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Poly(ß-malic acid) derivatives with non-charged hydrophilic lateral groups: synthesis and characterization

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Summary

Poly((3-hydroxypropyl ß malate) has been synthesized starting from aspartic acid and benzyloxy-1-propanol as precursors. This new polymer was characterized by ^{1}H and ^{13}C NMR, size exclusion chromatography and differential scanning calorimetry. Homopolymer is soluble in water. Two kinds of amphiphilic copolymers were also prepared and characterized. Such polymers have been synthesized with a view to preparing non-charged degradable associating networks or drug carriers.

Introduction

Necessary compatibility between polymeric materials and living organisms, and strict control of degradation and bioassimilation by living systems has led to the fast development of a new class of materials : biocompatible and biodegradable or hydrolyzable polymers. Biodegradable materials can be obtained from natural polysaccharides, polypeptides and bacterial polyesters, or from synthetic poly(anhydrides), poly (aminoacids), poly(orthoesters) or poly(esters) (1, 2). Among these synthetic biodegadable polymers, poly(α-hydroxyacids) such as poly(lactic acid), poly(glycolic acid) and poly(lactic acid-co-glycolic acid) have already found several applications in the biomedical field for surgery or therapy (3-6). However, their highly crystalline and hydrophobic nature has interfered with modulation of their degradation and of their properties. It has also been difficult to import functionality to these polymers. Bacterial polyesters and malic acid polymers belong to poly(ß-hydroxyacid) type polyesters which constitue an attractive family of materials for temporary therapeutic applications. These polymers bear lateral groups which can be neutral or functionalized, ionic or non-charged. Poly(ß-malic acid) repeating unit contains a pendant carboxylic group which can be turned under ionic form (carboxylate) or neutral ester group by an appropriate chemical modification (7-10). The effects of charges on biomedical polymers is very

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important in regard to their interactions with biological macromolecules, metabolites and cell membranes or biological fluids. These charges can be favorable or unfavorable according to the application and in many cases, neutral hydrophilic polymers are required. Poly(ethylene oxide) is a well-known water soluble non-charged polymer which is used in the biomedical field as homopolymer, block or graft copolymer (11-14) We were therefore interested in obtaining a water-soluble non-charged poly(ß-malic acid) derivative having hydroxy pendant groups instead of carboxylic acid lateral functions.

In this paper, we wish to report on the synthesis and characterization of a new ß-substituted ß-lactone, 4-(3-benzyloxypropyl)oxycarbonyl-2-oxetanone, which leads to the corresponding poly(3-hydroxypropyl malate) after specific deprotection of the benzyl groups. Moreover, we describe the synthesis and characterization of two copolymers, constituted by hydrophobic units (ethyladamantyl malate or butyl malate units) and hydrophilic units (3-hydroxypropyl malate units) for specific therapeutic applications.

Experimental

(RS)-bromosuccinic acid **1** was synthesized from aspartic acid as previously described (15). In all cases, SEC measurements have been done in THF with polystyrene standards. NMR spectra were carried at 200 MHz.

*Mixture of RS-3-(3-benzyloxypropyl)oxycarbonyl-3-bromopropanoic acid, **2** and RS-2-bromo-3-(3-benzyloxypropyl)oxycarbonylpropanoic acid, **3*** : was prepared from (RS)-bromosuccinic acid **1** as described elsewhere (15) using 3-benzyloxy-1-propanol to open the cyclic anhydride (Yield = 97 %). ^{1}H NMR ($CDCl_3$, δppm) : 1.86 (tt, 2H, CH_2-**CH_2**-CH_2, monoester **3**, 30 %); 1.9 (tt, 2H, CH_2-**CH_2**-CH_2, monoester **2**, 70 %); 2.87 (dd, 1H, **CH_2**-CO_2H, monoester **3**, 30 %); 2.92 (dd, 1H, **CH_2**-CO_2H, monoester **2**, 70 %); 3.15 (dd, 1H, **CH_2**-CO_2H, monoester **3**, 30 %); 3.23 (dd, 1H, **CH_2**-CO_2H, monoester **2**, 70 %); 3.46 (t, 2H, **CH_2**-O-$CH_2C_6H_5$, monoester **3**, 30 %); 3.49 (t, 2H, **CH_2**-O-$CH_2C_6H_5$, monoester **2**, 70 %); 4.17 (t, 2H, CO_2-**CH_2**, monoester **3**, 30 %); 4.27 (t, 2H, CO_2-**CH_2**, monoester **2**, 70 %); 4.4 (dd, 1H, CH-Br, monoester **2**, 70 %); 4.43 (s, 2H, **CH_2**C_6H_5); 4.48 (dd, 1H, CH-Br, monoester **3**, 30 %); 7.2 (s, 5H, C_6H_5); 8.9 (s, 1H, CO_2H).

*RS-4-(3-benzyloxypropyl)oxycarbonyl-2-oxetanone, **4*** : was prepared from the mixture of monoesters **2** and **3** according to the method previously described (15). After two chromatographies on silica gel (eluent : dichloromethane / petroleum ether, 9 / 1) the lactone **4** was obtained with 45 % yield. ^{1}H NMR ($CDCl_3$, δppm) : 1.93 (tt, 2H, CH_2-**CH_2**-CH_2); 3.44 (dd, 1H, **CH_2** lactone); 3.5 (t, 2H, **CH_2**-O-$CH_2C_6H_5$); 3.64 (dd, 1H, **CH_2** lactone); 4.22 (t, 2H, CO_2-**CH_2**);

4.44 (s, 2H, **CH_2**C_6H_5); 4.72 (dd, 1H, CH lactone); 7.25 (s, 5H, C_6H_5). ^{13}C NMR $CDCl_3$, δppm) : 28 (CH_2-**CH_2**-CH_2); 43 (**CH_2** lactone); 63 (**CH_2**-O-$CH_2C_6H_5$); 65 (CH lactone); 66 (CO_2-**CH_2**); 73 (**CH_2**C_6H_5); 128 (C_6H_5); 179 (C=O, ester); 189 (C=O, lactone). IR (ν, cm^{-1}) : 1844 cm^{-1} (C=O lactone); 1744 cm^{-1} (C=O ester).

Poly(3-benzyloxypropyl malate), ***5*** ***:*** was synthesized by anionic ring-opening polymerization of 3-benzyloxypropyl malolactonate **4** in presence of tetraethylammonium benzoate (10^{-3} eq. per mole of monomer) as initiator under nitrogen at 40 °C (16). Tg = -21 °C ; SEC : Mw = 4700, Ip = 1.4. ^1H NMR (CD_3COCD_3, δppm) : 1.8 (m, 2H, CH_2-**CH_2**-CH_2); 2.88 (m, 2H, CH_2 main chain); 3.4 (m, 2H, **CH_2**-O-$CH_2C_6H_5$); 4.10 (m, 2H, CO_2-**CH_2**); 4.32 (s, 2H, **CH_2**C_6H_5); 5.35 (m, 1H, CH main chain); 7.15 (s, 5H, C_6H_5). ^{13}C NMR (CD_3COCD_3, δppm) : 30 (CH_2-**CH_2**-CH_2); 36 (CH_2 main chain); 64 (**CH_2**-O-$CH_2C_6H_5$); 67 (CO_2-**CH_2**); 70 (CH main chain); 73 (**CH_2**C_6H_5); 128 (C_6H_5); 169 (C=O). IR (ν, cm^{-1}) : 1760 cm^{-1} (C=O ester).

Poly(3-hydroxypropyl malate), ***6*** : polymer **5** (500 mg) was dissolved in freshly distilled acetone (10 ml) and palladium (from Acros) (20 % weight) was added. The mixture was hydrogenolyzed at room temperature overnight. Polymer **6** was obtained after filtration over Celite and evaporation of the solvent under reduced pressure (16). Yield = 99 % ; Tg = -21 °C ; SEC : Mw = 4800, Ip = 1.8. ^1H NMR (CD_3COCD_3, δppm) : 1.74 (m, 2H, CH_2-**CH_2**-CH_2OH); 2.96 (m, 2H, CH_2 main chain); 3.52 (t, 2H, **CH_2**OH); 4.16 (m, 2H, CO_2-**CH_2**); 5.39 (m, 1H, CH main chain). ^{13}C NMR (CD_3COCD_3, δppm) : 32 (**CH_2**-CH_2OH); 36 (CH_2 main chain); 59 (**CH_2**OH); 64 (CO_2**CH_2**); 69 (CH main chain); 168 (C=O).

Poly(3-benzyloxypropyl malate-co-ethyladamantyl malate), (85 / 15), ***7*** : was obtained by copolymerization of lactone **4** (85 mol %) and (RS)-ethyladamantyl malolactonate (15 mol %) obtained as described previously (18). Tg = -15 °C ; SEC : Mw = 4600, Ip = 1.4. ^1H NMR (CD_3COCD_3, δppm) : 1.2-1.7 (m, 17H, adamantyl); 1.8 (m, 4H, O-CH_2-**CH_2**, propyl and ethyl); 2.9 (m, 2H, CH_2 main chain); 3.4 (m, 2H, **CH_2**-O-CH_2-$CH_2C_6H_5$); 4.15 (m, 4H, CO_2-**CH_2**, propyl and ethyl); 4.38 (s, 2H, **CH_2**C_6H_5); 5.38 (m, 1H, CH main chain); 7.2 (s, 5H, C_6H_5). ^{13}C NMR (CD_3COCD_3, δppm) : 30 (CO_2-CH_2-**CH_2**, ethyl and propyl); 36 (CH_2 main chain); 37 and 43 (adamantyl); 64 (**CH_2**-O-$CH_2C_6H_5$); 67 (CO_2-**CH_2**, propyl and ethyl); 70 (CH main chain); 73 (**CH_2**C_6H_5); 128 (C_6H_5); 169 (C=O). IR (ν, cm^{-1}) : 1760 cm^{-1} (C=O ester).

Poly(3-hydroxypropyl malate-co-ethyladamantyl malate), ***8*** : was synthesized using the same procedure as the one described for polymer **6**. Yield = 98 % ; Tg = -19 °C ; SEC : Mw = 3600, Ip = 1.4. ^1H NMR (CD_3COCD_3, δppm) :

1.42-1.8 (m, 17H, adamantyl); 1.8 (m, 4H, CO_2-CH_2-**CH_2**, propyl and ethyl); 2.9 (m, 2H, CH_2 main chain); 3.58 (m, 2H, **CH_2**-OH); 4.15 (m, 4H, CO_2-**CH_2**, propyl and ethyl); 5.4 (m, 1H, CH main chain). ^{13}C NMR (CD_3COCD_3, δppm) : 32 (**CH_2**-CH_2OH); 36 (CH_2 main chain); 37 and 43 (adamantyl); 59 (**CH_2**OH); 64 and 65 (CO_2-**CH_2**, propyl and ethyl); 69 (CH main chain); 169 (C=O).

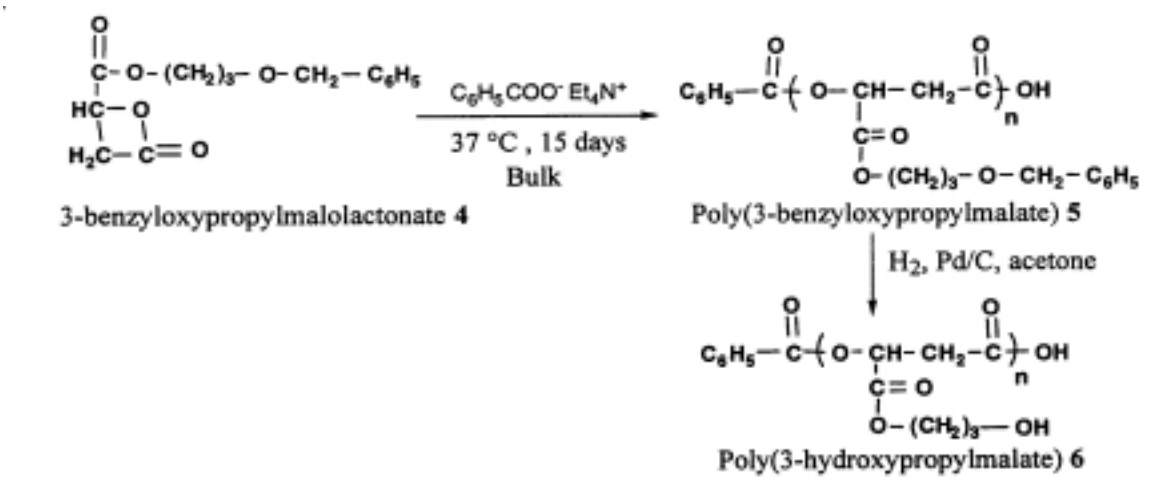
Poly(3-benzyloxypropyl malate-co-butyl malate), (30 / 70), ***9*** : was obtained by copolymerization of lactone **4** (30 mol %) and (RS)-4-butyloxycarbonyl oxetanone (70 mol %), obtained as described previously (17). Tg = -27 °C ; SEC : Mw = 3200, Ip = 1.3. ^{1}H NMR (CD_3COCD_3, δppm) : 0.8 (t, 3H, CH_3); 1.25 (m, 2H, **CH_2**-CH_3); 1.5 (m, 2H, O-CH_2-**CH_2** butyl); 1.8 (m, 2H, CH_2-**CH_2**-CH_2, propyl); 2.88 (m, 2H, CH_2 main chain); 3.4 (m, 2H, **CH_2**-O-$CH_2C_6H_5$); 4 (m, 2H, CO_2-**CH_2**, butyl); 4.1 (m, 2H, CO_2-**CH_2**, propyl); 4.4 (s, 2H, **CH_2**C_6H_5); 5.35 (m, 1H, CH main chain); 7.2 (s, 5H, C_6H_5). ^{13}C NMR (CD_3COCD_3, δppm) : 13 (CH_3); 19 (**CH_2**-CH_3); 30 (CH_2-**CH_2**-CH_2, butyl and propyl); 36 (CH_2 main chain); 64 (**CH_2**-O-$CH_2C_6H_5$); 67 (CO_2-**CH_2**, butyl); 69 (CO_2-**CH_2**, propyl); 70 (CH main chain); 73 (**CH_2**C_6H_5); 128 (C_6H_5); 169 (C=O). IR (ν, cm^{-1}) : 1760 cm^{-1} (C=O ester).

Poly(3-hydroxypropyl malate-co-butyl malate), ***10 :*** was synthesized using the same procedure as described for polymer **6**. Yield = 98 % ; Tg = -25 °C ; SEC : Mw = 3800, Ip = 1.3. ^{1}H NMR (CD_3COCD_3, δppm) : 0.8 (t, 3H, CH_3); 1.25 (m, 2H, **CH_2**-CH_3); 1.5 (m, 2H, O-CH_2-**CH_2** butyl); 1.8 (m, 2H, CH_2-**CH_2**-CH_2, propyl); 2.88 (m, 2H, CH_2 main chain); 3.4 (m, 2H, **CH_2**-OH); 4 (m, 2H, CO_2-**CH_2**, butyl); 4.1 (m, 2H, CO_2-**CH_2**, propyl); 5.35 (m, 1H, CH main chain). ^{13}C NMR (CD_3COCD_3, δppm) : 14 (CH_3); 20 (**CH_2**-CH_3); 30 (CH_2-**CH_2**-CH_2, butyl); 32 (**CH_2**-CH_2OH propyl); 36 (CH_2 main chain); 59 (**CH_2**OH); 64 (CO_2-**CH_2**, propyl); 66 (CO_2-**CH_2**, butyl); 70 (CH main chain); 169 (C=O).

Results and discussion

1. Poly (3-hydroxypropylmalate) :

Poly (3-hydroxypropylmalate) **6** was obtained by catalytic hydrogenolysis of poly (3-benzyloxypropylmalate) **5** which was prepared by anionic ring-opening polymerization of a ß-substituted ß-lactone **4** (Scheme 1). This lactone was synthesized in four steps starting from aspartic acid according to a previously reported synthesis route (15).



Scheme 1 : Synthesis of poly(3-hydroxypropylmalate) **6**

All polymers were characterized by ^{1}H and ^{13}C NMR spectra, size exclusion chromatography in THF and by differencial scanning calorimetry (Table 1). ^{1}H and ^{13}C NMR spectra of poly(3-benzyloxypropylmalate) **5** displayed the different peaks corresponding to the expected repeating unit structure.

Polymers	P 5	P 6	P 7	P 8	P 9	P 10
Mw[a]	4700	4800	4600	3600	3200	3800
Ip[a]	1.4	1.8	1.4	1.4	1.3	1.3
Tg (°C)[b]	-21	-21	-15	-19	-27	-25

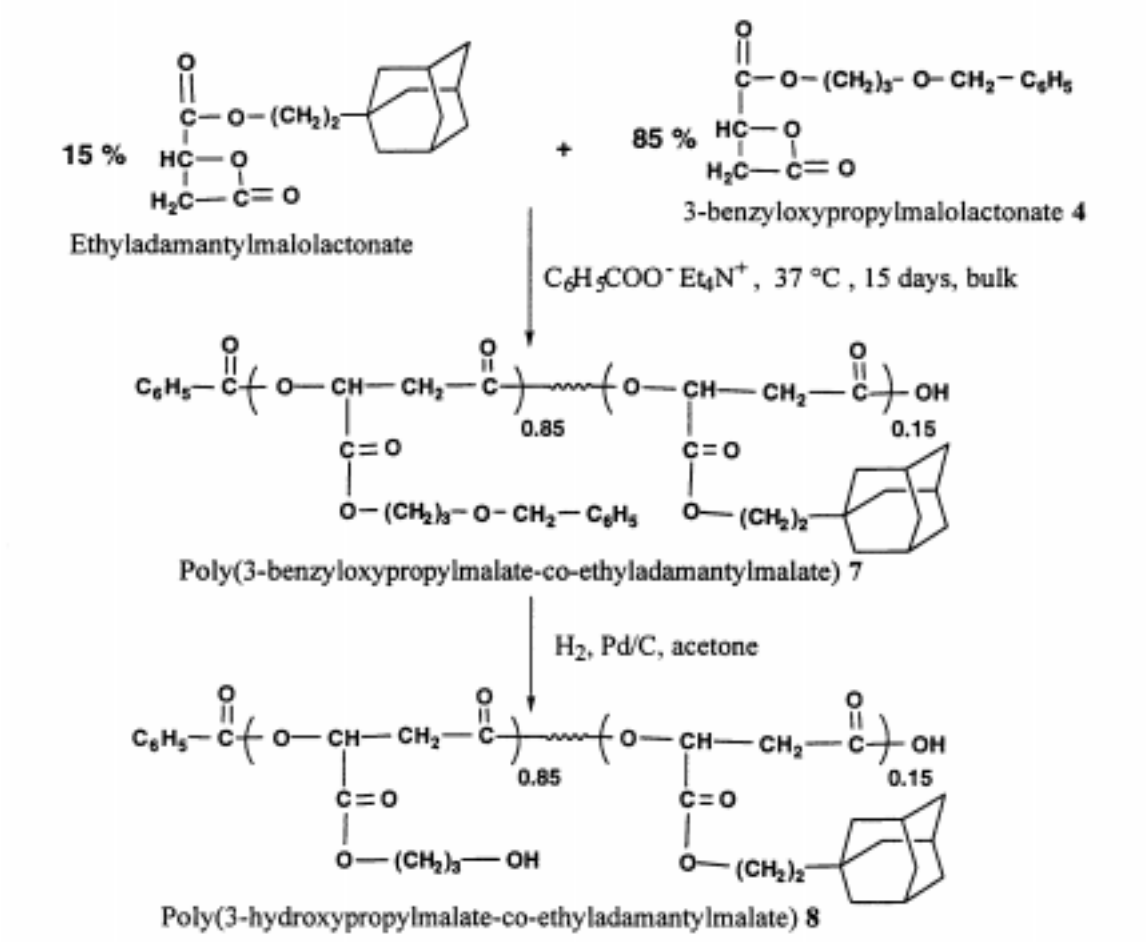
a - SEC in THF, Standards : PS b - Determined by DSC

Table 1 : Characteristics of synthesized homopolymer and copolymers

In all cases, deprotection of benzyl-protecting groups was possible by specific catalytic hydrogenolysis. The reaction was complete as determined by ^{1}H NMR ; no more benzyl signals were present in the spectra. Polymer **6** was amorphous (Tg at -21 °C) (Table 1) and soluble in water.

2. Poly(3-hydroxypropylmalate-co-ethyladamantylmalate) :

Poly(3-benzyloxypropylmalate-co-ethyladamantylmalate) **7** was synthesized by anionic ring-opening copolymerization of a mixture of 3-benzyloxypropylmalolactonate **4** (85 mol %) and ethyladamantylmalolactonate (15 mol %) (Scheme 2).

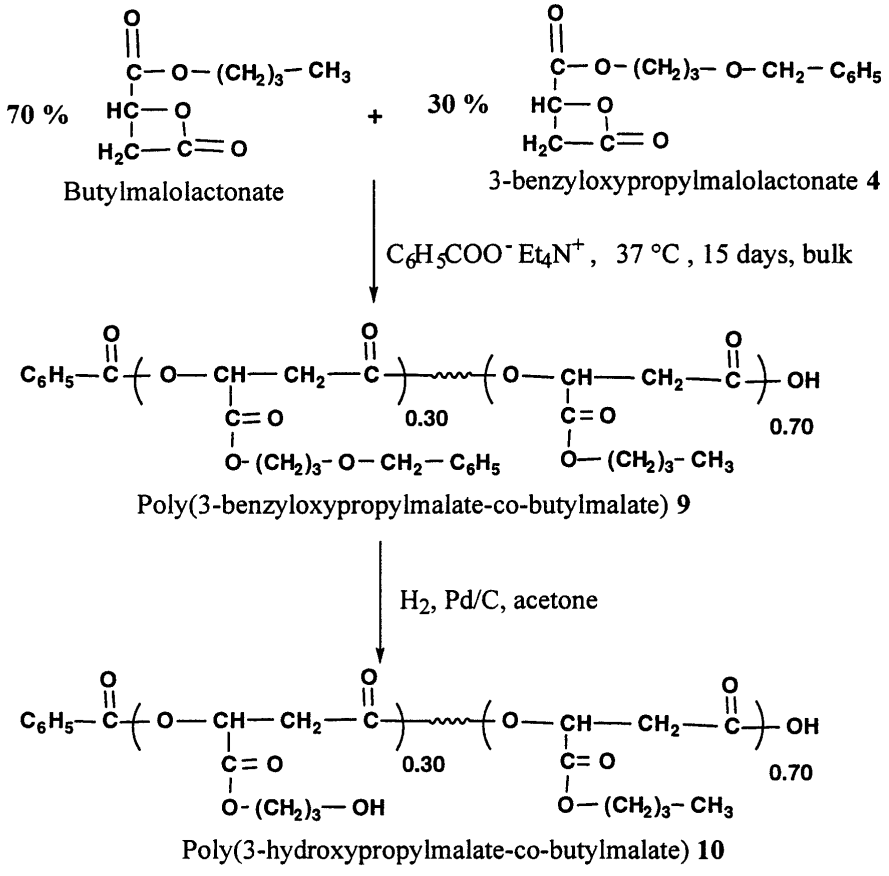


Scheme 2 : Synthesis of poly(3-hydroxypropylmalate-co-ethyladamantyl malate) **8**

^{1}H NMR spectrum displayed, the polymer composition was identical to the initial monomers feed. In addition, only one Tg was detected (Table 1). Poly(3-hydroxypropylmalate-co-ethyladamantylmalate) **8** was then obtained by catalytic hydrogenolysis without any chain degradation and all carboxylic pendant groups were deprotected as shown by ^{1}H NMR spectrum. Copolymer **8** was soluble in water.

3. Poly(3-hydroxypropylmalate-co-butylmalate) :

Poly(3-benzyloxypropylmalate-co-butylmalate) **9** was prepared by copolymerization of a mixture of 3-benzyloxypropylmalolactonate **4** (30 mol %) and butylmalolactonate (70 mol %), as described for copolymer **7**.



Scheme 3 : Synthesis of poly(3-hydroxypropylmalate-co-butylmalate) **10**

^{1}H NMR spectrum demonstrated that composition of the block copolymer was identical to the initial monomer feed. Moreover, DSC measurements displayed only one value of Tg at -27 °C and SEC results showed only one peak corresponding to copolymer **9** (Table 1). Copolymer **9** was then submitted to catalytic hydrogenolysis which gave access, without any chain degradation, to poly(3-hydroxypropylmalate-co-butylmalate) **10**, (Scheme 3) ; ^{1}H NMR displayed the chemical modification was complete.

In conclusion, we have shown the possibilities to prepare new materials of the poly(ß-malic acid) family, which have been expanded to hydrophilic non-charged pendant groups. Tailor making of copolymers bearing different functional pendant groups was possible. Their properties and performances in water at different pHs will be compared with the ones of corresponding charged copolymers. In order to obtain high molecular weights, purification of monomers will improved by using capillary supercritical CO_2 chromatography. Different copolymers (graft, block, random) are under study as hydrolyzable associating networks, macromolecular micelles and nanoparticules. The library of malolactonic acid esters has been enlarged to a novel monomer leading to polymers with new characteristics and properties.

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