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# Biodistribution properties of nanoparticles based on mixtures of PLGA with PLGA–PEG diblock copolymers

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**Abstract**

The basic characteristics and the biodistribution properties of nanoparticles prepared from mixtures of poly(lactide-co-glycolide) (PLGA) with poly(lactide-co-glycolide)–poly(ethylene glycol) (PLGA–PEG) copolymers were investigated. A PLGA(45)–PEG(5) copolymer of relatively low PEG content and a PLGA(5)–PEG(5) copolymer of relatively high PEG content were included in the study. Increasing the PLGA–PEG content of the PLGA/PLGA–PEG mixture, or when PLGA(45)–PEG(5) was replaced by PLGA(5)–PEG(5), a decrease in the size of the nanoparticles and an increase in the rate of PEG loss from the nanoparticles were observed. The blood residence of the PLGA/PLGA(45)–PEG(5) nanoparticles increased as their PLGA–PEG content was increased, reaching maximum blood longevity at 100% PLGA(45)–PEG(5). On the contrary, the blood residence of PLGA/PLGA(5)–PEG(5) nanoparticles exhibited a plateau maximum in the range of 80–100% PLGA(5)–PEG(5). At PLGA–PEG proportions lower than 80%, the PLGA/PLGA(45)–PEG(5) nanoparticles exhibited lower blood residence than the PLGA/PLGA(5)–PEG(5) nanoparticles, whereas at PLGA–PEG proportions higher than 80%, the PLGA/PLGA(45)–PEG(5) nanoparticles exhibited higher blood residence than the PLGA/PLGA(5)–PEG(5) nanoparticles. These findings indicate that apart from the surface PEG content, the biodistribution properties of the PLGA/PLGA–PEG nanoparticles are also influenced by the size of the nanoparticles and the rate of PEG loss from the nanoparticles.
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*Keywords:* Poly(lactide-co-glycolide)/poly(lactide-co-glycolide)–poly(ethylene glycol) nanoparticles; Biodistribution; Physicochemical characteristics

## 1. Introduction

Among the polymeric nanoparticles currently under investigation for controlled drug delivery and drug targeting applications, those receiving most attention are probably the nanoparticles based on the biocompatible and biodegradable poly(lactide)–poly (ethylene glycol) (PLA–PEG) and poly(lactide-co-glycolide)–poly(ethylene glycol) (PLGA–PEG) copolymers (Gref et al., 1995; Peracchia, 2003; Avgoustakis, 2004). Nanoparticulate drug carriers must show persistence in systemic circulation after intravenous (i.v.)

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administration in order to be useful for controlled drug delivery and/or targeting applications. The biodistribution properties of PLA–PEG or PLGA–PEG nanoparticles have been studied in experimental animals after labeling them with radioactive agents. Although the biodistribution data obtained may be influenced from the type of the label, which makes difficult a direct comparison of the data from different studies, the studies conducted have led to the establishment of certain facts for the biodistribution properties of the PLA–PEG and PLGA–PEG nanoparticles. First, the markedly increased blood circulation time and reduced liver uptake of the pegylated PLA or PLGA nanoparticles after i.v. administration to mice or rats compared to the non-pegylated nanoparticles has been demonstrated (Gref et al., 1994; Stolnik et al., 1994; Bazile et al., 1995). After intravenous administration, the PLA–PEG and PLGA–PEG nanoparticles remain in the systemic circulation for hours, whereas the PLA and PLGA nanoparticles are removed from blood within few minutes. It is generally accepted that the cells predominantly involved in the capture of nanoparticles administered intravenously are the macrophages of liver and spleen and circulating monocytes (MPS cells). Data reported by Zambaux et al. (2000) suggested, however, that the phagocytic circulating cells involved in the uptake of pegylated PLA nanoparticles were mainly neutrophilic granulocytes.

The composition of the pegylated nanoparticles affects the effectiveness of the PEG steric barrier and the size of the nanoparticles and, as a result, determines the biodistribution properties of the nanoparticles (Stolnik et al., 2001; Mosqueira et al., 2001; Avgoustakis et al., 2003). An increase in the PEG/PLA or PEG/PLGA ratio initially increased but later decreased blood circulation time of PLA–PEG or PLGA–PEG diblock nanoparticles prepared by the solvent displacement method (Stolnik et al., 2001; Avgoustakis et al., 2003). A possible explanation for these results may be that at relatively high PEG/PLA or PEG/PLGA ratios, although PEG still forms an effective steric barrier to opsonization on the surface of the nanoparticles, the size of the nanoparticles is sufficiently low to permit the nanoparticles to reach tissues that the bigger nanoparticles (those having relatively low PEG/PLA or PEG/PLGA ratios) cannot. In support to this explanation, recent observations indicated that very small particulates can pass through the sinusoidal fenestrations in the liver and gain access to the parenchymal cells of the liver (Stolnik et al., 2001).

PLGA–PEG nanoparticles were found to exhibit linear, dose-independent pharmacokinetics for a dose range of 150–1050 μg per mouse whereas the PLGA nanoparticles followed non-linear, dose-dependent pharmacokinetics in a similar doses range (Panagi et al., 2001). In addition to the prolonged blood residence (Avgoustakis et al., 2003), the dosage-independence of the pharmacokinetics of the PLGA–PEG nanoparticles would provide further advantages for their application in controlled drug delivery and targeting.

In order to increase drug entrapment efficiency, mixtures of PLA with PLA–PEG can be applied in the preparation of the nanoparticles instead of PLA–PEG alone (Quellec et al., 1998). The use of PLA (PLGA) with PLA–PEG (PLGA–PEG) mixtures allows for the easy adjustment of PEG content of the nanoparticles by simply mixing the appropriate amounts of PLA (PLGA) and PLA–PEG (PLGA–PEG). This provides an easy means to control the colloidal properties of the nanoparticles (Zambaux et al., 1999; Gref et al., 2000). In this work, we present data on the basic physicochemical and biodistribution properties of nanoparticles prepared from mixtures of PLGA with two different PLGA–PEG copolymers. The effect of mixture composition, i.e. type of PLGA–PEG copolymer and its weight fraction in the mixture, on nanoparticle biodistribution is reported.

## 2. Materials and methods

### 2.1. Materials

DL-Lactide (LE) and glycolide (GE) were purchased from Boehringer Ingelheim (Germany). They were recrystallized twice from ethyl acetate and dried under high vacuum at room temperature before use. Monomethoxypoly(ethyleneglycol) (mPEG, molecular weight 5000) was obtained from Sigma (St. Louis, MO) and dried under high vacuum at room temperature before use. Stannous octoate, sodium cholate and cholesterylaniline (5-cholesten-3β-[*N*-phenyl]amine, CA) were also obtained from Sigma. Sepharose CL-4B gel was purchased from Pharmacia (Sweden) and Biogel A15m from Bio-Rad. Tetrahydrofuran of HPLC grade and miscellaneous chemical reagents and solvents, all of analytical grade, were obtained from

Sigma, Merck (Germany), and SDS (France). The $Na^{125}I$ was provided by NCSR "Demokritos" (Greece) (source: MDS Nordion, Belgium).

### 2.2. Synthesis and characterization of PLGA–PEG copolymers

Poly(lactide-co-glycolide) and poly(lactide-co-glycolide)–monomethoxy(polyethyleneglycol) (PLGA–PEG) copolymers of different composition (PLGA/PEG molar ratio) were prepared by a melt polymerization process under nitrogen, using stannous octoate as catalyst (Beletsi et al., 1999). They were characterized with regard to their composition by $^1H$ NMR and their molecular weight and molecular weight distribution (polydispersity index, $PI = M_w/M_n$) by gel permeation chromatography (GPC) (Beletsi et al., 1999). The following copolymers were synthesized: (1) PLGA with molar composition LA:GA = 2.8, $M_w = 22 \times 10^3$ and PI = 1.9, (2) PLGA(45)–PEG(5) with $M_w = 51 \times 10^3$ and PI = 3.2 and (3) PLGA(5)–PEG(5) with $M_w = 9 \times 10^3$ and PI = 2.1. The number in parenthesis following each block in the word PLGA–PEG designates the molecular weight in kilodaltons of the respective block. The molecular weight and the polydispersity index of the mPEG used were measured by GPC to be $M_w = 5200$ and PI = 1.1, respectively.

### 2.3. Preparation of nanoparticles

$^{125}I$-cholesterylaniline ($^{125}I$-CA)-labeled nanoparticles were prepared from mixtures of PLGA with PLGA–PEG polymers using a solvent displacement technique (Panagi et al., 2001). Briefly, the mixture of polymers (100 mg) and the label were dissolved in acetone (2 ml) and the solution was transferred dropwise into a stirred solution of sodium cholate (20 ml, 12 mM) in phosphate buffered saline (PBS, pH 7.4). Acetone was allowed to evaporate and the resulting suspension of nanoparticles was carefully condensed to a final volume of 10 ml in a rotary evaporator (Buchi R114). The nanoparticles were purified by gel permeation chromatography using sepharose CL-4B and a stepwise gradient eluent from 100% phosphate buffered saline to 100% ethanol. The efficiency of label entrapment in the nanoparticles was always higher than 70%. The size and zeta ($\zeta$) potential of the nanoparticles were determined using photon correlation spectroscopy (PCS) and microelectrophoresis, respectively, in a Malvern Z-sizer 5000 instrument (five runs per sample). The $\zeta$ potential of the nanoparticles was measured in phosphate buffered saline (PBS), pH 7.4.

### 2.4. Determination of loss of PEG from PLGA/PLGA–PEG nanoparticles

The mPEG liberated from the PLGA/PLGA–PEG nanoparticles following incubation of the nanoparticles in phosphate buffered saline in a mildly shaking water bath (37 °C) for 3 h was determined by a colorimetric assay (Sims and Snape, 1980), as we have described elsewhere (Avgoustakis et al., 2002).

### 2.5. Biodistribution study

The tissue distribution of PLGA/PLGA–PEG nanoparticles labeled with $^{125}I$-cholesterylaniline following intravenous administration to female Swiss-De mice was determined as we previously described in detail (Avgoustakis et al., 2003). Briefly, the animals in groups of three were injected at random in the tail vein with 100 μl nanoparticles in PBS (300 μg polymer per mouse, 4.1 μCi). After 3 h, the mice were sacrificed by cervical dislocation, and their tissues (liver, spleen, lungs, muscle, bone (femur of left hind leg), intestines, kidney, urinary bladder, brain and thyroid) were excised, washed quickly with cold water to remove surface blood and counted for radioactivity. Blood samples (0.07–0.08 g) were obtained in duplicate by cardiac puncture in pre-weighed heparinized tubes. The radioactivity remaining in the tail was also measured and taken into consideration in the calculation of total radioactivity dose administered to the mice. The average residual radioactivity in tail was $6.95 \pm 3.05\%$ (standard deviation) of injected dose of radioactivity. The national regulations for the care and use of laboratory animals have been observed in this study.

## 3. Results

### 3.1. Physicochemical properties of the nanoparticles

The basic physicochemical characteristics of the nanoparticles are presented in Table 1. PLGA (0%

Table 1
Physicochemical characteristics of PLGA/PLGA–PEG nanoparticles

| Nanoparticle composition | PEG content (%, w/w) | Size (nm) | Size polydispersity | ζ Potential (mV) |
|---|---|---|---|---|
| PLGA(5)–PEG(5) (%, w/w) | | | | |
| 0 | 0 | 143.5 | 0.489 | −54.2 |
| 40 | 20 | 79.2 | 0.333 | −6.1 |
| 60 | 30 | 77.4 | 0.331 | −5.4 |
| 80 | 40 | 65.6 | 0.354 | −5.3 |
| 90 | 45 | 64.5 | 0.301 | −4.8 |
| 100 | 50 | 57.9 | 0.347 | −4.3 |
| PLGA(45)–PEG(5) (%, w/w) | | | | |
| 0 | 0 | 143.5 | 0.489 | −54.2 |
| 60 | 6 | 111.6 | 0.349 | −5.6 |
| 70 | 7 | 106.9 | 0.365 | −6.3 |
| 80 | 8 | 96.4 | 0.350 | −5.9 |
| 90 | 9 | 86.2 | 0.327 | −5.3 |
| 100 | 10 | 85.1 | 0.295 | −5.2 |

PLGA–PEG) nanoparticles were bigger and had a much lower ζ potential than the nanoparticles prepared from PLGA/PLGA–PEG mixtures. Increasing the PLGA–PEG content of the PLGA/PLGA–PEG mixtures caused a decrease in the size of the nanoparticles prepared from these mixtures. The nanoparticles prepared from PLGA/PLGA(5)–PEG(5) mixtures were smaller than the nanoparticles prepared from PLGA/PLGA(45)–PEG(5) mixtures.

The rate of PEG loss from the nanoparticles increased when the proportion of PLGA–PEG in the composition of the nanoparticles was increased or when the PLGA(45)–PEG(5) copolymer was replaced by the PLGA(5)–PEG(5) copolymer in the composition of the nanoparticles (Fig. 1).

### 3.2. Biodistribution of nanoparticles in mice

The radioactivity percent dose in blood, liver and spleen 3 h after the i.v. administration of labeled nanoparticles to mice is shown in Fig. 2. The percent dose remaining in blood circulation increased as the PLGA–PEG content of the PLGA/PLGA–PEG mixture, used to prepare the nanoparticles, was increased. In the case of nanoparticles prepared from PLGA/PLGA(45)–PEG(5) mixtures, the maximum percent dose in blood was obtained with the PLGA(45)–PEG(5) nanoparticles (i.e. with the 100% PLGA(45)–PEG(5) 'mixture'). However, in the case of nanoparticles prepared from PLGA/PLGA(5)–PEG(5) mixtures, the percent dose in blood increased with the PLGA(5)–PEG(5) content of the PLGA/PLGA(5)–PEG(5) mixture up to a PLGA(5)–PEG(5) content of 80%, but it did not change significantly with a further increase of the PLGA(5)–PEG(5) content of the PLGA/PLGA(5)–PEG(5) mixture. At PLGA–PEG proportions in the PLGA/PLGA–PEG mixtures 80% and higher, the nanoparticles prepared from PLGA/PLGA(45)–PEG(5) mixtures resulted to higher percent dose of radioactivity in blood than the nanoparticles prepared from PLGA/PLGA(5)–PEG(5) mixtures. The opposite was observed at PLGA–PEG proportions lower than 80%.

The percent radioactivity dose in liver decreased as the PLGA–PEG content of the PLGA/PLGA–PEG

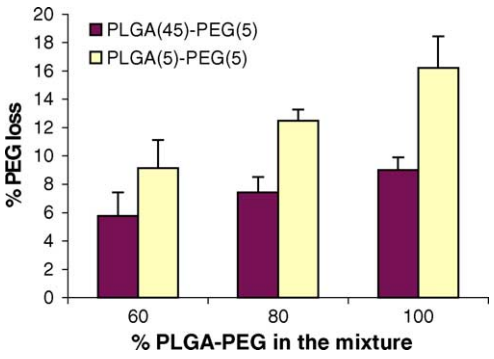


Fig. 1. PEG loss from PLGA/PLGA–PEG nanoparticles of different composition (different %, w/w, PLGA–PEG and different type of PLGA–PEG copolymer) upon incubation in phosphate buffered saline at 37 °C for 3 h (error bars indicate standard deviation, $N = 3$).

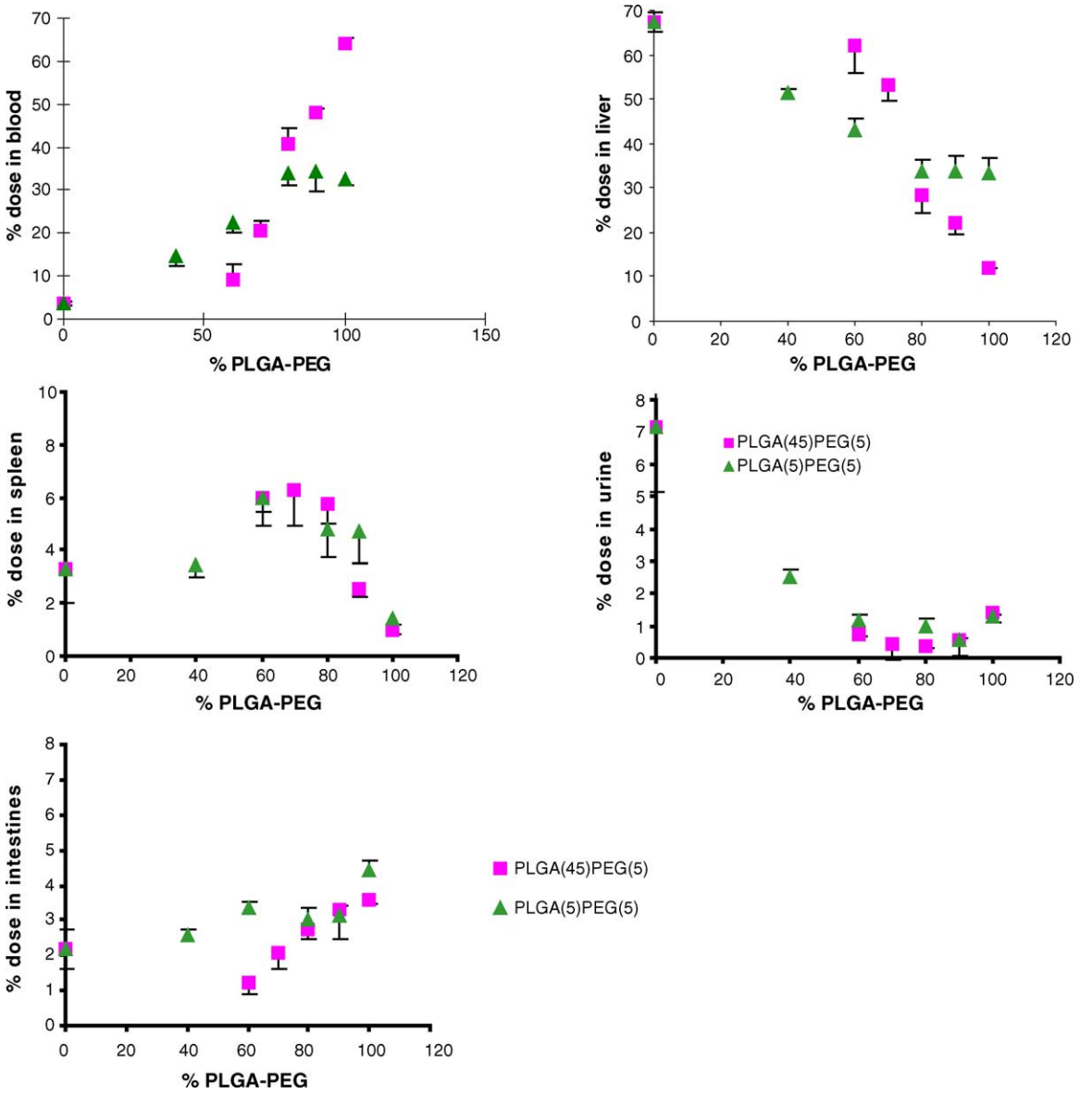


Fig. 2. Effect of nanoparticle composition (%, w/w, PLGA(45)–PEG(5) or PLGA(5)–PEG(5)) on the amount of radioactivity in blood, liver, spleen, urine and intestines 3 h after i.v. administration of $^{125}$I-CA-labeled PLGA/PLGA(45)–PEG(5) or PLGA/PLGA(5)–PEG(5) nanoparticles to mice (error bars indicate standard deviation, $N = 3$).

mixture, used to prepare the nanoparticles, was increased (Fig. 2). In the case of the nanoparticles prepared from PLGA/PLGA(45)–PEG(5) mixtures, the percent dose in liver decreased continuously with the PLGA(45)–PEG(5) content in the mixture, reaching a minimum with the PLGA(45)–PEG(5) nanoparticles (i.e. with the 100% PLGA(45)–PEG(5) 'mixture'). However, in the case of nanoparticles prepared from PLGA/PLGA(5)–PEG(5) mixtures, the percent dose in liver decreased with the PLGA(5)–PEG(5) content of the PLGA/PLGA(5)–PEG(5) mixture up to a PLGA(5)–PEG(5) content of 80%, but it did not change significantly with a further increase of the PLGA–PEG proportion in the PLGA/PLGA(5)–PEG(5) mixture.

At PLGA–PEG proportions in the PLGA/PLGA–PEG mixtures 80% and higher, the nanoparticles prepared from PLGA/PLGA(45)–PEG(5) mixtures resulted to lower percent dose of radioactivity in the liver than the nanoparticles prepared from PLGA/PLGA(5)–PEG(5) mixtures. The opposite was observed at PLGA–PEG proportions lower than 80%.

For both types of PLGA/PLGA–PEG mixtures, the percent dose of radioactivity in spleen initially increased and then decreased as the PLGA–PEG proportion in the PLGA/PLGA–PEG mixture, used to prepare the nanoparticles, was increased (Fig. 2).

The amount of radioactivity excreted in urine initially tended to decrease as the PLGA–PEG proportion in the PLGA/PLGA–PEG mixture, used to prepare the nanoparticles, was increased (Fig. 2). However, at PLGA–PEG proportions higher than 60%, no significant effect of PLGA–PEG proportion on the percent dose of radioactivity excreted in urine could be observed. The amount of radioactivity excreted in intestines tended to increase as the PLGA–PEG proportion in the PLGA/PLGA–PEG mixture was increased (Fig. 2). The cumulative (urine + intestines) excretion of radioactivity was low (<6%) for all types of nanoparticles, with the exception of PLGA nanoparticles, which resulted to higher excretion of radioactivity in urine.

In Fig. 3, the tissue distribution of radioactivity following the administration of nanoparticles prepared from mixtures rich in PLGA–PEG (≥80%, w/w, PLGA–PEG) is shown. In the case of the nanoparticles prepared from PLGA/PLGA(5)–PEG(5) mixtures, the tissue distribution of radioactivity essentially remains unaffected by the composition of the polymer mixture. The only exceptions were the radioactivity accumulation in spleen, where the percent radioactivity dose tended to decrease with increased PLGA(5)–PEG(5) content in the mixture, and in bones, where the percent radioactivity dose tended to increase with increased PLGA(5)–PEG(5) content in the mixture. On the contrary, in the case of the nanoparticles prepared from PLGA/PLGA(45)–PEG(5) mixtures, an increase of the PLGA(45)–PEG(5) proportion in the polymer mixture resulted to increased radioactivity accumulation in blood, which was accompanied by a decreased radioactivity accumulation in most other tissues (e.g. liver, spleen, muscle and bones).

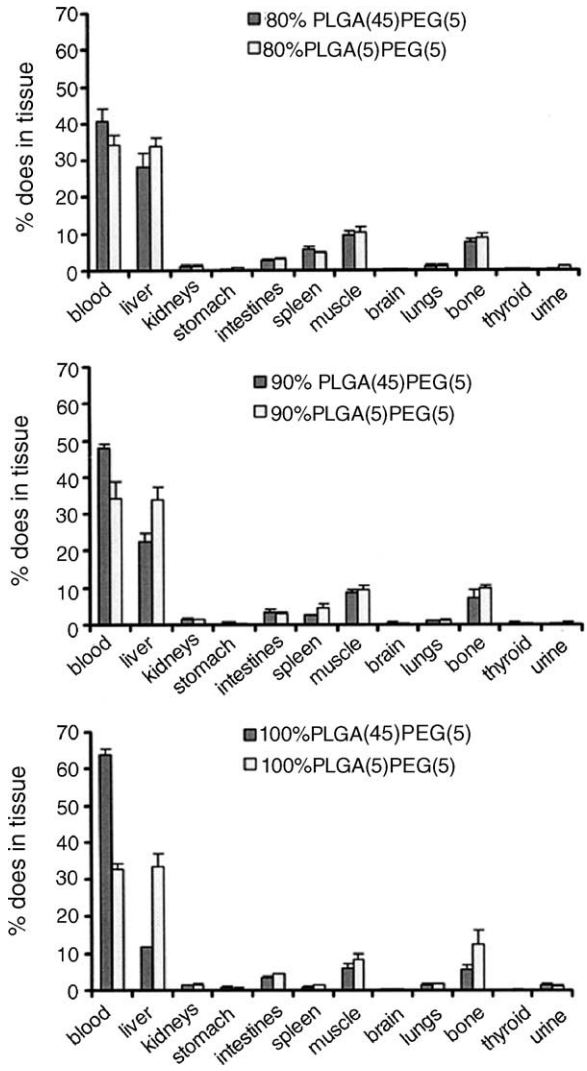


Fig. 3. Effect of nanoparticle composition (%, w/w, PLGA(45)–PEG(5) or PLGA(5)–PEG(5)) on the biodistribution of $^{125}$I-CA-labeled PLGA/PLGA(45)–PEG(5) and PLGA/PLGA(5)–PEG(5) nanoparticles 3 h after i.v. administration to mice (error bars indicate standard deviation, $N = 3$).

## 4. Discussion

In this work, the basic physicochemical characteristics and the biodistribution properties of nanoparticles prepared from mixtures of PLGA with PLGA–PEG copolymers were investigated. These mixtures may provide further flexibility in the selection of nanoparticle compositions in order to obtain nanoparticles

of suitable size and drug loading capacity. The PLGA/PLGA–PEG nanoparticles had $\zeta$ potential values relatively close to neutral due to the presence of PEG on their surface, which covers the surface charges, whereas the PLGA nanoparticles had a highly negative $\zeta$ potential. The PLGA/PLGA–PEG nanoparticles were smaller than the PLGA nanoparticles and their size decreased as their PLGA–PEG content was increased. Also, the size of the PLGA/PLGA(5)–PEG(5) nanoparticles was lower than the size of the PLGA/PLGA(45)–PEG(5) nanoparticles (Table 1). These data would indicate that PEG is able to moderate the association of polymer molecules during the formation of the PLGA/PLGA–PEG nanoparticles.

In our previous study, we investigated the biodistribution properties of PLGA–PEG nanoparticles having different PLGA/PEG molar ratio (Avgoustakis et al., 2003). In the present study, we investigated the biodistribution properties of nanoparticles prepared from mixtures of PLGA with PLGA–PEG. Two different types of PLGA–PEG diblock copolymers were included in the study; a PLGA(45)–PEG(5) copolymer of relatively low PEG content and a PLGA(5)–PEG(5) copolymer of relatively high PEG content. In order to investigate the biodistribution properties of the PLGA/PLGA–PEG nanoparticles, the nanoparticles were labeled with $^{125}$I-cholesterylaniline. We have shown that $^{125}$I-CA was firmly associated with the nanoparticles for at least 24 h and that free $^{125}$I-CA exhibited completely different biodistribution properties than the $^{125}$I-CA-labeled nanoparticles (Avgoustakis et al., 2003). Therefore, $^{125}$I-CA was considered to be a suitable label in order to follow the tissue distribution properties of the PLGA/PLGA–PEG nanoparticles during a period of 3 h.

All PLGA/PLGA–PEG nanoparticle compositions exhibited prolonged residence in blood circulation, compared to the conventional PLGA nanoparticles (Fig. 2). The major pathway for the removal of all nanoparticle compositions from blood during the period studied appeared to be the nanoparticle capture in MPS tissues, and especially in the liver (Fig. 2). It is characteristic that the nanoparticle compositions which exhibited high accumulation in blood also exhibited low liver uptake, and vice versa (Fig. 2). Also, comparatively little radioactivity (lower than 6% of injected dose) was excreted in urine and intestines during the 3 h period investigated (Fig. 2).

The blood residence of the PLGA/PLGA–PEG nanoparticles increased as their PLGA–PEG content was increased. For the PLGA/PLGA(45)–PEG(5) nanoparticles, the increase of blood residence with the PLGA(45)–PEG(5) content was continuous, reaching a maximum at the maximum possible PLGA(45)–PEG(5) content, i.e. at 100% PLGA(45)–PEG(5), whereas in the case of PLGA/PLGA(5)–PEG(5) nanoparticles the maximum blood residence was obtained with the mixture consisting of 80% PLGA(5)–PEG(5); the blood residence of the PLGA/PLGA(5)–PEG(5) nanoparticles did not change at PLGA(5)–PEG(5) proportions higher than 80% (Fig. 2).

It has been shown that the density of PEG on nanoparticle surface is increased when the PEG content of nanoparticles is increased (De Jaeghere et al., 1999; Avgoustakis et al., 2003). The increase of blood circulation time of the PLGA/PLGA–PEG nanoparticles with increasing PLGA–PEG content may be attributed to the increased PEG density on nanoparticle surface, which would result to the formation of a more effective steric barrier on nanoparticle surface, inhibiting nanoparticle opsonization and phagocytocis. At PLGA–PEG proportions lower than 80%, the PLGA/PLGA(45)–PEG(5) nanoparticles exhibited lower blood longevity than the PLGA/PLGA(5)–PEG(5) nanoparticles, probably because of their relatively low PEG content (for the same weight proportion of PLGA–PEG in the PLGA/PLGA–PEG mixture, the PEG content of the PLGA/PLGA(45)–PEG(5) mixtures is much lower than the PEG content of PLGA/PLGA(5)–PEG(5) mixtures) which would be inadequate to create an effective steric barrier on nanoparticle surface. Unexpectedly, at PLGA–PEG proportions higher than 80%, the PLGA/PLGA(45)–PEG(5) nanoparticles exhibited higher blood longevity than the PLGA/PLGA(5)–PEG(5) nanoparticles. Although, the latter loose their PEG chains more rapidly than the former due to copolymer degradation (Fig. 1), their PEG content is still high enough (Table 1) to prevent opsonization and sequestration by MPS. A reason why the PLGA/PLGA(5)–PEG(5) nanoparticles with high PLGA–PEG content ($\geq$80%) exhibited higher blood clearance than PLGA/PLGA(45)–PEG(5) nanoparticles with high PLGA–PEG content ($\geq$80%) might be their relatively small size, which could permit the nanoparticles to reach tissues that the bigger nanoparticles cannot. It may be possible that these

relatively small PLGA/PLGA(5)–PEG(5) nanoparticles can penetrate more efficiently than the bigger PLGA/PLGA(45)–PEG(5) nanoparticles through the fenestrae in the endothelial lining of the liver, which have a mean diameter of approximately 100 nm (Davis et al., 1994), and associate with parenchymal cells. Stolnik et al. (2001) reported that they have had results suggesting that small sterically stabilized particles can distribute mainly to the parenchymal cells of the liver after intravenous injection. Further evidence that the relatively small PLGA/PLGA(5)–PEG(5) nanoparticles are more highly distributed in the body may be considered to provide the little higher accumulation of these nanoparticles in bones (Fig. 3), which may result from the higher accumulation of these nanoparticles in bone marrow because of their relatively small size.

Although their PEG content increases with the proportion of PLGA(5)–PEG(5) in the mixture (Table 1), the tissue distribution of PLGA/PLGA(5)–PEG(5) nanoparticles prepared from PLGA/PLGA(5)–PEG(5) mixtures having PLGA(5)–PEG(5) proportion equal or higher than 80% essentially remains unaffected by the composition of the polymer mixture (Fig. 3). It appears that the PEG density on nanoparticle surface is already adequate at 80% PLGA(5)–PEG(5) content and a further increase on PLGA(5)–PEG(5) content does not increase the efficiency of the PEG corona on nanoparticle surface. However, the higher rate of PEG loss from the PLGA/PLGA(5)–PEG(5) nanoparticles as the PLGA(5)–PEG(5) proportion is increased (Fig. 1) may contribute to the absence of improvement in blood longevity of the PLGA/PLGA(5)–PEG(5) nanoparticles with an increase of their PLGA(5)–PEG(5) content from 80 to 100% (Fig. 3).

Apart from the blood residence properties (Fig. 2), the composition of the PLGA/PLGA–PEG mixture may influence the drug loading capacity of the PLGA/PLGA–PEG nanoparticles, through its effects on the hydrophobicity of the polymer matrix and the size of the nanoparticles. An increase of polymer hydrophobicity would enhance hydrophobic interactions of the polymer with hydrophobic drugs, increasing the entrapment efficiency of such drugs in the nanoparticles, and a higher nanoparticle size has been suggested to reduce drug leakage to the external aqueous phase during nanoparticle preparation, improving drug loading (Gorner et al., 1999). The blood residence data obtained in this study (Fig. 2) may have significant implications on the feasibility of development of PLGA/PLGA–PEG nanoparticulate formulations optimised with regard to both the blood residence and the drug loading properties by judicious selection of the composition of the nanoparticles. Thus, the data in Fig. 2 suggest that it is possible to prepare nanoparticles with low PEG content, which would generate favourable conditions for the entrapment of a hydrophobic drug, without compromising the prolonged blood circulation properties of the nanoparticles, by selecting a PLGA/PLGA(45)–PEG(5) composition containing more than 80% PLGA(45)–PEG(5). In the case that a hydrophilic drug is to be entrapped in the PLGA/PLGA–PEG nanoparticles, a more hydrophilic nanoparticle composition having high enough blood longevity, e.g. a nanoparticle composition having high content of PLGA(5)–PEG(5) or other, rich in PEG, copolymer, may be considered.

For both types of PLGA/PLGA–PEG mixtures, the percent dose in spleen initially increased and then decreased as the PLGA–PEG proportion in the PLGA/PLGA–PEG mixture was increased (Fig. 2). The initial increase of nanoparticle uptake in spleen is probably due to the reduced nanoparticle sequestration by the liver. That reduced liver uptake may lead to increased spleen capture was observed previously with pegylated PLA and PLGA particles (Stolnik et al., 1994) and other pegylated systems, such as the (MePEGcyanoacrylate–hexadecylcyanoacrylate) nanoparticles (Peracchia et al., 1999). The decrease in spleen uptake observed at high PLGA–PEG proportions (Fig. 2) may be due to the more efficient protection of the nanoparticles against phagocytosis from the denser PEG corona on nanoparticle surface.

## 5. Conclusions

The basic physicochemical characteristics of the nanoparticles and the PEG loss from the nanoparticles, prepared from mixtures of PLGA with PLGA–PEG copolymer, were significantly influenced by the composition of the PLGA/PLGA–PEG mixture, i.e. the type of PLGA–PEG copolymer and its proportion in the mixture. The composition of the PLGA/PLGA–PEG mixture also affected significantly the biodistribution properties of nanoparticles, probably through its effects on the density of PEG on nanoparticle surface, the size

of the nanoparticles and the rate of PEG loss from the nanoparticles.

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