

Efficient Synthesis, Characterization of Polymeric Material for Biomedical Application for the Benefits of Human Beings

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Abstract

Polydichlorophosphazenes (PDCP) were synthesized from hexachlorocyclotriphosphazenes (HCCP) by ring opening polymerization (ROP) in the presence of AlCl_3 as a catalyst. Poly[bis(salicylaldehyde)phosphazene](PSAP), Poly[bis (salicylaldehyde diethylamino)phosphazene] (PBADEAP), and poly[bis(5-nitrosalicylaldehyde diethylamino) phosphazenes] (PNSADEAP) were synthesized by the macromolecular substitution of PDCP chlorines. The polymers were characterized by ^1H NMR, ^{31}P NMR and GPC. Further, self assembly behaviors of PSAP, PSADEAP and PNSADEAP were investigated in different solvents with different concentration using optical microscope and SEM techniques. Self-Assembly behavior was used for biomedical applications such as drugs delivery applications for the benefits of mankind.

Keywords: Polyphosphazenes, synthesis, characteristics, self assembly behaviors.

1. Introduction

From the past few decades, the self assembly has attracted many researchers due to their applications such as pharmaceuticals, bioengineering, medicine, and materials science [1-5]. It was found that the polymers such as graft copolymers, dendrigraft copolymers, hyperbranched copolymers and dendrimers self assembled into different morphologies [6-10]. Polyphosphazenes are a novel class of polymers that gained tremendous attention in the past five decades due to their synthetic facility, fascinating properties and wide applications [11-15]. Polyphosphazenes connected with hydrophobic and hydrophilic side groups had special micellar properties and their biomedical applications due to their hydrolytic degradation [16-20]. A large number of polyphosphazenes were prepared by using alkoxy, aryloxy, organometallics and amines as side groups [21-30]. Polyphosphazenes attached with some side groups can self assemble in different forms such as fibers, vesicle, micelles, helical structures and macroscopic tubes [26-30]. Among polyphosphazenes, graft copolymer and amphiphilic polyphosphazenes are well known for their self-assembly behaviors [31-33]. Amphiphilic polymers are hard, durable and have different physicochemical and biochemical characteristics with various chemical formulation and structure [24-29]. Amphiphilic copolymers can self assemble into spherical, rod-like, lamellae, vesicles structure [30,31] and these copolymers can be applied in different fields such as shells for the protection of enzymes, drugs delivery systems, and transfection vector[32, 33]. Many researchers synthesized polyphosphazenes and investigated their self-assembly behaviors under various conditions and found their different morphologies [34-39]. But the relationship between the structures of the polyphosphazenes and their self-assembly behaviors still is open to discuss. In this article, PDCP was synthesized by the ring opening polymerization of HCCP. PSAP,

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PSADEAP and PNSADEAP were synthesized from PDCP by the replacement of chlorines in PDCP with aldehyde groups and (or) diethylamine. The self-assembly behaviors of PSAP, PSADEAP and PNSADEAP in different solvents were investigated using optical microscope and SEM techniques.

2. Experimental

2.1 Materials

Hexachlorocyclotriphosphazene (HCCP) is purchased from Across Organics. HCCP was recrystallized in n-hexane. To ensure the purity of monomer, HCCP sample is analyzed by ^{31}P NMR showed single sharp peak appeared [24, 25]. Salicylaldehyde, 5-nitrosalicylaldehyde and diethylamine purchased from Acros organics. Tetrahydrofuran (THF) is refluxed over potassium and distilled in nitrogen atmosphere.

2.2. Synthesis of PDCP from HCCP

Sample glass tubes are cleaned and dried. HCCP (1.10g, 3.16mmol) is weighed directly into ampule sample tube and then catalyst AlCl_3 (0.065g, 0.489mmol) is introduced and then sealed under vacuum. The sealed ampule tube is placed in oil bath at 250°C for 5 hrs. During heating, HCCP is converted into PDCP by changing physical states from clear melting mixture to highly viscous and mobile phase [24]. Reaction is shown in Scheme 1. After polymerization, PDCP is purified by dissolving in refluxed toluene and precipitated in refluxed n-hexane.

2.3 Synthesis of Poly[bis(salicylaldehyde)phosphazenes]

The synthesis of PSAP was based on the method reported in the literature with modification [27-32]. In this method, the flask was equipped with reflux condenser and flask was dried under vacuum. Salicylaldehyde (3.24g, 26.53mmol) (-Cl: -OH 1:4) was treated with NaH (0.64g, 26.67mmol -OH:-Na 1:1) to replace hydroxy group into sodiumoxy in THF and stirred overnight. The purified PDCP (0.77g, 6.64mmol, -Cl) was dissolved in refluxed THF (100ml). Then sodiumoxy salicylaldehyde was introduced dropwise into PDCP solution under nitrogen atmosphere. The reaction solution was stirred and refluxed at 67°C for 48hrs. Then resultant mixture filtered and filtrate was concentrated to remove excess THF under vacuum. The polymer was purified by repeated precipitation from THF into n-hexane thrice. At the end, polymer was dried under vacuum.

2.4. Synthesis of poly[bis(salicylaldehyde diethylamino)phosphazenes]

The synthesis of PSADEAP was preceded in the same way as in PSAP. The purified PDCP (0.45g, 3.69mmol) was dissolved in refluxed THF (100ml). A stoichiometric amount of salicylaldehyde (1.89g, 15.48mmol) (-Cl: -OH 1:4) and diethylamine (1.08g, 14.77mmol -Cl: -NH 1:4) were added to PDCP solution. Reaction was refluxed for 48hrs at 70°C then filtered. The PSADEAP was purified by repeated precipitation from THF into n-hexane twice. At the end, polymer was dried under vacuum till constant weight.

2.5. Synthesis of poly[bis(5-nitrosalicylaldehyde)phosphazenes]

The synthesis of PNSADEAP was preceded in the same way as in PSADEAP. The purified PDCP

(0.25g, 2.16mmol) was dissolved in refluxed THF (100ml). A stoichiometric amount of 4-nitro salicylaldehyde (1.44g, 8.62mmol) (-Cl: -OH 1:4) and diethylamine (0.63g, 8.61mmol -Cl: -NH 1:4) were added to PDCP solution. Reaction was refluxed for 48hrs at 70°C then filtered. The PSADEAP was purified by repeated precipitation from THF into n-hexane twice and then in methanol. At the end, polymer was dried under vacuum till constant weight.

3. Results and Discussion

3.1. Synthesis and Characterization

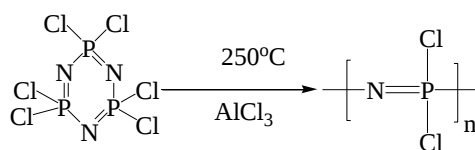
The purity of HCCP was confirmed by ^{31}P NMR before polymerization. It showed sharp single peak at 20ppm. The polymers PSAP, PSADEAP and PNSADEAP were synthesized by macromolecular substitution reaction in two steps. In the first step, PDCP was synthesized by thermal ring opening polymerization in the presence of AlCl_3 catalyst at 250°C for 5 hrs as shown in scheme 1. In the second step, the chlorine atoms of PDCP were substituted by the sodiumoxy salicylaldehyde for PSAP while in the preparation of PSADEAP the salicylaldehyde and diethylamine were added in excess amount to replace PDCP chlorines.

The synthesis products were investigated by ^1H NMR, ^{31}P NMR, GPC and DSC. The details were shown in Table 1. The ^1H NMR spectrum of PSAP showed peaks with the following shifts: 6.52-6.70ppm (2H of C_6H_4), 7.20-7.30ppm (2H of C_6H_4), 7.45-7.52ppm (2H of C_6H_4), 10.15-10.17ppm (1H of CHO) represented the diethylamine as shown in Figure 1 (1). ^{31}P NMR of PSAP showed chemical shift at -32ppm and GPC represented the molecular weight 18196 and curve shown in Figure 2(1).

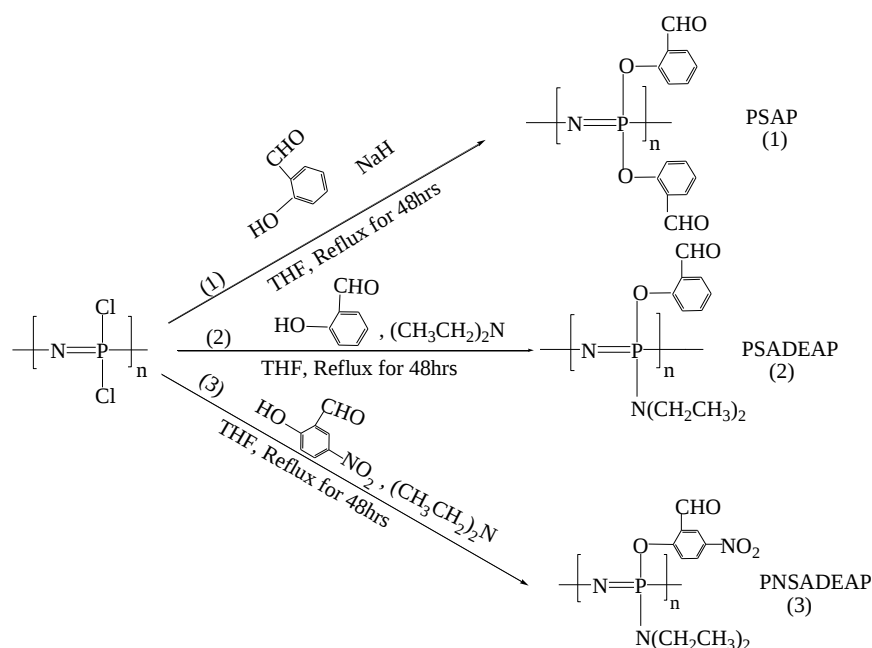
The ^1H NMR spectrum of PSADEAP showed peaks with the following shifts: 7.15-7.20ppm (2H of C_6H_4), 7.46-7.49ppm (2H of C_6H_4), 7.55-7.65ppm (2H of C_6H_4), 10.18-10.19ppm (1H of CHO) 3.52-3.85ppm (2H of CH_2) and from 2.46-2.51ppm (3H of CH_3) represented the diethylamine as shown in Figure 1 (2). ^{31}P NMR of PSADEAP showed peaks at -2ppm and GPC in Figure 2(2).

The ^1H NMR spectrum of PNSADEAP showed peaks with the following shifts: 6.75-6.80ppm (2H of C_6H_4), 8.05-8.10ppm (2H of C_6H_4), 8.30-8.32ppm (2H of C_6H_4), 10.19-10.20ppm (1H of CHO) 3.05-3.10ppm (2H of CH_2) and from 1.12-1.25ppm (3H of CH_3) represented the diethylamine as shown in Figure 1 (3). ^{31}P NMR of PNSADEAP showed peak at -1ppm and GPC in Figure 2(3).

There are some factors which effect the substitution reaction. First, traces of water or some other impurities in the reaction system, results cross linking and polymer cannot dissolve in any solvents, so all monomers and solvents must be dried and purified. Second, In PDCP, chlorine atoms are replaced up to limited point, then reaction is terminated that is indicated by the precipitation of the polymer. Third, steric hindrance decreased the speed of chlorine replacement because in polymer chain nitrogen and phosphorus atoms are at alternate position and each phosphorus atom connected with two organic side groups (salicylaldehyde).



Scheme 1. Synthesis of PDCP from HCCP in the presence of AlCl_3 catalyst



Scheme 2. Synthetic scheme of PSAP (1), PSADEAP (2) and PNSADEAP (3)

Table 1. Synthesis conditions of PSAP, PSADEAP and PNSADEAP

Polymers	HCCP		PDCP		Al-OH		DEA		M _w
	g	mmol	g	mmol			g	mmol	
PSA	1.10	3.16	0.77	6.64	3.24	26.53	-----	-----	18196
PSADEAP	0.65	1.87	0.45	3.69	1.89	15.48	1.08	14.77	6035
PNSADEAP	0.41	1.18	0.25	2.16	1.44	8.62	0.63	8.61	5545

Al-OH: aldehyde groups contained –OH. DEA: diethylamine, PDCP was synthesized using 5% AlCl_3 . (1) PSAP (–OH:–Na = 1:1 –Cl:–ONa = 1:4). (2) PSADEAP (–Cl:–OH = 1:4), (–Cl:–DEA 1:4).

^aThe ratio of PSADEAP and PNSADEAP side groups were calculated from ¹H NMR ($x+y=2$).

^bDMF with 0.3% NaNO_3 was used as eluent at 30°C M_w was determined by GPC versus narrow distributed PMMA standards.

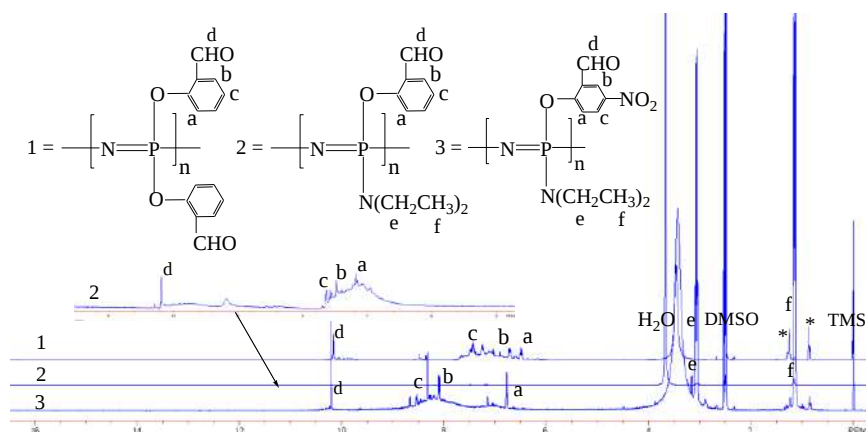


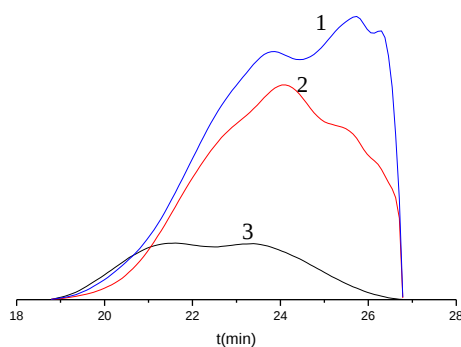
Figure 1. ^1H NMR of PSAP(1), PSADEAP(2) and PNSADEAP(3) Steric showed solvent peaks

Figure 2. GPC of PSAP (1), PSADEAP (2) and PNSADEAP (3)

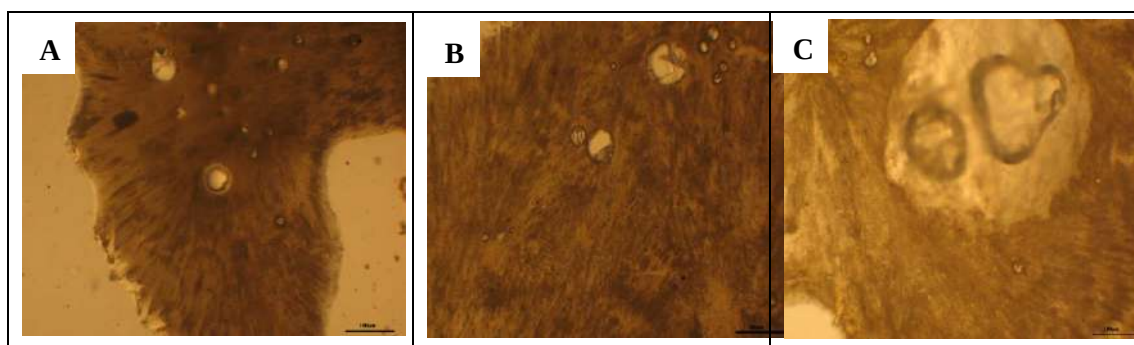
Thermal Stability

Table 2. DSC results of PSAP, PSADEAP, and PNSADEAP

Samples	T _g (°C)	T _c (°C)	T _m (°C)
PSAP	-51.96	48.70	72.07
PSADEAP	-38.28	125.98	91.02
PNSADEAP	-25.01	88.29	83.65

3.2 Self Assembly Behavior

The self assembly of behaviors of the PSAP, PSADEAP and PNSADEAP were observed by optical microscope and SEM images. It was noted that by changing the solvent and concentration of the polymer, morphology of the polymers was obviously changed. Firstly, Optical micrograph of PSAP, PSADEAP and PNSADEAP were taken using DMF and DMSO solvent with different concentrations such as 2mg/5ml, 2mg/10ml, 5mg/10ml and their images were given in Figure 3 (A to R) respectively. Optical micrographs of PSAP were taken using DMF (2mg/5ml) solvent as shown in Figure 3(A, B, C). After complete drying of the solvent, optical images were taken and it was noted that spots-like morphology appeared on the surface and to confirm their behavior, SEM images were taken. SEM image of the PSAP showed virus-like behaviors as shown in Figure 3(D,E,F). The optical image of PSADEAP small drop-like morphology as shown Figure 3(G,H,I) their SEM images were given in Figure 3(J,K,L). The optical microscope images of PNSADEAP as shown in Figure 3(M,N,O) and their SEM images were given Figure 3(P,Q,R,S). SEM images in using DMSO(3mg/10ml) as shown in Figure 3(T,U).





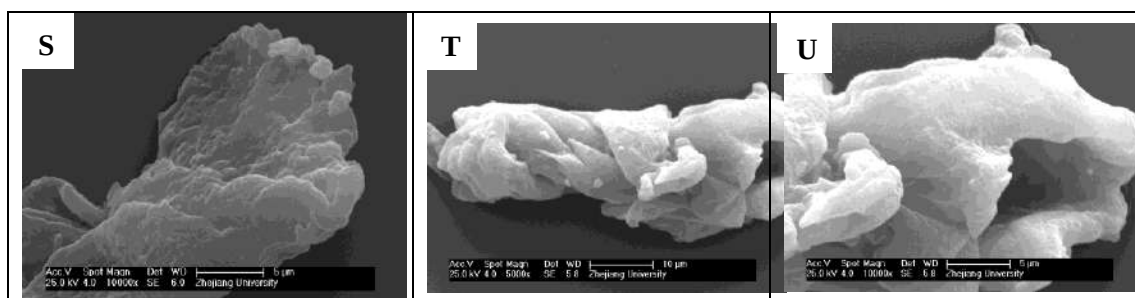
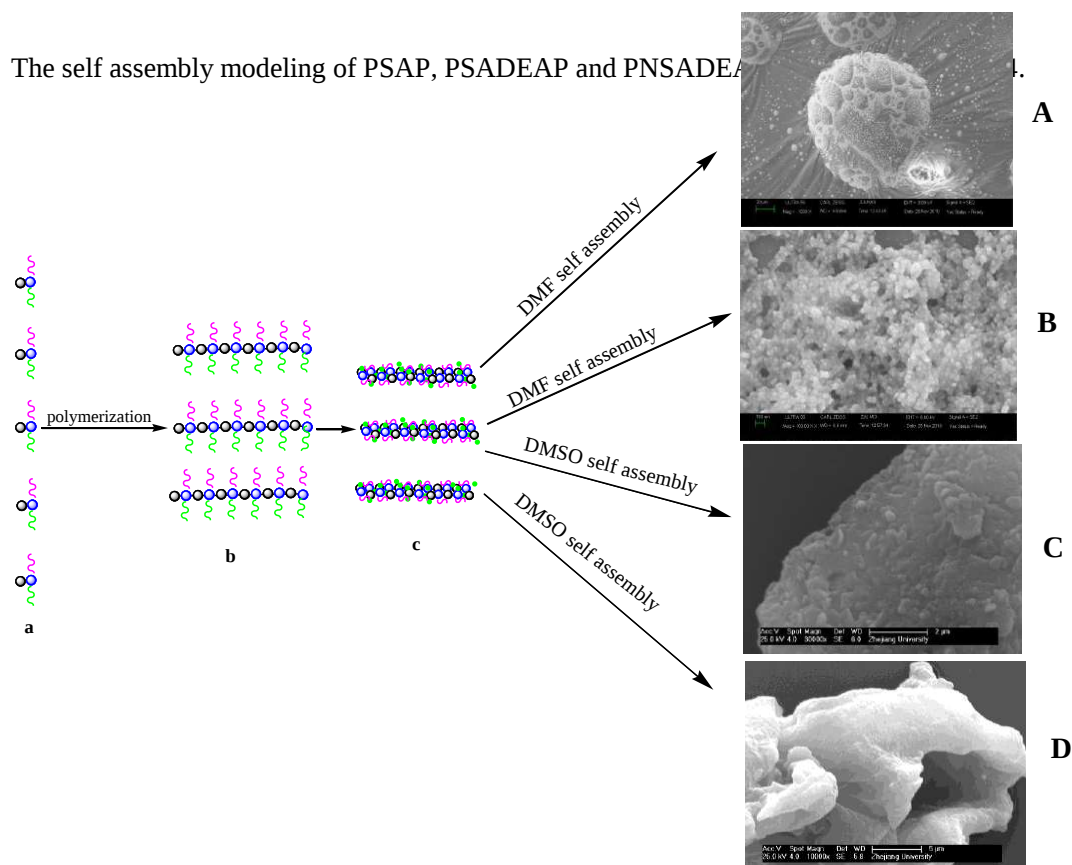


Figure 3. Optical micrograph and SEM images of PSAP (A to F), PSADEAP (G to L) and PNSADEAP (M to U) DMF (1mg/5ml) (2gm/5ml) (3gm/5ml).

The self assembly modeling of PSAP, PSADEAP and PNSADEAP



○ = nitrogen atom ○ = phosphorus atom, ∞ = PPOBADEAP backbone [-N=P-], ~ = diethylamine side group (purple) hydrophilic, ~ = p-oxybenzaldehyde side groups (green) hydrophobic (a) one molecule (b) PSAP, PSADEAP and PNSADEAP with side groups (c) After dissolving in solvents (d) self assembly after complete drying of solvents.

Figure 4. Possible schematic model for the self assembly of PSAP, PSADEAP, PNSADEAP in DMF, and DMSO solvents. (A) PSAP is DMF self assembly, (B) PSADEAP is DMF self assembly, (C) PNSADEAP is DMSO self assembly, (D) PNSADEAP is DMSO self assembly.

Conclusion

Polymeric materials are significant for the human beings in variety of ways such as PSAP, PBADEAP, PNSADEAP were synthesized by the macromolecular substitution of PDCCP chlorines with aldehyde groups and (or) diethylamine. The polymers were characterized by ^1H NMR, ^{31}P NMR and GPC. The self-assembly behaviors of the polymers were investigated by optical microscope and SEM techniques. Moreover, Self-assembly behavior can be applied for drugs

delivery applications for development of mankind.

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