J. Mex. Chem. Soc. **2006**, *50(4)*, 164-174
© 2006, Sociedad Química de México
ISSN 1870-249X

Article

Statistical copolymers of methacrylic acid derivatives with hydrophobic spacers and *N,N'*-dimethylaminoethylmethacrylate: New associating polyampholytes

Rosario Santos-Rosas[1], Angel Licea-Claveríe*[1] and Karl-Friedrich Arndt[2]

[1] Centro de Graduados e Investigación, Instituto Tecnológico de Tijuana, A.P.1166, 22000 Tijuana, B.C.; México. Tel/Fax: (++52)-(664)-623-3772. E-mail: aliceac@tectijuana.mx, aliceac19@hotmail.com. Address for international Airmail: PO BOX 431461, San Ysidro, CA 92143, U.S.A.
[2] Institut für Physikalische Chemie und Elektrochemie, Technische Universität Dresden, D-01062 Dresden, Germany

Recibido el 24 de julio del 2006; Aceptado el 20 de octubre de 2006

Abstract. Two series of statistical copolymers containing *N,N'*-(dimethylamino)ethylmethacrylate (DMAEM) and comonomers derived from methacrylic acid with three and five methylene-units as spacers, were prepared by free radical-copolymerization followed by selective saponification. These statistical copolymers prepared showed strong self-associative properties as revealed by viscosity, static and dynamic light scattering studies. The aggregates formed in aqueous solution show classical ampholytic behaviour with aggregate expansion both in acid and alkaline conditions and aggregate contraction at intermediate pH-values, near the isoelectric point of the system. The contraction of aggregates is shifted to higher pH-values when the content on hydrophobic acid comonomer is increased. The aggregates formed are stable to sonication above a concentration of 0.9 g/L and merge to larger structures at very high or very low pH-values. A conceptual model is proposed that accounts for the observed association behaviour of the copolymers. The statistical ampholytic copolymers studied are an easy to prepare alternative to block-copolymers for the formation of nano-sized aggregates for different applications.

Keywords: Polyampholytes, macromolecular aggregates, hydrophobic self-association, pH-sensitive copolymers

Resumen. Se prepararon dos series de copolímeros estadísticos conteniendo *N,N'*-dimetilaminoetil metacrilato (DMAEM) y comonómeros derivados del ácido metacrílico con tres y cinco metilenos como espaciadores, vía polimerización radicálica seguida de saponificación específica. Los copolímeros estadísticos obtenidos mostraron una fuerte tendencia a la autoasociación demostrada en experimentos de viscosidad y dispersión de luz estática y dinámica. Los agregados formados en solución acuosa mostraron comportamiento polianfolítico clásico, con expansión de agregados tanto en condiciones ácidas como en condiciones alcalinas y contracción de agregados a valores intermedios de pH, cercanos al punto isoeléctrico del sistema. La contracción de agregados se recorre a valores mayores de pH cuando se incrementa el contenido de comonómero hidrofóbico-ácido en el copolímero. Los agregados formados son estables al ultrasonido arriba de una concentración de 0.9 g/L y se agrupan en estructuras aun mayores a valores de pH muy altos o muy bajos. Se propone un modelo conceptual para explicar el comportamiento observado de autoasociación de los copolímeros. Los copolímeros anfolíticos estadísticos estudiados representan una alternativa fácil de preparar en comparación con los copolímeros en bloques para la formación de agregados nanométricos para aplicaciones diversas.

Palabras clave: Polianfolitos, agregados macromoleculares, autoasociación hidrofóbica, copolímeros sensibles al pH.

Introduction

Ampholytic or amphoteric polymers are a class of synthetic polyelectrolytes that contains both cationic and anionic groups in the same chain [1]. Investigation of polyampholytes has been an area of increasing interest because they are used as better models than polyelectrolytes for natural polymers like proteins [1-3], as ion exchange materials [1], as membranes [4] and in sewage treatment and flocculation [5]. One of the main advantages of these materials is their ability to expand in saline solutions, the so called antipolyelectrolyte behaviour [2]. The influence of different factors like temperature, ionic strength and pH on the behaviour of the polyampholytes in solution has been studied [1]. Solution behaviour of amphoteric polymers depends on the ratio of the acidic to basic groups incorporated in the polymer. In polyampholytes the electrostatic interactions can be repulsive or attractive. When the polymer has an excess of anionic or cationic groups, behaves as a polyelectrolyte [6]. A polyampholyte exist in aqueous solution as a polycation at low pH-values and as polyanion at high pH-values. At intermediate pH-values amphoteric polymers show a point where the ratio of positive to negative charges is the unity; this point is called the isoelectric point (IP) in analogy with proteins. The IP is characterized by a minimum of chain expansion in solution. The IP shifts to higher pH values when the base/acid ratio in the polymer is increased [7]. Classical examples of polyampholytes are the statistical copolymers of *N,N'*-(dimethylamino)ethylmethacrylate (DMAEM) with methacrylic acid (MAA). These copolymers have been studied 50 years ago [7, 8] and gave an insight into the general pH- and ionic strength-dependent behaviour of polyampholytes having weak acid and weak basic groups. The interest in this kind of copolymers was reborn in the nineties [2, 3, 9-11] with emphasis in diblock and triblock copolymer architectures and continued into this century [12-15] due to their capability to self assemble in water to aggregates of different morphologies. In some of these studies block-copolymers were compared to random or statistical analogs. For example Patrickios et al. [9] reported that while DMAEM-MAA-methylmethacrylate (MMA) triblock-copolymers aggregate to micellar structures depending on pH, random copolymeric-analogs do not. Hadjikallis et al. [14] came to a similar conclusion when the behaviour of blocky and random copolymers of MAA and 2-imidazoilethylmethacrylate were compared.

The self organization of hydrophobically modified water soluble polymers has been a focus of interest in past decades due to its applications as thickeners in food and as modifiers in paints [16, 17], as well as with respect to their biotechnological and pharmaceutical applications [8, 18]. In aqueous solution, association of hydrophobes covalently linked to water soluble polymers may occur intra- or intermolecularly. In concentrated solutions, interpolymer association would lead to bulk phase separation, whereas intrapolymer association leads to the formation of unimolecular micelles [19]. It has been reported that block-copolymers show the tendency to interpolymer association, whereas random-copolymers tend to associate intramolecularly [20]. However, recent studies showed that the preference of random copolymers to associate intramolecularly depends strongly on the fine chemical structure of the polymers [19, 21]. In the case of hydrophobically modified polyelectrolytes, hydrophobic self-association can occur competing with electrostatic repulsions within the same polymer chain or between different polymer chains, respectively; depending strongly on macromolecular architecture [21].

To our knowledge, the case of hydrophobically modified statistical polyampholytes has not been studied in deep. It is fairly possible that the hydrophobic self-association phenomenon may lead to the formation of micelles or other type of aggregates; however, the fact that electrostatic interactions may be repulsive or attractive depending on composition and pH needs to be evaluated carefully. Since the preparation of statistical copolymers is much easier than that of block-copolymers, this could open the possibility for a simple and versatile method to yield nano-sized structures as containers for several novel applications. This is the case for which this investigation was undertaken. For this, a series of statistical copolymers in a wide range of compositions containing the basic monomer DMAEM and two acidic monomers derivatives of methacrylic acid with hydrophobic spacers of different length (3 and 5 methylene groups) were synthesized. Extensive viscometric and light scattering studies were conducted to clarify the behaviour of these copolymers in aqueous solution as a function of pH in search of associative phenomena to occur.

Experimental

Copolymer synthesis

The basic monomer *N,N'*-(dimethylamino)ethylmethacrylate (DMAEM) was distilled under reduced pressure before use. The monomers with protected acid groups and hydrophobic spacers with 3 and 5 methylene units, 4-(methacryloyloxy)butanoate (M4MOB) and 6-(methacryloyloxy)hexanoate (M6MOH), respectively were synthesized as reported elsewhere [22]. Homopolymers of DMAEM, M4MOB, M6MOH and two series of copolymers of DMAEM with M4MOB and M6MOH, respectively, were synthesized by free radical polymerization in tetrahydrofurane (THF, Spectrum) as solvent under argon at 60 °C for 72 h using 1 mol% of *N,N'*-azobisisobutyronitrile (AIBN, Spectrum) as initiator (Figure 1-A). The feed ratio of monomers used for the copolymer synthesis was 75, 50 and 25 mol% of DMAEM in the mixture keeping the total monomer concentration of 1 M. The preparation of M4MOB-DMAEM (50-50) copolymer gives an example of the general procedure used for the copolymer synthesis: 3.91 g (0.021 mol) of M4MOB, 3.3 g (0.021 mol) of DMAEM and 0.06 g (0.00021 mol) of AIBN were dissolved in 42 mL of THF. The solution was transferred to a Schlenk-flask and was purged with argon and vacuum at -70 °C using three freeze-thaw cycles to eliminate any dissolved air. Afterwards the Schlenk-flask was filled with argon and was heated to 60 °C for 72 h to allow polymerization. The copolymer products where dissolved in THF and precipitated in petroleum ether (36-60 °C, Fermont). This process of purification was repeated three times. Finally the products were dried in vacuum oven at 40 °C for four days.

Deprotection of acid groups in copolymers

For the deprotection of the acid groups in the copolymers, a selective ester cleavage reaction was carried out at 115 °C for 24 h using dry pyridine as solvent as reported by Rogel-Hernández et al. [22] for homopolymers (Figure 1-B). The reaction on M4MOB-DMAEM (50-50) copolymer is described as an example of the general procedure used: In a three necked round bottom flask equipped with magnetic stir bar, reflux condenser, thermometer and argon inlet, 3.5 g (0.01 mol) of M4MOB-DMAEM (50-50) copolymer, a 4-fold excess of anhydrous lithium iodide (5.22 g, 0.04 mol, Acros) and 0.8 g (0.01 mol) of anhydrous sodium acetate (Aldrich) were dissolved in 30 mL of dry pyridine (Aldrich). The reaction mixture stirred for 24 h at 115 °C under argon. The solution was then cooled and the solvent removed under vacuum. The crude product was dissolved in deionized water under stirring and was dialyzed extensively for one week using cellulose ester dialysis tubing (MWCO: 500, Spectra/pore). The content of the dialysis tubing was concentrated using a rotary evaporator and the product was finally dried in vacuum oven at 40 °C for five days.

Nuclear magnetic resonance spectroscopy

^{1}H NMR-spectra were recorded using either a Varian Gemini II 200 spectrometer (200 MHz), for monomers and protected copolymers or a Bruker Avance 4000 NMR spectrometer (400 MHz) for deprotected homo- and copolymers. $CDCl_3$, C_3D_6O, CD_3OD or D_2O (all from Aldrich) were used depending on the solubility of the products. With exception of D_2O, tetramethyl-

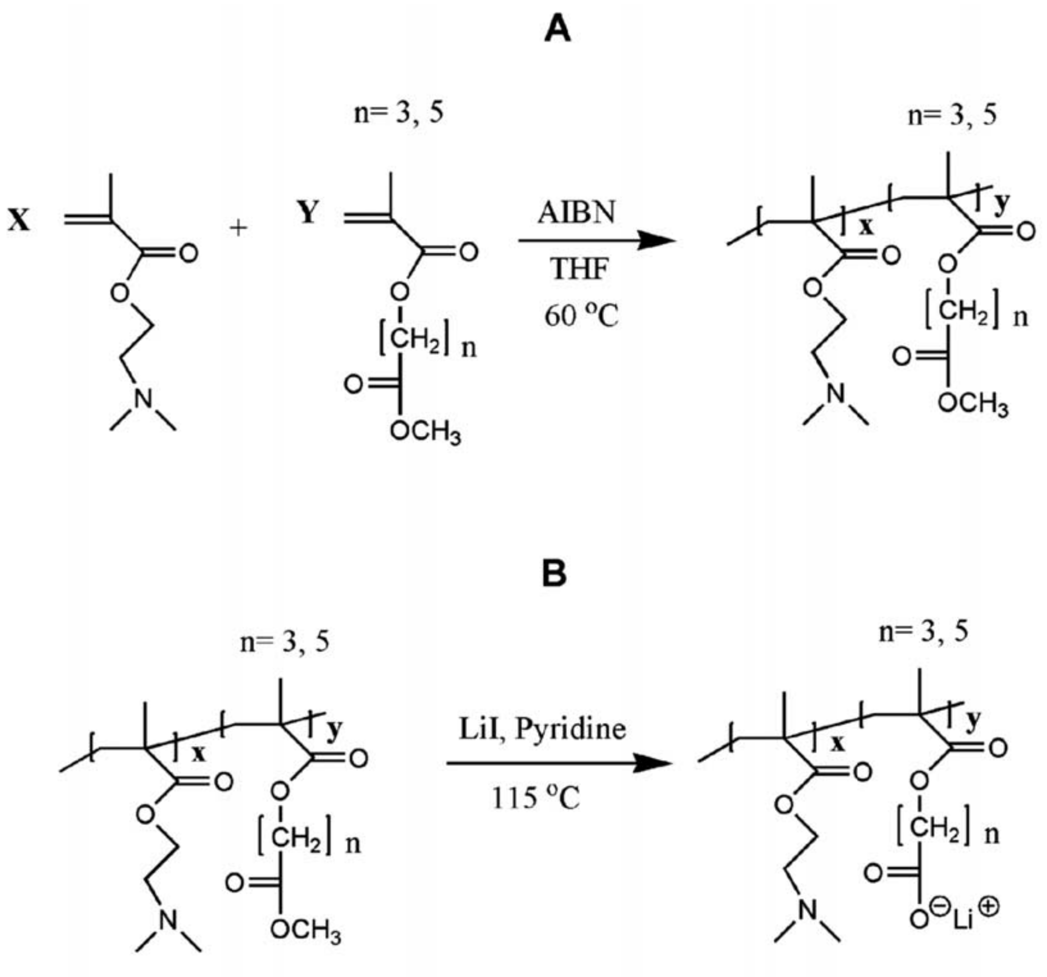


Fig. 1. Preparation method for ampholytic copolymers: A- Copolymerization to yield DMAEM/acid group protected copolymers. B-Selective saponification to yield ampholytic copolymers

silane (TMS) was added as standard to the deuterated solvents used.

Molecular weight determinations

Molecular weight and molecular weight distributions of protected copolymers were determined by gel permeation chromatography (GPC) using a Varian 9002 GPC equipped with refractive index (RI) and UV detectors. A mixed bed column (phenogel 5 linear) from Phenomenex was used for the separation. Calibration was carried out using polymethylmethacrylate standards ranging from 2 050 to 1 000 000 g/mol. The eluent was THF (HPLC grade, Spectrum) added with 1 vol% triethylamine (TEA, Aldrich) at a flow rate of 0.5 mL/min at 30 °C [23]. Since Mark-Howink parameters were not available for the copolymers, all molecular weights are reported as PMMA equivalents. The molecular weights of selected deprotected homo- and copolymers were determined by static light scattering (SLS) using the same equipment described in the subsection *Light scattering studies* (below) but using a 0.05 M NaCl aqueous solution as solvent.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed on a TA Instruments MDSC 2920. 18-23 mg of well dried samples (40 °C for 24 h in vacuum) was heated from -40 °C to 150 °C at a rate of 2 °C/min. The temperature was modulated to +/- 0.5 °C every 60 sec under nitrogen atmosphere. To clear thermal history, the curve of reversible heat at the second heating was evaluated at half height of the glass-transition event, and reported as the glass transition temperature (T_g).

Viscosity studies

Viscometric measurements were carried out using a Seide-Deckert viscometer at 25 °C. Stock solutions were prepared by dissolving designated amounts of polymer in buffer solutions prepared at constant ionic strength (I = 0.1 M) with pH values from 2 to 11, at room temperature. Two days were required for the polymers to dissolve completely. The solutions were filtered off using 0.2 mm polytetrafluoroethylene (PTFE) membrane filters before measurement. Three to five solutions from each sample in each buffer were prepared with concentrations ranging from 0.5 to 6.0 g/L. Each measurement was repeated five times and the actual pH of each solution was measured after the viscometric evaluation. The same solutions were used for light scattering measurements, both static and dynamic to have a better comparison of results.

For studies of formation and stability of aggregates using viscometry, a Cannon-Ubbelohde viscometer at 25 ^{o}C was used. Solutions in the concentration range of 0.6 to 1.0 g/L in the buffer of pH=7 were prepared and filtered off using 0.2 mm PTFE syringe filters.

Light scattering studies

Static light scattering measurements (SLS) were performed at room temperature on a modified static light scattering apparatus FICA 50 (SLS Systemtechnik, Germany) using a laser light source of 633 nm, in the angular range of 30-145^{o} in steps of 5^{o}. For these studies, buffer solutions of copolymers with nominal pH-values from 2 to 11, at a constant ionic strength (I=0.1M) were prepared, allowed to stand for 2 days and filtered off using 0.2 mm PTFE membrane filters. The refractive index increment needed for SLS studies was measured using a DR-1 differential refractometer (SLS Systemtechnik, Germany) at room temperature. The equipment was calibrated previously with a standard KCl solution. The concentration range studied was from 0.5 to 3.0 g/L using Buffer-solutions of nominal pH-values from 2 to 11.

Dynamic light scattering (DLS) measurements were performed at room temperature using a DLS 700 (Otsuka, Japan) at an angle of 90^{o}. For these measurements only the solution with the highest concentration for each copolymer (in each buffer) was used. The hydrodynamic diameter D_h of the macromolecules was calculated using the Stokes-Einstein equation for spheres, as usual [24].

Results and Discussion

Copolymer synthesis and characterization

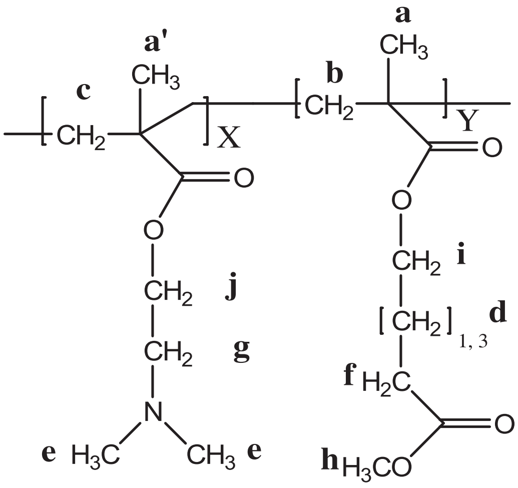
Copolymers with protected acid groups

Figure 1-A show the structures of the copolymers of DMAEM and methacrylic acid derivatives with hydrophobic spacers prepared. The main characterization parameters for the products obtained are given in Tables 1 and 2. All polymers were obtained in good yields, although the copolymers prepared using M4MOB were obtained in higher yields. Copolymer compositions were determined from ^{1}H NMR spectroscopy by integration ratio of the signals corresponding to methylene protons adjacent to the oxygen atom in both monomers taking the integration of the methoxy signal from the protected acid group as a reference (protons “i” and “j” referred to protons “h” in chemical structure insert on Table 2). All obtained NMR signals for homopolymers and selected copolymers are listed in Table 2. Only one example of every copolymer is listed since they show the same signals independent of the composition; only the integration value varied as a function of composition. The content on hydrophobic comonomer was close to the corresponding monomer feed mixture. Weight average molecular weights obtained by GPC at 30 ^{o}C are in the range of 4.0 x 10^{4} to 1.02 x 10^{5} g/mol and the samples are polydisperse (Table 1). The glass transition temperatures obtained for the series of copolymers including monomers

Table 1. Results of copolymerization and main properties of the copolymers at room temperature with protected acid groups

Copolymer (feed composition)	Yield [%]	Hydrophobic comonomer content [mol%]	M_w [g/mol]	Poly-dispersity index	T_g [K]
PolyDMAEM (100)	84	-	40 000	1.8	291.55
CopM4MOB-DMAEM (25-75)	96	22	100 200	-	280.75
CopM4MOB-DMAEM (50-50)	97	52	64 500	3.6	280.15
CopM4MOB-DMAEM (75-25)	98	75	42 300	2.0	277.75
PolyM4MOB (100)	93	100	76 600	2.1	273.15
CopM6MOH-DMAEM (25-75)	89	25	84 200	3.0	268.85
CopM6MOH-DMAEM (50-50)	91	44	88 800	3.2	264.15
CopM6MOH-DMAEM (75-25)	85	72	78 700	3.5	256.25
PolyM6MOH (100)	84	100	96 500	3.5	236.65

Table 2. ^{1}H-NMR chemical shifts (δ) of copolymers with protected acid groups. (δ-values in ppm)



	a, a'*	b	c	d	e	f	g	h	i	j
PolyDMAEM	0.9-1.0	-	1.9	-	2.3	-	2.6	-	-	4.0
PolyM4MOB	0.9-1.1	1.8	-	2.0	-	2.4	-	3.7	4.0	-
PolyM6MOH	0.8-1.0	1.4	-	1.5-1.7	-	2.3	-	3.6	3.9	-
CopM4MOB -DMAEM (50-50)	0.9-1.1	1.8	1.8	2.0	2.3	2.4	2.6	3.7	3.9	4.1
CopM6MOH -DMAEM (50-50)	0.9-1.1	1.4	1.8	1.5-1.7	2.3	2.3	2.6	3.7	3.9	4.1

* Two shifts within range because of atacticity

with 3 and 5 methylene spacers, show that the higher the content of the comonomers with spacer groups, the lower the value of T_g due to side chain flexibility introduced by the aliphatic spacer.

Copolymers with deprotected acid groups

The copolymers with protected acid groups were subjected to selective ester cleavage reactions aiming deprotection of the acid groups applying the methodology described by Rogel-Hernández et al. [22] (see Figure 1-B). The copolymers were obtained with deprotection yields that varied strongly with the starting copolymer composition (from 48 to 96%, Table 3). As a result the obtained amphoteric copolymers have to some extent also a content of hydrophobic non-ionic units in their structure. The composition of these copolymers was estimated by H^1-NMR measurements in D_2O or CD_3OD, depending on solubility (Table 3).

The amphoteric copolymers obtained after selective saponification were not soluble in THF anymore and the GPC analysis was not possible for this copolymer series using our equipment. We determined the molecular weights instead by SLS in aqueous NaCl solution for the copolymers that were water soluble at neutral pH. The obtained molecular weights can not be compared with those of the starting copolymers given the fact that the values of the starting copolymers are PMMA equivalents (Table 3). As expected, the T_g values of the deprotected copolymers increased dramatically with respect to the protected copolymers since by having free acid groups in addition to the tertiary amine groups there are many possibilities of interchain and intrachain attractive interactions like hydrogen bonding and acid-base interactions in the solid state.

Viscosity studies

It is known that the viscosity of dilute polymer solutions depends on the hydrodynamic volume of the polymer and their concentration [25]. At a constant ionic strength, the viscosity of a polyampholyte can also be related to its volume in solution; however a determination of the intrinsic viscosity of charged polymers is complicated due to their behavior at very high dilution, including the presence of an irreproducible maximum or even values of reduced viscosity that approach infinity [2]. An alternative for the calculation of intrinsic viscosity of this kind of polymers has been the evaluation of viscosity data using the Fuoss and Strauss equation [26]; however, recently it has been postulated that their use has been one of

Table 3. Results and main properties of copolymers at room temperature after deprotection reaction on acid groups.

Start composition of the copolymers	Yield* [%]	Content on monomer with free acid groups [mol%]	Content on monomer with protected acid groups [mol%]	Content on DMAEM [mol%]	M_w [g/mol]	R_g [nm]	T_g [K]
CopM4MOB-DMAEM (22-78)	48	11	7	82	98 200	20	301.9
CopM4MOB–DMAEM (52-48)	70	36	19	45	-	-	-
CopM4MOB-DMAEM (75-25)	63	48	44	8	143 000	42	-
PolyM4MOB (100)	96	96	4	-	98 000	-	362.7
CopM6MOH-DMAEM (25-75)	81	20	10	70	-	-	359.9
CopM6MOH–DMAEM (44-56)	80	35	35	30	120 000	-	373.7
CopM6MOH–DMAEM (72-28)	74	54	27	19	-	-	364.3
PolyM6MOH (100)	59	59	41	-	59 000	(14)	275.5

* Deprotection of acid groups

the capital errors of polyelectrolyte history [27]. Therefore we used single-point (concentration) reduced viscosity values to follow changes in coil dimensions of our copolymers as it is a common practice for charged polymers [2, 7, 28, 29]. Since the synthesized copolymers contain carboxylate and amine groups on the main chain, an effect of pH on the viscosity of the copolymers would be expected [6, 7]. The dependence of the reduced viscosity (η_{sp}/C_p) on the pH for the ampholytic copolymers with three methylene spacers (deprotected M4MOB-DMAEM copolymers) in buffer-solutions of constant ionic strength (I =0.1 M) are shown in Figure 2. The copolymers with lower content on partially hydrophobic acid comonomer were soluble over the entire pH-range studied, while the copolymer with 48% content on deprotected acid comonomer was insoluble at pH values lower than 6.5. The copolymers showed high values of viscosity both in acid and alkaline buffers suggesting that the chains adopt an extended polyelectrolyte configuration due to electrostatic repulsions between the groups of the same charge. They act as polycations at low pH values and as polyanions at high pH values. They show also a minimum in viscosity (isoelectric point, IP) at intermediate pH values due to the strong electrostatic attractions between groups of opposite signs suggesting that the copolymer chains are strongly collapsed. The IP of the copolymers shifted to lower pH values when the amino to acid ratio in the copolymer decreased. The homopolymers from M4MOB after deprotection (poly(4-methacryloyloxy-butanoic acid)) and DMAEM show typical polyelectrolyte behaviour with viscosity increase at high and low pH values respectively due to the presence of carboxylate groups in the former and quaternized amino groups in the later (Figure 2). The homopolymer DMAEM was soluble in all range of pH values studied while the polyacid from M4MOB precipitated at low pH values as already mentioned due to the presence of hydrophobic spacers. The deprotected polymer from M6MOH (poly(6-methacryloyloxy-hexanoic acid), here not shown) revealed also a viscosity maximum at high pH values and was not soluble at pH values lower than 5.2.

Light scattering studies

Static light scattering measurements were carried out to supplement the viscosity studies. They were conducted on the same solutions as for the viscosity experiments. The values of the radii of gyration (R_g), proportional to the polymer volume in solution, and the apparent molecular weights are reported in this investigation. Figure 3 shows that R_g of the copolymers behaves similarly to the viscosity in dependence of pH. R_g increases at pH values where the viscosity increases, and R_g droops at intermediate pH values, in analogy to viscosity. The increase of R_g on acid and alkaline buffers normally would indicate extended conformation of the copolymer chain and

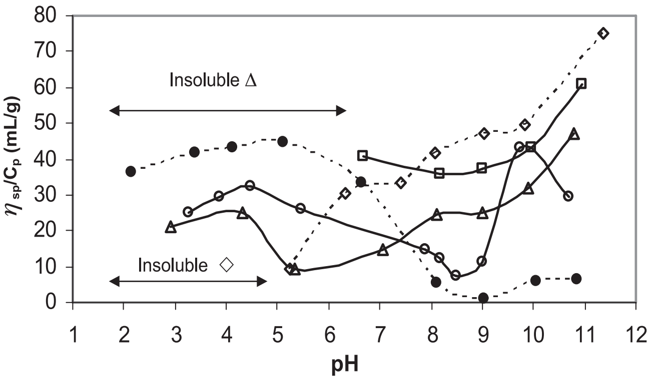


Fig. 2. Reduced viscosity in aqueous buffers (I = 0.1M) as a function of pH for deprotected polymers and copolymers originated from M4MOB and DMAEM: ◦ – CopM4MOB-DMAEM (22:78) [C_p= 1.8 g/L], Δ - CopM4MOB-DMAEM (52:48) [C_p= 1.8 g/L], □ – CopM4MOB-DMAEM (75-25) [C_p= 1.8 g/L], ◊ - PolyM4MOB [C_p= 0.5 g/L], • – PolyDMAEM [C_p= 0.5 g/L]. Curves draw as a guide for the eye only.

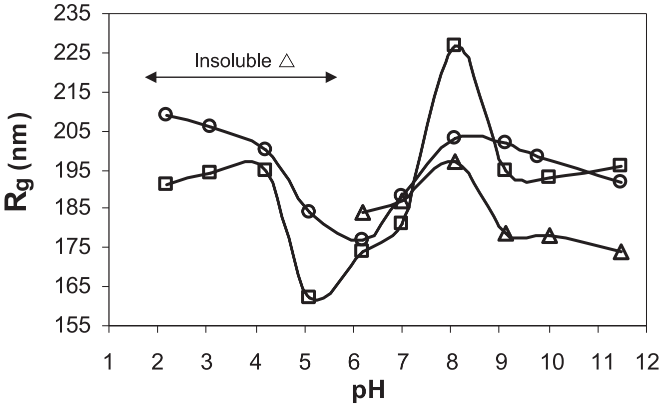


Fig. 3. Radius of gyration (R_g) in aqueous buffers (I = 0.1M) as a function of pH for deprotected copolymers originated from M4MOB and DMAEM: ∘ – CopM4MOB-DMAEM (22:78), □ - CopM4MOB-DMAEM (52:48), Δ – CopM4MOB-DMAEM (75-25). Curves draw as a guide for the eye only.

the reduction of R_g at intermediate pH buffers would indicate chain contraction. However the values of R_g from 175 to 210 nm do not corresponds with the size of a single copolymer chain. For example for the M4MOB-DMAEM (22-78) copolymer an R_g of 20 nm was measured in neutral water with added salt after deprotection reaction (0.05 N NaCl, Table 3). Moreover, the molecular weight of the same copolymer under the same conditions (neutral water, salt added) is M_w =98 200 g/mol, while in the buffer solutions at pH of 7.9, an apparent M_w of 9.95 million g/mol was measured (Table 4)! These results indicate that aggregation is occurring. In order to investigate further this behaviour we performed dynamic light scattering studies (DLS) in buffer solutions of different pH-values for the same copolymers. Figure 4 shows the, from diffusion coefficients calculated distributions of hydrodynamic diameters (D_h) for the copolymer M4MOB-DMAEM (22-78) after deprotection reaction at different pH values. As we can see the average values of D_h are much higher than the expectations for single macromolecules. Moreover, there are at least two distributions of sizes at each pH-value indicating the formation of aggregates that merge, in some cases, to even larger structures. In Table 4 the mean values of D_h for each population of aggregates and the results obtained by SLS are compared for the copolymer M4MOB-DMAEM (22-78) after deprotection reaction as a function of pH. The apparent molecular weights of the structures formed are in the millions, showing that more than 100 copolymer chains may aggregate into the structures formed. Taking the D_h values of the mid-size population of aggregates (hundreds of nm), we recognize the same trend observed for R_g and reduced viscosity: increase at low and also at high pH values while a decrease is observed at intermediate pH values. This further confirms the ampholytic behaviour of these macromolecular structures: expansion at low and at high pH-values, while contraction at intermediate pH-values, near the isolelectric point, is observed.

Self-association: Concentration dependence

Another question that arises at this point is about the concentration dependence of the aggregation capacity of these statistical ampholytic copolymers. It is well-known that the self-association of block-copolymers in a selective solvent into micelles depends not only on the chemical structure of the blocks but also on parameters like temperature and copolymer-concentration. In the case of concentration, above a so called critical micelle concentration (CMC) a micelle exists certainly, while below the CMC the association-equilibrium favors the molecular dissociation into single copolymers (unimers) [30]. In our case, we studied the reduced viscosity behaviour of the M4MOB-DMAEM (22-78) copolymer after deprotection reaction at three different concentrations: 0.60, 0.86 and 1.00 g/L. This study was performed in a buffer solution of pH=7.1 at ionic strength of 0.1 M. All solutions were

Table 4. Characteristics of macromolecular aggregates at room temperature from deprotected CopM4MOB-DMAEM(22:78) as a function of pH.

pH	Concentration (g/L)	M_w (10^6 g/ mol)	R_g (nm)	D_h (nm)			
3.7	2.5	16.6	209			284	2139
3.9	2.5	11.2	206	8	47	886	
4.5	2.0	13.7	200	10	65	734	
5.5	2.2	33.0	184	26		282	
7.9	2.3	9.95	177	37		225	
8.2	2.2	33.4	188	7	57	329	
8.5	2.5	30.6	203	9	80	877	
9.0	2.1	17.3	202	21	72	815	
9.3	2.0	12.1	198	18		123	1065
10.7	2.1	6.96	192	22		194	1286

Note 1: Single chain M_w = 98 200 g/mol, R_g = 20 nm
Note 2: Composition of copolymer after deprotection is: 82% DMAEM-11% free-acid M4MOB-7% M4MOB

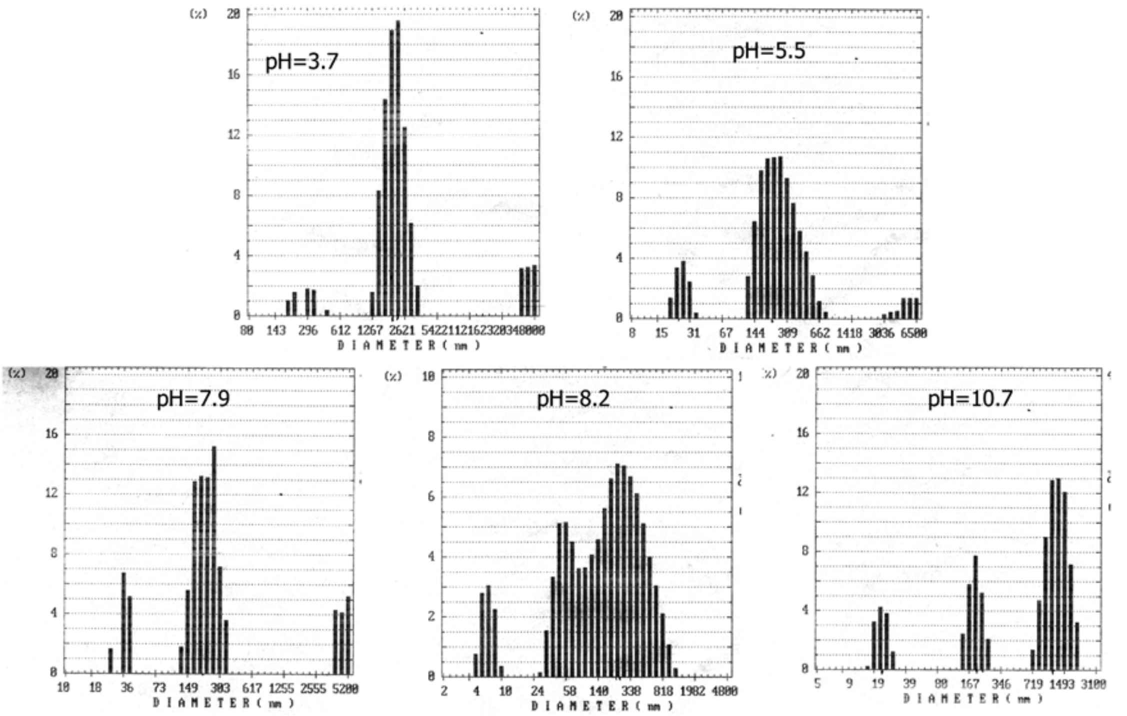


Fig. 4. Distribution of hydrodynamic diameters (D_h) for aqueous solutions of deprotected CopM4MOB-DMAEM (22:78) at different pH-values (room temperature).

first evaluated after 48 h of preparation and were filtered off using 0.2 mm syringe filters before measurement. After this original reduced viscosity measurement, the solutions were subjected to sonication for 20 min and the reduced viscosity was measured again. Finally the solutions were allowed to reach equilibrium again (48 h) and were evaluated for reduced viscosity for the third time. The results of these experiments are pictured in Figure 5. For the solutions at a copolymer concentration of 0.6 g/L we observed a large decrease in reduced viscosity after sonication, thus indicating a disruption of the structures formed initially. After equilibrium, the reduced viscosity recovers almost entirely its original value. In contrast, for a solution at a copolymer concentration of 1.0 g/L sonication did not resulted in any reduced viscosity change, while for the solution at a concentration of 0.86 g/L a slight decrease of reduced viscosity after sonication was observed accompanied with a partial recovery after equilibrium. These results suggests that a critical aggregate concentration behaviour may be encountered in the vicinity of 0.9 g/L insofar that at higher copolymer concentration the association equilibrium is totally shifted towards aggregate-formation, while at lower concentration the equilibrium allows for the existence of both aggregates and molecularly dissolved polyampholytes. This confirms also that the tendency to aggregation of the synthesized statistical copolymers is very strong in water even at concentrations as low as 0.9 g/L. This strong associative behaviour may result from the hydrophobic units (remaining protected acid units) in addition to the presence of hydrophobic spacers (protected and unprotected acid units) in the copolymer structure.

Association model

On the basis of the experimental results, we propose a conceptual model for the association of the statistical copolymers based on attractive hydrophobic interactions and attractive (ampholytic) or repulsive (electrolytic) ionic interactions

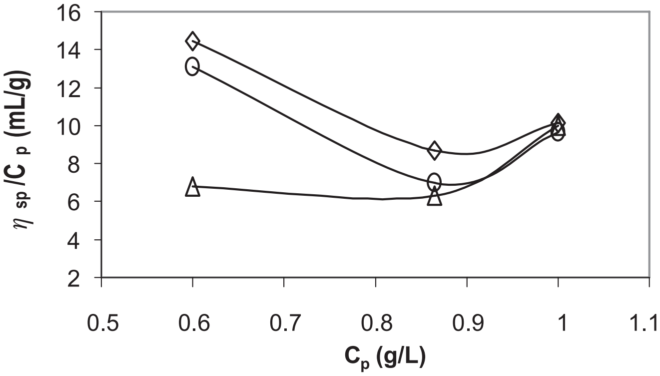


Fig. 5. Reduced viscosity as a function of polymer concentration for deprotected CopM4MOB-DMAEM (22:78) at pH=7.1 (I =0.1M, room temperature); Sonication experiment: ◊-Original value, Δ-Right after sonication, ◦- 48 h after sonication. Curves draw as a guide for the eye only.

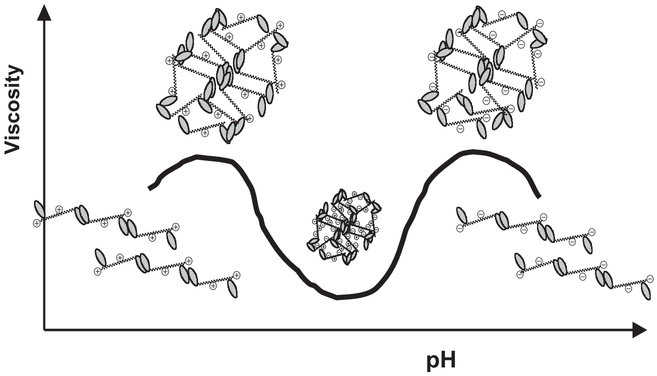


Fig. 6. Model of the associative behaviour in water of random hydrophobic copolyampholytes: ⊕ -Ionised basic units, ⊖-Ionised acid units, ⬭-Hydrophobic units

depending on the pH-value of the aqueous solution (Figure 6). At intermediate pH values (IP of the polyampholyte) all interactions hydrophobic and ionic, are attractive. As a result a collapsed aggregate is formed. The viscosity, R_g and D_h show lowest values. In some cases a precipitate is formed at this pH values. At lower pH values, a fraction of the units having basic groups (DMAEM) would be ionized. This will lead to repulsive cation-cation interactions resulting in expansion of the aggregate and the inclusion of more solvating water molecules. As a result, the viscosity, R_g and D_h increase its values. The aggregate is held together through hydrophobic interactions. At higher pH values, a fraction of the units having acid groups (deprotected M4MOB or M6MOH) would be ionized. This will lead to repulsive anion-anion interactions resulting in expansion of the aggregate and the inclusion of more solvating water molecules. As a result, the viscosity, R_g and D_h increase its values. The aggregate is held together through hydrophobic interactions. In some cases, at very high or very low pH values depending on copolymer composition, the viscosity goes through a maximum and drops again. This behaviour may be explained if we consider that the repulsive ionic forces may overcome the attractive hydrophobic interactions bringing the disruption of the aggregate structure. However, from DLS results we must emphasize that the strong association tendency of these copolymers is kept even in those conditions, since we observed that larger aggregates tend to be formed at very high or very low pH values (see Table 4 and Figure 4).

Effect of the spacer group

For this investigation we used methacrylic acid monomers having methylene spacer groups of 3 (M4MOB) and 5 units (M6MOH), respectively. How due these additional methylene units in the copolymer series influences the observed behaviour? Qualitatively we observed the same general features in both copolymer series regarding ampholytic and associative behaviour and regarding insolubility in water at certain pH-values if the acid comonomer content (protected and deprotected) was higher than 70%. In a previous work [22] we found that the acidity constants (pK_a) of methacrylic polymers with spacer groups increase with increasing number of methylenes in the spacer group. Therefore we expected a change in the properties that depend on the acidity of these units in the copolymers, specially the isoelectric point (IP) of the systems. Table 5 compares the main observed features of the pH-dependent aqueous solution behaviour for both copolymer series. Although, the composition of the copolymers is not the same in both series, is close enough to allow for some trends to be recognized. On the one hand we observe that the IP shifts to higher pH-values in the case of the M6MOH-copolymers, compare for this M4MOB-DMAEM (22-78) and M4MOB-DMAEM (52-48) copolymers with M6MOH-DMAEM (25-75) and M6MOH-DMAEM (44-56) copolymers, respectively. On the other hand, the maximum values of viscosity, A_2 and R_g in alkaline buffers shift also to higher pH-values for the M6MOH-copolymer series, compare M4MOB-DMAEM (52-48) and M6MOH-DMAEM (44-56) copolymers. If we compare our results with the IP reported for DMAEM-MAA copolymers systems, we find the following: for a random copolymer containing a 1 to 1 ratio of both units de IP is 6.4 [28], for a block copolymer with same composition the reported IP is 6.7 [3] and for a random terpolymer containing a ratio of 1:1:1 of DMAEM:MAA:MMA(methylmethacrylate) units the reported IP is 6.6 [9]. These results suggest that only the balance of ionisable units determines the IP. We must emphasize that the same authors report [9, 28] that block-copolymers show to the contrary of random copolymers, regions of pH where they are insoluble. In the case of a slightly more hydrophobic random copolymers in which instead of DMAEM, *N,N'*-diethylaminoethylmethacrylate (DEAEM) was used, the IP shifted to slightly higher pH-value of 7.0 for a 1:1 charge balanced sample [7]. Moreover, unbalanced random DEAEM-MAA copolymers showed regions of pH in which they were insoluble [7]. In our case, the nearly charge balanced copolymers we prepared: M4MOB-DMAEM (52-48) and M6MOH-DMAEM (44-56), showed IP values of 5.4 and 7.9 respectively; therefore 5 methylene units in the spacer are needed to exert a clear shift in the IP to higher values confirming that the IP is mainly determined by the charge balance and the respective acidity and basicity constants of the comonomers.

In the case of the copolymers prepared with high concentration on the hydrophobic acid comonomers: M4MOB-DMAEM (72-25) and M6MOH-DMAEM (72-28), the influence of the remaining protected acid units is so strong that insolubility superimposes to the expected features at intermediate and low pH-values showing that in this case ionic interactions are secondary to hydrophobic association.

Conclusions

Ampholytic copolymers containing DMAEM as basic units and methacrylic acid comonomers with aliphatic spacers of 3

Table 5. Comparison of pH-dependent expansion-contraction behaviour at room temperature in aqueous solution for copolymers after deprotection reaction.

Starting Copolymer	$[\eta_{sp}/C_p]^b$ in mL/g			R_g in nm			D_h^c in nm		
(Composition)[a]	Max	Min	Max	Max	Min	Max	Max	Min	Max
CopM4MOB-DMAEM	32.4	6.7	43.4	209	176	203	1910	160	866
(22-78)	(pH=4.4;	8.4;	10.5)	(pH=2.2;	6.2	8.1)	(pH=3.7;	7.9;	9.7)
CopM4MOB-DMAEM	24.5	9.2	47.4	195	162	228	——	£125	731
(52-48)	(pH=4.3;	5.4;	9.8)	(pH=4.1;	5.1	8.1)		(pH ≤ 5.4;	=8.2)
CopM4MOB-DMAEM	IS	≤41.6	61.0	IS	≤183	197	——	——	506
(75-25)		(pH ≤ 6.5;	=11.0)		(pH ≤ 6.2;	=8.1)			(pH= 10.0)
CopM6MOH-DMAEM	25.8	7.5	25.0	212	196	209	2698	401	1046
(25-75)	(pH=3.4;	8.1;	10.5)	(pH=4.4;	5.2-8.1	9.8)	(pH=6.1;	6.2-8.5;	10.4)
CopM6MOH-DMAEM	16.7	5.8	40.8	200	29	243	——	——	——
(44-56)	(pH=3.8;	7.9;	10.4)	(pH=3.3;	4.2	9.8)			
CopM6MOH-DMAEM	5.8	0	55.0	—	≤186	195	IS	≤519	940
(72-28)	(pH=2.8;	4-6;	10.8)		(pH ≤ 6.2;	=8.1)		(pH ≤ 6.3;	=10.5)[d]

a- For composition after deprotection see Table 3
b- C_p=1.8 g/L
c-Average-value from all distributions, C_p= 2.0 - 2.5 g/L
d-C_p= 4.0 – 6.0 g/L
IS= Value would lay in insoluble region
— Not evaluated

and 5 methylenes, as acid units were prepared by free radical polymerization followed by selective saponification of the protected acid groups. This synthetic methodology was effective to yield the desired statistical copolymers with appropriate molecular weights in a broad range of compositions. However, the saponification reaction being not complete resulted in different amounts of hydrophobic protected acid units remaining in the copolymers and contributing as additional sites for hydrophobic interactions.

In aqueous solutions the copolymers show a strong associative behaviour revealed by an increase in reduced viscosity and apparent molecular weights by SLS at least 100 times that of single macromolecules. The macromolecular aggregates formed are stable to sonication at copolymer concentrations higher than 0.9 g/L. The size of the aggregates change with changes in the pH of the solution in a similar fashion than ampholytic macromolecules as revealed by SLS and DLS measurements. They expand both at low and at high pH-values while they contract at intermediate pH-values. DLS studies showed further that at each pH-value at least two distributions of sizes of aggregates are formed. At very low and very high pH-values the aggregates merge into even larger structures demonstrating their strong associative tendency. On the one side, since the amount of ionic and hydrophobic interacting groups is composition dependent, the observed features depend also on the relative content of each type of unit present in the copolymer. On the other side, since the ionisation of the units depends on the acidity constant (pK_a) of the functional groups there is a dependency of the observed behaviour on the type of comonomer used, namely: using as acid comonomer one including a longer aliphatic spacer (5 methylenes, M6MOH), brings a shift in the pH of the contraction of the aggregates to higher values, in comparison to using M4MOB or a simple MAA, instead. All statistical copolymers prepared were able to form nano-sized aggregates in a range of pH-values, depending on their composition.

Nowadays that the formation of micelles and other aggregates for nano-technology purposes are subject of increased efforts worldwide using block-copolymers as target macromolecules, we find that statistical copolymers are an easier to prepare alternative for stable, nano-sized aggregates, provided that ionic and hydrophobic interactions are given through an adequate copolymer composition and pH.

Acknowledgements

The financial support of the following agencies is gratefully acknowledged: CONACYT-México (Project Nr. 28022-U) and Volkswagen Foundation-Germany (Project Nr. I/76 065). We appreciate the valuable technical support of I.A. Rivero and R. Somanathan (200 MHz-NMR), A. Zizumbo (DSC), S. Richter (DLS) and C. Meissner (SLS). We thank specially IBM-Almaden Research Center for 400 MHz-NMR measurements.

References

1. Bekturov, E.A.; Bakauova, Z.K. *Synthetic water-soluble polymers in solution*. Hüthig & Wepf Verlag, Basel-Heidelberg-New York, **1986**, p163.
2. Wen, S.; Stevenson, W.T.K. *Colloid Polym. Sci.* **1993,** *271*, 38-49.
3. Lowe, A.B.; Billingham, N.C.; Armes, S.P. *Macromol.* **1998,** *31*, 5991-5998.
4. Nonaka, T.; Matsumura, S.; Ogata, T.; Kurihara, S. *J. Membr. Sci.* **2003,** *212*, 39-53.
5. Corpart, J.M.; Candau, F. *Macromol.* **1993,** *26*, 1333-1343.
6. Kathmann, E.E.L.; White, L.A.; McCormick, C.L. *Macromol.* **1997,** *30*, 5297-5304.
7. Alfrey, T.Jr.; Fuoss, R.M.; Morawetz, H.M.; Pinner, H. *J. Amer. Chem. Soc.* **1952,** *74*, 438-441.
8. Ehrlich, G.; Dotty, P. *J. Amer. Chem. Soc.* **1954,** *76***,** 3764-3777.
9. Patrickios, C.S.; Hertler, W.R.; Abbott, N.L.; Hatton, T.A. *Macromol.* **1994,** *27*, 930-937.
10. Nath, S.; Patrickios, C.S.; Hatton, T.A. *Biotechnol. Prog.* **1995,** *11*, 99-103.
11. Goloub, T.; de Keizer, A.; Cohen-Stuart, M.A. *Macromol.* **1999,** *32*, 8441-8446.
12. Gohy, J.F.; Creutz, S.; Garcia, M.; Mahltig, B.; Stamm, M.; Jérôme, R. *Macromol.* **2000,** *33*, 6378-6387.
13. Bütün, V.; Armes, S.P.; Billingham, N.C. *Polymer* **2001**, *42*, 5993-6008.
14. Hadjikallis, G.; Hadjiyannakou, S.C.; Vamvakaki, M.; Patrickios, C.S. *Polymer* **2002**, *43*, 7269-7273.
15. Bieringer. R.; Abetz, V.; Müller, A.H.E. *Eur. Phys. Jnl. E* **2001**, *5*, 5-12.
16. Shalaby, S.W.; McCormick, C.L.; Buttler, G.B. *Water soluble polymers: Synthesis, solution properties and application*. Amer. Chem. Soc., ACS Symp Ser 467, Washington DC, **1991**.
17. Dubin, P.; Bock, J.; Davis, R.; Schulz, D.N.; Thies, C. *Macromolecular complexes in chemistry and biology*. Springer-verlag, Berlin, **1994**.
18. Morishima, Y. *Trends Polym. Sci.* **1994**, *2*, 31-36.
19. Yusa, S.; Kamachi, M.; Morishima, Y. *Langmuir* **1998**, *14*, 6059-6067.
20. Chang, Y.; McCormick, C.L. *Macromol.* **1993**, *26*, 6121-6126.
21. Noda, T.; Hashidzume, A.; Morishima, Y. *Macromol.* **2000**, *33*, 3694-3704.
22. Rogel-Hernández, E.; Licea-Claveríe, A.; Cornejo-Bravo, J.M.; Arndt, K.F. *Desig. Monom. Polym.* **2001**, *4*, 343-356.
23. Creutz, S.; Teyssié, P.; Jérôme, R.; *Macromol.* **1997**, *30*, 6-9.
24. Arndt, K.F.; Müller, G. *Polymer Charakterisierung*. Carl Hanser Verlag, München-Wien, **1996**, p182.
25. Arndt, K.F.; Müller, G. *Polymer Charakterisierung*. Carl Hanser Verlag, München-Wien, **1996**, p142.
26. Fuoss, R.M.; Strauss, U.P. *J. Polym. Sci.* **1948,** *3*, 246- .
27. Förster, S.; Schmidt, M. *Adv. Polym. Sci.* **1995**, *120***,**51-133.
28. Bekturov, E.A.; Kudaibergenov, S.E.; Rafikov, S.R. *J. Macromol. Sci., Rev. Macromol. Chem. Phys.* **1990**, *C30*, 233-303.
29. Lowe, A.B.; McCormick, C.L. *Chem. Rev.* **2002**, *102*, 4177-4189.
30. Hadjichristidis, N.; Pispas, S.; Floudas, G.A. *Block Copolymers: synthetic strategies, physical properties and applications*. Wiley-Interscience, Hoboken, NJ, **2003**, p.204.