Annex no. 2

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

1. NAME AND SURNAME: Wojciech Wałach

2. DIPLOMAS AND SCIENTIFIC DEGREES:

1989 **Master of Science, Enginieer**, specialization Polymer and Plastics Technology
Silesian University of Technology in Gliwice, Poland
Faculty of Chemistry

Diploma thesis:

„Polymerization of lactic acid lactide in the presence of anionic initiators"

Supervisor: Prof. Zbigniew Jedliński

1998 **Doctor of Chemical Sciences**

Silesian University of Technology in Gliwice, Poland
Faculty of Chemistry

Ph.D. thesis:

"Synthesis and structure of polymers and copolymers of 2,3-epoxypropanol"

Supervisor: Prof. Jolanta Maślińska-Solich

3. COURSE OF THE EMPLOYMENT – INFORMATION ABOUT PAST AND CURRENT EMPLOYMENTS IN SCIENTIFIC UNITS

Positions:

1989-1992	**Assistant** in Center of Polymers Polish Academy of Sciences in Zabrze
1992-1999	**Assistant** in Laboratory of Membrane Separation and New Materials in Institute of Coal Chemistry Polish Academy of Sciences in Gliwice
1999-2004	**Research scientist (adiunkt)** in Laboratory of Membrane Separation and New Materials in Institute of Coal Chemistry Polish Academy of Sciences in Gliwice
2004-2007	**Research scientist (adiunkt)** in Laboratory of nanostructural Materials in Institute of Coal Chemistry Polish Academy of Sciences in Gliwice
2007-2009	**Research scientist (adiunkt)** in Laboratory of Nano- and Microstructural Materials in Centre of Polymer and Carbon Materials Polish Academy of Sciences in Zabrze
2009 – to date	**Specialist** in Laboratory of Nano- and Microstructural Materials in Centre of Polymer and Carbon Materials Polish Academy of Sciences in Zabrze

4. PRESENTATION OF THE SCIENTIFIC ACHIEVEMENT

resulting from the article 16.2 of the act on scientific degrees and scientific titles and degrees and title in the field of art (Dz.U. [Journal of Laws] of 2003, No. 65, item 595, as amended)

a. TITLE OF SCIENTIFIC ACHIEVEMENT

The scientific achievement is the series of ten monothematic articles entitled:

"Branched polymers of oxiranes of controlled chain topology – synthesis and their properties"

b. List of selected publications constituting the scientific achievement with percentage contribution of habilitant to each work

Impact Factor (IF) – value for the year of publication

H1	W. Wałach, A. Kowalczuk, B. Trzebicka, A. Dworak Synthesis of high-molar mass arborescent-branched polyglycidol via sequential grafting, Macromol. Rapid Commun. 2001; **22**, 1272-1277.	IF=2.372 (contribution=70 %)
H2	A. Dworak, B. Trzebicka, A. Utrata, W. Wałach Hydrophobically modified polyglycidol – the control of lower critical solution temperature, Polym. Bull. 2003; **50**, 47-54.	IF=0.795 (contribution=40 %)
H3	W. Wałach, B. Trzebicka, J. Justyńska, A. Dworak High molecular arborescent polyoxyethylene with hydroxyl containing shell, Polymer 2004; **45**, 1755-1762.	IF=2.433 (contribution=60 %)
H4	B. Klajnert, W. Wałach, M. Bryszewska, A. Dworak, Dz. Shcharbin Cytotoxicity, haematoxicity and genotoxicity of high molecular mass arborescent polyoxyethylene polymers with polyglycidol-block-containing shells Cell Biol. Int. 2006; **30**, 248-252.	IF=1.363 (contribution=35 %)
H5	A. Dworak, W. Wałach Synthesis, characterization and properties of functional star and dendritic block copolymers of ethylene oxide and glycidol with oligoglycidol branching units, Polymer 2009; **50**, 3440-3447.	IF=3.573 (contribution=80 %)
H6	M. Libera, B. Trzebicka, A. Kowalczuk, W. Wałach, A. Dworak Synthesis and thermoresponsive properties of four arm, amphiphilic poly(*tert*-butyl-glycidylether)-block-polyglycidol stars, Polymer 2011; **52**, 250-257.	IF=3.438 (contribution=60 %)
H7	M. Libera, W. Wałach, B. Trzebicka, S. Rangelov, A. Dworak Thermosensitive dendritic stars of *tert*-butyl-glycidylether and glycidol – Synthesis and encapsulation properties, Polymer 2011; **52**, 3526-3536.	IF=3.438 (contribution=50 %)
H8	A. Dworak, A. Kowalczuk-Bleja, B. Trzebicka, W. Wałach Amphiphilic core-shell PEO stars by Williamson etherification reaction Polym. Bull. 2002; **49**, 9-16.	IF=0.711 (contribution=25 %)

H9	A. Mendrek, S. Mendrek, B. Trzebicka, D. Kuckling, W. Wałach, H.-J. Adler, A. Dworak Polyether core-shell cylinder – polymerization of polyglycidol macromonomers, Macromol. Chem. Phys. 2005; **206**, 2018-2026.	IF=2.111 (contribution=20 %)
H10	M. Jamróz-Piegza, W. Wałach, A. Dworak, B. Trzebicka Polyether nanoparticles from covalently crosslinked copolymer micelles, J. Coll. Int. Sci. 2008; **325**, 141-148.	IF=2.443 (contribution=35 %)

Total Impact Factor of 10 publications constituting the scientific achievement is 22,677

c. OVERVIEW OF THE SCIENTIFIC AIMS AND OBTAINED RESULTS PRESENTED IN THE SERIES OF PUBLICATIONS "Branched polymers of oxiranes of controlled chain topology – synthesis and their properties"

1. Introduction

The subject of this dissertation is the study of the methods and conditions of the controlled synthesis of branched polyoxiranes for different topologies of the macromolecule and their properties.

Although polymers of oxiranes have been known since the nineteenth century and the basics of branched polymers were developed by Flory and Stockmayer in the mid-twentieth century, the study of branched polymers of oxiranes and the description of the structure of such macromolecules have become possible only with the dissemination of living and controlled polymerization methods [1-8], allowing for the precise synthesis of linear and branched macromolecules.

The branching of the polymer chain entails changes in the properties of the polymer, which allows modification of the material properties to some extent without substantially changing the chemical composition [9, 10]. In comparison with their linear analogues, branched polymers exhibit better solubility, low solution viscosity, and a higher number of reactive end groups. These features have contributed to the development of research on branched polymers.

The control of the architecture, molar mass and number of end groups of the polymer enables the material properties to be better adjusted for specific applications [11]. For polyoxiranes, where the terminal groups are hydroxyl groups that can easily be used for modification, the controlled branching of the macromolecule, thereby increasing the number of end groups, provides new application possibilities. This possibility is particularly evident in many studies describing the potential use of hyperbranched polymers and copolymers of glycidol (oxirane with a hydroxyl group) for biomedical applications [12-21]; however, such polymers must have a sufficiently high uniformity of the chain structure. Research on methods for the synthesis of such materials is still needed. The literature describes methods for the synthesis of branched polyoxiranes, mainly related to hyperbranched structures; however, the ability to control the parameters of the chain structure, degree of branching, molar mass, and number of end groups is very limited [22-33]. The first work demonstrating that the synthesis of the polymers of glycidol is a complex process and leads to a mixture of oligomers with a complex composition and structure was published by Vandenberg [34, 35].

A major problem undertaken in the author's work is to identify the methods and appropriate synthesis conditions leading to different types of branched polyoxirane macromolecules with a controlled structure, composition, and number of end groups and low molar mass dispersion.

The commentary describes the most important works of the author on the research on the controlled synthesis and properties of branched polyoxiranes, where the chain branching units are:

- unit or polyglycidol block [H1, H2, H3, H4, H5, H7, H10]

- multifunctional initiator [H5, H6, H7]
- multifunctional terminator [H8]
- terminator with the functional group, which enable the polymerization of a macromonomer obtained in the termination step [H9]

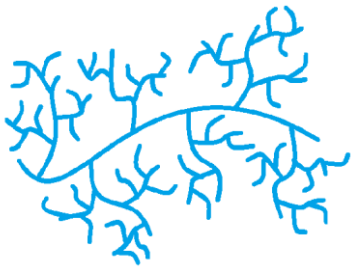
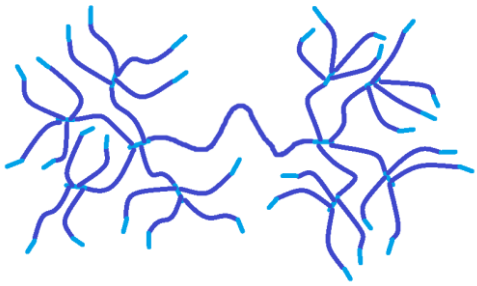
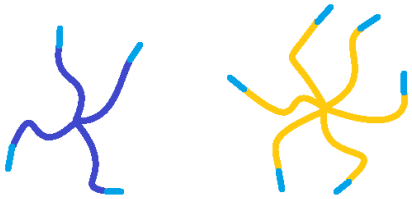
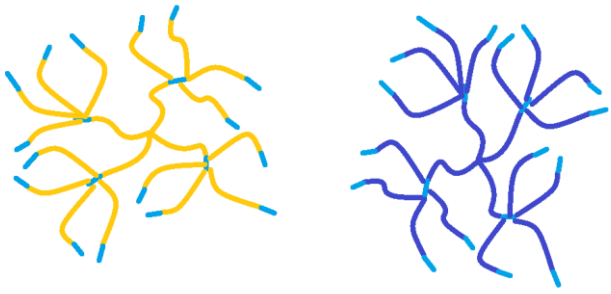
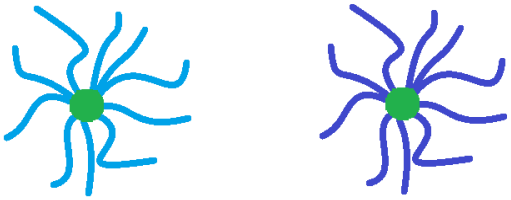
Some of the resulting polymers were biochemically tested to determine their effect on biological materials (cells, erythrocytes, DNA).

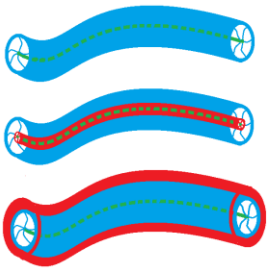
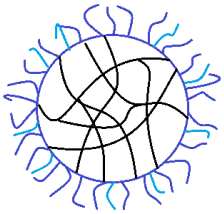
The key to the controlled synthesis of the described polymers is the anionic polymerization of oxirane monomers, which with the selection of the appropriate conditions may occur in a controlled manner. In most syntheses, the glycidol – oxirane with a hydroxyl group was used. The presence in the glycidol molecule of a hydroxyl group, which is reactive in the polymerization process, required the use of a monomer with a protected hydroxyl group, inactive during the polymerization, and easily and quantitatively recovered after the synthesis of the macromolecule. In the work, four oxirane monomers were used: ethylene oxide (EO), glycidol (Gl) (for synthesis 1-etoxyethyl glycidyl ether (EEGE) was used), and hydrophobic oxiranes: tert-butyl glycidyl ether (ETBG) and phenyl glycidyl ether (FGE).

For the chain-branching, in addition to glycidol, pentaerythritol, dipentaerythritol, inositol and *p*-chloromethylstyrene (monomer (CMS) and the polymer (PCMS)) were also used. To stabilize the nanoparticles by forming covalent bonds between the chains by the dimerization under the effect of UV light, double bonds in glycidyl cinnamate (CGL) were used.

Table 1. Schematic illustration of the chain structure of the macromolecules of polymers and copolymers synthesized in the frame of the work described in the commentary. The corresponding colors indicate the type of monomer from which the block of the polymer chain is based. A description is provided under the table.

Table 1. Branched polyoxiranes described in the work

Synthetic method, macromolecular structure, composition and nature	Structure
– grafting and multiple grafting – dendritic structure – homopolymer – hydrophilic [H1, H2]	
– grafting – dendritic structure of „pom-pom" type – copolymer – hydrophilic [H3, H4]	
– "core first" – stars 4 and 6 armed – copolymers – hydrophilic and amphiphilic [H5, H6]:	
– grafting – dendritic stars – copolymers – hydrophilic and amphiphilic [H5, H7]	
– termination – "arm first" – stars – homopolymeric arms, hydrophobic core – amphiphilic [H8]	

– polymerization of macromonomers – “bottle-brush” structure – copolymers – hydrophilic and amphiphilic [H9]	
– crosslinking of micelle – “core-shell” – copolymers – amphiphilic [H10]	

Description of table 1.

—(CH$_2$-CH$_2$-O)— poly(ethylene oxide) (PEO)	—(CH$_2$-CH-O)— lub —(CH$_2$-CH-O)— CH$_2$ CH$_2$ OH O)— polyglycidol (PGl)
—(CH$_2$-CH-O)— CH$_2$ O poly(phenyl glycidyl ether) (PFGE)	—(CH$_2$-CH-O)— CH$_2$ O H$_3$C—C—CH$_3$ H$_3$C poly(*tert*-butyl glycidyl ether) (PETBG)
—(CH$_2$-CH-O)— CH$_2$ O C=O HC CH poly(glycidyl cinnamate) (PCGl)	—(CH$_2$-CH)— CH$_2$— poly(*p*-chloromethylstyrene) (PCMS)

Most syntheses in this work involve a multi-step process, which uses a different method of branching of the macromolecule. For the synthesis of dendritic polymers, the grafting and multigrafting process was used ("graft" and "graft on graft") [H1-H4, H5, H7]. The stars were synthesized by the termination of a multi-functional terminator ("arm first") [H8] and initiation of the multi-functional initiator ("core first") [H5, H6]. For the synthesis of polymers of the "bottle-brush" structure [H9], the polymerization of the macromonomers method was used; however, the synthesis of stable nanoparticles was prepared by photocrosslinking of the micelles formed from the block copolymers with a modified polyglycidol block [H10].

All of the author's work that is described in the series focuses on determining the appropriate conditions for the synthesis, which enable control of the topology, size, number and type of functional groups and the molar mass dispersion of the macromolecules. The publications discussed in the commentary are collective work, in which the synthetic work and tests were performed or conducted under the supervision of the author or consultation was provided by the author concerning the conditions and methods of the synthesis.

2. Grafting of the polyglycidol chain

➢ Grafted polymers of glycidol [H1, H2]

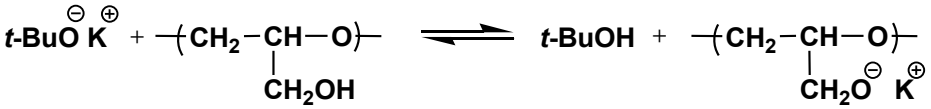
The study performed by the author focused on the use of the hydroxyl group in the repeating unit of the glycidol to branch the polyoxirane chain. The presence of the hydroxyl group in the monomer complicates the process of anionic polymerization of this monomer. Transfer reactions at the hydroxyl group of the monomer lead to a reduction in the molar mass of the polymer and the formation of low-molar-mass cyclic products, which in turn causes a loss of control [22]. The synthesis of hyperbranched polymers of glycidol using the slow monomer addition technique described by Frey [22, 27] can reduce the temporary concentration of glycidol and thereby reduce the reactions leading to cyclic products; however, the attainment of branched polyglycidol with a molar mass greater than 10 000 g/mol produces a polymer with significantly increased molar mass dispersion ($Đ_M$> 2) [27]. Beyond control is also the degree of branching DB, which is independent on the conditions and varies slightly from 0.53 to 0.59.

Irrespective of the polymerization method, the presence of the hydroxyl group leads to a highly branched polymer with a moderately high molar mass. The control of this process is difficult. In particular, the control of the density of branches, their distribution, and the length of the linear blocks connecting the branching points are beyond control.

In the work of the author of this dissertation, another method of the branching of polyoxirane chain was applied. To eliminate the transfer reactions, glycidol was used as the monomer with a protected hydroxyl group (1-etoxyethyl glycidyl ether – EEGE), and the generation of branching was achieved by repeated the grafting of a linear polyglycidol chain after the removal of the acetal protecting groups.

The synthesis was started from the linear polyglycidol with a molar mass of 10 300 g/mol ($Đ_M$ = 1.25) by initiating the polymerization of EEGE with potassium *tert*-butoxide in tetrahydrofurane. A linear polymer after deprotection of the hydroxyl groups and purification was used in the next step – grafting.

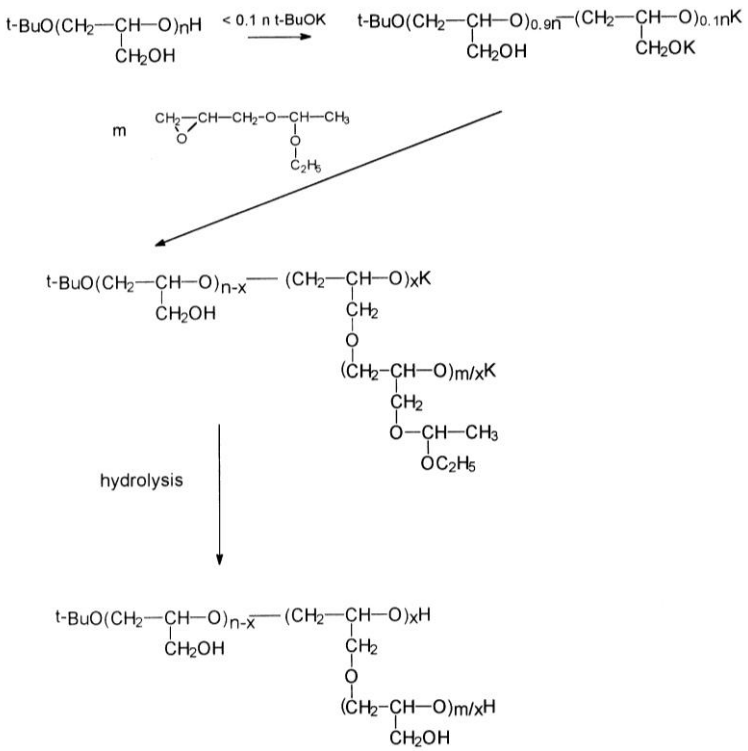
Homogeneous solutions of a polyfunctional macroinitiator – polyglycidol with alkoxide groups were obtained by the ionization of 10 % of all the hydroxyl groups of the linear polyglycidol. The alkoxide groups were obtained by reacting polyglycidol with potassium *tert*-butoxide, applying the equilibrium shown in scheme 2.1.



Scheme 2.1. Alcohol-alkoxide equilibrium in the potassium *tert*-butoxide/polyglycidol system

The resulting *tert*-butanol was removed by distillation under high vacuum together with almost all the DMSO. After being re-dissolved in DMSO, the polyfunctional macroinitiator was used for the polymerization of EEGE.

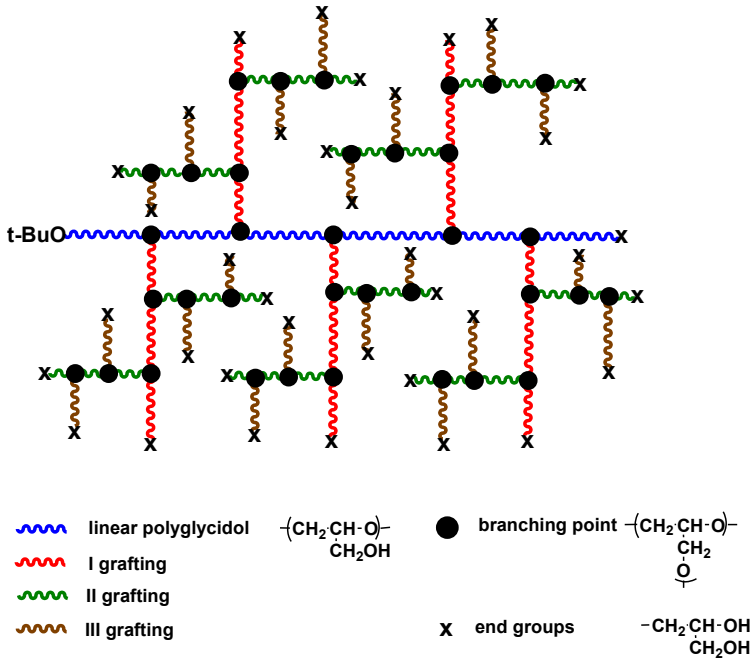
This reaction leads to the grafting of EEGE chains to the linear polyglycidol chain, and the exchange between all the alcohol and alkoxide groups of polyglycidol makes the propagation possible on each hydroxyl group. After reaching almost complete conversion of the monomer, the acetal protecting group is removed from pEEGE (Scheme 2.2).



Scheme 2.2. Grafting of the polyglycidol chain

The product after purification and analysis was used to initiate the polymerization of EEGE in the same manner as before, by performing a subsequent grafting reaction. The grafting process was performed three times, each time using the product from a previous grafting. In the last step, which resulted in a polymer with a molecular weight of up to two million, the molar mass of the macroinitiator was 740 000 g/mol, and to obtain a homogeneous initiating system, it was necessary to reduce the degree of ionization of the hydroxyl groups to approximately 7 %.

All the products of the following grafting after removal of the acetal protecting groups were analyzed by SEC-MALLS and ^{13}C NMR. The applied grafting method produced three generations of polymers with different molar masses and different degrees of branching. The subsequent grafting steps are shown in scheme 2.3. The different colors indicate chains resulting from the successive stages of grafting.



Scheme 2.3. Subsequent grafting steps of the grafting of polyglycidol macromolecule

The results of gel permeation chromatography analyses reveal that the applied method can well control the molar mass of the synthesized polyglycidol (Tab. 2.1). The increasing dispersion of the molar mass of the products for the subsequent grafting is most likely due to the increasing viscosity of the reaction mixture.

Table 2.1. Characteristics of the grafting product of polyglycidol

Grafting	Monomer conversion	$M_{n\ calcl.}$*	$M_{n\ (SEC\text{-}MALLS)}$	$Đ_M$
	%	g/mol	g/mol	
I	>99 %	79 500	82 000	1.25
II	>99 %	707 530	740 000	1.27
III	27 %	1 618 000	1 820 000	1.43

$Đ_M$ – dispersion of molar mass

* – Molar mass calculated from the reagent ratio and conversion of monomer.

Determination of the macromolecule architecture for each grafting product was possible due to the correlation of the results obtained from ^{13}C NMR spectroscopy and SEC-MALLS. From the spectroscopic analysis, the ratio of the end groups (K) to the linear units (L) was determined. Knowing that each branch (R) generates one terminal group (K), it is possible, based on the degree of polymerization DP determined from SEC-MALLS, to calculate the quantity of the linear unit using the formula

DP = L + R + K for sufficiently large DP R = K then L = DP - 2K.

Comparing the structure of the starting polymer (polyfunctional macroinitiator) and the product, the resulting share of grafted linear units in each step was also calculated, which characterizes the efficiency of grafting. The degree of branching of the polymer after each grafting was calculated using the following formula: DB = (K + R)/(L + M + R). The data are presented in table 2.2.

Table 2.2. Structure of the grafted polymers of glycidol

Grafting	The molar ration of end/linear units *	The share of grafted units	DB
		%	
I	1/8.5	76	0.19
II	1/11.2	84	0.15
III	1/1.2	89	0.63

* Data obtained from ^{13}C NMR.

The information obtained on the structure of the polymers demonstrates that using the appropriate conditions, a high degree of grafting of the polyglycidol chain, over 80 % of all linear units can be achieved. The degree of branching can be controlled by the number of monomer molecules per one center of growth – a smaller ratio results in shorter linear chains and less number of linear units.

To achieve control, it is necessary to provide a homogeneous reaction mixture at any stage of the synthesis in such a way that all of the hydroxyl groups in the system allow for a proton exchange reaction between alcohol and alkoxide groups.

Polymers of glycidol of a "graft- on-graft" structure were obtained with molar masses as high as 2 x 10^6 g/mol. These masses can be controlled, and their dispersion is low. In the literature, very few synthesis of polymers with such large molar masses together with the control of the process are described.

Modification of the above-described grafted polyglycidol by esterification is presented in publication [H2]. The reaction of hydroxyl groups with acetic anhydride in dimethylformamide in the presence of pyridine leads to polymers of glycidol in which approximately 30 % to 90 % of the hydroxyl groups have been esterified. The change in polarity changes the properties of the polymer and induces thermosensitivity of aqueous solutions of esterified polyglycidol, wherein the transition temperature depends not only on the polyglycidol degree of esterification and the molar mass but also on the structure of the macromolecules. Most likely, a more compact structure of the macromolecules leads to the esterification of the easily accessible, external hydroxyl groups, and this area is responsible for the interaction with the solvent. Therefore, the graft polyglycidol is more hydrophobic than a linear polymer, which reduces the cloud point. For the degree of esterification of approximately 45 %, the difference is substantial and reaches 40 °C.

The presented publications [H1, H2] describing the synthesis and modification of grafted polyglycidol demonstrate that it is possible to control the synthesis of branched polymers of glycidol, leading to different branched chain structures and different properties.

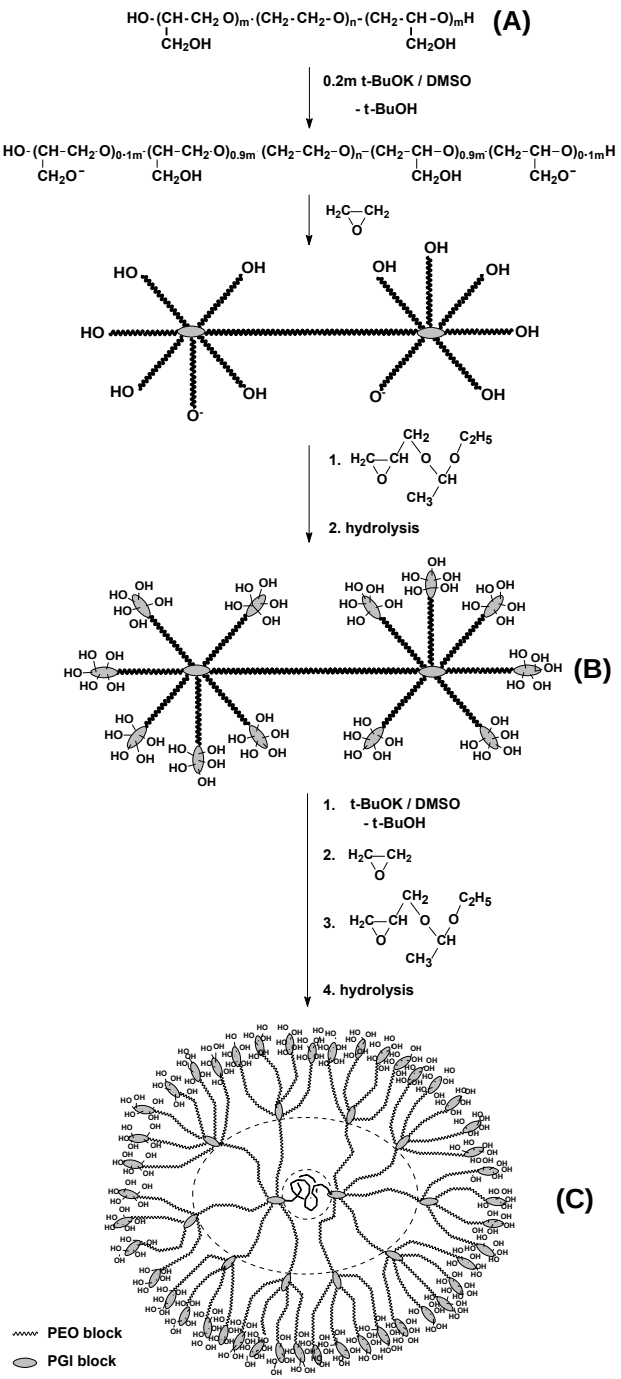
- **Branched copolymers of ethylene oxide and glycidol**

Copolymers of ethylene oxide of "pom-pom" chain structure [H3] [H4]

The studies described in previous publications lead to highly branched polymers. It is possible to control the degree of branching when the number of hydroxyl groups in the macromolecule is equal to the degree of polymerization. Subsequent research has been directed toward the development of methods of such controlled synthesis of functional oxirane copolymers in which the control of the degree of branching of the macromolecules and of the change in the number of hydroxyl groups are possible. First, the investigation was focused on the copolymerization of glycidol with ethylene oxide.

The study described in [H3] was started by applying the slow monomer addition method using the macroinitiator obtained from the poly(ethylene oxide) and glycidol as the monomer. It was observed that under these conditions, the dominant and competitive reaction to the chain growth was the chain transfer reaction to hydroxyl group of glycidol, making the control impossible.

The research was directed toward the multi-stage synthesis of ethylene oxide and the glycidol monomer [H3] with a protected hydroxyl group. In the first step of the synthesis, a triblock copolymer of poly(EEGE)-block-PEO-block-poly(EEGE) was prepared, where M_n of PEO block was 4 000 g/mol. After hydrolysis, the triblock copolymer – polyglycidol-block-PEO-block-polyglycidol was obtained (Product A – scheme 2.4). The amount of reactants was selected in such way that the average length of the polyglycidol blocks at full conversion of the monomer was approximately 6 units. Then, the average number of hydroxyl groups at the end of the chain was 7 (6 primary and one secondary), which allowed an average of 7 branches of the polymer chain to be created in the next step of the synthesis. Obtained in the first stage copolymer was used as a multifunctional macroinitiator for the polymerization of ethylene oxide and then EEGE. The macroinitiator was prepared similarly to the grafting of the homopolymeric polyglycidol chain. Both monomer conversion: ethylene oxide and then added EEGE was nearly 100 %, which guaranteed the formation of polymer blocks of suitable lengths. After deprotection of the hydroxyl groups in glycidol blocks, copolymerization was repeated once again, using the branched copolymer resulting from the previous step (product B) as the macroinitiator. The course of the synthesis is illustrated in scheme 2.4.



Scheme 2.4. Stages of the synthesis of branched copolymer of ethylene oxide and glycidol of "pom-pom" chain structure

An important part of the study was to confirm the control of the molar mass and the architecture of the synthesized polymers. After each step of the synthesis and removal of the acetal protecting groups in the polyglycidol block, the products were analyzed to determine the molar mass (SEC-MALLS) and the number and type of end groups (^{1}H NMR).

Confirmation of the structure of macromolecules demonstrated the lack of hydroxyl groups from the PEO block after the EEGE polymerization step

and the absence of secondary and primary hydroxyl groups from the polyglycidol block after polymerization of the ethylene oxide step.

Direct ^{1}H NMR analysis of the products did not allow the signals from the hydroxyl end groups to be distinguished; however, quantitative conversion of the hydroxyl groups into trichloroacetylcarbamate esters enabled the hydroxyl end groups to be differentiated and permitted quantitative analysis [36] and the calculation on that basis of the average length of the formed blocks at each stage of the synthesis.

Correlation of the results of the end group analysis and the results from the SEC-MALLS analysis with the values obtained from the calculation from the feed ratio revealed a very good convergence (Table 2.3). At any stage of the synthesis, the oligomeric fraction was not detected.

Table 2.3. Characteristics of the sequential products of the synthesis of copolymers of ethylene oxide and glycidol of "pom-pom" chain structure [H3]

Product	M_n	DP_{EO}	DP_{GL}	M_n	$Đ_M$	DP_{EO}	DP_{GL}
Scheme 4	Calculated from the feed ratio			SEC-MALLS		SEC-MALLS and ^{1}H NMR	
A	4 850	90	5.8	5 000	1.10	94	5.9
B	33 180	39	5.2	33 000	1.04	37	5.5
C	195 340	37	4.3	203 000	1.03	36	4.5

$Đ_M$ – dispersion of molar mass,
DP_{EO} and DP_{GL} – the average degree of polymerization of the block for ethylene oxide and glycidol in the outer shell; for the product A, the value DP_{EO} is the degree of polymerization of the PEO macroinitiator

For the successive products of the synthesis, the number of the corresponding elements in the macromolecule structure (the number of hydroxyl groups and branches) was also compared, calculated from the feed ratio of the substrates and obtained from the analysis (Table 2.4).

Table 2.4. Average number of hydroxyl groups and branches in copolymers of ethylene oxide and glycidol of "pom-pom" chain structure [H3]

Product	M_n	Average number of OH groups	Average number of branches	Average number of OH groups	Average number of branches
Scheme 4	SEC-MALLS	Calculated from the feed ratio		SEC-MALLS i ^{1}H NMR	
A	5 000	13.5		13.8	–
B	33 000	84	13.5	89	13.8
C	203 000	446	84	487	89

In addition to the consistency of data obtained from the calculations and analyses, an important expression of the control of degree of the polymerization is the dispersion of the molar mass

of the products. For the copolymers A, B and C, the dispersion is very small, and despite the increase in molar mass remains at a similar level (Table 2.3). The easy accessibility of active sites resulting from the less branched structure of the macromolecules and location of the propagation centers at the ends of mobile chains facilitates uniform growth in all the centers and allows a low molar mass dispersion to be obtained. For the process described in the previous paragraph [H1], more branched homopolymers of glycidol, of compact chain structure, where access to the active sites inside the packed macromolecule is more difficult, the dispersion was larger and reached a value of 1.43 upon increasing the degree of branching and the molar mass.

Copolymers of ethylene oxide and glycidol are hydrophilic and highly soluble in water and some organic solvents. Their easily controlled structure, the presence of a large number of hydroxyl groups in the outer shell of the macromolecules and their good solubility increases the chance of their potential use for biomedical purposes, such as in components of drug-delivery systems. The basis for the use of such carriers for biomedical purposes is the lack of toxicity of these polymers. In cooperation with the Department of General Biophysics, University of Lódź, studies were performed with these copolymers [H4] focusing mainly on their cytotoxicity, ability for hemolysis and interaction with DNA.

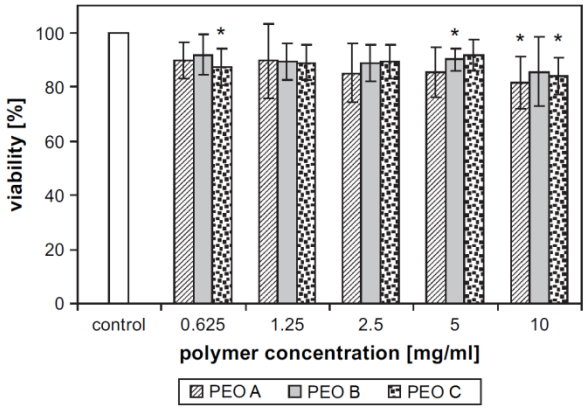


Fig. 2.1. Cytotoxicity of copolymers of ethylene oxide and glycidol versus their concentration [H4]. Copolymers A, B and C are characterized in tables 2.3 and 2.4

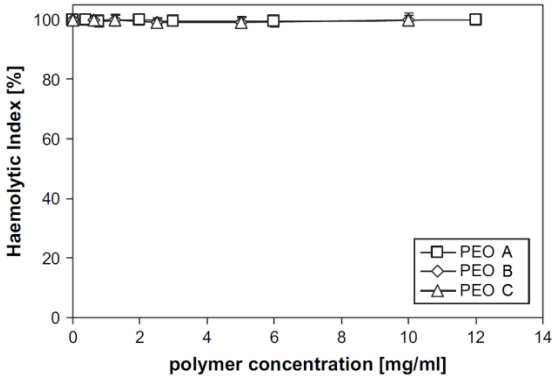


Fig. 2.2. Hemolytic Index of copolymers of ethylene oxide and glycidol versus their concentration [H4]. (100 % means that there is no release of hemoglobin from red blood cells) Copolymers A, B and C are characterized in table 2.3 and 2.4

Tests confirmed that there is no disadvantageous effect of copolymers on cells (cell line B14), even at concentration greater than 10 g/L (Fig. 2.1). These studies revealed no degradation of erythrocytes that have been in contact with solutions of the investigated polymers (Fig. 2.2). The effect of the copolymers on DNA was also studied. In the study, ethidium bromide was used, which intercalates DNA. The fluorescence of ethidium bromide intercalated between the base pair is very strong and weakens when another compound interacting with DNA releases the earlier intercalated ethidium bromide.

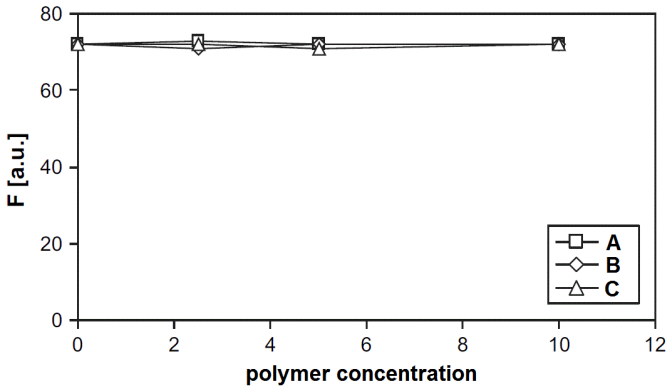


Fig. 2.3. The dependence of the fluorescence intensity F of complex DNA with ethidium bromide on the concentration of ethylene oxide and glycidol copolymers. Characteristics of the copolymers A, B and C in table. 2.3 and 2.4

The weakening of the emission should also be observed when the complex of DNA with ethidium bromide changes conformation or aggregates. For solutions of copolymers with concentrations of 10 g/L, there was no change in the fluorescence of DNA complex with ethidium bromide (Fig. 2.3), which indicates that the copolymers do not interact with DNA. These results are the basis for further research on the obtained copolymers for biomedical applications.

Star and dendritic copolymers of ethylene oxide and glycidol [H5]

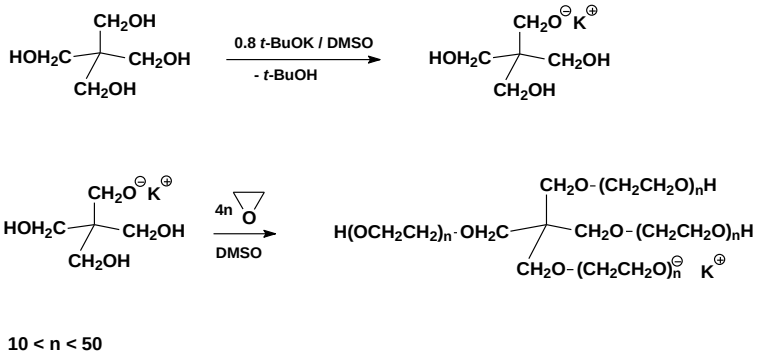
A well-controlled process for the synthesis of copolymers of ethylene oxide, wherein the initiator is a macromolecule with many hydroxyl groups was the starting point for investigating the synthesis of copolymers of ethylene oxide of a star macromolecule structure with uniformly cascade branching arms. Polymers with such a macromolecular structure are widely described in the literature. Potential applications of such materials are mainly mentioned for the preparation of drug nanocarriers [37], the nanoencapsulation of drugs [38, 39], supports for catalysts [40], and nano-reactors.

Obtaining the polymer star structure requires the use of a low-molecular-weight compound with more than two initiating groups. To control the synthesis of the star polymer, it is useful to maintain the homogeneity of the polymerization system during the initiation and chain propagation steps. For alkoxides, it is difficult to find suitable conditions for such a system because of the strong aggregation and loss of homogeneity in the preparation of a multifunctional initiator.

Because of the selection, the initiating system based on pentaerythritol – alcohol containing four primary hydroxyl groups was used. The homogeneous solution of tetrafunctional initiator was obtained by ionization of pentaerythritol in DMSO by potassium *tert*-butoxide – as for oligoglycidol blocks; however, the limit of the ionization degree in this case is approximately 25 % (one OH group of pentaerythritol molecule). Exceeding this value causes precipitation of alkoxide, and even substantial dilution of the system does not lead to a homogeneous solution. Similarly, as for ionization of the hydroxyl groups in oligoglycidol blocks, fast alcohol-alkoxide exchange in DMSO solution enables initiation of all the hydroxyl groups in the system.

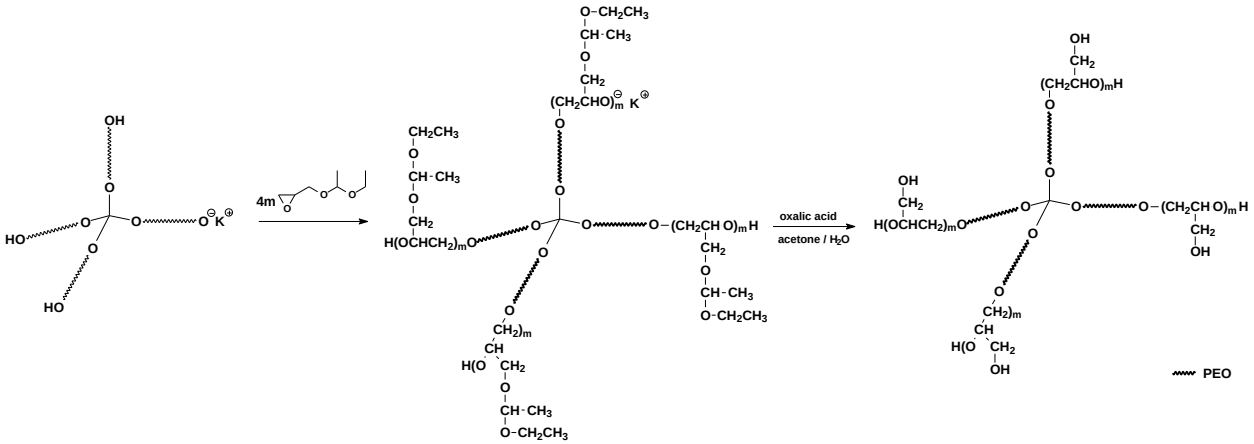
Syntheses of the copolymers were performed in such a way as to obtain two different populations of star structure and length of the arms. In any case, the first step of the synthesis was to obtain

a four-armed star of ethylene oxide (Scheme 2.5), wherein the three lengths of the star arm were selected – DP = 10, 30 and 50 units.



Scheme 2.5. The synthesis of star polymers of ethylene oxide

After the initiation of the polymerization of ethylene oxide with tetra-functional pentaerythritol and achieving complete conversion of the ethylene oxide monomer, the EEGE in an amount such that the ends of the arms formed oligomeric blocks with DP of approximately 5 units was added (Scheme 2.6). The hydrolysis with oxalic acid deprotected the hydroxyl groups in the glycidol blocks situated at the ends of the arms.



Scheme 2.6. The synthesis of star block copolymers of ethylene oxide and glycidol

The synthesis was performed similarly to that in the previously described copolymers of a "pom-pom" chain structure. The control of this step of the synthesis was confirmed by determining the molar mass of the copolymers applying SEC-MALLS and MALDI-TOF technique and comparing them with the molar masses calculated from the feed ratio (Tab. 2.5).

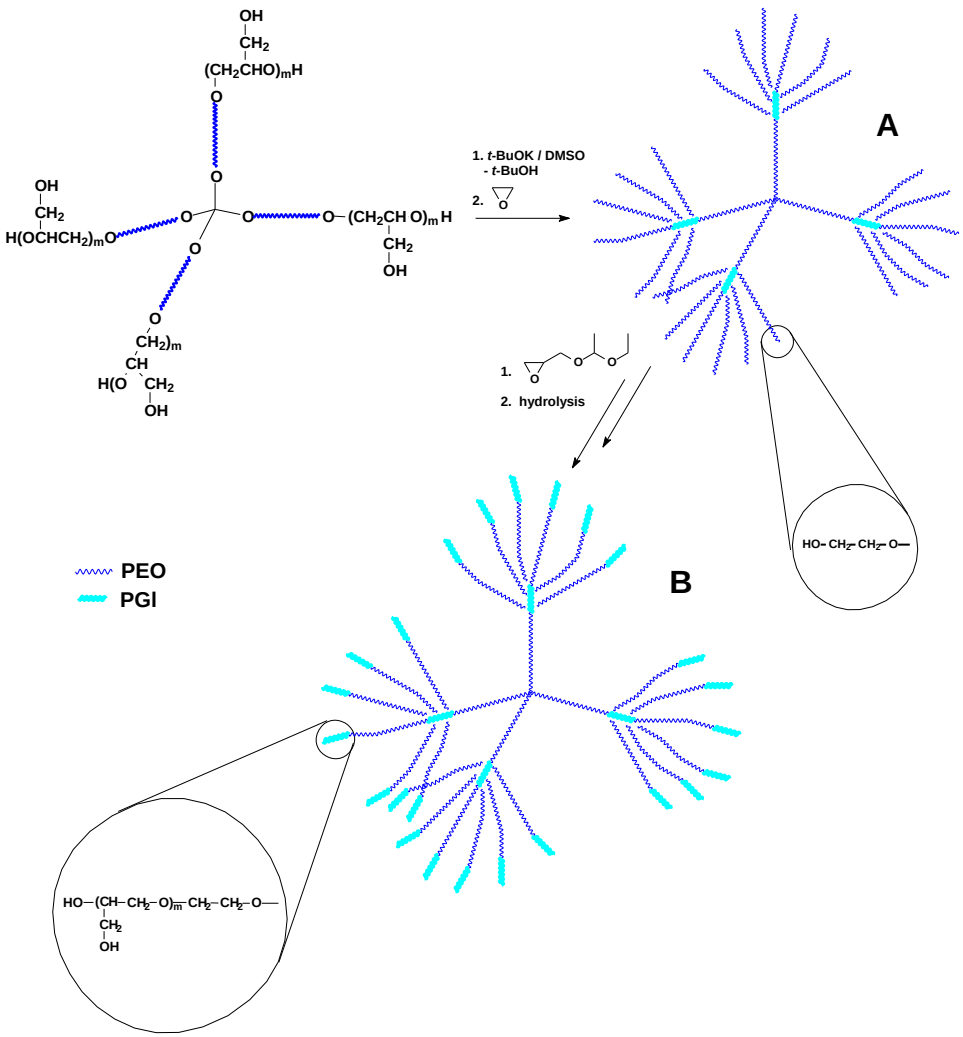
Table 2.5. Characteristics of star copolymers of ethylene oxide and glycidol

	DP_{EO}:DP_{GL}	M_n	DP_{EO}:DP_{GL}	M_n	$M_n/Đ_M$	
	Calculated from the feed ratio		Calculated from ^{1}H NMR		SEC-MALLS	MALDI-TOF
1.	10.0:6.2	3 720	10.0:6.1	3 600	5 100/1.11	3 670/1.06
2.	30.6:5.7	7 200	28.8:5.5	6 800	8 200/1.01	7 370/1.03
3.	50.6:4.3	10 320	51.9:4.4	10 570	9 200/1.01	

The small dispersion of molar masses also confirms the structural homogeneity of the copolymer macromolecules and shows good control of the synthesis.

Analysis of the trichloroacetylcarbamate ester end groups of the obtained copolymers performed by ^{1}H NMR spectroscopy confirmed the molar masses of the copolymers obtained from other analytical technique (SEC-MALLS, MALDI-TOF). This method allows calculation of the number and identification of the type of hydroxyl groups of the end groups of the copolymers (primary at the end of the poly(ethylene oxide), secondary at the end of the polyglycidol block and primary in the repeating glycidol unit). Using these values, the average length of the blocks and average molar mass of the copolymers were calculated. The good agreement of the results obtained using this technique with the data calculated from the feed ratio indicates the good control of the synthesis of the star copolymer.

The resulting star-shaped copolymers, after the ionization of part of the hydroxyl groups (less than 10 %), were used as multifunctional macroinitiators for the next step of the synthesis. In this case, even stronger than observed in previous experiments, the copolymers tended to form aggregates that were insoluble in DMSO in the form of films formed on the surface of the solution during evaporation of the solvent. Avoiding the increase of the local concentration of the macroinitiator in this step is a critical factor.



Scheme 2.7. Synthesis of the dendritic star copolymers of ethylene oxide and glycidol

The synthesis of branched arms performed in the first step of the synthesis of stars. Two series of dendritic copolymers were obtained: A – with branched PEO blocks arms and B – with branched copolymeric arms -PEO-block-polyglycidol (Scheme 2.7). The ratios of the reactants were selected such that for the branched arms of copolymers of both series, the degrees of polymerization of the PEO block were 10, 30, and 50, similarly to in the first step of the synthesis.

The characteristics of the products after hydrolysis are shown in table 2.6.

Table 2.6. Characteristics of the dendritic star copolymers of ethylene oxide and glycidol

Series/DP_{EO}	Mn	$Mn/Đ_M$	Number of hydroxyl groups in macromolecule	
	Calculated from the feed ratio	SEC-MALLS	Calculated from the feed ratio	Estimated from ^{1}H NMR
A/10	16 500	18 000/1.02	29	28
B/10	29 500	33 700/1.01	209	210
A/30	42 700	46 000/1.05	27	26
B/30	54 500	55 800/1.01	192	180
A/50	58 600	58 000/1.01	21	22
B/50	70 400	67 100/1.01	182	180

The copolymers of series A have only the primary hydroxyl group at the end of the PEO block (Fig. 2.4), which means that all of the hydroxyls, primary and secondary end groups of the oligoglycidol blocks are active in the initiating step.

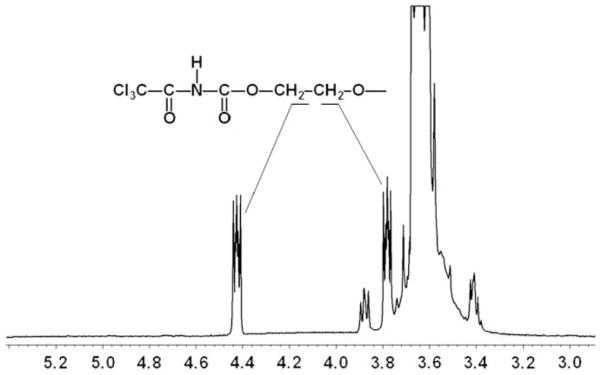


Fig. 2.4. ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of the dendritic star copolymer A/30 (see Table 2.6) after reaction with the trichloroacetylisocyanate

The convergence of data obtained from the analysis and calculation and the very low dispersion of the molar mass of the dendritic stars copolymers (Table 2.6) allow the synthesis of dendritic copolymers as well as the controlled process to be described. In this study, the dendritic star copolymers were examined for their thermal properties and their ability to solubilize hydrophilic compounds in hydrophobic solvents.

It was observed that the star and dendritic star copolymers of the degree of polymerization of EO with DP = 10 do not crystallize even at reduced temperature. Star-shaped copolymers with longer arms of PEO crystallize and have a melting point lower than their linear analogs of similar molar mass. This difference increases with increasing molar mass, and for the stars with a mass of approximately

10 000 g/mol, the difference is approximately 17 °C. This finding is most likely due to the branched structure of the macromolecule and the presence of the oligoglycidol elements that do not crystallize.

The branched structure, highly hydrophilic character of the oligoglycidol blocks and solubility of the star copolymers in low polar solvents, such as aromatic and chlorinated hydrocarbons, makes the solubilization of hydrophilic compounds by the tested copolymers possible. This process allows solutions of hydrophilic compounds to be obtained in a low polar solvent.

The results demonstrated that the insoluble in methylene chloride hydrophilic model – calmagite – dissolved in the tested solutions of copolymers, yielding a colored, slightly red solution (Fig. 2.5). The amount of solubilized calmagite depends strongly on the length of the arms of the copolymer – longer arms of copolymer solubilized more calmagite. Star copolymers dissolve more hydrophilic model compound than stars with branched arms. The solubilities of the model in the solutions of star copolymers and solutions of linear PEO homopolymer of similar molar mass were also compared. The branched copolymer structure of macromolecules can dissolve almost two times more calmagite than the linear PEO of similar molar mass.

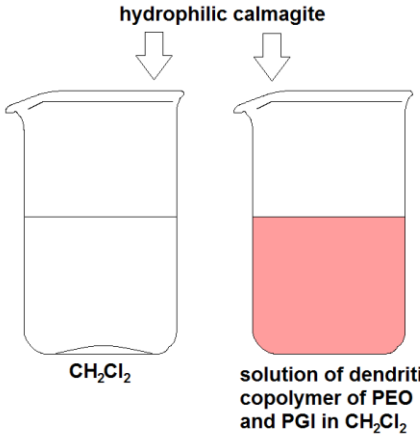


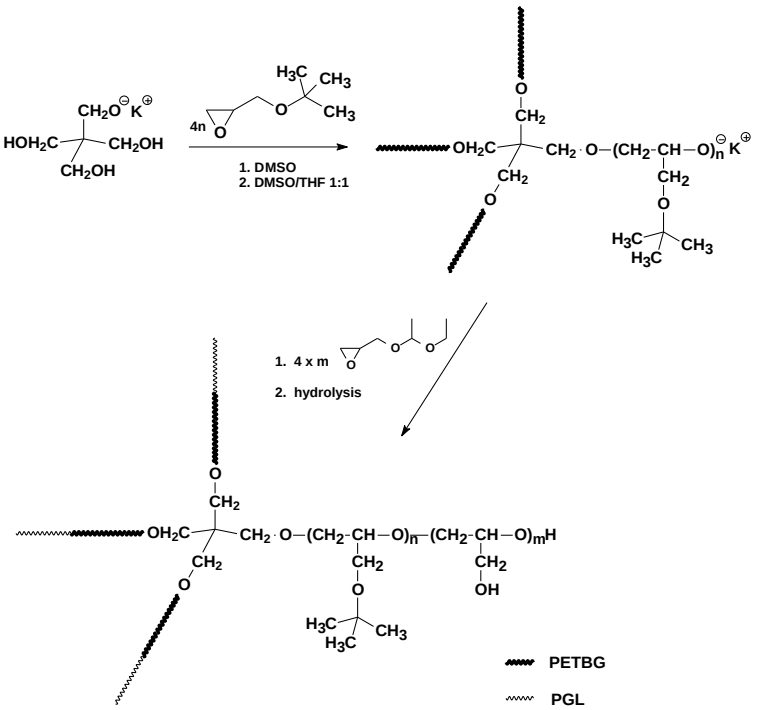
Fig. 2.5. Solubilization of calmagite in solution of dendritic star copolymers of ethylene oxide and glycidol

- **Star and dendritic copolymers of tert-butyl glycidyl ether (ETBG) and glycidol [H6] [H7]**

The branched copolymers of ethylene oxide and glycidol described in the preceding paragraph are highly hydrophilic and highly soluble in water. The lack of hydrophobic units prevents interactions with hydrophobic compounds and limits the application of such materials. To obtain branched polymers with similar architecture but constructed of hydrophobic and hydrophilic components, further research was performed aimed at obtaining star structures with hydrophobic blocks forming a core surrounded by hydrophilic polyglycidol blocks that provide water solubility. Polymers of this type, with the "core-shell" structure, are widely described in the literature [41-45] and can be used as nanocontainers of hydrophobic substance, allowing for delivery of substances insoluble in water. The problem that had to be solved was the selection of the appropriate hydrophobic oxirane comonomer and a ratio of reactants to prepare an appropriately loose hydrophobic core and a hydrophilic core insulating shell.

The synthetic method was very similar to the previously described syntheses. The selected hydrophobic comonomer – *tert*-butyl glycidyl ether (ETBG) is soluble in DMSO; however, as the chain grows, the polymer precipitates from the solution. This problem was solved, leading to initiation of the polymerization in the DMSO solution, while the further steps of polymerization were performed in a solution

of DMSO/THF. This procedure preserves the homogeneity of the polymerization system, allows for the fast exchange of the alkoxide – alcohol and facilitates the practically complete conversion of the monomer.



Scheme 2.8. Synthesis of star copolymers of poly(*tert*-butyl glycidyl ether) – block – polyglycidol arms

After the polymerization of the ETBG step to the living system, EEGE was added and the polymerization was continued to obtain the complete conversion of the second monomer (Fig. 2.8). Characterization of the products was performed for the stars directly after the completion of the polymerization of the EEGE block and after deprotection of the hydroxyl groups in the EEGE blocks. The results for the star copolymers of the poly(ETBG) -block-polyGL arms are shown in table 2.7.

Table 2.7. **.** Characteristics of the star copolymers of poly(*tert*-butyl glycidyl ether) – block – polyglycidol arms

	DP_{ETBG}:DP_{GL}		M_n			$Đ_M$	Weight % ratio of PGL
	Calculated from the feed ratio	^{1}H NMR	Calculated from the feed ratio	^{1}H NMR	SEC-MALLS		^{1}H NMR
1.	10:7	9.2:6.2	7 400	6 800	8 200	1.02	27
2.	10:12	9.0:11.5	8 900	8 200	9 100	1.01	41
3.	11:15	10.6:15.0	10 300	10 100	11 400	1.03	44
4.	10:17	9.5:16.9	10 400	10 100	11 600	1.03	50
5.	10:21	9.0:20.3	11 550	10 800	12 200	1.03	56

The syntheses were performed, selecting the ratios of the two monomers and the initiator to obtain copolymers with arms of similar length as the hydrophobic ETBG block and increasingly longer hydrophilic glycidol blocks. The results of the analyses indicate that this synthesis method allows well-defined copolymers of low molar mass dispersion and composition almost identical to the feed ratio of the comonomers to be obtained.

In addition to the synthetic investigation, the properties of the synthesized copolymers were also analyzed. Independent studies conducted by Dr. Agnieszka Kowalczuk defined the behavior of the copolymers in aqueous solutions. The dependency of the resulting structure and properties of the macromolecules of the studied copolymers on the synthesis was observed, as demonstrated below.

The molar ratio of the used comonomers strongly affects the properties of the obtained star copolymers in aqueous solutions. The copolymer with the shortest length of the polyglycidol block is insoluble in water. Copolymers with long hydrophilic polyglycidol blocks are thermoresponsive; these copolymers dissolve in water at room temperature, and during heating of the solutions of these copolymers, they become turbid. The transition temperature for these copolymers depends on the length of the hydrophilic block and falls in the range of 26-69 °C. The transmittance is plotted as a function of the temperature of the aqueous solutions of the soluble copolymers in figure 2.6.

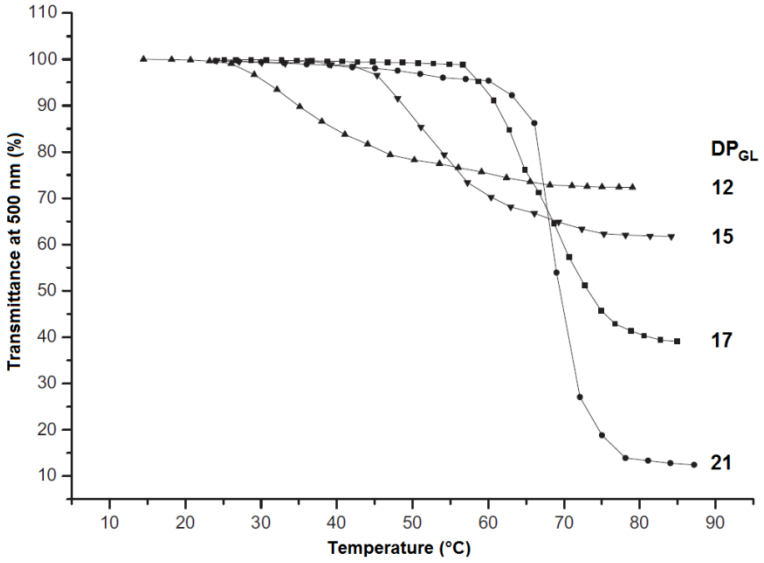


Fig. 2.6. Dependence of transmittance on the temperature of the water solution of star copolymers of poly(*tert*-butyl glycidyl ether) – block – polyglycidol arms of DP of glycidol block: 12, 15, 17 and 21

Below the transition temperature of the macromolecules, the water-soluble copolymers form micelles. Heating the polymer solution above the transition temperature causes aggregation of the micelles, and the solution becomes cloudy. The size of the micelles and the aggregates increase with increasing length of the hydrophilic polyglycidol blocks. The dependence of the hydrodynamic radius on the temperature is shown in figure 2.7.

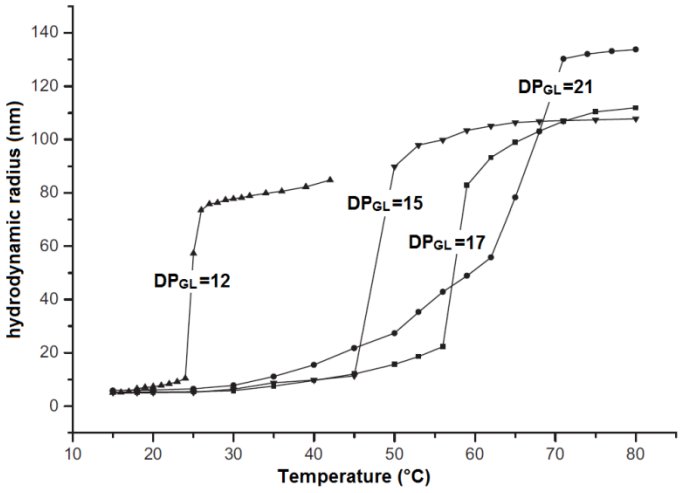
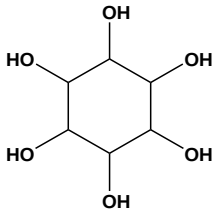


Fig. 2.7. The dependence of the hydrodynamic radius of star copolymers of arms of poly (*tert*-butyl glycidyl ether) – block – polyglycidol, with the DP of polyglycidol block:12, 15, 17 and 21 units in the water solution, on the temperature (concentration 2 g/L)

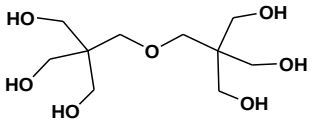
Investigation of the properties of the obtained copolymers reveals that controlling the synthesis of these copolymers allows control of several of the parameters responsible for their properties in a predictable and precise manner.

The hydrophilic polyglycidol blocks of the copolymers described in this section contain a significant amount of hydroxyl groups, which were used in the next stage of the research of the controlled synthesis of amphiphilic copolymers of "core-shell" structure.

The aim was to obtain more complex and branched star macromolecules of larger sizes using the previously obtained star copolymers for the synthesis. For this study, 4-armed star-shaped copolymers were used (for DP_{GL} = 7 and 12 – see Tab. 2.2.3.2. Pos. 1 and 2). To investigate the effect of the structure of copolymer macromolecules on the encapsulation ability of hydrophobic compounds, additional 6-armed star copolymers were synthesized in a similar manner as the four-armed, using inositol and dipentaerythritol as initiators.



inositol



dipentaerythritol

It was expected that the application of the macroinitiator with a star copolymer structure with different numbers of arms and hydroxyl groups allows to obtain in the next syntheses star-shaped copolymers of diverse dendritic arms and a hydrophobic core density and a number of hydrophilic arms creating the macromolecule shell.

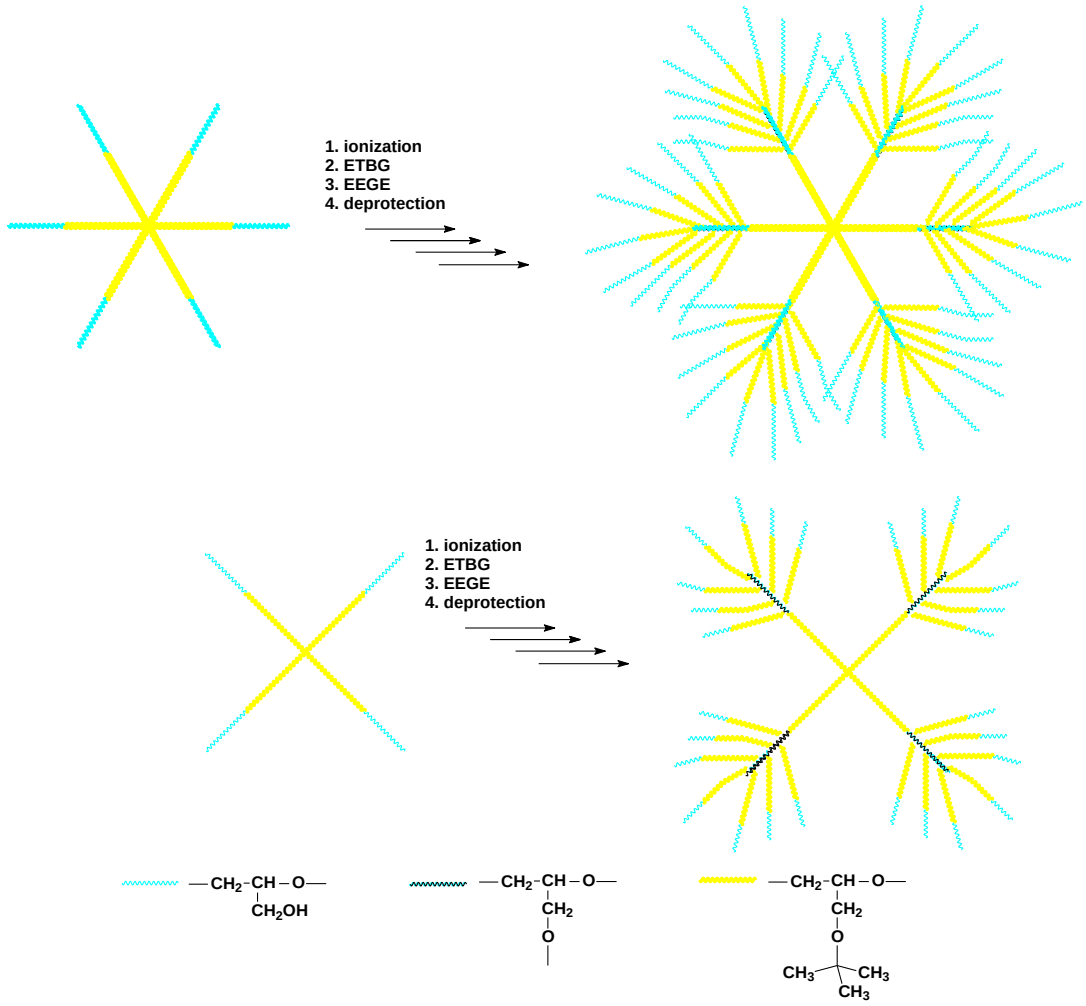
For the next step of the synthesis, four star copolymers were used as initiators: two 4-armed and two 6-armed. The characteristics of these copolymers are listed in table 2.8.

Table 2.8. Characteristics of 4- and 6-armed star copolymers of poly (*tert*-butyl glycidyl ether) – block – polyglycidol arms used for the synthesis of dendritic star copolymers

	Number of arms	DP_{ETBG}:DP_{GL}		M_n			$Đ_M$	Number of OH groups
	Initiator	Calculated from the feed ratio	^{1}H NMR	Calculated from the feed ratio	^{1}H NMR	SEC-MALLS		^{1}H NMR
1.	4 pentaerythritol	12:7	12:8	8 400	8 700	8 500	1.02	36
2.	4 pentaerythritol	10:12	9:11.5	8 900	8 200	9 100	1.01	50
3.	6 inositol	10:11	11:10.5	13 250	13 200	13 800	1.09	69
4.	6 dipentaerythritol	12:12	14:14	15 170	17 200	18 000	1.01	90

DP_{ETBG} and DP_{GL} are the degree of polymerization of *tert*-butyl glycidyl ether and glycidol, respectively, in the arms of the stars.

The synthesis was performed in the same way as for the star copolymers of ethylene oxide and glycidol; however, it was necessary to use a lower degree of ionization of the hydroxyl groups (less than 8 %) and to conduct the polymerization at a temperature above 60 °C. The course of the synthesis is illustrated in scheme 2.9.



Scheme 2.9. Synthesis of star copolymers of dendritic arms poly (*tert*-butyl glycidyl ether) -block-polyglycidol

As for the previous syntheses of amphiphilic star copolymers, it was necessary to add THF to the reaction mixture to maintain a homogeneous solution and achieve good control. The comonomer ratios were selected to obtain different lengths of the hydrophobic and hydrophilic blocks of the dendritic arms of the stars. The purpose of the selection of the monomer ratio applied was to obtain dendritic copolymers with varying thicknesses and densities of the hydrophilic outer shell. In all cases, very high monomer conversion rates above 97 % were achieved.

After hydrolysis of the acetal groups, four different star-shaped copolymers of dendritic arms with different contents of hydrophobic units were obtained. The characteristics of the copolymers are described in table 2.9.

Table 2.9. Characteristics of dendritic star copolymers of *tert*-butyl glycidyl ether and glycidol

Initiator *	Composition of arms of the shell DP_{ETBG}:DP_{GL}		M_n			$Đ_M$	Number [GL] in the shell (number of OH groups in the shell)
	Calculated from the feed ratio	^{1}H NMR	Calculated from the feed ratio	^{1}H NMR	SEC-MALLS		
1.	15:7	14:11	96 200	102 000	88 000	1.02	396 (432)
2.	9:11.5	9:13.5	110 000	117 000	116 000	1.03	675 (725)
3.	12:10	11.5:12	172 000	178 000	198 000	1.09	828 (897)
4.	8.5:12	8:12	197 000	191 000	226 000	1.06	1080 (1170)

* – the initiator used for the synthesis; number corresponds to the position of table 2.8.
DP_{ETBG} and DP_{GL} – degree of polymerization of the tert-butyl glycidyl ether and glycidol block, respectively

The differences in the determined values calculated from the feed ratio and from ^{1}H NMR spectra may result from the inaccuracy of the analytical method that uses the end group's signal of low intensity. The biggest difference between the molar mass determined from the end groups and the mass determined from the SEC-MALLS and calculated from the feed ratio occurs for the composition of item 1 in table 2.9, where the signal intensity is low, resulting from the low glycidol content and the small number of branches.

For all of the obtained copolymers, the molar mass dispersity is very low (less than 1.1), which indicates that, as in the previous case of the syntheses, the molar mass and composition of the copolymer can be very well controlled. An appropriate selection of these parameters allows control of the structure, size and density of the macromolecules, which directly affects the properties of the synthesized polymers. To illustrate the differences between the structure of 4- and 6-armed dendritic stars of different numbers and lengths of the hydrophilic blocks at the end of the branched arms, single arms of the copolymers from table 2.9 from position 1 and 4, are shown in figure 2.9.

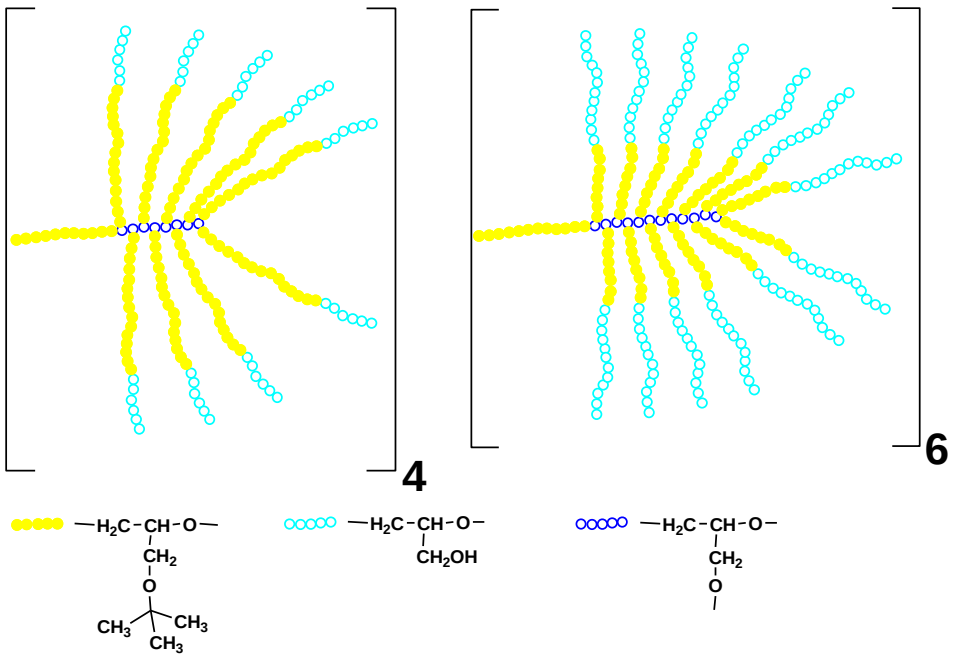


Fig. 2.9. Comparison of the arm structures of dendritic block copolymers of *tert*-butyl glycidyl ether and glycidol from position 1 and 4 of table 2.9

A larger number of hydrophilic blocks in the branches of the star (marked with blue circles in the figure) and six instead of four arms creates a more dense shell of the hydrophobic core.

The behavior of the obtained copolymers in solutions was investigated. All the copolymers dissolve well in dimethylformamide (DMF), a good solvent for both blocks, wherein the hydrodynamic radius increases from 6.4 to 8.0 nm, in accordance with the increasing molar mass of the copolymers.

Differences in the structure of these amphiphilic copolymers are visible in the interaction of these copolymers with water. The properties of the copolymers in water depend on the number of branches and the length of the hydrophilic polyglycidol blocks. The arithmetic product of the number of branches and the degree of polymerization of the blocks is the number of glycidol units in the shell of the macromolecule. The copolymer comprising the lowest number of glycidol units formed a cloudy solution, while the other, in which the number of repeating units of glycidol was greater, formed transparent solutions.

At room temperature, copolymers with the smallest molar mass and smallest number of glycidol units in the shell (pos. 1-3. Tab. 2.10) form aggregates in water, wherein the size of the aggregates decreases with an increasing number of glycidol units in the shell (Tab. 2.10). The copolymer with the thickest hydrophilic shell (the largest number of branches and the longest polyglycidol blocks, item 4 table 2.10) does not form aggregates in water.

Table 2.10. Characteristics of the star dendritic copolymers of *tert*-butyl glycidyl ether and glycidol in DMF and water solutions

Number of copolymer in table 2.9	Number of glycidol units in copolymer shell [GL]	R_h (nm)		
		DMF	H_2O	
		25 °C	25 °C	55 °C
1.	396	6.4	260	–
2.	675	7.2	20.0	89.0
3.	828	7.6	9.0	17.1
4.	1080	8.0	7.0	6.5

Thermosensitivity, frequently observed for amphiphilic copolymers, also occurs for the obtained copolymers. Copolymers 2 and 3 in table. 2.9 ([GL] = 675 and 828) are thermosensitive, wherein the transition temperature of their aqueous solution depends on the number of glycidol units in the shell. The copolymer with the highest number of glycidol units in the outer sphere of the macromolecules does not form aggregates in water (item 4 tab. 2.9) and is not thermosensitive.

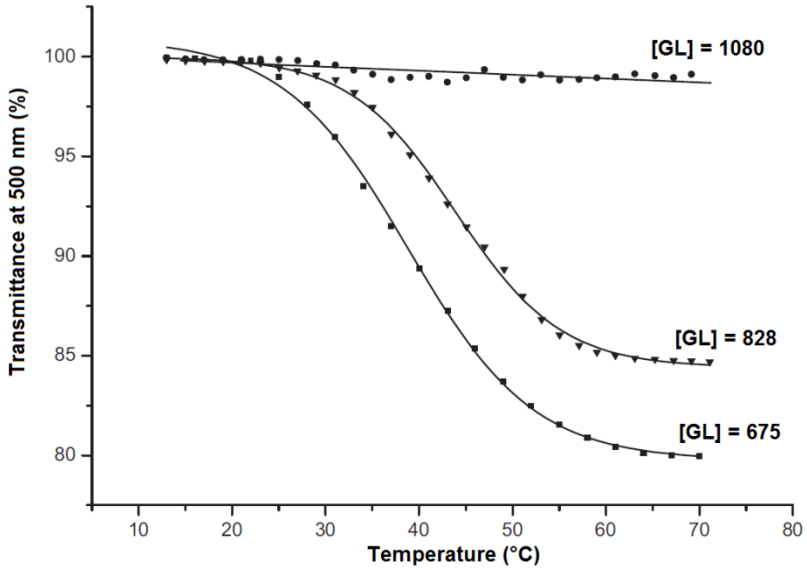


Fig. 2.9. The dependence of transmittance on the temperature of aqueous solutions of dendritic copolymers of *tert*-butyl glycidyl ether and glycidol. The value of [GL] corresponds to the number of glycidol units in the outer shell of the copolymer

The dependence of transmittance on the temperature of aqueous solutions of copolymers forming transparent solutions is illustrated in figure 2.9.

For the amphiphilic molecules, the main factor affecting the interaction with water is the mass percentage of the hydrophobic part in the molecule. For the investigated amphiphilic copolymers, the interaction with water is determined by the number of hydrophilic glycidol units in the shell, which depends on the degree of branching of the macromolecule and the degree of polymerization of the polyglycidol blocks. The number of glycidol units affects the degree of isolation of the hydrophobic core. If the shell is sufficiently thick and dense, the macromolecules are solvated and do not aggregate, which can be observed for the copolymer with 1080 glycidol units in the outer shell.

The effect of the macromolecular structure of the investigated copolymers is also evident in their ability to solubilize the water-insoluble hydrophobic model compound – pyrene. For the first three copolymers that form aggregates in water, the amount of pyrene molecules per copolymer macromolecule increases from 2.1 to 12.1; for the final copolymer, which does not aggregate in water, the dissolved pyrene molecules increased to above 53 per macromolecule of the copolymer. The ability to solubilize the hydrophobic compound is also determined by the appropriately extended hydrophilic copolymer shell that allows the hydrophobic core, which contains the hydrophobic pyrene, to be isolated. The studies described in this section demonstrate that by applying the precisely controlled synthesis of the polymers, polymeric materials with appropriate properties can be obtained.

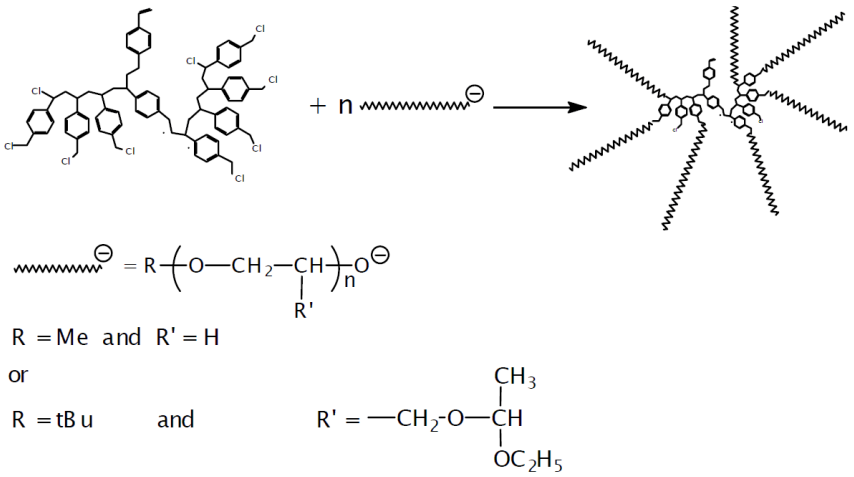
3. The structure of the polymer obtained by the termination of living polyoxirane chains

➢ Star copolymers of ethylene oxide and glycidol of core-shell structure [H8]

As described in section 2, the synthesis of stars using the "core-first" method, yielding copolymers with predictable and pre-planned structure of macromolecules, is based on the controlled polymerization of an oxirane monomer initiated by a multifunctional initiator. Another method to obtain polymers with a star structure is the "arm first" method, which consists of attaching the previously obtained polymer chains – arms with active end groups – to the multifunctional core. This method appears to be simpler; however, to achieve sufficient control, the termination reaction efficiency of the chain end groups with multifunctional active terminator must be sufficiently high.

In the author's studies on the synthesis of star copolymers of "core-shell" structure, the terminator obtained in the laboratory in research on the polymerization of *p*-chloromethylstyrene was used. Poly (*p*-chloromethylstyrene) with a molar mass of 1400 g/mol and dispersity 1.38, containing an average of nine chloromethylene groups in the macromolecule, was applied.

The synthesis of stars with a hydrophobic core and hydrophilic arms of poly (ethylene oxide) or polyglycidol was performed using a Williamson etherification reaction method. Polyoxirane chains with the alcoholate group at one chain end were terminated by poly(*p*-chloromethylstyrene) (Scheme 3.1).



Scheme 3.1. Termination of the living polyoxirane chains with branched poly(p-chloromethylstyrene) core

The reaction was tested for various lengths of poly(ethylene oxide) (Mn = from 750 to 5000 g/mol) and the polyglycidyl chain with a protected hydroxyl group (pEEGE) with Mn = 2300 g/mol. The reaction was monitored by SEC-MALLS analysis of regularly collected samples. SEC chromatograms of the samples that were acquired during the reaction of PEO of molar mass Mn = 2000 g/mol with a core of poly(*p*-chloromethylstyrene) are presented in figure 3.1.

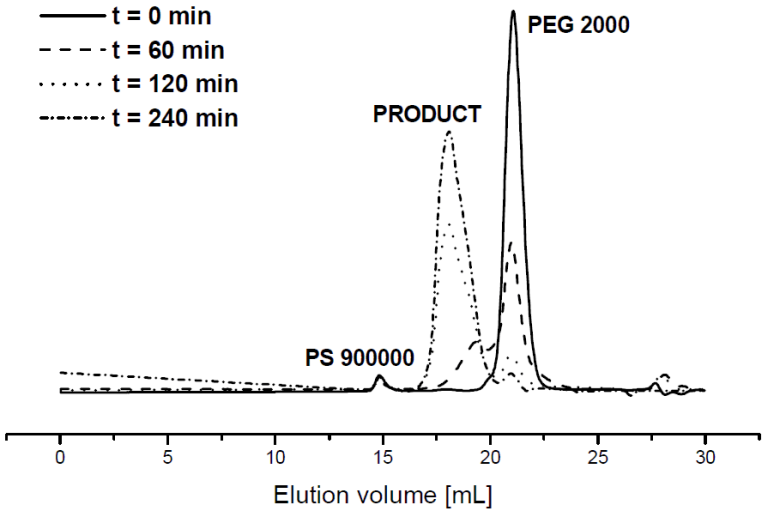


Fig. 3.1. SEC chromatograms of the reaction mixture PEO 2000 g/mol with a branched core of poly(*p*-chloromethylstyrene) for various reaction times

The yield for low-molar-mass compounds in the Williamson method is high, often above 80 %. The conducted investigation, however, demonstrates that in this case, the efficiency for polymers does not exceed 34 % of all the chloromethylene groups of the terminator. The yield is higher for shorter chains but decreases with increasing molar mass of the linear polyoxirane. The determined molar mass

of the products appears to be much higher than that expected from the calculations, considering the yield. The data are presented in table 3.1.

Table 3.1. The characterization of the products of termination of living polyoxirane chains with branched (*p*-chloromethylstyrene) core

Polymer and molar mass	M_n	M_n	$Đ_M$	Number of coupled cores	Number of arms
	Calculated from the feed ratio and yield	SEC-MALLS		Calculated from SEC-MALLS	
PEO 750	3 849	24 900	3.12	6.5	19
PEO 2 000	6 799	37 800	1.94	5.6	15
PEO 5 000	10 261	69 000	1.71	6.7	12
PEEGE 2 300	9 100	71 000	1.87	7.8	26

The higher molar mass of the termination product results from the coupling of hydrophobic cores during the etherification reaction. For confirmation, the cores of poly (*p*-chloromethylstyrene) were reacted with potassium *tert*-butoxide under similar conditions to those used in the synthesis of star copolymers. The crosslinked product was obtained.

The investigations did not establish the reason for the cores coupling during the etherification reaction. It is likely that the presence of the diamine (4,4'-dimethyl-2,2'-bipyridine), which is used in the synthesis of the poly(*p*-chloromethylstyrene) cores and is partly bonded to the polymer, may couple the cores by quaternization of both the nitrogen atoms of the diamine. A small nitrogen content in the purified core of poly(*p*-chloromethylstyrene) was confirmed by performing the elemental analysis of the polymers.

The studies of the synthesis of the star copolymers described under this point indicate that despite the use of a well-defined polymer chain and a core of low molar mass dispersity (less than 1.4), control of the molar mass of the stars and the uniformity of their composition cannot be achieved. This result occurs because of the low efficiency of termination likely caused by the relatively low concentration of alkoxide groups and isolation of chloromethylene groups already attached to the core polyoxirane chains. Another problem is the coupling of the cores and the formation of clusters containing between 5 to 8 cores.

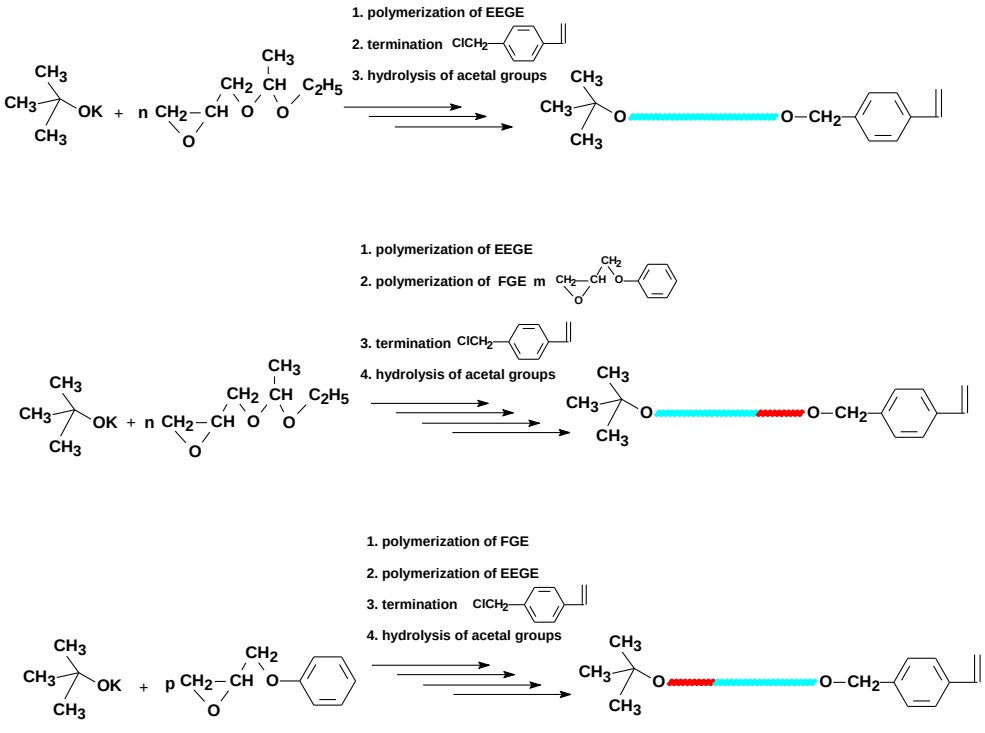
- **The synthesis of polymers of "bottle-brush" structure [H9]**

The previously described termination reaction of the polyoxirane chain with the polyfunctional terminator poly(*p*-chloromethylstyrene) does not allow good yields to be attained and does not lead to well-defined copolymers. The aim of the further study was to obtain branched macromolecules

of a "bottle-brush" structure with the polyoxirane chains using the monomeric *p*-chloromethylstyrene terminator. This path leads in a first step to the corresponding macromonomers and next, by the polymerization of such macromonomers, to the cylinder-like structure, where the core is linear styrene substituted by oxirane chains.

The research was directed to obtain three types of "bottle-brush" copolymers: two structures of amphiphilic character (a hydrophilic interior and a hydrophobic shell and a hydrophobic interior and a hydrophilic shell) and one with a hydrophilic interior and shell.

Phenyl glycidyl ether (FGE) was used for the synthesis of the hydrophobic blocks, and the hydrophilic polyglycidol blocks were obtained by polymerization of EEGE and hydrolysis of the acetal groups. The synthesis of macromonomers was performed using sequential block polymerization, wherein the second comonomer was added to the solution of the living polymer with active center after reaching a sufficiently high, close to 100 %, conversion of the first comonomer (Scheme 3.2). After the complete conversion of the second comonomer, *p*-chloromethylstyrene was added to the reaction mixture. Amphiphilic macromonomers were synthesized such that one the hydrophobic parts of the macromonomer chain consisting of pFGE was next to the termination group containing a polymerizable double bond, and in the second one, the hydrophobic part of the chain was separated from the double bond by the hydrophilic polyglycidol block.



Scheme 3.2. The synthesis of hydrophilic and amphiphilic polyoxirane macromonomers

The macromonomers were characterized before and after hydrolysis of the acetal groups in the chain of pEEGE using ^{1}H NMR and SEC-MALLS methods. The data obtained are presented in table 3.2.

Table 3.2. Characteristics of hydrophilic and amphiphilic polyoxirane macromonomers

		Macromonomers before hydrolysis of acetal groups in the PEEGE blocks			Macromonomers after hydrolysis of acetal groups in the PEEGE blocks		
		PEEGE-St	PEEGE-b-PFGE-St	PFGE-b-PFGE-St	PGl-St	PGl-b-PFGE-St	PFGE-b-PGl-St
Calculated from the feed ratio	*$DP_{PEEGE(Gl)}$	40	60	52	40	60	52
	DP_{PFGE}	–	8	7	–	8	7
	M_n	6 000	10 000	8 600	3 200	5 800	5 000
Calculated from the ^{1}H NMR using the intensity of styrene end groups	*$DP_{PEEGE(Gl)}$	55	100	85	60	110	95
	DP_{PFGE}	–	15	14	–	17	14
	M_n	8 100	17 000	14 700	4 600	10 800	9 400
Calculated from the ^{1}H NMR using the intensity of the initiator group (*t*-BuO)	*$DP_{PEEGE(Gl)}$	–	–	–	45	66	54
	DP_{PFGE}	–	–	–	–	10	10
	M_n	–	–	–	3 600	6 600	5 700
SEC-MALLS	M_n	7 000	10 600	9 000	4 100	7 200	6 000
	$Đ_M$	1.05	1.10	1.10	1.05	1.10	1.10

* – degree of polymerization of PEEGE block or PGL block after hydrolysis of acetal groups in PEEGE

The determinations of the degree of polymerization and the molar mass of the block macromonomers based on ^{1}H NMR spectra were performed using two methods. In one method, the signal of end *tert*-butyl groups was used, which is derived from the initiator; in the second method, signals of styrene end groups at the other chain end – from the attached terminator – were used. The obtained values are considerably different in some cases, wherein the molar mass calculated from the signal intensity of the *tert*-butyl groups, derived from the initiator, is closer to the values obtained from the feed ratios of the reactants and estimated by SEC-MALLS. In each case, the molar masses calculated from the intensity of signals of the styrene group (derived from terminator) are higher than the masses obtained from the SEC-MALLS method, which indicates that not all the chains are terminated by styrene groups derived from *p*-chloromethylstyrene. MALDI-TOF analysis of PEEGE-St macromonomer and PGL-St obtained after hydrolysis of acetal groups confirms the presence of two macromonomer populations – one with the styrene end group and one with a OH end group (Fig. 3.2). This finding demonstrates the incomplete termination of living chains by *p*-chloromethylstyrene but does not allow the yield of termination to be quantified.

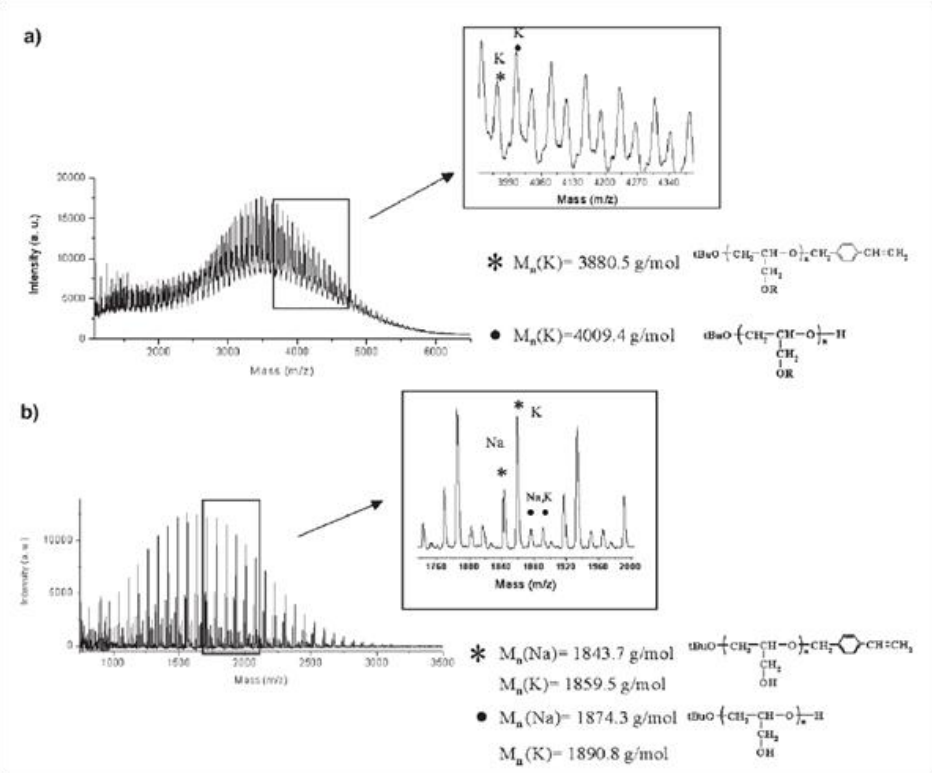
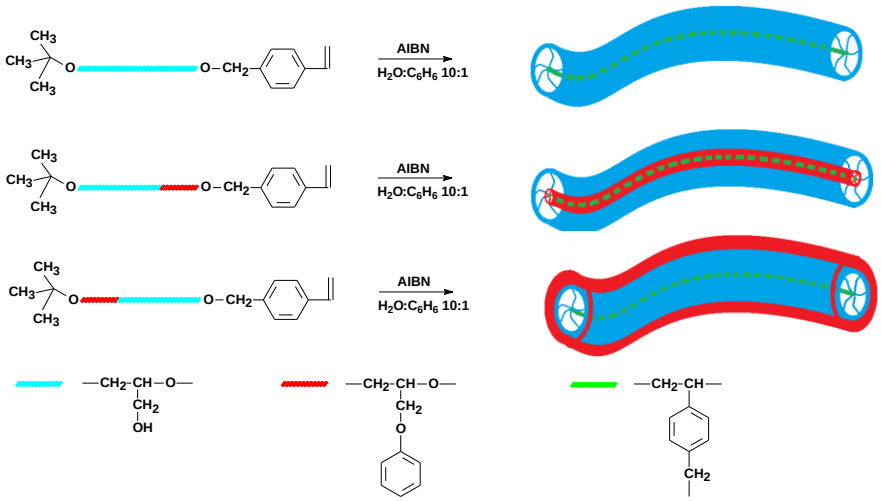


Fig. 3.2. MALDI-TOF spectrum: a) macromonomer PEEGE-St and b) macromonomer PGL-St – after deprotection of the EEGE block

After hydrolysis, three macromonomers were obtained: homopolymeric – PGL-St, a copolymer with a hydrophobic PFGE block next to the styrene group – PGL-b- PFGE-St and a copolymer, wherein the hydrophilic polyglycidol block was directly connected to the styrene end group – PFGE-b-PGl-St. Macromonomers subjected to free radical polymerization in water using AIBN as an initiator are shown in scheme 3.3.



Scheme 3.3. Free radical polymerization of polyoxirane macromonomers

Because of the insolubility of AIBN in water, an initiator was added to a mixture of the macromonomer/water in the form of a solution in benzene using such concentrations to always maintain the solvent ratio of water/benzene at 1/10. The polymerization occurs relatively rapidly, reaching conversion of approximately 50 % after 3 hours.

Attempts to achieve the higher conversion by performing the polymerization for a longer time or by adding a second portion of initiator did not change the molar mass or the conversion of the macromonomer. The results of the polymerization are presented in table 3.3.

Table 3.3. Characterization of the products of radical polymerization of polyoxirane macromonomers

Macromonomer	Concentration of macromonomer	Macromonomer conversion	Mn	$Đ_M$	DP of polymacromonomer
	%	%	SEC-MALLS		
PGl-St	10	50	74 000	1.4	18
	13	50	91 000	1.6	22
	25	49	140 000	1.9	34
	50	47	253 000	2.1	61
PGl-b-PFGE-St	10	50	143 000	1.4	18
	13	48	257 000	2.0	32
	25	50	473 000	2.1	60
PFGE-b-PGl-St	10	53	211 000	2.0	31
	13	53	370 000	2.6	47
	25	49	420 000	3.2	63

The polymerization products were separated from the unreacted macromonomer using two methods: fractionation and dialysis. Dialysis was proved to a more effective method. The resulting fractions having a molar mass similar to that of the macromonomers were analyzed by MALDI-TOF and ^{1}H NMR. Both analyses revealed that the separated fraction consisted of chains with molecular weights similar to the weight of the macromonomers but containing the hydroxyl group at the end instead of styrene. Thus, from the efficiency of the polymerization and analysis of the polymerizable fraction, it can be concluded that the efficiency of termination is approximately 50 % and that the conversion of the macromonomer, which is terminated by the styrene group, is almost 100 %.

The increase in the molar mass and molar mass dispersity of the obtained “bottle-brush” polymacromonomers with increasing initial macromonomer concentrations and high conversion

in radical polymerization of the macromonomer reaching 100 % may indicate that the macromonomer is polymerized in the aggregates of varying sizes swollen by benzene, as for the described in literature amphiphilic PEO macromonomers [46]. The local concentration of the polymerizable styrene group in such aggregates is much higher than that in the aqueous phase, and the rate of termination is controlled by diffusion due to the strong environment density. It is therefore possible, in a certain range, to control the molar mass of polymacromonomer by changing the initial concentration but at the expense of molar mass dispersity.

Polymerization of polyglycidol homopolymer macromonomers leads to hydrophilic polymers of "bottle-brush" structure of nearly equal length of arms (branches). Copolymer macromonomers provide polymers with a hydrophobic interior and hydrophilic shell (PGL-b-PFGE-St) or a hydrophilic interior and hydrophobic shell (PFGE- b-PGL-St). The proper selection of macromonomers in the synthesis determines the character of "bottle-brush" polymacromonomers as well as their properties and applications.

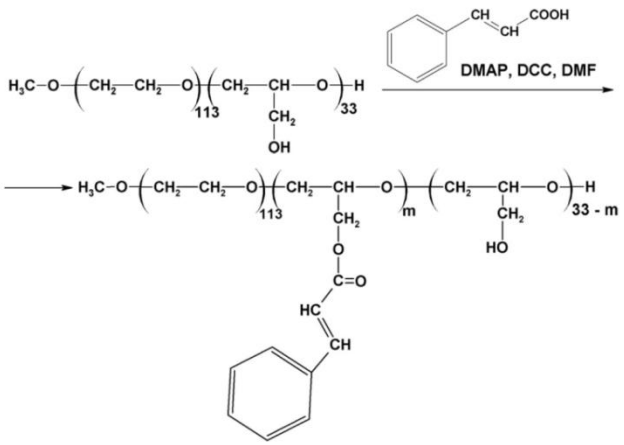
4. Polyether nanoparticles obtained by the cross-linking of polyoxirane chains using the hydroxyl groups in the polyglycidol block [H10]

Amphiphilic copolymers often associate in aqueous solution forming micelles, the size of which depends on many factors related to the structure of the copolymer such as the type and ratio of the comonomer in the amphiphilic chain, distribution of the blocks, presence of large hydrophobic end groups and degree of substitution of the comonomer unit with a pendant functional group.

Such micelles are not stable. One of the methods of stabilization of micelles in water is a coupling of chains by covalent bounds [47-49]. The potential applications of the resulting nanoparticles are very broad and depend on the type of copolymer and the method for stabilizing the micelles (stabilization of the core or shell). The presence of the hydroxyl group of the glycidol repeating unit allows the introduction into the chain of a group that is active in the chain coupling in the micelle and in the modification of the character of the micelles.

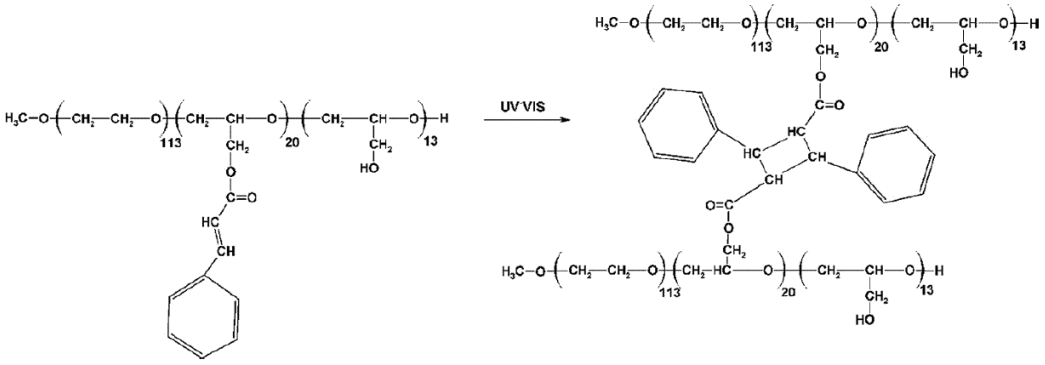
The aim of the study was to apply the organization of amphiphilic copolymers of oxirane in the water and form bonds between chains using hydroxyl groups in glycidol units to obtain stable nanoparticles with a "core-shell" structure.

For the study of the organization of amphiphilic copolymers, conducted in the laboratory by Dr. hab. Barbara Trzebicka, the synthesis method of block copolymers consisting of a poly(ethylene oxide) block and polyglycidol block of low molar mass dispersity ($Đ_M$ = 1.02) and a well-defined composition was elaborated. The synthesis was performed by initiating the polymerization of EEGE by the cesium alkoxide of monomethyl ether of poly(ethylene oxide) 5 000 g/mol. After hydrolysis of the acetal groups and complete characterization of the resulting copolymer, part of the hydroxyl groups was esterified with cinnamic acid (Scheme 4.1), which dimerized under the effect of UV radiation (Scheme 4.2).



Scheme 4.1. Modification of PEO-b-PGL by esterification with cinnamic acid

Esterification of the block copolymer Me-EO_{113}-b-Gl_{33} leads to the formation of approximately 20 units of glycidyl cinnamate in one chain, which allows for a stable covalent link of chains by the formation of dimers of two amino acid connected to two different chains. The presence of more cinnamate groups in the chain and its dimerization with cinnamate groups attached to other chains leads to crosslinking and stabilization of the micelles.



Scheme 4.2. Dimerization of cinnamate groups in the block copolymers of PEO-b-(GI-co-CGI)

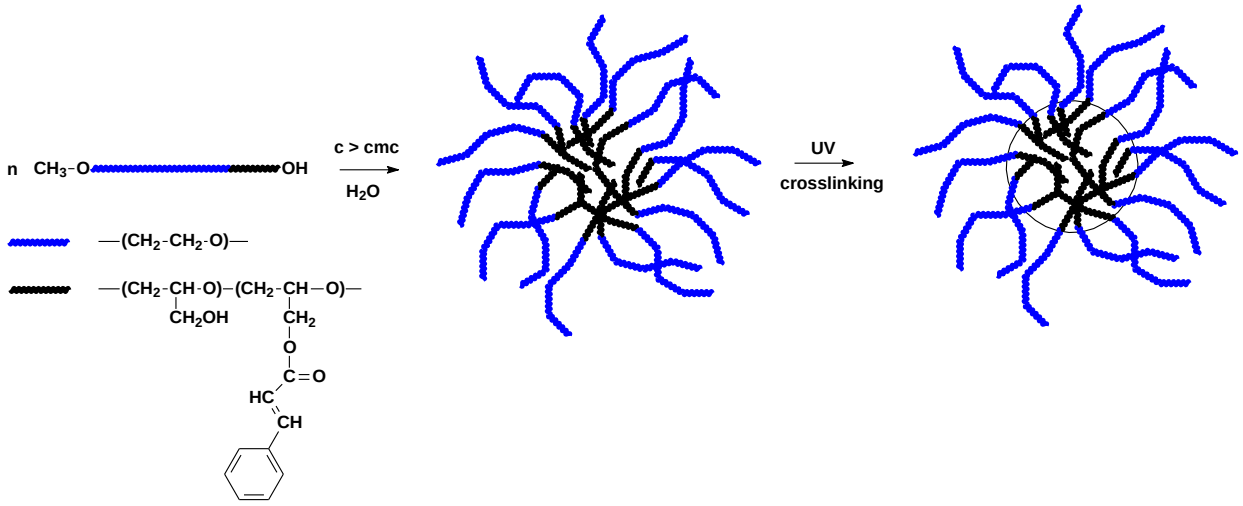
The characteristics of the modified copolymers are shown in table 4.1.

Table 4.1. Characteristics of the copolymers obtained by modification with cinnamic acid

copolymer	M_n	M_n	$Đ_M$	% of converted OH groups to cinnamate
	^{1}H NMR	SEC-MALLS	SEC-MALLS	
ME-EO_{113}-b-Gl_{33}	7 500	7 200	1.02	-
ME-EO_{113}-b-(Gl_{13}-co-GlC_{20})	10 100	9 900	1.02	60
ME-EO_{113}-b-(Gl_{17}-co-GlC_{16})	9 600	9 400	1.02	48

The amphiphilic copolymers obtained by esterification are soluble in water; however, after exceeding the critical micelle concentration, they form micelles. The PEO block that is present in the hydrophilic block of the copolymer should form a shell covering the micelles core composed of the hydrophobic blocks of cinnamic acid ester. Research results indicate that the hydrodynamic radius of the formed micelles depends on the degree of esterification (from 9.2 to 11.9 nm) and does not change with temperature in the range of 25 °C to 55 °C.

To obtain nanoparticles by the crosslinking of the copolymers, the aqueous solutions were prepared at a sufficiently high concentration (2.5 g/L) to form micelles (Scheme 4.3.).



Scheme 4.3. Formation and stabilization of amphiphilic PEO-b-(GI-co-CGl) copolymer micelles with cinnamate groups

These solutions were irradiated with UV light of 254 nm and tested for loss of double bonds in the cinnamate group by measuring the absorbance at a wavelength of 274 nm (Fig. 4.1).

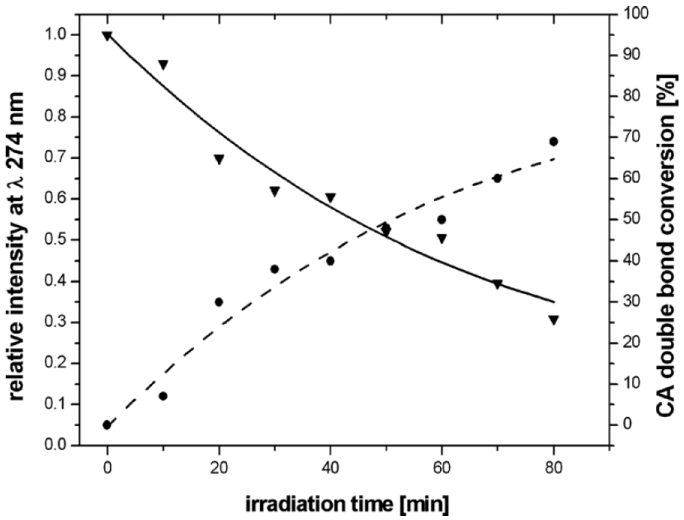


Fig. 4.1. The dependence of the relative intensity of the absorbance cinnamate groups in the copolymer and conversion of cinnamate groups depending on the UV exposure time

After 80 minutes of reaction, the amount of double bonds decreases to approximately 25 % of the initial quantity.

The formation of the nanoparticles was observed through the SEC analysis of samples collected during the UV exposure of the mixture (Fig. 4.2).

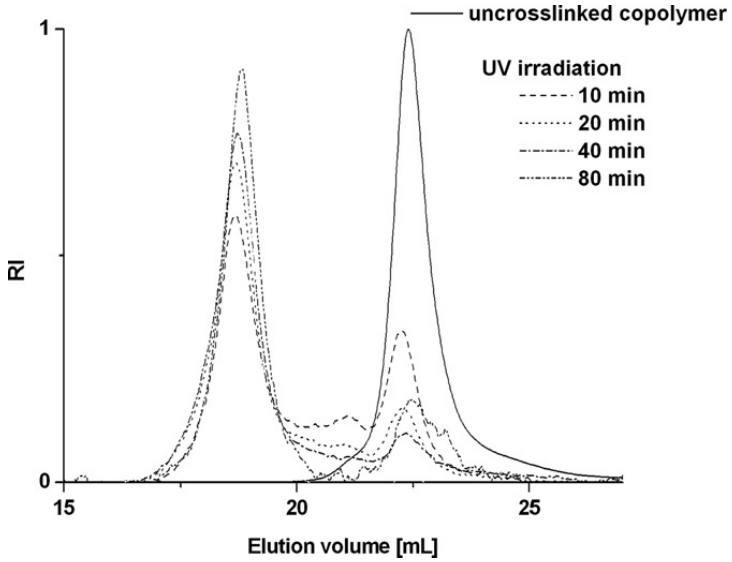


Fig. 4.2. SEC chromatograms of the copolymer after various exposure times of the micelles formed in water. Non-crosslinked micelles disintegrate under SEC analysis conditions and appear as a signal derived from the copolymer

The process of crosslinking of the micelles does not change their size and only slightly reduces the size dispersity. The distributions of the hydrodynamic radius of the non-crosslinked micelles and nanoparticles after crosslinking are shown in figure 4.3.

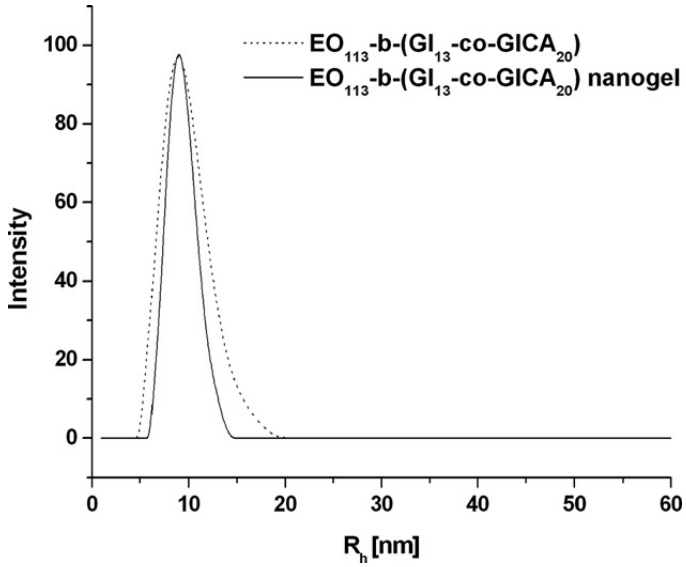


Fig. 4.3. Hydrodynamic radii R_h (25 °C, water) of copolymer Me-EO_{113}-b (GI_{13}-co-CGI_{20}) before and after stabilization of the micelle

Investigation allows stable nanoparticles with a size of approximately 9 to 12 nm with the hydrophilic shell of PEO chains and a hydrophobic core to be obtained. The size of the nanoparticles depends on the degree of substitution of the polyglycidol block.

5. Summary

The work described in the dissertation led to branched polymers and copolymers of oxiranes of diverse macromolecular architecture (star, dendritic star and "core-shell" structures; "pom-pom" and "bottle-brush" structures; hyperbranched; and nanoparticles). The method developed based on the use of the hydroxyl group of the glycidol unit to create branching allows for the controlled synthesis of branched polyoxiranes of predetermined architecture of a wide range of molar masses and low dispersity. The appropriate selection of the type of oxirane comonomers, synthesis methods and ratio of reactants allows the preparation of a material with desired properties that is suitable for applications through the control of the macromolecule parameters (the degree of branching, number of end groups, distribution of branches and distribution of hydrophilic and hydrophobic blocks in the macromolecule).

Polymers and copolymers containing glycidol units contain many terminal hydroxyl groups. By selecting the appropriate ratio of the reactants, polymers with suitable hydroxyl functional groups which can be easily used for modification, can be prepared.

The multiple grafting method of the polyglycidol chain developed by the author led to branched homopolymers of glycidol that were not previously described in the literature with high molar masses (1.8×10^6 g/mol) and low dispersity.

Some of the polymers described in the dissertation are now being used for research into the modification and preparation of surfaces with antifouling properties, to reduce proteins and cell adhesion.

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