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Functionalization of poly(oxyethylene phosphonate) under phase-transfer catalyst conditions

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Abstract

The feasibility of the reaction of poly(oxyethylene phosphonate) **1** with chloroacetone under phase-transfer catalysis conditions for the preparation of poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups in the side chains has been investigated. It has been established that the first stage of the reaction involves addition of the P–H to the carbonyl bond of chloroacetone resulting in poly(oxyethylene α-hydroxy phosphonate). During the second stage of the reaction, the oxirane formation proceeds via elimination of chloride ion. It is possible to direct the reaction to produce poly(oxyethylene phosphonate) **2**, bearing 86.2% α-hydroxyl groups, or to poly(oxyethylene phosphonate) **3**, bearing 91% oxirane groups. The structure of the products has been proved by means of ^{1}H, ^{13}C and ^{31}P NMR spectroscopy. Thus design poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups are of interest as polymer-carriers of drugs and polymers with their own bioactivity. The cytotoxicity of poly(oxyethylene phosphonate), poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups has been studied.

Keywords: Poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups; Phase-transfer catalysis; Cytotoxicity

1. Introduction

The macromolecular approach to improve some characteristics of widely used low-molecular mass bioactive compounds enables the formation of unique types of therapeutics. The polymeric drugs are macromolecules that contain a drug unit attached to the backbone chain or that exhibit drug action in the absence of such unit. The polymeric drugs present a new class of drugs with promising advantages such as greater effectiveness, lowered toxicity, higher resistance and controlled release compared with low-molecular mass drugs [1–5]. One of the major challenges in polymeric drugs is the possibility of targeting the delivery of a therapeutic agent to a specific disease site or organ in the body. Poly(oxyethylene phosphonate)s are a new class of biodegradable, biocompatible [6], water soluble polymers with low toxicity (IC_{50} 2.96 mg/ml) synthesized by transesterification of commercially available H-phosphonate diesters with diols [7,8] or poly(ethylene glycol)s [9–11], or by polymerization of 2,2-dihydro-2-oxa-1,3-2-dioxaphosphorinane [12]. These new polymers have been used as carriers for biologically active substances [13–17].

Polymers bearing hydroxyl group in their side chains have been widely applied as functional polymers such as biomedical materials and surfactants [18,19], since the hydroxyl group makes the polymers reactive, hydrophilic, and biocompatible [20]. Polymer materials bearing oxirane moieties are used for conjugation of bioactive substances due to high reactivity of oxirane group towards the amino function [21].

Presently, to our knowledge, there are no communications describing the synthesis of poly(oxyethylene phosphonate)s bearing α-hydroxyl or oxirane groups. Known data about the synthesis of low molecular mass analogues [22] report a reaction yielding epoxyphosphonate.

The aim of our work is to develop a method for the introduction of α-hydroxyl or oxirane groups into the macromolecule of poly(oxyethylene phosphonate)s, employing the reaction of poly(oxyethylene phosphonate)s

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1 with chloroacetone performed under phase-transfer catalysis conditions.

2. Experimental

2.1. Materials and measurements

Poly(ethylene glycol) (PEG 600), was dried at 120 °C under vacuum (0.2–0.3 mm Hg) for 8 h while simultaneously bubbling a stream of dry argon through the heated liquid. Dimethyl hydrogen phosphonate and cholsoroacetone were distilled prior to use. Dichloromethane and dichloroethane, were dried with P_2O_5 and distilled prior to use. Diethyl ether dried with $MgSO_4$ and distilled prior to use. K_2CO_3, was dried at 150 °C for 8 h. Tetramethylammonium chloride (TMACl), tetraethylammonium chloride (TEACl), tetrabutylammonium chloride (TBACl) were used as supplied. All reagents were supplied by Fluka.

^1H, ^{13}C and ^{31}P NMR spectra were measured on a Bruker 250 MHz instrument in $CDCl_3$ using tetramethylsilane as an internal standard.

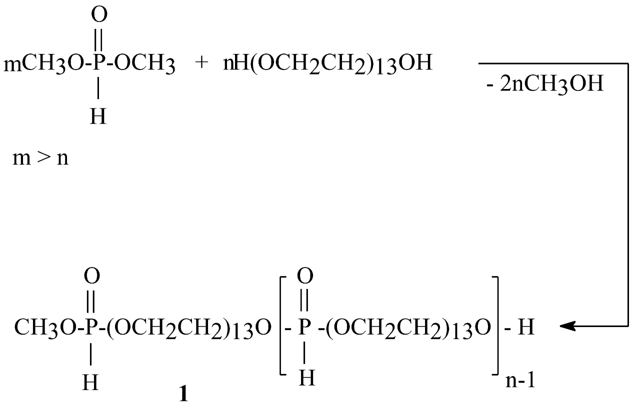
2.2. Synthesis of poly(oxyethylene phosphonate)s

Poly(oxyethylene phosphonate)s **1** were synthesized as described in Ref. [15] (Scheme 1).

NMR ^1H ($CDCl_3$): δ (ppm) = 3.59–3.68 (m, 5H, $OCH_2CH_2 + CH_3O$); 4.09–4.16 (m, 4H, $POCH_2CH_2$); 6.95 (d, $^1J_{(PH)} = 716.6$ Hz, 1H, P*H*); 6.87 (d, $^1J_{(PH)} = 709.1$ Hz, 1H, P*H*).

NMR $^{13}C\{H\}$ ($CDCl_3$): δ (ppm) = 65.50 (d, $^2J_{(PC)} = 6.5$ Hz, $POCH_2$); 69.88 (d, $^3J_{(PC)} = 5.8$ Hz, $POCH_2CH_2$); 70.22 (OCH_2CH_2).

NMR ^{31}P ($CDCl_3$): δ (ppm) = 10.46 (dquintet, $^1J_{(PH)} = 716.2$ Hz, $^3J_{(PH)} = 9.9$ Hz); 11.17 (dsextet, $^1J_{(PH)} = 709.7$ Hz, $^3J_{(PH)} = 10.5$ Hz).



1a n = 13; Mn = 8400
1b n = 10; Mn = 6400

Scheme 1.

The number average molecular weights ($\bar{M}_n$) of the synthesized poly(oxyethylene phosphonate)s were determined by the ratio of the integral intensities of P–H protons in the repeating units towards that of the end groups. The spectral data and the estimated values for the number average molecular weights of two example polymers **1a** and **b** are listed in Table 1.

Poly(oxyethylene phosphonate)s prepared by us gave polydispersities in the range of 1.15–1.20 [17].

2.3. Synthesis of poly(oxyethylene phosphonate) bearing α-hydroxyl groups

Poly(oxyethylene phosphonate) **1a** (6.46 g, 1×10^{-2} mol) and CH_2Cl_2 (10 ml) were added in a two-necked round-bottom flask equipped with a magnetic stirrer, reflux condenser, thermometer and dropping funnel. The mixture was stirred at room temperature for 15 min, after which K_2CO_3 (2.07 g, 1.5×10^{-2} mol) and TMACl (0.01 g, 1×10^{-4} mol) were added. At 30 °C and vigorous stirring chloroacetone (0.93 g, 1×10^{-2} mol) was added dropwise. The reaction mixture was stirred for 1 h at 30 °C and then refluxed for another hour. The mixture was filtered and the filtrate was poured in diethyl ether. The isolated polymer was dried at 40 °C under vacuum (15 mm Hg). The product **2a** (7.1 g) is oil. Products **2b** and **c** were obtained using TEACl and TBACl as catalysts (Table 2).

2.4. Synthesis of poly(oxyethylene phosphonate) bearing oxirane groups

Poly(oxyethylene phosphonate) **1b** (6.46 g, 1×10^{-2} mol) and $ClCH_2CH_2Cl$ (10 ml) were added in a two-necked round-bottom flask equipped with a magnetic stirrer, reflux condenser, thermometer and dropping funnel. The mixture was stirred at room temperature for 15 min, after which K_2CO_3 (2.07 g, 1.5×10^{-2} mol) and TMACl (0.08 g, 7×10^{-4} mol) were added. At 30 °C and vigorous stirring chloroacetone (0.93 g, 1×10^{-2} mol) was added dropwise. The reaction mixture was stirred for 1 h at 30 °C and then for another hour at 55–60 °C. The mixture was filtered and the filtrate was poured in diethyl ether. The isolated polymer was dried at 40 °C under the vacuum (15 mm Hg). The product **3a** is oil. Yield 7.0 g. Oxirane groups, 91.0%; PH-phosphonate groups, 4.0%; α-hydroxyl groups, 5.0%.

Products **3b,c** were obtained using the same procedure but decreasing the TMACl amount to 3×10^{-4} and 2×10^{-4} mol, respectively (Fig. 3).

Poly(oxyethylene phosphonate) **1b** (6.40 g, 1×10^{-2} mol), $ClCH_2CH_2Cl$ (10 ml), K_2CO_3 (2.03 g, 1.5×10^{-2} mol), TBACl (0.28 g, 1×10^{-3} mol) and chloroacetone (0.93 g, 1×10^{-2} mol) reacted under the same conditions. The product **3d** is oil. Yield 6.87 g. Oxirane groups, 91.0%; PH-phosphonate groups, 4.0%; α-hydroxyl groups, 4.0%; phosphate structure < 1.0%.

Table 1
Number-average molecular weights ($\bar{M}_n$) of poly(oxyethylene phosphonate)s (POEP) **1a** and **b**

POEP	^{1}H NMR δ (ppm) P–H protons		Integral intensity of P–H protons		$n = \text{I/II}$	$\bar{M}_n$
	In repeating unit	In end unit	In repeating unit (I)	In end unit (II)		
1a	6.95	6.87	2.01	0.152	13.29	8400
1b	6.96	6.85	2.00	0.195	10.25	6400

2.5. NMR data for **2a** and **3a**

^{1}H NMR (CDCl$_3$): δ (ppm) = 1.23 (d, $^3J_{(PH)} = 15.4$ Hz, 3H, CH_3); 3.10–3.50 (m, 4H, OCH_2CH$_2$ + CH_2Cl); 3.80–4.10 (m, 4H, POCH_2CH$_2$); 4.50 (s, 1H, OH); ^{13}C {H} NMR (CDCl$_3$): δ (ppm) = 19.66 (CH$_3$); 49.43 (d, $^2J_{(PC)} = 18.4$ Hz, CH$_2$Cl); 65.15 (dd, $^2J_{(PC)} = 5.4$ Hz, POCH$_2$); 69.43 (d, $^3J_{(PC)} = 5.8$ Hz, POCH$_2$$CH_2$); 70.10 (O$CH_2CH_2$); 71.43 (d, $^1J_{(PC)} = 177.7$ Hz, P–C).

^{31}P NMR (CDCl$_3$): δ (ppm) = 24.82 (quintet, $^3J_{(PH)} = 7.1$ Hz).

These NMR data are characteristic for the repeating unit bearing α-hydroxyl group. The content of the repeating units is: in **2a**, 86.2%; in **3a**, 5.0%.

^{1}H NMR (CDCl$_3$): δ (ppm) = 1.41 (d, $^3J_{(PH)} = 11.5$ Hz, 3H, CH_3); 2.61 (t, $^3J_{(PH)} + {}^1J_{(PH)} = 5.1$ Hz, 1H, CH_2,); 3.05 (t, $^3J_{(PH)} + {}^1J_{(PH)} = 5.2$ Hz, 1H, CH_2); 3.59–3.68 (m, 4H, OCH_2CH$_2$); 4.09–4.16 (m, 4H, POCH_2).

^{13}C{H} NMR (CDCl$_3$): δ (ppm) = 17.23 (d, $^2J_{(PC)} = 15.2$ Hz, CH$_3$); 51.09 (d, $^1J_{(PC)} = 203.4$ Hz, P–C); 51.31 (CH$_2$); 65.45 (d, $^2J_{(PC)} = 6.5$ Hz, POCH$_2$CH$_2$); 69.88 (d, $^3J_{(PC)} = 5.8$ Hz, POCH$_2$$CH_2$); 70.22 (O$CH_2CH_2$).

^{31}P{H} NMR (CDCl$_3$): δ (ppm) = 22.89.

These NMR data are characteristic for the repeating unit bearing oxirane group. The content of the repeating units is: in **2a**, 3.4%; in **3a**, 91%.

2.6. Cytotoxicity assay

In each experiment 1×10^4 cells per well were seeded in 96-well plate (Costar, Corning Incorporated). After 24 h incubation period (cells form half-confluent monolayer) the cultures were treated with eight decimal geometric concentrations [23] (six wells per concentration) of each test extract, diluted in fresh medium. One 96-well plate per test-extract was used in each experiment. After 24 h treatment, each plate was examined under inverted microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Alterations in monolayer morphology were registered by an adapted to an inverted microscope digital camera and a computer program EDC-1000E IS. Cytotoxicity was expressed as a concentration-dependent reduction of the uptake of the vital dye Neutral Red [24]. Optical density was measured at wave length 540 nm by Organon Teknika Reader 530. Relative cell viability, expressed as a percentage of the untreated negative controls, was calculated for each concentration.

Table 2
Reaction of poly(oxyethylene phosphonate) with chloroacetone in the presence of tetraalkylammonium chloride

Entry	Catalyst	Content (%)		
		P–H groups	α-Hydroxy groups	Oxirane groups
2a	TMACl	10.4	86.2	3.4
2b	TEACl	5.6	73.8	20.6
2c	TBACl	5.0	85.4	9.6

Conditions: reaction time 1 h at 30 °C and 1 h at 40 °C, catalyst 1 mol%, solvent CH$_2$Cl$_2$. The content of P–H, α-hydroxy and oxirane groups was estimated by ^{31}P{H} NMR spectroscopy.

2.7. Statistical analysis

Probit regression analysis ('Statistica 4.3' software package) was applied to determine the concentrations required to reduce cell viability by 50% (IC$_{50}$ values). Probit regression analysis transforms cell viability, expressed as a percentage, into standardized normally distributed values (probits) and produces a linearized model of the relationship between cell viability and the concentrations (expressed as decimal logarithms) of the test substances [25].

3. Results and discussion

3.1. Synthesis of poly(oxyethylene phosphonate)s bearing α-hydroxyl groups

Due to the presence of the highly reactive P–H group poly(oxyethylene phosphonate)s are applicable as synthetic intermediate. The reaction of poly(oxyethylene phosphonate) **1a** with chloroacetone under phase-transfer catalysis conditions proceeds in two stages: at 40 °C the reaction results mainly in the formation of poly(oxyethylene α-hydroxyl phosphonate) **2**; at 60 °C the main product is poly(oxyethylene phosphonate)s bearing oxirane groups.

In the ^{1}H NMR spectrum of **2a** besides the signals characteristic for **1** there are a doublet at $\delta = 1.23$ ppm with a coupling constant of 15.4 Hz which can be assigned to the CH$_3$ protons in α-hydroxyl phosphonate groups [26] and a singlet at $\delta = 4.50$ ppm for the OH groups. The characteristic signal for the protons in the CH$_2$Cl group is overlapped with the signal for the CH$_2$OCH_2 protons, which is a multiplet at 3.10–3.50 ppm. It should be pointed out that the integral intensity of the signal at $\delta = 6.67$ ppm with a coupling constant of 715.1 Hz, characteristic for P–H protons in the repeating unit, is strongly reduced. This shows that P–H group has been added successfully to the carbonyl bond of chloroacetone. The $^{13}C\{H\}$ NMR spectrum of **2a** also confirmed the statement made-above. The signal at 19.66 ppm can be assigned to the CH_3 carbon atom, the doublet at 49.43 ppm with a coupling constant of 18.4 Hz can be assigned to the carbon atom in the CH_2Cl group, and the signal at 71.43 ppm with a coupling constant of 177.7 Hz is characteristic for the carbon atom bound to the phosphorus atom. This signal is partially overlapped by the signal for the OCH_2CH_2 carbon atoms in the poly(ethylene glycol) fragments at 70.10 ppm. The $^{31}P\{H\}$ NMR spectrum of **2a** (Fig. 1) shows resonances for three types of phosphorus atoms at: (i) $\delta = 24.82$ ppm, a multiplet (from ^{31}P NMR) which can be assigned to the phosphorus atom in the repeating units with a α-hydroxyl phosphonate group [26] with a calculated content of 86.2%; (ii) $\delta = 10.42$ ppm which is a doublet of quintets with coupling constants $^1J(P,H) = 715.8$ Hz and $^3J(P,H) = 9.9$ Hz, characteristic for the phosphorus atom in the repeating unit with P–H group with a content of 10.4% and (iii) $\delta = 22.89$ ppm, a multiplet assigned to the phosphorus atom connected with the oxirane group [22]. Based on the $^{31}P\{H\}$ NMR spectrum of **2a** the content of repeating units bearing α-hydroxyl group is 86.2%.

We suggest that the polymer-analogous reaction proceeds by the mechanism accepted for the reaction between dialkyl hydrogen phosphonates and chloroacetone [22].

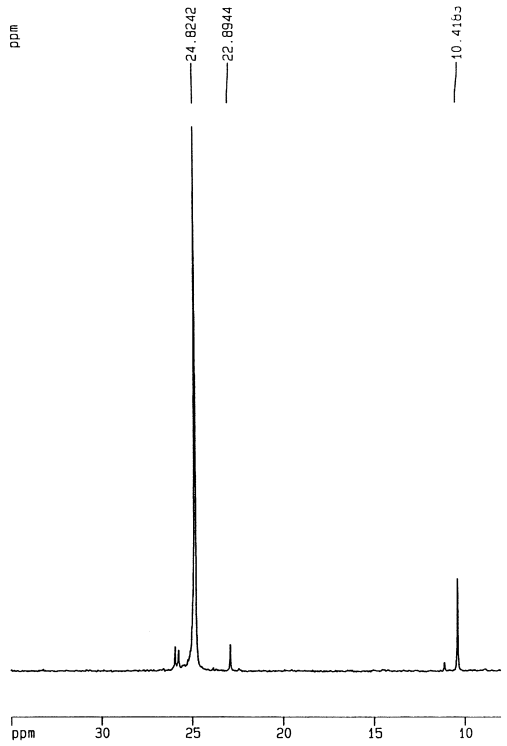


Fig. 1. $^{31}P\{H\}$ NMR spectrum of poly(oxyethylene phosphonate) bearing α-hydroxyl groups (**2a**).

The effect of the catalyst type was investigated. The reaction progress was monitored by $^{31}P\{H\}$ NMR spectroscopy. The results of these studies are summarized in Table 2.

The experimental results revealed that TBACl was the most effective catalyst, of these studied, for the addition reaction of chloroacetone to **1**. The degree of reaction completion was found to be almost 95%. TEACl showed comparable activity to that of TBACl for the addition reaction while it accelerated the conversion of the α-hydroxyl groups to oxirane groups to a higher extent. In the presence of TEACl the content of the oxirane groups was 20.64%. Under these conditions (temperature about 40 °C and catalyst concentration 1 mol%), TMACl is also more active in the addition reaction than in the cyclization one, thus the ratio between the α-hydroxyl to oxirane groups was estimated to be 26:1. In the next section, it will be shown that changing some of the experimental conditions (increasing the temperature and catalyst concentration) the ratio also changed in favor of oxirane groups content.

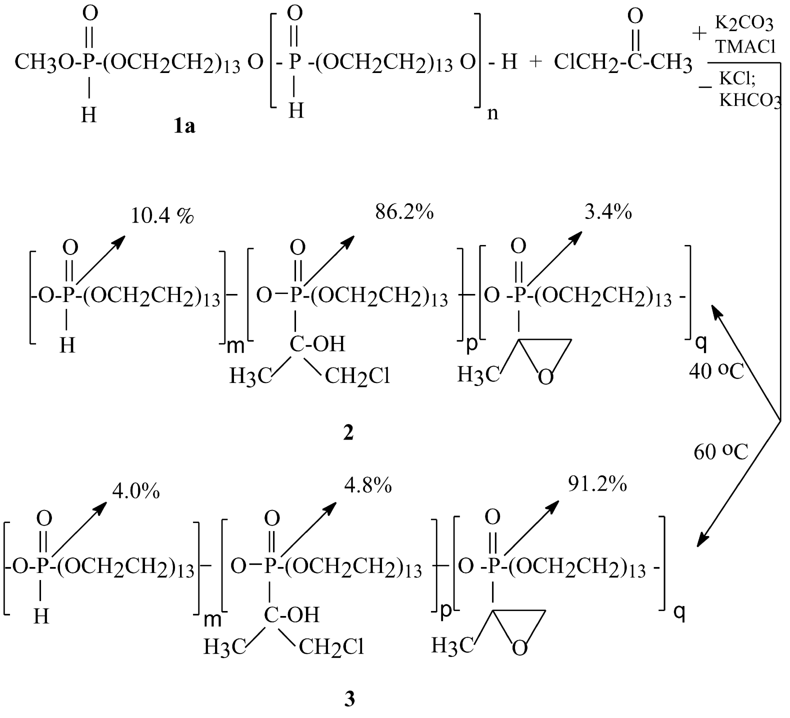
It should be pointed out that the side reactions leading to the formation of phosphates and vinyl phosphates [22] are strongly suppressed. In the $^{31}P\{H\}$ NMR spectra of the **2a**–**c** there are no signals in the region 1.0–1.5 ppm, characteristic for these types of phosphorus atoms.

3.2. Synthesis of poly(oxyethylene phosphonate)s bearing oxirane groups

It was established that the reaction of poly(oxyethylene phosphonate) **1b** with chloroacetone under phase-transfer catalysis conditions at 60 °C resulted in a product with a higher content of oxirane groups (see Scheme 2). In the ^1H NMR spectrum of **3** there is a signal at 1.41 ppm, a doublet with a coupling constant $^3J(P,H) = 11.5$ Hz characteristic for the protons in CH_3 groups bound to the oxirane ring [22]. Triplets at 2.61 and 3.05 ppm can both be assigned to the CH_2 protons of the oxirane ring. The signal at 17.23 ppm, a doublet with a coupling constant $^2J(P,C) = 15.2$ Hz in the $^{13}C\{H\}$ NMR spectrum of **3** can be assigned to the CH_3 carbon atom [26]. The doublet at 51.09 ppm with a coupling constant $^1J(P,C) = 203.4$ Hz corresponds to the carbon atom bound to the phosphorus atom. The signal at 22.97 ppm in the $^{31}P\{H\}$ NMR spectrum of **3a** (Fig. 2) can be assigned to the phosphorus atom bearing the oxirane ring [22].

Experimental conditions were found under which the end product of the reaction was a polymer bearing mainly oxirane groups (91%), i.e. temperatures in the range of 55–60 °C and a catalyst concentration in the order of 10 mol%. It was established that increasing the concentration of TMACl from 2 to 7 mol%, the yield of the oxirane groups increased from 44 to 91% (Fig. 3).

Using tetrabuthylammonium chloride as catalyst



Scheme 2.

(10 mol%) resulted in the same content of oxirane groups (91%) in the reaction product. It was also observed that the higher concentrations of TBACl promoted to some extent side reactions resulting in the formation of phosphates structures [22]. In $^{31}P\{H\}$ NMR spectrum of **3d** (see Section 2.4) there is a signal at 1.2 ppm, characteristic for a phosphorus atom in phosphate structures, with a low concentration (< 1.0%). The experimental data undoubtedly show that when the reaction of dialkyl phosphonates with α-halogenated carbonyl compounds is carried out under phase-transfer catalysis conditions, the side reactions that usually accompany this reaction are strongly suppressed [22].

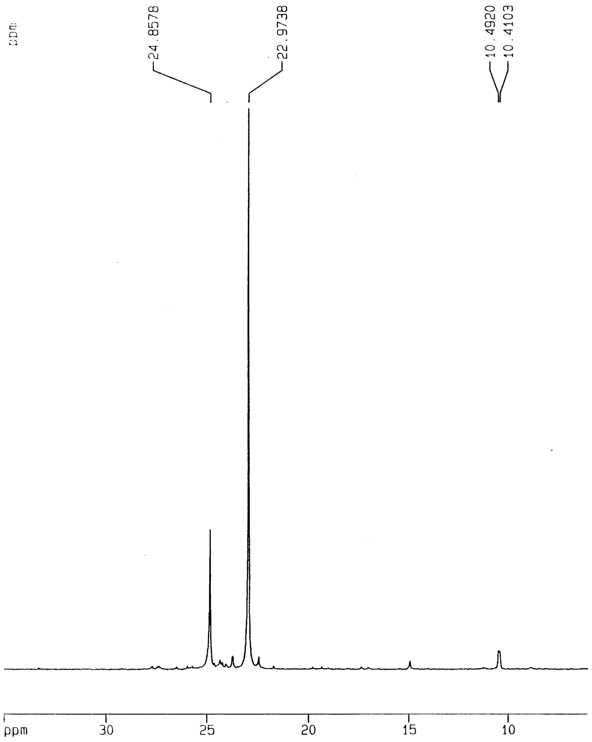


Fig. 2. $^{31}P\{H\}$ NMR spectrum of poly(oxyethylene phosphonate) bearing oxirane groups (**3a**).

Dichloroethane has a higher boiling point and it replaced dichloromethane as solvent. The reaction temperature was raised up to 60 °C (see Section 2.4). That favored the preparation of a polymer with higher content of oxirane groups compared to the product obtained in dichloromethane. Thereby in our study we demonstrated that lower temperatures favor formation of α-hydroxyl groups while higher temperatures promote the cyclization reaction. Furthermore, this fact allowed to be defined more accurately the reaction mechanism, which was predicted by us for the reaction between low molecular compounds [22]. Thus, in the first stage of the reaction (the low-temperature one) the addition of the P–H bond to the carbonyl of chloroacetone occurs, during the second stage the oxirane formation proceeds. There were no data for substitution reaction in the $ClCH_2-$ group.

3.3. Cytotoxicity assay

The toxicity of the poly(oxyethylene phosphonate), poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups has been studied for the first time (Table 3).

The calculated IC_{50} values show that poly(oxyethylene

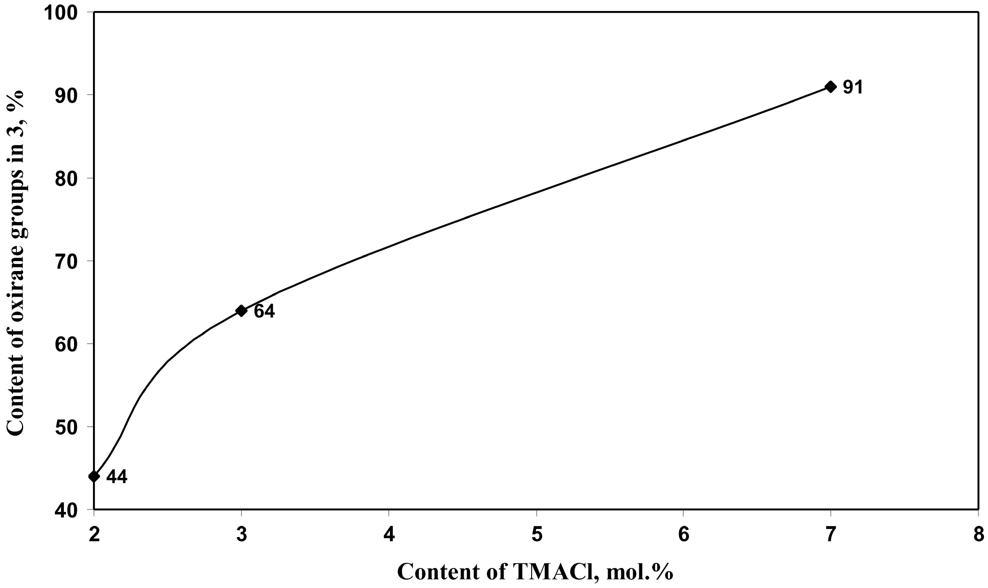


Fig. 3. Content of oxirane groups in **3** vs. concentration of TMACl.

phosphonate) with molecular weight 8400 (**1a**) exhibits lower toxicity in comparison with **2a** and **3a**. The observed toxicity of **2a** is higher compared to that of **1a** and **3a**.

Poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups are of interest as carriers of low-molecular mass bioactive substances and as polymers with their own bioactivity. The data about the cytotoxicity of the products are quite promising for biomedical application of these poly(oxyethylene phosphonate) derivatives, especially those bearing oxirane groups in the side chain, due to their water solubility and reactivity towards amino and hydroxyl groups under mild conditions. Poly(oxyethylene phosphonate) **2** bearing α-hydroxyl groups in the side chain is also of interest as synthetic intermediate for the preparation of poly(oxyethylene phosphonate)s bearing keto groups in the side chain. Poly(oxyethylene phosphonate) **3** bearing oxirane groups in the side chain can be further modified to poly(oxyethylene phosphonate)s bearing 1,3-dioxolan-2-on groups.

Table 3
IC_{50} concentartion of **1a**, **2a** and **3a**

Substance	IC_{50} (mg/ml)
1a	2.96
2a	0.01
3a	1.24

4. Conclusion

It was established that the polymer-analogous reaction between poly(oxyethylene phosphonate)s and chloroacetone under phase-transfer catalysis conditions can be used for the preparation of poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups in the side chain. The mole ratio between these functionalities depends on the reaction conditions. At low temperature and catalyst concentration (40 °C and 1 mol%, respectively) the product contains mainly α-hydroxyl groups (74–86%), while increasing the temperature to 60 °C and catalyst concentration up to 10 mol% the oxirane groups are predominant (91%). Thus designed poly(oxyethylene phosphonate)s bearing α-hydroxyl and oxirane groups are of interest as drug carriers or as intermediates in the synthesis of poly(oxyethylene phosphonate)s bearing keto and 1,3-dioxolan-2-on groups.

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