Polymer Vol. 37 No. 14, pp. 3141–3145, 1996

0032-3861/96/$15.00+0.00

Synthesis and evaluation of polyphosphazene derivatives with ω-methylpoly(ethylene oxide) side-groups

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(Received 20 December 1994; revised 3 October 1995)

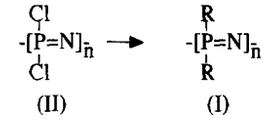
Polyphosphazene derivatives have been prepared which contain monomethyl-poly(ethylene oxide) side-groups in addition to glycine ethyl esters or 2-ethyl-(*O*-glycyl)lactate substituents. Differential scanning calorimetry (d.s.c.) showed that polyphosphazene derivatives with poly(ethylene oxide) side-groups of sufficient length were partially crystalline. The rate of degradation of the polyphosphazene derivatives was investigated by means of h.p.l.c.-analysis. The rate of degradation could be controlled by variation of the amount of poly(ethylene oxide) substituted, or variation of the molecular weight of the poly(ethylene oxide) side-groups. Introduction of a small amount of hydrolizable groups had however a more pronounced effect on the degradation rate. Copyright © 1996 Elsevier Science Ltd.

(Keywords: polyphosphazenes; (bio)degradation; hydrolysis)

INTRODUCTION

Biodegradable polymers show great promise as basic materials for the design of drug delivery system[1–3]. One family of polymers where a large variability in properties is feasible are the polyphosphazenes. Polyphosphazenes (I) are polymers with an inorganic backbone consisting of alternating nitrogen and phosphorous atoms, linked by alternating single and double bonds. Starting from the poly[(dichloro)phosphazene] (II), a variety of polymers with variable properties can be prepared by nucleophilic displacement reactions[4–6] (*Figure 1*).

One important factor for the use of polymers as biomaterials is their biocompatibility and biodegradability. Allcock and coworkers, who extensively explored the field of polyphosphazene synthesis, reported that amino-substituted polyphosphazenes (e.g. R,R′ = ethyl esters of amino acids, imidazol, methylamine) are susceptible towards hydrolytic degradation[7–9] and hold promise as biodegradable materials. Crommen *et al.*[10,11] have already demonstrated that the rate of degradation of polyphosphazene derivatives depends on the nature of the amino acids and that introduction of small amounts of 2-ethyl-(*O*-glycyl)lactate ester cosubstituents resulted in a drastic increase of the rate of degradation.



(II) (I)

Figure 1 Structure of poly[(dichloro)phosphazene] (II) and poly-[(organo)phosphazene] (I)

In this paper we describe the synthesis and evaluation of polyphosphazene derivatives with poly(ethylene oxide) (PEO) side-groups. The introduction of PEO side-groups will influence the hydrophilicity of the final polymers and is anticipated to affect the hydrolytic degradation. The effect of the degree of substitution of the poly(ethylene oxide) side-groups as well as the influence of the molecular weight on the rate of degradation was examined. Finally, a small percentage of hydrolytical labile groups, ethyl-2-(*O*-glycyl)lactate, was introduced to further increase the rate of degradation.

MATERIALS AND METHODS

Poly[(dichloro)phosphazene] $[PNCl_2]_n$ ($M_w = 8.4 \times 10^5$; molecular weight distribution 1.6) was obtained as a cyclohexane solution (12 wt%) from Ethyl Corporation (Baton Rouge, USA) and was used as received.

Ethyl glycinate hydrochloride was obtained from Janssen Chimica (Beerse, Belgium). It was dried before use in a vacuum cabinet over P_2O_5 at 60°C for 6 h.

α-Hydroxy-ω-methylpoly(ethylene oxide), M_W 750 and 5000, was obtained from Fluka (Bornem, Belgium), α-hydroxy-ω-methylpoly(ethylene oxide) M_W 2000 was obtained from Aldrich (Bornem, Belgium). The polyethers were dried before use in a vacuum cabinet over P_2O_5 at 60°C for 6 h.

All organic solvents were obtained from Janssen Chimica. They were dried and distilled prior to use.

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Triethylamine (Janssen Chimica) was purified over tosylchloride, subsequently over ninhydrin and finally distilled from CaH_2.

I.r. spectra were recorded on a Perkin Elmer (1600 Series) *FT* i.r. ^{1}H-n.m.r. spectra and ^{13}C-n.m.r. spectra were recorded on a 360 MHz (Brüker WH-360) n.m.r. apparatus.

Molecular weights were determined by means of gel permeation chromatography (g.p.c.) using a Waters styragel mix column (10 μ), THF containing 0.25 g l^{-1} Bu_4NBr as mobile phase (flow: 1 ml min^{-1}), refractive index detection, and polystyrene standards.

Thermal analysis of the polymers was carried out using a Perkin-Elmer differential scanning calorimeter (DSC-7).

For the determination of the degree of substitution of the ethylglycinate side-groups the method of Goedemoed[13] was used.

Preparation of polymer pellets

Pellets (ϕ = 10 mm, h = 1.5–2 mm) were prepared using a mould which allowed processing of four devices at the same time. 4 × 200 mg of polymer was placed into cavities (d = 1 cm) of the mould preheated at 45°C (Fontijne press, Vlaardigen, Holland).

In vitro *degradation studies*

For each type of polymer, a number of pellets (wt = ca 150 mg, d = 1 cm, h = 1.5–2 mm) were incubated in 20 ml phosphate buffer solution (Sørensen buffer, pH 7.4, containing 0.01% (wt/v) NaN_3) at 37°C. Release of glycine, ethylglycinate and α-amino-ω-methylpoly(ethylene oxide) was determined by means of FmocCl-h.p.l.c. analysis as described before by Crommen[10].

SYNTHESIS OF THE POLYPHOSPHAZENE DERIVATIVES

Synthesis of poly[(monomethylpoly(ethylene oxide))-co-(glycine ethyl ester)]phosphazenes

α-Amino-ω-methyl-PEO was prepared, following the procedure described before by Loccufier *et al.*[13].

As an example is given the preparation of a polyphosphazene containing 10% monomethyl-PEO M_W 750 and 90% glycine ethyl ester side-groups.

To a solution of 0.5 g (4.31 mmol) poly[(dichloro)phosphazene] in 50 ml dry toluene, cooled to 0°C, α-amino-ω-methyl-PEO (0.65 g, 0.86 mmol) dissolved in 30 ml dry toluene and 0.12 ml (0.87 mmol) purified triethylamine was added. Stirring was continued at 0°C for 5 h, then at room temperature for another 15 h. Meanwhile, dried ethylglycinate hydrochloride (3.35 g, 0.024 mol) was transferred into a 100 ml flask containing dry toluene (50 ml) and triethylamine (3.34 ml). The mixture was stirred and refluxed for 4 h. It was allowed to cool to room temperature and filtered. The poly[(chloro)-*co*-(α-amino-ω-methyl-PEO)] solution was cooled to 0°C and the ethylglycinate solution with 1.08 ml (7.76 mmol) triethylamine was added dropwise. The solution was stirred for an additional 5 h at 0°C and at room temperature for 15 h. After removal of the insoluble hydrochloride salts by filtration, the polymer solution was concentrated to 40 ml. Dropwise addition of this concentrate in 300 ml dry pentane yielded a solid polymer. Further purification occurred by reprecipitation from THF in pentane. The polymer was dried under vacuum (yield: 57.5%).

N.m.r. 1: ^{1}H n.m.r. ($CDCl_3$): $[NP(NH(CH_2CH_2{}^1O)_m$-$CH_3^2)_{0.2}(NHCH_2^3COOCH_2^4CH_3^5)_{1.8}]_n$: δ = 1.2 ppm ($3H^5$); δ = 2.2 ppm (NH); δ = 3.3 ppm ($3H^2$); δ = 3.6 ppm ($4H^1$); δ = 3.7 ppm ($2H^3$); δ = 4.1 PPM ($2H^4$).

N.m.r. 2: ^{13}C n.m.r. ($CDCl_3$): $[NP(NH(C^1H_2CH_2O)_m$-$C^2H_3)_{0.2}(NHC^3H_2C^4OOC^5H_2C^6H_3)_{1.8}]_{0.2}$: δ = 14.012 ppm (C^6); δ = 42.902 ppm (C^5); δ = 60.358 ppm (C^3); δ = 70.452 ppm (C^1); δ = 172.506 ppm (C^4); C^2 cannot be observed.

I.r. (film on KBr): cm^{-1}: 3345 (NH); 3000 (CH); 1740 (C=O ester); 1130 (P=N).

Synthesis of poly[ethyl-2-(O-glycyl)lactate)-co-(α-amino-ω-methylpoly(ethylene oxide))-co-(glycine ethyl ester)phosphazenes]

As an example is given the preparation of a polyphosphazene containing 1% of GlylacOEt (ethyl-2-(*O*-glycyl)lactate) and 5% of α-amino-ω-methyl-PEO side-groups. Ethyl-2-(*O*-glycyl)lactate was prepared as described before by Crommen *et al.*[10].

To a suspension of dried ethyl-2-(*O*-glycyl)lactate ammoniumoxalate (0.08 g, 0.34 mmol) in 40 ml anhydrous acetonitrile 0.050 ml (0.36 mmol) triethylamine was added. The mixture was stirred until a clear solution was obtained. To this solution was added 0.050 ml (0.36 mmol) triethylamine and 200 ml dry THF. This mixture was added to a solution of 2 g (0.017 mol) poly[(dichloro)-phosphazene] in 200 ml ice-cooled dry THF. Stirring was continued for 19 h. Then 8.6 g (1.72 mmol) α-amino-ω-methyl-PEO M_W 5000, dissolved in 300 ml dry THF, and 0.24 ml (1.72 mmol) triethylamine was added to the poly[(chloro)-*co*-(ethyl-2-(*O*-glycyl)lactate) phosphazene] solution. Stirring was continued for 3 h at 5°C. The reaction was stirred for an additional 15 h. Meanwhile a solution of 12.4 g (0.089 mol) glycine ethyl ester hydrochloride and 12 ml (0.089 mol) triethylamine was refluxed in 200 ml dry THF for 4 h. After removal of the insoluble triethylammonium salts, the solution was added to the poly[(chloro)-*co*-(ethyl-2-(*O*-glycyl)lactate)-*co*-(α-amino-ω-methyl-PEO) phosphazene] solution. The solution was stirred for 5 h at 5°C and an additional 15 h by room temperature. After removal of the triethylammonium salts by centrifugation, the polymer solution is concentrated by vacuum evaporation at 30–35°C. The polymer was precipitated in pentane and reprecipitation from dry THF into heptane. (yield 87.7%).

N.m.r. 3: ^{1}H n.m.r. ($CDCl_3$): $[NP(NH(CH_2CH_2^1O)_m$ $CH_3^2)_{0.1}(NHCH_2COOCH(CH_3)COOCH_2CH_3)_{0.02}$ (NH $CH_2^3COOCH_2^4CH_3^5)_{1.88}]_n$: δ = 1.22 ppm ($3H^5$); δ = 3.4 ppm ($3H^2$); δ = 3.5–3.8 ppm ($4H^1 + 2H^3$); δ = 4.1 ppm ($2H^4$). Small amounts of GlyLacOEt (<3%) side-groups cannot be detected by n.m.r. spectroscopy. The degree of substitution was determined by h.p.l.c.-analysis.

N.m.r. 4: ^{13}C n.m.r. ($CDCl_3$): $[NP(NH(C^1H_2CH_2O)_m$ $C^2H_3)_{0.1}(NHCH_2COOCH(CH_3)COOCH_2CH_3)_{0.02}$ (NH $C^3H_2C^4OOC^5H_2C^6H_3)_{1.88}]_n$: δ = 13.994 ppm (C^6); δ = 42.826 ppm (C^5); δ = 60.497 ppm (C^3); δ = 70.444 ppm (C^1); δ = 172.518 ppm (C^4); C^2 cannot be observed; no peaks of the GlyLacOEt side-groups can be detected.

I.r. (film on KBr): cm^{-1}: 3362 (NH); 2886 (CH); 1742 (C=O ester); 1205 (C-O-C); 1130 (P=N).

RESULTS AND DISCUSSION

Synthesis of the polyphosphazene derivatives

A number of phosphazene polymers containing monomethyl-PEO and ethylglycinate side-groups were synthesized. In some polymers, small amounts of a hydrolizable group: ethyl-2-(O-glycyl)lactate, were also introduced. The general procedure for the preparation of these polymer derivatives is given in *Figure 2*.

In a first step poly[(dichloro)phosphazene] was allowed to react with a selected amount of ethyl-2-(*O*-glycyl)lactate. Due to the small amounts of ethyl-2-(*O*-glycyl)lactate introduced, the reaction was followed by means of HPLC analysis, to determine the depletion of the depsipeptide from the reaction medium and hence to control the degree of substitution. These results are comparable with the theoretical values as can be seen in *Table 1*.

In a second (or first) step, the α-amino-ω-methyl-PEO was added. Different molecular weights of monomethyl-PEO were used: M_W 750, M_W 2000 and M_W 5000. Finally, remaining chlorides were substituted by subsequent reaction with an excess of ethylglycinate. In this way it was possible to synthesize a range of PEO substituted polyphosphazenes. These variations in side-group ratios alter the various properties of these polymers, particularly the hydrophilic character of the polymers and their degradation characteristics. The structure of the various polyphosphazene derivatives is given in *Table 2*.

Molecular weights of the polyphosphazene derivatives were determined by means of gel permeation chromatography (g.p.c.). THF with 0.25% tetrabutylammonium bromide (Bu_4NBr) was used as solvent. Without the presence of Bu_4NBr no molecular weights could be obtained due to adsorption of the amine substituted derivatives onto the polystyrene phase. Still, in the presence of this salt, it was not always possible to obtain accurate molecular weight data. The molecular weight data obtained are given in *Table 3*.

Comparison of these values with the molecular weight data of the original poly[(dichloro)polymer] indicates that substitution is accompanied by some decrease in chain length.

Incorporation of PEO side-groups can lead to the formation of water soluble polymers. The degree of substitution necessary for the polymers to become water soluble is listed in *Table 4*.

For polymers carrying PEO of M_W 2000 and 5000 water solubility is reached at low *DS*. However, for the polyphosphazene derivatives with PEO M_W 750 about 30% substitution is required to obtain water solubility at room temperature.

Thermal analysis

D.s.c. analysis revealed that most polyphosphazene

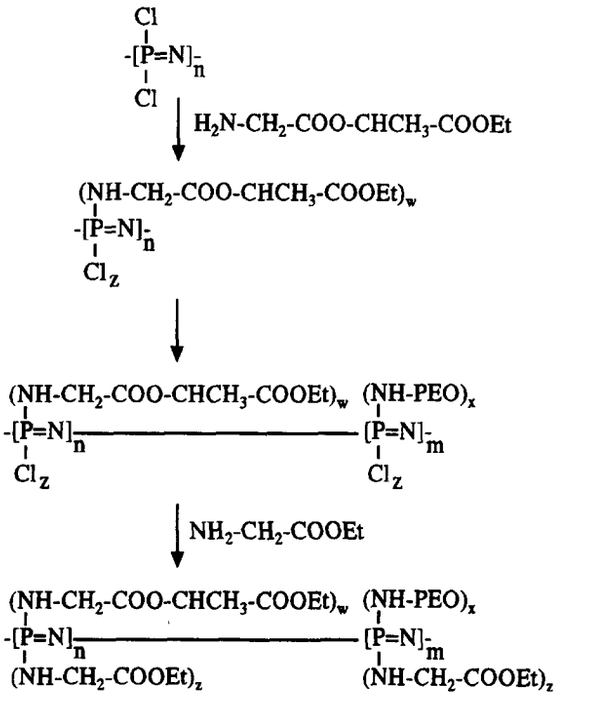

Figure 2 General procedure for the synthesis of poly[(organo)-phosphazenes]

Table 1 Degree of substitution of ethyl-2-(*O*-glycyl)lactate, theoretical and experimental

Degree of subst. (theoretical)	Degree of subst. (experimental)
1%	1%
3%	2.9%

Table 2 Characteristics of the polyphosphazene derivatives

Polymer	M_W PEO	% GlyLacOEt	% PEO	% GlyOEt
(I)	750	0	10	90
(II)			40	60
(III)			60	40
(IV)	2000	0	10	90
(V)			15	85
(VI)	5000	0	5	95
(VII)		1	5	94
(VIII)		3	5	92
(IX)		0	2	98
(X)			4	96
(XI)			6	94
(XII)			8	92
(XIII)			10	90

Table 3 Molecular weight data of poly[(poly(ethylene oxide))-*co*-(amino acid ester)phosphazenes]

Polymer	M_n	M_w	M_w/M_n
$[PNCl]_n$	541.000	621.000	1.15
(IV)	142.000	205.000	1.44
(V)	157.000	224.000	1.43
(VI)	554.000	836.000	1.51
(VII)	105.000	165.000	1.57

Table 4 Minimal degree of substitution required for water solubility

M_W PEO	Degree of subst. required for water solubility
750	30
2000	8
5000	2

Table 5 D.s.c. data of the polymers

Polymer	T_g (°C)	T_m (°C)
(I)	−52.7	6.0
(II)	−40.0	23.5
(III)	−49.5	31.2
(IV)	−19.9	40.0
(V)	−19.8	38.4
(VI)	−22.6	44.7
(VII)	−34.6	45.3
(VIII)	−35.1	45.3

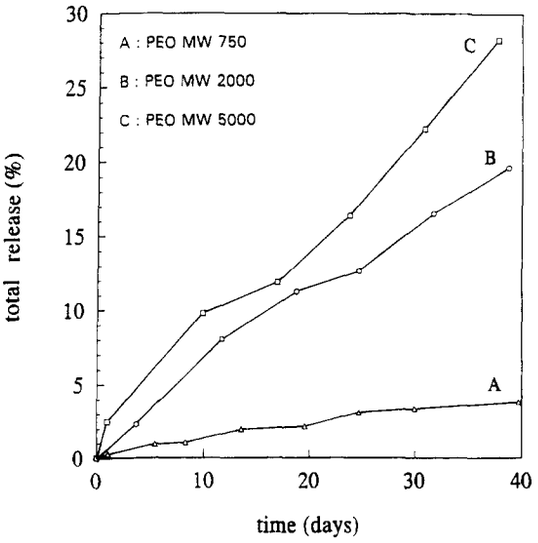

Figure 3 Release of side-groups from polyphosphazene derivatives substituted with poly(ethylene oxide) of different molecular weight

derivatives with PEO side-groups showed crystallinity. D.s.c. results of the polymers are given in *Table 5*.

A major increase in T_g can be observed by introducing a small amount of hydrolizable side-groups. Comparing the polyphosphazene derivatives, containing 10% and 60% PEO of M_W 750, one can see that increasing the amount of PEO has a large influence on the melting point. The influence of the crystallization time and crystallization temperature on the melting point of the polyphosphazene derivatives is minimal. Crystallization occurs almost immediately. This behaviour is typical for PEO.

In vitro *degradation studies*

Pellets (d = 10 mm, h = 1.5 mm) of polymers III–XV prepared by heat compression were immersed in phosphate buffer pH 7.4 at 37°C. The release of side-groups (glycine, ethylglycinate and α-amino-ω-methyl-PEO) was monitored by h.p.l.c. analysis. The influence of the molecular weight of the PEO, introduced on the polyphosphazene chain was examined. Three polyphosphazene derivatives, each substituted for 10% by poly(ethylene oxide) of molecular weight (750, 2000 and 5000) were examined. The degradation profile can be seen in *Figure 3*.

Introducing PEO of molecular weight 750 does not have a large influence on the hydrolytic degradation. However, the release rate did raise from 4% to about 30% after 40 days by introducing PEO side-groups of molecular weight 5000. The polyphosphazene derivative

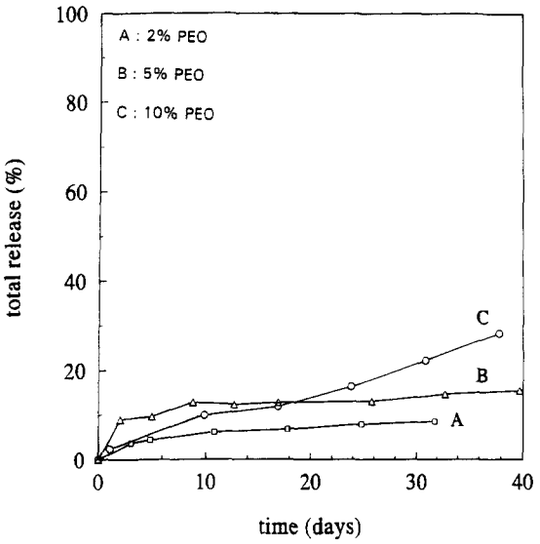

Figure 4 Release of side-groups of polyphosphazenes substituted with different amounts of poly(ethylene oxide) molecular weight 5000

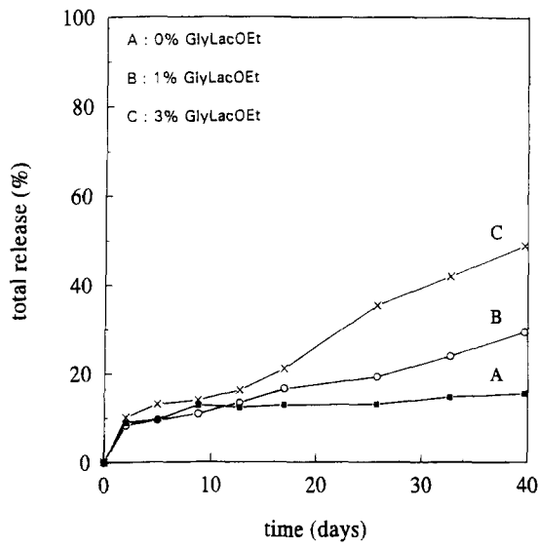

Figure 5 Release of side-groups from polyphosphazenes containing hydrolytic sensitive groups

having PEO side-groups of molecular weight 2000, gives a release pattern, closer to that for the PEO M_W 5000 substituted polymer. 20% of the side groups were released after 40 days.

The influence of different degrees of substitution by PEO side-groups, of the same molecular weight, on the degradation rate was also examined. We can see from *Figure 4* that only in the later stage of the degradation process (after about 15 days), a higher degree of substitution results in an increase of side-group release.

Introduction of a small amount of hydrolytic sensitive groups has a more pronounced effect on the hydrolytical degradation as can be seen in *Figure 5*. The difference in rate of degradation is also more pronounced in the later stage of the degradation.

CONCLUSION

Polyphosphazenes having α-amino-ω-methyl-PEO side-groups, are an interesting class of hydrolytical degradable

polymers. The rate of degradation can be adjusted by the introduction of PEO, or by variation of the M_W of PEO. However, the effect of introducing a small amount of hydrolizable 2-ethyl-(*O*-glycyl)lactate groups has a more pronounced influence on the degradation rate, due to the catalytic effect of the formed carboxylic acid side-groups.

ACKNOWLEDGEMENTS

The authors are grateful to the Belgian Institute for Encouragement of Research in Industry and Agriculture (I.W.O.N.L.) for providing a research fellowship to Vandorpe. They also express their gratitude to Ethyl Corporation (Baton Rouge, USA) for providing the poly[(dichloro)-phosphazene]. This work was supported in part by the European Union, Brite Euram project BRE 0053.

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