Synthesis and Aqueous Solution Characterization of Catalytically Active Block Copolymers Containing Imidazole

Martin R. Simmons[†] and Costas S. Patrickios*[,‡]

Department of Chemical Engineering, University of Manchester Institute of Science and Technology (UMIST), P.O. Box 88, Manchester M60 1QD U.K.

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Introduction

There has been an intense research activity in the 1960s through the 1970s in pursuit of synthetic polymer enzyme mimics, also termed *synzymes* or *plastic enzymes*.[1−4] This research involved the synthesis of homopolymers, graft and random copolymers containing nucleophilic groups such as imidazole or pyridine. Although these polymers exhibited the ability to hydrolyze activated esters, their molecular weight and composition were poorly controlled. Recent efforts aim at revisiting this work with increased attention on the control over the polymer structure which has resulted in enzyme mimics with improved activity and selectivity.[5−8]

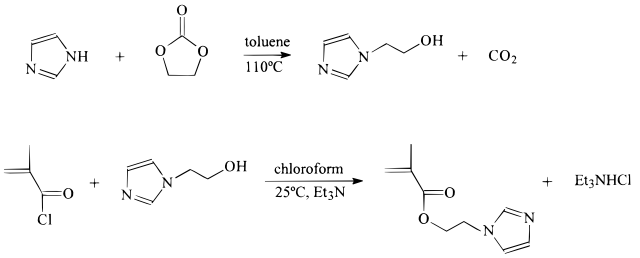
In an effort to elucidate the critical structural features for catalytic activity in methacrylate copolymers, this investigation presents the synthesis and characterization of the hydrolytic activity of copolymers containing imidazole, of precise size and composition. The imidazole-containing monomer, 2-(1-imidazolyl)ethyl methacrylate (ImEMA), confers to the block copolymers catalytic activity, particularly at neutral and alkaline pH, due to the nucleophilicity of imidazole. The second monomer, 2-(dimethylamino)ethyl methacrylate (DMAEMA), imparts to the block copolymers aqueous solubility at neutral and slightly alkaline pH.

Results and Discussion

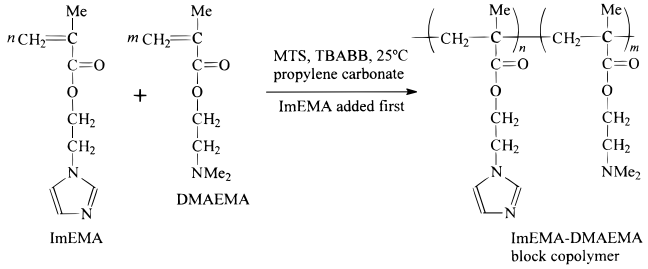
While DMAEMA is commercially available, ImEMA must be synthesized. Thus, imidazole was reacted with an excess of ethylene carbonate in refluxing toluene to give 2-(1-imidazolyl)ethanol at ca. 50% yield,[9−11] which was reacted to an excess of methacryloyl chloride in chloroform in the presence of triethylamine at room temperature to give ImEMA in nearly-quantitative yield (ca. 90%) (Scheme 1). ImEMA was purified by vacuum distillation over calcium hydride.

A series of ImEMA−DMAEMA diblock copolymers were prepared using group transfer polymerization (GTP)[12−14] at room temperature. The initiator used was 1-methoxy-1-trimethylsiloxy-2-methyl-1-propene (MTS) while tetrabutylammonium bibenzoate (TBABB)[14] served as the polymerization catalyst. Due to the insolubility of the ImEMA homopolymer[15] in the most usual GTP solvent, tetrahydrofuran (THF), a more polar solvent, propylene carbonate, was used. To the best of our knowledge, this solvent has been reported as a suitable

Scheme 1. Synthesis of ImEMA Monomer from Imidazole, Ethylene Carbonate, and Methacryloyl Chloride



Scheme 2. Synthesis of ImEMA−DMAEMA Diblock Copolymers by Sequential GTP



GTP solvent only once, namely for the GTP of α-methylene-γ-butyrolactone to give a polymer with M_n = 31 500 and M_w/M_n = 1.52.[13] It was determined in preliminary block copolymerizations in propylene carbonate that the ImEMA monomer should be polymerized first (Scheme 2); when DMAEMA was polymerized first, no polymerization of the second, ImEMA monomer was observed. This observation is puzzling because we could prepare the statistical copolymer of DMAEMA and ImEMA (see below).

Quantitative yields were obtained in all polymerizations. The polymers were recovered from their polymerization solutions by precipitation with petroleum ether, followed by vacuum-drying. Prior to the addition of the precipitant, acetone (or THF) was introduced to secure the miscibility of the propylene carbonate solution of the polymer with petroleum ether. The molecular weights, polydispersities, and copolymer compositions of the resulting block copolymers are presented in Table 1. An ImEMA homopolymer and a statistical copolymer of ImEMA and DMAEMA were also prepared. Narrow molecular weight distributions were obtained in all cases (M_w/M_n < 1.2) as determined by gel permeation chromatography in THF (PMMA standards, RI detector, Polymer Laboratories PL Mixed "E" column) and water (Bis−Tris buffer with $NaNO_3$, 1 M at pH 6.5, PEG standards, RI detector, Pharmacia Biotech "Superdex" column). The copolymer compositions determined using ^{1}H NMR spectroscopy agreed well with those expected from the comonomer feeds. The ^{1}H NMR spectra of the two homopolymers and the equimolar block copolymer are presented in Figure 1.

Our copolymers constitute the third reported example of diblock copolymers in which both block components are polybases. The first example has been presented recently by Bütün *et al.*[16] who have also employed GTP (in THF) to prepare block copolymers based on DMAEMA and 2-(diethylamino)ethyl methacrylate. DMAEMA

* To whom correspondence should be addressed.
[†] Present address: Hexcel Composites, Duxford, Cambridge CB2 4QD, U.K.
[‡] Present address: Department of Natural Sciences, University of Cyprus, P.O. Box 537, 1678 Nicosia, Cyprus.

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Table 1. Molecular Weights, Polydispersities, and Copolymer Compositions of the ImEMA−DMAEMA Copolymers Determined Using Gel Permeation Chromatography and ^{1}H NMR Spectroscopy

polymer code	polymer formula	theor[a] MW	GPC in THF[b] M_n	GPC in THF[b] M_w/M_n	GPC in H_2O pH 6.5[c] M_n	GPC in H_2O pH 6.5[c] M_w/M_n	ImEMA content (mol %) theor	ImEMA content (mol %) actual[d]
MS103	$(ImEMA)_{22}$	4060	*e*		*f*	*f*	100	100
MS110	$(ImEMA)_6$-*b*-$(DMAEMA)_{57}$	10130	12900	1.08	5230	1.02	10	11
MS107	$(ImEMA)_{11}$-*b*-$(DMAEMA)_{50}$	9930	12110	1.09	5190	1.02	18	21
MS111	$(ImEMA)_{11}$-*b*-$(DMAEMA)_{19}$	5060	7870	1.17	4150	1.06	37	38
MS122	$(ImEMA)_{11}$-*b*-$(DMAEMA)_{11}$	3810	4770	1.12	2790	1.07	50	51
MS123	(ImEMA-*co*-$DMAEMA)_{11}$[g]	3810	2690	1.10	2040	1.14	50	47
MS124	$(ImEMA)_{11}$-*b*-$(DMAEMA)_6$	3020	2340	1.09	1450	1.09	65	74

[a] Initiator fragment (100 g mol^{-1}) included. [b] PMMA standards, PL mixed "E" column, RI detector. [c] PEG standards, Superdex column, pH 6.5 in 50 mM Bis−Tris buffer and 1 M $NaNO_3$, RI detector. [d] As determined by ^{1}H NMR spectroscopy in $CDCl_3$. [e] Insoluble in THF; end-group analysis by ^{1}H NMR indicated an M_n of 4240. [f] Insoluble in water at pH 6.5; aqueous GPC at pH 2.5 in 0.1 M malonic acid and 1 M $NaNO_3$ gave an M_n of 1960 and an M_w/M_n of 1.37. [g] Random copolymer.

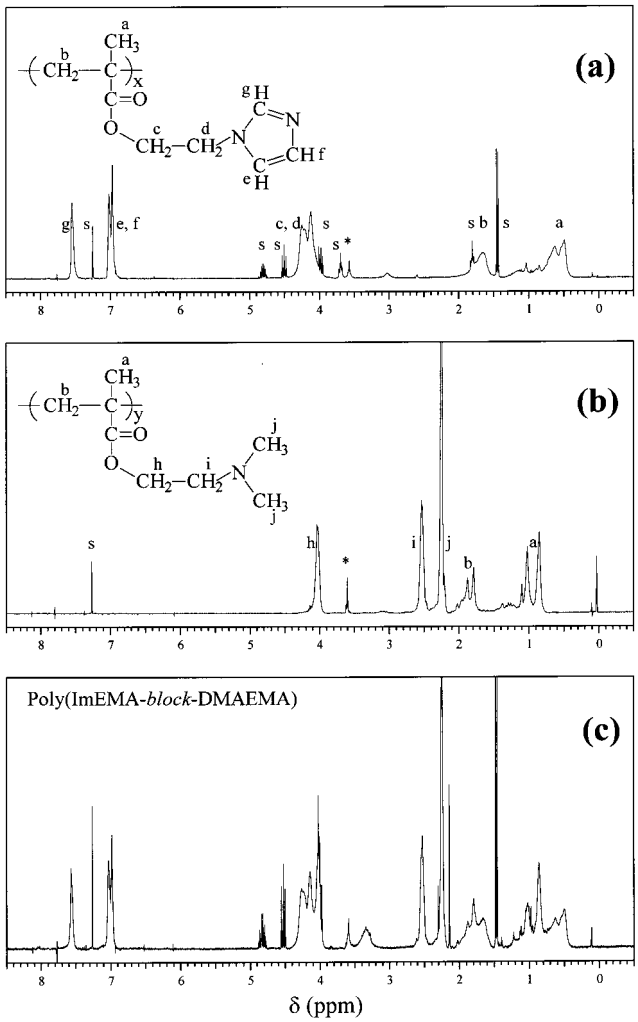


Figure 1. Proton NMR spectra of (a) the $(ImEMA)_{22}$ homopolymer, (b) the $(DMAEMA)_{20}$ homopolymer, and (c) the $(ImEMA)_{11}$-*b*-$(DMAEMA)_{11}$ diblock copolymer.

has already been used for the preparation by GTP of diblock[17,18] and ABC triblock[19,20] copolymers with methyl methacrylate and methacrylic acid. The second example has been provided by Saunders and co-workers[21] who have employed "living" ring-opening metathesis polymerization (ROMP) to prepare diblock copolymers of norbornenes, functionalized with 1-imidazole and a secondary amine.

Figure 2 shows the hydrogen ion titration curves of the two homopolymers, $(ImEMA)_{22}$ and $(DMAEMA)_{20}$, and of the two equimolar copolymers, the block $(ImEMA)_{11}$-*b*-$(DMAEMA)_{11}$ and the statistical $(ImEMA)_{11}$-*co*-$(DMAEMA)_{11}$. The effective p*K*s of polyImEMA and polyDMAEMA, taken as the midpoints of the two titration curves, are ca. 5 and 7.5, respectively. Due to the relative proximity of these two constants, the titration curves of the copolymers do not present one distinct inflection at each p*K*, but rather exhibit continuous transitions in the pH range between the two p*K*s. The titration curves of the two copolymers are almost identical with an effective p*K* around 6.2.

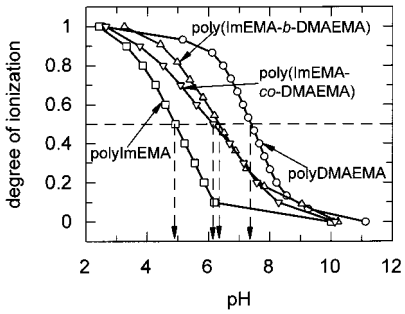
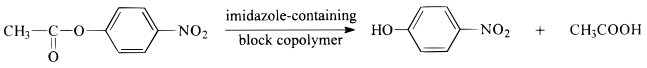


Figure 2. Hydrogen ion titration curves of the two homopolymers, $(ImEMA)_{22}$ and $(DMAEMA)_{20}$, and of the equimolar block copolymer $(ImEMA)_{11}$-*b*-$(DMAEMA)_{11}$ as well as of the statistical copolymer (ImEMA-*co*-$DMAEMA)_{11}$.

Scheme 3. Hydrolysis of *p*-nitrophenyl Acetate Catalyzed by the Presence of Imidazole-Containing Copolymers



The presence of imidazole in the polymers leads to catalytic properties. We have evaluated the ability of our copolymers to hydrolyze a simple ester, *p*-nitrophenyl acetate, as shown in Scheme 3. One of the hydrolysis products, *p*-nitrophenol, absorbs strongly at 400 nm and provides a convenient means to follow the progress of the reaction spectrophotometrically (Lambda 10 Perkin-Elmer UV/vis spectrophotometer). Figure 3 shows the initial rate of *p*-nitrophenyl acetate hydrolysis at pH 7 (buffered by 25 mM Bis−Tris) in the presence of the various copolymers at a 0.05% polymer concentration and an initial *p*-nitrophenyl acetate concentration of 1.0 mM. Clearly, a higher imidazole concentration leads to a higher activity. It is noteworthy that pure buffer has also some catalytic activity. All the copolymers exhibit a higher activity, by approximately a factor of 2, than the ImEMA homopolymers, as determined previously at pH 5 (they precipitate above pH 5.8) and at the same substrate and equivalent imidazole concentrations.[15] This can be attributed to the strong nucleophilicity of unprotonated imidazole[22] as well as its enhanced hydrophobicity which can lead to the more

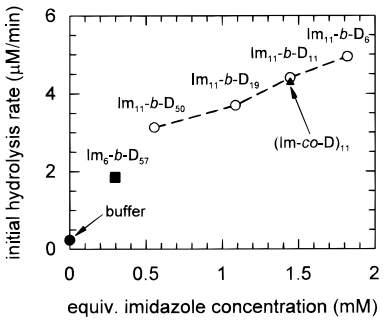


Figure 3. Initial hydrolysis rate of *p*-nitrophenyl acetate at pH 7 in the presence of various polymers at a 0.05% polymer concentration and an initial *p*-nitrophenyl acetate concentration of 1.0 mM. The *p*-nitrophenol hydrolysis product is highly colored with an absorbance maximum at 400 nm. Key: Im, ImEMA; D, DMAEMA.

efficient binding of the polymer to the hydrophobic substrate. With an effective p*K* of 5, polyImEMA is 50% protonated at pH 5 while it is only 1−10% protonated at pH 7.

Another important observation from Figure 3 comes from the comparison of the initial hydrolysis rates in the presence of the block and statistical copolymers. The two copolymers present identical behavior, suggesting that the polymer architecture has no effect on catalysis in this case. This is opposite to our expectation that the insolubility of the ImEMA units at neutral pH would lead to micelle formation of the block copolymer in water, which would certainly accelerate hydrolysis. Our aqueous GPC results at pH 6.5 in Table 1 show that no micellization takes place. We repeated the experiments at pH 7.2 in Tris buffer with 1M $NaNO_3$ using the Pharmacia Biotech "Superdex" 200 HR 10/30 column and observed the partial (5% based on the refractive index signal) formation of small aggregates (dimers) in the block copolymers. Thus, it appears that the hydrophobicity of ImEMA is not sufficient to lead to extensive micellization at the present range of copolymer molecular weights. This is consistent with the marginal hydrophobicity of imidazole with an octanol−water partition coefficient of 1.[22] Future work will involve the introduction of more hydrophobic comonomers, which will ensure the aqueous solution micellization of the block copolymers to enhance catalytic activity.

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