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Polyglycidol — how is it synthesized and what is it used for?

Summary — This paper presents a short review on the synthesis and properties of polyglycidol (PGL) and its derivatives and on selected medical applications of polyglycidol-containing materials. These materials are often used in the fabrication of medical diagnostic tests and biosensors as well as in bioseparation, biocatalysis and drug delivery systems. Various methods for the polymerization of glycidol (cationic, anionic) are described. Regardless of the synthesis method, each glycidol polymerization process yields branched macromolecules. However, glycidol with protected hydroxyl group can be anionically polymerized, which yields linear polyglycidol after deprotection of the hydroxyl groups. Modifications of the polyglycidol hydroxyl side and end groups and the syntheses of polyglycidol-containing copolymers with various architectures are discussed. A macromonomer, the polyglycidol derivative, α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol was used as a surfmer in emulsion polymerization of styrene in water. This synthesis method produces core-shell microspheres [P(S/PGL)] that possess a very low (usually less than 1.06) diameter dispersity parameter D_w/D_n (where D_w and D_n denote the weight and number average diameters, respectively). The relationships between the concentration of macromonomer in the polymerization mixture and the concentration of polyglycidol in the particle interfacial layer, final particle diameters and the suitability of the particles for binding biomolecules are discussed. Selected applications of the polyglycidol macromonomer and P(S/PGL) microspheres for the preparation of some materials are described.
Keywords: polyglycidol, macromonomer, microspheres, surfmer, diagnostic tests.

POLIGLICYDOL — SYNTEZA I ZASTOSOWANIE
Streszczenie — Artykuł stanowi krótki przegląd metod syntezy i właściwości poliglicydolu (PGL) oraz jego pochodnych a także opisuje wybrane zastosowania medyczne materiałów z ich udziałem. Materiały takie są wykorzystywane często do wytwarzania diagnostycznych testów medycznych i biosensorów, jak również w bioseparacji, biokatalizie i systemach dostarczania leków. Omówiono kationową i anionową metodę polimeryzacji glicydolu. Niezależnie od sposobu jej przeprowadzenia, uzyskuje się produkt o rozgałęzionej strukturze łańcucha. Liniowe polimery glicydolu mogą być natomiast otrzymane w wyniku anionowej polimeryzacji glicydolu zawierającego zablokowaną grupę hydroksylową, po przeprowadzeniu deprotekcji. Przedstawiono sposób modyfikacji końcowych oraz bocznych grup hydroksylowych poliglicydolu oraz syntezę kopolimerów glicydolu o różnej architekturze makrocząsteczki. Opisano zastosowanie makromonomeru α-*tert*-butoksy-ω-winylobenzylo-polyglycidolu jako surfmeru w emulsyjnej polimeryzacji styrenu w wodzie. W taki sposób wytworzono mikrosfery [P(S/PGL)] typu rdzeń-otoczka (*core-shell*) o bardzo małej (zwykle poniżej 1,06) dyspersji rozmiarów D_w/D_n (gdzie D_w i D_n oznaczają, odpowiednio, liczbowo średnią i wagowo średnią średnicę mikrosfer). Przedstawiono zależności pomiędzy stężeniem makromonomeru w mieszaninie reakcyjnej a stężeniem poliglicydolu w granicznej warstwie cząstek, ostatecznym rozmiarem powstających cząstek oraz przydatnością tak otrzymanych mikrosfer do wiązania związków biologicznych. Opisano także wybrane sposoby wykorzystania makromonomeru poliglicydolowego oraz uzyskanych mikrosfer P(S/PGL) do syntezy materiałów do zastosowań biomedycznych.
Słowa kluczowe: poliglicydol, makromonomer, mikrosfery, surfmer, testy diagnostyczne.

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INTRODUCTION

In early chemistry research, researchers concentrated on developing methods that were suitable for the synthesis and modification of small molecules. During the first half of the twentieth century, when the concept of large

molecules was formulated and supported with experimental data, many researchers shifted their attention to the synthesis of macromolecules, *i.e.*, processes in which small molecules are used as building blocks. Progress in this field has led to the development of methods that allow for the controlled synthesis of macromolecules with the required molar mass and molar mass distribution as well as controlled microstructure. The development of polymers, which are materials that are composed of these macromolecules, has resulted in an almost countless number of products that are found in many important applications.

In the last few decades, one can observe increasing interest in processes during which objects considerably larger than macromolecules (with dimensions ranging from approximately tenths of nanometers to hundreds of micrometers) are formed. These objects usually possess complex internal structures.

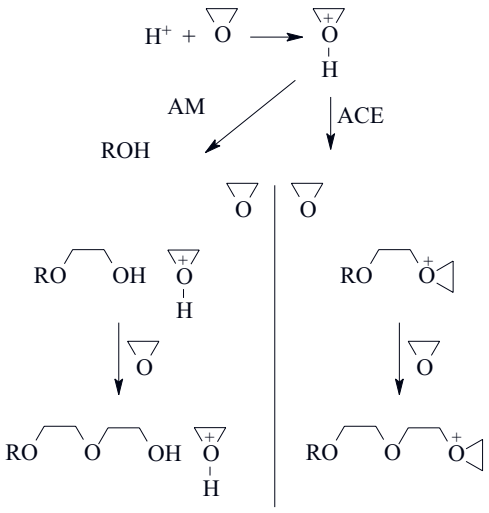
This paper provides examples of studies on syntheses conducted at molecular, macromolecular and particle levels. These studies focused on glycidol (GL), polyglycidol (PGL) and polyglycidol derivatives. The main chain of polyglycidol is similar to that of poly(ethylene oxide), which is a polymer that is found in many applications in fundamental biomedical studies and in pharmaceutical and medical applications, including the formulation of drug carriers, the preparation of implants and the modification of surfaces of elements of therapeutic and diagnostic devices that are in contact with tissue and/or body fluids. However, the advantage of using linear polyglycidol rather than linear poly(ethylene oxide) is related to presence of -OH functional group in each polyglycidol monomeric unit. These hydroxyl groups are suitable for further modifications that allow the properties of polyglycidol-containing materials to be changed in a simple manner.

This mini-review presents the reactions involved in the synthesis of linear and branched polyglycidol as well as polyglycidol macromonomers and in the one-batch synthesis of core-shell particles in which the shells are enriched in polyglycidol segments. Results from investigations of the properties of polyglycidol macromolecules, macromolecular assemblies and polyglycidol-containing particles are discussed. In addition, the potential applications of some of these products in medicine are presented.

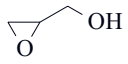
2,3-EPOXYPROPANOL-1 (GLYCIDOL) AND ITS POLYMERS

Oxiranes are relatively easy to obtain, inexpensive monomers that have a high reactivity. The majority of oxiranes polymerize both under the action of anionic and cationic initiators. The mechanisms of polymerization have been studied in detail and are known [1—3].

The polymerization of oxiranes initiated by the cationic initiators may proceed through two routes (Scheme A). The „classical" active chain end mechanism (ACE), which has been demonstrated to occur in most cases, assumes propagation *via* onium active centers at the chain end [4], thus giving rise to side reactions, first of all cyclization, and strong deviations from the living character. However, if hydroxyl group-containing compounds are present in the system, the reaction proceeds *via* the activated monomer mechanism (AM), in which the active centers are located at the monomer rather than at the chain end [5].



Scheme A. Cationic polymerization of oxiranes; activated monomer (AM) (left) and active chain end (ACE) (right) mechanisms



(I)

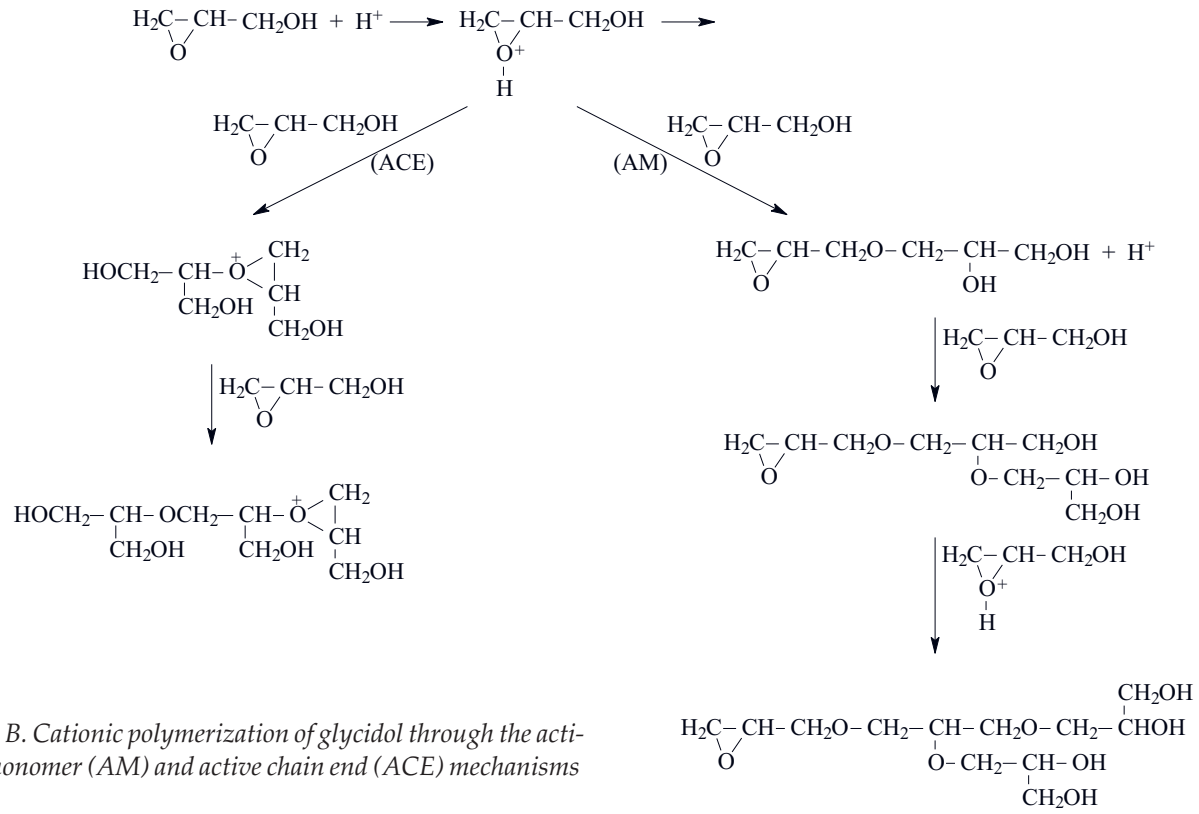
Glycidol [Formula (I)] is a unique oxirane.

The glycidol molecule contains a hydroxyl group adjacent to the oxirane ring, which strongly influences the mechanism, the course of polymerization and the properties of the obtained polymers.

The presence of a hydroxyl group bound to each glycidol molecule makes the activated monomer mechanism possible. The fraction of chains formed by this mechanism during the polymerization of glycidol has been investigated [6, 7], but regardless of the quantitative data, it is clear that this process (Scheme B) yields highly branched polymers.

Branched polymers also result from the anionic polymerization of glycidol [8] (Scheme C). Here, the fast proton exchange between the alcoholate anion and the alcohol group, which is considerably faster than the chain growth, is responsible for the multiplication of active centers and chain branching.

The latter process has been optimized to obtain highly branched polymers (degree of branching close to 100 % according to Frey's definition [9]) and to some extent to control the molar masses („slow monomer addition") [8, 10, 11].



Scheme B. Cationic polymerization of glycidol through the activated monomer (AM) and active chain end (ACE) mechanisms

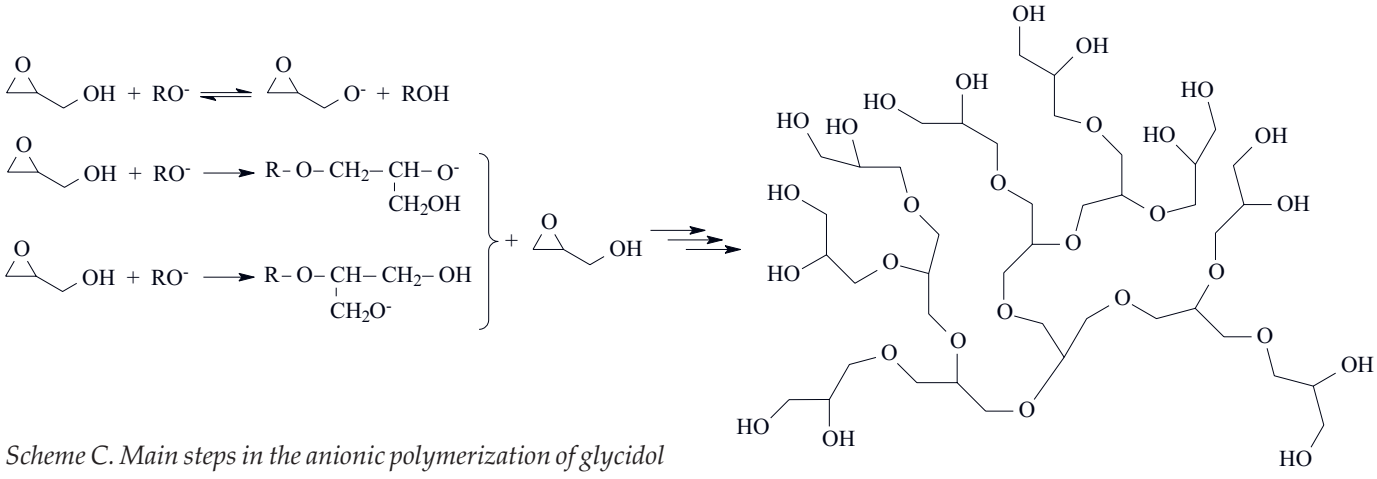
Regardless of the mechanism, the polymerization of glycidol itself yields branched macromolecules because the numerous hydroxyl groups that are present in the monomer and in the formed macromolecules considerably influences the process. Moreover, control over the process is rather limited. An upper molar mass limit of branched polymer *ca.* 20 000 g/mol can hardly be exceeded, which is most likely due to the transfer of active centers not thoroughly studied.

To obtain well-defined linear macromolecules of polyglycidol, the hydroxyl groups should be protected. The protecting groups must be inert during polymerization and easily cleavable after the growth is completed or otherwise interrupted. Among the numerous hydroxyl protecting groups known in organic chemistry, four were applied in this case: the *tert*-butyl [12—14], allyl [12], trimethylsilyl [15] and the 1-ethoxyethyl [16] groups. The last one is by far the most frequently used.

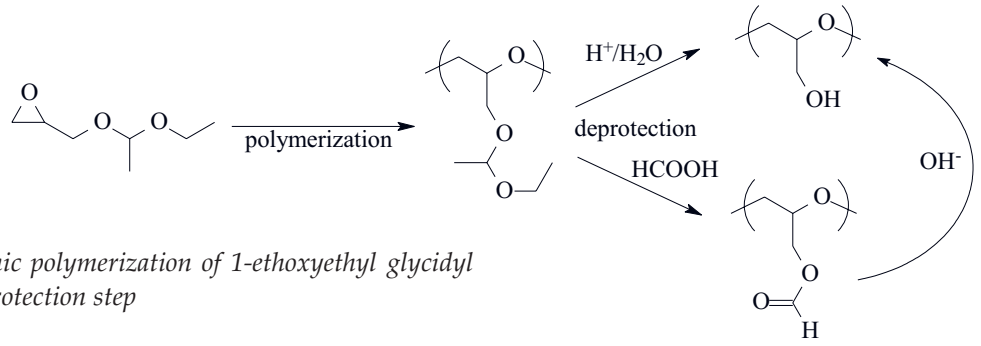
When the coordinative Tsuruta catalyst ($ZnEt_2/H_2O$) is used to polymerize 1-ethoxyethyl glycidyl ether, molar masses of up to 2 million are easily obtainable [17, 18]. Otherwise, the anionic polymerization of 1-ethoxyethyl glycidyl ether (Scheme D) leads to lower molar masses; however, when this limit is not exceeded, the polymerization can essentially be considered to be living.

The 1-ethoxyethyl protecting group may easily be removed under mild acidic conditions or converted to formic ester and then hydrolyzed to polyglycidol without causing measurable degradation of the polyether chain [13].

Polyglycidol is a highly hydrophilic polymer, and it is miscible with water in any proportion. For the subject of



Scheme C. Main steps in the anionic polymerization of glycidol



Scheme D. Anionic polymerization of 1-ethoxyethyl glycidyl ether and the deprotection step

this report, note that polyglycidol exhibits good biocompatibility, both in solution [19] and as a crosslinked layer [20].

The living character of the anionic polymerization of protected glycidol opens considerable synthetic possibilities, which are typical for living anionic polymerization processes. For example, block copolymers with ethylene oxide [21, 22] or propylene oxide [23] may be easily obtained by a subsequent monomer addition [24, 25].

The hydroxyl group in the repeating chain units of polyglycidol is reactive. This property may be used to modify the chains to obtain thermoresponsive polymers with an easily tunable transition point [26—28]. The hydroxyl groups may also be used for crosslinking, which leads to the formation of hydrogels with a controllable degree of swelling (up to 6000 %) that sometimes exhibit thermoresponsivity [21, 29].

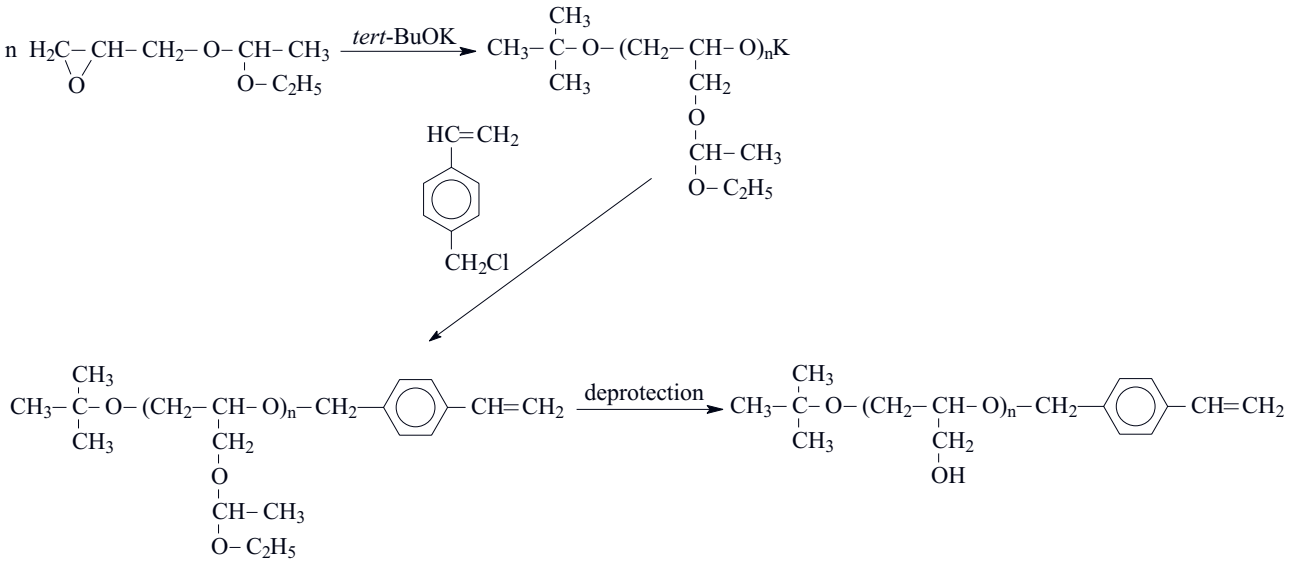
The abovementioned living character of the anionic polymerization of protected glycidol implies that the active alcoholate centers live so long that they may be reacted with electrophiles after the desired degree of polymerization has been reached. In this way, different functionalities may be introduced as the end groups.

The termination of the living poly(1-ethoxyethyl glycidyl ether) chains with *p*-chloromethylstyrene attaches styryl end groups to the polymer chains (Scheme E). These end groups may be attached either directly, or *via* a linker — hydrophilic (*e.g.*, PEO segment) or hydrophobic [*e.g.*, poly(phenyl glycidyl ether)] chain.

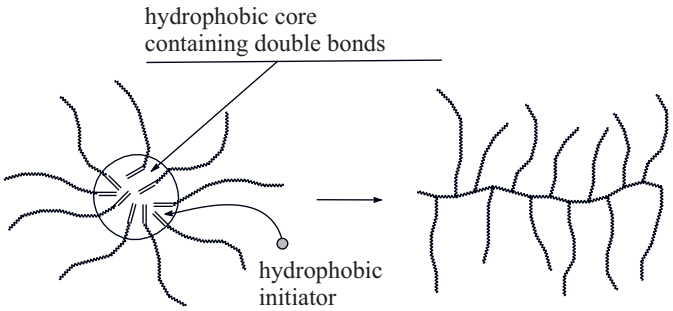
The ethoxyethyl groups of poly(1-ethoxyethyl glycidyl ether) can be easily hydrolyzed [30]. The hydrolysis (Scheme E) converts the poly(1-ethoxyethyl glycidyl ether) macromonomer, which is soluble exclusively in organic solvents (*e.g.* methylene chloride), into a water-soluble polyglycidol macromonomer (α-*tert*-butoxy-ω-vinylbenzylpolyglycidol).

The macromonomers obtained using the aforementioned route are amphiphilic. The „tail", which contains polyglycidol, is highly hydrophilic, whereas the „head", which is the styryl unit, is hydrophobic. This structure is typical for surfactants, which, because of their ability to self-organize in aqueous solution and their specific interactions with hydrophobes, are widely applied as micelle forming species or soaps.

Although the termination of the living poly(1-ethoxyethyl glycidyl ether) with *p*-chloromethylstyrene does not quantitatively lead to the styrene-terminated macromonomer, the obtained macromonomer may be polymerized in water with a surprisingly high yield to high molar mass comb-like polystyrene-graft polyglycidol



Scheme E. Synthetic pathway for obtaining the polyglycidol macromonomer



Scheme F. Polymerization of the macromonomer that contains hydrophobic chain ends in water

[31]. This behavior is frequently attributed to the self--organization of the amphiphilic macromonomer, which leads to the high local concentration of the hydrophobic reactive styryl groups, forming is a domain that is easily penetrable by the hydrophobic radical initiator (Scheme F).

More important, it has been demonstrated that the surfactant-like behavior of the styrene-terminated polyglycidol macromonomer stabilizes the emulsion of styrene in water, which enables the synthesis of polystyrene microspheres under surfactant-free conditions [32].

SYNTHESIS AND PROPERTIES OF POLY(STYRENE/ /POLYGLYCIDOL) MICROSPHERES [P(S/PGL)]

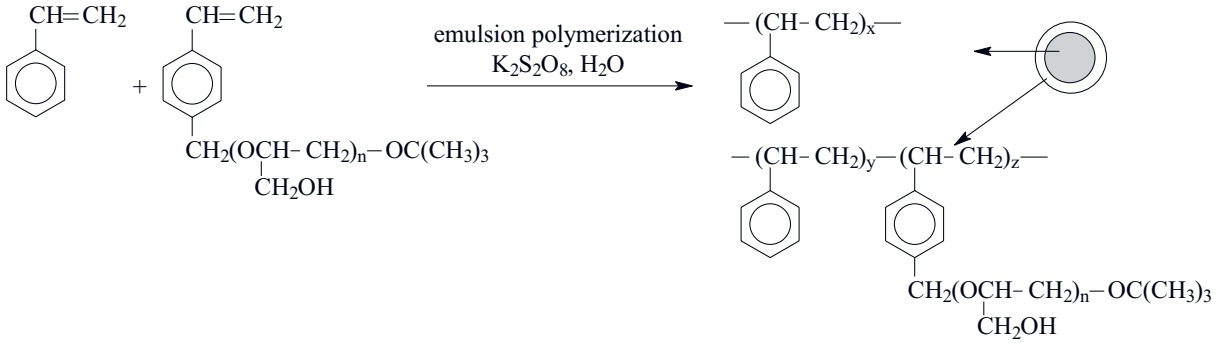
The amphiphilic macromonomer, α-*tert*-butoxy-ω-vinylbenzylpolyglycidol, which is obtained as shown in Scheme E, can be used in the emulsion polymerization of hydrophobic polymers, for example, styrene, as a surfmer (*i.e.*, as a compound capable of functioning as a surfactant and a monomer capable of copolymerizing with styrene). In this process, the surface active species that contain polyglycidol segments are covalently bound in the interfacial layer of growing particles.

The emulsion copolymerization of polyglycidol macromonomer (after removal of the ethoxyethyl protecting groups; see Scheme E) with styrene, which was conducted in water and initiated by potassium persulfate, yields uniform particles [P(S/PGL)], as shown in Scheme G.

Emulsion polymerization is particularly suitable for synthesizing particles with diameters in the range of 100—1000 nm. At the end of the polymerization process, particles are obtained that usually have a very low diameter distribution (diameter dispersity parameter defined as the ratio of the weight and number average diameters D_w/D_n is very close to 1.0). The uniform particles can be easily separated from the polymerization mixture by centrifugation [33, 34]. A comprehensive description of the synthesis of P(S/PGL) particles was described in our previous paper [35]. Table 1 provides the parameters for the syntheses of P(S/PGL) microspheres and their characteristics.

The data presented in Table 1 clearly indicate that the diameters of the particles decreased with increasing concentration of the PGL macromonomer in the monomer feed. This result indicates that polyglycidol is present in the interfacial layer of the particles. For a given mass of particles, their total surface is larger when the diameters of the particles are smaller; therefore, it is possible to accommodate the larger amount of polyglycidol in the interfacial layer. In our studies, the concentration of the macromonomer in the polymerization feed was greater than the critical micelle concentration. Polymerization of these micelles swollen with styrene should yield primary particles that have an interfacial layer enriched in polyglycidol. Moreover, it is reasonable to assume that oligoradicals that contain polystyrene and polyglycidol segments can be adsorbed onto the surface of growing particles, which therefore contributes to the increase in the fraction of polyglycidol in the particle interfacial layer.

X-ray photoelectron spectroscopy (XPS) measurements revealed a significant increase of polyglycidol in the interfacial layer of microspheres as the concentration of macromonomer increased in the polymerization mixture. Comparing the fraction of polyglycidol in the interfacial layer (measured by XPS) with the fraction of polyglycidol in the bulk microspheres (determined by elemental analysis) led to conclusion that almost the entire amount of polyglycidol was present in the interfacial



Scheme G. Emulsion copolymerization of styrene and α-tert-butoxy-ω-vinylbenzylpolyglycidol yielding poly(styrene/α-tert-butoxy-ω-vinylbenzylpolyglycidol) microspheres [P(S/PGL)]

T a b l e 1. Basic properties of P(S/PGL) and PS microspheres

Symbol of particles	[PGL] in monomer feed mol. %	D_n, nm*) (SEM)	D_w/D_n	D_h, nm**) (PCS)	[PGL] in the whole particle mol. %	[PGL] in interfacial layer, mol. %	[-OSO_3-] mol/m^2
PGL with $\overline{M}_n$ = 2700 ($\overline{M}_w/\overline{M}_n$ = 1.03)							
P(S/PGL)1	$2.89 \cdot 10^{-5}$	650	1.008	765	0.023	0.216	$1.65 \cdot 10^{-6}$
P(S/PGL)2	$5.78 \cdot 10^{-5}$	350	1.007	410	0.028	0.255	$8.77 \cdot 10^{-7}$
P(S/PGL)3	$3.85 \cdot 10^{-4}$	260	1.007	294	0.053	0.423	$4.85 \cdot 10^{-7}$
P(S/PGL)4	$2.88 \cdot 10^{-4}$	220	1.022	259	0.070	0.426	$4.13 \cdot 10^{-7}$
PGL with $\overline{M}_n$ = 3000 ($\overline{M}_w/\overline{M}_n$ = 1.08)							
P(S/PGL)5	$5.78 \cdot 10^{-5}$	300	1.006	316	0.069	0.278	$1.05 \cdot 10^{-6}$
Emulsion polymerization of styrene without PGL macromonomer							
PS	0	529	1.011	535	0	0	$3.58 \cdot 10^{-6}$

*) The number average diameter of microspheres (D_n) and dispersity parameter (D_w/D_n) were determined by measuring the diameters of at least 700 microspheres in different microphotographs.

**) The hydrodynamic diameter (D_h) was determined based on measurements of the diffusion coefficients of spherical particles measured using photon correlation spectroscopy (PCS) and calculated according to the Einstein-Stokes equation [36].

layer of the P(S/PGL) particles, which revealed their core-shell morphology (as shown in Scheme G).

XPS analysis provides information on the chemical composition of the *ca.* 5 nm thick interfacial layer of the particles. For P(S/PGL)3 and P(S/PGL)4 particles, an assumption that the outer layer contains pure polyglycidol indicates that this layer is 2 nm thick. However, TEM images (Fig. 1) revealed that the thickness of the interfacial layer is close to 30 nm [37]. Therefore, the outer layer of microspheres cannot be composed of pure polyglycidol, and there is a concentration gradient of polyglycidol along the radius of the particles.

The polyglycidol fraction in the particle interfacial layer did not exceed 42 mol. % even when the fraction of polyglycidol in the polymerization mixture was greater than $3.85 \cdot 10^{-4}$ mol. %. The poly(styrene-*co*-polyglycidol) chains with a content of polyglycidol monomeric units exceeding 45 % are water-soluble and should primarily remain in the continuous phase, not incorporated into the surface of the particles.

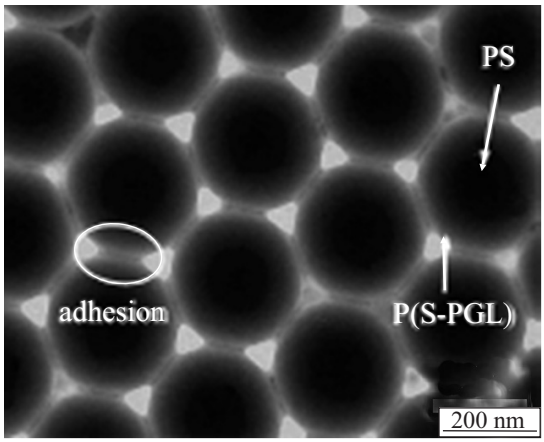


Fig. 1. TEM image of P(S/PGL)4 particles showing the core-shell morphology. Reproduced from Ref. [36] with permission

The P(S/PGL) particles are negatively charged because of the presence of sulfate end-groups that are formed during the initiation of polymerization with potassium persulfate. The surface concentration of -OSO_3^- groups decreased with the fraction of polyglycidol in interfacial layer, which was presumably due to the screening of anions by the water-swollen polyglycidol-rich interfacial layer.

The number average diameter of the P(S/PGL) microspheres in the dry state, which was measured from SEM or TEM images, is smaller than the hydrodynamic diameter determined by PCS. This result reflects the difference between the particles with swollen and dried PGL layers.

It appeared that the P(S/PGL) particles are thermoresponsive [36].

Although neither polystyrene nor polyglycidol homopolymers are thermoresponsive, the thicknesses of the shells of microspheres that contain polyglycidol and polystyrene segments reversibly change with the temperature in a quite narrow temperature range in the vicinity of 45 °C. This transition temperature depends on the concentration of NaCl in the continuous medium. For a higher salt concentration, the transition temperature is lower, and the decrease in the particle diameters is greater [36].

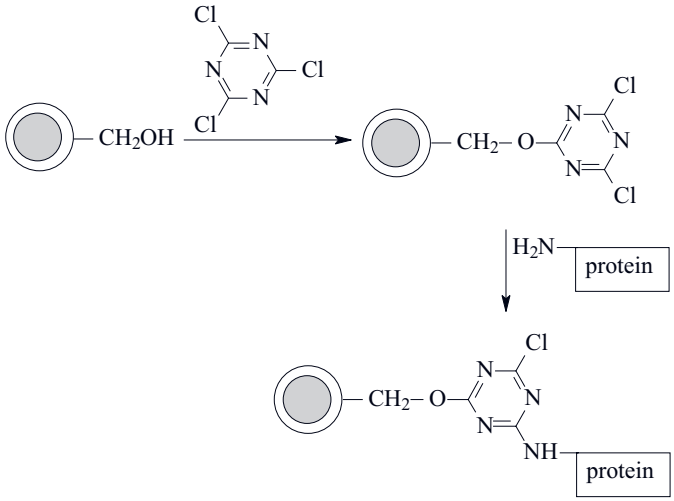
The high mobility of polyglycidol chains in the particle interfacial layer should be important for protecting the surface against the adsorption of biomolecules. The reduction of an uncontrolled protein adsorption is especially desired in the case of particles used in medical diagnostics when microspheres are exposed to contact with various components of analyzed body fluids (*i.e.*, with proteins of human serum) because an extensive adventitious adsorption leads to a loss of analytical selectivity.

The spin-lattice relaxation time in ^{13}C NMR experiments (T_1) was used as a measure of chain mobility in microspheres. It was observed that the T_1 relaxation times for polyglycidol chains in the interfacial layer of particles suspended in water and for polyglycidol chains in solution are very close, which reflects the high mobility of polyglycidol chains in the particle interfacial layer [38]. This result suggests that protein adsorption onto the P(S/PGL) particles should be low. Indeed, studies on the adsorption of proteins present in high concentration in human serum (for example, serum albumin, γ-globulins and fibrinogen) revealed that the protein repellency is enhanced with an increasing fraction of polyglycidol in the interfacial layer of the particles. For example, the maximal surface concentration (Γ_{max}) of human serum albumin adsorbed onto P(S/PGL)4 particles was approximately ten-times less than the maximal surface concentration of the same protein adsorbed onto the pure polystyrene particles [39].

Medical diagnostic tests require particles with irreversibly bound proteins, which do not leak into the liquid environment even after long storage times. Therefore, covalent protein binding is preferred. To covalently immobilize proteins via their amine groups, the hydroxyl groups of the polyglycidol in the interfacial layer of the particles were activated with 2,4,6-trichloro-1,3,5-triazine (TCT) (Scheme H).

The covalent immobilization of various proteins onto the majority of substrates is usually accompanied with protein adsorption [40].

The difference between the total amount of bound protein and the amount of protein that could be „washed out" is equal to amount of the covalently immobilized protein. Studies on the interactions of human serum albumin and γ-globulins with the P(S/PGL) particles containing TCT-activated polyglycidol hydroxyl groups revealed that these proteins were 100 % covalently immobilized [41]. The covalent-only attachment of proteins is



Scheme H. Covalent binding of proteins onto the P(S/PGL) microspheres via a TCT linker

especially advantageous in the case of particles dedicated for diagnostic applications because eliminating non-specific protein adsorption enhances the selectivity of diagnostic tests.

SELECTED APPLICATIONS OF POLYGLYCIDOL MACROMONOMERS AND POLY(STYRENE/POLYGLYCIDOL) MICROSPHERES FOR BIOMEDICAL PURPOSES

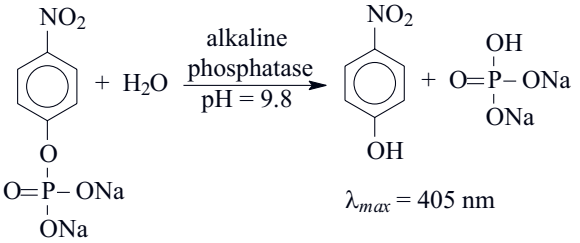
The implantation of stents and other metal prostheses usually induces inflammation. The primary event of this disadvantageous process consists of protein deposition onto the surface of the implants. Modifying stainless steel and gold by „grafting from" polymers formed by the radical photo-polymerization of polyglycidol macromonomer makes the surfaces of these materials superhydrophilic (contact angle of water equal to 0°). It has also been observed that surfaces with tethered chains of polymerized polyglycidol macromonomer prevent the adsorption of anti-bovine serum albumin [42]. However, the same surfaces activated with TCT are suitable for the covalent binding of bovine serum albumin (BSA). The covalently immobilized BSA specifically binds anti-BSA by antigen-antibody interactions, but it is resistant to the adsorption of anti-myoglobin [42].

The aforementioned considerations indicate that the modification of steel and gold by tethering polyglycidol chains onto their surface yields materials that especially suitable for the fabrication of a new class of metal implants that devoid of some deficiencies of the presently employed ones.

Although there are currently many diagnostic tests available on the market, new ones with improved selectivity and sensitivity are still needed. The simplest diagnostic test consists of mixing a suspension of particles with immobilized biomolecules (usually antigens/or antibodies) with a sample of the analyzed liquid. In the presence of an analyte to be detected (specific antibody or antigen), the particles aggregate as a result of the antigen-antibody interactions. Aggregation is usually detected by the naked eye. The sensitivity of aggregation tests (sometimes called agglutination tests) could be increased using optical methods based on light scattering, such as nephelometry or turbidimetry. However, in many instances, the sensitivity of these methods is insufficient. The direct detection of *H. pylori* requires an unpleasant sampling of tissue from the stomach or duodenum walls. The presence of *Helicobacter pylori* in the digestive tract of many humans (from 30 to 70 % of the population in various countries) often causes stomach and duodenum ulcers and cancer. The detection of antibodies against *H. pylori* in the blood serum of patients can be considered to be indirect proof of a patient's infection with this pathogen. The tests that are present on the market are quite simple, but they do not allow for the quantitative determination of the level of antibodies against *H. pylori*. Remembering that P(S/PGL) microspheres are negatively

charged due to presence of the sulfate end groups in polymer chains and that proteins are also charged (except when the pH is equal to the protein isoelectric point), we developed a test based on monitoring the changes in the electrophoretic mobility of P(S/PGL) with immobilized *H. pylori* antigens after exposure to blood sera with anti-*H. pylori* [43]. It was observed that the binding of *H. pylori* antibodies to P(S/PGL)4 microspheres with immobilized *H. pylori* antigens caused changes in the electrophoretic mobility that were strongly dependent on the concentration of *H. pylori* antibodies, which could be used for quantitative determination of the antibodies.

Proteins immobilized on the surface of particles should remain biologically active during prolonged periods of storage and handling. To investigate this possibility, we selected alkaline phosphatase, which is the enzyme responsible for hydrolysis of phosphate groups. In the test reaction, the hydrolysis of *p*-nitrophenyl phosphate catalyzed by phosphatase yields *p*-nitrophenol and disodium phosphate (Scheme I). The concentration of



Scheme I. Hydrolysis of p-nitrophenyl phosphate catalyzed by alkaline phosphatase

p-nitrophenol can be easily determined by measuring the absorption of UV light at λ_{max} = 405 nm, *i.e.*, at the wavelength corresponding to the maximum of absorption of *p*-nitrophenol.

The activity of alkaline phosphatase covalently immobilized onto P(S/PGL)5 particles was compared to the activity of the same enzyme adsorbed on the surface of polystyrene [P(S)] microspheres. The surface concentrations of the enzyme attached to the P(S/PGL)5 and P(S) particles were close to and equal to 0.66 and 0.60 mg/m^2, respectively. The absorption of *p*-nitrophenol produced in the reaction catalyzed by covalently immobilized and/or adsorbed phosphatase as a function of the enzyme concentration averaged over the entire sample volume is shown in Figure 2. The plots in this figure indicate that the phosphatase adsorbed onto the P(S) microspheres is approximately four-times less reactive than the enzyme covalently immobilized onto the P(S/PGL)5 microspheres.

The enzyme molecules adsorbed onto the P(S) microspheres are presumably more prone to conformational changes that affect the structure of active centers, which interferes with the access of substrates or with release of

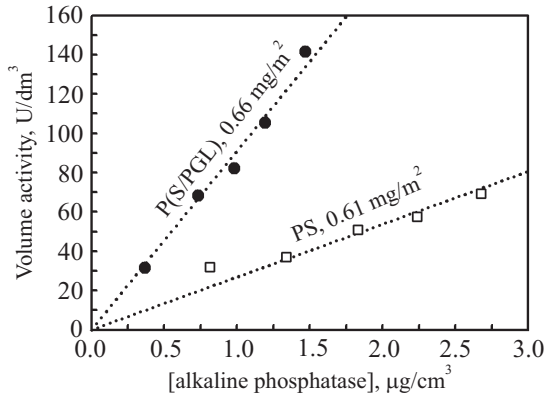


Fig. 2. Absorbance of p-nitrophenol produced in the reaction catalyzed by alkaline phosphatase as a function of the phosphatase covalently immobilized onto P(S/PGL)5 and adsorbed onto the P(S) microspheres. The lines are fitted to the experimental points using the least squares method

products more than those covalently immobilized onto the hydrophilic polyglycidol-enriched surface.

CONCLUSIONS

The development of relatively simple methods allows for the synthesis of branched and/or linear polyglycidol in a controlled manner. Synthesized polyglycidol macromonomers function as surfmers in emulsion copolymerization with styrene. The obtained microspheres that possess a hydrophilic interfacial polyglycidol-enriched layer are suitable for the covalent immobilization of proteins with practically complete elimination of uncontrolled protein adsorption. The covalently immobilized proteins are more active than proteins adsorbed onto the hydrophobic polystyrene microspheres. Consequently, the synthesis of microspheres with a polyglycidol-rich outer layer provides possibilities for designing new types of sensitive diagnostic tests based on the changes in the electrophoretic mobility of microspheres. Moreover, the particles with a polyglycidol-enriched surface can serve as suitable enzymes supports that are used as indicators of biochemical reactions, biochemical catalysis tools or components of biosensors.

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