A Mechanistic Study of the Hydrolytic Stability of Poly(2-(dimethylamino)ethyl methacrylate)

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ABSTRACT: The hydrolytic stability of poly(2-(dimethylamino)ethyl methacrylate) was investigated and compared with the stability of its monomer 2-(dimethylamino)ethyl methacrylate (DMAEMA), with 2-(dimethylamino)ethyl isobutyrate (DMAEIB), representing the repeating unit in the polymer, and with the related 3-(dimethylamino)propyl methacrylate (DMAPMA) (H_0/pH range −0.5 to +12, at 37 °C, in aqueous solution). At pH < 3, the unsaturated DMAEMA and DMAPMA were more stable than the saturated DMAEIB. At pH 4−8, DMAEMA and DMAEIB were equally stable, but less stable than DMAPMA. This has been ascribed to a coordination of the protonated dimethylamino group and the ester carbonyl, rendering the ester more susceptible to nucleophilic attack of a hydroxyl ion. At alkaline pH (> pK_a) no differences in stability between the compounds were found. P(DMAEMA), either in its free form or complexed to DNA, was substantially more stable to hydrolytic degradation than DMAEMA and DMAEIB. Fluorescence measurements performed with a copolymer of DMAEMA and dansyl ethyl methacrylamide showed that the dielectric constant (ϵ_r) experienced in the environment of the polymer backbone, was low (about 7). This microenvironment might be the reason for the hydrolytic stability of the polymer, since the hydrolysis of the monomer decreased substantially with decreasing ϵ_r of the medium. Accelerated degradation (80 °C, pH 1 and 7) of p(DMAEMA) and poly(2-(dimethylamino)ethyl acrylate), p(DMAEA), showed that p(DMAEA) was more sensitive to hydrolysis. This can be explained by the assumption that, due to the lack of the methyl group, the ϵ_r in the environment of the acrylate backbone is higher than the ϵ_r in the environment of the p(DMAEMA) backbone.

Introduction

Water-soluble cationic polymers (e.g. DEAE dextran,[1] poly(L-lysine),[2] poly(ethyleneimine),[3] and derivatized chitosan[4,5]) are currently under investigation as DNA-binding agents to be used in nonviral gene delivery systems. Recently, we have shown that 2-(dimethylamino)ethyl methacrylate- (DMAEMA-) based (co)-polymers are able to bind plasmid DNA via electrostatic interactions. The formed polymer/plasmid complexes (polyplexes) are taken up by cells, resulting in the synthesis of the protein for which the plasmid encodes.[6−8] Although the mechanism by which cationic polymers facilitate the expression of the transgene is far from clear, it is obvious that dissociation of the polyplex has to occur intracellularly. The dissociation process can be caused by physical methods (e.g. by anionic macromolecules present in the cells) or, in the case of p(DMAEMA), by chemical hydrolysis of the side groups of the polymer. Hydrolysis of cationic 2-(dimethylamino)-ethyl esters results in anionic acid groups, thereby reducing the overall charge of the polymer, which can result in a reduced polymer/plasmid interaction. Also, for designing stable pharmaceutical formulations of polymer/plasmid which fulfill the requirements defined for pharmaceutical products, insight into the hydrolytic (in)stability of the polymer is necessary.

In this paper, the hydrolytic stability of p(DMAEMA) was investigated and compared with the stability of relevant low molecular weight compounds, namely its monomer DMAEMA (Figure 1a), a compound more representative for the repeating unit in this polymer, 2-(dimethylamino)ethyl isobutyrate (DMAEIB, Figure 1b), and a DMAEMA analogue with a longer side chain group, 3-(dimethylamino)propyl methacrylate (DMAPMA, Figure 1c). Moreover, the stability of p(DMAEMA) was compared with the stability of the corresponding acrylate polymer and its monomer 2-(dimethylamino)-ethyl acrylate (DMAEA, Figure 1d). By using fluorescently labeled p(DMAEMA), insight was obtained into the possible reasons for the remarkable stability of the polymer in comparison with the low molecular weight compounds.

Experimental Section

Materials. The following compounds were used as received: isobutyric acid (IBA, Fluka, Bornem, Belgium), acrylic acid (AA, Fluka), methacryloyl chloride (98%, Fluka), 2-(dimethylamino)ethanol (DMAE−OH, Aldrich, Bornem, Belgium), 2-(dimethylamino)propanol (DMAP−OH, 99%, Aldrich), acetonitrile (chromatography grade, Biosolve, Netherlands) and 2-(dimethylamino)ethyl acrylate (DMAEA, 98%, Aldrich). 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 98%, Fluka) and methacrylic acid (MAA, Fluka) were purified by distillation under reduced pressure.

Methods. NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Varian G-300 (300 MHz) spectrometer (Varian, NMR Instruments, Palo Alto, CA). Chemical shifts (δ) are given in ppm.

FTIR Spectroscopy. FTIR-spectra were recorded with a Bio-Rad FTS-25 spectrometer (Bio-Rad, Cambridge, MA).

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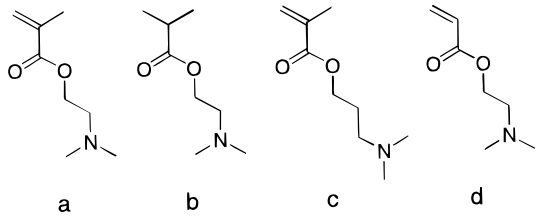

Figure 1. Chemical structure of 2-(dimethylamino)ethyl methacrylate (DMAEMA, a), 2-(dimethylamino)ethyl isobutyrate (DMAEIB, b), 3-(dimethylamino)propyl methacrylate (DMAPMA, c), and 2-(dimethylamino)ethyl acrylate (DMAEA, d).

Liquid compounds were measured in $CHCl_3$ solution. For each sample 64 scans were recorded between 4000 and 400 cm^{-1} with a resolution of 2 cm^{-1}. HCl salts of the compounds were made by adding a 5−10-fold excess of HCl−ether to a solution of the compound in diethyl ether. After evaporation of the solvent, the excess HCl was removed by storing the product over KOH pellets overnight in vacuo.

Synthesis of 2-(Dimethylamino)ethyl Isobutyrate (DMAEIB). To a solution of DMAEMA (3 mL, 17 mmol) in CH_2Cl_2 (50 mL) was added 50 mg of 10% palladium on carbon (Pd/C). Hydrogenation was carried out overnight in a Parr apparatus at a constant pressure of 50 psi of hydrogen, and at ambient temperature. Pd/C was removed by filtration over Celite and CH_2Cl_2 was evaporated under reduced pressure: yield 95%; purity 99% (RP-HPLC). The compound was characterized by ^{1}H NMR: δ (ppm) 4.17 (s, 2H, OCH_2), 2.54 (m, 3H, NCH_2 + CHCO), 2.24 (s, 6H, $N(CH_3)_2$), 1.15 (d, 6H, $C(CH_3)_2$).

Synthesis of 3-(Dimethylamino)propyl Methacrylate (DMAPMA). To an ice-cooled solution of pyridine (20 mL) were added DMAP−OH (2.4 mL, 20 mmol), CH_2Cl_2 (10 mL), and methacryloyl chloride (4.0 mL, 40 mmol) under magnetic stirring, after which a solid was formed. Addition of CH_2Cl_2 (40 mL) resulted in a clear solution. After 5 h at ambient temperature, all DMAP−OH had reacted according to TLC (eluent ethyl acetate/triethylamine, 95/5, v/v). The solvents and excess methacryloyl chloride were removed by evaporation under reduced pressure. After being dissolved in $CHCl_3$, the product was extracted by an ice-cooled 0.5 M HCl solution in water. The water layer was brought to pH 10 with 4 M NaOH, and the product was extracted with $CHCl_3$. After this was washed with a saturated NaCl solution, the organic layer was dried on Na_2SO_4 and filtered. The product was isolated by evaporation under reduced pressure, giving a 62% yield with a purity of 96% (RP-HPLC), and characterized by ^{1}H NMR: δ (ppm) 6.08 (s, 1H, C=CH), 5.54 (s, 1H, C=CH), 4.18 (t, 2H, OCH_2), 2.36 (t, 2H, NCH_2), 2.22 (s, 6H, $N(CH_3)_2$), 1.93 (s, 3H, $C{=}CCH_3$), 1.84 (m, 2H, $OCH_2CH_2CH_2N$).

Synthesis of 2-[[(5-Dimethylamino)naphthalene-1-sulfonyl]amino]ethyl Methacrylamide (Dansyl Ethylmethacrylamide, DaEMam). DaEMam was synthesized (overall yield 48%) and purified as described before.[9]

Synthesis of p(DMAEMA), p(DMAEA), and p(DMAEMA-*co*-DaEMam). P(DMAEMA) and p(DMAEA) were synthesized by a radical polymerization of the corresponding monomers in toluene initiated by AIBN as described before.[7] P(DMAEMA-*co*-DaEMam) was synthesized similarly from DMAEMA and DaEMam (DMAEMA/DaEMam 50/1, mol/mol; M/AIBN 400/1, mol/mol). Molecular weights of the different polymers relative to dextran were determined by gel permeation chromatography (GPC), as described previously.[7] For p(DMAEMA), p(DMAEA), and p(DMAEMA-*co*-DaEMam) the M_w (and M_n) were 75 kD (17 kD), 21 kD (9 kD), and 94 kD (11 kD), respectively. To investigate whether DaEMam was copolymerized with DMAEMA, GPC analysis of p(DMAEMA-*co*-DaEMam) was performed by a combination of UV (330 nm) and RI detection. A UV peak with the same retention time as a peak observed with RI detection, proved that the copolymer was indeed synthesized. The amount of DaEMam incorporated into p(DMAEMA-*co*-DaEMam) was determined by UV analysis at 328 nm of a p(DMAEMA-*co*-DaEMam) solution in 20 mM Hepes buffer (pH 7.4) and 0.15 M NaCl and found to be 64/1 (mol/mol), which is close to the feed ratio (50/1).

Fluorescence Spectroscopy. Fluorescence measurements were performed on a LS-50B luminescence spectrophotometer (Perkin-Elmer, Norwalk, CT). A solution of 50 μg/mL p(DMAEMA-*co*-DaEMam) was prepared in 5 mM Hepes buffer and 0.9% NaCl, and the pH of the medium was adjusted by addition of an appropriate amount of a concentrated HCl solution or NaOH solution. The emission maximum (λ_{max}) was determined as a function of the pH using an excitation wavelength of 330 nm, an emission filter at 390 nm, and bandwidths of 5 nm. By fitting the obtained data to a modified Henderson−Hasselbalch equation (eq 1), an average pK_a of

$$\mathrm{pH} = \mathrm{p}K_a + A \log\left(\frac{\lambda - \lambda_H}{\lambda_{OH} - \lambda}\right) \quad (1)$$

p(DMAEMA-*co*-DaEMam) was estimated. λ is the measured value of λ_{max}, and λ_H and λ_{OH} are values of λ_{max} at pH $\ll$ pK_a or pH $\gg$ pK_a, respectively.

The free fluorophore DaEMam was diluted to 1.75 μg/mL in dioxane/water mixtures (0, 20, 45, 70, 82, and 100% w/w dioxane/water, corresponding to dielectric constants of 78.4, 60.8, 38.5, 17.7, 9.53 and 2.21[10]). Fluorescence of DaEMam was measured by scanning the emission wavelength between 400 and 600 nm at an excitation wavelength of 330 nm.

RP-HPLC. The RP-HPLC system consisted of a pump Model 510, an autoinjector Model 717, and a variable wavelength absorbance detector Model 486 (all Waters, Milford, MA). The used columns were a thermostated (30 °C) LiChrosphere 100 RP-18 column (5 μm, 125 × 4 mm i.d.) and a RP-18 guard column (4 × 4 mm) (Merck, Darmstadt, Germany).

The mobile phase consisted of a water/acetonitrile solution (95/5 w/w for DMAEA degradation studies, 90/10 w/w for DMAEMA, DMAPMA and DMAEIB degradation studies, and 80/20 w/w to analyze the degradation of polymer samples) completed with triethylamine (final concentration 10 mM) and brought to pH 2 with perchloric acid (final concentration 25 mM). The flow rate was 1.0 mL/min, the injection volume was 100 μL, and the detection wavelength was 210 nm.

Degradation of DMAEMA, DMAPMA, DMAEIB, and DMAEA was monitored by disappearance of these compounds. To check the chromatographical method, the formation of degradation products, MAA (in the case of DMAEMA and DMAPMA), MIB (from DMAEIB), and AA (from DMAEA), was observed too. The decrease of the parent compounds was found to be in accordance with the appearance of degradation products, which proves the stability indicating capability of the method.

The determination of polymer degradation at 37 °C was based on the formation of DMAE−OH. After derivatization with benzoyl chloride as described below, the compound could be detected at 232 nm. The presence of polymer did not interfere with the analysis. The recovery of DMAE−OH from spiked samples was quantitative.

Degradation of DMAEMA, DMAPMA, DMAEIB, and DMAEA. Degradation of DMAEMA, DMAPMA, DMAEIB, and DMAEA at Different pH Values. Stock solutions (10 mM) of DMAEMA, DMAPMA, and DMAEA were made in DMSO. In 50 mL infusion bottles placed in a thermostated bath (37 °C), 0.5 mL of stock solution was diluted to a final concentration of 100 μM with the appropriate buffer (H_0/pH −0.5−12). Buffers used were perchloric acid at H_0/pH −0.5 to +0.5, hydrochloric acid at pH 1.1−2.0, acetate at pH 3−6, phosphate at pH 7−8, borate at pH 8.5−9.5, carbonate at pH 10−11, NaOH at pH 11.5, and glycine/NaOH at pH 12. Buffer concentrations were 10 mM, except in the H_0/pH range −0.5−1.1 (concentration 100 mM). H_0 values were calculated according to Hammett.[11,12] The ionic strength (μ) was adjusted to 0.3 M with NaCl.

Periodically, a 1.0 mL sample was added to 100 μL of 1 M acetate buffer (pH 3) to inhibit further degradation and stored

Table 1. pK_a values of DMAEMA, DMAPMA, DMAEIB, and DMAE−OH, and average pK_a Values of P(DMAEMA) at μ = 0.15 M, 25 °C (n = 3, sd = 0.1)

compound	(average[a]) pK_a value	compound	(average[a]) pK_a value
DMAEMA[b]	8.4	DMAEA[b]	8.3
DMAPMA[b]	8.9	p(DMAEMA) 4 kD[c]	7.8
DMAEIB[b]	8.3	p(DMAEMA) 100 kD[c]	7.5
DMAE−OH[b]	9.3	p(DMAEMA) 550 kD[c]	7.4
DMAP−OH[b]	9.3		

[a] Average pK_a: pH at which 50% of the dimethylamino groups in p(DMAEMA) are protonated. [b] Solutions of the compounds (40 μL in 20 mL of 0.15 M NaCl) were acidified with 0.1 M HCl and titrated with 0.1 M NaOH. [c] Solutions of 2−3 mg/mL polymer were prepared in 0.15 M NaCl, acidified with 0.1 M HCl, and titrated with 0.1 M NaOH.

at 4 °C prior to analysis. The influence of pH on k_{obs} was determined in the H_0/pH range −0.5 to +11.9 for DMAEMA, pH 1−12 for DMAPMA, and pH 1.3 and 7 for DMAEA.

The degradation experiments with DMAEIB were performed essentially the same as the degradation experiments with DMAEMA and DMAPMA. However, due to a higher detection limit (caused by a lower extinction coefficient of DMAEIB compared to DMAEMA and DMAPMA), 0.5 mL of stock solution (500 mM in DMSO) was diluted to 5.0 mM with the appropriate buffer (buffer concentration was 100 mM, μ = 0.3 M). The influence of pH on k_{obs} was determined in the pH range 1.1−11.9.

Degradation of DMAEMA at Different Dielectric Constants. A stock solution of 10 mM DMAEMA in DMSO was diluted to a final concentration of 100 μM in different dioxane/buffer solutions (0 and 70% w/w dioxane/(buffer pH 11), buffer concentration 10 mM, μ = 0.3 M). The solutions (in 50 mL infusion bottles) were placed in a thermostated bath (37 °C). Periodically, a 1.0 mL sample was added to 100 μL of 1 M acetate buffer (pH 3) to inhibit further degradation and stored at 4 °C prior to analysis.

Degradation of Polymers. Degradation of p(DMAEMA). A p(DMAEMA) solution (15.6 μg/mL) was prepared in the appropriate buffer (H_0/pH 0−12, 10 mM, μ = 0.3 M) in 50 mL infusion bottles placed in a thermostated bath (37 °C).

The degradation of p(DMAEMA) was followed by the formation of 2-(dimethylamino) ethanol. Because direct analysis of this compound by gas chromatography was not possible, a derivatization method with benzoyl chloride was developed. Periodically 1.0 mL was added to 100 μL of 1 M acetate buffer (pH 3) to inhibit further degradation. Then 100 μL of each sample was acidified with 10 μL of 4 M HCl and lyophilized. Then, 100 μL of pyridine containing 10% (v/v) benzoyl chloride was added. After 15 min, the pyridine was evaporated by a nitrogen flow. The crude product was dissolved in 0.5 mL of water and washed with 1 mL of CH_2Cl_2. The water phase was analyzed by RP-HPLC.

Accelerated Degradation of p(DMAEMA) and p(DMAEA). Stock solutions of 6 mg/mL polymer were prepared in buffers of pH 1 and pH 7. These solutions were divided in 5 mL portions in infusion bottles which were put at 80 °C. Periodically, the contents of a bottle were dialyzed against water, lyophilized, dissolved in D_2O, and analyzed with ^{1}H NMR.

Results and Discussion

pK_a Measurements. The pK_a values of DMAEMA, DMAEIB, DMAPMA, DMAEA, DMAE−OH, and DMAP−OH were determined by titration of acidified solutions with NaOH (see Table 1). The pK_a value of DMAE−OH is about 1 pH unit higher than the pK_a values of DMAEMA, DMAEIB, and DMAEA, whereas the pK_a values of DMAP−OH and DMAPMA only differ slightly. Comparable differences in pK_a between dimethylethyl and dimethylpropyl compounds have been reported before and might be explained by a difference in the preferential conformation of the compounds.[13] When DMAEMA (or DMAEIB, or DMAEA) adapts a cyclic conformation instead of a stretched conformation, the free electron pair of the amino group is somewhat delocalized by an interaction with the ester carbonyl and thus less accessible for protonation (Figure 2).[14] This results in a lower pK_a.

Figure 2. Ring conformation of DMAEMA (R: $C(CH_3)=CH_2$) or DMAEIB (R: $CH(CH_3)_2$).

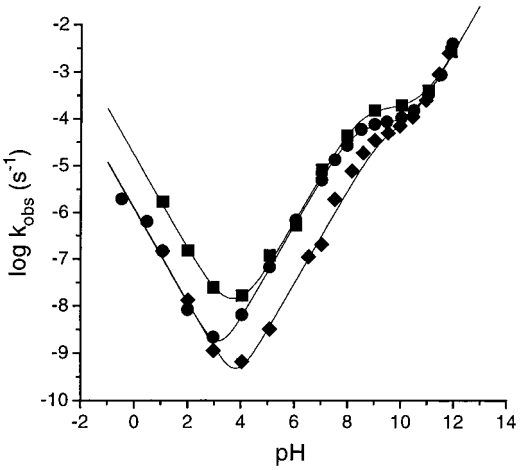

Figure 3. log k_{obs}-pH profile of DMAEMA (●), DMAEIB (■) and DMAPMA (◆), measured at 37 °C. The curves are fitted to eq 2, and the macro- and microconstants are shown in Table 2.

Table 1 shows that the average pK_a of p(DMAEMA) (=pH at which 50% of the dimethylamino groups are protonated) is 0.6−1 pH units below the pK_a of DMAEMA and DMAEIB. The presence of protonated side chains reduced the protonability of the remaining dimethylamino groups, a phenomenon which has been reported before for polymers.[15] With increasing molecular weight, the average pK_a value slightly decreased, indicating more interference of neighboring groups.

Hydrolysis of DMAEMA, DMAEIB, and DMAPMA. In the studied H_0/pH ranges, the concentrations of DMAEMA, DMAEIB, and DMAPMA decreased according to pseudo-first-order kinetics. Sampling was done over a range of 2−3 times the estimated half-life. For each degradation reaction at a fixed pH, a plot was made of the natural logarithm of the residual compound fraction vs time. The reaction rate constant k_{obs} was calculated from the slope of this plot. The intraday and interday variation in k_{obs} of DMAEMA were determined under standard degradation conditions at pH 9.0, and were found to be 0.2% and 3.4% respectively. The intraday variation is usually higher and typically on the order of 1−5%, and the interday variation is on the order of 5−10%.[16,17]

The log k_{obs}−pH profiles of DMAEMA, DMAEIB, and DMAPMA are shown in Figure 3, and were fitted according to a model developed by Van der Houwen et al.,[18] which accounts for changes in k_{obs} caused by protolytic equilibria. When protolytic equilibria occur, certain parts of the pH profile cannot be ascribed to a single reaction, but to a series of kinetically indistinguishable reactions. Depending on the degree of (de)-

Table 2. Macroreaction Constants (measured at 37 °C) and Calculated Microreaction Constants (±SE) of DMAEMA, DMAPMA, and DMAEIB

macroreaction constant	DMAEMA	DMAPMA	DMAEIB
M_0 (M^{-1} s^{-1})	$(1.2 \pm 0.2) \times 10^{-6}$	$(1.2 \pm 0.3) \times 10^{-6}$	$(17 \pm 3) \times 10^{-6}$
M_1 (s^{-1})	$<10^{-9}$	$<10^{-10}$	$(7.9 \pm 3.4) \times 10^{-9}$
M_2 (M s^{-1})	$(5.4 \pm 0.6) \times 10^{-13}$	$(0.31 \pm 0.05) \times 10^{-13}$	$(6.4 \pm 0.8) \times 10^{-13}$
M_3 (M^2 s^{-1})	$(1.9 \pm 0.6) \times 10^{-23}$	$(0.13 \pm 0.10) \times 10^{-23}$	$(1.0 \pm 0.4) \times 10^{-23}$
pK_a	8.2 ± 0.2	9.3 ± 0.4	8.4 ± 0.2

microreaction constant	DMAEMA	DMAPMA	DMAEIB
k_{DH}^{H} (M^{-1} s^{-1})	$(1.2 \pm 0.2) \times 10^{-6}$	$(1.2 \pm 0.3) \times 10^{-6}$	$(17 \pm 3) \times 10^{-6}$
k_{DH}^{s} (s^{-1})[a]	$<10^{-9}$	$<10^{-10}$	$(7.9 \pm 3.4) \times 10^{-9}$
k_{DH}^{OH} (M^{-1} s^{-1})[a]	23 ± 2	1.3 ± 0.2	27 ± 3
k_{D}^{OH} (M^2 s^{-1})	0.21 ± 0.06	0.043 ± 0.032	0.085 ± 0.034

[a] Obtained by neglecting the other kinetically indistinguishable reaction.

protonation, different species are present, which degradations are defined by their own micro reaction constants k_0, k_H, and k_{OH}. The microreaction constants can be combined to macroreaction constants M_x. DMAEMA, DMAEIB, and DMAPMA have one ionizable function in the pH range studied. This results in two species: the protonated form, DH^+, and the deprotonated form, D. Therefore, the log k_{obs}−pH profiles of these compounds were fitted using eq 2 and are shown

$$k_{obs} = \frac{M_0[H^+] + M_1 + \frac{M_2}{[H^+]} + \frac{M_3}{[H^+]^2}}{1 + \frac{K_a}{[H^+]}} \qquad (2)$$

in Figure 3. K_a is the proton dissociation constant of the compound, and M_0 to M_3 represent eqs 3−6,

$$M_0 = k_{DH}^{H} \qquad (3)$$

$$M_1 = k_{D}^{H} K_a + k_{DH}^{s} \qquad (4)$$

$$M_2 = k_{D}^{s} K_a + k_{DH}^{OH} K_w \qquad (5)$$

$$M_3 = k_{D}^{OH} K_a K_w \qquad (6)$$

respectively. Superscripts H, S, and OH refer to the proton-, solvent-, and hydroxyl-catalyzed reactions respectively, K_w is the autoprotolysis constant of water (which is 2.37×10^{-14} M^2 at 37 °C[19]). In Table 2 the macro- and microreaction constants of DMAEMA, DMAPMA and DMAEIB are shown.

At pH 0.5−3, specific proton acidic ester hydrolysis occurred. The k_{DH}^{H} values were derived from eq 3. Table 2 shows that the k_{DH}^{H} values of DMAEMA and DMAPMA are a magnitude of order lower than the k_{DH}^{H} of DMAEIB. In this pH range, the unsaturated DMAEMA and DMAPMA were more resistant toward hydrolysis than the saturated DMAEIB. The conjugated double bond in DMAEMA and DMAPMA stabilizes the ground state through resonance stabilization. As a result, the activation energy necessary to reach the transition state increases, which may be the reason for the slower hydrolysis of DMAEMA and DMAPMA at acidic pH.[20a] This is in accordance with Van Dijk et al., who reported that methyl methacrylate was more stable at low pH in comparison with its saturated analogue methyl isobutyrate.[21] The extra CH_2 group in DMAPMA (compared to DMAEMA) had no influence on the hydrolytic stability.

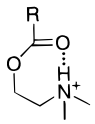

Figure 4. Ring conformation of protonated DMAEMA (R: $C(CH_3)=CH_2$) or DMAEIB (R: $CH(CH_3)_2$).

At pH 3−4, the mechanisms described by M_1 occur: degradation occurs via proton-catalyzed hydrolysis of the deprotonated compound and solvent hydrolysis of the protonated compound. Assuming that $(k_D^H K_a) \ll k_{DH}^s$, values for k_{DH}^s could be calculated. However, due to the low hydrolysis rates, the errors in the values for M_1 and k_{DH}^s are relatively high which indicates that the processes described by M_1 did not contribute significantly to the degradation. Therefore, only upper limits can be estimated, which are 10^{-9}, 10^{-10}, and 1×10^{-8} for DMAEMA, DMAPMA, and DMAEIB respectively (Table 2).

At pH 4−8, k_{obs} is mainly determined by M_2: degradation occurs via hydroxyl hydrolysis of the protonated compound and solvent catalyzed hydrolysis of the deprotonated compound. With the assumption $(k_D^s K_a) \ll (k_{DH}^{OH} K_w)$, an estimated value for k_{DH}^{OH} was calculated (Table 2). In this pH range, DMAPMA was more stable than DMAEMA and DMAEIB toward hydrolysis. A possible explanation is that the DMAE group is able to approach the carbonyl oxygen (Figure 4), rendering the ester group more susceptible to nucleophilic attack of a hydroxyl ion. In DMAPMA, this coordination might be hindered by the extra CH_2 group. In the literature, for 3-(dimethylamino)propyl methanoate and for 2-(dimethylamino)ethyl methanoate, similar differences in hydrolysis rate have been described and supported with IR measurements.[13] FTIR spectra of DMAEMA and DMAPMA in $CHCl_3$ show a C=O stretch vibration at 1714 cm^{-1}. When these compounds were in the protonated form, the C=O stretch vibrations shifted. However, the shift of C=O in DMAEMA·HCl (1725 cm^{-1}) was greater than the shift of C=O in DMAPMA·HCl (1719 cm^{-1}). This could mean that intramolecular interactions are indeed more pronounced in DMAEMA as compared to DMAPMA.

M_3 describes the mechanism that takes place at pH > 10, where the deprotonated species predominate and hydroxyl-catalyzed hydrolysis[20b] most frequently takes place. Table 2 shows that, for the deprotonated com-

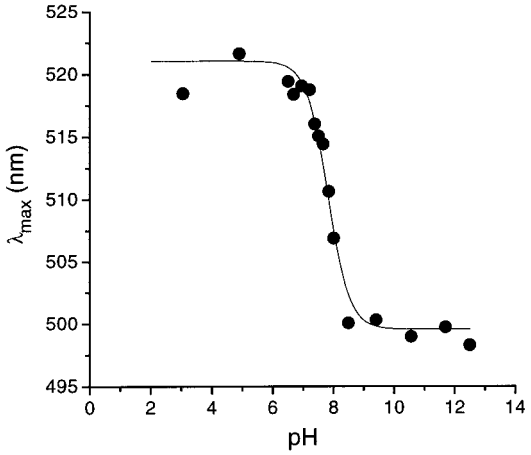

Figure 5. Wavelength of emission maximum (λ_{max}) of p(DMAEMA-*co*-DaEMam) as a function of the pH (25 °C). The curve is fitted to eq 1.

pounds, k_{DH}^{OH} values are about a factor 10−300 greater than k_D^{OH} values. This can be explained by the electrostatic attraction between the protonated amino groups and the hydroxyl ions at pH 4−8. However, at pH ≫ pK_a, this interaction does not occur, resulting in a lower k_D^{OH} value.

From the fits of the pH profiles, the protolytic dissociation constants (K_a) were calculated (Table 2). The values obtained in this way agree very well with the pK_a values determined by acid−base titration (Table 1).

Fluorescence Measurements. A DMAEMA polymer in which the fluorophoric dansyl moiety was incorporated was synthesized via copolymerization of DMAEMA with DaEMam.

The wavelength of the emission maximum (λ_{max}) of polymer bound DaEMam shifted to a lower value with increasing pH (blue shift or Stokes' shift, Figure 5). This Stokes' shift of emission spectra is caused by changes in the polarity (or in the dielectric constant) of the environment of the fluorophore.[22] Since the λ_{max} of the fluorophore alone was independent of the pH in the pH range 5−12 (pK_a = 3.8 ± 0.1), the observed differences in fluorescence characteristics of p(DMAEMA-*co*-DaEMam) must be caused by differences in the dielectric constant of the microenvironment of the dansyl group. If the curves of Figure 5 are fitted to a modified Henderson−Hasselbalch equation (eq 1), the average pK_a of p(DMAEMA-*co*-DaEMam) could be estimated to be 7.8 ± 0.1, which is in good agreement with the average pK_a value determined by acid−base neutralization (Table 1).

The dielectric constant of the environment of the dansyl group in p(DMAEMA-*co*-DaEMam) can be estimated as a function of the pH, by comparing Figure 5 with a calibration curve (not shown) of λ_{max} of the free fluorophore DaEMam as function of the dielectric constant (ϵ_r) of the solvent (different dioxane/water mixtures): when the tertiary amines of the p(DMAEMA-*co*-DaEMam) were fully protonated (at pH ≪ average pK_a) the dansyl group was fully exposed to water, and when the amines were neutral (pH ≫ average pK_a), this group was almost fully excluded from water. At high pH, the dansyl group was most likely trapped into the collapsed polymer, and experienced an environment (the polymer backbone) with a very low dielectric constant ($\epsilon_r \approx 7$). It might be hypothesized that the dielectric constant in the environment of the polymer backbone (including the ester groups) is not dependent on the pH, since the hydrophilic protonated amino group is to a certain extent positioned away from the polymer backbone.

In addition, the pK_a of the monomer, DMAEMA, was determined in different dioxane/water mixtures. It was found that the pK_a decreased from 8.2 at $\epsilon_r = 78$ to 6.5 at $\epsilon_r = 9.5$. Therefore, besides the explanation given in the literature,[15] the lower dielectric constant of the microenvironment of the polymer backbone might be an alternative explanation for the lower average pK_a value of the polymer compared to the pK_a value of the monomer.

Degradation of p(DMAEMA) and p(DMAEA). The stabilities of p(DMAEMA) and p(DMAEMA) complexed to the plasmid pCMV-*LacZ*[23] were studied in the pH range 1−11 at 37 °C. However, after 6 months incubation time, no detectable amounts of the degradation product DMAE−OH were found in both cases, demonstrating that the ester was orders of magnitude less sensitive to hydrolysis than the monomer. In a previous study it also was shown that the hydrolytic stability of the methacrylate ester in methacrylated dextran was strongly increased after polymerization.[24]

On the basis of the fluorescence measurements, a lower dielectric constant in the environment of the polymer backbone (including the ester) compared to the dielectric constant in the bulk was suggested. Therefore, the hydrolysis of DMAEMA in water/dioxane mixtures with varying compositions (and thus varying dielectric constant) was studied. In aqueous solution pH 11 ($\epsilon_r \approx 78$), k_{obs} was 2.95×10^{-4} s^{-1}. When dioxane was present in a concentration of 70% w/w dioxane/(buffer pH 11) ($\epsilon_r \approx 18$), k_{obs} was 4.96×10^{-6} s^{-1}, which is about 60 times smaller. Indeed, the lower dielectric constant might be an explanation for the substantially lower hydrolysis rate of the ester in the polymer.

To accelerate the hydrolysis of the ester groups in the polymer, degradation experiments under more extreme conditions (80 °C and pH 1 or 7) were performed with p(DMAEMA). The hydrolysis of p(DMAEA), a more hydrophilic acrylate analogue of p(DMAEMA) was also evaluated, which was expected to be less stable to hydrolysis than the polymethacrylate.[25] As a control, the stabilities of DMAEA and DMAEMA were evaluated at pH 1.3 and 7. Table 3 shows that at both pH values, DMAEA is a factor of 5−7 less stable to hydrolysis than DMAEMA.

Figure 6 shows that even at high temperature p(DMAEMA) was quite stable; after 12 days only ±4% and 15% of the side groups were hydrolyzed at pH 1 and 7, respectively. On the other hand, the more hydrophilic p(DMAEA) degraded faster under these conditions (about 10% and 45% after 1 h at pH 7 and at pH 1, respectively) as expected. Interestingly, the degradation

Table 3. k_{obs} (s^{-1}) of DMAEMA, DMAEA, P(DMAEMA), and P(DMAEA) at Different pH Values and Temperatures

	aged at 37 °C		aged at 80 °C		estimated for 37 °C	
pH	DMAEMA	DMAEA	p(DMAEMA)	p(DMAEA)	p(DMAEMA)	p(DMAEA)
1.3	2.3×10^{-8}	1.6×10^{-7}	1×10^{-8}	5×10^{-5}	3×10^{-10}	1×10^{-8}
7.0	5.2×10^{-6}	3.0×10^{-5}	7×10^{-8}	1×10^{-6}	2×10^{-9}	3×10^{-8}

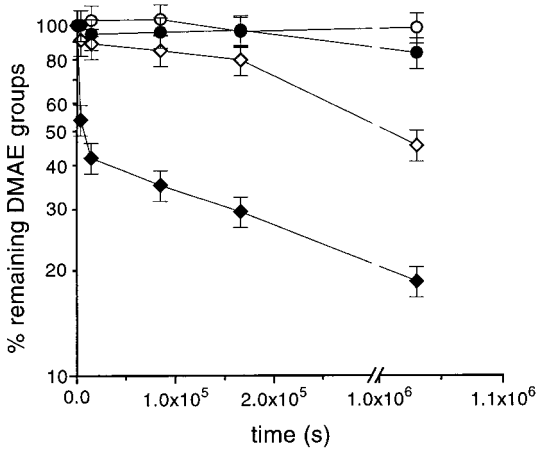

Figure 6. Degradation of p(DMAEMA) (circles) and p(DMAEA) (diamonds) at 80 °C and pH 1 (closed symbols) or pH 7 (open symbols).

rate of p(DMAEA) at pH 7 decreased with time. This can be explained by the formation of acrylic acid groups which resulted in a decrease in the overall positive charge of the polymer. As a result, the electrostatic attraction of hydroxyl ions decreased, which resulted in a slower hydrolysis of remaining ester groups. From the curves, values for k_{obs} were estimated. Taking into account that k_{obs} is reduced ~2.5-fold with a temperature decrease of 10 degrees, an estimation of the k_{obs} value at 37 °C can be made (Table 3). Comparing the estimated values for the polymers and measured k_{obs} values of the monomers at pH 1 and pH 7, reveals that the hydrolytic stability of both DMAEMA and DMAEA have increased substantially after polymerization (about 10−100 fold at pH 1 and 1000−3000-fold at pH 7).

Conclusions

This study shows that the ester in DMAEMA is rather unstable toward hydrolysis (at pH 7.4 and 37 °C, $t_{1/2}$ = 17 h). However, after polymerization the ester groups in the polymer are quite insensitive toward hydrolysis, even under more extreme condition (80 °C, pH 1 and 7). This might be explained by the low dielectric constant in the microenvironment of the polymer backbone (including the ester groups) as demonstrated by fluorescence and pK_a measurements.

These data show that, by polymerization of monomers, completely new characteristics evolve which have large consequences for stability, due to changes in dielectric constant in the vicinity of the polymer backbone.

References and Notes

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