

# Synthesis and Properties of Novel Cationic, Temperature-Sensitive Block-Copolymers

Smeet Deshmukh, Lev Bromberg, and T. Alan Hatton  
Department of Chemical Engineering  
Massachusetts Institute of Technology, Cambridge, MA 02139

**Abstract**—Facile, one-step synthesis of self-assembling, cationic block copolymers of poly(2-N-(dimethylaminoethyl) methacrylate) (pDMAEMA) and PEO-PPO-PEO (Pluronic®) is developed. The copolymers are obtained via free-radical polymerization of DMAEMA initiated by Pluronic-radicals generated by cerium (IV). The copolymers possess surface activity, are polycationic at pH<7.1, and self-assemble into micelle-like aggregates when neutralized. Potential applications of the novel copolymers for DNA transfection in gene therapy are discussed.

**Index Terms**— poly(2-N-(dimethylaminoethyl) methacrylate), Pluronic®, cerium (IV), free-radical polymerization, self-assembly, gene therapy.

## INTRODUCTION

THE development of gene therapy relies on designing optimal systems for nucleic acid transfer and expression, cationic polymers being a promising alternative to viral vectors [1]. Because the latter are toxic and immunogenic, non-viral strategies toward gene transfection are becoming more and more attractive. A variety of synthetic, polycationic polymers have become available; they demonstrate adequate safety profiles and can complex large plasmids, but their rather low transfection efficiency remains a major drawback [2]. Polycations bind to nucleic acids due to the formation of cooperative systems of salt bonds between the cationic groups of the polycation and phosphate groups of the DNA. Great efforts have been undertaken to improve the efficiency of the polycations, however satisfactorily effective polymer systems are still to be achieved. One of the promising strategies toward creating more effective polycationic systems that complex DNA (forming so-called polyplexes) involves the attachment of hydrophilic polymers such as poly(ethylene oxide) (PEO). The modification of the polyplexes with PEG shields positive surface charges and creates a steric barrier against aggregation with albumin, complement factors, or cellular components in the bloodstream. The modification of such polyplexes may reduce the potential of non-specific interactions, such as opsonization or deposition due to cationic surface charges. Block-copolymers from cationic polymers and PEO form

complexes with DNA that are more stable than complexes of the unmodified polycations and DNA [3,4,5]. Furthermore, copolymers of PEO and poly(propylene oxide) (PPO) such as PEO-PPO-PEO (tradename Pluronic®), when bonded to polycations, provide additional advantages to the resulting copolymers from the efficiency standpoint [6,7]. The Pluronic-polycation copolymers and oligonucleotides spontaneously associate into polyion complex micelles with a hydrophobic core from neutralized polyions and a hydrophilic shell from PEO. Such complexes are sufficiently small, stable in solution despite complete neutralization of charge, and exhibit elevated levels of transgene expression [7].

The present work aimed at development of a novel polycationic system that is capable of self-association at ambient temperatures and yet possesses surface activity. We chose poly[2-(dimethylamino)ethyl methacrylate] (pDMAEMA) as a polycationic component because of its known capacity to complex DNA [8]. Relatively hydrophobic Pluronic L92 (PPO content, 80%) was chosen as a surface-active building block of our copolymers because of its high modification activity toward cell membranes. The synthetic route explored herein involves free-radical polymerization of DMAEMA initiated by the Pluronic-radicals generated by the redox reaction of the Pluronic with cerium (IV). It appears that the facile, one-step synthesis results in desired block-copolymers capable of self-assembly in aqueous solutions.

## EXPERIMENTAL SECTION

### A. Materials

Pluronic® L92 (average composition,  $\text{EO}_8\text{PO}_{52}\text{EO}_8$ ) was received from BASF Corp. and used without further treatment. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 98%), ammonium cerium (IV) nitrate (98.5%), 2,2'-azobisisobutyronitrile (AIBN, 98%), and nitric acid (70%) were all obtained from Aldrich Chemical Co. and were used without further purification. Deuterated solvents were obtained from Cambridge Isotope Laboratories, and all other chemicals were received from commercial sources and were of highest purity available.

### B. Polymer Synthesis

The polymerization with the cerium ion redox system and DMAEMA monomer was carried out in a three-necked flask equipped with a stirrer, a reflux condenser, and a nitrogen inlet/outlet. In a typical polymerization procedure, DMAEMA (5.0 g), Pluronic L92 (5.0 g), and 1 mL of 70% nitric acid

were dissolved in 70 mL of deionized water and the solution was deoxygenated by nitrogen flow and thermostatted at 55 °C while stirring for 1 h. The reaction was started by injecting 3.278 g (6 mmol) of cerium (IV) ammonium nitrate dissolved in 3 mL of 0.5 M HNO<sub>3</sub>. The reaction was allowed to proceed for 8 h under nitrogen flow and stirring. The resulting polymer was precipitated by addition of 1 M NaOH, redissolved in 0.01 M HCl and dialyzed against a large excess of 0.01 M HCl solution in deionized water using pleated dialysis tubing (regenerated cellulose, molecular weight cut-off, 6,000). In order to prove the suggested mechanism of polymerization via formation of polyether-radicals due to the presence of cerium (IV), a control experiment without Ce<sup>4+</sup> was conducted. Namely, a polymerization in a mixture of DMAEMA and Pluronic L92 identical to the described above was induced by the addition of 1 mmol AIBN in acetone. A viscous solution was obtained after the polymerization conducted at 55 °C for 8 h. The solution was exhaustively dialyzed against 0.01 M HCl. It was also observed that on the addition of Ce<sup>4+</sup> solution to DMAEMA in the same range of concentrations used in the above polymerization, no polymer was formed within 8 h at 55 °C, indicating the absence of homopolymerization.

### C. Procedures

Proton NMR spectra of polymer solutions in D<sub>2</sub>O/DCl were acquired on a Bruker AMX400 spectrometer. To obtain the apparent pK<sub>a</sub> of the polymers, bulk titration was performed at 25±1 °C using a 736 GP Titrino potentiometric titration system (Metrohm Ltd, Herisau, Switzerland). Titration of pDMAEMA-L92 in its acidic form was carried out in a degassed 75 mM NaCl solution with 0.1 M NaOH as a titrant, at 0.5 wt% polymer concentration. The titration was carried out slowly for several hours to allow for proper equilibration. The potential at the surface of shear ( $\zeta$ -potential) of the 0.5 wt% salt-free aqueous polymer solutions with pH adjusted to a desired value by adding 1 M NaOH or HCl, was measured using a ZetaPals Zeta Potential Analyzer (Brookhaven Instruments Co.) with built-in software employing the Smoluchowski  $\zeta$ -potential model. A micro DSC III (SETARAM, France) with an ultra-low background noise (<0.2 mW) was employed in the DSC measurements. The liquid samples weighing 70-100 mg were placed in a fluid-tight batch vessel and ran against deionized water as a reference. The heating-cooling cycling scans were performed with scan rates of 1 °C/min. The heat flow vs temperature signal was used to obtain the maximum peak temperature (T<sub>m</sub>). Using PeakFit Version 4.11 (SYSTAT, Inc., Richmond, CA) software, the thermograms were fitted by a linear baseline calculated using the entire temperature range and then subtracted from the signal. Molecular weight determination was accomplished using a Bruker OminiFlex Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometer applying 2,5-dihydroxybenzoic acid as a matrix.

Dynamic light scattering experiments were performed at 25 °C with a Brookhaven Instruments BI-200SM having

Goniometer Alignment software, a BI-9000 Correlator and a Spectra Physics He-Ne Model 127 laser operating at a scattering angle of 90° and a wavelength of incident light of 633 nm at a power of 50 mW. A 10 mL quartz scattering cell was thermostatted using refractive index matching silicone oil, and temperature was controlled to within 0.02 °C.

Surface tension measurements were accomplished using a Kruss USA Model K11 Tensiometer applying Wilhelmy plate method.

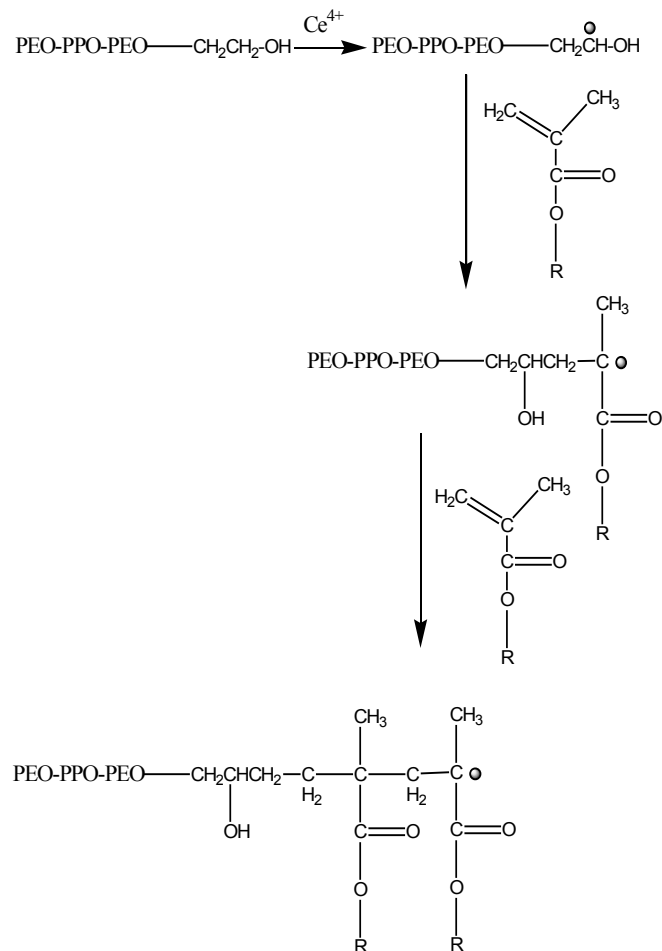
## RESULTS AND DISCUSSION

### D. Polymer Synthesis and Structure

The polymerization of DMAEMA was carried out using a redox system consisting of Ce<sup>4+</sup> ions and Pluronic. The cerium ion-alcohol redox reaction leads to the formation of a radical on the carbon atom linked with the hydroxyl group [9]. It has been shown that in the case of a polymeric alcohol, PEG, as a reducing agent the radical formation takes place at the terminal carbon atom and not along the polyether chain [10]. Drawing an analogy between PEG and PEO-PPO-PEO copolymers, we depicted the polymerization as shown in Scheme 1. In the first step, a redox reaction occurs between Ce<sup>4+</sup> and the -CH<sub>2</sub>OH terminal group of the Pluronic, generating a free radical in a position of the hydroxide. The formed radical is transferred from the polymer chain to the DMAEMA monomer, and the polymerization propagates. Formation of diradicals is possible, but their relative content is very minor, according to the literature data [9-11]. The presence of diradicals, if any, can lead to the formation of copolymers of higher molecular weight, which we did not observe in the present study.

Using <sup>1</sup>H NMR spectroscopy, we were able to verify the structure of the pDMAEMA-L92 copolymers resulting from polymerization (Schemes 1,2). As is seen in Table 1, the proton ratios measured by NMR corresponded to the proposed structure quite well. The average number of DMAEMA units bonded to the one L92 segment was calculated from the ratio of the backbone methylene protons in these units, respectively (Table 1). At y=1 (one Pluronic segment), we obtained the expression  $x/84=0.9/3.3$ , and thus  $x=23$ , i.e., one Pluronic segment is bound to 23 DMAEMA units on average. Using average molecular weight of L92 of 3720, we then obtain the average molecular weight of the pDMAEMA-L92 copolymer to be 7320, the weight ratio of the pDMAEMA-to-L92 in the copolymer to be 50:50, and nitrogen content to be 19.5 wt%. The latter number was confirmed by elemental analysis (found, 19.1%). Importantly, no protons belonging to the methylene or methyl groups of the polyether was observed in the NMR spectrum of the sample synthesized in the absence of Ce<sup>4+</sup> followed by the Pluronic removal by dialysis (see spectrum C in Fig.1). These observations confirm the mechanism of the copolymer formation via cerium (IV)-induced initiation

(Scheme 1).



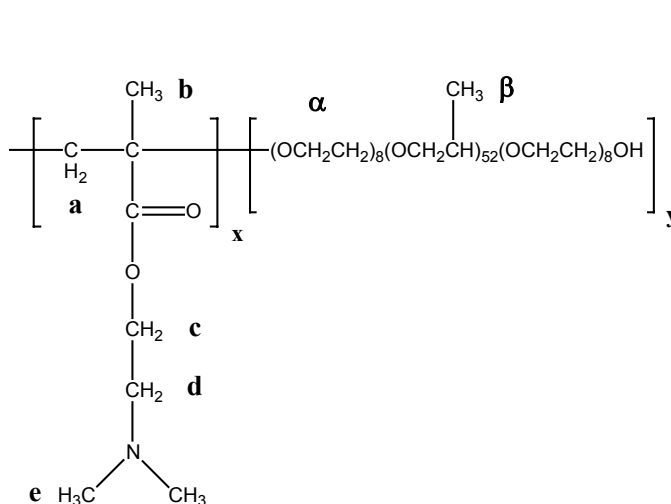
Scheme 1. Initiation and propagation steps in the free-radical polymerization leading to pDMAEMA-Pluronic block-copolymers.

Molecular mass of thus obtained pDMAEMA-L92 block copolymer was characterized by MALDI-TOF (Fig.2). Area of masses below 20 kDa yielded weight average weight of Pluronic of 3647, which is satisfactorily close to the nominal molecular mass of 3720. A peak at 3487 Da, uncharacteristic of the parent L92 spectrum, appeared in the mass spectrum of pDMAEMA-L92. This peak can be assigned to the charged pDMAEMA block. At a mass of the DMAEMA unit of 157 Da, the length of the pDMAEMA block ( $x=22$ ) is in excellent agreement with  $x=22.9$  obtained from NMR. The weight-average molecular weight of pDMAEMA-L92 was found to be 6246 with the polydispersity of 1.05. It should be noted that both the procedure of purification by dialysis (which eliminates the lower molecular weight species) and the MALDI method (which is insensitive to the large polymer species) contributed to the low polydispersity result.

TABLE I  
STRUCTURE OF COPOLYMERS OBTAINED FROM <sup>1</sup>H NMR

Structural Element	Protons <sup>a</sup>	Calculated	Found from NMR
DMAEMA	a:b:c:d:e	1.0:1.5:1.0: 1.0:3.0	1.0:1.1:1.1: 1.0:3.2
L92	α:β	1.08:1.00	1.06:1.00
DMAEMA/ L92, mol/mol	a:α	x:84y	0.9:3.3 x=22.9 y=1

<sup>a</sup> For proton designations, see Scheme 2.



Scheme 2. Structure of the pDMAEMA-L92 copolymer.

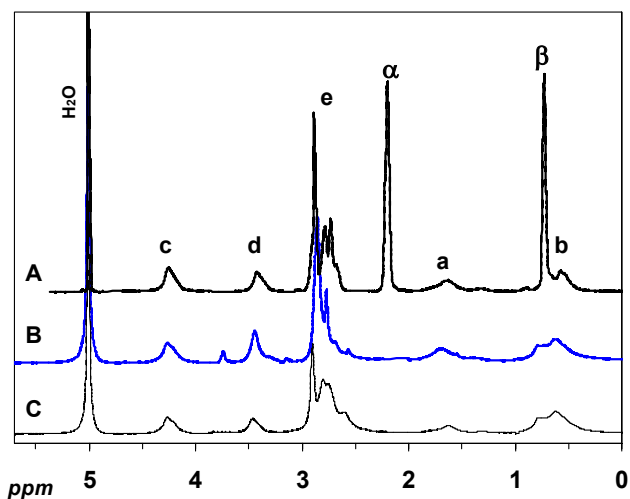
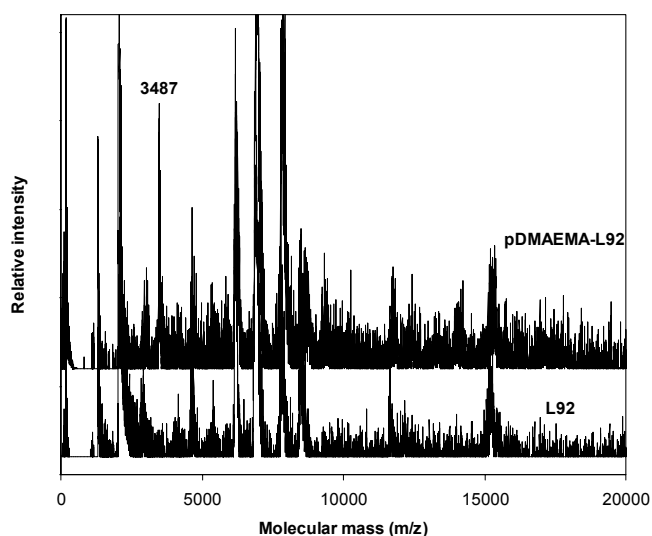


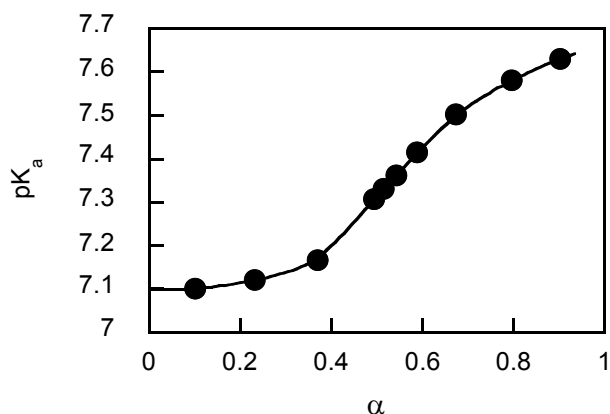
Fig.1. <sup>1</sup>H NMR (400 MHz) spectra of pDMAEMA-L92 (A), pDMAEMA (B), and pDMAEMA/L92 blend after dialysis (C) in D<sub>2</sub>O/5 mM DCl. Protons are labeled as shown in Scheme 1.



**Fig.2.** MALDI-TOF spectra of pDMAEMA-L92 copolymer and the original Pluronic L92. Peak pertaining to the pDMAEMA block is labeled (mass 3487).

### B. Polyelectrolyte properties and self-assembly

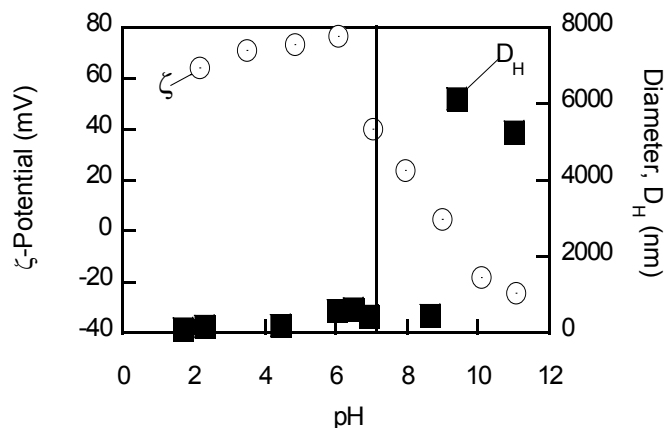
In order to assess the polyelectrolyte properties of the novel pDMAEMA-L92 copolymers, potentiometric titration of their aqueous solutions was conducted. The titration curves yielded clear inflection points, which enabled calculation of the apparent  $pK_a$  and ionization degree ( $\alpha$ ) of the titratable amino groups [12] (Fig.3). The intrinsic dissociation constant ( $K_o$ ) was found by extrapolating  $pK_a$  to  $\alpha=0$  in the  $pK_a$  vs  $\alpha$  curves. The  $pK_o$  was found to be 7.1, which is less than  $pK_a=7.4-7.8$  of the pDMAEMA polymer or of the DMAEMA monomer ( $pK_a=8.4$ ) [13]. The shift to more acidic  $pK$  can be explained by the effect of Pluronic lowering the dielectric constant of the microenvironment of the amino groups.



**Fig.3.** Relationship between the apparent dissociation constant ( $pK_a$ ) and the degree of ionization ( $\alpha$ ) for pDMAEMA-L92 copolymers.  $T = 25$  °C. The parameters were calculated from experimental potentiometric titration data using Henderson-Hasselbach expression [12].

Fig.4 shows the pH-dependent electrokinetic mobility

(expressed in terms of  $\zeta$ -potential) and hydrodynamic diameter of the pDMAEMA-L92 polymer. As is seen, the reduction in the positive charge above  $pK_o$  of the tertiary amino groups of pDMAEMA leads to the loss of the colloidal stability of the polymer and appearance of large aggregates. The observed negative values of  $\zeta$ -potential at very basic pH can be explained by the presence of carboxylic groups on the polymer, appearing due to the partial hydrolysis of the DMAEMA [13].

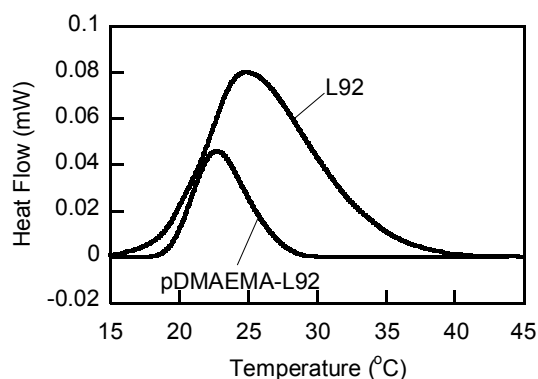


**Fig. 4.**  $\zeta$ -Potential ( $\zeta$ ) and effective hydrodynamic diameter ( $D_H$ ) of the pDMAEMA-L92 aggregates as functions of pH. The vertical line shows the  $pK_o$  of the tertiary amino groups.  $T=25$  °C, salt-free solutions throughout.

Aggregation in aqueous solutions of pDMAEMA-L92 appeared to be temperature-dependent, as illustrated by DSC endotherms (Fig.5). Pronounced endothermic peaks were observed in both Pluronic and pDMAEMA-L92 solutions, indicating cooperative transitions over a 20-40 °C range. The solutions under study exhibited no significant differences between the first and second heating/cooling cycles, which indicated the complete reversibility of these transitions, related to the micellization of the polymers. Positive changes in both enthalpy and entropy observed upon aggregation are typical for entropy-driven aggregation processes due to hydrophobic associations. Considering aggregation as a multimeric temperature-induced reaction, we can relate the apparent standard enthalpy of aggregation,  $\Delta H_o^*$ , to the characteristic temperature  $T^*$  taken as a temperature of the maximum of the endothermic peak due to aggregation ( $T_m$ ), via the Gibbs-Helmholz equation:

$$\left[ \frac{\partial(1/T_m)}{\partial \ln C} \right] = \frac{R}{\Delta H_o^*}$$

Here  $C$  is the total polymer concentration in solution. The above equation predicts that  $\ln C$  should vary linearly with the reciprocal of  $T_m$  with a slope  $\Delta H_o^*/R$ . The  $T_m$  values determined experimentally for solutions of Pluronic L92 and pDMAEMA-L92 as functions of the total polymer concentration  $C$  in terms of the above equation. The linear



**Fig. 5.** DSC heating scans of 1.0 wt% aqueous solutions of pDMAEMA-L92 and of the parent Pluronic L92. Scanning rate, 1 °C/min, pH 4.5.

regressions fit the data well, with  $R^2 > 0.98$  in all cases. Assuming average molecular weights of L92 and pDMAEMA to be 3720 and 6296 Da, respectively, we estimated the corresponding  $\Delta H_0^*$  values to be 560 and 490 kJ/mol, respectively. A slightly lesser  $\Delta H_0^*$  value in the case of pDMAEMA-L92 might originate from the higher overall hydrophilicity of these polymers due to the hydrophilic, partially charged pDMAEMA block.

In conclusion, the self-assembly in the pDMAEMA-L92 is thermodynamically analogous to the one in Pluronic solutions, which is well-documented [14]. Surfactant properties of the pDMAEMA-L92 were further studied by the surface tensiometry. The surface tension of 1 wt % pDMAEMA-L92 solution was found to be 35.7 mN/m at 17 °C. This value is quite comparable to the surface activity typical seen in Pluronic aqueous solutions [15].

## CONCLUSIONS

A simple synthetic procedure of the free-radical polymerization of DMAEMA initiated by Pluronic-radicals that are generated by cerium (IV) results in block-copolymers of pDMAEMA and Pluronic. The copolymers are cationic and are self-assemble in aqueous solutions at pH exceeding  $pK_a$  of the tertiary amino groups. Micelle-like aggregation is observed in the pDMAEMA-L92 by differential scanning calorimetry. Studies are currently underway to characterize interaction of the newly developed polymers with polyanions including DNA.

## REFERENCES

- [1] M. A. Ilies, W. A. Seitz, and A.T. Balaban, "Cationic lipids in gene delivery: principles, vector design and therapeutical applications", *Curr. Pharm. Des.*, 2002, 8 (27), 2441-2473.
- [2] T. Merdan, J. Kopecek, and T. Kissel, "Prospects for cationic polymers in gene and oligonucleotide therapy against cancer", *Adv. Drug Deliv. Rev.*, 2002, 54 (5), 715-758.
- [3] A. V. Kabanov and V. A. Kabanov, "Interpolyelectrolyte and block ionomer complexes for gene delivery: physicochemical aspects", *Adv. Drug Deliv. Rev.*, 1998, 30 (1-3), 49-60.

- [4] V. Toncheva, M. A. Wolfert, P. R. Das, D. Oupicky, K. Ulbrich, L. W. Seymour, and E. H. Schacht, "Novel vectors for gene delivery formed by self-assembly of DNA with poly-(lysine) grafted with hydrophilic polymers", *Biochim. Biophys. Acta*, 1998, 1380, 354-368.
- [5] P. Dubruel and E. H. Schacht, "Effect of polyethylene oxide blocks or grafts on the physicochemical properties of poly-(2-N-(dimethylaminoethyl)methacrylate) DNA complexes of polycations and DNA", *Bioact. Compat. Polym.*, 2000, 15, 279-296.
- [6] P. Lemieux, S. V. Vinogradov, C. L. Gebhart, N. Guerin, G. Paradis, H. K. Nguyen, B. Ochiatti, Y. G. Suzdaltseva, E. V. Baratakova, T. K. Bronich, Y. St-Pierre, V. Y. Alakhov, and A. V. Kabanov, "Block and graft copolymers and NanoGel copolymer networks for DNA delivery into cell", *J. Drug Target*, 2000, 8 (2), 91-105.
- [7] C. L. Gebhart, S. Sriadibhatla, S. Vinogradov, P. Lemieux, V. Alakhov, and V.A. Kabanov, "Design and formulation of polyplexes based on Pluronic-polyethyleneimine conjugates for gene transfer", *Bioconjug. Chem.*, 2002, 13 (5), 937-944.
- [8] P. van de Wetering, N. M. Schuurmans-Nieuwenbroek, W. E. Hennink, and G. Storm, "Comparative transfection studied of human ovarian carcinoma cells in vitro, ex vivo and in vivo with poly-(2-N-(dimethylaminoethyl methacrylate)-based polyplexes", *J. Gene Med.*, 1999, 1 (3), 156-165.
- [9] K. Tauer, M. Antonietti, L. Rosengarten, and H. Miller, "Initiators based on poly(ethylene glycol) for starting heterophase polymerizations: generation of block copolymers and new particle morphologies", *Macromol. Chem. Phys.*, 1998, 199, 897-908.
- [10] P. Tauer, J. Westbroek, P. De Strycker, P. Dubruel, E. Temmerman, and E.H. Schacht, "Flow-through cell for on-line amperometric determination of Ce(IV) during polymerization reactions", *Anal. Chem.*, 2002, 74, 915-920.
- [11] S. Nagarajan, K. Sabdham, and V. Spinivasan, "Redox polymerization process: an efficient tool for the synthesis of block copolymers", *J. Polym. Sci., Polym. Chem.*, 1995, 33, 2925-2933.
- [12] L. Bromberg, M. Temchenko, and T.A. Hatton, "Smart microgel studies. Polyelectrolyte and drug-absorbing properties of microgels from polyether-modified poly(acrylic acid)", *Langmuir*, 2003, 19(21), 8675-8684.
- [13] P. van de Wetering, N.J. Zuidam, M. J. van Steenberg, O. A. G. J. van der Houwen, M. J. M. Underberg, and W. E. Hennink, "A mechanistic study of hydrolytic stability of poly(2-(dimethylamino)ethyl methacrylate)", *Macromolecules*, 1998, 31, 8063-8068.
- [14] L. Bromberg, M. Temchenko, G.D. Moeser, and T.A. Hatton, "Thermodynamics of temperature-sensitive polyether-modified poly(acrylic acid) microgels", *Langmuir*, to appear.
- [15] I. F. Paterson, B. Z. Chowdhry, and S. A. Leharne, "Investigation of naphthalene solubilization in aqueous solutions of ethylene oxide-b-propylene oxide-b-ethylene oxide copolymers", *Langmuir*, 1999, 15, 6187-6194.

**Smeeta Deshmukh** received her B. Chem. Eng. (2002) from University's Department of Chemical Technology (Mumbai) and is currently a graduate student at MIT. She has completed her Chemical Engineering Practice School Program. She is working on responsive gels for separation of biomolecules in microchannels.

**Lev Bromberg** received his M.Sc. in Chemical Engineering ('82) and Ph.D. in Macromolecular Chemistry ('88) from Moscow State University and conducted postdoctoral studies at the Weizmann Institute of Science and Massachusetts Institute of Technology. He served as a senior and principal scientist at Gel Sciences, Inc., Abbott Laboratories, Periodontix, Inc., and Cabot Corporation. He authored over 170 publications and patents and currently is a research scientist at MIT. His major field of study is intelligent polymers and gels for biomedical and hi-tech applications.

**T. Alan Hatton** received his B.Sc. and M.Sc. degrees in Chemical Engineering at the University of Natal, Durban, South Africa, and a Ph.D. from the University of Wisconsin, Madison. He has been at the faculty of MIT since 1982 and is currently the Ralph Landau Professor of Chemical Engineering Practice, and Director of the David H. Koch School of Chemical Engineering Practice. His research interests focus on the exploitation of responsive colloidal systems for chemical and biological applications.