J Mater Sci: Mater Med (2008) 19:1453–1458
DOI 10.1007/s10856-007-3315-z

New stimuli-responsive polymers derived from morpholine and pyrrolidine

Diego Velasco · Carlos Elvira · Julio San Román

Received: 7 September 2007 / Accepted: 16 October 2007 / Published online: 29 November 2007

Abstract The preparation of three new ionizable monomers: *N*-ethyl morpholine metacrylate (EMM), *N*-ethyl morpholine methacrylamide (EMA) and *N*-ethyl pyrrolidine metacrylamide (EPA) and their respective homopolymers poly-EMM, poly-EMA and poly-EPA prepared by radical polymerization in solution, is described. The systems have been characterized by NMR and FTIR spectroscopic techniques, determined their glass transition temperatures by DSC and their respective pKs. Moreover, crosslinked samples were prepared by bulk polymerization using *N,N*-methylene bisacrylamide (BAam) and the trifunctional 1,3,5-triacryloylhexa-hydro-1,3,5-triazine (135-T) as crosslinkers. The studies of swelling kinetics were carried out in different pH buffer solutions (2, 7.4 and 10) in a thermostatic bath at 37 °C showing hydration degrees that go from 2,600% to about 200% depending on the pH and on the crosslinker used. The systems seem to be suitable for the preparation of smart hydrogels for drug delivery and Tissue Engineering.

1 Introduction

Stimuli-responsive polymers are defined as systems that undergo relatively to abrupt chemical or physical changes in response to small external changes in the environmental conditions [1]. pH responsive conformations with solubility changes is a common behavior in biopolymers [2]. In this sense, pH responsive polymers are characterized of having ionizable pendant groups that can accept or donate protons, being cationic or anionic respectively. Cationic polymers are very interesting not only because of their pH sensitivity [3] and their possible applications in drug delivery, but also due to their ability to bind or complex with other systems with anionic character [4, 5]. In this sense, cationic polymers have been evaluated as alternative vectors to virus in gene therapy [6]. Cationic lipids and polymers such as poly(L-lysine) [7], poly(ethylene imine) [8], chitosan [9, 10] and poly(dimethylamino ethyl) methacrylate [11], are under investigation as DNA delivery systems. Some studies have been focussed on the nature and influence of the charge of the cationic groups on the transfection efficiency from biodegradable polyphosphoramidates [12]. However, improvements are necessary as the present generation of non-viral carriers is far less efficient in transfection than viral vectors. In this sense, in a previous work we prepared a methacrylic derivative from ethylpyrrolidine that exhibited both temperature and pH responsiveness [13], and also has shown complexation ability with DNA. In this article the preparation of three hydrophilic ionizable polymers derived from the ionizable groups pyrrolidine and morpholine is described. The new ionizable polymers are the methacrylic derivative of ethyl morpholine, poly-EMM, and two methacrylamides, poly(*N*-ethyl morpholine methacrylamide), poly-EMA, poly(*N*-ethyl pyrrolidine methacrylamide), poly-EPA (see Fig. 1). The morpholine derivatives present a tertiary amine group, which is ionizable and therefore sensitive to the pH, but in addition the presence of a oxygen in the heterocycle provides a high hydrophilicity to the polymeric system, and probably a very positive contribution to the biocompatibility of the whole system.

D. Velasco · C. Elvira · J. San Román (✉)
Department of Biomaterials, Institute of Polymer Science and Technology, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain
e-mail: jsroman@ictp.csic.es

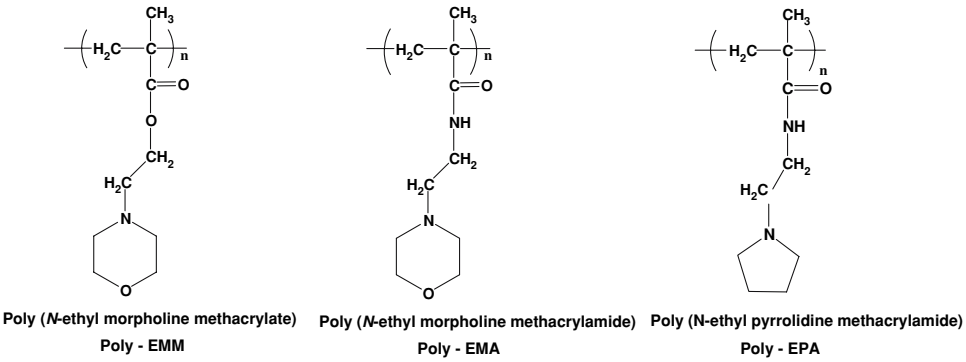

Fig. 1 Chemical structures of poly(*N*-ethyl morpholine metacrylate) (poly-EMM), poly(*N*-ethyl morpholine methacrylamide) (poly-EMA) and poly(*N*-ethyl pyrrolidine metacrylamide) (poly-EPA)

Polymers containing morpholine or *N*-acryloylmorpholine have shown excellent properties as soluble supports in the synthesis of oligonucleotides [14], and have been applied as copolymers with *N*-acryloylsuccinimide for the formation of complexes with oligonucleotides [15], and also in the preparation of amphiphilic copolymers with *tert*-butylacrylamide in DNA detection [16]. Synthesis and characterization of the morpholine polyacrylic systems, as well as their pH sensitivity in terms of pKb determination are described. Their swelling behavior has been evaluated at different pHs using crosslinked polymers. Crosslinkers with different functionalities have been used in the preparation of the pH sensitive hydrogels showing significant differences in the equilibrium hydration degrees. Evaluation of their complexation with DNA, and the possibilities of application of supercritical carbon dioxide technologies will be considered for new applications of the systems described and analysed in this article.

2 Materials and methods

4-(2-Hydroxyethyl) morpholine (Fluka), 4-(2-aminoethyl) morpholine (Aldrich), *N*-(2-aminoethyl) pyrrolidine (ABCR) and ammonium persulphate (APS) (Aldrich) were used as received. Methacryloyl chloride (Fluka) and triethylamine (Scharlau) were distilled and freshly used (b.p. = 99 °C and 89 °C, respectively). *N,N*-Methylene bisacrylamide (Aldrich) and 1,3,5-triacryloylhexa-hydro-1,3,5-triazine (Aldrich) were used as received.

2.1 Synthesis of monomers and polymers

The new monomers were synthesized by reacting the corresponding hydroxyl and amine derivatives with methacryloyl chloride at low temperature and in the presence of triethylamine.

2.1.1 Synthesis of EMM

N-Ethyl morpholine methacrylate (EMM) was synthesized by using the following procedure: to a solution of 10 g of 4-(2-hydroxyethyl) morpholine (0.076 mol) in chloroform (75 mL) with 10.6 mL (0.076 mol) of triethylamine, a solution of 9.5 g (0.09 mol) of methacryloyl chloride in 25 mL of chloroform was added dropwise at 0 °C under N_2 atmosphere with magnetic stirring. After 6 h of reaction solution was washed four times with aqueous NaOH (5 wt%), dried over anhydrous Na_2SO_4 and then removed the solvent at reduced pressure.

2.1.2 Synthesis of EMA

Similarly the monomer *N*-ethyl morpholine methacrylamide (EMA) was synthesized as EMM by using the following amounts of reagents: 10 g of 4-(2-aminoethyl) morpholine (0.077 mol), 10.7 mL (0.077 mol) of triethylamine and 8.8 g (0.08 mol) of methacryloyl chloride.

2.1.3 Synthesis of EPA

Similarly the monomer *N*-ethyl pyrrolidine methacrylamide (EPA) was synthesized as EMM by using the following amounts of reagents: 10 g of *N*-(2-aminoethyl) pyrrolidine (0.087 mol), 12.2 mL (0.087 mol) of triethylamine and 10.1 g (0.10 mol) of methacryloyl chloride.

The corresponding monomers EMM, EMA and EPA were homopolymerized at 50 °C under oxygen-free N_2 atmosphere in mixtures of water/isopropanol ([monomer] = 1 mol/L) using APS (1.5×10^{-2} mol/L) as radical initiator. After desired reaction time polymer samples were dialysed against water in a Spectra/Por® (Spectrum Laboratories Inc.) using membranes with a molecular weight cut-off 3,500 and then lyophilized. Average molecular weight and molecular weight distributions were determined

by size exclusion chromatography (SEC) by using polymer solutions (5 mg/mL) in *N,N*-dimethyl formamide (DMF). Measurements were carried out at 1 mL/min flow using Ultrastyragel columns of 500, 10^4 and 10^5 Å (Polymer Laboratories) at 70 °C and using a differential refractometer as detector. The calibration was performed with poly(styrene) (PS) standards in the range of 2,990–1,400,000 D and polydispersity values lower than 1.1.

2.2 Spectroscopic characterization

The monomers and the respective homopolymers were characterized by 1H Nuclear Magnetic Resonance spectroscopy, NMR. Spectra were recorded in 5% deuterated chloroform ($CDCl_3$) solutions on a Varian XLR-300 spectrometer. Fourier Transformed Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) was performed on a Perkin–Elmer 1720 Spectrometer (4 cm^{-1}, 32 scans).

2.3 Thermal analysis

The glass transition temperatures, Tg, of the polymers were determined by Differential Scanning Calorimetry, DSC, using a Perkin–Elmer DSC-7 calorimeter. Typical sample weights were 6–8 mg. Thermal transition temperature measurements were conducted by heating the samples from −60 to 200 °C at 10 °C/min. All the systems were analysed in triplicate.

2.4 Determination of the dissociation constants (pKb)

The pKb of the corresponding monomers and homopolymers were determined by acid–base titration of a 100 mg of monomer or homopolymer solution in a 25 mL aqueous 0.1 M NaCl. Diluted aqueous solutions of NaOH were used to complete the titration using small volumes in order to avoid the modification of the ionic strength. About 1–3 mL of a 0.1 N HCl solution was added to ensure the ionization of the amine groups of the homopolymers. The changes of pH were measured with a Schott GC841 pHmeter. Determination of the corresponding compounds were carried out in triplicate. The diagrams drawn in Fig. 2 are the results of the average of the values obtained in three separate experiments. The standard deviation is lower than 3% in all the cases.

2.5 Preparation of crosslinked samples and swelling studies

The crosslinked polymers were prepared by bulk polymerization in Teflon moulds of the corresponding

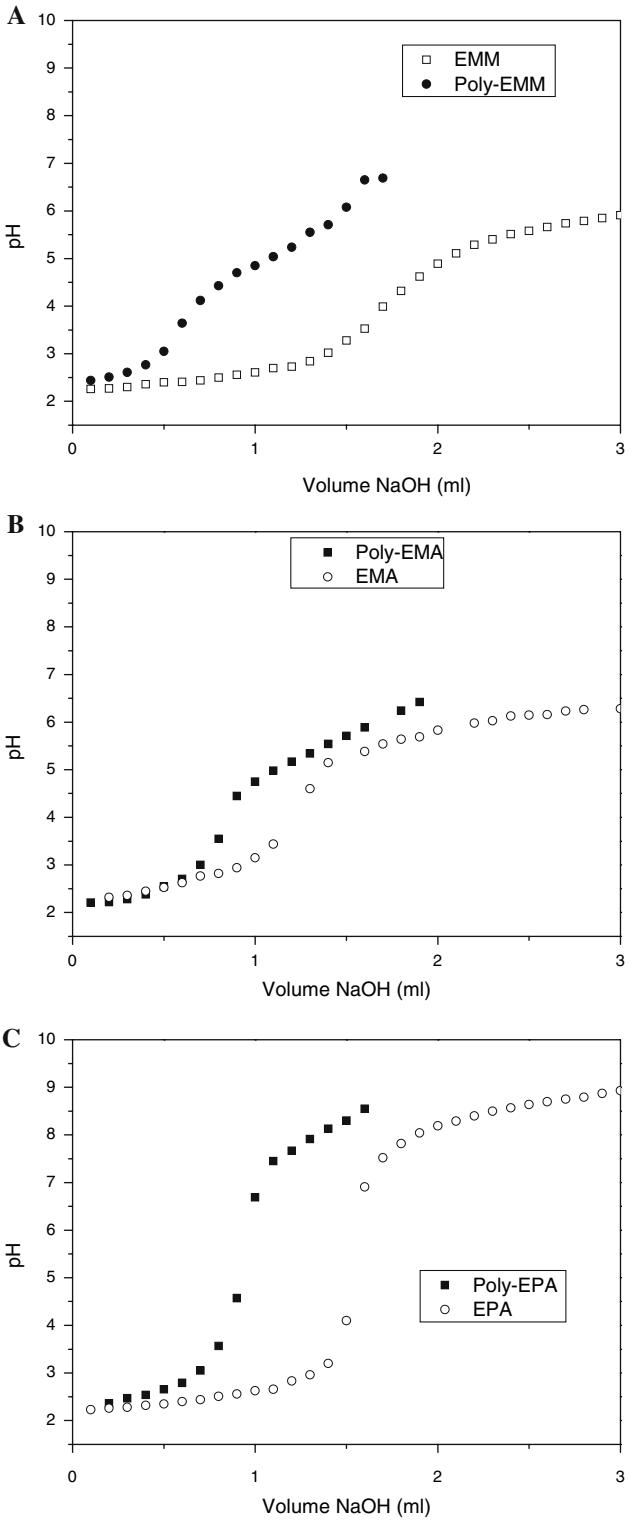

Fig. 2 Titration diagrams for the determination of pKb, pH versus NaOH volume of (**a**) EMM and poly-EMM, (**b**) EMA and poly-EMA and (**c**) EPA and poly-EPA

monomers in the presence of two different crosslinker (2 wt%), the difunctional *N,N*-methylene bisacrylamide (B-Aam) and the trifunctional 1,3,5-triacryloylhexa-hydro-

1,3,5-triazine (135-T) and using ammonium persulfate as initiator (2 wt%) . The prepared polymer films were washed with water in order to remove residual monomers and then were vacuum dried. Swelling studies were performed on crosslinked hompolymers obtained by bulk polymerization in Teflon moulds with bis-acrylamide or 135-T as crosslinkers, using APS as initiator. The obtained polymer films were immersed in buffer solutions (pH 2, 7.4, 10) at 37 °C weighing them at different times until equilibrium was attained. The experiments were carried out by measuring the weight gain as a function of immersion time in 10 mL of buffer solution. Measurements were taken until equilibrium hydration degree was reached, considered when three consecutive determinations gave the same weight (±0.001 g). All the experiments were followed with three independent samples, being the results the average of all the data collected. The deviation of data in all the cases is lower than 2% and it is indicated in the corresponding diagrams.

3 Results and discussion

3.1 Preparation of the polymers

The yield of the monomers synthesis was about 65% whereas the homopolymers was 85%. Average molecular weight M_w determined by SEC were: 69,400 for poly-EMM, 121,900 for poly-EPA and 105,120 for poly-EMA, with a polydispersity of 2.6, 1.5 and 1.6, respectively.

3.2 Spectroscopic characterization

^{1}H-NMR Signals assignments ($CDCl_3$) (ppm) and FTIR signal assignments, stretching vibrations, υ (cm^{-1}) (see also Fig. 1):

EMM. ^{1}H-NMR:(CH_2=) 6.1 and 5.55, (αCH_3) 1.9, (OCH_2) 4.28, (CH_2N) 2.65, (CH_2NCH_2 cycle) 2.5, (CH_2OCH_2 cycle) 3.7. IR: υ (C–H) 2959–2810, υ (C=O) 1716, υ (C=C) 1637.

Poly-EMM. ^{1}H-NMR: (CH_2–C) 1.8, (αCH_3) 1 and 0.8, (OCH_2) 4.1, (CH_2N) 2.7, (CH_2NCH_2 cycle) 2.55, (CH_2OCH_2 cycle) 3.7. IR: υ (C–H) 2954–2810, υ (C=O) 1724, υ (O=CH_2) 1150, υ (N–CH_2) 1244.

EMA. ^{1}H-NMR: (CH_2=) 5.7 and 5.35, (αCH_3) 1.99, (NH) 6.44, ($NHCH_2$) 3.45, (CH_2N) 2.55, (CH_2NCH_2 cycle) 2.45, (CH_2OCH_2 cycle) 3.71. IR: υ (NH) 3297, υ (C–H) 2970–2812, υ (C=O) 1650, υ (C=C) 1615.

Poly-EMA. ^{1}H-NMR: (CH_2–C) 1.75, (αCH_3) 1.05 and 0.95, (NH) 6.62, ($NHCH_2$) 3.25, (CH_2N) 2.5, (CH_2NCH_2 cycle) 2.5, (CH_2OCH_2 cycle) 3.75. IR: υ (NH) 3361, υ (C–H) 2970–2812, υ (C=O) 1633, υ (O–CH_2) 1142, υ (N–CH_2) 1202.

EPA: (CH_2=) 5.7 and 5.35, (αCH_3) 1.98, (NH) 6.48, ($NHCH_2$) 3.44, (CH_2N) 2.65, (CH_2NCH_2 cycle) 2.55, (CH_2CH_2 cycle) 1.8. υ (NH) 3331, υ (C–H) 2963–2788, υ (C=O) 1655, υ (C=C) 1613.

Poly-EPA. ^{1}H-NMR: (CH_2–C) 1.8, (αCH_3) 1.1 and 0.98, (NH) 6.9, ($NHCH_2$) 4.25, (CH_2N) 3.3, (CH_2NCH_2 cycle) 2.6, (CH_2CH_2 cycle) 1.8. IR: υ (NH) 3346, υ (C–H) 2964–2814, υ (C=O) 1630, υ (O–CH_2) 1141, υ (N–CH_2) 1203.

3.3 Thermal analysis

The Tgs of the corresponding polymers, higher than 37 °C in all cases, were found to be 130 °C for poly-EMA, 46 °C for poly-EMM and 141 °C for poly-EPA, much higher in the case of the methacrylamides compared to their corresponding methacrylates (the methacrylate derivative of ethyl pyrrolidine has a Tg of 27 °C), due to the rigidity associated to the amide bonds as well as the possible formation of intra- and intermolecular hydrogen bonding.

3.4 pK_a

The ionizable character of the prepared polymers was studied by the determination of the dissociation constants pKb, also determined for the monomers (see Fig. 2). The presence of tertiary amine groups in the systems prepared confers the partial or total ionization of the polymer with pK_b values that between 3.5 and 5.6. The monomers pK_b values obtained were 4.2 for EMA, 4.0 for EMM and 5.2 for EPA. The pKb of their respective homopolymers were 4.1, 3.6 and 5.6 indicating that both monomers and the respective polymers will be ionised an acidic pH as is expected due to their cationic character. The macromolecular nature does not seem to affect the ionization parameters of the molecules linked to the polymer entity respect to the free monomers. This can be expected because of the functional groups are located in the side substituents of the polymer chains, but it is clear that the interactions between neighbouring groups, have not influence on the ionization process of the corresponding functions.

3.5 Swelling studies

Figure 3 shows the swelling isotherms at 37 °C, of the poly-EMA crosslinked with B-Aam, poly-EMM crosslinked with 135-T and poly-EPA with B-Aam at different pH as examples of the performed swelling kinetics studies, where is also reflected the pH sensitivity of the prepared polymer systems, similar at physiological and basic pH.

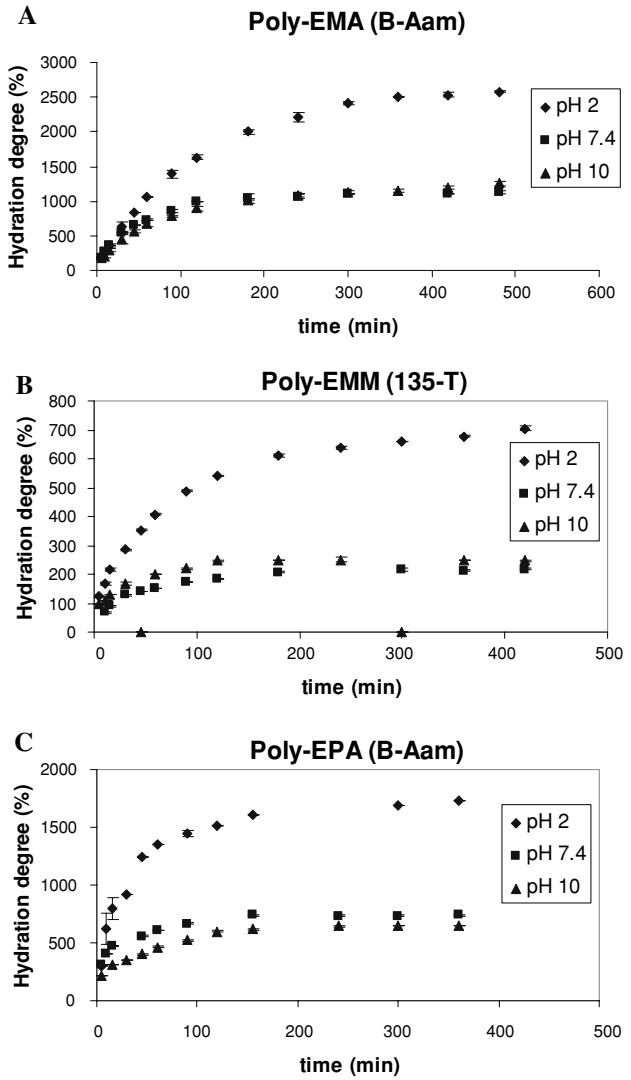

Fig. 3 Swelling isotherms at 37 °C of (**a**) poly-EMA (B-Aam), (**b**) poly-EMM (135-T) and (**c**) poly-EPA (B-Aam)

In order to know the transport mechanism, the initial swelling data was fitted using the following equation [17]:

$$\frac{M_t}{M_{\text{eq}}} = kt^n \tag{1}$$

where M_t is the mass of water uptake at time t, M_{eq} is the equilibrium water uptake, and k and n are constants, which are characteristic parameters of the specific (bioactive agent/dissolution medium) system. A value of $n = 0.5$ indicates Fickian diffusion and $n = 1$ implies case II transport; values of n between these limits define anomalous or non-Fickian transport, where both diffusion and polymer relaxation control the overall rate of water uptake simultaneously. The values of diffusion exponent n are presented in Table 1. It was found that n is closed to 0.5 showing Fickian behavior in most of the hydrogels with the exceptions of poly-EPA (B-Aam) and poly-EMM (135-T)

Table 1 Diffusional exponent n (calculated from equation 1) for water transport of the prepared hydrogels with different crosslinkers (temp. 37 °C)

Crosslinker	Polymer	pH 2	pH 7.4	pH 10
B-Aam	Poly-EMM	0.62 ± 0.02	0.42 ± 0.01	0.41 ± 0.02
	Poly-EMA	0.71 ± 0.02	0.54 ± 0.02	0.55 ± 0.02
	Poly-EPA	0.55 ± 0.05	0.25 ± 0.01	0.31 ± 0.01
135-T	Poly-EMM	0.46 ± 0.01	0.37 ± 0.03	0.28 ± 0.01
	Poly-EMA	0.53 ± 0.02	0.46 ± 0.02	0.42 ± 0.02
	Poly-EPA	0.69 ± 0.04	0.45 ± 0.02	0.46 ± 0.08

at pH 7.4 and 10 that n lies between 0 and 0.5, and some cases at acidic pH (poly-EMA (B-Aam) and poly-EPA (135-T)) that n is in between 0.5 and unity.

Figure 4 shows the swelling at equilibrium at hydrogels for different pH. It can be observed that the highest H% are attained in the polymers crosslinked with B-Aam going from 2,600% at acidic pH to 1,300% at basic pH in

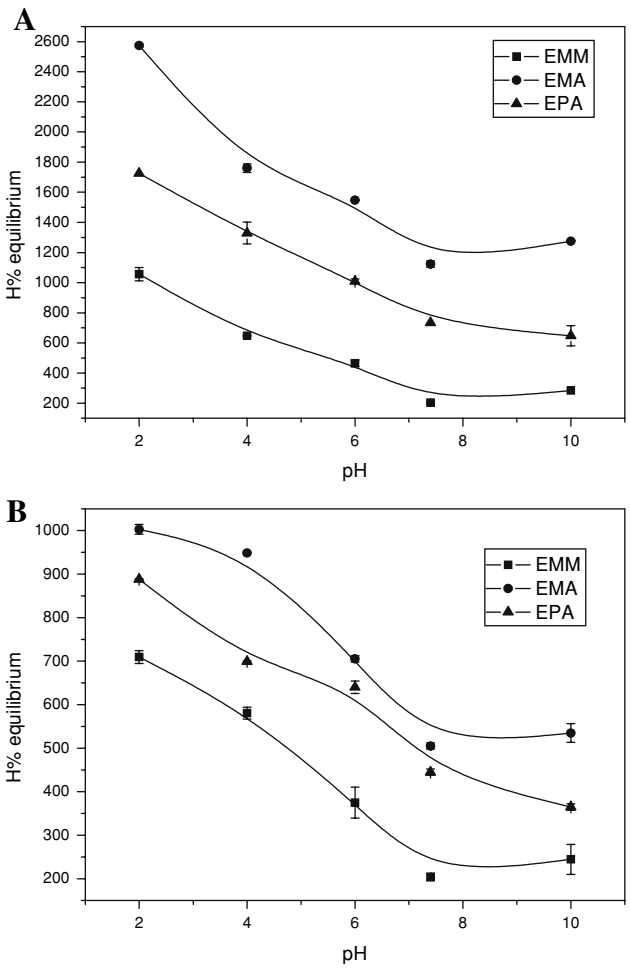

Fig. 4 Swelling at equilibrium versus pH of crosslinked polymers with (**a**) B-Aam, and with (**b**) 135-T

the case of poly(EMA). When polymers were crosslinked with 135-T the H% was lowered as a consequence of the higher crosslinked density that prevents the accessibility of water molecules to the hydrophilic chemical groups (the highest H% is about 1,000 for poly-EMA and the lowest about 200 for poly-EMM). It can also be of interest that the swelling profiles are different depending on the crosslinker. Polymer ionization (at acidic pH) causes higher H% as the polymer is solvated by more water molecules in comparison to non-ionised hydrogel (neutral and basic pH), due to the ionic repulsion of the protonated amine groups which collapses or deswell at higher pH because of the deionization. Poly-EMA is the most swollen polymer due to the presence of also hydrophilic amide and ether groups in its structure, amide groups are also present in poly-EPA system, whereas poly-EMM posses an ester group.

The pH sensitivity of the polymers prepared makes these systems as promising candidates as pH drug delivery carriers, as well as possible vectors for gene therapy. DNA–polymers complexation studies will be reported in the near future.

4 Conclusions

Three new cationic polymers (methacrylic and methacylamides) derived from morpholine and pyrrolidine have been synthesized and characterized. The polymeric systems prepared, were evaluated to pH responsiveness by studying the pKa values of the linear homopolymers and the swelling behavior of crosslinked samples. The hydrogels exhibited swelling profiles dependent not only on the pH, ranging from 2,600% to 200%, but also on the crosslinker used. These systems are candidates for their application as pH drug delivery carriers and as DNA non-viral vectors in gene therapy.

Acknowledgements Financial support from EU project SURFACET, the NoE, EXPERTISSUES, the CICYT project MAT 2004-01654 and the Fundación Domingo Martínez are acknowledged. D. Velasco thanks the grant I3P from the CSIC.

References

1. S. CHATERJI, I. K. KWON and K. PARK, *Prog. Polym. Sci.***32** (2007) 4302
2. E. S. GIL and S. M. HUDSON, *Prog. Polym. Sci.***29** (2007) 1173
3. A. S. LEE, A. P. GAST, V. BÜTÜN and S. P. ARMES, *Macromolecules***32** (1999) 4302
4. S. C. CHEN, Y. C. WU, F. L. MI, Y. H. LIN, L. C. YU and H. W. SUNG, *J. Control Rel.***96** (2004) 285
5. H. STORRIE and D. J. MONEEY, *Adv. Drug Deliv. Rev.***58** (2006) 500
6. T. G. PARK, J. H. JEONG and S. W. KIM, *Adv. Drug Deliv. Rev.***58** (2006) 467
7. E. LAI and J. H. van ZANTEN, *Biophys. J.***80** (2001) 864
8. T. MERDAN, K. KUNATH, H. PETERSEN, U. BAKOWSKY, K. H. VOIGT, J. KOPECEK and T. KISSEL, *Bioconjug. Chem.***16** (2005) 107
9. A. BOZKIR and O. M. SAKA, *Drug Deliv.***11** (2004) 107
10. H. DAI, X. JIANG, G. C. Y. TAN, Y. CHENG, M. TORBENSON, K. W. LEONG and H. Q. MAO, *Int. J. Nanomedi***1** (2006) 507
11. F. J. VERBAAN, P. K. KLOUWENBERG, J. H. VAN STEENIS, J. SNEL, O. BOERMAN, W. E. HENNINK and G. STORM, *Int. J. Pharm.***304** (2005) 185
12. P. C. ZHANG, J. WANG, K. W. LEONG and H. Q. MAO, *Biomacromolecules***6** (2005) 54
13. N. GONZALEZ, C. ELVIRA and J. SAN ROMAN, *Macromolecules***38** (2005) 9298
14. G. M. BONORA, A. BALDAN, O. SCHIAVON, P. FERRUTI and F. M. VERONESE, *Tetrahedron Lett.***37** (1996) 4761
15. C. MINARD-BASQUIN, C. CHAIX, F. D'AGOSTO, M. T. CHARREYRE and C. PICHOT, *J. Appl. Polym. Sci.***92** (2004) 3784
16. B. De LAMBERT, C. CHAIX, M. T. CHARREYRE, A. LAURENT, A. AIGOUI, A. PERRIN-RUBENS and C. PICHOT, *Bioconjug. Chem.***16** (2005) 265
17. J. MACK, T. OKANO, and S. W KIM, in "Hydrogels in Medicine and Pharmacy-Polymers", edited by N. A. PEPPAS, Vol. II (CRC Press, Boca Raton, USA, 1988) p. 65