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Biodegradable Materials for Drugs Delivery Applications for Social Benefit of Mankind

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Abstract
Biodegradable and non-toxic poly[bis(p-oxy azo benzoic acid diethylamino) phosphazenes] (POABADEAP) was synthesized in two steps. In first step, the polydichlorophosphazenes (PDCP) was synthesized from hexachlorocyclotriphosphazene (HCCP) by thermal ring opening polymerization in the presence of $AlCl_3$ as catalyst and in second step chlorines from PDCP were substituted by hydroxy groups of p-hydroxy azobenzoic acid and then diethylamine respectively. The synthesis results were proved by ^1H NMR and GPC. Hydrolytic degradation of POABADEAP was studies and it was found that polymer degraded smoothly for the biomedical application such as drugs delivery and for social benefits of human beings.
Keywords: Polyphosphazenes, synthesis, characteristics, azobenzene, biomedical applications, drugs delivery, hydrolytic degradation, Social Benefit

1. Introduction

Recently, many types of polymeric and composite materials have attracted attentions due to potential applications such as biodegradability, drugs delivery and tissue engineering. The characteristics and applications of any materials were indicated their importance **[1-10]**. Among these polymeric materials, polyphosphazenes indicated unique properties and applications as biodegradable and biocompatible precursor due to Phosphorus and Nitrogen backbone. Polyphosphazenes have nitrogen and phosphorus backbone [-N=P-] and phosphorus is pentavalent and can easily connected with two organic side groups and is biodegradable, nontoxic, biocompatible and friendly environment **[3, 11-14]**.

It was observed many researchers that properties and applications of polyphosphazenes are depended upon side groups such as alkoxy, aryloxy and amines **[3, 15-18]** such as hydrophobic and hydrophilic. Due to side group

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connecting facility different types of polyphosphazenes have been synthesized and utilized for different types of applications.

Polyphosphazenes were connected with hydrophobic and hydrophilic groups such as amines and amide side groups such as amino acid, amino acid esters, lactate, glucosyl, glycolate, glyceryl, and imidazole hydrolyze easily while with other hydrophilic side groups such as alkoxy, and aryloxy protect the backbone to control the degradation mechanics **[4-6, 19,20]**.

It has been proved that polyphosphazenes with the combination of hydrophilic and hydrophobic side groups indicated controlled degradation rate for drugs release applications. Some researcher found that esters as side groups degraded very easily and produced alcohol, amino acid, phosphate and ammonia after hydrolysis **[7,8, 21-23]** and have applications as drug delivery and tissue engineering **[3, 24-26]**.

For biomedical applications, such as drugs delivery applications has attracted researchers due to their significance, importance and material used for environment **[9,10, 27]**. From the past few decades, many polymeric materials were studied for self-assembly behavior and among them polyphosphazenes is relatively new class that is easily biodegradable and biocompatible because of their inorganic backbone nitrogen and phosphorus atoms [-N=P-] **[11,12, 28].** Polyphosphazenes represented big advantages for the synthesis and applications of biological compounds due to biodegradability, structural design and functional of nitrogen-phosphorus backbone **[20-24, 29]**. Some polyphosphazenes such as thermosensitive polyphosphazenes, thermosensitive amphiphilic graft polyphosphazenes were synthesized and utilized for biodegradability in drug delivery systems **[25-30]**.

In this study, we synthesized biodegradable and biocompatible poly[bis(p-oxy azobenzoic acid diethylamino)phosphazene] (POABADEAP) by thermal ring opening polymerization in the presence of $AlCl_3$ as catalyst and in second step chlorines from PDCP were substituted by hydroxy group of azobenzoic acid and diethylamine respectively and later hydrolytic degradation study was investigated for drugs delivery application for social benefits of this human beings.

2. Experimental

2.1 Materials

Hexachlorocyclotriphosphazene (HCCP) is purchased from Across Organics. HCCP was recrystallized in n-hexane. P-hydroxybenzaldehyde purchased from Acros organics. Diethylamine is distilled and stored under nitrogen atmosphere. Tetrahydrofuran (THF) is refluxed and distilled under inert atmosphere.

2.2. Synthesis of PDCP from HCCP

Clean glass tubes are dried in oven and then HCCP (1.23g, 3.54mmol) is weighed directly into sample tube and then catalyst $AlCl_3$ (0.065g, 0.489mmol) is introduced. The tube is evacuated and then sealed under vacuum. The sealed tube is placed in oil bath at 250°C for 5 hrs. During heating, HCCP converted into PDCP by changing physical states from clear melting mixture to highly viscous and mobile phase **[24]**. Reaction is shown in Figure 1. Sample tube is broken and connected with schlenk line. PDCP is purified by dissolving in refluxed toluene (10ml) through sonication and precipitated in refluxed n-hexane. After this, n-hexane is removed, unreacted HCCP trimer is weighed and the amount of PDCP is calculated (0.86g, 70%). Finally, PDCP is dissolved in refluxed tetrahydrofuran THF in the presence of inert atmosphere **[26]**.

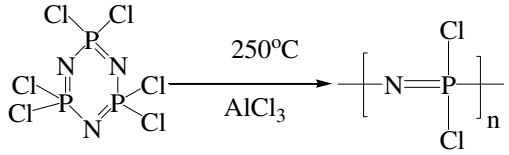

Figure 1. Synthesis of PDCP from HCCP in the presence of catalyst

2.3. Synthesis of Poly[bis(p-oxy azobenzoic acid diethylamino)phosphazene]

The synthesis of POABADEAP is based on the method reported in the literature **[27-30]** with modification. In typical synthesis, three neck flask is equipped with reflux condenser and flask is dried under vacuum by heating at flame to remove moisture and oxygen and then nitrogen gas introduced respectively. PDCP (0.86g, 4.73mmol) is purified by dissolving in toluene and precipitating in n-hexane, finally dissolved in THF **[28]**. In another flask, p-hydroxy azo benzoic acid (Cl:OH, 1:2)is dried under vacuum for 5 hours at 50°C and dissolved in refluxed THF. After this, p-hydroxy benzoic acid solution transferred into PDCP solution under inert atmosphere. The details are given in Table 1. Finally, diethylamine (Et_2N) is added dropwise into the mixture respectively. Reaction mixture is stirred and refluxed at 67°C for 48hrs shown in Figure 2. The resultant mixture filtered, and residue washed with water many times to remove salt and impurities. Residue dried in vacuum oven for 24 hours. Excess THF from filtrate is removed by rotatory evaporator, added into n-hexane dropwise and stirred by magnetic stirrer. Flask cooled down by ice bags. Stirring process stopped and n-hexane poured out. Again, the resultant product dissolved in minimum amount of THF (10ml) and added into fresh n-hexane (100ml) dropwise and this process repeated for 2-times and then precipitated in ethanol (100ml) 2-times to ensure purification.

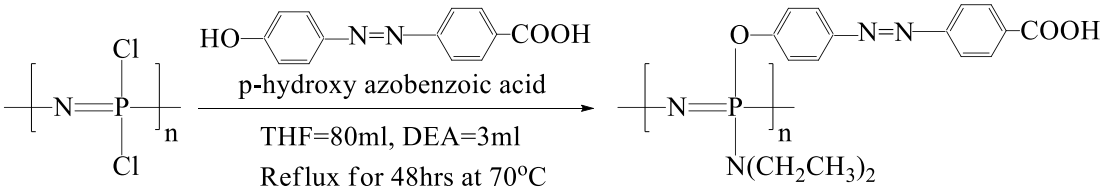

Figure 2. Synthesis of poly[bis(p- oxy azobenzenoic acid diethylamino)phosphazenes]

2.4. Equipments

Molecular weight and molecular weight distribution were determined by gel permeation chromatography (GPC). The eluent was DMF at a flow rate of 1.0 ml min^{-1} and calibrations were narrow-distribution polystyrene standards. ^{1}H NMR, was obtained from a 400 MHz AVANCE NMR spectrometer (model DMX400). For protons, the chemical shifts were relative to tetramethylsilane at δ=0 ppm.

3. Results and Discussion

HCCP monomer was purified by recrystallization and sublimation and finally analyzed and confirmed by $^{31}PNMR$ indicated peak at 20ppm. PDCP was synthesized from HCCP by thermal ring opening polymerization at 250°C under vacuum using catalyst detail is given in table 1 **[24]**. It has been studied that the product phase depends on the amount of catalysts and reaction time, when catalyst amount is more than 3%, then viscous phase PDCP was obtained and content of catalyst less than 2% result mostly crosslinked product. Reaction time also varied from 3hours to 10 hours. PDCP purified in refluxed n-hexane because HCCP can easily dissolve in n-hexane while PDCP could not dissolve. After purification of PDCP, we calculated the amount of PDCP (60-70% yield). Reaction is shown in Figure 1. In this reaction, $AlCl_3$ acts as initiator for thermal ring opening polymerization, but in excess amount of catalyst, reaction stopped due to formation of stable acid base adduct with HCCP. Reaction time and catalyst depends upon each other, it is concluded that when catalyst amount increased then reaction time decreased and vice versa **[24]**.

Table 1. Synthesis conditions of POABADEAP

Sample	HCCP		$AlCl_3$		PDCP		p-hydroxyazo benzoic acid		DEA	Mw
	g	mmol	g	mmol	g	mmol	ml	mmol	ml	
1	1.230	3.54	0.065	0.489	0.86	7.42	10.0	46.68	3.0	18589

Reaction temperature: PDCP was synthesized at 250°C at 5hrs and POABADEAP was synthesized at 70°C. The glass transition temperature of the POABADEAP was Tg = 33.27°C, crystalline temperature Tc = 48.90 °C, melting temperature Tm = 42.30 °C.

But here our purpose to replace chlorine groups with some suitable side groups to control the degradation rate for biomedical applications such as drugs delivery etc. In second step, POABAP is synthesized by the replacement of chlorine from PDCP with hydroxyl group of p-hydroxy azo benzoic acid in THF solvent and later dimethylamine (DEA) was introduced.by refluxing 48 hours. Then polymer was filtered and crystalized in n-hexane. Initially, PDCP was purified and dissolved in THF, and after this p-hydroxy azobenzoic acid dissolved in THF and triethylamine was introduced respectively.

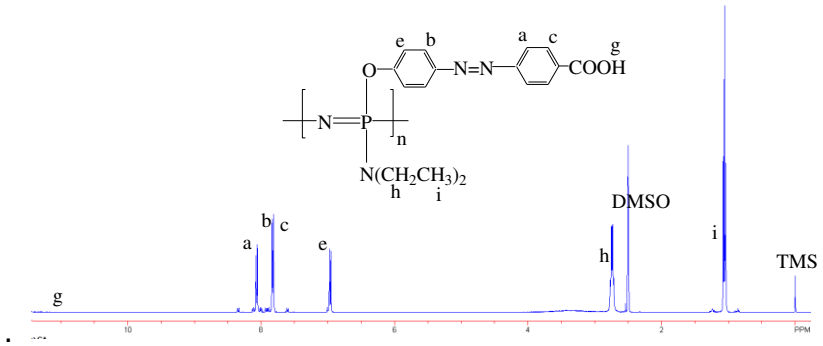

Figure 3. ^{1}H NMR of poly[bis(p- oxy azo benzenoic acid diethylamino)phosphazenes]

The ^{1}H NMR spectrum of POABADEAP showed peaks with the following shifts: 6.70-7.30ppm (2H of C_6H_4), 7.40-7.50ppm (2H of C_6H_4), 7.59-7.75ppm (2H of C_6H_4), 11.18ppm (1H of COOH) 3.50-3.83ppm (2H of CH_2) and from 2.45-2.50ppm (3H of CH_3) represented the diethylamine as shown in Figure 3. ^{31}P NMR of PSADEAP showed peaks at -2ppm and GPC results demonstrated that its molecular weight was 57589 and Mn 14929 as shown in Figure 4. Thus, NMR and GPC results proved the successful synthesis of POABADEAP.

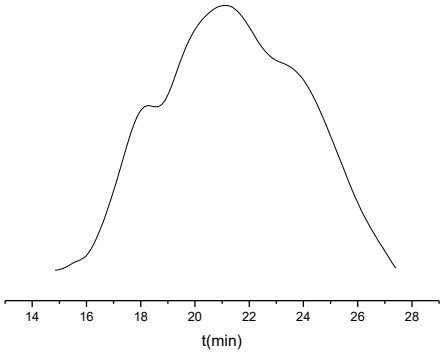

Figure 4. GPC of poly[bis(p- oxy azo benzenoic acid diethylamino)phosphazenes]

3.1. Hydrolytic Degradation

Degradation of polyphosphazene initiated by the hydrolysis of side groups followed by the backbone chain. The degradation of PPPOABADEAP was studied by the release of side groups and decrease in molecular weight of the polymer chains **[4,5,15]**. Hydrolytic degradation was studied at 37^{o}C in acidic, basic and neutral medium.

It was observed that time-dependent degradation of the present POABADEAP was examined in different pH Acidic, Basic and neutral media buffer solutions at 37 ^{o}C. The POABADEAP (20 mg) were dissolved in 2 ml of 0.5 M buffer solution (acetate buffer of pH 5, phosphate buffer of pH 7.4, and carbonate buffer of pH 10), which was incubated in a water bath at 37 ^{o}C as shown in Figure 5. Time-dependent hydrolytic behavior of the polymers was determined in terms of molecular weight decrease of the polymers by GPC **[26]**. Hydrolytic degradation was investigated by ^{31}PNMR.

After degradation, the hydrolytic degradation products like phosphate and ammonia and amino acids were confirmed by formation of yellow precipitate with silver nitrate and intense violet color with ninhydrin **[4,5,16]**. The degradation rate depends upon side groups and it increases with the number of unreacted chlorine atoms. Further, degradation was studied by ^{31}P NMR. In the possible mechanism of degradation of polyphosphazene, unreacted chlorine atoms and other side groups are hydrolyzed followed by cleavage of the backbone. The common products of hydrolysis could be carboxylic acids, ammonium, phosphate and alcohol. Possible degradation mechanism of PPPOABADEAP is shown in Figure 6.

The production of amnio acid was investigated by Ninhydrin test and showed bluish violet color.

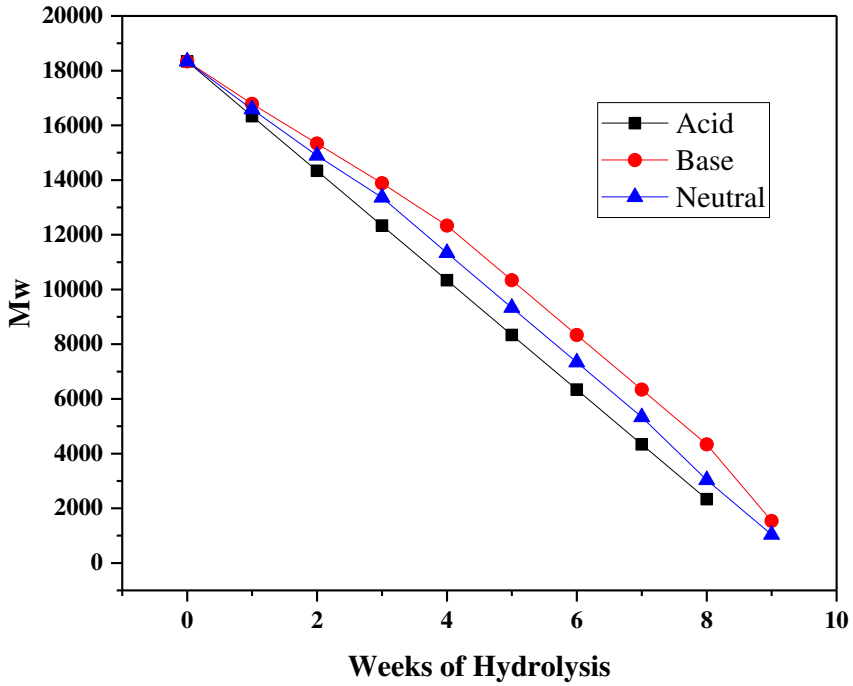

Figure 5. Hydrolysis of PPPOABADEAP in acidic, basic and neutral

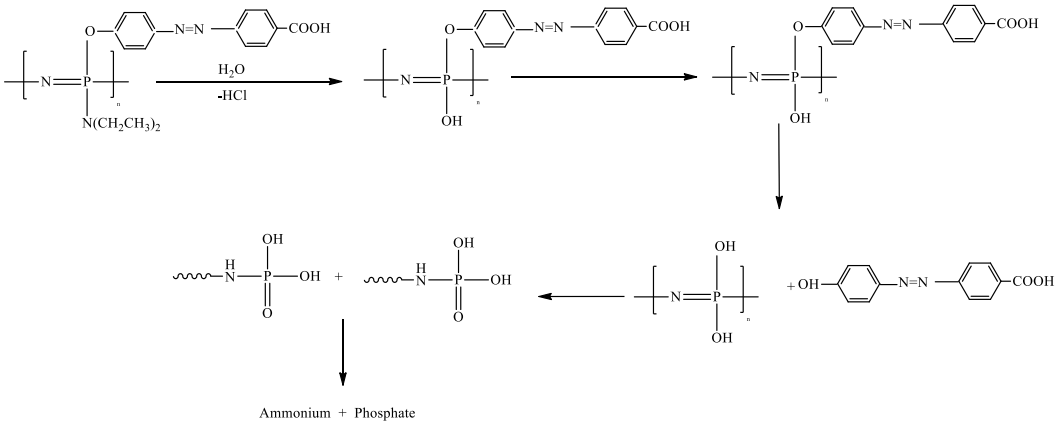

Figure 6. Possible degradation mechanism of PPPOABADEAP

Conclusion

A biodegradable and biocompatible poly[bis(p- oxy azobenzoic acid diethylamino)phosphazenes] (POABADEAP) were synthesized in two steps. In first step, the polydichlorophosphazenes (PDCP) was synthesized from hexachlorocyclotriphosphazene (HCCP) by thermal ring opening polymerization in the presence of $AlCl_3$ catalyst and in second step chlorine atoms from PDCP were substituted by hydroxy groups of 4-hydroxy azo azobenzoic acid and diethylamine respectively. Hydrolytic degradation results showed that end product was phosphate and ammonium. Degradation mechanics will be utilized for drugs delivery application for the social benefits of this mankind.

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