Poly(dichlorophosphazene) As a Precursor for Biologically Active Polyphosphazenes: Synthesis, Characterization, and Stabilization

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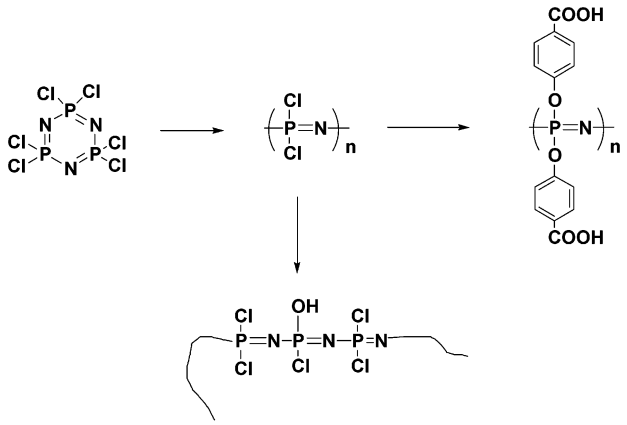
ABSTRACT: Water-soluble polyphosphazenes emerge as an important class of biologically active macromolecular compounds dictating the need for the development of their well-defined and controlled synthesis. The synthetic pathway leading to biologically active polyphosphazenes involves preparation and chemical transformation of inorganic macromolecular precursor—poly(dichlorophosphazene), PDCP. Synthesis, stabilization, and characterization of this hydrolytically sensitive, reactive intermediate are the focus of the present study. Ring-opening polymerization reaction leading to PDCP has been investigated under strictly controlled conditions by NMR, viscometry, and direct multiangle laser light scattering—GPC methods. A substantial dependence of the molecular weight on degree of conversion and the formation of branched polymer structures have been observed even at early stages of the polymerization process. A new approach has been suggested for the stabilization of PDCP solutions involving the use of diethylene glycol dimethyl ether (diglyme). This stabilization technique allows highly reliable direct analysis of PDCP using chromatographic methods and greatly simplifies PDCP conversion in the organo-substituted polymer.

Introduction

Water-soluble polyphosphazenes present considerable interest as an emerging class of biologically active macromolecular compounds. Poly[di(carboxylatophenoxy)-phosphazene] (PCPP) and other polyphosphazene polyelectrolytes demonstrate powerful immunostimulating properties and have been widely investigated as vaccine adjuvants and materials for protein microencapsulation.[1−7] The unprecedented control of biodegradation profiles, targeting, and other critical biological characteristics in phosphazene polymers result from the combination of unique phosphorus—nitrogen backbone and structural diversity of side groups. It has been shown that biologically relevant properties of polyphosphazenes are also greatly affected by their macromolecular characteristics. Immunostimulating activity of PCPP, for example, can be modulated through changes in the molecular weight parameters.[8] Thus, it is of critical importance to develop synthetic methods that enable a well-defined synthesis of this valuable class of polymers.

Synthetic pathways leading to the biologically active polyphosphazenes, however, imminently require synthesis and derivatization of poly(dichlorophosphazene) (PDCP)—a unique reactive inorganic macromolecule with extreme hydrolytic sensitivity (Scheme 1).[9,10] One of the main challenges in polyphosphazene chemistry is the understanding and control of hydrolytic reactions involving chlorine atoms of this polymer. Such reactions result in undesirable processes of polymer degradation and cross-linking in the presence of even trace amounts of water. Although a number of valuable studies have been reported on the preparation of PDCP by thermal ring-opening polymerization of hexachlorocyclotriphosphazene,[11−14] the reaction is only understood in a qualitative sense,[9] and some fundamental parameters, such as molecular characteristics of PDCP and their dependence on the reaction conditions, remain to be investigated. Adding to the problem is the scarcity of direct PDCP molecular characterization methods due to the polymer's uncontrollable behavior in GPC analysis.[11]

Scheme 1. Synthetic Pathway to the Biologically Active Polyphosphazenes, Such as PCPP, Involves Synthesis and Derivatization of Reactive Precursor, PDCP[a]



[a] Extreme hydrolytic sensitivity of PDCP can lead to uncontrolled degradation or cross-linking of the polyphosphazene.

In this paper we focused on the synthesis of reactive polyphosphazene precursor, PDCP, the first stage in the production of biologically active water-soluble polyphosphazenes. We investigated ring-opening polymerization of hexachlorocyclotriphosphazene using a highly controlled environment, stabilized PDCP formulations, and direct analysis by the multiangle laser light scattering—GPC method. Special attention was given to the earlier stages of the polymerization process to provide maximum information on the mechanism of polymerization and the effect of reaction conditions on the molecular weight characteristics.

Experimental Section

Materials. Hexachlorocyclotriphosphazene (trimer, Phosnic 390) (Nippon Fine Chemicals, Japan) was purified in portions

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of 700−800 g by vacuum distillation (35 mmHg). Approximately 500 g of the purified product with the boiling point in the range of 145−155 °C was collected after each distillation. To ensure homogeneity of hexachlorocyclotriphosphazene, these portions were combined together, melted, and stirred under nitrogen at 150 °C. Purified hexachlorocyclotriphosphazene (approximately 10 kg) was then transferred to Teflon trays, cooled to room temperature, disintegrated, sealed, and used for polymerization without additional treatment.

2-Methoxyethyl ether (diethylene glycol dimethyl ether, diglyme), anhydrous, 99.5%, and 0.0015% water, and tetrahydrofuran (THF), anhydrous, 99.99%, and 0.0009% water (both from Aldrich Chemical Co., Inc., Milwaukee, WI), were used as received and handled under an anhydrous nitrogen atmosphere. Sodium propyl paraben, U.S.P./N.F. grade (Spectrum Quality Products, Inc., Gardena, CA), and propyl 4-hydroxybenzoate (propyl paraben), 99.5+% (Aldrich Chemical Co., Inc., Milwaukee, WI), were dried prior to use in a vacuum oven at 80 °C for 2 h.

Analytical Methods. Characterization of PDCP was performed using diglyme as a mobile phase. The chromatographic system was equipped with a Waters 510 HPLC pump (Waters, Milford, MA), one in-line filter−0.5 μm high-pressure filter (Rainin, Woburn, MA), and a Waters model U6K universal liquid chromatograph injector. A Waters Styragel HR 5E column was used with a Waters Styragel guard column (Waters, Milford, MA). The column size was 7.8 mm i.d. × 30 cm. The temperature was maintained at 35 °C in a Waters column oven. An aqueous chromatographic system for characterization of PCPP included a Waters 510 HPLC pump and a Waters 717 autosampler (Waters, Milford, MA), the Ultrahydrogel guard column, Ultrahydrogel 2000 (pore size 2000 Å), Ultrahydrogel 250 (pore size 250 Å), and Ultrahydrogel 120 (pore size 120 Å) columns (Waters, Milford, MA) connected in a series. A phosphate buffer solution (PBS) (pH 7.4)—Dulbecco's phosphate buffered saline (Sigma Chemical Co., St. Louis, MO)—was used as a mobile phase. 0.01% NaN_3 was added to mobile phase to prevent biological degradation of the columns. Both aqueous and organic HPLC systems also included a multiangle laser light scattering (MALLS) detector (DAWN DSP-F, Wyatt Technology, Santa Barbara, CA) connected in series to a Waters 410 refractive index detector. The aqueous chromatographic system also included a Waters 486 tunable UV/vis absorbance detector set at 254 nm. Astra 2.1 data capture and processing software (Wyatt Technology, Santa Barbara, CA) was used to calculate molecular weight averages based on MALLS detection data.

PDCP samples were prepared at a concentration of 0.1% w/v in an Inert Atmosphere Box (Vacuum/Atmospheres Co., Hawthorne, CA). The samples were further protected from ambient air by the use of Mininert Valves (Supelco, Bellafonte, PA) and a 100 μL Hamilton SampleLock syringe (Hamilton, Reno, NV) for injection. The concentration of the stock solution was measured by ^{31}P NMR instrument. The refractive index increment (dn/dc) of PDCP was determined by injecting known concentrations of PDCP and using Astra 2.1 software assuming 100% mass recovery and known RI calibration constant. The refractive index increment was determined to be 0.106 mL/g. ^{31}P and ^{1}H NMR spectra were recorded using a Bruker AM 360 NMR with an Oxford magnet operated at 145 and 360 MHz, respectively.

Polymerization. Polymerization was conducted in 1 L titanium pressure reactor equipped with heater, thermocouple, pressure gauge, stirrer, and high-torque magnetic drive (Parr Instrument Co., Moline, IL). Temperature, stirring speed, and the electrical current drawn by the stirrer motor were monitored using a model 4841 proportional controller (Parr Instrument Co., Moline, IL). The reactor was loaded with 300 g of purified hexachlorocyclotriphosphazene, evacuated under vacuum for 1 h (5 mmHg), pressurized with anhydrous nitrogen (30 psi), and heated to 235 °C. Polymerization was monitored by changes in the electrical current drawn by the motor, which at a constant stirring rate should correlate to the viscosity of the reaction mixture. The reaction was continued until the desired reaction time/viscosity was achieved. The polymerization reaction was then stopped by cooling the reactor to 120 °C. 300 mL of diglyme was then transferred to the reactor under anhydrous conditions to prevent solidification of the reaction mixture. Polydichlorophosphazene (PDCP) solution in diglyme was separated from the unreacted and undissolved trimer by decantation under anhydrous conditions. Concentration of PDCP in solution and degree of conversion were determined by ^{31}P NMR in a mixture diglyme/deuterated chloroform (1:3) using trimethyl phosphate as an internal reference.

Polymer Derivatization. PDCP was derivatized to the stable poly(organophosphazene), PCPP, for further analysis. A typical procedure used for the derivatization of PDCP is described below. A reagent mixture containing sodium propyl paraben and propyl paraben (molar ratio 1:1) was prepared in the presence of diglyme. 85 g of propyl paraben was mixed with 19.7 g of diglyme, and the mixture was heated with constant stirring until melted. 96 g of sodium propyl paraben was then added to the melt, and the heating was continued to dissolve sodium propyl paraben. The obtained solution was then diluted with 190 mL of diglyme and added to the three-neck reaction flask charged with 130 g of polydichlorophosphazene solution in diglyme (0.2 mol/L) while stirring. The reaction mixture was refluxed for 8 h under nitrogen and then cooled to 95 °C. 100 g of KOH (16 N aqueous solution) was slowly added with vigorous stirring to the reaction mixture to bring about the hydrolysis and subsequent precipitation of PCPP. 20 mL of water was then added to facilitate a good phase separation. The liquid organic layer was decanted, and the precipitate was dissolved in 300 mL of 15% aqueous sodium chloride solution and then reprecipitated by addition of 600 mL of deionized water. The aqueous layer was decanted; the precipitate redissolved in 150 mL of deionized water and finally precipitated by addition of 150 mL of ethanol. The PCPP precipitate was filtered and dried. Polymer structure was verified by ^{31}P NMR and ^{1}H NMR; the molecular weight was determined by GPC−MALLS as described below.

Results and Discussion

Design of the Polymerization System. Ring-opening polymerization (Scheme 1) of hexachlorocyclotriphosphazene (trimer) can be affected by multiple factors, such as presence of water and acidic contaminants in the system, purity of trimer, insufficient agitation, and even catalytic activity by the glass wall of the polymerization tube.[9] Thus, every effort was made to minimize the impact of such factors and to design the appropriate polymerization device to provide a much needed accurate and quantitative description of the system.[9]

To minimize the level of potential impurities, such as hydroxyl-containing trimer species, hexachlorocyclotriphosphazene was purified by distillation under nitrogen to produce "polymerization grade" material. The purity of hexachlorocyclotriphosphazene was verified by ^{31}P NMR (single peak at 20.6 ppm) and GC measurements (single peak).

The polymerization reaction was conducted in a highly inert titanium pressure reactor. The selection of such a system was supported by the following considerations: (a) titanium is chemically inert, ideally suited for reactions in acidic environment and eliminates catalytic effect of glass or impurities generated as a result of steel corrosion; (b) the amounts of water, hydrochloric acid, and other volatile impurities are controlled by high-temperature purging and solvent transfer, and high pressure and vacuum techniques are used to minimize the level of impurities; (c) the polymerization progress is continuously monitored by measuring the electric current drawn by the stirring motor, which at a constant stirring rate corresponds to the

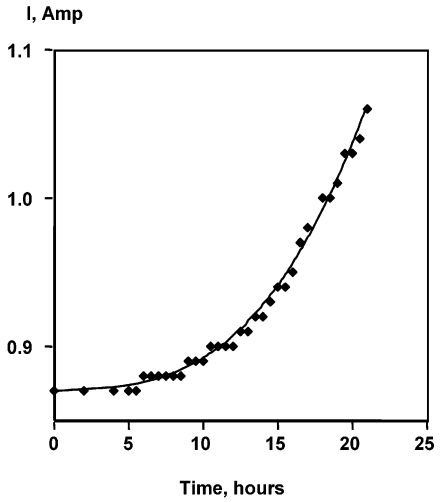


Figure 1. Typical kinetic curve for the polymerization of hexachlorocyclotriphosphazene as monitored by changes in the electrical current drawn by the stirrer motor. Changes in the current (*I*) at a constant stirring rate correspond to changes in the viscosity of polymerization mixture (235 °C, nitrogen, 35 psi).

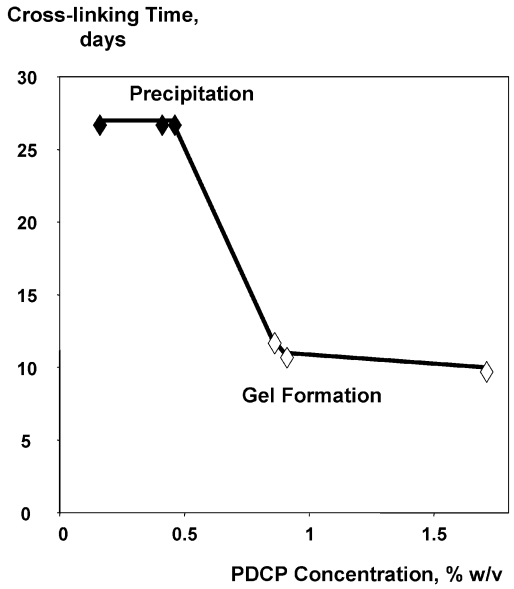


Figure 2. Cross-linking of PDCP in anhydrous THF. Time of visual gel formation or precipitation vs polymer concentration (20 °C).

changes in the viscosity of the system; (d) the reaction mixture is effectively stirred at all stages of the process to minimize inhomogeneity and local overheating.

A typical polymerization process included melting of trimer in the reactor under a nitrogen blanket, purging the reaction content with anhydrous nitrogen to remove traces of moisture or hydrochloric acid, and performing the reaction at 235 °C with the intensive stirring. After the completion of the reaction, solvent was added to the reaction mixture to prevent the solidification or heterogeneous system formation during the cooling stage. No formation of gel or particulates was observed in the reaction mixture under the conditions used in the current study.

Polymerization Kinetics. Polymerization was monitored by measuring changes in the electrical current drawn by the stirring motor, which at a constant stirring rate correlates to the viscosity of the reaction mixture. A typical kinetic curve for the polymerization reaction is presented in Figure 1. As seen from the figure, the reaction is characterized by two main stages. Initially, in the first 7−10 h, no significant change in the viscosity of the reaction mixture was noticed. Following this initial period, a gradual increase in the viscosity of the polymerization mixture was observed with time. No abrupt changes in the viscosity profiles were detected, which is consistent with the fact that we have not observed any gelation at these stages of polymerization. More detailed discussion of the amperage−conversion−molecular weight characteristics is given below. It is noteworthy that the kinetic profiles were similar for the polymerization reactions conducted in the reactors of different sizes and at various reaction scales. 1 L, 2 L, and 5 gal reactors were used with the reaction scale ranging from 200 g to 20 kg hexachlorocyclotriphosphazene loading.

Stabilization of PDCP Solutions. The key feature of polyphosphazene chemistry—high reactivity of PDCP toward nucleophilic compounds—allows versatile polymer derivatization. To date, hundreds of polyphosphazene structures have been synthesized via the macromolecular substitution route. However, this unique advantage of polyphosphazene chemistry also presents one of its main challenges. PDCP reactions with water lead to the formation of cross-linked material or polymer degradation. Both of these processes result either in products not useful for further substitution or difficulties with molecular weight control and reproducibility.

The PDCP cross-linking process was investigated in the anhydrous aprotic solvents in the sealed glass flask under nitrogen. Figure 2 shows that storage of sealed PDCP solutions even in THF with water content of 0.0009% (Karl Fisher titration) resulted in polymer cross-linking within days. The process of polymer hydrolysis manifested itself in polymer precipitation or gelation depending on its concentration (Figure 2). The precipitate or gel material collected in our experiments was not soluble in any solvents, indicating the existence of covalent cross-linking. As expected, the border between these two physical states (gel−precipitate), which can be clearly detected in Figure 2 by an abrupt change in the cross-linking time, is in the vicinity of the overlap concentration for this polymer, 0.6%.

PDCP cross-linking has been reported previously for other solvents, such as benzene, toluene, chlorobenzene, monoglyme (1,2-dimethoxyethane), and acetone, and attempts to exclude water from the system did not retard the cross-linking process appreciably.[9,15−17] Methods for PDCP stabilization described in the patent literature are typically limited to the use of chloro-substituted compounds, such as stannic chloride, titanium chloride, etc.[17] However, high reactivity of these compounds can potentially cause side reactions, contamination of the final product, undesired polymer modification, and interference with analytical methods as well as environmental concerns.

We found that diglyme has a remarkable stabilizing effect on the PDCP behavior in solution. Figure 3 shows that the addition of diglyme to the THF solution gradually increased the stability of PDCP. In pure diglyme there were no signs of cross-linking detected over a period of more than 4 years. GPC analysis of PDCP and its derivatization into poly[di(carboxylatophenoxy)phosphazene] did not show any indication of cross-linking or degradation even after 4 years of storage. This phenomenon cannot be correlated to the

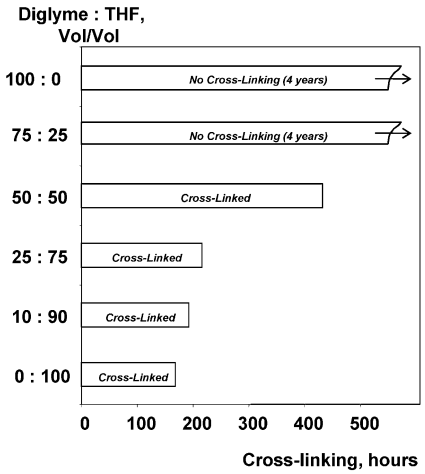


Figure 3. Stabilizing effect of diethylene glycol dimethyl ether (diglyme) on PDCP solution in THF. Time of cross-linking vs solvent ratio (PDCP concentration: 1.58% w/v; 20 °C).

content of water in the system—the amount of water in diglyme was actually higher than in THF (0.0015% vs 0.0009% based on Karl Fisher titration).

The nature of the stabilizing effect of diglyme on PDCP is not clear. It is not uncommon that solvents with different properties, often loosely defined, may give rise to substantial stability and reactivity changes. These changes are generally due to solute−solvent interactions, which stabilize (or destabilize) the reacting components or intermediates to a different extent. It is worth mentioning that diglyme is considered the shortest homologue of poly(ethylene oxide) (PEO)—a powerful cation coordination molecule.[18] This similarity manifests itself in the tridentate cation coordination and crystallization conformation similar to long-chain PEO, which in turn separates diglyme from other common ether solvents, such as THF or dioxane.[18] This observation can be of critical importance since it was suggested that degradation of polyphosphazene backbone might involve protonation of the nitrogen atoms in the polyphosphazene skeleton with the formation of cationic species.[19,20] Thus, it is possible that the stabilization effect is due to the "inhibition" of such charged groups on the polyphosphazene backbone, which act as intermediates in the hydrolysis process. Alternatively, it is highly probable that the solvation of water molecules by diglyme makes them "unavailable" for the reaction with PDCP. This hypothesis can be supported by the well-known ability of oligo(ethylene oxides) to coordinate water molecules.[21]

The stabilizing effect of diglyme also appears to be of importance also due to this solvent's high polarity and excellent solvation of the alkoxides, including the fluorinated ones.[22] Thus, diglyme can be suggested as a universal solvent in the pathway of PDCP production, storage, and substitution without the need for additional difficult-to-control intermediate purifications of PDPP.

Characterization of PDCP Using MALLS−GPC. Stabilized PDCP−diglyme formulations were used for the direct HPLC analysis of PDCP. Characterization of chloro-substituted polyphosphazene using conventional chromatographic techniques has encountered a number of problems associated with its hydrolytic instability and high reactivity.[12,23−25] Because of the PDCP spontaneous cross-linking in the column and notorious distortions in its HPLC profiles, it is still most common to determine the molecular weight of this polymer by converting

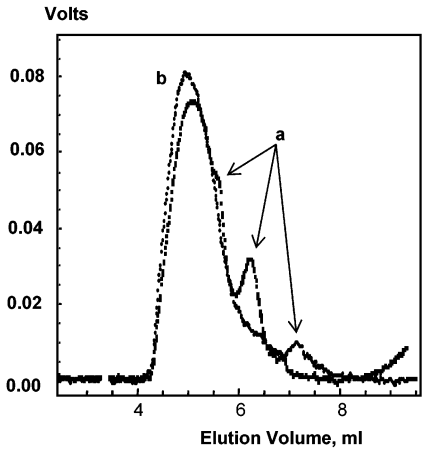


Figure 4. HPLC profiles of poly(dichlorophosphazene), PDCP, before (a) and after (b) column priming with three injections of PDCP (diglyme, 37 °C, Waters Styragel HR 5E column).

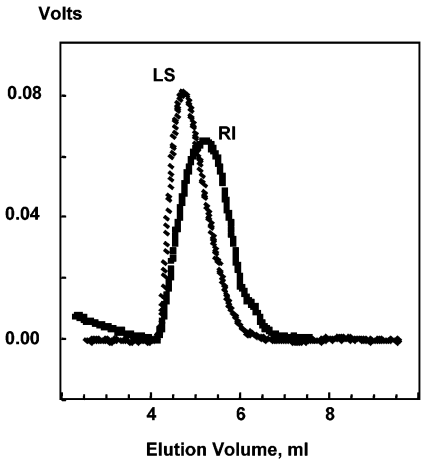


Figure 5. Light scattering (LS) PDCP in organic phase contrasted with RI signal (RI) (aqueous phase: PBS, pH 7.4, Waters Ultrahydrogel column set; organic phase: diglyme, 37 °C, Waters Styragel HR 5E column).

it to a hydrolytically stable poly(organophosphazene).[11,26] The extent to which these results can be used for the characterization of PDCP due to the possibility of scrambling the molecular weight parameters during the nucleophilic substitution reactions still remains a subject of discussion.[25]

Chromatographic characterization of PDCP was conducted in anhydrous diglyme with sample handling performed under anhydrous conditions. Some distortions in light scattering GPC profiles expressed in the form of multiple small peaks on the low molecular weight shoulder of chromatograms were initially observed during the analysis (Figure 4, curve a). It has been found, however, that these were rather nonreproducible distortions that could be completely eliminated by priming columns with 2−3 injections of PDCP samples (Figure 4, curve b). Typical multidetection GPC chromatographs including light scattering (LS) and refractive index (RI) profiles for PDCP are presented in Figure 5. GPC profiles obtained after such treatment of the column were reproducible and steep dependence of molecular weight on the elution volume proved the size-exclusion mechanism of chromatography (Figure 6, curve a).

Molecular Weights of PDCP after Conversion into Stable Derivative, PCPP. The molecular weight of PDCP was also determined indirectly—by transform-

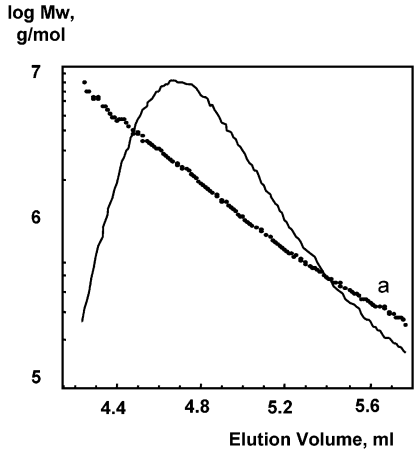


Figure 6. Size-exclusion mechanism of PDCP separation is confirmed by a strong dependence of logarithm of molecular weight on elution volume (a). The LS signal is superimposed (diglyme, 37 °C, Waters Styragel HR 5E column).

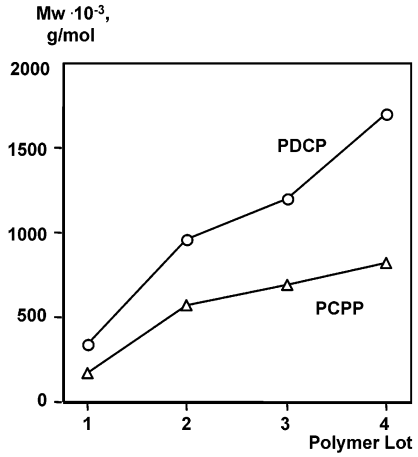


Figure 7. Weight-average molecular weights of four PDCP samples before derivatization and the same samples after their chemical derivatization to PCPP. GPC−light scattering analysis was performed in anhydrous diglyme as a mobile phase for PDCP and in aqueous phosphate buffer, pH 7.4, for PCPP.

ing it into stable organic derivative and performing GPC analysis of such stabilized polymer. PDCP was converted into water-soluble derivative (PCPP) using the reaction with sodium propyl hydroxybenzoate with subsequent hydrolysis under basic conditions. The weight-average and number-average molecular weights of four different samples of PDCP and the same samples after conversion to PCPP were determined by GPC−light scattering in diglyme and phosphate buffer solution (pH 7.4) correspondingly. The results are presented in Figures 7 and 8. The graphs reveal correlation between molecular weight characteristics for PCPP and PDCP for both types of averages. This indicates the absence of experimental factors capable of the random scrambling of the molecular weights during the substitution reaction and validates the results of the direct analysis of PDCP. The method of direct MALLS−GPC characterization of PDCP was further used for the quantitative description of the polymerization system.

Viscosity−Conversion−Molecular Weight Dependence. As described above, a typical curve for the polymerization process (Figure 1) demonstrates a gradual increase in the viscosity of the polymerization mixture with time. The molecular weight parameters and actual yields of PDCP corresponding to the different stages on

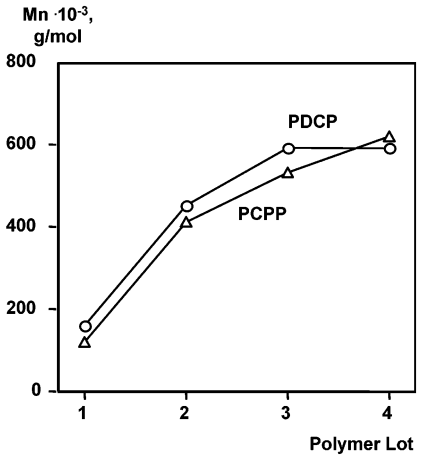


Figure 8. Number-average molecular weights of four PDCP samples before derivatization and the same samples after their chemical derivatization to PCPP. GPC−light scattering analysis was performed in anhydrous diglyme as a mobile phase for PDCP and in aqueous phosphate buffer, pH 7.4, for PCPP.

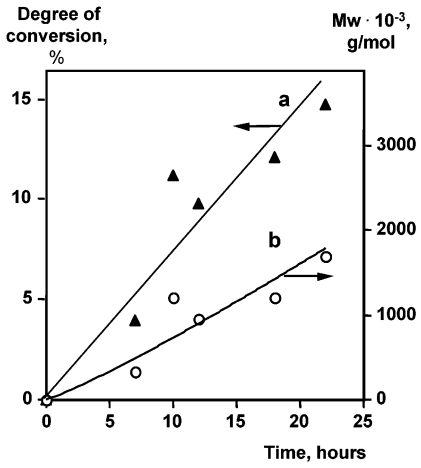


Figure 9. Degree of conversion (a) and molecular weight (b) vs reaction time for the thermal polymerization of hexachlorocyclotriphosphazene (235 °C, nitrogen, 35 psi).

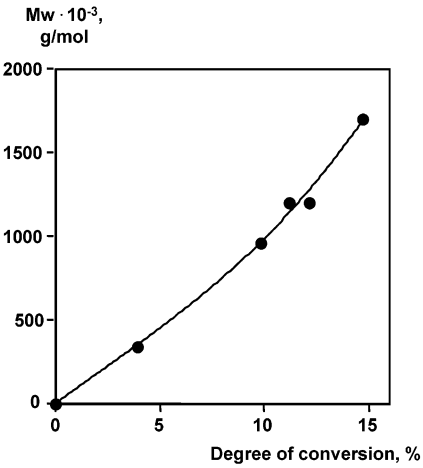


Figure 10. Weight-average molecular weight of poly(dichlorophosphazene) vs degree of conversion of hexachlorocyclotriphosphazene (235 °C, nitrogen, 35 psi).

the kinetic profile were measured in a series of polymerization reactions stopped at various time points. The results of this study are given in Figure 9. As seen from the figure, the increase in viscosity corresponds to the increase in both concentration of polymer (degree of

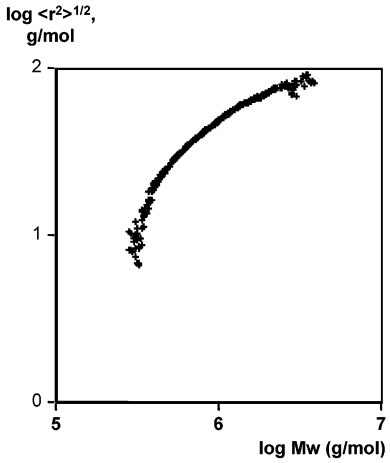


Figure 11. Logarithm of root-mean-square radius $\langle r^2 \rangle^{1/2}$ vs logarithm of molecular weight for the PDCP.

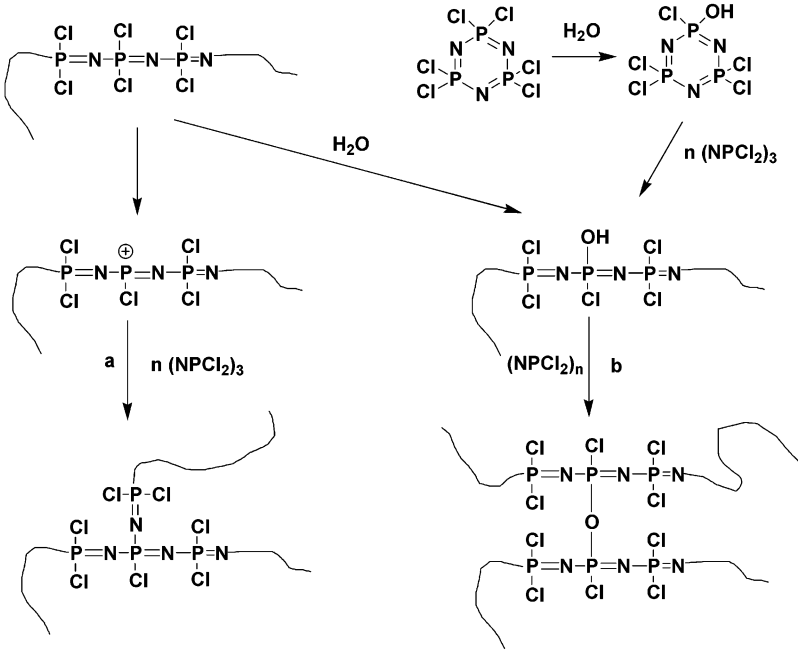
conversion, curve a) and weight-average molecular weight of the polymer (curve b). Figure 10 shows gradual growth in the molecular weight with increase in the degree of conversion. These results, although useful in terms of polymer molecular weight control, are somewhat unexpected on the basis of the previous data obtained for the noncatalytic ring-opening polymerization.[11,25]

Formation of Branched Structures and Mechanism of Cross-Linking. One of the most interesting and practically important characteristics of the ring-opening polymerization is the formation of cross-linked material at the later stages of the reaction. The question remains as to the exact mechanism of such polymer cross-linking. Valuable information can be obtained from the analysis of the reaction mixture for the presence of nonlinear polyphosphazene structures. Light-scattering−HPLC analysis of PDCP solutions showed no evidence of microgel formation in the system. However, conformation plots (logarithmic plots of root mean radius vs molecular weight) showed significant departure of the linearity indicating the presence of branched material (Figure 11). Since branched polymers are typically characterized by the smaller slope in the conformational plot, such deviation in the linearity suggests branching inhomogeneity of the sample.[27] It is seen from the figure that PDCP fractions of higher molecular weight are characterized with a higher degree of branching (smaller slope).[27]

The evidence of branching reactions in the system can be critical for the explanation of the molecular weight increase with conversion described in the previous subchapter. As the concentration of polymer grows, the probability of branching reactions increases, leading to the rise in the molecular weight, and, later, to the formation of completely cross-linked polymer. Previously reported results on the apparent independence of the molecular weights on the conversion[11] might not be in contradiction with our present findings since authors were not reporting the absolute molecular weights, but rather relative values for the derivatized polymers (traditional GPC analysis based on polymer standards calibration).

The presence of branched PDCP molecules suggests the existence of different reaction mechanisms even at the early stages of the polymerization process. A commonly accepted mechanism of ring-opening polymerization involves initiation via ionization of P−Cl bond on hexachlorocyclotriphosphazene to P^+Cl^-, followed by a cationic chain growth process, which results in linear polyphosphazene molecules.[9] Two mechanisms have been proposed for the cross-linking reactions.[9,11,12] The first is similar to the cationic process described above with the chain initiation starting on the PDCP molecule, as shown in Scheme 2, reaction a. The second involves intermolecular condensation reactions between P−Cl and P−OH groups through the formation of P−O−P bonds (Scheme 2, reaction b). A P−OH bond required for the last reaction can be produced either through the interaction of water with hexachlorocyclotriphosphazene with subsequent copolymerization of such hydroxyl group containing trimer with regular monomer or through the reaction of PDCP with water in the polymerization reactor. A number of hydrolyzed trimer

Scheme 2. Cross-Linking and Branching Reactions in Polyphosphazene Chemistry



species and isomers have been described previously.[20] In our experiments, neither additional trimer purification nor repeated purging of the molten reaction mixture with anhydrous nitrogen before or during the process of polymerization helped to eliminate or decrease branching of PDCP samples. Thus, the results discussed above provide experimental support for the "polymerization based" mechanism of cross-linking (a).

Conclusions

A synthetic pathway leading to the production of biologically active polyphosphazenes involves preparation of hydrolytically sensitive macromolecular intermediate, PDCP, and requires careful control of the reaction conditions. The polymerization reaction is characterized with the increase in the molecular weight of the synthesized polymer and the existence of branching reactions in the system. The remarkable stabilizing effect of diglyme, resulting in the prevention of PDCP cross-linking is of critical importance both for the polyphosphazene production and for the development of direct methods of PDCP analysis. The PDCP stabilization approach has already been successfully applied to the commercial production of the well-defined biologically active PCPP. Macromolecular transformation of hydrolytically sensitive PDCP to water-soluble biologically active polyphosphazenes will be the subject of our next report.

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