Chain dimensions in free and immobilized brush states of polysulfobetaine in aqueous solution at various salt concentrations

Y Terayama¹, H Arita¹, T. Ishikawa¹, M Kikuchi², K Mitamura², M Kobayashi², N L Yamada³ and A Takahara*¹,2,4

¹Graduate School of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0385, Japan
²JST, ERATO Takahara Soft Interfaces Project, 744 Motooka, Nishi-ku, Fukuoka 819-0385, Japan
³Neutron Science Laboratory, High Energy Accelerator Research Organization, 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan
⁴Institute for Materials Chemistry and Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0385, Japan

E-mail: takahara@cstf.kyushu-u.ac.jp

Abstract. The chain dimensions of free and immobilized polysulfobetaine in aqueous solution at various salt concentrations were investigated by size exclusion chromatography with multiangle light scattering and neutron reflectivity measurement, respectively. The dependence of the z-average mean square radius of gyration ($<S_z^2>^{1/2}$) on the weight-average molecular weight ($M_w$) of free poly(3-(N-2-methacryloyloxyethyl-N,N-dimethyl)ammoniatopropanesulfonate) (MAPS) in aqueous solution at salt concentrations of 74, 100, 200, and 500 mM was described by the perturbed wormlike chain model using the chain stiffness parameter $\lambda^{-1}$, the molar mass per unit contour length $M_c$, and the excluded volume effect $B$. $B$ increased from 0 to 1.8 nm with increasing salt concentration to 500 mM due to the screening of attractive electrostatic interaction between ammonium cations and sulfonyl anions by salt ions. The swollen structure of the poly(MAPS) brush in D$_2$O changed from a shrunken state to a relatively extended state with increasing salt concentration from 0 to 500 mM NaCl/D$_2$O solution. The thickness of the swollen poly(MAPS) brush in 500 mM NaCl/D$_2$O was 9.0 times greater than $2<S_z^2>^{1/2}$ of free poly(MAPS) due to high osmotic pressure generated by the excluded volume effect of densely grafted polymer chains.

1. Introduction

Aqueous solution of poly(3-(N-2-methacryloyloxyethyl-N,N-dimethyl)ammoniatopropanesulfonate) (MAPS) exhibits a unique salt dependency compared with the typical polyelectrolyte. In deionized water, the poly(MAPS) chain is insoluble due to the strong attractive inter- and intramolecular electrostatic interaction between ammonium cations and sulfonyl anions. With increasing salt concentration in aqueous solution, especially above theta concentration, poly(MAPS) shows enhanced solubility in aqueous solution and greater chain dimensions because the attractive electrostatic interaction among sulfobetaine groups is neutralized by the screening effect of salt ions [1]. Kato et al.
estimated the mean square radius of gyration $<\xi^2>$, and theta concentration of poly(MAPS) to be 60 mM NaCl aqueous solution using static light scattering for polymers ranging from $5 \times 10^3$ to $3 \times 10^5$ in weight-average molecular weight ($M_w$); however, the molecular weight distribution (MWD) of the polymers was not clarified in their report. Polymers with broad MWD are unfavorable for the estimation of chain dimensions. In the present study, we precisely estimated the theta concentration and mean square radius of gyration $<\xi^2>$ of poly(MAPS) in NaCl aqueous solution using size-exclusion chromatography combined with multiangle light scattering (SEC-MALS) for polymers with a wide range of molecular weight ($M_w = 1 \times 10^3$ – $3 \times 10^5$) and narrow MWD ($M_w/M_n < 1.2$).

On the other hand, when poly(MAPS) is covalently tethered to a solid surface to form a brush, the thickness of the brush layer in the solution is affected by the solvent quality and ionic strength [2]. The poly(MAPS) brush chains in aqueous salt solution would be considerably extended in the perpendicular direction against the substrate over the radius of gyration of the corresponding free polymer due to the reduction in attractive intra- and intermolecular interaction and excluded volume effect. In this study, the effect of salt concentration on the swollen thickness of the poly(MAPS) brush with relatively narrow MWD was estimated by neutron reflectivity (NR) measurement and compared with the dimensions of unbound poly(MAPS). The depth profiles of the swollen brush structures were also examined from the scattering length density curves.

2. Experimental

Surface-initiated atom transfer radical polymerization (ATRP) of MAPS [3] from (2-bromo-2-methyl)propionoxyhexyltrimethoxy-silane-immobilized silicon wafer and quartz plate was carried out using CuBr and 2,2-dipyridyl in 1-hexyl-3-methylimidazolium chloride / 2,2,2-trifluoroethanol (TFE) (1/9, wt/wt) mixture at 60°C for 12 h under dry argon atmosphere to give a poly(MAPS) brush with $M_w$ of 2.69×10$^5$, relatively narrow MWD ($M_w/M_n = 1.23$), and thickness of 41 nm as determined by ellipsometry in air.

Free poly(MAPS) was prepared by means of free radical polymerization in TFE using 2,2'-azobis(isobutyronitrile) as an initiator and was fractionated to 10 parts with different molecular size by a recycling-preparative HPLC system (Japan Analytical Industry Co., Ltd., LC-9104) equipped with an RI detector (JAI RI-7s) using two columns (JAIGEL GS620-50L, GS320-50L) in 200 mM aqueous NaCl solution as an eluent at a flow rate of 20 mL min$^{-1}$ at room temperature.

SEC was carried out using an RI detector (Shimadzu, RID-10A) equipped with three columns (Tosoh, TSKgel G3000PWXL, G5000PWXL, and G6000PWXL) in aqueous NaCl solutions as the eluent at a flow rate of 0.8 mL min$^{-1}$ at 40°C. $M_w$ and $z$-average mean square radius of gyration ($<\xi^2>$) of the fractionated poly(MAPS) were determined by SEC-MALS (Wyatt Technology, DAWN-EOS, wavelength: $\lambda = 690$ nm) at 25°C. The Rayleigh ratio $R(90)$ at a scattering angle of 90° was determined using pure toluene. The sensitivity and dead volume of 16 detectors were corrected using the scattering intensity of 0.30 wt% NaCl aqueous solution of a poly(2-(N-2-methacryloyloxyethyl-N,N-dimethyl)ammoniothanesulfonate) standard with $M_w = 4.82 \times 10^2$ g mol$^{-1}$ and $M_w/M_n = 1.10$. Polymer solutions with mass concentration of about $1 \times 10^{-3}$ g cm$^{-3}$ were injected into SEC columns using a sample loop of 100 µL. The specific refractive index increment ($dn/dc$) of the poly(MAPS) ($M_w = 1.36 \times 10^3$ g mol$^{-1}$, $M_w/M_n = 3.07$) in 74, 100, 200, and 500 mM NaCl aqueous solution at 25°C measured by a differential refractometer (Otsuka Electronics DRM-1020, wavelength: $\lambda = 633$ nm) was 0.121, 0.121, 0.120, and 0.117 g cm$^{-1}$, respectively. These $dn/dc$ values were used for the analysis of SEC-MALS even though the laser wavelength was different from that of DRM, because the difference between $dn/dc$ values at $\lambda = 633$ nm and $\lambda = 690$ nm is negligible.

NR measurement was carried out using the ARISA-II time-of-flight type reflectometer at BL16 in MLF/J-PARC providing 25-Hz-pulsed neutron radiation at ~120 kW. The wavelength of the incident neutrons was tuned at 0.2–0.88 nm by a disk chopper. A neutron beam was irradiated from the quartz to the interface between D$_2$O and poly(MAPS) brush on quartz glass and the reflected neutrons were...
collected by a 2D scintillation detector. The incident slits were adjusted to maintain a 50-mm footprint size on the sample surface at any incident angle (typically 0.30, 0.75, 1.20°). The NR profiles were analyzed by fitting the calculated reflectivity from the model scattering length density (SLD) profiles to the data, using Parratt32 software.

3. Results and discussion

3.1. Chain dimensions of free poly(MAPS) in aqueous solution at various salt concentrations

The chain dimensions of free poly(MAPS) in 74, 100, 200, and 500 mM NaCl aqueous solutions were investigated by SEC-MALS measurement using 10 samples with various $M_w$ ranging from $1.11 \times 10^4$ to $5.45 \times 10^6$ and with narrow MWD ($M_w/M_n < 1.2$) prepared by fractionation using a recycling-preparative HPLC system. Figure 1(a) shows typical SEC curves of poly(MAPS) and the plot of $M_w$ versus retention volume ($RV$) in 74 and 500 mM NaCl aqueous solutions calculated from the SEC curves of 10 samples with different $M_w$. Despite the same $M_w$, the SEC curve of poly(MAPS) in 500 mM NaCl aqueous solution appeared at a lower RV region compared with the 74 mM solution. The calibration curve for the 500 mM NaCl aqueous solution clearly shifted to a lower RV region compared to that for the 74 mM solution, indicating that the poly(MAPS) chains in the 500 mM NaCl aqueous solution had expanded.

The $<S^2>_z^{1/2}$ of the poly(MAPS) in 74, 100, 200, and 500 mM NaCl aqueous solution was estimated using equation (1):

$$P(q)^{-1/2} = 1 + \frac{1}{6} \left< S_z^2 \right> q^2$$

and

$$q = \frac{4\pi n_0 \sin(\theta/2)}{\lambda}$$

where $q$, $P(q)$, $n_0$, and $\theta$ are the scattering vector, single particle scattering function, refractive index of solvent, and scattering angle of detector, respectively. Figure 1(b) shows typical plots of $P(q)^{-1/2}$ vs. $q^2$ for the poly(MAPS) at different RV from 18.9 to 22.3 mL when 74 mM NaCl aqueous solution was used as an eluent. The $<S^2>_z^{1/2}$ was determined by the initial slope between $P(q)^{-1/2}$ vs. $q^2$.

Figure 2(a) shows the dependency of $<S^2>_z^{1/2}$ on $M_w$ of poly(MAPS) in 74, 100, 200, and 500 mM NaCl aqueous solution at 25°C. In general, $<S^2>_z^{1/2}$ is related to $M_w^\alpha$, where the scaling exponent $\alpha$ varies with solvent quality; $\alpha = 0.5$ indicates theta conditions. We found that 74 mM NaCl aqueous solution revealed the scaling exponent to be 0.5 from the slope of logarithmic plots of $<S^2>_z^{1/2}$ and $M_w$, as shown in figure 2(a). Therefore, we established 74 mM NaCl aqueous solution as the theta concentration of poly(MAPS). Kato et al. previously determined the theta concentration for poly(MAPS) by static light scattering at 30°C to be 60 mM of NaCl aqueous solution [1]. The slight difference between 74 mM in our study and 60 mM in theirs might be attributed to the broader MWD of poly(MAPS) compared with our experiment.

![Figure 1](image.png)

**Figure 1.** (a) SEC curves of fractionated poly(MAPS) ($M_w = 1.21 \times 10^6$, $M_w/M_n = 1.17$) in 74 and 500 mM aqueous NaCl solution, together with the absolute calibration curves of the poly(MAPS) in aqueous NaCl solution with 74 (circles) and 500 mM (squares) determined by SEC-MALS. (b) Angular dependence of $P(q)^{-1/2}$ for the indicated RV of the poly(MAPS) in 74 mM aqueous NaCl solution at 25°C by SEC-MALS.
Figure 2. (a) $M_w$ dependence of $<S^2>_{z\frac{1}{2}}$ for the poly(MAPS) in 74, 100, 200, and 500 mM aqueous NaCl solution at 25°C as experimental data, and in 60 mM at 30°C as reported by Kato et al. [2]. The solid curves are the theoretical values for the perturbed wormlike chain model as calculated by equation (2). (b) Murakami Plot of $[M_w/<S^2>_z]^{1/2}$ vs. $M_w^{-1}$ for the poly(MAPS) in 74 mM aqueous NaCl solution at 25°C.

For the chain dimensions of the polymer under theta conditions, the mean square radius of gyration $<S^2>_{KP}$ can be described by the unperturbed wormlike chain (Kratky-Porod chain) model followed by equation (2) [4]:

$$<S^2>_{KP} = \frac{L}{6\lambda} - \frac{1}{4\lambda^2} + \frac{1}{8\lambda^2L^2}[1 - \exp(-2\lambda L)]$$  \hspace{1cm} \text{(2)}$$

where $\lambda$ and $L$ are the chain stiffness parameter (Kuhn segment length) and the contour length, respectively. When $\lambda L$ is greater than 2, the maximum error between the theoretical and exact values is less than 1%. Therefore, equation (2) approximates equation (3) (Murakami plot) [5]:

$$\left[\frac{M}{<S^2>_{z\frac{1}{2}}} \right]^{1/2} = \left(6\lambda M_L \right)^{1/2} \left(1 + \frac{3M_L}{4\lambda M_L} \right)$$  \hspace{1cm} \text{(3)}$$

where $M$ and $M_L$ are the molecular weight of polymer and the molar mass per unit contour length, respectively, and are defined as $L = M/M_L$. The $\lambda$ and $M_L$ can be evaluated from the slope and intercept of the plot of $[M/<S^2>_z]^{1/2}$ vs. $M^{-1}$ using equation (3). Figure 2(b) shows the plot of $[M_w/<S^2>_z]^{1/2}$ vs. $M_w^{-1}$ for the poly(MAPS) in 74 mM NaCl aqueous solution. These plots were well aligned on the theoretical line described by equation (3) using 5.1 nm of $\lambda$ and 2470 g mol$^{-1}$ of $M_L$ corresponding to the experimental $M_w/<S^2>_{z\frac{1}{2}}$. The estimated $\lambda = 5.1$ nm of poly(MAPS) in 74 mM NaCl aqueous solution was larger than the $\lambda = 1.5$ nm of PMMA under theta conditions [6]. The contour length per MAPS monomer is 0.11 nm, which is smaller than the 0.125 nm of the PMMA chain with the all-trans conformation. These results indicated that the experimental $M_w$ dependence of $<S^2>_{z\frac{1}{2}}$ under theta conditions was quantitatively described by the unperturbed wormlike chain model.

The $M_w$ dependence of $<S^2>_{z\frac{1}{2}}$ in 100, 200, and 500 mM should be described by the unperturbed wormlike chain model modified by the quasi-two-parameter (QTP) theory using the excluded volume strength ($B$) [7-10]. Figure 2(a) shows the experimental plots of $<S^2>_{z\frac{1}{2}}$ in 100, 200, 500 mM and the corresponding theoretical straight line calculated using an unperturbed wormlike chain model based on the QTP theory. All $<S^2>_{z\frac{1}{2}}$ were well represented by theoretical lines when $B$ in 100, 200, and 500 mM was 0.3, 1.2, and 1.8 nm, respectively. The increase in $B$ parameter indicated that the intramolecular excluded volume effect increased with increasing salt concentration from 100 to 500 mM due to the screening effect of salt ions in the aqueous solution. The experimental $M_w$ dependence of $<S^2>_{z\frac{1}{2}}$ for the poly(MAPS) in NaCl aqueous solution at all salt concentrations was quantitatively described in terms of the perturbed wormlike chain model using the three parameters of $\lambda$, $M_L$, and $B$.}


3.2. Swollen state of poly(MAPS) brush on the substrate at aqueous solution interface

Figure 3. NR curves (left) of poly(MAPS) brush in (a) D₂O, (b) 100 mM NaCl/D₂O, (c) 500 mM NaCl/D₂O. Their corresponding neutron SLD profiles (center) and volume fraction profiles of MAPS (right) along with the distance from the quartz surface. The fitting line of the volume fraction profile in 500 mM poly(MAPS) was calculated using the parabolic function \( \phi = \phi_0(1-(z/h)^2) \), where \( \phi_0 \) is the distance from the substrate \( z = 0 \), and \( h \) is the cut-off thickness of the swollen brush layer.

Figure 3 shows the NR curves of the poly(MAPS) brush in D₂O, 100 mM, and 500 mM NaCl/D₂O and their corresponding fitting curves calculated by assumed SLD profiles consisting of a quartz layer (3.40×10⁻⁴ nm⁻²), initiator layer (0.60×10⁻⁴ nm⁻²), gradient brush layer, and D₂O layer (6.38×10⁻⁴ nm⁻²). The SLD in D₂O increased drastically from 1.70×10⁻⁴ to 6.38×10⁻⁴ nm⁻² at 85 nm. With increasing salt concentration to 100 mM, the SLD profile gradually increased from 3.05×10⁻⁴ to 6.38×10⁻⁴ nm⁻² at 120 nm. With a further increase in salt concentration to 500 mM, the SLD reached 150 nm with increasing SLD from 4.0×10⁻⁴ to 6.38×10⁻⁴ nm⁻². Swollen brush thickness \( h \) was estimated at 130 nm by the volume fraction profile using the parabolic function fitting. In D₂O, the brush chains interact with neighboring polymer chains due to the attractive electrostatic interaction of sulfobetaine groups to form shrunken structures. The brush chain length in 100 mM NaCl/D₂O was slightly increased in the direction of the D₂O interface due to screening of the electrostatic interaction by salt ions, while inside the brush chain a shrunken state was maintained. On the other hand, the brush chain was further extended at 150 nm in 500 mM NaCl/D₂O. The volume fraction profile of MAPS in 500 mM NaCl/D₂O shows a parabolic function curve indicating that the brush chain was completely swollen in the aqueous solution [11].

Free poly(MAPS) dissolved in the NaCl aqueous solution above 74 mM NaCl aqueous solution; however, the near substrate region of poly(MAPS) brush chains in 100 mM NaCl/D₂O seemed to remain in a shrunken state. We supposed that densely-grafted poly(MAPS) chains strongly interacted with each other to generate strong attractive electrostatic interaction among sulfobetaine groups. On the other hand, the poly(MAPS) brush in 500 mM NaCl/D₂O was swollen because concentrated salt ions effectively screened the electrostatic interaction inside the brush chains. The region of the brush chains reached 131 nm from the substrate, which was 9.0 times greater compared with 14.6 nm of \( 2\langle S^2 \rangle_z^{1/2} \) of free poly(MAPS) corresponding to the same \( M_n \) of the estimated poly(MAPS) brush in 500 mM NaCl aqueous solution. The graft density of the poly(MAPS) brush \( \sigma \) was 0.26 chains nm⁻² calculated by \( \sigma = \frac{dN_A\times L \times 10^{-21}}{M_n} \) where \( d, N_A, \) and \( L \) are the assumed bulk density, Avogadro’s number, and the dry thickness of the brush, respectively, i.e., the occupied area of a single brush chain was 3.84 nm², which is smaller than the chain dimensions of the free poly(MAPS) in 500 mM NaCl aqueous solution. We supposed that high osmotic pressure among the densely grafted polymer chains resulted in the relatively extended structure in aqueous solution.
Salt concentration dependency of chain dimensions in aqueous solution varies according to the chemical structure of ionic groups of polyelectrolytes. The uniqueness of poly(MAPS) can be identified by comparing the salt concentration dependency with that of other polyelectrolyte chain conformations. For example, poly(2-methacryloyloxyethyl phosphorylcholine) (MPC) brush, which is a zwitter-type polyelectrolyte as well as poly(MAPS), in salt aqueous solution maintains a relatively extended chain structure over a wide range of ionic strength in the solution probably due to weak electrostatic static interaction among the phosphobetaine groups [12, 13]. In the case of a typical anionic polymer, poly(styrenesulfonate sodium salt) (SSNa), poly(SSNa) brush in deionized water forms a stretched structure due to the strong repulsion between sulfonate monomers [14], while the brush takes on a more coiled conformation, similar to electronically neutralized chains, in 5.0 M NaCl aqueous solution by screening of electrostatic interchain repulsion. In contrast, the poly(MAPS) brush in aqueous media transform from collapsed to extended conformation with increase in salt concentration due to the reduction in attractive electrostatic interaction among sulfobetaine groups by hydrated salt ions. This unique solution behavior could be applicable to friction or adhesion control of the surface.

4. Conclusion
The thickness of the swollen poly(MAPS) brush estimated by NR measurement increased at increasing salt concentration from 0 to 500 mM. However, the brush chains near the substrate in 100 mM NaCl aqueous solution remained in a shrunken state due to the strong attractive electrostatic interaction among betaine groups, even though the 100 mM NaCl aqueous solution would be a good solvent for free poly(MAPS) because the theta concentration was 74 mM. This attractive electrostatic interaction was further screened in 500 mM NaCl/D$_2$O solution to give an electrically neutral polymer brush. The swollen thickness of the poly(MAPS) brush achieved a value 9.0 times greater than $2S_z^{1/2}$ of free poly(MAPS), indicating that the brush chains in 500 mM NaCl/D$_2$O extended in the perpendicular direction from the substrate due to high osmotic pressure generated by densely grafted polymer chains.

Acknowledgements
The present work is supported by a Grant-in-Aid for the Global COE Program, “Science for Future Molecular Systems” from the Ministry of Education, Culture, Science, Sports and Technology of Japan. YT acknowledges support from the Japan Society for the Promotion of Science. The experiments at J-PARC were performed under the approval of the Neutron Scattering Program Advisory Committee of IMSS, KEK (Proposal No. 2009S08).

References
[1] Kato T and Takahashi A 1996 Ber. Bunsenges. Phys. Chem. 100 784
[2] Terayama Y, Kikuchi M, Kobayashi M, Hino M and Takahara A 2009 J. Phys: Conf. Ser. 184 012011
[3] Terayama Y, Kikuchi M, Motoyasu K and Takahara A, 2011 Macromolecules, 44, 104
[4] Benoit H and Doty P 1953 J. Phys. Chem. 57 958
[5] Murakami H, Norisuye T and Fujita H 1980 Macromolecules 13 345
[6] Tamai Y, Konishi T, Einaga Y, Fujii M and Yamakawa H 1990 Macromolecules 23 4067
[7] Yamakawa H and Stockmayer W H 1972 J. Chem. Phys. 57 2843
[8] Domb C and Barrett A J 1976 Polymer 17 179
[9] Yamakawa H and Shimada J 1985 J. Chem. Phys. 83 2607
[10] Shimada J and Yamakawa H 1986 J. Chem. Phys. 85 591
[11] Milner S T, Witten T A and Cate M E 1988 Macromolecules 21 2610
[12] Kobayashi M, Terayama Y, Hino M, Ishihara K and Takahara A 2009 J. Phys: Conf. Ser. 184 012010
[13] Matsuda Y, Kobayashi M, Annaka M, Ishihara K and Takahara A, 2008 Langmuir, 24, 8772
[14] Tran Y, Auroy P, and Lee L-T 1999 Macromolecules 32 8952