an increase of the composition of MCGPC in copolymers as well as poly(MPC-*co*-BMA).

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