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A novel degradable poly(β-amino ester) and its nano-complex with poly(acrylic acid)

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Abstract

A novel degradable cationic polymer with discrete charge based on water-soluble poly(β-amino ester) was synthesized by Michael addition of tetraethylene pentamine (TEPA) to ethylene glycol diacrylate (EGDA), which is denoted as PTE. There are primary, secondary and tertiary amines in PTE molecule as branched polyethylenimine. The nitrogen content of PTE is 13.9 mmol/g at molar feed ratio of 1:1, lower than that of polyethylenimine of 23 mmol/g, so PTE is a polycation with discrete charge relative to polyethylenimine with excess high charge density. Acid–base titration indicated that PTE was protonated in the ranges from both pH 5.0 to pH 7.4 and pH 7.4 to pH 11. The changes of pH, viscosity and ^{1}H NMR spectra showed that PTE was degraded in water first rapidly and then slowly. In aqueous solution, PTE self-assembled with weak anionic polymer, poly(acrylic acid) (PAA), into nanoparticles. The particle size first increased and then decreased with increasing the mass ratio of PTE to PAA, which is defined as θ, from 0.1 to 2. At θ of more than 0.8 or less than 0.6, complex particles with diameter less than 200 nm were obtained. At θ of 0.8, the UV–vis absorbance of complex solution at first day was obviously higher than that at seventh day, while at θ of 0.2, two absorbance curves at these two time points were almost superimposed, indicating that small complex particles were more stable than large ones because of electrostatic repulsion.

Keywords: Degradable; Complex; Poly(β-amino ester)

1. Introduction

Formation of interpolymer complex can be achieved through specific interactions such as electrostatic interactions, hydrogen bonding and hydrophobic interactions etc [1–4]. The interpolymer complex resulting from these interactions possesses distinguished characteristics that are different from those of individual components [5]. Polyelectrolyte complex is one kind of interpolymer complex formed by electrostatic interaction of oppositely charged polyelectrolytes in solution [6,7]. It is of important interest because of its similarity to biological system and has potential applications, for example, the design of drug delivery system, protein separation, anticoagulant coating, and membrane for separating materials or even as skin substitute [8,9]. Polyelectrolyte complex formed by polycation and DNA has been extensively used to gene therapy study and in this kind of polyelectrolyte complex, polycation is an important component called as gene delivery vector [10,11].

As a necessary precursor of polyelectrolyte complex, polycation exerts significant effect on formation and property of complex [9]. In biomedical applications, charge density of polycation has opposite effects. For example, in terms of gene carrier, on one hand, high charge density facilitates formation of compact DNA/polycation complex to protect DNA from enzymatic disruption. On the other hand, it intensifies cytotoxicity of carrier because polycation exhibits charge related toxicity to cell and baffles the release of DNA from complex as well as its nuclear entrance, which result in low transfection efficiency [12]. Among studied polycations, the highest charge density, 23 mmol/g, is characteristic of polyethylenimine, which makes polyethylenimine exhibit very strong

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complexing ability but accompanied by significant cytotoxicity after polyethylenimine is protonated in aqueous solution. To solve these conflicts, degradable crosslinked polyethylenimines were prepared [13–18]. Although degradable crosslinked polyethylenimine decreased cytotoxicity to some extent, its final degradation product is still polyethylenimine, which has a certain amount of charge preventing the release of DNA and can not be degraded further [19]. It is possibly a promising way for solving this question to develop charge-discrete degradable polycation with lower charge density than polyethylenimine in order to realize synchronously low cytotoxicity and high efficiency.

Nitrogen-grade profile in polycation is also an important factor affecting polyelectrolyte complex characteristics. For example, primary amines of polyethylenimine are reported to participate in forming complexes with DNA by an ionic interaction between amine and phosphate groups, while secondary and tertiary amines present a so-called ''proton sponge effect'' due to their protonation at endosomal environments, which makes polyethylenimine/plasmid complex easily break through the endosomal compartment in case of degradation [15,20]. So coexistence of all grades of amine groups is possibly an advantageous condition for gene transfection.

Degradable polycation whose degraded products are of low molecular weight and low charge amount is an excellent alternative to nondegradable polycation in solving charge related conflicting effects on gene transfection [21–24]. If degradable polycation is used as precursor of polyelectrolyte complex, loaded polyanion is expected to be released gradually. Poly(β-amino ester) is one kind of excellent degradable polycation almost without detectable cytotoxicity [25–33]. In comparison with other polycations, optimized poly(β-amino ester) is more promising to be exploited in the clinical therapy owing to its combination of low cytotoxicity with high transfection efficiency. But when poly(β-amino ester) was prepared, monomers containing secondary amines were commonly used and accordingly, the produced poly(β-amino ester) contained more tertiary amine groups than primary and secondary amine groups. It was found that polycation only with tertiary amines produced low transfection efficiency [34]. Therefore optimizing the transfection performance of poly(β-amino ester)s is probably realized by selecting more appropriate monomers, especially the monomers containing amine groups.

In this work, we synthesized a degradable charge-discrete cationic polymer based on water-soluble poly(β-amino ester), named as PTE, by Michael addition reaction using cheap and easily obtained TEPA with high cationic charge density as containing amino groups monomer and EGDA as containing vinyl group monomer. In each structural unit of PTE, there are two degradable ester bonds and the atomic weight sums of TEPA and EGDA units are adjacent 189 and 170, respectively. The protonizing characteristic of PTE was studied using acid–base titration. The degrading characteristic was studied by monitoring pH, viscosity and ^{1}H NMR of PTE aqueous solution. The polyelectrolyte complex of PTE with PAA was prepared, in which PAA was selected as a model polyanion because PAA has wide applications as biomaterials and was commonly used as precursors to study interpolymer complex formation [2,3,5]. We suppose if PTE can complex with PAA, it will be complexed more easily with stronger polyacids, for example, poly(sulfonic acid) and poly(phosphorous acid) including DNA. Particle size analysis and UV–vis spectroscopy were used to study complex formation.

2. Experimental section

2.1. Chemicals

Acrylic acid (Kelong, Chengdu, China) was distilled under vacuum over cuprous chloride prior to use. TEPA (Kelong, Chengdu, China) was purified by drying with NaOH followed by vacuum distillation. Ethylene glycol (Waigang, Shanghai, China) was dried over 4A molecular sieve and then distilled under vacuum. Potassium peroxydisulfate (Beijing Chemical Company, Beijing, China) was recrystallized from water. Other reagents were used as-received.

2.2. Synthesis method

2.2.1. Synthesis of EGDA

EGDA was prepared from acid catalyzed esterification between ethylene glycol and acrylic acid. The synthetic method was similar to that of ethylene glycol dimethacrylate described elsewhere with some modifications [35]. In brief, ethylene glycol (0.15 mol), acrylic acid (0.36 mol), hydroquinone (3.19 g), 4-methyl-benzene sulfonic acid (2.07 g), and 80 mL cyclohexane were mixed at room temperature, then reacted at 110 °C until no water was separated from flask for about 6 h. The reaction mixture was neutralized with sodium hydroxide aqueous solution, washed with salt water until pH equaled 7 and dried by anhydrous sodium sulfate. The mixture was added with hydroquinone (3.19 g) and distilled to remove solvent at 110 °C under gradually elevated vacuum with circulating water vacuum pump. Crude EGDA was obtained with the yield of 86%. To further refine the product, the above crude product was distilled with oil vacuum pump and EGDA was collected at 66–67 °C under 266 Pa. Collected product was resolved in 30 mL CH_2Cl_2, neutralized with sodium hydroxide aqueous solution, washed with salt water to pH of 7, dried with anhydrous sodium sulfonate and distilled at 35 °C under vacuum to remove CH_2Cl_2. ^{1}H NMR (in $CDCl_3$) analysis confirmed the EGDA structure (Fig. 1). The peaks at $\delta =$ 6.42 ppm (1H, dd), 6.14 ppm (1H, dd) and 5.85 ppm (1H, dd) are assigned to CH_2=CH− protons. The peak at $\delta =$ 4.29 ppm (2H, s) is assigned to −$COOCH_2$− protons.

2.2.2. Synthesis of PTE

2.2.2.1. Dichloromethane as solvent. TEPA (2.50 g) in 10 mL CH_2Cl_2 was added to 50-mL round-bottomed flask fitted with a Teflon stir bar, a H_2O reflux condenser and a dropping funnel. EGDA (2.25 g) in 10 mL CH_2Cl_2 was added drop wise to TEPA solution with continuous stirring in 30 min at room temperature. Then the reaction was carried out at 50 °C for 48 h.

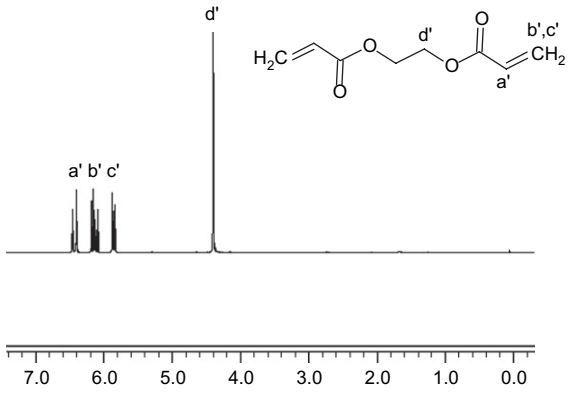


Fig. 1. ^{1}H NMR spectrum of EGDA (in $CDCl_3$).

The mixture was concentrated and added with ethyl ether under rigorous stirring. The obtained yellow precipitate was washed first with anhydrous ethyl ether then acetone for three times and dried under vacuum at 40 °C overnight. Synthesized PTE by this way was used to complex with PAA.

2.2.2.2. Chloroform as solvent. When dichloromethane was used as reaction solvent, precipitate was formed at certain reaction degree. When chloroform was used as solvent, reaction mixture kept homogeneous during the whole reaction course. So in order to monitor polymerization, PTE was synthesized again in chloroform. TEPA (2.50 g) in 10 mL $CHCl_3$ was added to 50-mL round-bottomed flask fitted with a Teflon stir bar, a H_2O reflux condenser and a dropping funnel. EGDA (2.25 g) in 10 mL $CHCl_3$ was added drop wise to TEPA solution with continuous stirring in 30 min at room temperature. Then the reaction was carried out at 35 °C for 12 h then 50 °C for 48 h. During reaction, aliquots of sample were withdrawn for ^{1}H NMR measurement.

2.2.3. Synthesis of PAA

Acrylic acid (10.09 g), potassium peroxydisulfate (0.11 g) and 100 mL deionized water were added to 250-mL mononeck round-bottom flask equipped with a water reflux condenser and a Teflon stir bar. The mixture was stirred to homogeneous phase at room temperature, then reacted at 60 °C for 7 h and 100 °C for 6 h. Obtained solution was directly used to prepare stock solution for complexation. In order to measure its FT-IR absorption spectrum, after the mixture was cooled to room temperature, water separator was equipped and 40 mL cyclohexane was added to flask as water-carrying reagent. Water separation was carried out at 110 °C under refluxing until no water was separated. After cyclohexane was distilled, 50 mL absolute alcohol was added to flask and distilled at 110 °C to remove remnant water. Concentrated alcohol solution of PAA was precipitated with ethyl ether. The resultant PAA was dried under vacuum at 85 °C for 24 h. The viscosity average molecular weight (M_η) was estimated as 1.1×10^5 from intrinsic viscosity of the polymer in 2 M NaOH aqueous solution at a constant temperature of 298 ± 0.1 K, using the Mark–Houwink–Sakurada equation [36].

2.3. Complexation of PTE with PAA

2.3.1. Preparation of stock solutions

PTE (1.48 g) was dissolved in 2000 mL deionized water to obtain 0.74 g/L PTE aqueous solution. PAA (0.73 g/L) aqueous solution was prepared by diluting PAA polymerization solution in deionized water.

2.3.2. Complexation of PTE with PAA

Varied amounts of PTE solution were added slowly to 30 mL PAA solution under gentle vortexing. After addition, the mixture was vortexed for 5 min again. Resultant solutions were analyzed using UV–vis photospectrometer and particle size analyzer. The mass ratio of PTE to PAA was represented as θ. The θ values studied here were 0.1, 0.2, 0.6, 0.8, 1 and 2. Complex solutions were left at room temperature and at certain intervals aliquots of supernatant liquid were withdrawn to determine complex stability by UV–vis spectroscopy.

2.4. Measurements

2.4.1. ^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded on Brucker Avance DPX 300 NMR spectrometer (Brucker, Germany) using $CDCl_3$ as solvent for EGDA, D_2O or $CDCl_3$ for PTE.

2.4.2. GPC

Aqueous GPC was performed using a Waters 515 HPLC pump and a Waters 2410 refractive index detector.

2.4.3. Protonation characteristic of PTE

Protonation characteristic of PTE was determined by acid–base titration. PTE (0.5 g) was dissolved in 10 mL 150 mmol/L NaCl solution and then added drop wise with 0.58 mL 37% HCl aqueous solution under ice cooling and continuous stirring. The obtained PTE·HCl salt solutions were titrated with 0.1 mol/L and 0.3 mol/L NaOH aqueous solution separately. The titrations of 10 mL 150 mmol/L NaCl solution and 10 mL 0.69 mol/L HCl solution containing 150 mmol/L NaCl were used as references. The pH changes were measured with Leici PHS-25 numerical pH meter (Leici Scientific Instrument Co., Shanghai, China) at room temperature.

2.4.4. Degradation of PTE

Degradation of PTE was determined by monitoring changes of pH, viscosity and ^{1}H NMR of PTE aqueous solutions. PTE was dissolved in 150 mmol/L NaCl aqueous solution and was incubated statically at 37 °C. At certain intervals, aliquots of the solutions were withdrawn to measure pH, viscosity and ^{1}H NMR.

2.4.5. Size analysis of complex nanoparticles

When PTE and PAA solutions were mixed, complex was formed immediately. The sizes of complex particles were measured by dynamic light scattering at room temperature on Malvern MASTERSIZER 2000 (Malvern Instruments Ltd., Malvern, U.K.). Samples were inhaled with Hydro 2000MU

with deionized water used as dispersion medium. Between two measures, the instrument was washed three times with deionized water.

2.4.6. UV–vis spectroscopic analysis of complex solution

Complex solutions formed at various θ values had different transparences. Otherwise, if complex solutions were not stable, complex particles would aggregate and precipitate. So the transparences of supernatant complex solutions were monitored to study the stability of complex nanoparticles. The stability of complex nanoparticle was characterized spectroscopically on UV-2401PC photospectrometer (Shimadzu, Japan).

2.4.7. FT-IR analysis

Fourier transform infrared spectroscopy (FT-IR) was carried out on Nicolet MX-1E FT-IR Spectrometer. Anhydrous samples of PAA, PTE and their complex were ground to fine power, then mixed with KBr and pressed into thin pieces for measurement.

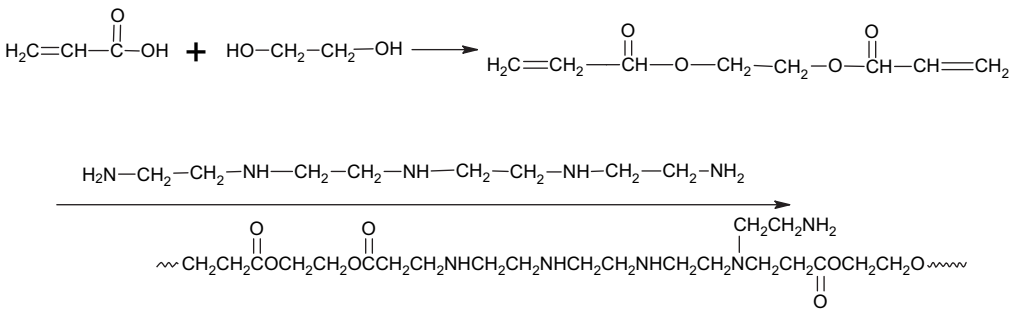
3. Results and discussion

3.1. Synthesis of PTE

PTE is a kind of poly(β-amino ester) obtained through Michael type addition polymerization (Scheme 1). During the reaction, there are possibly two side reactions: one is the polymerization of EGDA, the other is nucleophilic substitution of amine groups of TEPA to ester group of EGDA. In order to avoid side reactions, reaction temperature was kept at 50 °C. Conventional poly(β-amino ester) was synthesized for more than two days. In order to explore whether amidation side reaction took place, reaction process was monitored by ^{1}H NMR. When CH_2Cl_2 was used as solvent, the reaction mixture became more and more yellow, viscous and opaque because of the increase of PTE molecular weight. When reaction solvent was $CHCl_3$, the content was always homogeneous. So monitoring polymerization process was carried out in $CHCl_3$. ^{1}H NMR spectra changes are shown in Fig. 2. When reaction proceeded for 20 h, there was no more vinyl group, which indicated that the synthesis of PTE needs less time than conventional poly(β-amino ester). Amidation side reaction occurred with continued reaction. When reaction proceeded for 44 h, in ^{1}H NMR spectrum there appeared characteristic proton peaks corresponding to semi-ester and amide methylene groups, which indicated the existence of amidation side reaction. But amidation side reaction went more slowly than addition polymerization because addition polymerization first took place. From ^{1}H NMR spectrum, it was found that there was no proton peak characteristic of ethylene glycol, which showed that amidation side reaction only produced semi-ester group. So amidation reaction didn't change the polymerization degree of PTE but favorable to produce weakly degraded amide group (Scheme 2). However, continual amidation possibly took place during drying at higher temperature, which resulted in production of crosslinking as accompanied by ethylene glycol. In entire reaction, there was 20.6% ester group into hydroxymethyl group.

In theory, although both primary and secondary amines have reaction activity, more secondary amines possibly participate in reaction because the nucleophilic capability of secondary amine is higher than that of primary amine. After addition reaction, part of secondary amines were converted to tertiary ones and some primary amines into secondary ones. So PTE had a nitrogen-grade profile including primary, secondary and tertiary amines. Tertiary and secondary amines include two types, respectively (Fig. 2). For type I tertiary amine, amine molar number was calculated through α position hydrogen number from TEPA divided by 4. For type II tertiary amine, calculating way was α position hydrogen number from TEPA divided by 2. Molar number of primary amine was obtained through α position hydrogen number divided by 2, in which α position hydrogen number was obtained by subtracting methylene proton derived from EGDA from total proton between 2.44 and 2.52 ppm. According to above method, the relative contents of tertiary, secondary and primary amines are 29%, 51% and 20%, respectively. Initial contents of these amines are in turn 0%, 60% and 40%. So there are 20% primary amines participating in reaction were transferred into secondary amines. Then 29% secondary amines were transferred into tertiary amines. This result was in accordance with theoretical analysis for reaction activity of primary and secondary amines.

Molecular weight (M_w) of PTE was 8110 and polydispersity index was 4.55 (Fig. 3), which were obtained with aqueous GPC. However, GPC is prone to underestimate the molecular weight and overrate the molecular weight



Scheme 1. Synthetic procedure and ideal molecular structure of PTE.

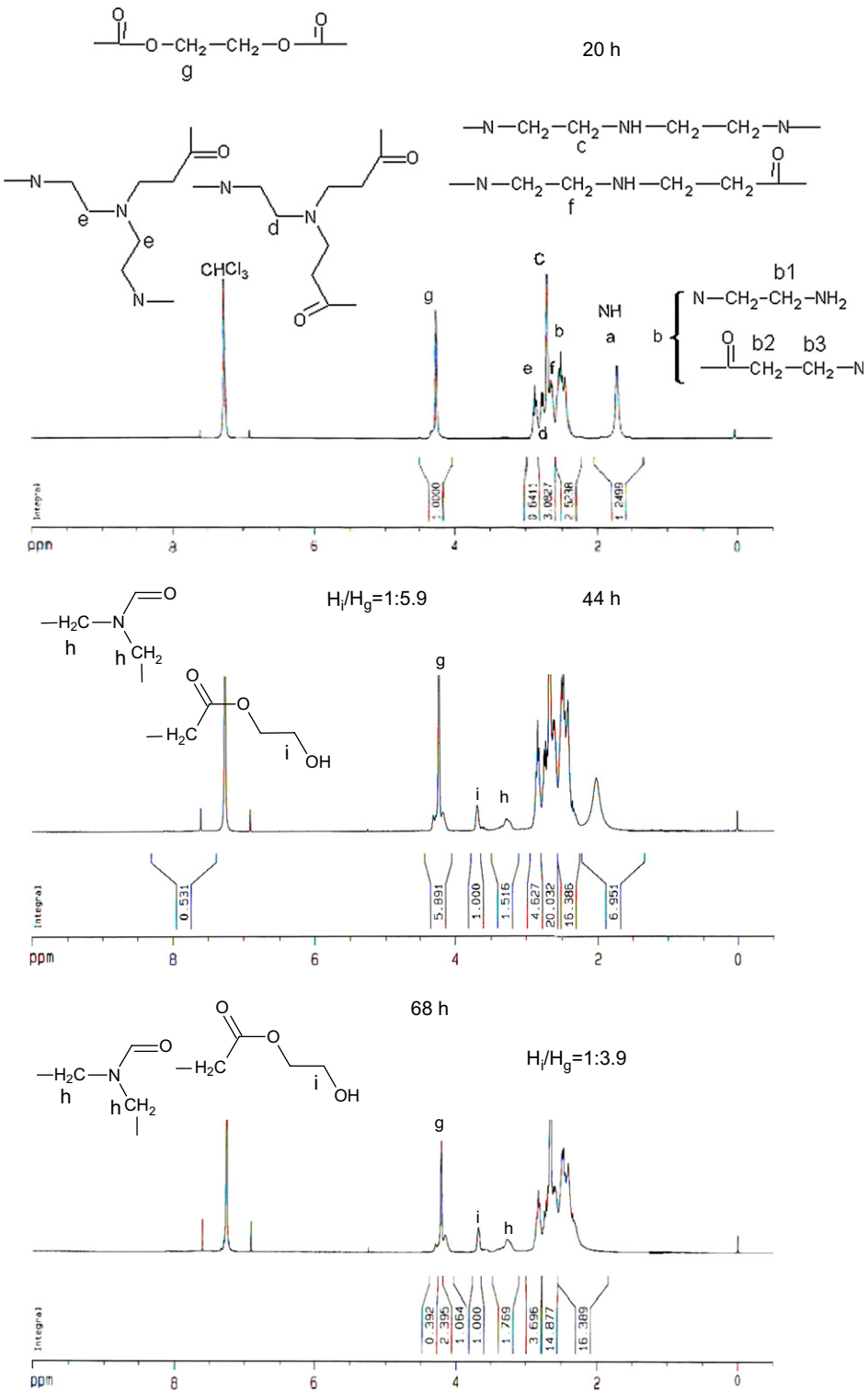
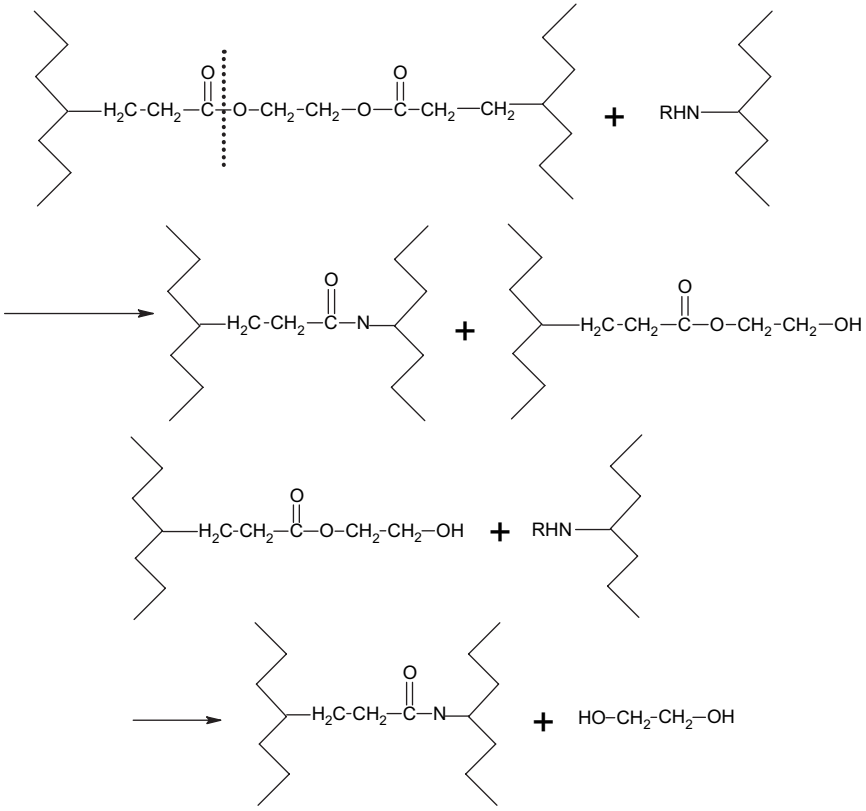


Fig. 2. ^{1}H NMR spectrum changes (in $CDCl_3$) of PTE as synthesized using $CHCl_3$ as solvent.

distribution of weak polycation due to interaction between polycation and GPC column [37].

3.2. Protonation characteristic of PTE

PTE is a weak polycation. Its protonation characteristic exerts important effect on ionic complex formation because polyelectrolyte complex is formed through electrostatic interaction following protonation. In biomedical applications as gene therapy, the protonation characteristic of polycation affects significantly the protection of DNA and transfection efficiency. If polycation has a wide pH range of protonation, there will be some free amine groups left after complexation at a higher pH value of this range. The remanent amine groups



Scheme 2. Amidation side reaction during synthesis of PTE.

continue to protonize at an environment with decreasing pH value. Thus, the complex exhibits environmental sensitivity. The effect, known as ‘‘proton sponge effect’’, makes polyethylenimine produce high transfection efficiency without additional endosome disrupting agent. In contrast, polylysine as gene carrier often resulted in low transfection efficiency when no adjuvant additive was used together because of absence of this effect [38]. Protonation characteristic of PTE was determined by acid–base titration as shown in Fig. 4. Upon titration, NaCl aqueous solution shows abruptly high pH value. Then pH value slowly changes with continued titration due to approximation to limiting pH value of NaOH solution. The titration curve of HCl solution presents typical S shape. In initial titration, NaOH is neutralized, which results in relatively stable pH value. When HCl is exhausted, pH value rises abruptly followed by gradual change at higher pH value with the same cause as NaCl solution. For titration of PTE, pH value slowly changes from first to last, which indicates that the ammonium groups of PTE deprotonize at different pH values because different types of ammonium groups have respective combining force to hydrogen ion. Therefore, PTE has proton buffering capability in the environment as cell endosome. The buffering capability in this range was considered to be in relation with tertiary amine groups. After Michael addition reaction, there appeared tertiary amine groups, which contributed to wide buffering range.

In order to eliminate the effect of dilution on titration, both 0.1 and 0.3 mol/L NaOH were used to titrate PTE solution. As

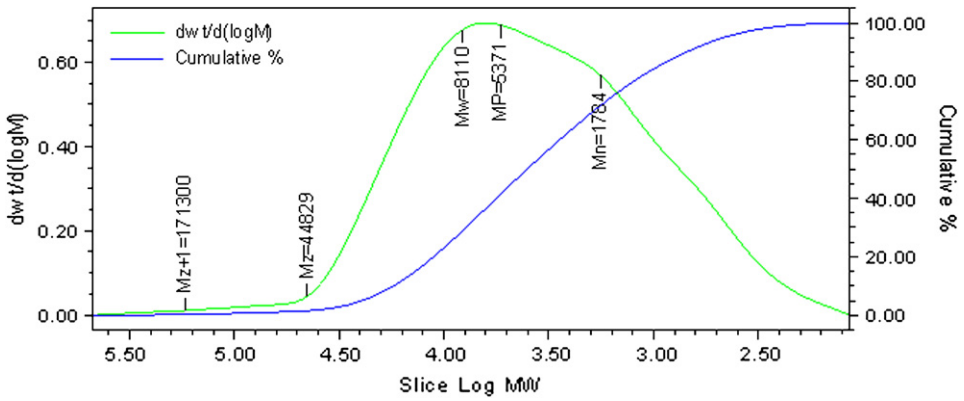


Fig. 3. GPC profile of PTE with H_2O as eluant.

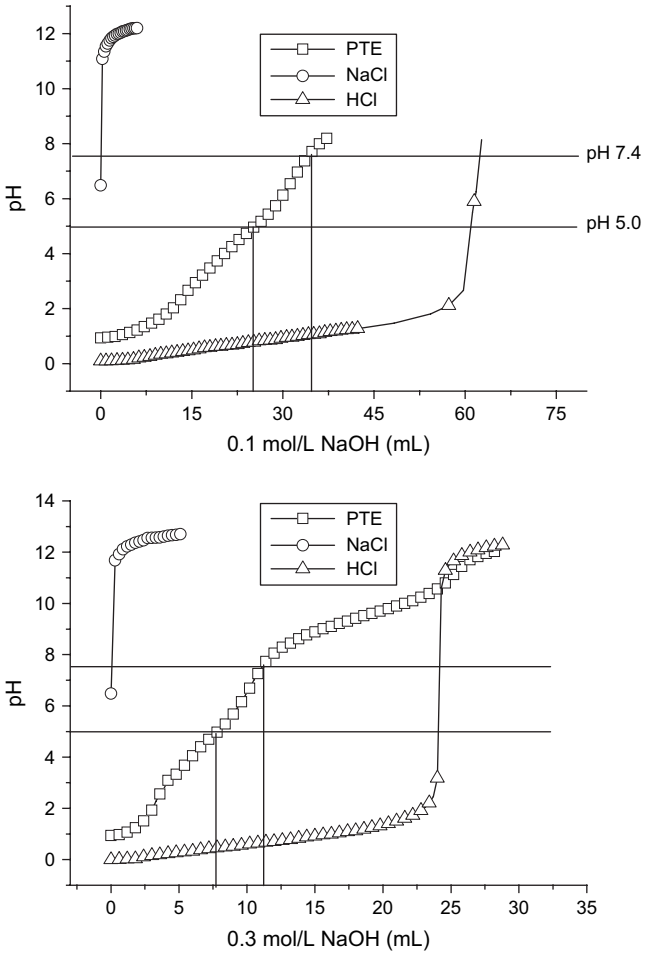


Fig. 4. Titrations of 10 mL 50 g/L PTE, 150 mmol/L NaCl and 0.69 mol/L HCl aqueous solutions with NaOH solution.

depicted in Fig. 4, there was about 2 mL 0.3 mmol/L NaOH solution consumed between pH 5.0 and pH 7.4, which corresponds to above 6 ml 0.1 mmol/L NaOH. It was seen that titrations performed with 0.1 and 0.3 mmol/L NaOH, respectively, have little difference and they all manifest that PTE is protonated within the pH range where polyethylenimine presents ''proton sponge effect''.

3.3. Degradation of PTE

Degradable polycations have been found to be degraded rapidly in first several hours [25,33,39]. There are a lot of ester groups and alkaline and nucleophilic amine groups on PTE backbone, which prefigures the easy degradation of PTE due to amidation or hydrolyzation. ^{1}H NMR spectrum, pH and viscosity were monitored. As shown in Fig. 5, at time of 0.5 h, there appear characteristic peaks of semi-ester, ethylene glycol and amide. Semi-ester and amide could be produced in polymerization, however, ethylene glycol was possibly produced during degradation in aqueous solution. At time of 9 h, characteristic peak of ester becomes very small but the peak of ethylene glycol obviously increases. At time of 24 h, ester disappears completely, semi-ester decreases obviously and ethylene glycol continues to increase. At time of 48 h, there are small amount of semi-ester groups. By analyzing relative peak area, degradation of PTE in aqueous solution results by and large from hydrolyzation rather than amidation because of unchanged peak area of amide. During degradation, amino group plays an important catalytic role as shown in Scheme 3. Protonation of PTE increased pH value of solution and promoted hydrolyzation of PTE. Changes of pH during degradation are shown in Fig. 6. When PTE is degraded, carboxylic acid groups appear and solution pH descends. As shown in Fig. 7, viscosity of PTE aqueous solution gradually decreases, indicating reduction of molecular weight. Decreasing viscosity is also characteristic of hydrolyzation rather than amidation, because hydrolyzation results in decreasing molecular weight while amidation do not decrease but, instead, possibly increase molecular weight. Main pH and viscosity changes occurred at first day, indicating that PTE had an abrupt degradation at first day. During degradation, amine groups contributed to catalytic effect. But produced carboxylic acid protonated amine groups and reduced their catalytic action. So the degradation of PTE became more and more slow.

In biomedical applications, degradable polycations effectively solve the conflicting effects of molecular weight and charge density on transfection efficiency and toxicity, because degraded products have low amount of charge. Moreover part of amine groups of degraded products is also protonated by neonatal carboxylic acid groups further decreasing charge amount. So degradable polycations can easily release bioactive complexed polyanion due to weakened electrostatic interaction.

PTE should be kept airproof due to its strong moisture absorption ability and easy degradation.

3.4. Complexation of PTE with PAA

Complexation of polycation with DNA is widely reported. Polyelectrolyte complexation nanoparticle is produced as driven by electrostatic interaction in dilute solution. When one component A is added into the other charged contrarily component B, extensive A molecular chain becomes collapsed into an initial charged nuclear due to electrostatic compress. The nuclear grows by adsorbing charged contrarily component A and changes its charge type. Then B and A components are adsorbed onto nuclear in turn until one of them is exhausted. The ultimate nanoparticle charges what excess component charges. This process is depicted in Scheme 4.

Complex formation was characterized by FT-IR. FT-IR spectra of PAA, PTE and their complex are shown in Fig. 8. In spectrum of PAA, due to association of carboxylic acid group a broad peak of COO–H stretching vibration is observed to be contacted with CH_2 stretching band. Carbonyl group stretching vibration occurs at 1714.5 cm^{-1}. Peaks at 1454.1 cm^{-1} and 1416.2 cm^{-1} are attributed to CH and CH_2 bending vibration. Peak at 1247.2 cm^{-1} is attributed to C–O stretching vibration. In spectrum of PTE, NH stretching vibration occurred at 3422.3 cm^{-1}. Symmetric and asymmetric stretching vibrations of CH_2 occur separately at 2953.4 cm^{-1} and 2829.3 cm^{-1}. Peak

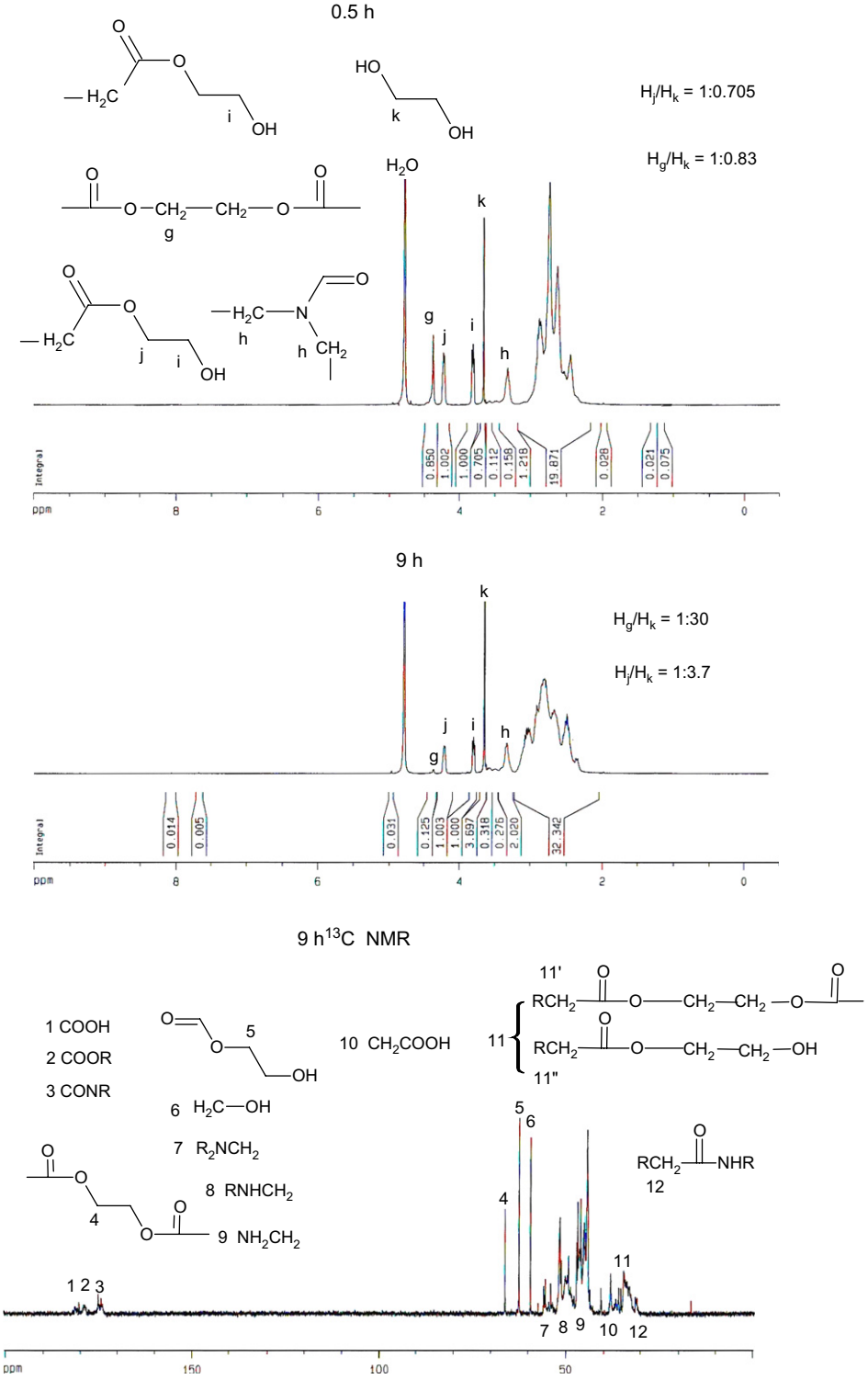


Fig. 5. Changes of ^{1}H and ^{13}CNMR spectra during PTE degradation in aqueous solution.

at 1726.9 cm^{-1} is attributed to carbonyl stretching vibration. Peaks at 1648.9 cm^{-1} and 1549.5 cm^{-1} are characteristic of amide and amine. Peaks at 1450.9 cm^{-1} and 1380.7 cm^{-1} are attributed to CH_2 bending vibration. Peak at 1185.1 cm^{-1} is attributed to C–O stretching vibration. In the spectrum of complex, peak between 3000.0 and 3432.3 cm^{-1} becomes narrower than that of PAA because association peak of carboxylic acid groups is weakened due to the formation of complex. Peak characteristic of $=NH_2^+$ at 1560.8 cm^{-1} appears.

The charge density of PTE is 13.9 mmol/g and significantly higher than those of chitosan and polylysine, although lower than that of polyethylenimine. So PTE is called as charge-discrete polycation with high charge density. When PTE solution was mixed with PAA solution, they would self-assemble

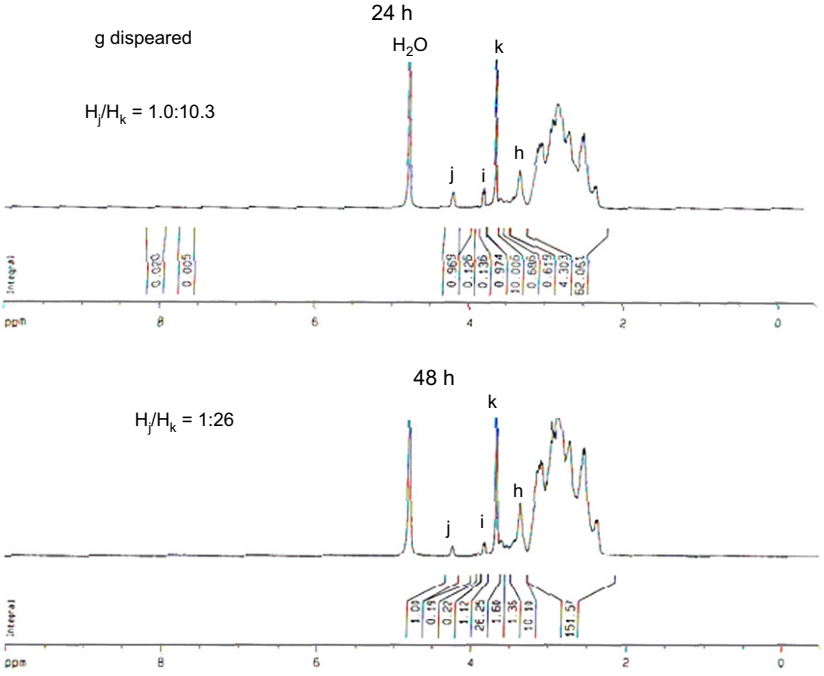
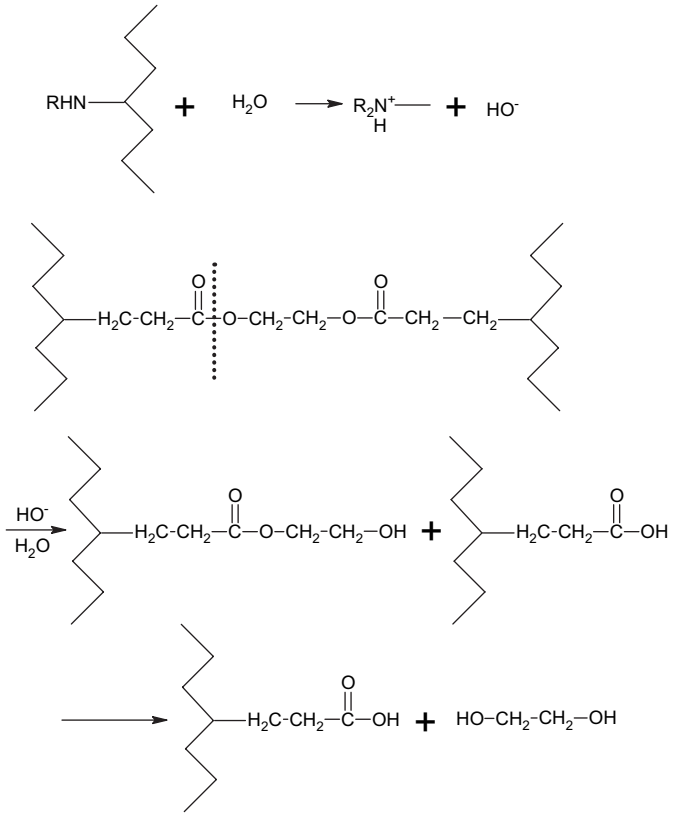


Fig. 5 (*continued*)

under electrostatic interaction into complex. Dynamic light scattering was used to measure complex particle size. From Figs. 9 and 10, it is seen that complex formed at $\theta = 0.8$ has the broadest size distribution. As revealed in Fig. 11, complex formed at $\theta = 0.8$ has the highest average particle diameter and the curve of particle diameter against θ appears unimodal. At optimal θ values, complex particles with the diameter of about 120 nm can be obtained. When PTE was complexed with PAA at higher or lower θ values, one of them was excess. The excess component endowed complex particles with negative or positive charge. Electrostatic repulsion between charged particles kept complex particles from aggregating. At θ of 0.8, the amount of ionized amine groups almost equaled that of ionized carboxylic acid groups and approximately neutral complex particles were formed. They would aggregate into bigger particles under surface tension.



Scheme 3. Degradation process of PTE in aqueous solution.

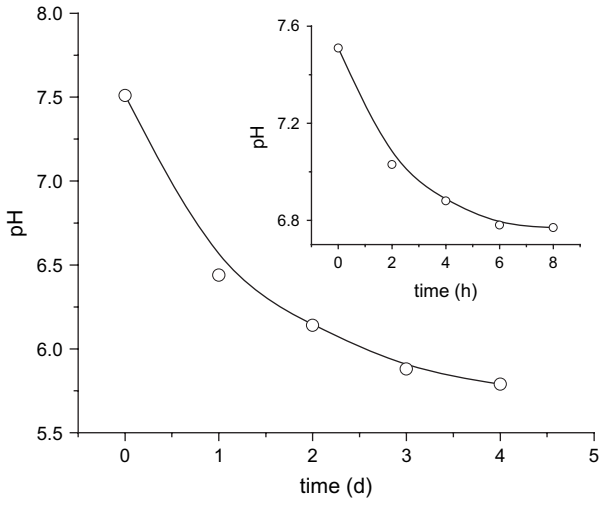


Fig. 6. The pH change of 49.67 g/L PTE aqueous solution containing 150 mmol/L NaCl.

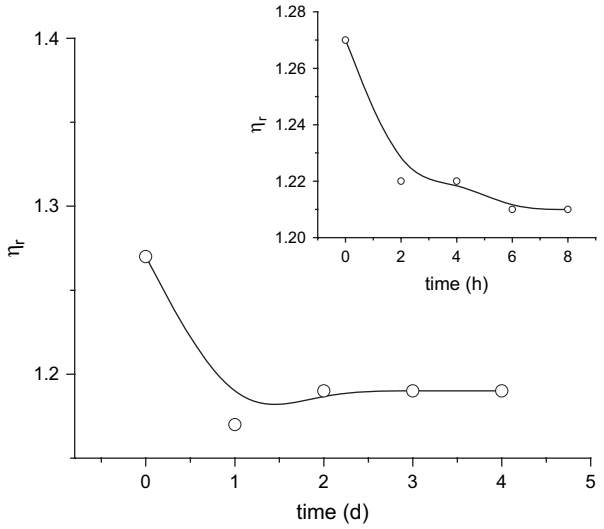


Fig. 7. The η_r change of 49.67 g/L PTE aqueous solution containing 150 mmol/L NaCl.

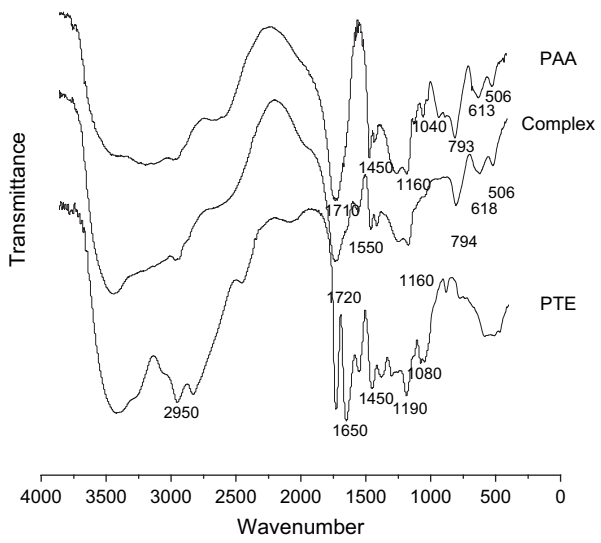


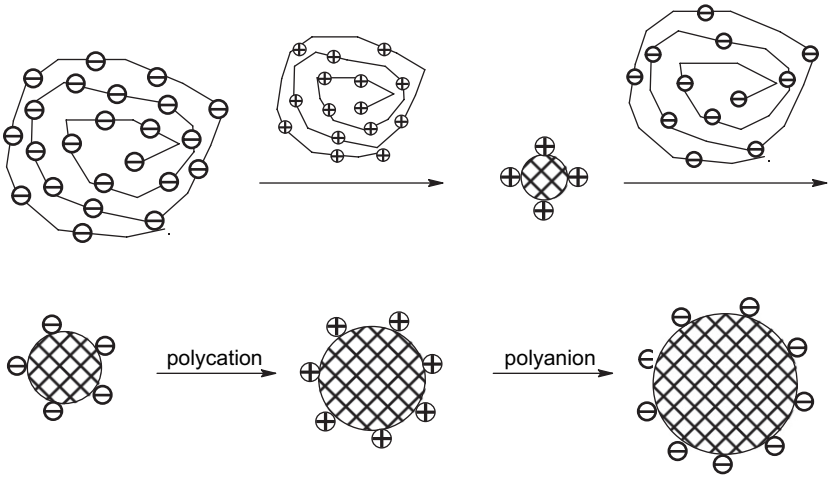
Fig. 8. FT-IR spectra of PAA, PTE and their complex.

UV–vis was used to measure the apparent absorbance of complex solution to validate complex size and the absorbance of supernatants of complex solution to estimate the stability of complex solution. Fig. 12 gives the UV–vis spectra of complex solutions at various θ values. From these figures, it can be seen that when θ increases from 0.1 to 0.8, apparent absorbance gradually increases, while from 0.8 to 2, apparent absorbance gradually decreases. This is related to light scattering and reflecting by complex particles. At θ of 0.8, complex particles are biggest, so they strongly scatter and reflect light. The shorter the wavelength of incident light, the more intensive it's scattering and reflection become. So every curve decreases monotonically. No exception was found for relation between UV–vis spectra and complex particle size, so UV–vis spectroscopy was a good adjuvant tool characterizing complex particle size.

The stability of PTE/PAA complex solutions was characterized by UV–vis spectroscopy as depicted in Fig. 13. When complex formed at $\theta = 0.8$, the absorbance curve of complex solution at seventh day is significantly lower than that at first day. In contrast, when formed at $\theta = 0.2$, the absorbance curve of complex solution at first day completely overlaps with that at seventh day. At seventh day precipitate was found at the bottom of complex solution formed at $\theta = 0.8$, while not for that at $\theta = 0.2$. This indicates that the stability of complex at $\theta = 0.2$ exceeds that at $\theta = 0.8$. Due to absence or insufficiency of electrostatic repulsion, complex particle formed at $\theta = 0.8$ was big and easy to aggregate together resulting in precipitate. Contrarily, complex particle formed at $\theta = 0.2$ was small and stable.

4. Conclusion

An idea of degradable charge-discrete cationic polymer with high charge density was put forward to obtain a new



Scheme 4. Mechanism of complex of PTE with PAA.

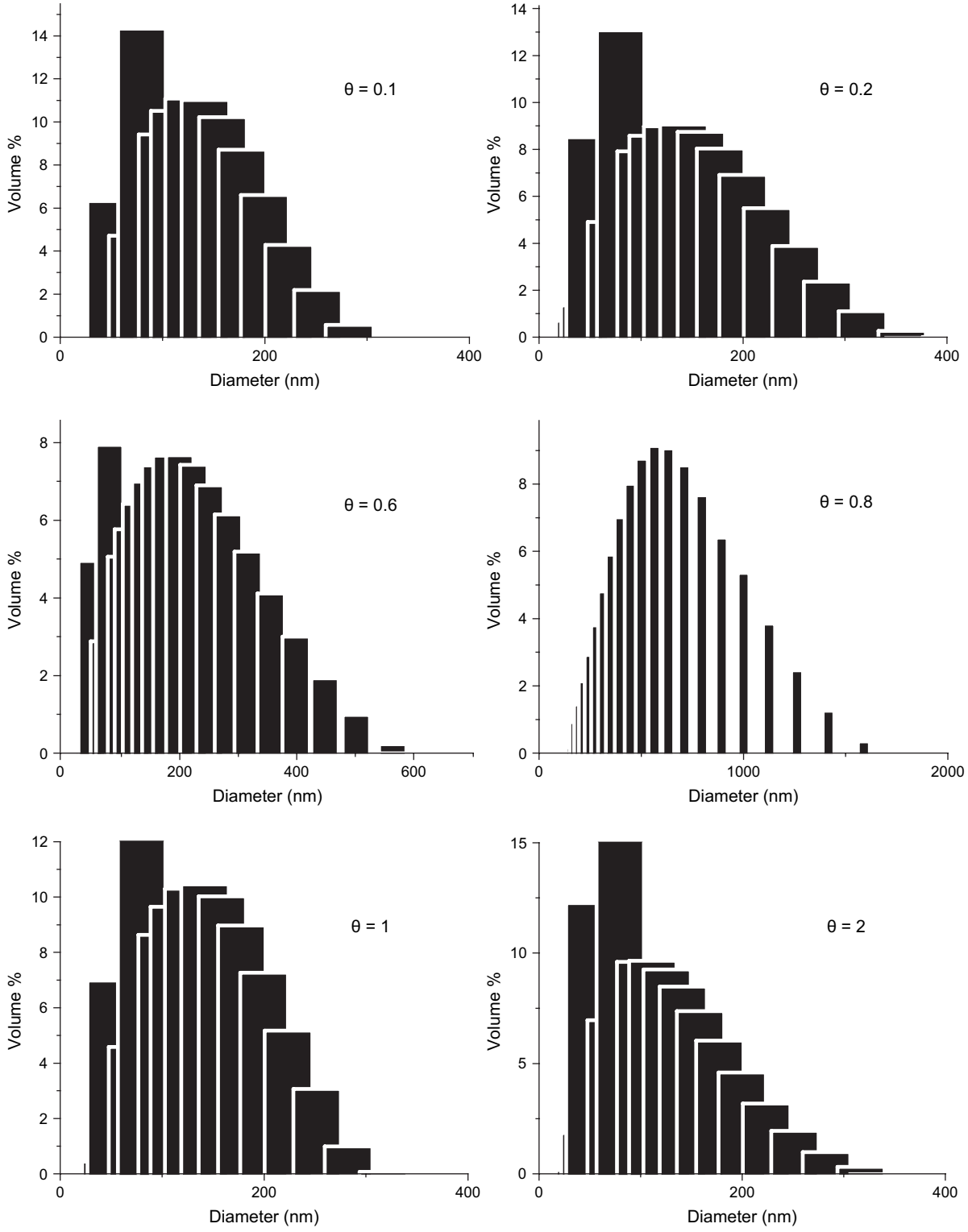


Fig. 9. The volume distributions of complex particles at various θ values.

generation of gene vector with high transfection efficiency and without all-round toxicity. Based on this idea a dissolvable polymer in water, PTE, was synthesized by Michael type addition reaction. PTE can protonize both in pH 7.4–11 and pH 5–7.4 indicating that PTE has similar "proton sponge effect" as polyethylenimine. Different from polyethylenimine, PTE is degradable because of existence of ester groups as indicated by the reduced pH and viscosity of the PTE aqueous solution during incubation. In ^{1}H NMR spectra, with the proceeding degradation, ester

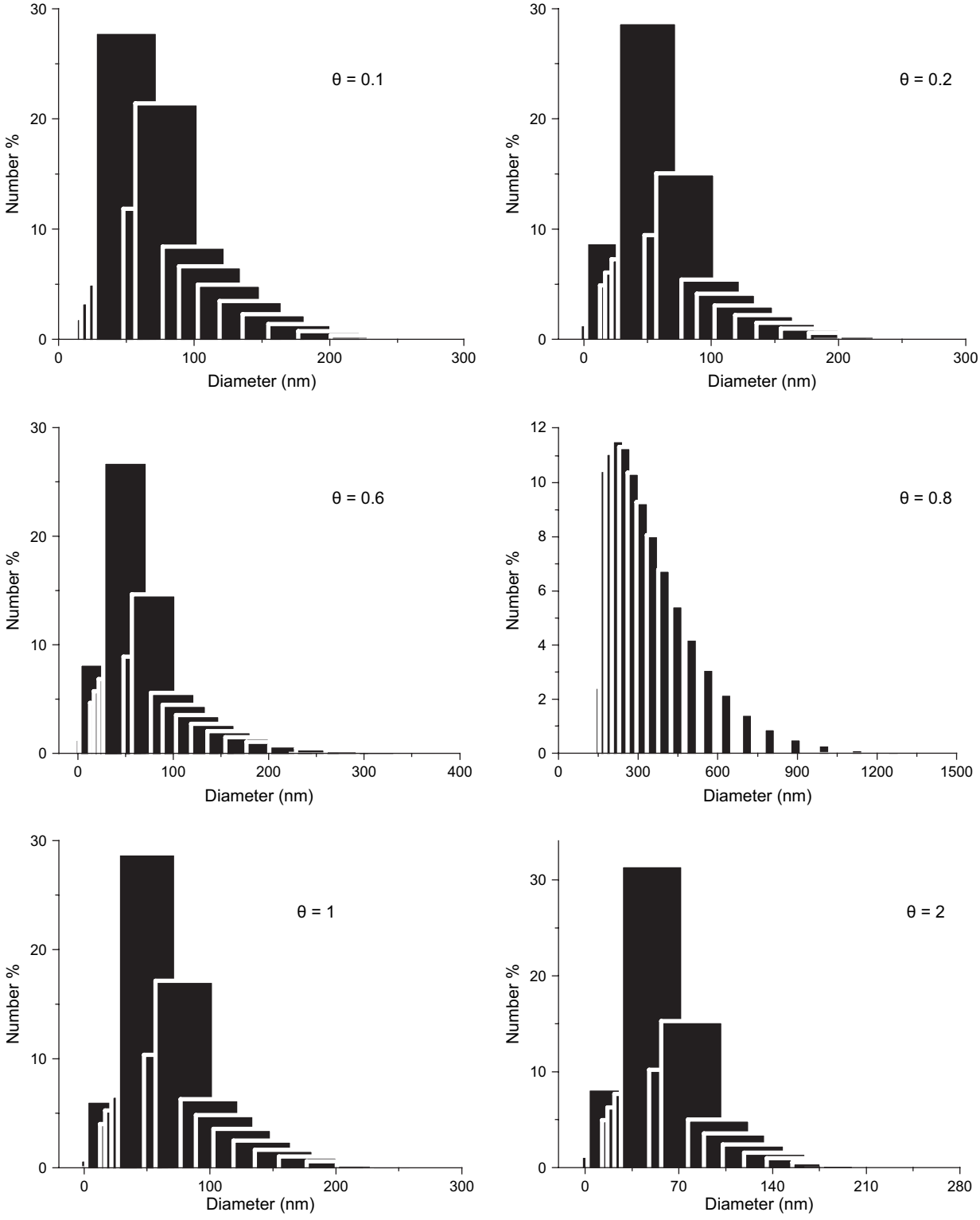


Fig. 10. The number distributions of complex particles at various θ values.

characteristic peak decreased gradually. Different from degradable crosslinked polyethylenimine, PTE has more degradable ester groups, which makes it degraded more rapidly. In comparison with conventional poly(β-amino ester)s, PTE has more amino groups and broader nitrogen-grade profile. PTE was complexed with PAA, a weak polyanion, into nanometer-graded particles in aqueous solutions, which implicated that PTE complexed with stronger polyacid more easily. At θ values of more than 0.8 or less than 0.6, complex particles with diameter less than 200 nm were obtained. At θ of 0.2, UV–vis absorbance curve almost had no change in 7 days, indicating that small stable complex

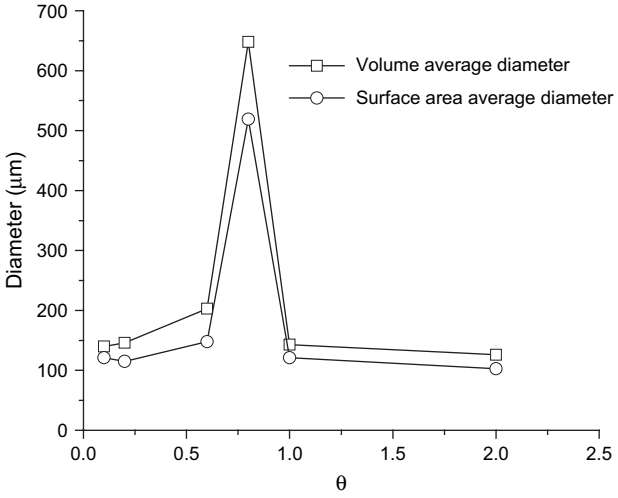
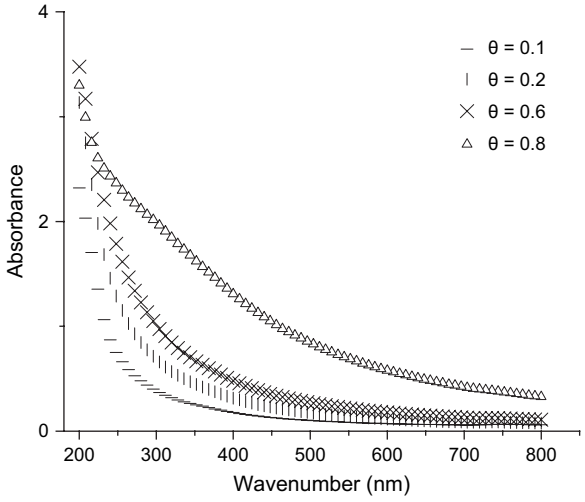


Fig. 11. Diameters at various θ values.



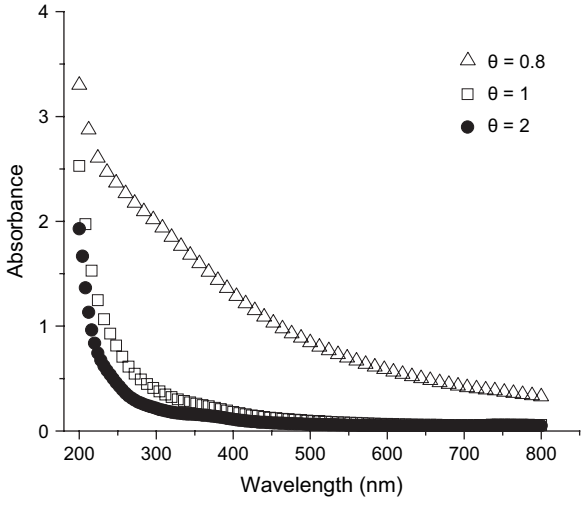
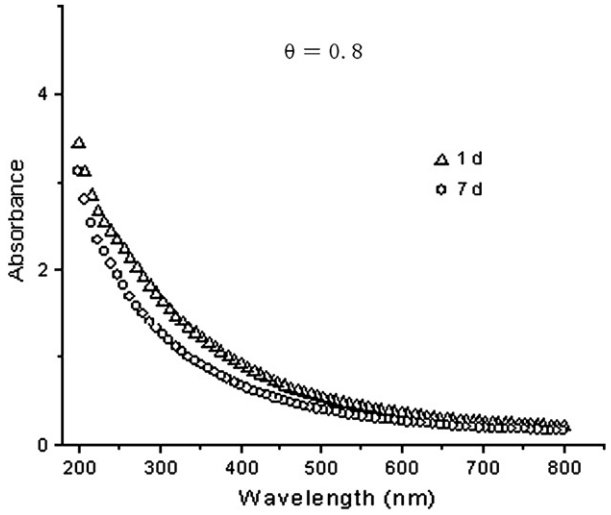


Fig. 12. UV–vis spectra of complex solution at various θ values.

particles could be obtained when either of components was excess. In conclusion, PTE is a potential gene vector and we are seeking to testify it.



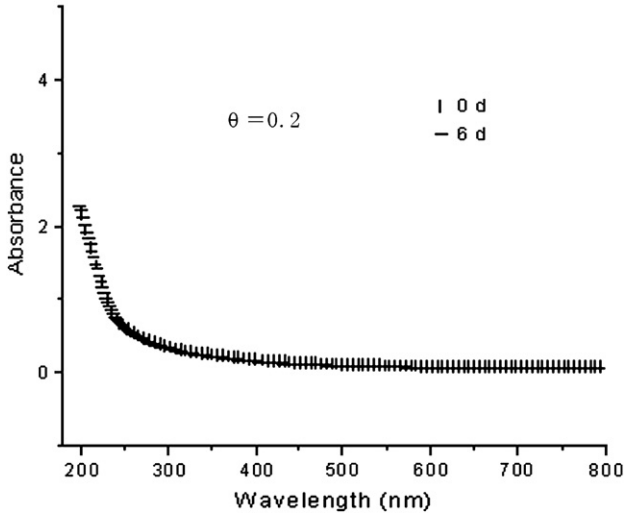


Fig. 13. The UV–vis absorbance changes of complexation solution at θ of 0.2 and 0.8.

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