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IN VITRO RELEASE OF MODEL COMPOUNDS WITH DIFFERENT HYDROPHILICITY FROM POLY(ESTER-ANHYDRIDE) MICROSPHERES

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Abstract: Poly(ester-anhydride) microspheres were prepared using emulsion solvent evaporation technique from two copolymers obtained by polycondensation of sebacic acid (SBA) and oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl groups (OSAGE). The SBA content in copolymers was 90 or 70 w/w %, respectively. The size of microspheres obtained was in the range of 2-4 μm (small microspheres) or 12-31 μm (large ones) and depended on stirring conditions used in emulsion formulation process, as well as on concentration of polymer solution used. Poly(ester-anhydride) microspheres were loaded with three model compounds (rhodamine B, *p*-nitroaniline and piroxicam) with different water solubility. The loading efficiency was dependent on kind of model compound, polymer composition and size of microparticles. Each of three model compounds also exhibited different release profiles. Rhodamine was released very quickly. The release of *p*-nitroaniline was more prolonged, but showed only little dependence on microsphere size and polymer composition, whereas the piroxicam-loaded microspheres exhibited the most interesting release profile, showing that release rate as well as transport mechanism can be adjusted by changing microsphere size and poly(ester-anhydride) compositions.

Keywords: biodegradable microspheres, emulsion solvent evaporation, poly(ester-anhydride), release kinetic, drug delivery

Microspheres are a useful type of delivery system for administration of drugs, since at one fell swoop they can be used to encapsulate, protect and control release of a wide variety of drugs (1-4). They are especially useful due to that they are easily administered by injection. Biodegradable microspheres do not require surgical removal after drug exhaustion (5, 6). The drug release rate from biodegradable microspheres can be controlled by manipulation with the particle size (7-9) the polymer degradation rate, the polymer degradation and erosion mechanism as well as other factors (10, 11). Tamada and Langer (12) classified degradable polymers into surface eroding and bulk eroding ones. The bulk erosion would be expected for polyesters whereas polyanhydrides exhibit a surface erosion profile controlled by the hydrophobic nature of the polymer backbone. Surface eroding polyanhydrides may simplify the drug release kinetics because water penetration into the microsphere interior is minimized, and the drug release rate becomes dependent predominantly on the polymer erosion rate (8, 13, 14). Poly(ester-anhydride)s combine the individual properties of two different classes of biodegradable polymers. The differences in degradation behavior (depending on the poly(ester-anhydride) copolymers ratio) can be used to influence release profiles of encapsulated bioactive compound (11, 15). However, drug release and polymer degradation are sometimes not correlated, depending on the relative hydrophobicity and compatibitity of the comonomers as well as the drug loaded. Thus, the interaction between the polymer and the drug often influenced the mechanism of drug release due to non-uniform drug distribution (8). One of critical factors determining drug release rate is microsphere size. In many cases, smaller microspheres release the active compound faster due to increased surface area/volume ratio (7). However, the effects of microsphere size can be more complex. The small microspheres used to solidify more quickly and exhibit more homogeneous drug distribution than large ones (especially when drug and polymer matrix differ in hydrophilicity) resulting in slower

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releasing rate. Specific physicochemical characteristics of a drug, such as molecular weight, molecular volume and polarity, are also expected to affect the interaction between drugs and polymers, both during formulation and release experiments, and may also influence the release mechanism (8, 16-18).

Recently, we have obtained functional poly(ester-anhydride) microspheres with relatively narrow size distribution by emulsion solvent evaporation technique (19). In this paper, we present the studies on loading the microspheres with model compounds with different hydrophilicity and their release characteristics.

The aim of this work was to evaluate the influence of polymer composition and size of microparticles on loading efficiency and release profiles of model compound with different hydrophilicity. Three low molecular weight model compounds: rhodamine B (water solubility 8 mg/mL), *p*-nitroaniline (water solubility 1 mg/mL) and piroxicam (water solubility 0.1 mg/mL) were chosen to investigate the role of different "compatibility" of loaded compound with polymer matrix on the drug loading and release mechanism.

EXPERIMENTAL

Materials

Succinic acid 99% (Aldrich), sebacic acid 98% (ACROS Organic), allyl glycidyl ether 99% (Aldrich) and acetic anhydride (POCH S.A.), poly(vinyl alcohol) (MW = 88000 g/mol, 88% hydrolyzed, ACROS Organic), rhodamine B and *p*-nitroaniline (Fluka), piroxicam (Aldrich) and phosphate buffer solution pH = 7.41 (LabStand Poznań) were used as supplied. Solvents were purified according to known procedures.

Synthesis of poly(ester-anhydride)s

Poly(ester-anhydride)s were synthesized in two-step reaction from sebacic acid (SBA) and oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl groups (OSAGE), according to procedure described earlier (20). Copolymers purposed for the preparation of microspheres in this work were synthesized with use of 70 or 90% w/w of SBA. SBA and OSAGE (total amount 10 g) were refluxed in 100 mL of acetic anhydride, under nitrogen for 30 min, to yield mixed prepolymers. An excess of acetic anhydride and acetic acid formed as a by-product were next removed under vacuum. In the second step, mixed prepolymers were condensed to yield poly(ester-anhydride)s. Polycondensation was carried out at 150^{o}C for 2 h under high vacuum (finally 0.01 mm Hg). The poly(ester-anhydride)s obtained were dissolved in methylene chloride, precipitated in petroleum ether and dried at 40^{o}C under vacuum. The polymers obtained were stored at -18^{o}C prior to be used for microspheres formulation.

Microsphere preparation

The poly(ester-anhydride)s obtained were formulated into unloaded (blank) and model compound loaded microspheres using emulsion solvent evaporation technique. Parameters of the process such as: concentration of polymer solution and speed of homogenizer were chosen basing on the research described previously (19) to obtain small or large microparticles.

Blank microspheres

The polymer solution in methylene chloride (20 ml, concentration 10 or 50 mg/mL) was emulsified in 400 mL of aqueous solution (0.5 % w/w) of poly(vinyl alcohol) (PVA), using ULTRA-TURRAX T18 homogenizer (3000 or 18000 rpm), for 30 s. The emulsion was then stirred with magnetic stirrer at 1100 rpm at room temperature for 3 h to evaporate the organic solvent. After that, microspheres were collected by centrifugation at 5000 rpm for 5 min, washed 3 times with distilled water, lyophylized and stored in freezer. The yield of microparticles obtained were ca. 40% for small particles and above 70% for large ones.

Model compound loaded microspheres

The procedure for preparation of *p*-nitroaniline or piroxicam loaded microspheres was similar to that used for preparation of blank particles. *p*-Nitroaniline or piroxicam (10% w/w 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.

For encapsulation of water soluble rhodamine B, water-oil-water (W/O/W) technique was used. Rhodamine B (3% w/w with respect to the mass of polymer) was dissolved in 0.8 mL of distilled water. The aqueous solution was then emulsified in 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 rpm, for 30 s. The outer emulsion was obtained using 3000 rpm when large microspheres were prepared or 18000 rpm when small microsphe-

res were prepared. The solidification and isolation of microspheres were performed similarly to blank ones.

Characterization of the particles

Shape, size distribution of microspheres were estimated using optical microscope (OM) DELTA Optical ME 100 and PHAMIAS 2003 v.1.3 B software, as described earlier (19). The morphological characterization of microspheres was carried out on a TESLA BS 340 scanning electron microscope (SEM). The differences in thermal properties of poly(ester-anhydride) blank or loaded microspheres were observed by differential scanning calorimetry (DSC), using 822e DSC Mettler Toledo apparatus. Samples were tested at the temperature ranging from -70 to 250^{o}C at a heating rate of 10^{o}C/min.

Hydrolytic degradation

Hydrolytic degradation of blank microspheres was performed in phosphate buffer solution of pH = 7.41 (PBS) at 37^{o}C. Microspheres (ca. 0.2 g) were placed in vials containing 20 mL of PBS. The vials were incubated at 37^{o}C for various time (1 h to 14 days). After incubation, the samples were separated by centrifugation, washed with water, dried in vacuum at 50^{o}C and weighted to estimate weight loss.

***In vitro* model drug release**

Drug release studies were performed during their hydrolytic degradation in PBS at 37^{o}C under static conditions. Microspheres (~5 mg) containing model compound were placed in vials and suspended in 1.5 mL of PBS. The vials were incubated at 37^{o}C. At certain time point (1 h to 30 days), the samples were centrifuged, 1 mL of supernatant was removed and the medium was supplemented with fresh PBS. Vials were briefly vortexed to resuspense the microspheres. Concentration of model compounds in the supernatant was determined by measuring the UV absorbance at the λ_{max} = 553 nm for rhodamine B, 377 nm for *p*-nitroaniline and 394 nm for piroxicam. All these experiments were performed in triplicate.

Cumulative release (Su) of model compound was calculated in respect to mass of microspheres, according to eq. (1) and (2):

$$Su = \Sigma m_{SMn-1} + 1.5 m_{SMn} \quad (1)$$

$$m_{SMn} = (C \cdot r)/m_m \quad (2)$$

where: Su – cumulative amount of model compound released from 1 mg microspheres within defined time [μg/mg]; m_{SMn} – the mass of model compound in the n-th sample of supernatant (taken after a specified time) in respect to 1 mg of microspheres [μg/mg]; C – concentration of the model compound in a buffer solution in the n-th sample [μg/mL]; r – dilution of the buffer solution used for the analysis of UV; m_m – weight of microspheres [mg]

Absorbance spectra of model compounds, obtained from fresh solution and supernatant sampled at various times during the release experiment, were identical in shape indicating that there was no degradation of model compounds during the release period.

Furthermore, the model compound release mechanism was determined by fitting the experimental data to Korsmeyer-Peppas equation model (21, 22). This model is used to analyze release of pharmaceutical polymeric dosage forms when the release mechanism is not well known or when more than one type of release phenomena are involved (18).

Drug loading efficiency

Total amount of model compound contained in microspheres was obtained in separate experiment by accelerated degradation in PBS at 70^{o}C. 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.

The actual loading of model compound (LA) and encapsulation efficiency (EE) were calculated from the weight of the initial – drug loaded microspheres and the amount of model compounds used and incorporated, according to equation (3) and (4).

$$LA = (mSM / mm) \quad (3)$$

$$\% EE = (LA / LTh) \times 100 \quad (4)$$

where: L_A – actual loading of model compound [μg/mg]; m_{SM} – the weight of model compound [μg] encapsulated in microspheres; m_m – the weight of model compound loaded microspheres [mg]; L_{Th} – theoretical loading of model compound for piroxicam and *p*-nitroaniline, L_{Th} = 100 μg/mg, for rhodamine B L_{Th} = 30 μg/mg.

LA of piroxicam and *p*-nitroaniline were also directly determined by dissolving 1 mg of loaded microparticles in chloroform and subsequent determination of amount of piroxicam and *p*-nitroaniline in organic solutions by UV.

LA values determined in PBS solutions after accelerated degradation of microspheres (Table 1) and these determined in organic solutions were sim-

Table 1. Microsphere size, actual and theoretical loading and encapsulation efficiency of various microsphere samples and various model compounds.

Kind of		$D_n \pm S$ (D_v/D_n)	$L_A \pm SD^a$ [μg/mg]	L_{Th} [μg/mg]	$EE \pm SD^a$ [%]
model compound	microspheres				
Blank particles	PSAGESB90 large	17.75 ± 5.04 (1.37)	-	0	-
	PSAGESB70 large	24.87 ± 7.17 (1.22)	-		-
	PSAGESB90 small	2.57 ± 0.64 (1.19)	-		-
	PSAGESB70 small	1.98 ± 0.45 (1.16)	-		-
Rhodamine B	PSAGESB90 large	27 ± 5.98 (1.48)	9.4 ± 0.8	30	31.3 ± 2.7
	PSAGESB70 large	31.44 ± 7.51 (1.52)	4.1 ± 0.7		13.7 ± 2.3
	PSAGESB90 small	3.04 ± 0.97 (1.29)	10.9 ± 1.2		36.3 ± 4.0
	PSAGESB70 small	2.39 ± 0.11 (1.21)	6.1 ± 0.6		20.3 ± 2.0
p-Nitroaniline	PSAGESB90 large	15.59 ± 4.95 (1.32)	61.9 ± 3.3	100	61.9 ± 3.3
	PSAGESB70 large	23.77 ± 8.89 (1.33)	56.3 ± 2.3		56.3 ± 2.3
	PSAGESB90 small	2.05 ± 0.54 (1.22)	12.1± 1.2		12.1 ± 1.2
	PSAGESB70 small	2.32 ± 0.65 (1.27)	48.8 ± 3.1		48.8 ± 3.1
Piroxicam	PSAGESB90 large	12.64 ± 4.77 (1.44)	16.2 ± 0.9	100	16.2 ± 0.9
	PSAGESB70 large	14.19 ± 5.29 (1.37)	29.3 ± 1.0		29.3 ± 1.0
	PSAGESB90 small	4.01 ± 1.16 (1.25)	7.3 ± 0.7		7.3 ± 0.7
	PSAGESB70 small	2.47 ± 0.78 (1.27)	29.7 ± 1.0		29.7 ± 1.0

EE – encapsulation efficiency (% ± SD^a); L_A - actual model compound loading (μg/mg ± SD^a); L_{Th} - theoretical model compound loading; [a]Standard deviation, calculated on three different batches

ilar to Su values obtained after relatively long release period.

RESULTS AND DISCUSSION

Functional poly(ester-anhydride)s (PSAGESB) with allyl pendant groups (Fig. 1) were obtained by polycondensation of sebacic acid (SBA) and oligo(3-allyloxy-1,2-propylene succinate) (OSAGE). The detailed characteristics of poly(ester-anhydride)s were described earlier (20, 23). Copolymers purposed for the preparation of microspheres in this work were synthesized with use of 70 or 90% w/w of SBA in the reaction mixture.

Formulation of poly(ester-anhydride) microspheres

Microspheres were prepared using emulsion solvent evaporation technique (O/W). Poly(vinyl alcohol) (PVA) was used as emulsion stabilizing agent. Incorporation of rhodamine B (the most hydrophilic substance) into microspheres required the use of inner W/O emulsion prepared by dispersion of an aqueous rhodamine B solution in polymer solution in methylene chloride. The inner emulsion was then dispersed in 0.5% of aqueous PVA solution to form W/O/W emulsion. Such method (W/O/W) is commonly used for encapsulation of hydrophilic substance into microspheres (1). During

the preparation of microspheres such parameters as concentration of polymer solution and speed of homogenizer were changed to prepare small particles with diameter within the range Dn = 1.9–4.2 μm or large particles with Dn = 12.4–31.4 μm, respectively. From each poly(ester-anhydride)s small and large microspheres (Fig. 1) loaded with one of three model compounds with different hydrophilicity as well as unloaded (blank) microspheres were prepared (Table 1).

Microspheres with the highest diameters were obtained from PSAGESB70 solution with concentration of 50 mg/mL, whereas the smallest particles were prepared from PSAGESB70 solution with concentration of 10 mg/mL. The size of microparticles was dependent also on the kind of model compound loaded, besides of basic factors of preparation conditions. The sizes of microspheres containing *p*-nitroaniline were most similar to blank microparticles, probably due to its relatively good compatibility with the polymer matrix. The presence of *p*-nitroaniline did not influence solidification of microspheres during the solvent evaporation from the dispersion. The compatibility of *p*-nitroaniline with poly(ester-anhydride)s was confirmed by thermal analysis. DSC thermograms were performed for blank and model compounds loaded, large microspheres. The presence of model compounds in microspheres caused decreasing of melting temperature (T_m) of poly(ester-anhydride)s as well as reducing of the heat of fusion (ΔH_m), compared to T_m and ΔH_m registered for blank microparticles. These changes were least significant for microspheres loaded with *p*-nitroaniline (Table 2).

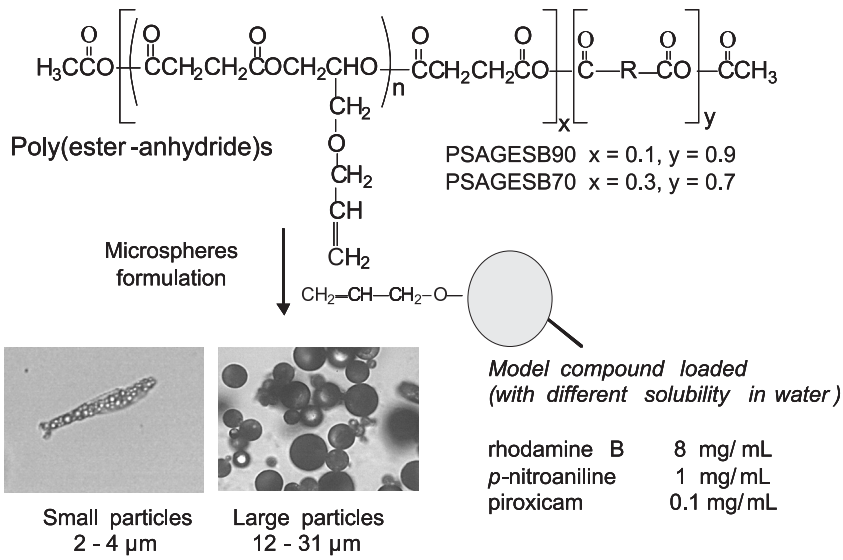

Figure 1. The scheme of preparation of model compounds loaded poly(ester-anhydride) microspheres

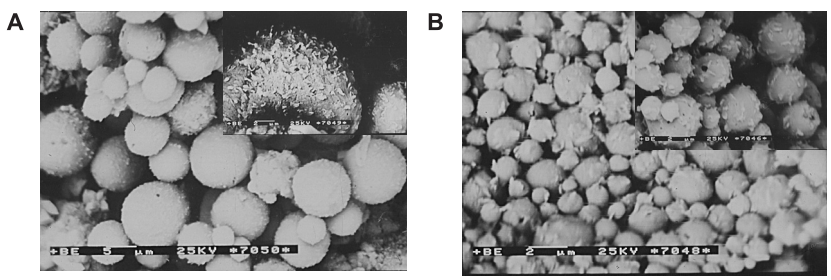

Figure 2. Scanning electron microphotographs of (**A**) large PSAGESB70 microspheres loaded with piroxicam (**B**) small PSAGESB90 microspheres loaded with rhodamine B

Table 2. Thermal properties of large microspheres (blank and loaded) made of poly(ester-anhydride)s.

Kind of		Thermal properties	
polymer	model compound	T_m [°C]	ΔH_m [J/g]
PSAGESB90	-	79.8	85.0
	p-Nitroaniline	74.2	77.9
	Piroxicam	71.5	57.8
	Rhodamine B	73.7	65.3
PSAGESB70	-	78.9	81.2
	p-Nitroaniline	76.4	79.5
	Piroxicam	71.0	55.3
	Rhodamine B	72.9	61.8

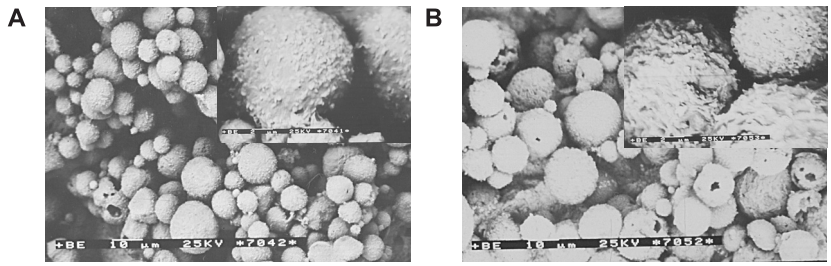

Figure 3. Scanning electron microphotographs of large microspheres loaded with *p*-nitroaniline (**A**) made of PSAGESB90, (**B**) made of PSAGESB70

Piroxicam and rhodamine B did not affect the dimension of small microspheres, but influenced the size of large ones. Large microspheres containing piroxicam were slightly smaller than respective blank ones, whereas large microspheres loaded with rhodamine B were, in turn, slightly larger than the blank ones (Table 1). The kind of the model compound had also an impact on the surface morphology of microspheres. The surface of microspheres containing the active substances (observed by SEM) were not smooth and uniform (homogeneous) in contrast to surface of blank particles. Active substances were visible on the surface of microspheres in form of cambers coated with polymer or uncoated crystals. Piroxicam crystals (as the needles completely uncovered by polymer) were very well visible on the surface of microspheres (Fig. 2A). Less piroxicam crystals were observed at the surface of small microspheres than at the surface of large ones. Rhodamine B (in form of many pins or cambers covered by polymer) was visible at the surface of small and large microspheres with similar surface density (Fig. 2B). *p*-Nitroaniline was poorly visible at the surface (Fig. 3), suggesting that it is better dispersed within the polymer matrix than piroxicam or rhodamine B.

Hydrolytic degradation of blanc microspheres

Blank microspheres obtained from PSABESB90 and PSAGESB70 were subjected to hydrolytic degradation. The degradation of the microparticles was run for 14 days in the PBS at 37°C. The hydrolytic degradation was followed by determination of the weight loss of the samples. All microspheres degraded completely to products soluble in water over two weeks whereas they lost nearly 90% of its initial weight within first 7 days of immersion in PBS. Microspheres obtained from PSAGESB90 degraded slower than those obtained from PSAGESB70. It was also stated that small microspheres degraded slightly faster (especially in the first period) than the large ones (Fig. 4). The dependence of chemical copmposition of poly(ester-anhydride)s and size of microparticles on rate of hydrolytic degradation can influence release characteristics of such microspheres. It is discussed in the next part of this article.

Drug loading and *in vitro* drug release

Three model compounds exhibiting various degrees of water solubility (Fig. 1) were encapsulated in poly(ester-anhydride) microspheres. The encapsulation efficiency was dependent on the kind of model compound as well as on the kind of copolymers (ester to anhydride ratio) and the size of microparticles (small or large ones) (Table 1). Piroxicam, having the lowest solubility in water showed encapsulation efficiency in the range 7–30%. The loading efficiency of this hydrophobic compound was higher in PSAGESB70 than in PSAGESB90. The loading efficiency of piroxicam in microspheres obtained from PSAGESB70 was independent on the size of microparticles, whereas in microspheres obtained from PSAGESB90 significant dependence on size was observed (small microparticles contained 2-fold less of piroxicam). The *p*-nitroaniline with medium solubility in water showed the highest encapsulation efficiency in the range 12–62%. It was found that *p*-nitroaniline loading as well as its encapsulation efficiency increased with increasing the size of the

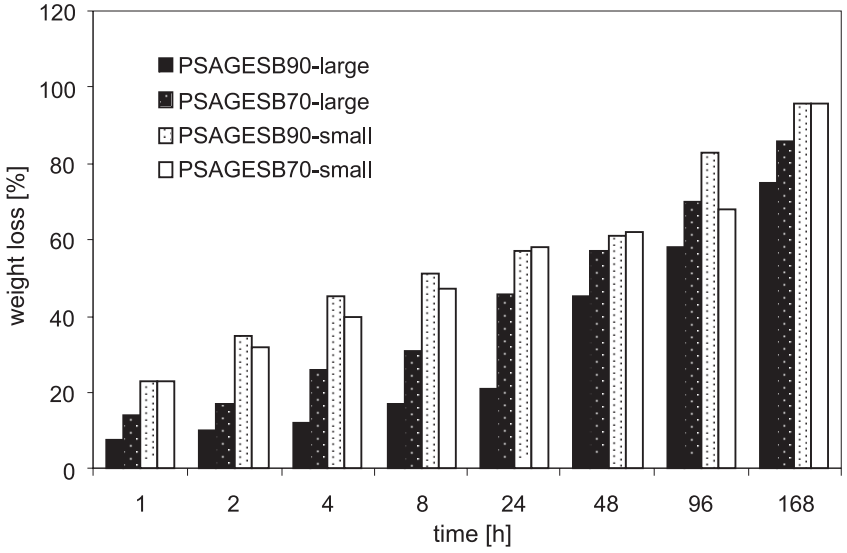

Figure 4. Effect of polymer composition and size of microparticles on the hydrolytic degradation of blank microspheres in PBS at 37°C

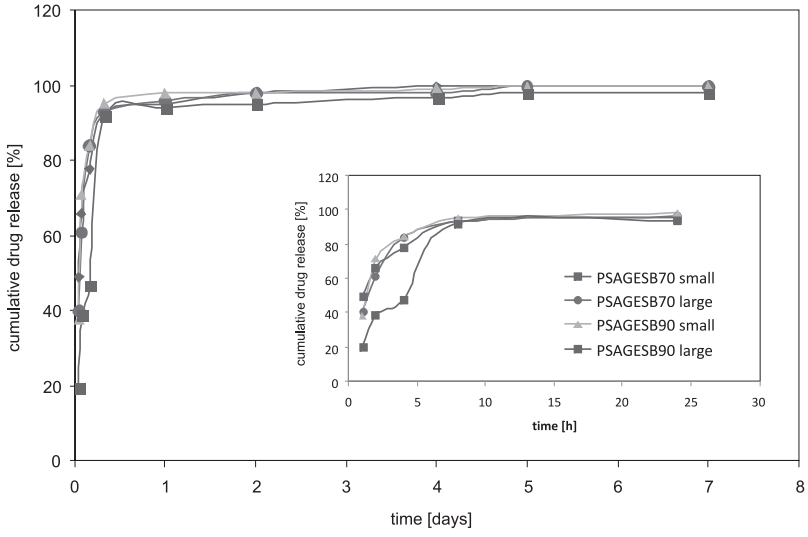

Figure 5. Cumulative release profiles of rhodamine B from various microspheres as a function of time

microspheres. For PSAGESB70 containing more oligoester fragments, the loading of *p*-nitroaniline was only slightly dependent on the size of microspheres, whereas for PSAGESB90 this dependency was strong (Table 1). The small microspheres obtained from PSAGESB90 contained 5-fold less of *p*-nitroaniline than the large ones. These results indicated better compatibility of piroxicam and *p*-nitroaniline with PSAGESB70 than with more hydrophobic and crystalline PSAGESB90. Compared to piroxicam and *p*-nitroaniline, which were better encapsulated in PSAGESB70, rhodamine B was more efficiently loaded in microspheres obtained from PSAGESB90. Amount of rhodamine B encapsulated in PSAGESB70 microspheres was 2-3-fold smaller than in microspheres obtained from PSAGESB90. This hydrophilic substance was also better encapsulated in small particles than in larger ones, probably due to their rapid precipitation. Encapsulation efficiency of rhodamine B was in the range 14-37%, however, the theoretical loading of rhodamine B was only 30 μg/mg (drug/polymer) compared to 100 μg/mg for *p*-nitroaniline and piroxicam. Lower loading typically results in higher encapsulation efficiency, due to smaller concentration gradients driving the drug out of the polymer/solvent droplets. Thus, the rhodamine encapsulation efficiency cannot be directly compared.

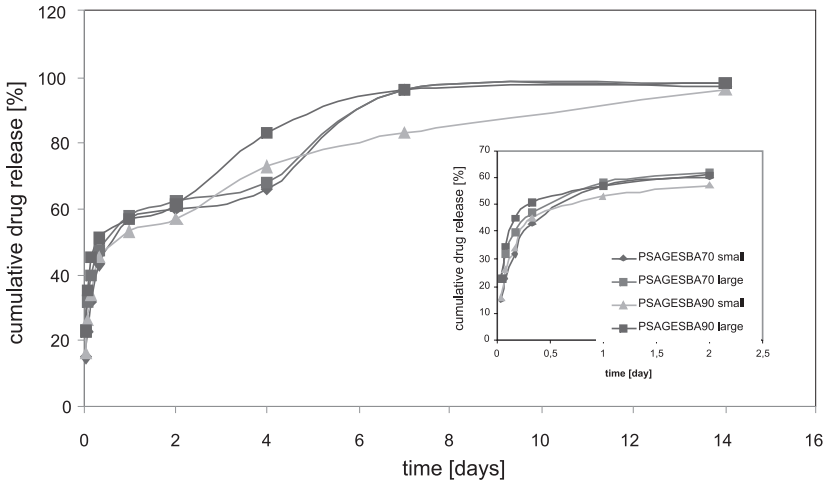

Figure 6. Cumulative release profiles of *p*-nitroaniline from various microspheres as a function of time

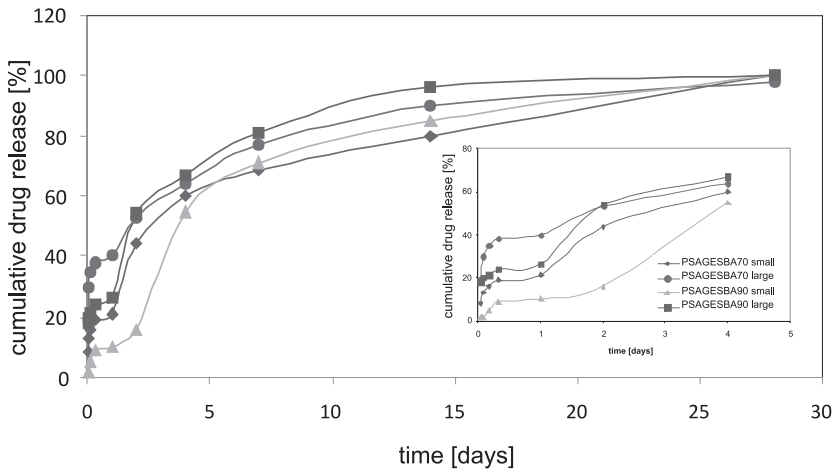

Figure 7. Cumulative release profiles of piroxicam from various microspheres as a function of time

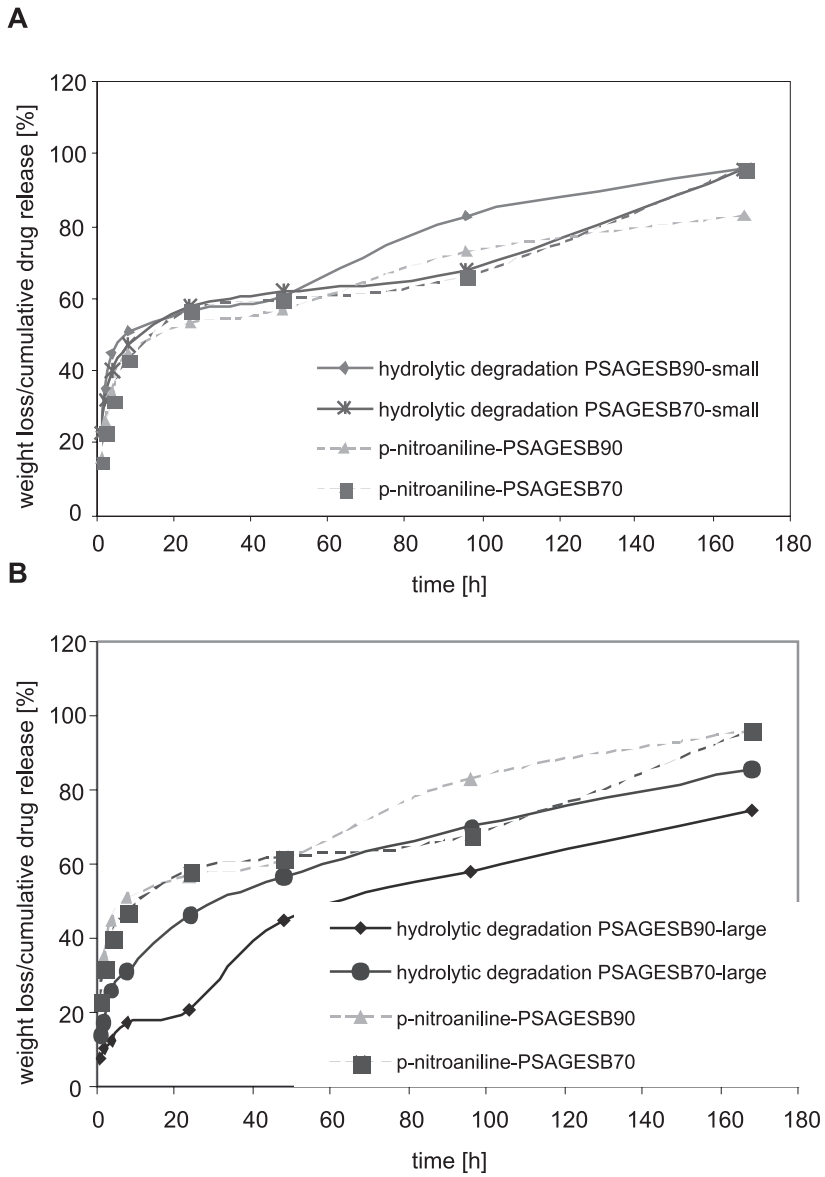

Figure 8. The comparison the release profiles of *p*-nitroaniline with hydrolytic degradation of small (**A**) and large (**B**) microspheres

The profiles of rhodamine B, *p*-nitroaniline and piroxicam release from small or large microspheres composed of two poly(ester-anhydride)s differed with SBA and OSAGE content, are shown in Figures 5, 6 and 7, respectively. The effect of polymer composition and microsphere size on drug release was different for the tree model compounds.

Rhodamine B release from microspheres was very rapid and nearly complete within 24 h. The rate of release was faster than the rate of hydrolytic degradation of blank microparticles. This hydrophilic substance was released practically independently on microsphere size and polymer compositions (Fig. 5), only for large microspheres obtained from PSAGESB90 somewhat slower release was observed during the first 8 h.

Gradual release of *p*-nitroaniline was observed within 7 days (only small microspheres obtained from PSAGESBA90 released *p*-nitroaniline slightly longer) but nearly 60% of the initial amount of this compound was released within the first 2 days (Fig. 6). The strong dependence of the rate of release on

Table 3. Kinetic parameters (n and K) estimated for Korsmeyer-Peppas model for release of different model compounds from various microspheres during the first period (ca. 60% release) and for total release.

Kind of		60 % of release[A)]			total release[B)]		
model compound	microspheres	n	K	r^{2} [C)]	n	k	r^{2} [C)]
Rhodamine B	PSAGESB90 large	0.70	21.18	0.98	0.49	24.83	0.88
	PSAGESB70 large	0.44	43.10	0.94	0.27	48.11	0.81
	PSAGESB90 small	0.42	43.98	0.85	0.27	49.33	0.73
	PSAGESB70 small	0.31	50.91	0.98	0.21	54.64	0.86
p-Nitroaniline	PSAGESB90 large	0.23	58.26	0.89	0.23	58.30	0.94
	PSAGESB70 large	0.25	57.21	0.95	0.23	55.5	0.97
	PSAGESB90 small	0.31	53.12	0.91	0.28	49.91	0.95
	PSAGESB70 small	0.36	54.71	0.95	0.31	49.55	0.95
Piroxicam	PSAGESB90 large	0.28	37.56	0.93	0.30	39.29	0.93
	PSAGESB70 large	0.22	45.98	0.91	0.23	46.85	0.96
	PSAGESB90 small	0.67	14.31	0.94	0.66	14.40	0.96
	PSAGESB70 small	0.46	30.24	0.93	0.43	29.90	0.97

[A)] 60% of release (4 days for piroxicam, 2 days for *p*-nitroaniline and 8 h for rhodamine B); [B)] total release (30 days for piroxicam, 14 days for *p*-nitroaniline and 24 h for rhodamine B); [C)] r^{2} – correlation coefficient.

the size of microparticles as well as on polymer composition was not observed also in this case. The *p*-nitroaniline release profiles showed similar burst effect (ca. 40% of cumulative release within the first few hours) for all studied microspheres followed by a time-lag when only a little amount of drug was released. Longer time-lag (up to 4 days) was observed for microspheres obtained from PSAGESB70 compared to 2 days lag period for PSAGESB90 microspheres. The *p*-nitroaniline release from small microparticles correlated with hydrolytic degradation of blank particles independently on polymer composition (Fig. 8A), but the release profile from the large particles correlated with hydrolytic degradation profile only for PSAGESB70 (Fig. 8B). The release of *p*-nitroaniline from large microspheres obtained from PSAGESB90 was faster than their hydrolytic degradation (Fig. 8B), probably due to better compatibility of *p*-nitroaniline with PSAGESB70 than with PSAGESB90.

Piroxicam release was the slowest one and in contrast to *p*-nitroaniline or rhodamine B, varied significantly with polymer composition and microsphere size. Generally, it could be stated that the small microspheres released the piroxicam slower than the large ones (bellow 80% of piroxicam was released within 14 days from small particles, whereas above 90% from larger ones). During the first 4 days, in which 53-65% of this model compound was released, the rate of release was higher for microspheres composed from PSAGESB70 (Fig. 7). The piroxicam release profiles showed a burst effect followed by a short „lag" period and next more rapid release phase between 2-7 days. Small microparticles exhibited much smaller burst effect than the large ones. Below 10% of cumulative release was observed within the first 8 h for small PSAGESB90

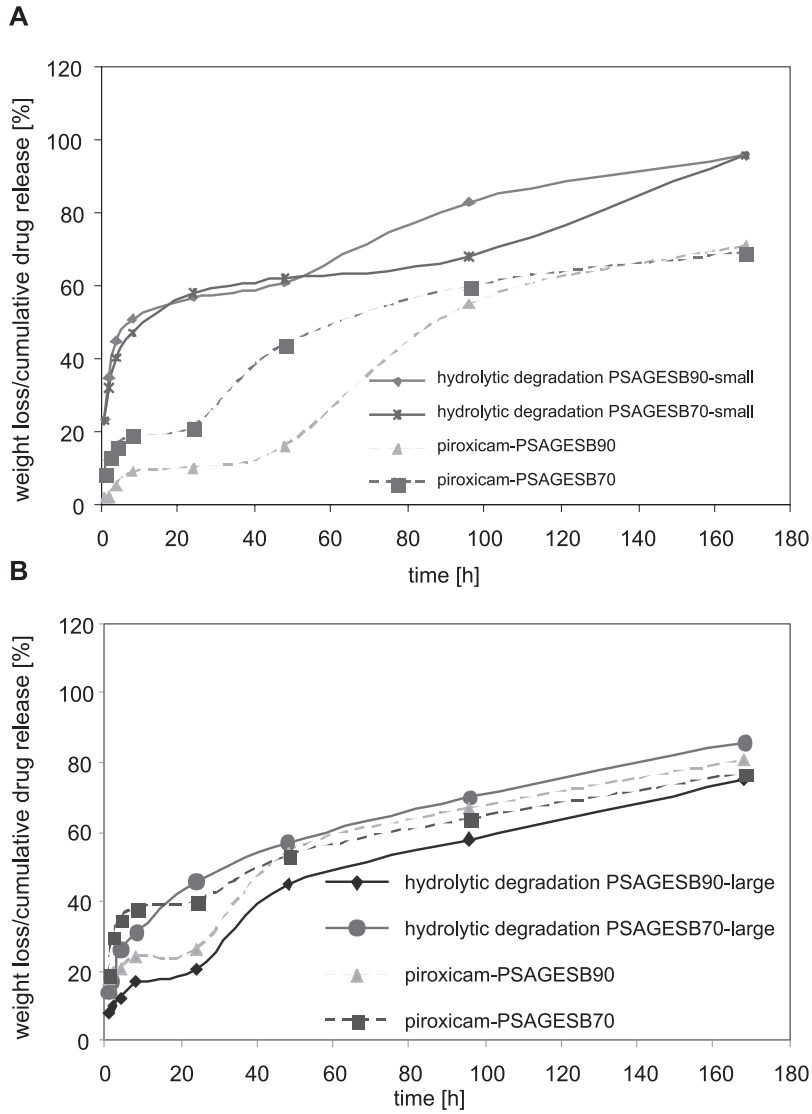

Figure 9. The comparison the release profiles of piroxicam with hydrolytic degradation of small (**A**) and large (**B**) microspheres

microspheres, whereas nearly 40% of piroxicam was released during that period from large PSAGESB70 microspheres. The release of piroxicam from large microparticles was better correlated with hydrolytic degradation of blank particles than the release of this compound from small microspheres (Fig. 9). The release of piroxicam from small particles was slower than hydrolytic degradation of blank microspheres (Fig. 9A). It is probably due to more hydrophobic nature of piroxicam-loaded particles compared to blank ones.

Kinetics of model compound release

The mechanism of drug release may be studied by using different release models. Modeling of the controlled release of drugs from polymeric devices has been a subject of considerable research over the past 30 years. Higuchi proposed (24) a simple relationship that described drug release from a matrix as a function of time (eq. 5).

$$M_t/M_8 = K\, t^{1/2} \quad (5)$$

where M_t/M_8 is the amount of released drug (%) at time t. The relative release of drug is proportional to the square root of time. Korsmeyer and Peppas (21, 25) extended the Higuchi model to a more general form (eq. 6).

$$M_t/M_8 = K\, t^n \quad (6)$$

where K is a kinetic constant incorporating structural and geometrical characteristics of the drug-loaded devices, and n is the release exponent, indicative of

the mechanism of drug release. This model is used to analyze the release from polymeric dosage forms when the release mechanism is not well known or when more than one type of release phenomena are involved. For drug-loaded slab or film system, values of n = 0.5 or less correspond to a purely Fickian diffusion mechanism (18). When n = 1.0, the rate of release is independent on time and the drug release follows zero-order kinetics. The value of n between 0.5 to 1.0 is an indicator of the superposition of two independent mechanism of drug transport (anomalous transport) (26, 27). For a spherical drug carriers, the threshold of the n-value distinguishing between Fickian and non-Fickian diffusion mechanism has been slightly modified and thus n-values between 0.43 and 0.85 represent anomalous transport (28).

Kinetic parameters K and n for microspheres loaded with three different model compounds were calculated and summarized in Table 3. The kinetic parameters were calculated for total release profile and for first period in which ca. 60% of model compound was released. The equation (6) is valid for the first 60% of the release, especially when the diffusion play the important role in release mechanism (29). As seen in Table 3, the n-values were higher than 0.43 for large microspheres loaded with rhodamine B and for small microspheres loaded with piroxicam. It suggested that the release of a low-molecular weight hydrophilic compounds from large microparticles and low-molecular weight hydrophobic compounds from small ones followed anomalous transport mechanism related to non-Fickian model. The n-value was higher for microspheres obtained from PSAGESB90 containing more anhydride than ester fragments. It indicated release mechanism closer to zero order release kinetics. The results achieved in this study indicated that the size of microspheres as well as polymer composition can play the important role in release mechanism of hydrophilic or hydrophobic drugs from poly(ester-anhydride) microspheres.

It was stated that for microspheres containing *p*-nitroaniline, a low-molecular weight compound with medium hydrophilicity, the n-values were below 0.43, suggesting that the release mechanism of such compounds is Fickian-diffusion controlled. The kinetic parameters (n and K-values) estimated for Korsmeyer-Peppas model for all microparticles loaded with *p*-nitroaniline were similar, independently on size of microparticles and composition of the polymer. It correlated with release profiles of *p*-nitroaniline showed in Figure 6.

Those results indicated that the release of encapsulated drugs from poly(ester-anhydride) microspheres depended on kind of encapsulated drugs, chemical composition of polymers (due to different compatibility of drug with polymer matrix and due to various rate of hydrolysis of polymer), and also on particle size. Therefore, by adjusting these parameters it is possible to obtain different release rates and profiles, which might be of interest for different therapeutic purposes and modalities of administration.

CONCLUSIONS

The present study demonstrates that the emulsion solvent evaporation method can be useful to formulate small or large microspheres from functional poly(ester-anhydride)s, loaded with model compounds with different hydrophilicity. The kind of model compound loaded influenced size and surface morphology of microparticles obtained. The main objective of this study was to evaluate the influence of polymer composition (OSAGE and SBA content) and size of microspheres on loading efficiency and release profiles of model compounds. The highest encapsulation efficiency was obtained for *p*-nitroaniline, probably due to its good compatibility with polymers. Piroxicam and *p*-nitroaniline, (hydrophobic compounds) were better encapsulated in PSAGESB70,whereas rhodamine B (hydrophilic compound) was more efficiently loaded in PSAGESB90 microspheres. The piroxicam loaded microspheres exhibit the most interesting release profiles, showing that elongation of release as well as reduction of burst effect can be achieved by decreasing microsphere size. The release of hydrophobic compounds from small poly(ester-anhydride) microparticles followed anomalous transport mechanism, for PSAGESB70, closer to Fickian diffusion and for PSAGESB90 closer to zero order release kinetics. When rhodamine B or *p*-nitroaniline were encapsulated, it may be difficult to tailor release profiles by controlling microsphere size and polymer composition. An understanding of the factors affecting drug release mechanisms from poly(ester-anhydride) microspheres allow to predict the behavior of biologically active substances, eventually incorporated into microparticulate systems and to design particular polymer-drug systems for various purposes.

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Declaration of interest

The author reports no conflicts of interest. The author alone is responsible for the content and writing of this article.

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