

## ORIGINAL ARTICLE

# Solution properties of poly(*N*-methylethylene imine), a highly hydrophilic polycation

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The synthesized linear poly(*N*-methylethylene imine) (PMEI; weight-average molecular weight, 26 kDa) has been characterized in terms of its solubility, titration behavior, lower critical solution temperature (LCST)- and upper critical solution temperature (UCST)-type phase separations, self-aggregation and aggregation behaviors with polystyrene (PS) latex particles. PMEI is soluble in several solvents (that is, from nonpolar *n*-hexane above 45 °C to polar water at room temperature) and exhibits a UCST-type phase separation in toluene at 36 °C. The pKa value of PMEI is 7.28. An aqueous PMEI solution exhibited LCST-type phase behavior, and the LCST was determined to be 116 °C with the aid of the salting out effect. PMEI chains formed aggregates with negatively charged PS latex particles, and the aggregate structure was dependent on the PMEI concentration. In the vicinity of the isoelectric point, the PMEI-PS aggregates grew larger and precipitated, and at higher PMEI concentrations, the aggregates were positively charged and uniformly dispersed. In conclusion, PMEI is expected to be used as a highly hydrophilic and versatile polycation.

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## INTRODUCTION

In aqueous solutions, poly(ethylene imine) (PEI) tends to be protonated and forms aggregates with polyanions, such as DNA. Therefore, attempts have been made to use PEI as a gene delivery polymer.<sup>1–5</sup> In recent years, PEI has also attracted attention as an effective carbon dioxide absorbent to capture CO<sub>2</sub> from the ambient environment for global warming abatement.<sup>6,7</sup>

Linear poly(ethylene imine) ( $[-\text{CH}_2\text{CH}_2\text{NH}-]_x$ ) possesses a unique structural characteristic (that is, nitrogen inversion) where the hydrogen atom and lone pair on the same nitrogen atom readily exchange positions. The transition free energies were determined to be as small as 4 kcal mol<sup>-1</sup>.<sup>8</sup> Therefore, PEI undergoes not only internal rotations around the C–C and C–N bonds but also nitrogen inversion (that is, rapidly changes both conformation and configuration). These characteristic features of PEI prevent us from controlling its tacticity via synthetic methods. The probabilities of the *meso* (*P<sub>m</sub>*) and *racemo* (*P<sub>r</sub>*) forms defined by the adjacent NH sites depend on the intramolecular interactions and reach the equilibrium values under a given environment. Two types of intramolecular N–H ··· N attractions strongly influence the configurational properties including the characteristic ratio ( $\langle r^2 \rangle / nl^2$ ) and the *meso* and *racemo* probabilities.<sup>8</sup> If the two attractions are as strong as those evaluated from molecular orbital calculations (–1.54 and –0.58 kcal mol<sup>-1</sup>, hydrogen bond strength = 100%),  $\langle r^2 \rangle / nl^2 = 2.9$  and  $P_m = 1 - P_r = 0.63$ , and the PEI chain tends to include a helical form with 18 bonds per turn due to the intramolecular attractive interactions.<sup>9</sup> Without the interactions

(hydrogen bond strength = 0%),  $\langle r^2 \rangle / nl^2 = 6.3$  and  $P_m = 0.52$ , and the polymeric chain would be close to an atactic random coil ( $P_m \approx P_r \approx 1/2$ ). In aqueous solutions, PEI is most likely protonated and positively charged. Therefore, electrostatic interactions are also influential.

If the hydrogen atom of the NH site is replaced by an alkyl group (R), the *N*-alkylated PEI chain loses the N–H ··· N attractions and only acts as an electron donor,<sup>9</sup> and the intramolecular attractions may be switched to intermolecular ones with the electron-accepting sites of media. Therefore, the chain dimension would increase, which would result in improved solubility. This prediction may be contradictory to intuitive understanding because the NH-to-NR replacement renders the polymeric chain more organic like (that is, more hydrophobic). Although a long or bulky substituent must disturb the intermolecular interactions, its methyl-substituted polymer (poly(*N*-methylethylene imine) (PMEI,  $[-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)-]_x$ )) may be an appropriate candidate to confirm our hypothesis. In fact, PMEI is highly soluble in cold water,<sup>10</sup> and linear PEI is soluble in hot or cold water only under acidic conditions.<sup>11,12</sup>

To render a hydrophobic polymer water soluble, the polymer can be copolymerized with poly(ethylene oxide) (PEO,  $[-\text{CH}_2\text{CH}_2\text{O}-]_x$ ),<sup>13</sup> which differs from PEI only in the heteroatom (O or NH) and is soluble in a number of solvents (that is, nonpolar cyclohexane to polar water). The oxygen atom of PEO or the N(CH<sub>3</sub>) group of PMEI acts as an electron donor, and the NH site of PEI acts as both an electron donor and acceptor. The wide solubility of PEO is partially due to its conformational characteristics because PEO can change its

conformational preference based on its surroundings (conformation-adjustable property). With increasing solvent polarity, its intramolecular C–H · · · O attractions are shifted to intermolecular attractions,<sup>14–16</sup> and the gauche conformation appears to be enriched in the C–C bond.

In addition to the conformation-adjustable property, PEI and PMEI exhibit the previously mentioned configuration-adjustable characteristics due to nitrogen inversion. In solution, the former and latter adjustable properties decrease the enthalpy and increase the entropy, respectively. Therefore, the free energy of mixing decreases, and the solubility is enhanced. The relationship between the solubility and nitrogen inversion of PEI and PMEI will be discussed in the next section. In comparison with PEI, the PMEI chain that does not possess intramolecular N–H · · · N interactions, will more readily adapt a conformation and configuration based on its surroundings. Therefore, we can expect PMEI to be a highly hydrophilic polycation.

Few water-soluble polymers exist but these polymers have been used in cosmetics, detergent, oral care and water treatment.<sup>17</sup> Hydrophilic polycations are much rarer. Poly[2-(trimethylammonio) ethyl methacrylate] ([–CH<sub>2</sub>–C(CH<sub>3</sub>)(COOCH<sub>2</sub>CH<sub>2</sub>N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>)–]<sub>x</sub>) is a typical water-soluble polycation, and its quaternary nitrogen atom is positively charged and electrostatically interacts with water (negative enthalpy). In addition, its long side chain possesses a large degree of freedom with respect to internal rotation (positive entropy). In contrast to these polycations, PMEI may dissolve in water in the unique manner stated above.

For PMEI, a few synthetic methods, molecular weight measurements and solubilities in some solvents have been previously reported.<sup>10,18</sup> In this study, we have experimentally elucidated the solution properties of PMEI to determine the suitability of using PMEI as a hydrophilic polycation for various applications.

### Theoretical basis

The configurational entropy ( $S_{\text{conf}}$ , designated occasionally as conformational entropy) quantitatively represents differences in the conformational and configurational freedoms of a polymeric chain between a given state (for example, solution and melt) and the perfect crystal. In the latter state, the molecular chain lies in only a single conformation and has a null configurational entropy. The  $S_{\text{conf}}$  value can be calculated from<sup>19</sup>

$$S_{\text{conf}} = \frac{R}{x} \left[ \ln Z + T \frac{d(\ln Z)}{dT} \right] \quad (1)$$

**Table 1** Configurational entropies of PMEI, PEI, PEO and PPO at 25 °C that were calculated using the RIS scheme

Polymer	Condition	$S_{\text{conf}}$ (J K <sup>-1</sup> mol <sup>-1</sup> )	$TS_{\text{conf}}$ (kJ mol <sup>-1</sup> )
PMEI	As MO calc. predicted <sup>a</sup>	24.3	7.25
PEI	HBS = 100%	19.0	5.66
	HBS = 0%	26.2	7.81
PEO	In gas phase	20.9	6.23
	In organic solvents	20.1	5.99
	In water	17.8	5.31
Isotactic PPO	As MO calc. predicted	15.9	4.74

Abbreviations: HBS, hydrogen bond strength; MO, molecular orbital; PEI, poly(ethylene imine); PEO, poly(ethylene oxide); PMEI, poly(*N*-methylethylene imine); PPO, poly(propylene oxide); RIS, rotational isomeric state.

<sup>a</sup>With conformational energies evaluated from zero-potential energies of *N,N,N',N'*-tetramethylethylenediamine:  $E_7 = 1.27$ ,  $E_8 = 0.04$ ,  $E_9 = -0.42$ ,  $E_{11} = 0.34$ ,  $E_v = \infty$ ,  $E_{v'} = -0.27$ ,  $E_w = 1.69$ , and  $E_{w'} = 3.09$  kcal mol<sup>-1</sup>. Note that 1 cal = 4.184 J.

where  $R$  is the gas constant,  $x$  is the degree of polymerization and  $T$  is the absolute temperature. The partition function ( $Z$ ) is given by

$$Z = J^* \left( \prod_{j=1}^n U_j \right) J \quad (2)$$

where  $n$  is the number of skeletal bonds,  $U_j$  is the statistical weight matrix of the  $j$ -th bond,  $J^* = [1 \ 0 \ 0 \dots]$ , and  $J$  is the column matrix whose elements are set to 1. The partition function can be computed by the rotational isomeric state (RIS) scheme with conformational energies.<sup>20,21</sup> In principle, a polymer with a larger configurational entropy possesses a lower melting point and higher solubility. For PEO, some conformational energy sets reflect the gas phase and solvent effects of common organic solvents and water.<sup>16</sup> Therefore, Table 1 includes the  $S_{\text{conf}}$  data on PEO at 25 °C under different environments, and the entropy tends to decrease with increasing medium polarity, which is most likely due to the polar solvents capturing the PEO chain more strongly than the nonpolar media.

The nitrogenous polymers (that is, PEI and PMEI) undergo rapid nitrogen inversion. Therefore, the partition function, which is the sum of the Boltzmann factors of all of the possible configurations and conformations, is modified to<sup>8,22</sup>

$$Z = J^* \left( \prod_{i=1}^x W_i \right) J \quad (3)$$

where

$$W_i = \begin{pmatrix} V_i^{ll} & V_i^{ld} \\ V_i^{dl} & V_i^{dd} \end{pmatrix} \quad (4)$$

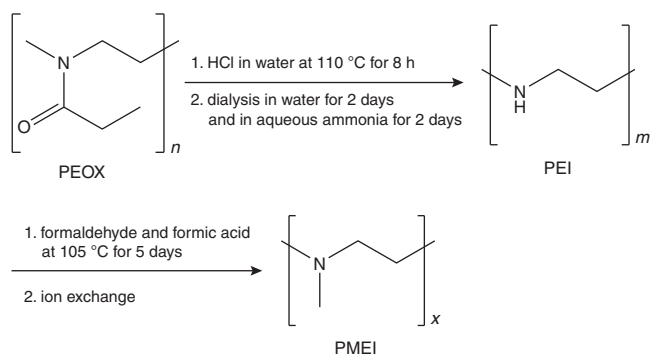
Here,  $V_i^{\alpha\beta}$  ( $\alpha = ll, dd, ld$  or  $dl$ ) is the product of statistical weight matrices of the  $i$ -th monomer unit. The  $l$  and  $d$  configurations are based on the concept of Flory's pseudoasymmetry.<sup>23</sup>

The  $S_{\text{conf}}$  value of PMEI was computed with conformational energies evaluated from zero-point energies that were previously derived from *N,N,N',N'*-tetramethylethylenediamine, which is a monomeric model compound of PMEI. The conformational energies have been defined analogous to those of poly(1-methylphosphirane).<sup>24</sup> For the energy values, see the footnote in Table 1. Preferably, the conformational equilibrium and related properties should be discussed with the Boltzmann factor calculated from the Gibbs free energy. In general, however, the differences in zero-point energies between conformations are similar to those in the free energies at room temperature.<sup>24</sup> The  $S_{\text{conf}}$  value of PMEI was determined to be 24.3 J K<sup>-1</sup> mol<sup>-1</sup>, which is larger than that of PEO in water by 6.5 J K<sup>-1</sup> mol<sup>-1</sup>. The entropy difference ( $\Delta S_{\text{conf}}$ ) yields a free energy of  $-T \Delta S_{\text{conf}} = -1.9$  kJ mol<sup>-1</sup>. If the intramolecular hydrogen bonds of PEI are as strong as the MO calculations indicate (hydrogen bond strength = 100%),<sup>8</sup> the  $S_{\text{conf}}$  value is 19.0 J K<sup>-1</sup> mol<sup>-1</sup>. If the hydrogen bonds completely disappeared (hydrogen bond strength = 0%), the PEI chain would be free with a larger  $S_{\text{conf}}$  of 26.2 J K<sup>-1</sup> mol<sup>-1</sup>. For the sake of comparison, Table 1 includes the  $S_{\text{conf}}$  value (15.9 J K<sup>-1</sup> mol<sup>-1</sup>) of a hydrophobic polyether (poly(propylene oxide)<sup>25</sup>). Comparison of the data listed in Table 1 indicates that the nitrogen inversion tends to increase the configurational entropy and enhance the solubility.

## MATERIALS AND METHODS

### Chemicals

Commercially available chemicals were used as obtained [poly(2-ethyl-2-oxazoline), abbreviated as PEOX, weight-average molecular weight ( $M_w$ ) ~ 500 000 Da, polydispersity index = 3–4 (Sigma-Aldrich Japan, Tokyo,



**Scheme 1** Synthesis of PMEI from PEOX via linear PEI.

Japan); 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Tokyo Chemical Industry, Tokyo, Japan); hydrochloric acid (35–37 wt%), aqueous ammonia (25 wt%), aqueous formaldehyde solution (36–38 wt%), formic acid, methanol, ethanol, chloroform, chloroform-*d*, *N,N*-dimethylformamide, dimethyl sulfoxide, toluene, acetone, diethyl ether, tetrahydrofuran (THF), *n*-hexane, *tert*-butyl methyl ether, sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), potassium chloride (KCl), sodium chloride (NaCl), potassium hydroxide (KOH), ultrapure water, and phosphate, carbonate and phthalate pH standard solutions (Wako Pure Chemical Industries, Osaka, Japan); polystyrene (PS) latex aqueous solution (standard sample of average diameter =  $119 \pm 2$  nm and zeta potential =  $-53.8$  mV (National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan); cellulose dialysis membrane Cellu-Sep T1/Nominal MWCO: 3500 (Membrane Filtration Products, Seguin, TX, USA); ion-exchange resin DIAION SA10AOH (Mitsubishi Chemical, Tokyo, Japan)]. In the static light scattering (SLS) measurements, spectrophotometric grade chloroform and toluene (Wako Pure Chemical Industries, Osaka, Japan) were used.

### Synthesis of linear PEI

The procedures for synthesizing PMEI from PEOX via linear PEI are shown in Scheme 1. Poly(2-ethyl-2-oxazoline) (10.0 g, 0.101 mol), hydrochloric acid (35–37 wt%, 80 ml), and distilled water (60 ml) were stirred at 110 °C for 8 h in a flask equipped with a Dimroth condenser and magnetic stirrer.<sup>26</sup> After cooling to room temperature, the reaction mixture was neutralized with aqueous ammonia (25 wt%), moved into the cellulose dialysis membrane, and soaked in distilled water for 2 days and in aqueous ammonia (25 wt%) for 2 days to yield a white precipitate in the membrane. The precipitate was collected by suction filtration and dried under reduced pressure to yield a white solid, which was identified as linear PEI by  $^1\text{H}$  and  $^{13}\text{C}$  NMR analyses (yield 74%).

### Synthesis of PMEI

The as-prepared linear PEI (2.0 g, 47 mmol), an aqueous formaldehyde solution (36–38 wt%, 20 ml), and formic acid (20 ml) were stirred at 105 °C for 5 days in a flask equipped with a reflux condenser.<sup>10</sup> After cooling to room temperature, the solution was mixed with hydrochloric acid (35–37 wt%, 12 ml), stirred for 30 min, concentrated and dried *in vacuo*. The product was dissolved in distilled water, filtered to remove the byproduct (that is, paraformaldehyde) and placed in a column filled with an ion-exchange resin that was previously conditioned to remove chlorine ions. The column eluate was concentrated and dried *in vacuo* overnight to yield a brown oily product, which was identified as PMEI based on  $^1\text{H}$  and  $^{13}\text{C}$  NMR analyses (yield 68%).

### NMR measurements

The  $^1\text{H}$  ( $^{13}\text{C}$ ) NMR spectra were recorded at 500 (125.7) MHz on a JEOL JNM-ECA500 spectrometer in the Center for Analytical Instrumentation at Chiba University. The NMR solvent was chloroform-*d*, which was also used for chemical shift calibration. The free induction decays were accumulated 64 (84–1024) times. In general, the pulse duration and recycle delay were 6.15 (3.3–3.7)  $\mu\text{s}$  and 10.0 (2.0) s, respectively. In the  $^{13}\text{C}$  NMR measurements, broadband proton decoupling was used under the conditions written in the parentheses.

### Fourier transform infrared

The samples for Fourier transform infrared measurements were prepared as follows: a lump of PEOX or PEI was crushed and pressed using a load of 20 kN for 60 s into a thin film. This film was placed between two KBr plates and pressed again with a load of 3 kN for 30 s. Although PMEI is a viscous liquid, it was placed directly between the KBr plates and pressed. The Fourier transform infrared spectra of these samples were measured at room temperature with a JASCO FT/IR-4200ST spectrometer under the following conditions: resolution,  $4\text{ cm}^{-1}$ ; accumulation, 128 times; measurement range,  $350\text{--}7800\text{ cm}^{-1}$ . The recorded data underwent  $\text{CO}_2$ -signal reduction and baseline correction using splines.

### SLS measurements

The SLS experiments were conducted on an Otsuka Electronics DLS-8300CU photometer equipped with a He–Ne laser (wavelength, 632.8 nm) and installed in a Yamato ADS101SMA clean bench. The sample cell, syringes and glassware were exposed to acetone vapor for cleaning before the experiment.

The as-synthesized PEI underwent molecular weight fractionations. PEI (2.4 g) was dissolved in ethanol (50 ml), and the solution was poured into *tert*-butyl methyl ether (800 ml), warmed and allowed to stand for 5 days to afford a precipitate, which was collected by filtration and dried under reduced pressure at 50 °C for 3 days to yield the first fraction. The filtrate was mixed with *tert*-butyl methyl ether (200 ml), and the above handling was repeated to collect the second fraction. The remaining solution was evaporated and dried to yield the third fraction.

Five chloroform solutions (concentration, 0.6651, 1.2175, 1.7709, 2.0684 and 2.8086  $\text{mg ml}^{-1}$ ) of PEOX, four chloroform solutions (0.8175, 1.703, 2.474 and 3.522  $\text{mg ml}^{-1}$ ) of the first fraction of PEI, and three toluene solutions (0.3171, 0.6342 and 1.022  $\text{mg ml}^{-1}$ ) of PMEI were prepared, and their concentrations were determined gravimetrically. Immediately before the measurement, the solutions were purified by filtration through a Merck 0.22  $\mu\text{m}$  polytetrafluoroethylene membrane filter and poured into a cylindrical glass cell. The excess Rayleigh ratio was measured with a scattering angle range of  $30^\circ \leq \theta \leq 130^\circ$  at intervals of  $5^\circ$  with the sample temperature maintained at  $38.1 \pm 0.1$  °C for PEOX,  $25.3 \pm 0.1$  °C for PEI or  $36.1 \pm 0.1$  °C for PMEI using a Lauda RE206 thermostat. The differential refractive index increment ( $dn/dc$ ) was measured with an Otsuka Electronics DRM-3000 differential refractometer equipped with a tungsten lamp (wavelength 633 nm). The  $dn/dc$  values of the PEOX and PEI chloroform solutions were 0.0984 and 0.1127  $\text{ml g}^{-1}$ , respectively.

### Size exclusion chromatography

A size exclusion chromatography measurement of PMEI was carried out by the DJK Corporation (Tokyo, Japan) using a Shodex GPC-104 system equipped with two Shodex GPC LF-404 columns and a refractive index detector under the following conditions: eluting solvent, HFIP including sodium trifluoroacetate (5  $\text{mmol l}^{-1}$ ); polymer concentration, 0.2 wt/vol %; column temperature, 40 °C; flow rate, 0.3  $\text{ml min}^{-1}$ . The molecular weight was related to the retention time ( $\tau$ ) using poly(methyl methacrylate) (PMMA) standards with molecular weights ( $M^{\text{PMMA}}$ s) of 202, 645, 1970, 6830, 20 300, 63 000, 202 000, 509 000, 955 000 and 1 677 000 Da according to a cubic polynomial (that is,  $\log M^{\text{PMMA}} = A\tau^3 + B\tau^2 + C\tau + D$ , where  $A$ ,  $B$ ,  $C$  and  $D$  are empirical parameters). The number-average ( $M_n$ ) and weight-average ( $M_w$ ) molecular weights were calculated by the built-in software.

### Solubility test

The solubilities of PMEI were investigated using various solvents including *n*-hexane, toluene, diethyl ether, chloroform, THF, acetone, ethanol, methanol, *N,N*-dimethylformamide, dimethyl sulfoxide, water and HFIP. The polymer was mixed with a given solvent to achieve a concentration of 10  $\text{mg ml}^{-1}$  and then stirred at room temperature for 1 h. When the polymer did not completely dissolve at room temperature, the mixture was heated to a given temperature with agitation. When the polymer remained undissolved, the polymer was determined to be insoluble.

### Measurement of the acid dissociation constant (pK<sub>a</sub>)

PMEI was dissolved in water to achieve a concentration of  $6.21 \times 10^{-2}$  monomer mol l<sup>-1</sup>. As hydrochloric acid ( $1.25 \times 10^{-2}$  mol l<sup>-1</sup>) was added dropwise to the solution, the pH value was monitored using a Yokogawa PH81 pH meter, and the titration curve was plotted. To calibrate the pH meter, equimolar amounts of phosphate (pH = 6.86 at 25 °C), carbonate (10.01) and phthalate (4.01) buffer solutions were used. From the inflection point of the titration curve (that is, the minimum of its first derivative), the equivalence point was determined. During the measurement, the solution temperature was maintained in a range of 23.3–24.7 °C, and the intermediate value was 24.0 °C, where the ion product of water is exactly equal to 10<sup>-14</sup>.

### Cloud point measurement

A certain amount of a given salt was added to an aqueous solution of PMEI (0.5 wt%) in a test tube. The test tube, which was sealed, was immersed in a Haake K20 thermostat, and the temperature was varied in a gradual manner. The turbidity of the solution was observed with the naked eye to detect the cloud point (*T*<sub>c</sub>).<sup>27</sup> The heating-cooling cycle was repeated three times around the cloud point to determine the *T*<sub>c</sub> value without ambiguity. The following inorganic salts were used to exert the salting out effect: NaCl, KCl, KOH and Na<sub>2</sub>SO<sub>4</sub>.

### Particle size and zeta potential measurements

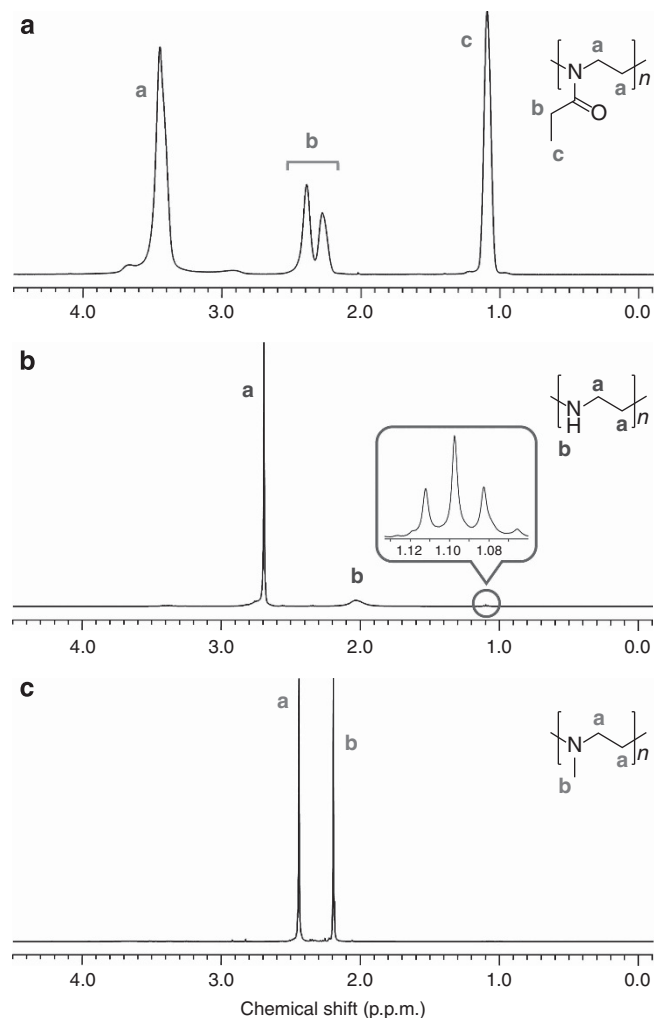
The dynamic light scattering (DLS) and zeta potential measurements of the aggregates of the PMEI and PS latex particles were performed using an Otsuka ELSZ-1000ZSCK zeta potential and particle size analyzer equipped with a He-Ne laser (632.8 nm) in the Center for Analytical Instrumentation at Chiba University. The PMEI concentrations ranged from 0 to 100 μmol l<sup>-1</sup>, and the PS latex concentration was 0.30 g l<sup>-1</sup>. Both aqueous solutions were mixed, subjected to sonication for 5 min, and moved to a sample cell. Exactly 10 min after the sonication, the measurement began. The sample temperature was set to 37 °C, and the scattering angle was fixed at 165° (DLS) or 15° (zeta potential). The recorded DLS data were analyzed by the built-in software.

## RESULTS AND DISCUSSION

### Purities and molecular weights of PEI and PMEI

Figure 1 and Supplementary Figure S1 show the <sup>1</sup>H and <sup>13</sup>C NMR spectra, respectively, for PEOX, PEI and PMEI. As shown in Figure 1b, a tiny peak from the remaining PEOX was observed at 1.1 p.p.m., and a comparison of the integrated intensity between the peaks at 1.1 and 2.7 (peak a) p.p.m. suggests that 99.0% of the PEOX was converted to PEI. As shown in Figure 1c, the intensity ratio between peaks a (2CH<sub>2</sub>) and b (CH<sub>3</sub>) was evaluated to be 4.00: 3.06. It is important to note that this ratio also depends on the integration range and baseline correction. The <sup>13</sup>C NMR spectrum of PEI (Supplementary Figure S1) does not contain a peak corresponding to the methylene group shielded magnetically by the tertiary amine (that is, there was no branching), and the <sup>13</sup>C NMR spectrum of PMEI does not contain a CH<sub>2</sub> signal affected by the residual NH group. Although small peaks stemming from impurities were observed in the spectra, the synthesized PEI and PMEI are sufficiently pure for the subsequent characterizations. The FTIR spectra of the three polymers (Supplementary Figure S2) are in good agreement with those previously reported<sup>28,29</sup> and indicate the formation of sufficiently pure PEI and PMEI.

One of the best methods for directly determining the molecular weights of polymers is the SLS method. Therefore, we carried out SLS measurements for the starting material (PEOX), the precursor (PEI) as well as PMEI. The Berry plot,<sup>30</sup> which is a square-root Zimm plot<sup>31</sup> for chloroform solutions of PEOX at 38.1 °C (Supplementary Figure S3), yielded the following molecular parameters: *M*<sub>w</sub>, 6.1 × 10<sup>5</sup> Da; radius of gyration ( $\langle S^2 \rangle^{1/2}$ ), 34 nm; second virial



**Figure 1** <sup>1</sup>H NMR spectra for (a) PEOX (the starting material), (b) PEI and (c) PMEI. The signals were assigned as indicated. PEI, poly(ethylene imine); PEOX, poly(2-ethyl-2-oxazoline); PMEI, poly(*N*-methylethylene imine). A full color version of this figure is available at *Polymer Journal* online.

coefficient (*A*<sub>2</sub>),  $3.9 \times 10^{-4}$  cm<sup>3</sup> g<sup>-2</sup> mol. The *M*<sub>w</sub> value is somewhat larger than that specified by the manufacturer (that is,  $5 \times 10^5$  Da).

However, chloroform solutions of the as-synthesized PEI and PMEI resulted in such weak SLS that no significant data were obtained. Therefore, the PEI sample was subjected to molecular weight fractionation. The first fraction yielded comparatively intense scattering. The Berry plot (Supplementary Figure S4) yielded *M*<sub>w</sub> =  $3.2 \times 10^4$  Da,  $\langle S^2 \rangle^{1/2} = 12$  nm and *A*<sub>2</sub> =  $1.7 \times 10^{-3}$  cm<sup>3</sup> g<sup>-2</sup> mol. However, the Berry-plot data for the second fraction (not shown) were so scattered that we obtained *M*<sub>w</sub> ~ 7.1 kDa with difficulty. For the above reason, a SEC measurement of PMEI was performed using HFIP containing sodium trifluoroacetate as the eluting solvent because HFIP has been used with a trifluoroacetate salt in SEC measurements of poly(*N*-alkylethylene imine).<sup>18</sup> From the molecular weight distribution of PMEI (Supplementary Figure S5), the *M*<sub>n</sub> and *M*<sub>w</sub> values were determined to be 17 and 26 kDa, respectively, and therefore, the polydispersity index was 1.5.

The weight-average degree of polymerization (*x*<sub>w</sub> (= *M*<sub>w</sub>/*M*<sub>0</sub>, *M*<sub>0</sub>: formula weight of monomeric unit)) of the starting material (that is, PEOX) was  $6.2 \times 10^3$  (by SLS), and *x*<sub>w</sub> of the first fraction of PEI was  $7.4 \times 10^2$  (by SLS). Therefore, the ratio of the former to the latter,



which corresponds to the average number of chain scissions, is 8.4, which is larger than that previously reported (4.3).<sup>32</sup> To attain complete conversion to PMEI, the linear PEI was heated at 105 °C for 5 days with formaldehyde and formic acid, and the  $x_w$  value of the resulting PMEI was  $4.6 \times 10^2$  (by SEC).

### Solubility

The solubilities of PMEI in several common solvents were investigated. PMEI is soluble in chloroform, THF, acetone, ethanol, methanol, *N,N*-dimethylformamide, HFIP and water at room temperature and in *n*-hexane above 45 °C but insoluble in diethyl ether and dimethyl sulfoxide. Tanaka *et al.*<sup>10</sup> reported that PMEI does not dissolve in *n*-hexane. However, our experiments indicated that PMEI is soluble in *n*-hexane above 45 °C.

Linear PEI is soluble in hot water, cold water at a low pH, methanol and ethanol but insoluble in benzene and diethyl ether.<sup>12</sup> Similar to PEO, PMEI is soluble in a variety of solvents. The solubility of PMEI in toluene is unusual and will be discussed below.

### Self-aggregation of PMEI

In our solubility test, the toluene solution containing PMEI exhibited an upper critical solution temperature (UCST)-type phase separation.<sup>33,34</sup> The solution was turbid at room temperature but became transparent at 36 °C. Therefore, we attempted SLS measurements of the toluene solution at 36.1 °C (as close to 36 °C as possible). The square-root Zimm plot (Supplementary Figure S6) afforded the following data:  $M_w = 1.09 \times 10^6$  Da;  $\langle S^2 \rangle^{1/2} = 41$  nm;  $A_2 = -3.4 \times 10^{-4}$  cm<sup>3</sup> g<sup>-2</sup> mol.

The square-root Zimm plot appears to be slightly convex upward. The apparent molecular weight of the self-aggregates of PMEI was larger than one million. Fujita<sup>35,36</sup> derived the following equation as an analytical method for huge polymers:

$$\frac{1}{y(u)} = \frac{1}{M_w} + bZ(u) + \text{higher terms in } Z \quad (5)$$

where

$$y(u) = \lim_{c \rightarrow 0} \frac{R(\theta)}{Kc} \quad (6)$$

$$b = \frac{32\pi^2 \langle S^2 \rangle_z}{3\lambda^2 M_w} \quad (7)$$

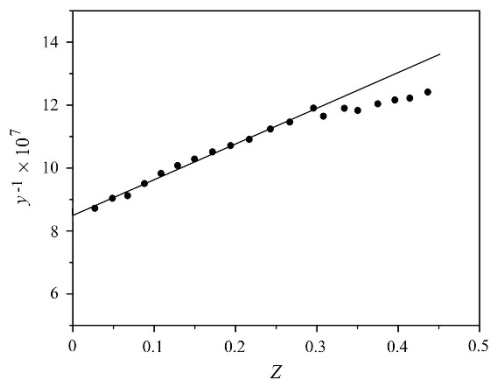
$$Z(u) = \left[ y(u)u^{4/3} \right]^{-1} \int_0^u y(u)u du \quad (8)$$

and

$$u = \sin^3 \frac{\theta}{2} \quad (9)$$

where  $R(\theta)$  is the excess Rayleigh ratio,  $K$  is the optical constant,  $c$  is the polymer concentration,  $\theta$  is the scattering angle,  $\lambda$  is the wavelength of light, and  $\langle S^2 \rangle_z$  is the  $z$ -average mean-square radius of gyration.

The  $Z(u)$  values were numerically calculated from the data observed in a range of  $30 \leq \theta \leq 130^\circ$ . To exclude the effect of the higher terms in equation (5), we fitted a straight line to the data in the small  $Z$  range (Figure 2), and the intercept and slope of the line yields  $M_w^{-1}$  and  $b$ , respectively. From these data, we derived  $M_w = 1.17 \times 10^6$  Da and  $\langle S^2 \rangle_z^{1/2} = 71$  nm. The apparent  $M_w$  value is very large compared with that of a single PMEI chain (26 kDa). The SLS and SEC data are summarized in Table 2.



**Figure 2** Fujita plot for PMEI in toluene at 36.1 °C. The variables ( $y$  and  $Z$ ) are defined in equations (6) and (8), respectively. PMEI, poly(*N*-methylethylene imine).

We also attempted SLS measurements of the toluene solution over 36 °C (for example, approximately 40 °C) but could not observe sufficiently intense scattering. Therefore, the aggregates were broken into pieces, and a uniform phase was formed. The upper critical solution temperature was 36 °C.

### pKa value

The titration curve for PMEI is shown in Supplementary Figure S7. The equivalence point was located at 26.8 ml of titrant (that is, hydrochloric acid). According to the Henderson–Hasselbalch equation,<sup>37</sup>

$$\text{pOH} = \text{pK}_b + \log_{10} \frac{[\text{HB}^+]}{[\text{B}]} \quad (10)$$

where B represents the base, the pH value at the half equivalence point corresponds to  $\text{pK}_a = 14 - \text{pK}_b$ . At 13.4 ml on the abscissa axis, the titration curve gives  $\text{pH} = \text{pK}_a = 7.28$ , and therefore,  $\text{pK}_b = 6.72$ . The  $\text{pK}_a$  value is close to those of *N*-methylmorpholine (7.38) and *N*-methylimidazole (7.13).<sup>38</sup> The Henderson–Hasselbalch equation gives  $[\text{HB}^+] = 0.76 [\text{B}]$  at  $\text{pH} = 7.4$  (physiological condition, equal to the pH value of human blood). Although the protonation degree ( $\alpha$ ) is defined as  $[\text{HB}^+]/([\text{HB}^+] + [\text{B}])$ , 43% of the  $-\text{N}(\text{CH}_3)-$  groups of PMEI will be protonated at a pH of 7.4. Under near-neutral conditions (that is,  $\text{pH} = 7.4$ ), the PMEI chain is sufficiently protonated to act as a polycation.

The  $\alpha$  values of PEIs in different shapes under physiological conditions have been reported (that is, linear PEI, 0.28 (salt-free) and 0.45 (with 0.1 M NaCl);<sup>39,40</sup> branched PEI, 0.26–0.35<sup>41</sup> and 0.31 (salt-free)<sup>42</sup> and 0.21 (with 0.1 M NaCl);<sup>43</sup> comb-like PEI, 0.49 (under 0.1 M ionic strength)<sup>44</sup>). The  $\alpha$ -value of PMEI is larger than or comparable to those of these PEIs.

### Phase separation of lower critical solution temperature type of PMEI aqueous solution

For the pure aqueous solution containing PMEI, no cloud point ( $T_c$ ) was observed between 0 and 90 °C. Therefore, some inorganic salts were added to the solution to induce a phase separation with the aid of the salting-out effect.<sup>45</sup> The PMEI concentration was 0.50 wt%, and the salts included NaCl, KCl, KOH and Na<sub>2</sub>SO<sub>4</sub>. The addition of salt to the aqueous solution led to a lower critical solution temperature (LCST)-type phase separation (see Figure 3b). In Figure 3a, the observed cloud points are plotted as a function of the salt concentration. The effect of the phase separation (that is, the ability to strengthen the hydrophobic interaction) can be expressed as the slope

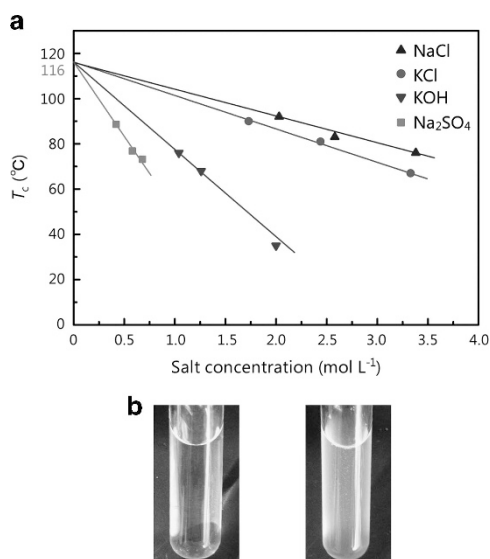
**Table 2** SLS and SEC data for PEOX, PEI and PMEI

Polymer	Temp (°C)	dn/dc (ml g <sup>-1</sup> )	SLS			SEC	
			M <sub>w</sub> (kDa)	A <sub>2</sub> (cm <sup>3</sup> g <sup>-2</sup> mol)	<S <sup>2</sup> > <sup>1/2</sup> (nm)	M <sub>n</sub> (kDa)	M <sub>w</sub> (kDa)
PEOX	38.1	0.0984	610	3.9 × 10 <sup>-4</sup>	34		
PEI							
First fraction	25.3	0.1127	32	1.7 × 10 <sup>-3</sup>	12		
Second fraction			7.1				
PMEI							
Isolated chain						17	26
Self-aggregate <sup>a</sup>	36.1		1090	-3.4 × 10 <sup>-4</sup>	41		
Self-aggregate <sup>b</sup>			1170		71		

Abbreviations: PEI, poly(ethylene imine); PEOX, poly(2-ethyl-2-oxazoline); PMEI, poly(*N*-methylethylene imine); SEC, size exclusion chromatography; SLS, static light scattering.

<sup>a</sup>From the Berry plot.

<sup>b</sup>From the Fujita plot.



**Figure 3** LCST-type phase separations of PMEI in water. (a) Cloud points ( $T_c$ ) of aqueous PMEI solutions including different inorganic salts as a function of the salt concentration. The data points for the individual salts converge at a single point (116 °C) on the axis of the ordinate. (b) Phase separation behavior of an aqueous PMEI solution containing KCl (3.33 mol l<sup>-1</sup>) at temperatures that were 2 °C lower (left) and higher (right) than the cloud point. LCST, lower critical solution temperature; PMEI, poly(*N*-methylethylene imine). A full color version of this figure is available at *Polymer Journal* online.

of the  $T_c$  versus salt concentration plot and evaluated in the order of NaCl < KCl << KOH < Na<sub>2</sub>SO<sub>4</sub>. For aqueous solutions of PEO, the hydrophobic effect has been estimated to be (anion) Cl<sup>-</sup> < SO<sub>4</sub><sup>2-</sup> and (cation) Na<sup>+</sup> < K<sup>+</sup>.<sup>45</sup> These trends (that is, the so-called Hofmeister series) are consistent with our experimental observations. In Figure 3a, the data points for the individual salts appear to converge to a single point on the axis of the ordinate at a salt concentration of 0. The convergent point is expected to yield the LCST of the pure aqueous solution. On the basis of linear least squares fitting, the temperature was determined to be 116 °C. Hoogenboom *et al.*<sup>18</sup> reported that an aqueous poly(*N*-ethylethylene imine) solution exhibits a cloud point of 81.7 °C. In addition, poly(*N*-propylethylene imine) is insoluble in water. The LCST of poly(*N*-alkylethylene imine) tends to decrease as

the side-chain length increases. The LCSTs of aqueous solutions of PEO were determined to be 100–110 °C,<sup>46,47</sup> which is similar to that of PMEI.

#### Dynamic light scattering and zeta potential measurements

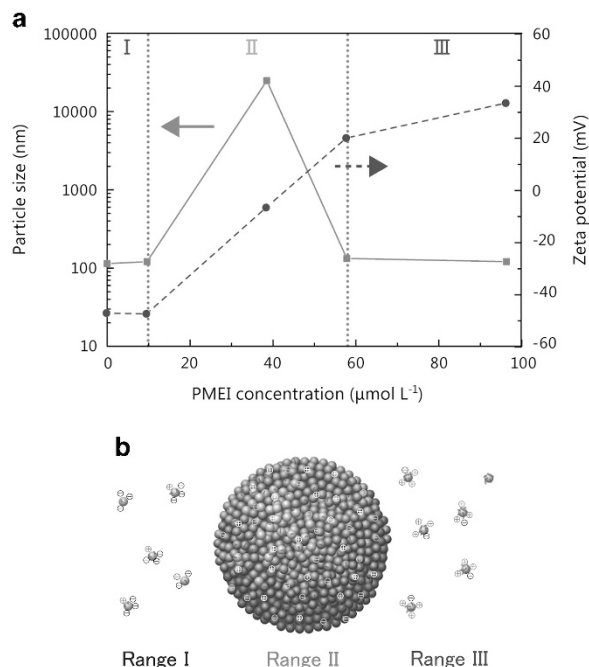
To investigate the behaviors of PMEI with negatively charged substances, PMEI was mixed in an aqueous PS latex solution, which is the standard substance for particle size calibration. The hydrodynamic diameter ( $D_h$ ) and zeta potential ( $\zeta$ ) are 119 ± 2 nm and -53.8 mV, respectively. The PMEI chain was not sufficiently large to generate any detectable DLS. Therefore, no  $D_h$  value for only PMEI dissolved in water could be determined.

Figure 4 shows the  $D_h$  and  $\zeta$  values plotted as a function of the PMEI concentration ( $c_{\text{PMEI}}$ ). In the range of  $0 \leq c_{\text{PMEI}} \leq 10 \mu\text{mol l}^{-1}$  (monomer ratio of PS to PMEI (abbreviated as MR)  $\geq 290$ ),  $D_h = 121$  nm and  $\zeta = -47.3$  mV. These data are close to those of the pure PS latex particle and invariant over a long period of time. At  $c_{\text{PMEI}} = 38.5 \mu\text{mol l}^{-1}$  (MR = 75), the  $\zeta$  value increased to -6.6 mV, and therefore, the isoelectric point is positioned in the region. The  $D_h$  value of the PMEI and PS latex (PMEI-PS) aggregates was as large as  $2.48 \times 10^4$  nm and further increased in size with time until the aggregates were deposited. At  $c_{\text{PMEI}} = 57.8 \mu\text{mol l}^{-1}$  (MR = 50), the  $D_h$  value decreased to 133 nm and remained constant and independent of time. In addition, the  $\zeta$  potential was positive (+20.1 mV). At  $c_{\text{PMEI}} = 96.3 \mu\text{mol l}^{-1}$  (MR = 30),  $D_h = 121$  nm (equal to the initial value) and  $\zeta = +33.5$  mV.

The experimental results can be interpreted as illustrated in Figure 4. In range I ( $c_{\text{PMEI}} \leq 10 \mu\text{mol l}^{-1}$ ), the PS latex particles absorb the PMEI chains on the surface but remain negatively charged to repulse each other and uniformly disperse. In range II ( $10 \leq c_{\text{PMEI}} \leq 60 \mu\text{mol l}^{-1}$ ), the PMEI-PS particles, which are close to (or equal to) neutral, combine with each other to form huge aggregates. In range III ( $c_{\text{PMEI}} \geq 60 \mu\text{mol l}^{-1}$ ), the PMEI-PS particles are positively charged and dispersed due to the electrostatic repulsions. In ranges II and III, PMEI clearly behaves as a flocculant and a dispersant, respectively.

#### CONCLUSIONS

In our previous studies,<sup>8,9</sup> PMEI has been theoretically suggested to be a hydrophilic polycation due to its conformational- and configurational-adjustable properties. To demonstrate our prediction, we investigated the solution properties of PMEI and determined that



**Figure 4** Aggregation behaviors of PMEI with PS latex particles in water: (a) particle size (hydrodynamic diameter) and zeta potential as a function of the PMEI concentration ( $c_{\text{PMEI}}$ ) and (b) schematic illustration of the aggregation behaviors: range I ( $c_{\text{PMEI}} \leq 10 \mu\text{mol l}^{-1}$ ); range II ( $10 \leq c_{\text{PMEI}} \leq 60 \mu\text{mol l}^{-1}$ ); range III ( $c_{\text{PMEI}} \geq 60 \mu\text{mol l}^{-1}$ ). PMEI, poly(*N*-methylethylene imine); PS, polystyrene. A full color version of this figure is available at *Polymer Journal* online.

PMEI exhibits broad solubility similar to PEO and a high protonation capacity similar to PEI. In addition, PMEI forms aggregates with negatively charged macromolecules and acts as either a polymer flocculant or a polymer dispersant depending on the given conditions. An aqueous solution containing the as-synthesized PMEI exhibited an LCST of 116 °C, and a toluene solution has a UCST of 36 °C. In conclusion, PMEI is expected to be useful as a highly hydrophilic polycation for various applications.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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