Versatility of Potential Biomedical Use of Functional Polysuccinates

Jan Łukaszczyk, Piotr Benecki, Katarzyna Jaszcz, Monika Śmiga*

Silesian University of Technology, Department of Physical Chemistry and Technology of Polymers, 44-100 Gliwice, ul. M. Strzody 9, Poland

Summary: Polysuccinates with pendant allyl groups (PSAGE) were synthesized by melt copolymerization of succinic anhydride with allyl glycidyl ether and eventually other glycidyl ethers. It was found that PSAGE could be crosslinked by radical copolymerization with methyl methacrylate. Oxidized PSAGE considered as multifunctional epoxy resin was cured with use of glutaric anhydride to form solid material susceptible to hydrolytic degradation to water-soluble non-toxic products. Comb-like amphiphilic polysuccinates containing both pendant poly(oxyethylene) chains and epoxy groups have been synthesized as well and checked for their solubility in water. Properties of PSAGE-type polymers suggests their potential use as biomaterials and polymeric drug carries.

Keywords: biodegradable biomaterials; drug carriers; functional polysuccinates

Introduction

Increasing use of synthetic biodegradable polymers in medicine and pharmacy is a result of intensive studies on their synthesis and characteristics as well as on their new applications. Besides well known practical applications of the polymers in medical equipment, apparatus, single use devices etc. at the present they are used for more sophisticated purposes as biomaterials and polymer drug carriers. Polymer biomaterials are both termoplasts and thermosets, while for preparation of polymeric prodrugs non-crosslinkable polymers are used almost exclusively. Generally biodegradable polymers for medical application should respond to the same criteria of biocompatibility and biofunctionality as non-degradable polymers. Additionally the rate and the mechanism of biodegradation and the nature of degradation products must be taken into consideration.

At the present the most often used biodegradable polymers are thermoplastic polyesters derived from lactic or glycolic acids or caprolactone [1]. Since some time however growing interest in functional biodegradable polymers could be observed [2].

Recently we have described the synthesis of functional polysuccinates with pendant allyl groups [3,4], which could be utilized directly or after oxidation of double bonds. Polyesters composed of succinic acid, naturally present in living tissues, and of allyloxyglycerin could be

 DOI: 10.1002/masy.200450651

considered as potentially biodegradable and useful in medical applications.

The aim of this work was to recognize and to demonstrate some of possible modes of the use of functional polysuccinates in different areas of medicine.

Results and discussion

The functional polysuccinates with pendant allyl groups (PSAGE) were obtained by melt copolymerization of succinic anhydride (SA) and allyl glycidyl ether (AGE) at 120^0C in the presence of benzyltrimethylammonium chloride (BTMAC) and some water added [3,4]. Their alternating structure, i.e. lack of oligoether blocks was confirmed by NMR and MALDI-TOF spectra as well as by determination of double bonds content [3].

The content of allyl groups i.e. functionality of PSAGE could be easily adjusted by replacing a part of AGE with other glycidyl ethers, i.e. by changing AGE/GE feed ratio, where GE is the sum of glycidyl ethers. Biodegradable aliphatic polyester chain and pendant allyl groups enable consideration of potential biomedical application of PSAGE-type polymers or oligomers: in biodegradable bone cements, as biodegradable termosets for temporary implants and as amphiphilic polymeric drug carriers.

Biodegradable bone cements

In spite of low reactivity and even retarding effect of allyl groups in radical copolymerization [6], which is due to easy abstraction of allyl hydrogen and resonance stabilization of resulting radical, allyl ethers are used in some industrial systems, e.g. unsaturated polyester resins and coatings [7,8] cured by radical mechanism. This enable consideration of the solutions of PSAGE-type polyesters in low viscosity monomers as potential biodegradable thermoset resins, which could be cured in site of application like classical acrylate bone cements [9] or bone substitutes proposed so far [10]. Composition of PSAGE with some MMA is viscous liquid, which could be self-cured after addition of small amount of benzoyl peroxide (BPO) and N,N-dimethylamino-p-toluidine (DMPT) in reasonable time and with moderate exothermic effect, i.e. with peak temperature below 90^0C, which is acceptable for acrylic bone cement [11]. Selected results of initial study of curing of PSAGE-MMA compositions initiated by redox system: BPO-DMPT are shown in tab.1. Due to susceptibility of polysuccinate chain to hydrolytic degradation, the composition of PSAGE and MMA or other monomer could be considered eventually as biodegradable bone cement for

temporary, resorbable support of damaged bone tissue, though improving of some properties of cured materials requires further studies.

Table1. Influence of the cement composition and initial curing temperature on hardening and on selected properties of the material

Components/Curing parameters/Properties		Cement composition/Properties		
PSAGE M_n=14800, MWD = 1.26 [g]		1	1	1
MMA [g]		0.75	0.75	1
BPO [wt.%]		4.37	2.23	1.96
DMPT [wt.%]		0.53	0.27	0.23
Initial temp.23°C	Setting time [min.]	3	4.25	8
	Peak temperature [°C]	48	45.6	-
Initial temp.37°C	Setting time [min.]	1.5	3.25	-
	Peak temperature [°C]	48.1	42.1	-
Compressive stress [MPa] at strain 20%		5.87	5.47	9.91
Extractable fraction [%]		25.1	40.0	16.9

Thermoset resins for pressureless casting

Polysuccinates with various degree of unsaturation were synthesized by replacing a part of AGE with butyl glycidyl ether. Polyesters containing 40-91 mol% of unsaturated repeating units derived from AGE were oxidized to respective poly(epoxypolyester)s. Pendant allyl groups in PSAGE were epoxidized quantitatively by *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 solution at room temperature as described elsewhere [5]. It was found that duration of the process required for full epoxidation (24-96h) increases, while decreasing degree of unsaturation, i.e. the content of AGE in the feed and thus in the polymer obtained. In spite of disappearance of double bonds observed in ^{1}H NMR spectra, the epoxide content (EC) determined for the resins obtained (EC = 0.14-0.41 eq/100g) was always lower than calculated theoretical one ($EC_{theor.}$ = 0.18-0.48).

Epoxyfunctional polysuccinates obtained (EPSAGE) have been considered as multifunctional epoxy resin, which could be cured with use of dicarboxylic acid anhydrides. One may expect that the resin cured with succinic or glutaric anhydrides is susceptible to hydrolytic degradation producing diglycerin ether or glycerin and succinic acid or succinic and glutaric one.

All end products of the hydrolysis, especially both acids naturally present in the body could be considered as well tolerated by living tissue [12].

Selected epoxyfunctional polysuccinates were cured with both anhydrides mentioned above, but due to technical problems with homogenization of the resin and SA (Tm = 120^{0}C), in systematic studies only GA was used due to its lower melting temperature (Tm = 54-55^{0}C). Appearant mechanical properties as well as other properties of various samples appeared to be diversified and dependent on functionality of initial resin and on the content of anhydride hardener. Selected properties of cured resins are gathered in tab.2.

Table 2. Influence of the composition of epoxyfunctional polysuccinates cured with glutaric anhydride (GA)* on their properties.

Composition of cured resin		Shore hardness	Tg	Water sorption	Swelling in CH_2Cl_2 vapors	Weight loss [%] during degradation**	
AGE/GE feed ratio	Amount of GA [mol/1mol of epoxy groups]	[^{0}Sh A or D]	[^{0}C]	[%]	[%]	28 days	70 days
1.0	0.0	82D	36.2	14.1	76	76.7	99.7
	0.2	-	31.2	12.0	-	74.4	97.1
	0.4	71D	-	7.4	79	62.1	96.4
	0.6	73D	34.9	2.6	79	11.9	98.5
	0.85	79D	46.7	2.1	69	82.4	99.8
0.6	0.0	-	8.6	10.5	203	48.2	99.9
	0.2	78A	18.4	7.7	169	-	-
	0.4	83A	-	5.6	149	-	-
	0.6	85A	14.9	3.2	129	81.6	98.4
	0.85	86A	17.6	3.0	106	-	-
0.4	0.0	-	1.0	9.8	214	29.6	99.3
	0.2	62A	6.0	12.6	194	27.5	100
	0.4	74A	-	9.9	192	11.5	95.0
	0.6	81A	3.7	6.8	155	30.6	97.9
	0.85	75A	4.7	7.1	151	92.0	99.9

*resins cured at elevated temperature at 150^{0}C according to procedure described elsewhere [4, 5],
**accelerated degradation at 70^{0}C in PBS, pH = 7.4

As could be expected, the resins with higher functionality and cured with higher amount of GA were harder and displayed higher Tg values and lower sorption capacity in swelling experiments. Cured resins of low functionality expressed with EC values after curing were soft

and weak. This was observed also in fractographic analysis of the samples before and after partial hydrolytic degradation in phosphate buffer solution (pH 7.4). Only the sample of cured resin with highest functionality displayed fracture surface typical for glassy material, while the same material after some time of degradation as well as samples of cured resins with lower functionality displayed fracture surface typical for soft, weak material [5].

Unexpectedly thermal hardening of epoxyfunctional polysuccinates was observed even without addition of an anhydride, probably due to the polymerization and/or other reactions of pendant epoxy groups. There were observed also differences in the rate of hydrolytic degradation followed by determination of weight loss, but there was no simple relation between the composition and rate of degradation. The rate of degradation is probably connected with the network density (related closely to AGE/GE ratio in the initial resin), with the character of crosslinks (ether or ester ones) and additionally with hydrophilicity of the samples. As could be expected the highest rate of weight loss was observed for the samples with highest content of ester bonds, i.e. for those cured using 85% of GA in relation to EC. All the samples studied appeared to be susceptible for full degradation to water-soluble products. NMR studies of degradation products confirmed the presence of succinic and glutaric acids as well as glycerin and butanol [5], though the presence of diglycerin ether and soluble oligomers cannot be excluded.

The results obtained so far suggest that optimized compositions of EPSAGE and GA could be used for fabrication of resorbable, temporary implants, when thermoplastic biodegradable polymers cannot be used due to high costs of the mold.

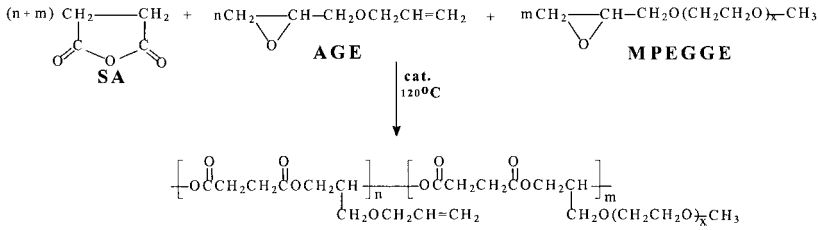
Drug carriers for designing polymeric prodrugs

The comb-like epoxyfunctional polysuccinates with pendant poly(oxyethylene) chains were obtained in two ways:

1) in direct polyreaction with use of poly(ethylene glycol) macromonomers
2) by covalent grafting of poly(ethylene glycol) derivatives onto functional polymer

On the first way, the comb-like polyesters with poly(oxyethylene) and allyl pendant groups were synthesized from SA, AGE and ω-methoxy poly(ethylene glycol) glycidyl ether (MPEGGE). MPEGGEs were obtained in the reaction of epichlorohydrin with sodium alcoholate groups of ω-methoxy poly(ethylene glycol)s (MPEGs) with different molecular weight (nominal MW=750-5000). Amphiphilic comb-like polyesters were synthesized in melt as described above

for PSAGE. The GEs were used in 20 % molar excess in relation to SA. MPEGGE/GE feed ratio was changed within the range of 0.1-0.7.



MPEGGE/GE ratio in the resulting polymers (found by ^{1}H NMR and calculated from iodine number (IN)) was equal to MPEGGE/GE feed ratio for polyesters synthesized with use of macromonomers with lower molecular weight (MW of MPEG equal to 750 or 1100). In the polyesters synthesized with use of macromonomers with higher molecular weight, MPEGGE/GE ratio in polymers were higher than in feed (tab.3).

Table 3. Characteristics of comb-like polyesters with pendant allyl groups

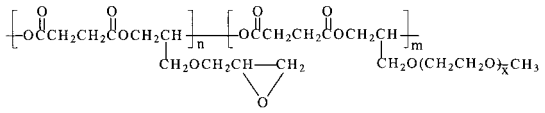
MW of MPEG [Da]	MPEGGE/GE* feed ratio [mol/mol]	IN [g I_2/100g]	M_n (GPC) [Da]	M_w/M_n	MPEGGE/GE* in polyester calculated from		Solubility in water**
					^{1}H NMR	IN	
750	0.7	10.43	16700	1.30	0.73	0.71	+
1100	0.1	65.87	18700	1.43	0.13	0.12	+/-
	0.5	24.14	16100	1.22	0.44	0.40	+
2000	0.1	47.33	22300	1.65	0.14	0.13	+/-
	0.3	7.08	22700	1.27	0.66	0.61	+/-
	0.5	4.62	22400	1.21	0.74	0.71	+
	0.7	3.05	21250	1.22	---	0.79	+
5000	0.1	18.86	33300	1.38	0.21	0.18	+/-
	0.5	10.47	39800	1.21	---	0.30	+

*GE=MPEGGE+AGE, ** + water-soluble, +/- water-dispersible

The molecular weights (M_n values, GPC), of the comb-like polyesters were within the range of ca. 16700-39800 Da (tab.3) and were dependent on the length of the poly(oxyethylene) grafts, but practically did not depend on the degree of grafting.

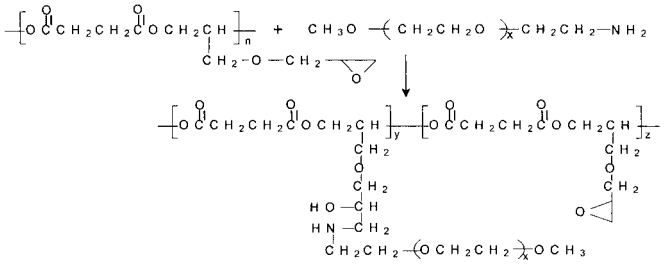
The length and content of poly(oxyethylene) grafts in the polyesters was found to influence the solubility in water (tab.3).

The allyl groups in comb-like polyesters were oxidized to epoxy ones. Oxidation led to respective polyesters with oxirane groups in side chains.



The length of the poly(oxyethylene) grafts and degree of grafting influenced the conversion of double bonds. Oxidation of the polyesters with the longest graft chains and with the highest degree of grafting was the most difficult. Elongation of the reaction time, increase of the polymer concentration in reaction solution or higher excess of MCPBA allowed however to achieve complete oxidation of allyl groups. Oxidized polyesters exhibited solubility characteristics similar to that before oxidation. In the second way the comb-like functional polyesters were obtained by covalent grafting of $MPEGNH_2$ onto epoxidized PSAGE. $MPEGNH_2$ was obtained (yield 33%) from ω-metoxy-poly(ethylene glycol), nominal MW=2000, by means of modified Gabriel synthesis in the reaction of tosylated MPEG with potassium phthalimide and next with hydrazine [13].

The grafting of $MPEGNH_2$ onto functional polymer was carried out in methylene chloride, at room temperature for 48 or 96 hours.



The molecular weight of the poly(epoxyester) before grafting (calculated from end groups analysis (acid value: AV=37 mg KOH/1g, hydroxyl value: HV=12 mg KOH/1g)) was 2289 Da. The conversion of epoxy groups calculated from ^1H NMR was 6% when the reaction was run 48h and 20% after 96h. Calculated molecular weight of obtained comb-like polymers was ca. 3740 in the first case and 6740 in the second case. The products were water-dispersible.

Conclusions

Pendant allyl groups in the polysuccinates could be utilized directly (in radical copolymerization with low viscosity monomers) or could be utilized indirectly (after transformation into other functional groups, e.g. epoxy ones).

Polysuccinates with allyl pendant groups could be used probably in formulation of biodegradable, injectable bone cements, but problems with low reactivity of functional groups must be solved.

Epoxidized polysuccinates, i.e. respective poly(epoxyester)s could be useful as thermoset resins for presureless casting of temporary medical implants of desired individual shape.

Poly(epoxyester)s derived from functional polysuccinates could be modified in such a way, that they would become water-soluble, while preserving a part of their epoxy groups useful for coupling drugs to form polymeric prodrugs.

Biocompatibility of the end products of hydrolytic degradation may be predicted in case of medical implants made of poly(epoxyester)s cured with glutaric anhydride (succinic acid, glutaric acid, glycerin), while only expected for other biomaterials or drug carriers based on polysuccinates.

Acknowledgement

Financial support of European Graduate College "Advanced Polymer Materials" is gratefully acknowledged.

[1] D. Bendix, *Polym. Degrad. Stab.*, **1998**, *59*, 12; B.L. Seal, T.C. Otero, A. Panitch, *Mater. Sci. Eng.* **2001**, *R34*, 147.
[2] H. Chung, D. Xie, A.D. Puckett, J.W.Mays, *Eur. Polym. J.*, **2003**, *39*,1817; H. Uyama, M. Kuwabara, T. Tsujimoto, S. Kobayashi, *Biomacromolecules*, **2003**, *4*, 211.
[3] J. Łukaszczyk, K. Jaszcz, *React. Funct. Polym.*, **2000**, *43*,25.
[4] J. Łukaszczyk, K. Jaszcz, *Macromol. Chem. Phys.*, **2002**, *203*, 301.
[5] J. Łukaszczyk, K. Jaszcz, *Polym. Adv. Technol.*, **2002**, *13*, 871.
[6] G. Odian, *Principles of Polymerization*, 2nd ed., Wiley-Interscience, New York 1981, p.251; J.C. Bevington, T.N. Huckerby, B.J. Hunt, A.D. Jenkins, *J. Macromol. Sci.-Pure Appl. Chem.*, **2001**,*38*, 981.
[7] H.J. Traenckener, H.U. Pohl, *Angew. Makromol. Chem.*, **1982**, *108*, 619.
[8] G. Rokicki, E. Szymańska, *J. Appl. Polym. Sci.*, **1998**, *70*, 2031.
[9] D.H. Kohn, P. Ducheyne, "Materials for Bone and Joint Replacement", in: Cahn R.W., Haasen P., Kramer E.J. (Eds.), "*Materials Science and Technology*", vol.14, "*Medical and Dental Materials*", VCH, Weinheim 1992.
[10] M.D. Timmer., S.B. Jo, C.Y. Wang, C.G. Ambrose, A.G. Mikos, *Macromolecules*, **2002**, *35*, 4373; A.K. Burkoth, K.S. Anseth, *Biomaterials*, **2000**, *21*, 2395.
[11] ISO/FDIS 5833:2001 "*Implants for surgery – Acrylic resin cements*".
[12] S.Rose, S.Bullock, *The Chemistry of Life*, Penguin Books, London 1991.
[13] S. Furukawa, N. Katayama, T. Lizuka, I. Uwabe, H. Okada, *FEBS Lett.* **1980**, *2*, 239.