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Synthesis, Spectroscopic Studies and Antimicrobial Activity of Phosphazenes Derivatives

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The monosubstituted, fullysubstituted trimericphosphazenes, poly(dichloro)phosphazenes and poly(diorgano)phosphazene with *para*-substituted anilino side groups have been synthesized. The *p*-substituents is fluorine groups. The poly(diorgano)phosphazene was prepared by two routes. A direct synthetic route to the poly(diorgano)phosphazene was prepared by polycondensation of mono and fullysubstituted monomer. Second route is the substitution of the chlorine atoms in poly(dichloro)-phosphazene by organic groups, giving a poly(diorgano)phosphazene. The monomers and polymers were analyzed by elemental analysis, IR, ^{1}H, ^{13}C and ^{31}P NMR spectra. The number average molecular weight, M_n, mass average molecular weight, M_w and polydispersity index values of compounds **3** and **4** were found to be 722 967 g mol^{-1}, 755 005 g mol^{-1} and 1.044 g mol^{-1} and 557 448 g mol^{-1}, 699 315 g mol^{-1} and 1.25, respectively. TG data was shown to be stable of compound **4** against thermo-oxidative decomposition. The weight loss of compound **4** was found to be 50 % at 500 °C and 34 % at 800 °C, respectively. The antimicrobial activities of the monomers and polymer have been screened *in vitro* against the organisms *Escherichia coli* ATCC 11230, *Staphylo-coccus aureus* ATCC 6538, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* La 2971, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium smegmatis* CCM 2067, *Bacillus cereus* ATCC 7064, *Listeria monocytogenes* ATCC 15313, *Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra* DSM 70403, *Hanseniaspora guilliermondii* DSM 3432 and *Debaryomyces hansenii* DSM 70238. It is observed that the polymer has pronounced effect on the microbial activities of the monomer. The polymer has higher antimicrobial effect than the monomer.

Key Words: Phosphazene, Polyphosphazene, Polycondensation, Spectroscopic studies, Antimicrobial activities.

INTRODUCTION

Polyphosphazenes are exceptional in macromolecular chemistry because of their extremely versatile adaptability for applications. Generally, the structure of a polymer is determined by the monomer and its resulting

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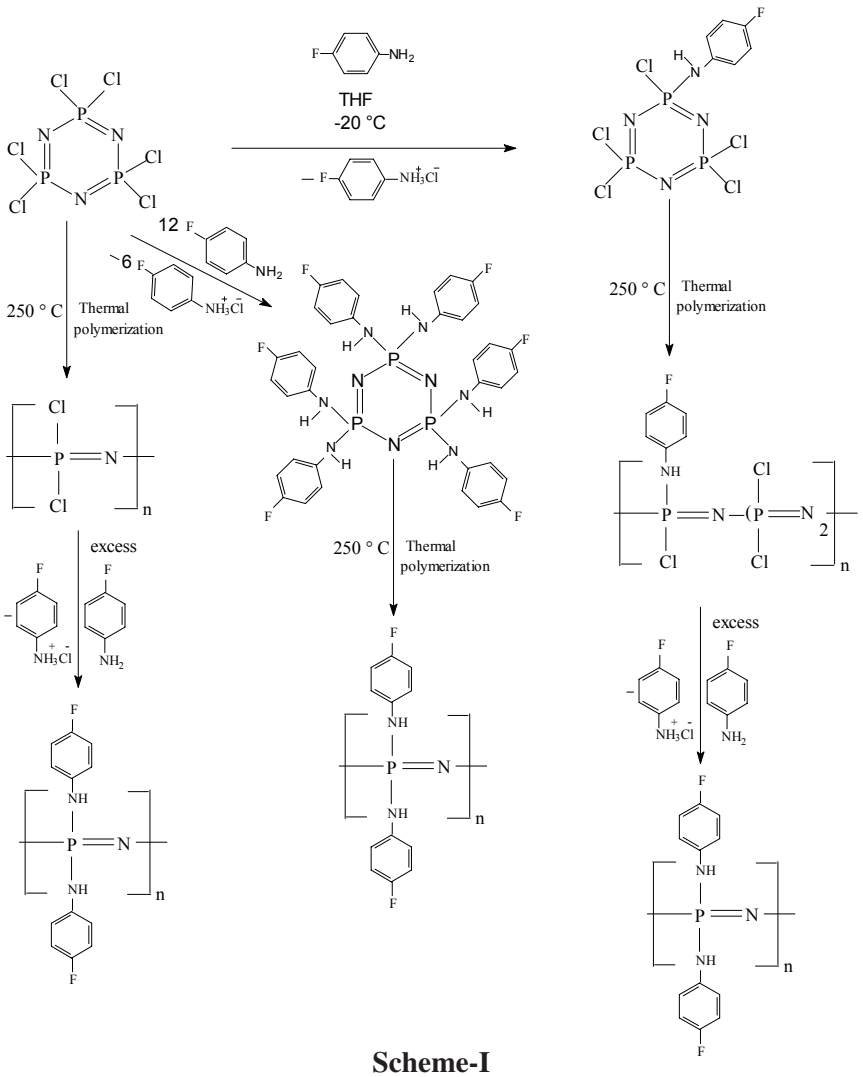
properties tend to be random rather than specifically engineered. Polyphosphazenes do not comply with this rule and from the same precursor an almost unlimited number of specific structures can be selected. Polymerization of hexachlorocyclotriphosphazene to give poly(dichloro)-phosphazene was reported by stokes[1]. However, the polymer was always in the form of an insoluble, crosslinked gum and consequently unsuitable for any applications.Allcock and Kugel[2,3] filed a patent claiming the preparation of a soluble poly(dichloro)phosphazene and the substitution for producing poly(diorgano)phosphazenes which were stable and suitable for applications.

Two general processes are usually exploited for the preparation of poly(dichloro)phosphazene, based on the oldest one ring-opening polymerization of low molecular weight inorganic cyclic compounds hexachlorocyclotriphosphazene or octachlorocyclotetraphosphazene and the new by polycondensation reactions of linear oligomers[4-9]. The new method of poly(dichloro)phosphazene synthesis at ambient temperature involves the initiation of trichlorotrimethylsilylphosphorane with small amounts of PCl_5 in CH_2Cl_2 to give a polymer with narrow polydispersities and molecular weight controlled by altering the ratio of monomer to initiator[4]. Poly-(dichloro)phosphazene can be synthesized by the solution polycondensation reaction of phosphinoyliminotrichloro-phosphorane under an atmospheric pressure of 240-290 °C, with pure $P(O)Cl_3$ elimination[6]. The synthesis, identification and purity degree determination of chloro cyclophosphazenes is very important. These compounds are substrates of the synthesis of poly(diorgano)phosphazenes, the class of polymeric materials having a wide application in many fields of technique and medicine[8,10,11]. Properties of synthesized polymers depend strongly on properties of an initial poly-(dichloro)phosphazene and its syntheses depend on purity and preparation of hexachlorocyclotriphosphazene[12] or other cyclic homologues influence on the yield of obtained soluble poly(dichloro)phosphazene was performed using IR and ^{31}P NMR spectroscopy[13].

In this paper, mono, fully and poly(diorgano)phosphazene compounds were synthesized (**Scheme-I**) and then subjected to in *in vitro* assays of antimicrobial activity. Poly(dichlorophosphazene) was synthesized by the thermal reaction of hexachlorocyclotriphosphazene in the presence of $AlCl_3$ as catalyst and non catalyst. Using the poly(dichlorophosphazene), poly*bis*(4-floroanilino)phosphazene, was prepared. The structure of the monomers and polymer were confirmed using data obtained from element analysis, FT-IR, ^1H NMR, ^{13}C NMR, ^{31}P NMR, TGA, DTA and HPLC techniques.



Scheme-I

EXPERIMENTAL

Hexachlorocyclotriphosphazene was purchased from Aldrich. It was recrystallized from hexane and purified by fractional vacuum sublimation at 55 °C before use. Benzene, tetrahydrofuran and hexane were purchased from Merck and distilled over sodium/benzophenone and stored over molecular sieves. 4-Floroaniline was purchased from Merck and was used as received. Silica gel (Aldrich, 70-230 mesh, 60 Å) was used as received and all reactions were monitered by using Kieselgel 60 F 254 (silica gel) precoated TLC plates. All reactions and manipulations were carried out under an atmosphere of dry argon by using standard Schlenk techniques or an inert atmosphere glovebox.

The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker DPX FT-NMR spectrometer operating at 400, 101.6 and 161.99 MHz, respectively. The ^{1}H and ^{13}C chemical shifts were measured using $SiMe_4$ as an internal standard and the ^{31}P chemical shifts, using 85 % H_3PO_4 as an external standard. Infrared absorption spectra were obtained from a Perkin Elmer BX II spectrometer in KBr discs and were reported in cm^{-1} units. Carbon, nitrogen and hydrogen analyses were performed on a Leco CHNS-932 CHN analyzer. Melting points were measured on a Electro Thermal IA 9100 apparatus using a capillary tube. Thermal data were obtained by using a NETZSCH Simulton Thermal Analyzer STA 409 EP. The number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PDI) were determined by high pressure liquid chromatography (HPLC) of Shimadzu VP-10A. For HPLC investigations were used a SGX (100 Å and 7 mm diameter loading material) 3.3 mm i.d. × 150 mm columns; eluent: DMF (0.2 mL/min), polystyrene standards. A refractometric detector (at 25 °C) was used to analyze the product. The thermogravimetric measurements were made between 20-1200 °C (in air, rate 10 °C/min).

Molecular weight calibration was carried out using mstyragel columns with narrow molecular weight polystyrene standards.

Synthesis of 2-(4-fluoroanilino)-2,4,4,6,6,-pentachlorocyclo-2λ^5,4λ^5,6λ^5-triphosphazene (1): 4-Fluoroaniline (3.19 g, 0.028 mol) in dry THF (50 cm^3) was added dropwise to a stirred solution of hexachlorocyclo-triphosphazene (5.0 g, 0.014 mol) in dry THF (100 cm^3) at -20 °C for over 1 h, with argon being passed over the reaction mixture. After the mixture had been allowed to come to ambient temperature, it was boiled under reflux (4 h) using a condenser fitted with a $CaCl_2$ drying tube. The precipitated amine hydrochlorides were filtered off and the solvent removed by rotary evaporation. The crude product was subjected to column chromatography (silica gel, 120 g, eluent; $CHCl_3$) for separating compound (**1**). Crystallized from CH_2Cl_2/*n*-hexane (3:1), white crystal, m.p. 54 °C, 3.76 g (62 %) yield. Found (%): C, 17.38; H, 1.06; N, 11.13. Calcd. (%) for $C_6H_5N_4P_3Cl_5F$: C, 17.20; H, 1.19; N, 11.47. IR (KBr, cm^{-1}) ν(N-H) 3180 m, ν(C=C) 1509 s, ν(C-N) 1377 m, ν(P=N) 1202 s, ν(P-Cl) 591, 520 s. ^{31}P NMR ($CDCl_3$); δ ppm, 13.62 (t, 1P, PClNH-ArF, $^{2}J_{PNP}$: 48 Hz), 22.59 (d, 2P, PCl_2, $^{2}J_{PNP}$: 48 Hz). ^{1}H NMR ($CDCl_3$); δ ppm, 5.52 (s, 1H, NH), 7.07 (m, 2H, Ar-H), 7.19 (m, 2H, Ar-H). ^{13}C NMR ($CDCl_3$); δ ppm, 116.78 (d, 1C, $^{3}J_{PNC}$: 22.8 Hz), 123.82 (d, 1C, $^{4}J_{PNC}$: 15.2 Hz), 123.9 (s, 1C), 132.69 (s, 1C), 159.35 (d, 1C, $^{2}J_{PNC}$: 2 Hz), 161.78 (d, 1C, $^{5}J_{PNC}$: 2 Hz). MS (highest peak in multiplet, based on Cl^{35}) : m/z; 425 (M + 3H, 25), 422.5 (M^+, 65), 421.7 (M-H, 100), 386 (M-HCl, 35), 312 (M-NHC_6H_4F, 45).

Synthesis of 2,2,4,4,6,6,-hexa(4-fluoroanilino)cyclo-2λ^5,4λ^5,6λ^5-triphosphazene (2): 4-Fluoroaniline (18.65 g, 0.168 mol) in dry THF (50

cm^3) was added dropwise to a stirred solution of hexachlorocyclotriphosphazene (5.0 g, 0.014 mol) in dry THF (150 cm^3) at -20 °C for over 1 h, with argon being passed over the reaction mixture. After the mixture had been allowed to come to ambient temperature, it was boiled under reflux (12 h) using a condenser fitted with a $CaCl_2$ drying tube. The precipitated amine hydrochlorides were filtered off and the solvent removed by rotary evaporation. The crude product was subjected to column chromatography (silica gel, 150 g, eluent; $CHCl_3$) for separating compound (**1**). Crystallized from CH_2Cl_2/*n*-hexane (3:1), white solid, m.p. 122 °C, 7.45 g (65 %) yield. Found (%): C, 53.14; H, 3.85; N, 14.96. Calcd. (%) for $C_{36}H_{30}N_9P_3F_6$: C, 54.33; H, 3.77; N, 15.84. IR (KBr, cm^{-1}) ν(N-H) 3218 m, ν(C=C) 1510 s, ν(C-N) 1382 m, ν(P=N) 1213 s. ^{31}P NMR ($CDCl_3$); δ ppm, 13.62 (t, 1P, PClNH-ArF, $^2J_{PNP}$: 48 Hz), 22.59 (d, 2P, PCl_2, $^2J_{PNP}$: 48 Hz). ^{1}H NMR ($CDCl_3$); δ ppm, 5.40 (d, 1H, NH, $^2J_{PNH}$: 20 Hz), 6.93 (m, 2H, Ar-H), 7.09(m, 2H, Ar-H). ^{13}C NMR ($CDCl_3$); δ ppm, 115.10 (s, 1C), 115.33 (s, 1C), 122.20 (d, 1C, $^3J_{PNC}$: 22.8 Hz), 131.77 (d, 1C, $^4J_{PNC}$: 2.8 Hz), 157.65 (d, 1C, $^2J_{PNC}$: 9.6 Hz), 160.78 (d, 1C, $^5J_{PNC}$: 10 Hz). MS (highest peak in multiplet, based on Cl^{35}): m/z; 795 (M + 3H, 25), 422.5 (M^+, 65), 421.7 (M-H, 100), 386 (M-HCl, 35), 312 (M-NHC_6H_4F, 45).

Synthesis of poly(dichloro)phosphazene (3)

Method I: Purified hexachlorocyclotriphosphazene (2.0 g, 0.005 mol) and $AlCl_3$ (0.40 g, 0.006 mol) as catalyst were placed in a Pyrex ampoule in an argon-filled dry box. The ampoule was then evacuated for 0.5 h at a pressure of 0.1 mm Hg and then isolated from the vacuum line. The ampoule was sealed and then polymerized at 250 °C during 1-5 h. When the ampoule was cooled to room temperature, the resultant product was a transparent viscous liquid without any solidified material. The ampoule was cut into small pieces in argon filled glove box and dry benzene (100 mL) was added. The polymer (**3**) was isolated from the benzene solution with *n*-hexane. Experimental details are given Table-1. IR (KBr, cm^{-1}) ν(P=N) 1255 s ν(P-Cl) 766 s, 572s.

Method II: Hexachlorocyclotriphosphazene (2.0 g, 0.005 mol) was placed in a Pyrex ampoule in an argon-filled dry box. The ampoule was then evacuated for 0.5 h at a pressure of 0.1 mm Hg and then isolated from the vacuum line. The ampoule was sealed and then polymerized at 250 °C during 1-30 h. When the ampoule was cooled to room temperature, the resultant product was a transparent viscous liquid without any solidified material. The ampoule was cut into small pieces in argon filled glove box and dry benzene (100 mL) was added. The polymer (**3**) was isolated from the benzene solution with *n*-hexane. Experimental details are given Table-1. IR (KBr, cm^{-1}) ν(P=N) 1255 s ν(P-Cl) 766 s, 578 s.

TABLE-1
BULK POLYMERIZATION OF
HEXACHLOROCYCLOTRIPHOSPHAZENE AT 250 ºC

Time (h)	Poly(dichloro)phosphazene			
	Catalyst yield (%)	Soluble polymer yield (%)	Non-catalyst yield (%)	Soluble polymer yield (%)
1	80.2	68.8	2.3	0.8
2	90.4	70.4	4.3	2.1
3	94.1	64.3	8.7	4.8
4	98.2	53.2	10.8	6.3
5	100.0	38.4	13.7	8.1
10	-	-	16.6	11.2
15	-	-	35.7	15.3
20	-	-	63.8	20.2
25	-	-	87.4	27.1
30	-	-	100.0	30.4

Synthesis of poly[*bis*(4-fluoroanilino)phosphazene] (4)

Method I: 4-Fluoroaniline (19.13 g, 0.172 mol) in dry THF (50 cm^3) was added dropwise to a stirred solution of poly(dichloro)phosphazene (5.0 g) in dry THF (150 cm^3) at -20 °C for over 1 h, with argon being passed over the reaction mixture. After the mixture had been allowed to come to ambient temperature, it was boiled under reflux (24 h) using a condenser fitted with a $CaCl_2$ drying tube. The precipitated amine hydrochlorides were filtered off and the solvent removed by rotary evaporation. The product (**4**) was isolated from the water solution with chloroform. Brown solid, m.p. > 300 °C (decomposed), 9.98 g (87 %) yield. IR (KBr, cm^{-1}) ν(N-H) 3366 m, ν(C=C) 1506 s, ν(C-N) 1370 m, ν(P=N) 1209 s.

Method II: Compound **2** [2,2,4,4,6,6,-hexa(4-fluoroanilino)cyclo-2λ^5,4λ^5,6λ^5-triphosphazene] (5.0 g, 0.006 mol) was placed in a Pyrex ampoule in an argon-filled dry box. The ampoule was then evacuated for 0.5 h at a pressure of 0.1 mm Hg and then isolated from the vacuum line. The ampoule was sealed and then polymerized at 250 °C for 30 h. When the ampoule was cooled to room temperature, the resultant product was a transparent viscous liquid without any solidified material. The ampoule was cut into glove box and dry THF (100 mL) was added. The solvent was removed using a rotary evaporator and the solid product (**4**) was dried under vacuum. Brown solid, m.p. > 300 °C (decomposed), 3.5 g (70 %) yield. (KBr, cm^{-1}) ν(N-H) 3366 m, ν(C=C) 1507 s, ν(C-N) 1370 m, ν(P=N) 1208 s.

Method III: Compound **1** [2-(4-fluoroanilino)-2,4,4,6,6,-pentachloro-cyclo-2λ^5,4λ^5,6λ^5-triphosphazene] (5.0 g, 0.006 mol) was placed in a Pyrex ampoule in an argon-filled dry box. The ampule was then evacuated for 0.5 h at a pressure of 0.1 mm Hg and then isolated from the vacuum line. The ampoule was sealed and then polymerized at 250 °C for 30 h. When the ampoule was cooled to room temperature, the resultant product was a transparent viscous liquid without any solidified material. The ampoule was cut into small pieces in argon filled glove box and dry THF (100 mL). Excess 4-fluoroaniline (7.0 g, 0.063 mol) in dry THF (50 cm^3) was added dropwise to a stirred solution of poly[1-(4-fluoroanilino)-1-chloro-2,2-dichloro]-phosphazene at -20 °C for over 1 h, with argon being passed over the reaction mixture. After the mixture had been allowed to come to ambient temperature, it was boiled under reflux (24 h) using a condenser fitted with a $CaCl_2$ drying tube. The precipitated amine hydrochlorides were filtered off and the solvent removed by rotary evaporation. The product (**4**) was isolated from the water solution with chloroform. Brown solid, m.p. > 350 °C, 2.18 g (23 %) yield. IR (KBr, cm^{-1}) ν(N-H) 3364 m, ν(C=C) 1506 s, ν(C-N) 1370 m, ν(P=N) 1209 s.

RESULTS AND DISCUSSION

The infrared vibration bands with the wave numbers of 3180 m, 3218 m and 3366 m due to ν(N-H), 1509 s, 1510 s and 1506 s due to ν(C=C), 1377 m, 1382 m due to ν(C-N) and 1202 s, 1197 s and 1209 cm^{-1} due to ν(P=N) were observed for compounds **1**, **2** and **4**, respectively (Fig. 1). The ν(P=N) and ν(P-Cl) vibration bonds were observed at 1255s and 766 s, 578 s cm^{-1}, respectively for compound **3** (Fig. 2). Absorption bands of hexachlorocyclotriphosphazene P=N bond (1215 cm^{-1}) are close to absorption bands of octachlorocyclotetraphosphazene (1310 cm^{-1}), decachlorocyclopentaphosphazene (1355 cm^{-1}), dodecachlorocyclohexaphosphazene (1325 cm^{-1}), tetradecachlorocycloheptaphosphazene (1310 cm^{-1}), higher cyclic homologues or polymer (1305 cm^{-1}) and acyclicmonophosphazenes (1230-1160 cm^{-1})[13]. The P=N vibration bands of monomer (**2**) observed in a lower frequency such as 23 cm^{-1} than the same band of polymer (**4**). In the poly-(dichloro)phosphazene (**3**), the chlorine substituent caused the intensities of ν(P=N) band increased in comparison with poly[*bis*(4-fluoroanilino)-phosphazene (**4**) which possesses electron withdrawing 4-fluoroanilino group.

The N-H protons are observed δ = 5.52 ppm singlet and δ = 5.40 ppm doublet ($^2J_{PNH}$: 20 Hz) for compounds **1** and **2**. The phenyl protons resonate at δ = 6.93 ppm multiplet, 7.09 multiplet ppm and δ = 7.07 ppm multiplet, 7.19 ppm multiplet, respectively, for compounds **1** and **2**.

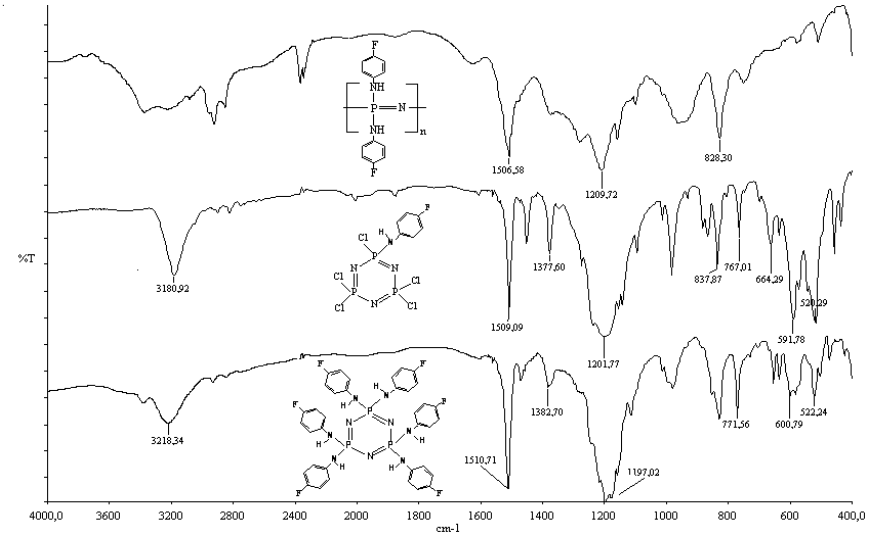


Fig. 1. FT-IR spectra of poly(diorgano)phosphazene, monosubstituted phosphazene and fullysubstituted phosphazene

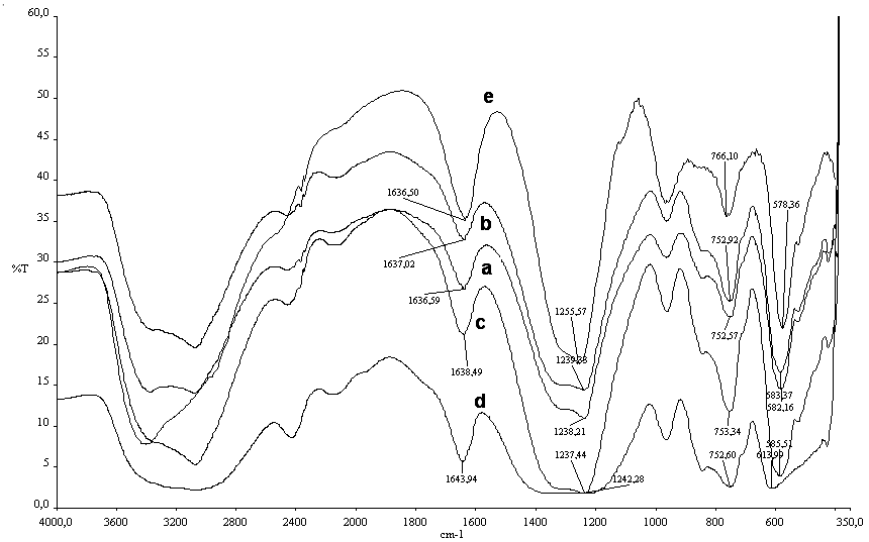


Fig. 2. FT-IR spectra of monomer and poly(dichloro)phosphazenes in mixtures: a: 1 h (80 % of polymer), b: 2 h (90.4 % of polymer), c: 3 h (94.1 % of polymer), d: 4 h (98.2 % of polymer), e: 5 h (100 % of polymer)

According to the ^{13}C NMR spectra compounds (**1**) and (**2**) have 6 signals. ^{13}C NMR data; for compound **1**, 116.78 (d, 1C, $^{3}J_{PNC}$: 22.8 Hz), 123.82 (d, 1C, $^{4}J_{PNC}$: 15.2 Hz), 123.9 (s, 1C), 132.69 (s, 1C), 159.35 (d, 1C, $^{2}J_{PNC}$: 2 Hz), 161.78 (d, 1C, $^{5}J_{PNC}$: 2 Hz) and for compound **2**, 115.10 (s, 1C), 115.33 (s, 1C), 122.20 (d, 1C, $^{3}J_{PNC}$: 22.8 Hz), 131.77 (d, 1C, $^{4}J_{PNC}$: 2.8 Hz), 157.65 (d, 1C, $^{2}J_{PNC}$: 9.6 Hz), 160.78 (d, 1C, $^{5}J_{PNC}$: 10 Hz) have been observed.

The ^{31}P NMR spectra of compounds trimer, monosubstituted trimer (**1**), fully substituted trimer (**2**), poly(dichloro)phosphazene (**3**) and poly-[*bis*(4-floroanilino)phosphazene] (**4**) were interpreted as the result of a simple A_3, AB_2, A_3, A_n and A_n spin system. The ^{31}P NMR spectra showed, that higher cyclic phosphazene homologues were not found in a residue after the polymerization process of trimer (only signals at δ = -5.21 ppm and 6.31 ppm were observed for **3** and **4**) (Fig. 3).

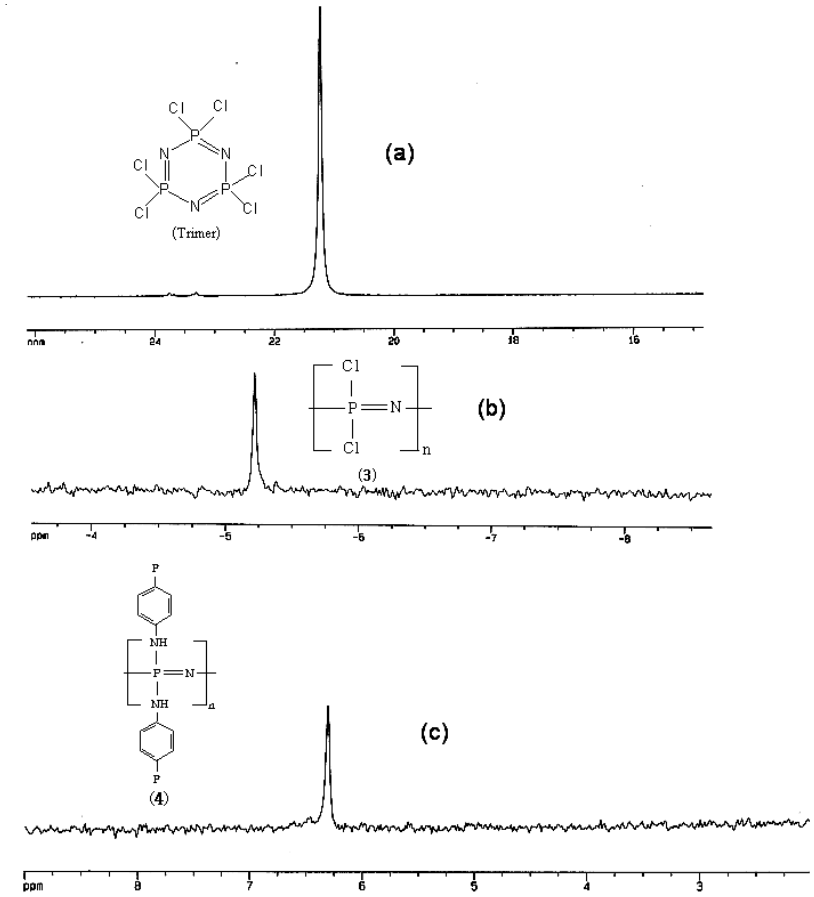


Fig. 3. ^{31}P NMR spectra of monomer (a), poly(dichloro)phosphazene (b) and poly(diorgano)phosphazene (c)

Molecular weight distribution of polyphosphazene] (3 and 4): According to molecular weight distribution, the number average molecular weight (M_n), mass average molecular weight (M_w) and polydispersity index (PDI) values of poly(dichloro)phosphazene (**3**) and poly[*bis*(4-fluoroanilino)phosphazene] (**4**) were found to be 736444 g/mol, 770105 g/mol, 1.060 and 557 448 g/mol, 699 315 g/mol, 1.254, respectively. At the molecular weight distribution of compounds **3** and **4**, one peaks were observed. The compounds **3** and **4** weight was high molecular weight (M_n: 722 967 g mol^{-1}, M_w: 755 005 g mol^{-1} and PDI: 1.044; M_n: 557 448 g mol^{-1}, M_w: 699 315 g mol^{-1} and PDI: 1.25).

Thermal analyses of poly[*bis*(4-fluoroanilino)phosphazene] (4): The TGA and DTA of the prepared poly(diorgano)phosphazene was measured under air atmosphere in the temperature ranges 20-1200 °C, in order to investigate the thermal stability. Fig. 4 shows the TG and DTA traces for the poly[*bis*(4-fluoroanilino)phosphazene] (**4**). According to TG traces, compound **4** was started to degradation at 136 °C. 50 % of mass compound **4** was lost at 136 and 381 °C. The weight loss of the compound **4** was found to be 84 % at 845 °C.

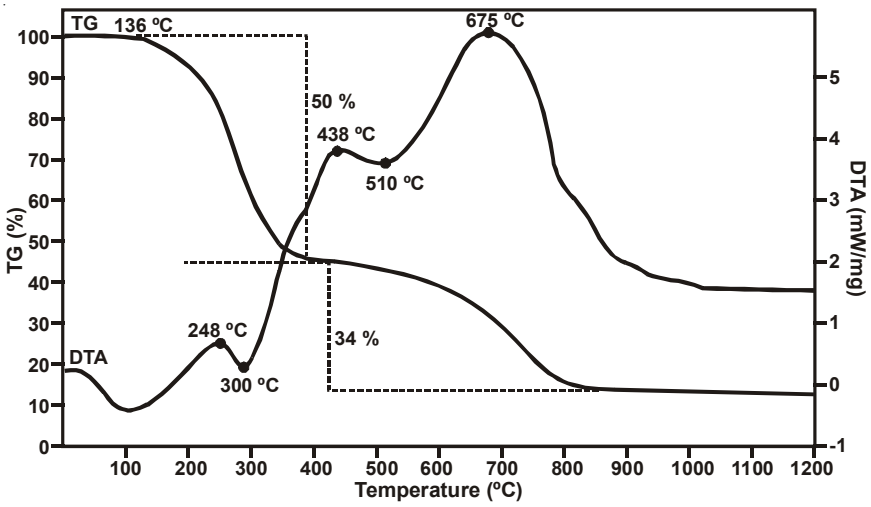


Fig. 4. TG and DTA curve of compound **4**

DTA traces of compound (**4**) showed the phase transition endotherms at 300 and 500 °C. Since, the weight loss was observed of 50 % on the TG curve, these two endothermic reactions were shown any deviation. It was expected that these thermal effects were structural rearrangement reactions. The highly exothermic thermal effects occurring between about 400-450 °C and 650-700 °C are probably due to the oxidation of the polymer. In this

temperature range there is a weight loss of 34 % on TG curve which represents the formation of CO_2, H_2O and NH_3 products. Further decomposition, between 400 and 700 °C, occurs to give a product which is stable up to 1200 °C as appeared on TG-DTA curves. The peaks appearing in the mentioned temperature range have a weight loss was not observed. Thermal decomposition of some groups with the simultaneous oxidation processes are complicated affair in which more then one reaction may occur over this temperature range. Thermal effects occuring between 400 and 700 °C are exothermic and complicated. Fig. 4 indicate that the compound **4** decomposed at high temperatures.

Screening for antimicrobial activities: The bacteria *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* La 2971, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium smegmatis* CCM 2067, *Bacillus cereus* ATCC 7064 and *Listeria monocytogenes* ATCC 15313, the yeast cultures *Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra* DSM 70403, *Hanseniaspora guilliermondii* DSM 3432 and *Debaryomyces hansenii* DSM 70238 were used in this study as the test microorganisms.

The compounds dissolved in DMSO to a final concentration of 30 mg/mL. Empty sterilized tube were each impregnated with 20 µL of solution. All the bacteria mentioned above were incubates at 30 ± 0.1 °C for 24 h by inoculution into Nutrient Broth (Difco) and the yeasts studied were incubated in Malt Extract Broth (Difco) for 48 h. An inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller-Minton Agar (Oxoid) plates (1 mL inoculum / plate). The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35 °C (24 h) for bacteria and at 25 °C (72 h) for yeast. On each plate on appropriate reference antibiotic disc was applied depending on the test microorganisms[16,17]. The data reported in Table-2 are the average data of three experiments.

Table-2 shows antimicrobial activities of of the tested compounds. As can clearly be seen from Table-2, all compound show antibacterial activity against both Gram-positive and Gram-negative bacteria and the yeast cultures in this study. In classifying the antibacterial activity as Gram-positive or Gram-negative, it would generally expected that a much greater number would be active against Gram-positive than Gram-negative bacteria[14]. However, in this study, the compounds were active against both Gram positive and Gram negative bacteria and the yeast cultures.

When the results obtained were compare to those of standard antibiotics. It was determined that compound **4** has the highest antimicrobial effect against all tested bacteria. *Staphylococcus aureus* is more susceptible to

TABLE-2
ANTIMICROBIAL ACTIVITIES OF COMPOUNDS (**1**, **2** AND **4**) AND SOME STANDARD ANTIBIOTICS

Microorganisms	Inhibition zone (mm)											
	Compounds			Antibiotics								
	1	2	4	P10	SAM20	CTX30	VA30	OFX5	TE30	NY100	KETO20	CLT10
Escherichia coli	15	16	19	18	12	10	22	30	28	-	-	-
Staphylococcus aureus	16	18	22	13	16	12	13	24	26	-	-	-
Klebsiella pneumoniae	13	15	17	18	14	13	22	28	30	-	-	-
Pseudomonas aeruginosa	14	15	18	8	10	54	10	44	34	-	-	-
Proteus vulgaris	11	11	13	10	16	18	20	28	26	-	-	-
Bacillus cereus	17	15	20	14	12	14	18	30	25	-	-	-
Mycobacterium smegmatis	12	14	16	15	21	11	20	32	24	-	-	-
Listeria monocytogenes	17	20	18	10	12	16	26	30	28	-	-	-
Micrococcus luteus	15	15	17	36	32	32	34	28	22	-	-	-
Candida albicans	18	16	22	-	-	-	-	-	-	20	21	15
Kluyveromyces fragilis	14	18	22	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	15	18	20	-	-	-	-	-	-	18	22	16
H. guilliermondii	14	17	19	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	14	14	16	-	-	-	-	-	-	16	14	18

Inactive (-), moderately active (8-13); higher active (> 14) Includes diameter of disc (6 mm).

P10: Penicillin G (10 Units), SAM20: Ampicillin 10 µg, CTX30: Cefotaxime 30 µg, V30: Vancomycin 30 µg, OFX 5: Oflaxacin 5 µg, TE30: Tetracyclin 30 µg, N30: Nystatin 100 µg KETO20: etaconazole 20 µg; CLT10: Clotrimazole 10 µg.

the all compounds, as compared to the standard antibiotics except for OFX5 and TE30. Against the acid-fast bacterium *Mycobacterium smegmatis*, all compounds have higher antibacterial effect than that of CTX30. *Micrococcus luteus* is resistant to the all compounds. *Proteus vulgaris* is weak antibacterial effect against the compounds than those of standard antibiotics except for P10. All compounds against *Bacillus cereus, Enterobacter aerogenes* and *Escherichia coli* have shown strong effects. Also, the compounds are influenced the other bacteria in different levels.

In general, all compounds have shown high antiyeast activity against the yeast cultures used in this study. Especially, it is determined that compound **4** is the most effectual compound. *Candida albicans* and *Kluyveromyces fragilis* are more suspectible to the compound **4**, as compared to the standard antibiotics NY100, KETO20 and CLT10. The other yeast cultures are influenced in different levels.

The compounds differ significantly in their activity against tested microorganisms. These differences may be attributed to fact that the cell wall in Gram-positive bacteria of a single layer, whereas the Gram-negative cell wall is multi-layered structure and the yeast cell wall is quite complex[17].

Conclusion

The poly(dichloro)phosphazene is synthesized by the thermal reaction of hexachlorocyclotriphosphazene in the presence of $AlCl_3$ as catalyst and non-catalyst. Poly*bis*(4-fluoroanilino)phosphazene was prepared by the reaction of poly(dichloro)phosphazene with 4-fluoroaniline and their the structures, chemical and physical properties were determined. M_n and M_w values of compounds **3** and **4** synthesized at DMF medium were found to be weight 722 967 g mol^{-1}, 755 005 g mol^{-1} and 557 448 g mol^{-1}, 699 315 g mol^{-1} and PDI:1.044 and 1.25, respectively. According to TG and DTA, the poly*bis*(4-fluoroanilino)phosphazene was resistant enough against thermooxidative degradation. Antimicrobial activity *in vitro* were determined by using cup-plate diffusion method. The compounds demonstrating broad spectrum activities, may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health of infectious diseases.

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