

Highly Porous Crosslinked Poly(ester-anhydride) Microspheres with High Loading Efficiency

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Abstract Microparticles with diameter within the range of $D_n = 26\text{--}38\ \mu\text{m}$ were prepared from functional poly(ester-anhydride)s with different amount of allyl groups in the side chains, using emulsion solvent evaporation technique. Porous structure was obtained as the effect of photocrosslinking of allyl groups. 2,2-Dimethoxy-2-phenylacetophenone (DMPA) (0.5 wt%–10 wt%) was used as a photoinitiator. The crosslinking was carried out by UV irradiation during the solvent evaporation. Effectiveness of the crosslinking was characterized by the content of insoluble part of samples and it was in the range of 18%–75%. Porosity of microparticles (in the range of 76%–88%) depended on the functionality of poly(ester-anhydride)s and amount of the photoinitiator used. The most porous particles were obtained with use of 0.5 wt% of DMPA. Their flow ability expressed by Carr's index was excellent, and their theoretical mass mean aerodynamic diameters were acceptable for use in pulmonary drug delivery. The most porous particles were loaded with *p*-nitroaniline, theophylline or doxycycline. The loading efficiencies of drugs in porous microspheres were higher compared to nonporous ones. The porosity of loaded microparticles was slightly decreased, however their flow ability was still very good.

Keywords: Functional poly(ester-anhydride); Crosslinkable microspheres; Allyl groups; Photocuring; Porous structure.

INTRODUCTION

Porous polymeric microspheres have been widely used in drug delivery^[1–5] or tissue regeneration^[6–10]. Microspheres with a porous structure are much more appreciated than the non-porous ones in many cases. For example, because of their large size and low density, porous microspheres can be aerosolized more easily than smaller non-porous ones, resulting in higher respirable fractions of inhaled therapeutics^[3, 11, 12]. Nowadays, drugs are administered *via* pulmonary route for two main purposes: local therapies and systemic adsorption^[3]. Inhalable innovative drug delivery systems such as microspheres are expected to reduce direct contact of highly concentrated drug formulations with the lung tissue and to minimize drug concentration fluctuation through sustained release^[13]. The pulmonary delivery route has been attractive for systemic drug administration, due to the large surface area, high permeability and slow mucociliary clearance of lung tissues^[14]. Porous polymeric microspheres can be also used in gastroretentive drug delivery systems^[15–19]. In oral controlled release systems, gastrointestinal transit time is extremely important for extended drug absorption. Several approaches have been proposed to retain oral dosage forms in the stomach. Those methods include bioadhesive systems, swelling and expanding systems, and floating systems^[20]. Porous hydrophobic microspheres possessed good floating behavior and are expected to remain buoyant on the gastric contents, because their density is lower than that of the gastric content ($\sim 1.004\ \text{g/cm}^3$)^[21].

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Among synthetic biodegradable polymers, poly(lactide-*co*-glycolide) PLGA is most frequently used to prepare microspheres for drug delivery systems^[4, 22]. From a technological standpoint, PLGA-based porous microspheres have been typically obtained by the double emulsion technique in presence of porogen^[3, 23]. Relatively simple methods, as salt leaching or gas foaming, typically used for pore generation, indicated however, several limitations such as irregular pores structure and difficulties in particle size control^[24]. Uniform porous structures can be achieved using osmotic agents, however in this case the poor control of drug encapsulation efficiency and unsatisfactory speed of release of protein are observed^[24–26].

Recently, we obtained porous microspheres from functional poly(ester-anhydride)s with allyl groups in side chains. Such microparticles were successfully prepared by means of solvent evaporation technique combined with UV irradiation in the presence of a photoinitiator. Porous structure was achieved as the effect of photocrosslinking, without any additional substances acting as porogens^[27]. Poly(ester-anhydride)s are attractive polymers for use in drug delivery, due to their degradation and release characteristics^[28–33]. Moreover, the microenvironmental pH resulting from polyanhydride degradation is higher than in the case of PLGA degradation, what is potentially less detrimental to biologically active compounds as well as less irritating for tissue^[34, 35]. A major drawback of PLGA systems is the accumulation of acid degradation products inside degrading matrices, which is associated with a drop of local pH^[35, 36]. A second problem with the use of large porous microspheres as drug carriers is poor encapsulation efficiency of many drugs^[37]. In our latest studies it was stated that introduction of crosslinked structure to porous microspheres resulted in increase of the drug loading efficiency in comparison to uncrosslinked microparticles^[27]. In this work the phenomenon was studied in detail with use of various model drugs.

The aim of this work was to evaluate the influence of functionality of poly(ester-anhydride)s and resulting photocrosslinking degree on the porosity, flow ability and drug loading efficiency of obtained microparticles. The influence of drug incorporation on porosity and flow ability of selected microspheres was also estimated.

EXPERIMENTAL

Materials

Succinic acid 99% (Aldrich), allyl glycidyl ether 99% (Aldrich), sebacic acid 99% (Aldrich), 2,2-dimethoxy-2-phenylacetophenone (ACROS Organics), acetic anhydride (POCH S.A.), poly(vinyl alcohol) ($M_w = 88000$ g/mol, 88% hydrolyzed) (ACROS Organics), *p*-nitroaniline (Fluka), theophylline (Aldrich), doxycycline (Sigma) were used as supplied. Solvents were purified according to known procedures.

Synthesis of Poly(ester-anhydride)s

Poly(ester-anhydride)s were synthesized in a two-step reaction from sebacic acid (SBA) and oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl groups (OSAGE), according to the procedure described earlier^[38]. Copolymers purposed for the preparation of microspheres in this work were synthesized with use of 60 wt% or 80 wt% of SBA and 40 wt% or 20 wt% of OSAGE, respectively.

Microspheres Preparation

The poly(ester-anhydride)s obtained were formulated into porous microspheres using emulsion solvent evaporation technique parallel with UV irradiation. Parameters of the process such as: concentration of polymer solution and speed of homogenizer were chosen basing on the research described previously^[39] to obtain microparticles with mean diameter ~ 30 μm . The polymer solution in methylene chloride (20 mL, concentration 50 mg/mL) was emulsified in 400 mL of aqueous solution (1 wt%) of poly(vinyl alcohol) (PVA), using ULTRA-TURRAX T18 homogenizer (3000 r/min), for 30 s. 2,2-Dimethoxy-2-phenylacetophenone (DMPA) used as a photoinitiator (0.5 wt%–10 wt% in respect to the polymer) was introduced to the polymer solution. The emulsion was stirred with magnetic stirrer at 1100 r/min at room temperature for 3 h to evaporate the organic solvent. In that time irradiation was performed with use of UVItec (LF-180S, England) lamp ($\lambda = 254$ nm). After that microspheres were collected by centrifugation at 5000 r/min for 5 min, washed 3 times with distilled water, lyophilized and stored in a freezer. Three batches were produced and evaluated for each formulation conditions.

The yields of microparticles obtained were ~70%.

***p*-Nitroaniline Loaded Microspheres**

p-Nitroaniline (10 wt% in respect to the mass of polymer) was dissolved in methylene chloride polymer solution and then the organic phase was emulsified in aqueous PVA solution. The solidification and isolation of drug loaded microspheres were performed similarly to blank ones.

Doxycycline Loaded Microspheres

For encapsulation of water soluble doxycycline, water-oil-water (W/O/W) technique was used. Doxycycline (15 wt% with respect to the mass of polymer) was dissolved in 5 mL of distilled water. The aqueous solution was then emulsified in organic phase (20 mL of polymer solution in methylene chloride), to form W/O inner emulsion. The inner emulsion was next emulsified in 400 mL of aqueous solution of PVA, forming W/O/W emulsion. The inner emulsion was obtained using ULTRA-TURRAX T18 homogenizer at 9000 r/min, for 30 s. The solidification and isolation of microspheres were performed similarly to blank ones.

Theophylline Loaded Microspheres

Encapsulation of theophylline, was performed similarly to encapsulation of doxycycline with use of W/O/W technique, but theophylline (15 wt% with respect to the mass of polymer) was dissolved in 5 mL of 1% acetic acid solution, because it was insufficiently soluble in distilled water. Such aqueous solution was then emulsified in organic phase (20 mL of polymer solution in methylene chloride) and next the W/O inner emulsion was emulsified in 400 mL of aqueous solution of PVA, forming W/O/W emulsion. The solidification and isolation of microspheres were performed similarly to blank ones.

Characterization of Microspheres

Particle morphology and size

Shape, size and size distribution of microspheres were estimated using optical microscope (OM) DELTA Optical ME 100 and PHAMIAS 2003 v.1.3 B software. The diameters of at least 100 particles obtained were measured for each sample at the images with magnification 100x and 400x. Then, number average diameters (D_n), volume average diameters (D_v), standard deviation (S) and dispersity index (D_v/D_n) were calculated ($D_n = \sum N_i D_i / \sum N_i$, $D_v = \sum N_i D_i^4 / \sum N_i D_i^3$, where N_i is the number of particles having diameter D_i). The mean size and size distribution were also determined by a laser diffraction particle size analyzer (Fritsch Analysette 22).

The morphological characterization of microspheres was carried out on Hitachi S-4800 UHR or Hitachi TM 3000 scanning electron microscopes (SEM), after sputter coating of samples with gold. The number average diameters of pores (with standard deviation) were calculated by measuring at least of 50 pores at the surface of at least 10 microparticles on SEM images.

Crosslinking efficiency

Effectiveness of the crosslinking was characterized by the content of insoluble fraction. Samples of crosslinked microspheres after lyophilization (~0.2 g) were weighed (W_1) and then extracted with 5 mL of chloroform for 24 h. Next, the insoluble parts of samples were dried at 40 °C under vacuum and again weighed (W_2). The content of insoluble part of sample (gel content) was calculated as W_2/W_1 . All experiments were performed in triplicate.

Micrometric properties

All microspheres were characterized for their micrometric properties such as: powder density (estimated as the tapped and untapped ones), porosity, compressibility index, flow and aerodynamic properties. All experiments were performed in triplicate. Tapped density was estimated as follows: microspheres were loaded into 0.3 mL section of a 1 mL plastic pipette capped with teflon cap and tapped approximately 300 times until the volume of the powder did not change. Tapped density was calculated from the difference between the weight of the pipette before and after loading, divided by the volume of powder after tapping. The porosity (P) of microspheres was calculated according to Eq. (1):

$$P (\%) = 100 (1 - \rho_T / \rho_{BP}) \quad (1)$$

where ρ_T is the tapped density of microspheres, ρ_{BP} is the bulk density of poly(ester-anhydride), estimated by pycnometric method in methanol.

The porosity and pore size distribution were also determined with use of mercury intrusion porosimetry (Poremaster 60, Quantachrome), with pressure in the range of 15–60 MPa.

The aerodynamic diameter (d_{ac}) of the particles (defining the mechanism of particle deposition in the respiratory system^[40]) was estimated on the basis of the definition (Eq. 2)

$$d_{ac} = d_v (\rho_T / X \rho_0)^{1/2} \quad (2)$$

where d_v is a volume-equivalent diameter, ρ_0 is a reference density of 1 g/cm³ and X is the dynamic shape factor, which is 1 for a sphere^[25, 40].

To get information about flow properties of microspheres, the compressibility index (Carr's index) was estimated through the relative percent difference between tapped and untapped density, according to Eq. (3)^[25].

$$\text{Carr's index (CI)} = 100 (1 - \rho_{UT} / \rho_T) \quad (3)$$

where ρ_{UT} and ρ_T are untapped and tapped density of the powder, respectively.

On the basis of Carr's index value, powder flow ability is defined as: excellent (IC = 5–12), good (IC = 12–18), fair (IC = 18–21), poor, fluid (IC = 21–25), poor, cohesive (IC = 25–32), very poor (IC = 32–38), extremely poor (IC > 40).

Loading efficiency studies

The actual loading of drug (L_A) and loading efficiency (LE) were calculated, according to Eq. (4) and Eq. (5), from the weight of drug loaded microspheres and the amount of incorporated drug. Amount of drug incorporated was directly determined by UV spectrometry ($\lambda_{\max} = 377$ nm for *p*-nitroaniline, $\lambda_{\max} = 271$ nm for doxycycline and theophylline) in phosphate buffer solution (PBS) after hydrolytic degradation of ~0.1 g loaded microparticles. The degradation was performed at 37 °C in 20 mL of PBS with pH = 7.4 for 30 days. In this time all microparticles were degraded to products soluble in PBS. The same conditions of the hydrolytic degradation were also applied for unloaded microspheres, to obtain blank test in order to verify whether the UV spectrum of the post-degradation solution of blank microspheres do not overlap with the UV spectra of model compounds. Post-degradation solutions of unloaded microspheres do not show the absorbance within the range of bands characteristic for model compounds. Absorbance spectra of model compounds, obtained from fresh and post-degradation solutions, were identical in shape, indicating that there was no degradation of model compounds during the hydrolytic degradation of microspheres.

$$L_A = (m_{SM} / m_m) \quad (4)$$

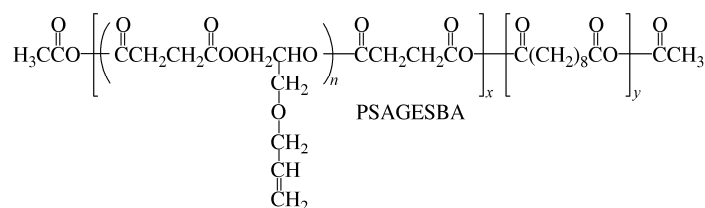
$$\text{LE (\%)} = (L_A / L_{Th}) \times 100 \quad (5)$$

where L_A is the actual loading of drug (μg/mg), m_{SM} is the weight of drug (μg) entrapment in microspheres, m_m is the weight of loaded microspheres (mg) and L_{Th} is theoretical loading of drug ($L_{Th} = 100$ μg/mg for *p*-nitroaniline, $L_{Th} = 150$ μg/mg for doxycycline and theophylline). All these experiments were performed in triplicate.

RESULTS AND DISCUSSION

Characteristics of Poly(ester-anhydride)s

Functional poly(ester-anhydride)s with allyl pendant groups were obtained by polycondensation of oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl groups (OSAGE) and sebacic acid (SBA). Copolymers purposed for the preparation of crosslinkable microspheres were synthesized with use of 60 wt% (PSAGESBA60) or 80 wt% (PSAGESBA80) of SBA in the reaction mixture.



Molecular weight of poly(ester-anhydride)s determined by GPC was $M_n = 2300$ g/mol for PSAGESBA60 and $M_n = 1600$ g/mol for PSAGESBA80. The materials were crystalline solids with melting temperature $T_m = 72$ °C for PSAGESBA60 or $T_m = 79$ °C for PSAGESBA80, soluble in chlorinated solvents. PSAGESBA80 was more crystalline than PSAGESBA60. Obtained poly(ester-anhydride)s were hydrolytically degradable. In the first period of hydrolytic degradation, PSAGESBA60 degraded significantly faster than PSAGESBA80, probably due to fast hydrolysis of anhydride bonds in whole volume of sample (bulk degradation) compared to mainly surface degradation process of PSAGESBA80. The detailed study on hydrolytic degradation of such poly(ester-anhydride)s was also described earlier^[31].

Poly(ester-anhydride)s based on SBA and OSAGE could be considered as biocompatible. The copolymers of SBA have been approved by FDA (The Food and Drug Administration) for use in delivery of the chemotherapeutic agent for the treatment of brain cancer^[41]. Succinic acid is naturally present in living tissues. The poly(3-allyloxy-1,2-propylene succinate) of $M_n = 3600$ Da was tested by us on rat alveolar macrophage cell line using the MTT assay to determine its cytotoxicity, as well as the NO production level, which is an indicator of cell activation. Obtained data show that it is non-toxic, and that the viability of cells ranges from 86% to 100%^[42].

Preparation of Microspheres

Microspheres were prepared using emulsion solvent evaporation technique. Poly(vinyl alcohol) was used as emulsion stabilizing agent. The crosslinking was done by UV irradiation during the solvent evaporation. 2,2-Dimethoxy-2-phenylacetophenone (DMPA) in amount of 0.5 wt%–10 wt% in respect to the mass of polymer was used as photoinitiator. The microspheres obtained had diameters within the range $D_n = 25.6$ –37.9 μm .

Table 1. Characteristics of microspheres obtained from PSAGESBA60 and PSAGESBA80, with use of different amounts of DMPA

Polymer	DMPA (wt%)	D_n^a (μm)	S^a (μm)	D_v/D_n^a	Gel content ^b (%)	Powder density (g/cm^3)		IC ^c	d_{ae}^d
						Untapped ^e	Tapped ^e		
PSAGESBA80 (80% SBA)	–	23.85	14.22	1.30	0	–	0.71	–	–
	0.5	26.27	7.68	1.21	18 ± 2	0.13	0.14	7	10.89
	2	29.18	12.77	1.28	25 ± 2	0.13	0.20	35	16.70
	5	36.41	9.30	1.17	39 ± 3	0.11	0.26	57	21.67
	10	37.31	13.67	1.29	71 ± 4	0.10	0.28	64	25.33
PSAGESBA60 (60% SBA)	–	27.70	9.85	1.21	0	–	0.69	–	–
	0.5	25.58	12.92	1.19	35 ± 3	0.14	0.16	12	12.07
	2	28.03	16.58	1.32	44 ± 2	0.12	0.22	45	17.35
	5	31.65	15.18	1.31	49 ± 3	0.10	0.26	61	21.14
	10	37.94	7.76	1.25	75 ± 4	0.09	0.27	67	24.15

DMPA-2,2-dimethoxy-2-phenylacetophenone, used as a photoinitiator;

^a D_n -number average diameters, D_v -volume average diameters, S -standard deviation, and D_v/D_n -dispersity index calculated from optical microscope images;

^b The content of insoluble part of sample characterized the effectiveness of crosslinking;

^c Carr's index (IC), calculated from tapped and untapped density on the basis of Eq. (3);

^d Aerodynamic diameter (d_{ae}) calculated on the basis of Eq. (2);

^e Powder density was measured three times. The standard deviation in both tapped and untapped density was below 0.02 g/cm^3 .

Crosslinked microspheres appeared to be larger than the uncrosslinked ones, obtained without irradiation. The D_n of crosslinked particles was statistically slightly higher when more DMPA was used (Table 1). The arithmetic mean diameter of microspheres determined from laser diffraction particle size analyzer was always slightly higher than those calculated from optical microscopic images, whereas their geometric mean diameter was lower.

The size distribution of microspheres calculated as D_v/D_n from microscopic images (Table 1) or determined by laser diffraction particle size analyzer was independent on crosslinking process. The representative size distribution data, obtained from laser diffraction particle size analyzer are shown in Fig. 1.

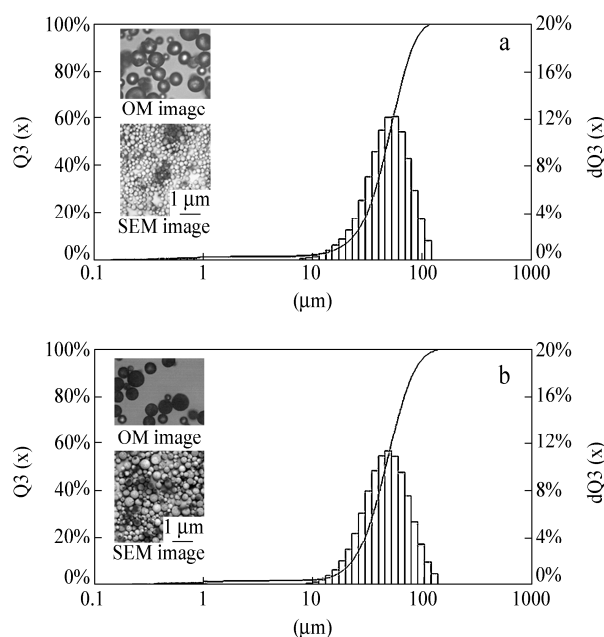


Fig. 1 Size distribution (determined by laser diffraction particle size analyzer) with representative OM and SEM images of (a) non porous (obtained without DMPA) and (b) porous (obtained with use of 2% of DMPA) PSAGESBA 80 microspheres

Effectiveness of crosslinking was characterized by the content of insoluble part of samples (gel content) and it was in the range of 18% to 75% (Table 1). The usage of higher amount of DMPA resulted in higher gel content in microspheres. Microparticles obtained from PSAGESBA60 were always more crosslinked than those obtained from PSAGESBA80.

Uncrosslinked and crosslinked microspheres obtained from PSAGESBA80 were tested on human fetal osteoblast cells using the MTS assay to determine their cytotoxicity. The obtained preliminary data show that they are non-toxic and that crosslinking process and presence of DMPA had no influence on the viability of cells (the specific results will be published separately).

Porosity Characteristics

Crosslinked microparticles, contrary to uncrosslinked ones were porous. The porous structure was obtained as the effect of photocrosslinking^[27]. Photopolymerization begins instantly when the UV irradiation is applied, causing the formation of crosslinked skin at the surface of microspheres. Methylene chloride closed inside of microparticles acts as a porogen and during evaporation destroys the crosslinked structure thus creating the pores.

The porous structure of crosslinked microspheres caused their lower powder density. The powders densities (estimated as the tapped and untapped once) of obtained microspheres are collected in Table 1. The tapped

density of crosslinked porous particles was in the range 0.14–0.28 g/cm³ and was much lower than the tapped density of non-porous uncrosslinked particles, which was equal to about 0.7 g/cm³. The powder density (tapped and untapped one) of porous crosslinked particles depended on the amount of DMPA used. The tapped density was the lowest for microspheres obtained with the smallest amount of DMPA. Such microspheres were the most porous ones. The porosity of obtained microspheres was calculated from the difference between the powder density (tapped one) and bulk density of poly(ester-anhydride). It was also estimated by mercury intrusion technique. The results are presented in Table 2.

Table 2. Porosity characteristics of microspheres obtained with different amounts of DMPA

Polymer	DMPA (wt%)	V_{total}^c (cm ³ /g)	P_{total} (%)		P_{intra}^c (%)	Pore size (μm)	
			Cal. ^a	MIP ^c		SEM ^b	MIP ^c
PSAGESBA80 (80% SBA)	–	0.65	40	46	–	–	–
	0.5	3.82	88	81	36	2.45 ± 0.68	1.22
	2	3.67	81	81	34	2.99 ± 0.55	2.05
	5	2.61	78	76	34	2.95 ± 0.46	2.03
	10	2.53	76	63	17	2.91 ± 0.22	2.50
PSAGESBA60 (60% SBA)	–	0.89	42	52	–	–	–
	0.5	3.17	87	77	27	3.46 ± 0.96	1.24
	2	3.02	79	78	25	5.58 ± 0.92	2.22
	5	3.01	78	76	27	5.94 ± 1.21	2.66
	10	2.65	77	71	11	4.32 ± 1.43	3.80

V_{total} – specific volume of pores, P_{total} – total porosity, P_{intra} – intraparticle porosity

^a Calculated from tapped and bulk (ρ_{BP}) density on the basis of Eq. (1), $\rho_{\text{BP}} = 1.18$ g/cm³ for PSAGESBA80,

$\rho_{\text{BP}} = 1.20$ g/cm³ for PSAGESBA60;

^b Calculated from SEM images;

^c Determined by mercury intrusion porosimetry (MIP)

As seen in Table 2, the porosity of microparticles depended mainly on the amount of DMPA used and depended only slightly on the functionality of poly(ester-anhydride). The highest porosity was observed for microspheres obtained from PSAGESBA80 with use of 0.5 wt% DMPA. The insoluble part of sample (gel content) in such microspheres was only 18%. The regular porous structure of microspheres with open pores can be observed on SEM images presented in Fig. 2.

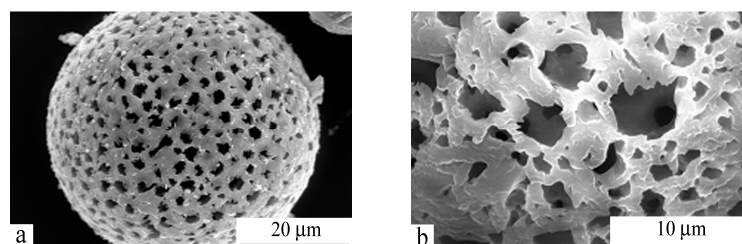


Fig. 2 SEM images of PSAGESBA80 microspheres obtained with use 0.5 wt% of DMPA (a) surface morphology, (b) inner structure

Open-pore structure was observed at the surface (Fig. 2a) as well as inside (Fig. 2b) microspheres. It was also stated that the pores inside were slightly larger than those at the surfaces. The surface morphology of microspheres obtained from poly(ester-anhydride)s with different functionality and with use of different amount of DMPA was presented in Fig. 3. As it can be seen, the microspheres obtained from PSAGESBA80 possessed more regular porous structure than those obtained from PSAGESBA60. The size of pores at the surface of PSAGESBA80 microspheres, determined from SEM images, were in the range of 0.5–3.5 μm, whereas at the surface of PSAGESBA60 microspheres much bigger pores appeared (even above 7 μm) and their distribution was less regular.

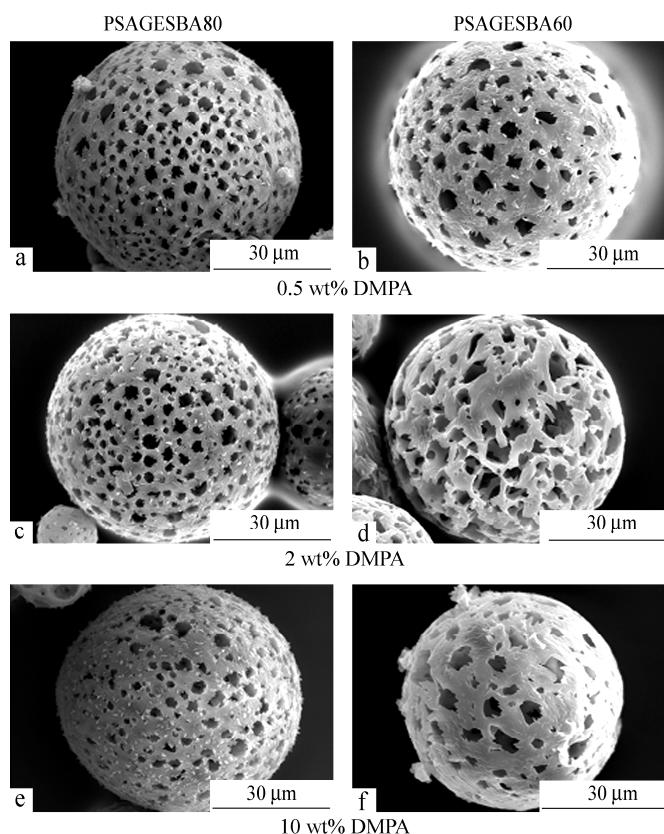


Fig. 3 SEM microphotographs of porous microspheres obtained with use of different amounts of DMPA: (a, b) 0.5 wt%, (c, d) 2 wt%, (e, f) 10 wt% (Left panel: microspheres obtained from PSAGESBA80, right panel: microspheres obtained from PSAGESBA60.)

The mean diameters of pores calculated from SEM images (~500 pores were measured) are collected in Table 2. Generally pores observed by SEM at the surface of PSAGESBA60 microspheres were statistically larger than those observed at the surface of PSAGESBA80 microparticles. The pore sizes varied as a function of DMPA content (so due to crosslinking degree), especially for PSAGESBA60 microspheres. These results were confirmed by mercury intrusion porosimetry. The pore size distribution curves obtained for all porous microspheres have bimodal character with two maximum peaks at ~2–3 μm (corresponding to intraparticle pores) and ~20 μm (corresponding to interparticle volume). In the pore size distribution curves obtained for non-porous particles, there was observed always just one peak corresponding to interparticle voids^[27]. Figure 4 presents the intraparticle pore size distribution curves for selected microspheres obtained from PSAGESBA80 and PSAGESBA60, with use of various amount of DMPA.

The median pore diameters calculated from mercury intrusion porosimetry data are presented in Table 2. The median pore diameters were always smaller for PSAGESBA80 microspheres than for those obtained from PSAGESBA60 with use the same amount of DMPA. In Table 2, total porosity (P_{total}) and intraparticle porosity (P_{intra}) of microspheres, as well as the total specific volume, determined by mercury intrusion porosimetry, were also collected. Total porosities determined by mercury intrusion porosimetry corresponded to porosity values calculated from tapped and bulk density. As can be seen in Table 2, P_{intra} of microspheres obtained from PSAGESBA80 was bigger than P_{intra} of PSAGESBA60 microspheres obtained with the same amount of DMPA. This finding indicates that the porosity and pore size of microspheres can be controlled by the amount of photoinitiator and functionality of poly(ester-anhydride) used. This approach to controlling the porous structures of polymeric microspheres could find many useful applications.

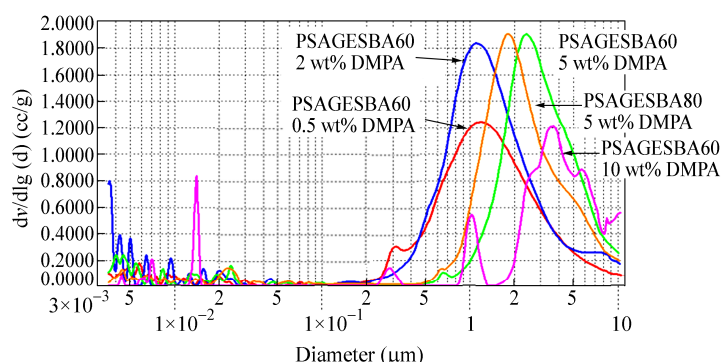


Fig. 4 Pore size distribution curves for selected microspheres obtained with different amounts of DMPA

Flow Ability and Aerodynamic Properties

The most porous particles obtained with use of 0.5 wt% DMPA possessed the lowest tapped density and simultaneously the highest untapped one (Table 1). Hence such microspheres displayed also low value of Carr's index $IC = 7$ for PSAGESBA80 and $IC = 12$ for PSAGESBA60 respectively (Table 1). The very low values of IC indicate their good flow ability. Furthermore aerodynamic diameter (d_{ac}) values estimated by normalizing the volume average diameters of microspheres with the tapped density according to Eq. (2), was reduced to about $10\ \mu\text{m}$ (Table 1). The aerodynamic diameter is regarded as a key factor for pulmonary delivery, especially for inhaled polymer microspheres with the appropriate value in the range of $1\ \mu\text{m}$ to $5\ \mu\text{m}$. The d_{ac} values of microspheres obtained with use of 0.5 wt% DMPA can be one more reduced (below $5\ \mu\text{m}$) by obtaining microspheres with the same tapped density and smaller volume average diameters (below $20\ \mu\text{m}$). The studies done until now indicate that the porosity of microspheres is strictly connected with their size. Although small microspheres with diameter $2\text{--}3\ \mu\text{m}$ are not porous, microspheres with diameter above $10\ \mu\text{m}$ indicate high porosity and possess low tapped density. The appropriate flow and aerodynamic properties of such microspheres indicate their usability in pulmonary drug delivery, however the aerosolization properties and pulmonary deposition studies have to be done. More crosslinked microparticles, obtained with use of more than 0.5 wt% of DMPA possessed higher d_{ac} and IC parameters (Table 1), so they are not suitable for pulmonary drug delivery. Such microparticles however were also very porous and can be used in other biomedical applications, for example injectable or gastroretentive drug delivery systems as well as regenerative medicine to fabricate biodegradable scaffolds.

Characteristics of Drug Loaded Microspheres

The study on loading efficiency was performed with use of *p*-nitroaniline, doxycycline and theophylline. *p*-Nitroaniline was used as a model of drug soluble in methylene chloride, which can be incorporated into microspheres by use of simple O/W (oil in water) emulsion technique. *p*-Nitroaniline was entrapped in microparticles with various crosslinking degree and various porosities. The loading efficiencies were dependent on the kind of copolymers and also on the crosslinking degree (gel content). The *p*-nitroaniline was better entrapped in PSAGESBA80 than in PSAGESBA60 microspheres (Fig. 5). It is in accordance with our previous studies^[39]. We found however that loading efficiencies of *p*-nitroaniline in crosslinked particles were relatively higher than in non-crosslinked ones (Fig. 5). It is connected with faster solidification of crosslinked microspheres and thus closing higher amount of *p*-nitroaniline inside. The higher loading efficiencies can be also caused by regular porous structures of crosslinked microspheres. The same effect was observed by Lee *et al.* for PLGA microspheres with regular porous structure obtained with use of BSA as osmotic agent^[24]. However, in our study crosslinking appears to be the main factor responsible for the increase of loading efficiency, rather than regular porous structure. It is proved by the increase of that efficiency with the increase of crosslinking degree (Fig. 5).

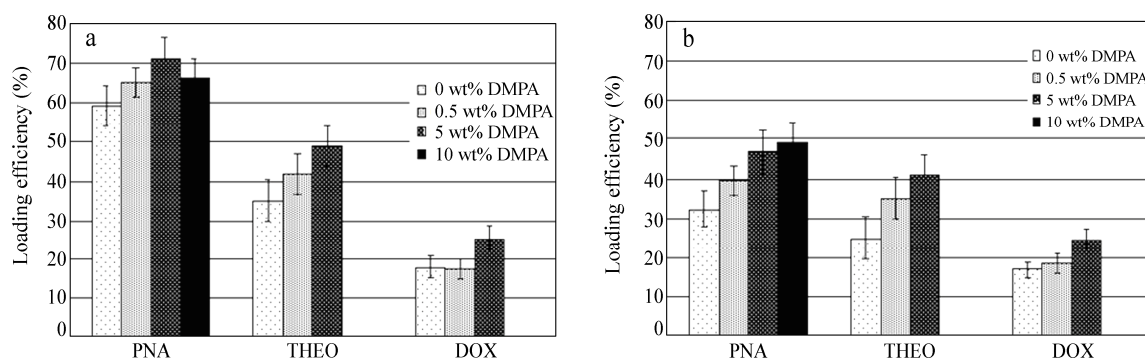


Fig. 5 Effect of DMPA content on loading efficiency of *p*-nitroaniline (PNA), doxycycline (DOX) and theophylline (THEO) in (a) PSAGESBA80 and (b) PSAGESBA60 microspheres

The porosity of *p*-nitroaniline loaded microparticles was slightly decreased (compared to unloaded microparticles), however their powder density was still low and their flow ability was very good (Table 3).

Loading of drugs insoluble in methylene chloride (doxycycline and theophylline) required the use of double emulsion system (W/O/W). The performed experiments indicated that the use of W/O/W technique also allowed to obtain macroporous microspheres (by photopolymerization of allyl groups), but this technique, as it was expected, influenced the porosity characteristics. The total porosity of unloaded microspheres obtained with use of W/O/W technique was slightly lower than the porosity of those obtained with simple O/W method (Table 3).

Table 3. Comparison of properties unloaded and loaded microspheres obtained from PSAGESBA80 with use of 0.5 wt% of DMPA

Kind of microspheres	Powder density (g/cm ³)		P_{total}^a (%)	IC ^b	V_{total}^c (cm ³ /g)	P_{total}^c (%)	Pore size ^c (μm)	
	Tapped ^d	Untapped ^d					median	mode
Unloaded O/W	0.14	0.13	88	7	3.82	81	2.03	1.82
Unloaded W/O/W	0.22	0.20	81	10	1.82	62	3.98	4.61
Loaded with PNA	0.20	0.18	83	11	2.65	76	2.22	2.01
Loaded with theophylline	0.23	0.21	80	9	1.91	70	4.01	4.70
Loaded with doxycycline	0.21	0.18	82	16	2.01	74	3.05	3.53

V_{total} - specific volume of pores, P_{total} - total porosity

^a Calculated from tapped and bulk density on the basis of Eq. (1);

^b Carr's index (IC) calculated from tapped and untapped density on the basis of Eq. (3);

^c Determined by mercury intrusion porosimetry;

^d The standard deviation in powder density was below 0.02 g/cm³.

The use of double emulsion can generate pores in microspheres without any additional treatment, which may disturb the process of pores creation by photopolymerization and cause irregularity of porous structure. However, the study on crosslinked microspheres obtained by W/O/W technique indicated that such microspheres can be successfully loaded with doxycycline or theophylline and the crosslinking process has a positive impact on loading efficiencies of drugs also in this case (Fig. 5). Microspheres loaded with doxycycline or theophylline indicated high porosity and good flow ability (Table 3), however their porosity is slightly lower than the porosity of microparticles loaded with *p*-nitroaniline (obtained with use O/W technique). The data collected in Table 3 indicated that the use of W/O/W technique also influenced the size of pores created in microparticles. The median and mode diameters of pores were always higher for microspheres obtained by W/O/W technique than for those obtained with use simple O/W emulsion. The surface morphology and porous structure were also different for those microparticles (Fig. 6).

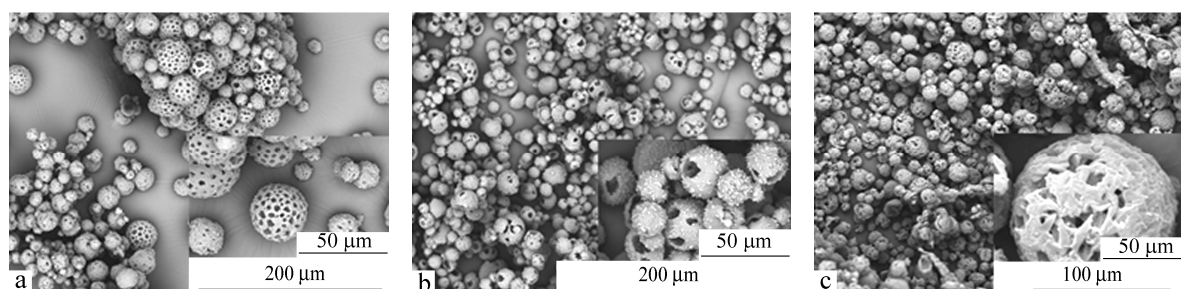


Fig. 6 SEM microphotographs of porous microspheres obtained from PSAGESBA80 with use of 0.5 wt% of DMPA, loaded with (a) *p*-nitroaniline, (b) doxycycline and (c) theophylline

As it can be seen, the microspheres loaded with *p*-nitroaniline, possessed more regular porous structure (similar to unloaded microparticles obtained by O/W technique) than those loaded with doxycycline or theophylline obtained with use of W/O/W method. The SEM observations confirmed also lower porosity (determined by mercury intrusion porosimetry) of doxycycline or theophylline loaded microspheres, however it can be stated that the porosity of such microspheres is sufficient to achieve good flow ability (Table 3).

CONCLUSIONS

Functional poly(ester-anhydride)s with allyl pendant groups can be used to formulate photocrosslinked porous microspheres with diameters within the range of 26–38 µm. The effectiveness of the crosslinking process depends on the content of pendant allyl groups as well as on the amount of photoinitiator used. Crosslinked microspheres are porous, contrary to uncrosslinked ones. Porosity of microparticles (in the range of 76%–88%) depends on functionality of poly(ester-anhydride)s and amount of the photoinitiator. The most porous particles (with the lowest crosslinking degree) are obtained with use of the lowest amount of photoinitiator (0.5 wt% of DMPA), from poly(ester-anhydride) containing the lowest amount of allyl groups. Their flow ability expressed by Carr's index is excellent. The high porosity of microspheres would enable their eventual use in biomedical applications *e.g.* in pulmonary drug delivery, gastroretentive floating systems or tissue engineering. Crosslinked porous microparticles indicate higher loading efficiencies of model drugs than non-crosslinked ones. The porosity of drug loaded microparticles is slightly lower, however their flow ability is still very good.

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