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Research paper

Radiolabelled biodegradable microspheres for lung imaging

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Abstract

The effect on lung accumulation of modifying the surface compositions of ^{99m}Tc poly(lactide-co-glycolide) (PLGA) and ^{99m}Tc poly(ethylene glycol)-poly(lactide-co-glycolide) (PEG–PLGA) microspheres with different surfactants was assessed after intravenous injection into rats. Microspheres were prepared with PLGA or PEG–PLGA by the emulsion solvent evaporation method using polyvinyl alcohol (PVA), polyethylene glycol (PEG), albumin (BSA) or poloxamer 188 as surfactant, in the external aqueous phase. Commercial human albumin microspheres (Sferotec®, HAM) were used as reference. According to the European Pharmacopeia, >80% of ^{99m}Tc-HAM in the size range 10–50 μm, must be accumulated in the lung 15 min after intravenous administration. By modifying the surfactant, the resulting lung accumulation was 99% for ^{99m}Tc-HAM, and more than 50% for PLGA microspheres prepared with poloxamer 188 (1 and 4%), reaching 67% with 8% Poloxamer 188 and around 30–39% for PLGA and PEG–PLGA microspheres prepared with the other surfactants. PLGA microspheres made with 8% poloxamer 188 gave good quality lung images under a gamma camera for the first few minutes, subsequently liver radioactivity masked lung images.

Keywords: Lung imaging; Lung uptake; Lung targeting; Radiolabelling; ^{99m}Tc-biodegradable microspheres; Poly(lactide-co-glycolide); Poly(ethylene glycol)-poly(lactide-co-glycolide)

1. Introduction

Lung perfusion imaging is based on the trapping of large particles in the capillary bed of the lung. Particles larger than 10 μm are expected to lodge in the capillaries in the first pass of circulation through the pulmonary artery following intravenous administration. The two established radiopharmaceutical agents used for lung perfusion scintigraphy are ^{99m}Tc macroaggregated albumin (^{99m}Tc-MAA) and albumin microspheres (^{99m}Tc-HAM), with a particle size between 10–90 μm and 10–50 μm [1] respectively. Particles larger than 10 μm should be located in the lung, particles over 100 nm accumulate preferentially in the liver and spleen (0.2–3 μm), while smaller particles (<30 nm) tend to accumulate in a relatively higher concentration in bone-marrow [2].

Protein microspheres in the size range 5–30 μm, such as albumin [3–6], casein [3] and gelatin [7] have been reported to be accumulated almost entirely in the lung following intravenous injection. The authors proposed that as these microspheres target the lung so effectively, they could be useful as drug delivery systems to the pulmonary area.

In addition, many reports have appeared in the literature suggesting that synthetic biodegradable particles could be used as carrier systems for site-specific drug delivery. However their major disadvantage systems is rapid elimination, mainly by Kupffer cells in the liver and macrophages in the spleen. Much effort has been made in recent years to obtain biodegradable nanospheres and microspheres with reduced reticuloendothelial system (RES) uptake and prolonged blood circulation time [8–12]. The strategies followed until now have been focused on obtaining more hydrophilic particles to avoid opsonization. To modify particulate systems, extensive investigations have been carried out with nanospheres and small microspheres coated with albumin [11,13], or more recently, with amphiphilic block copolymers such as poly(ethylene glycol)-poly(lactide-co-glycolide) (PEG–PLGA) [8,9] or methoxy-poly(ethylene glycol)-poly(lactide) (PEG–PLA) [10,11]. All of these surface modifications have increased the blood circulation time but PEG- or methoxy-PEG–PLGA gave the best results.

The above studies have mainly been focused on nanoparticles prepared with polylactic acid and its copolymers, as

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an injectable colloidal drug carrier. Although Sato et al. [14] have reported a high lung accumulation of taxol-loaded PLGA microspheres containing isopropyl myristate (taxol-IPM–PLGA–MS), little attention has been paid to the possibility of using these types of microparticles as radiopharmaceuticals or target delivery systems to the lung, for diagnostic or therapeutic purposes, respectively.

Blood cells, MAA and HAM are the few human-source radiopharmaceuticals still on the market. As is well known, medicinal products derived from human or animal sources have caused several undesirable effects. They must therefore be carefully controlled, as human plasma derivatives could not only transmit hepatitis and the AIDS virus, but also carry unknown potential risks as happened with bovine spongiform encephalopathy (BSE).

The aim of this research was to develop biodegradable microspheres using synthetic polymers such as poly(lactic-co-glycolic acid) (PLGA) for diagnostic lung perfusion imaging (radiolabelled microspheres) or lung regional therapy. Biodistribution of ^{99m}Tc-PLGA microspheres was studied in the rat, along with the effect of surface modification on their biodistribution using ^{99m}Tc-HAM (SFEROTEC®) as reference.

2. Materials and methods

2.1. Materials

Poly(DL-lactide-co-glycolide) (PLGA) (molar ratio 63:37) and weight average molecular weight (Mw) 41 kDa, and methoxypoly(ethylene glycol)-poly(lactide-co-glycolide) (PEG-PLGA) (13:57:30), Mw 47 kDa, were synthesized using stannous octoate as catalyst and characterized (GPC, NMR) in our laboratory, as previously described [15,16].

Poloxamer 188 (Pluronic® F-68) and polyvinyl alcohol (PVA) (Mw 30–70 kDa) were purchased from Sigma Chemical Company, Inc. (St. Louis, MO). Polyethylene glycol 10000 was supplied by Merck, (Schuchardt, Germany).

A SFEROTEC® kit for labelling human serum albumin (HSA) microspheres with ^{99m}Tc was purchased from Sorin Biomedica Diagnostics S.p.A. (Vercelli, Italy).

2.2. Microspheres preparation

The different microsphere formulations were prepared by the emulsion solvent evaporation method. A polymer solution in dichloromethane (DCM) was emulsified with 50 ml of aqueous surfactant solution using a homogenizer (Silverson L4RT, 3/4′ tip). The DCM was then evaporated under magnetic stirring at room temperature. A description of the method is given in Table 1.

The microspheres were isolated by centrifugation and washed several times with water. Microspheres were freeze dried and stored in a desiccator at a temperature below 5°C.

2.3. Microsphere characterization

The particle size distribution was obtained by laser diffractometry using a Coulter® LS100. All the results reported here were based on the size-volume distribution.

Morphologically, microspheres were characterized by SEM (Jeol JSM-6300, Tokyo).

2.4. Surface chemistry analysis

Samples of polymer, surfactant, and freeze-dried microspheres were analyzed in powder form.

X-ray photoelectron spectra were obtained using a Physical Electronics PHI-5500 ESCA system, with a standard, non-monochromatic X-ray Al source operated at 300 W. For all samples, a survey spectrum was recorded over a binding energy range from 0 to 1100 eV, using a pass energy of 180 eV. A higher precision single element spectrum was recorded for:

1. Carbon, C1s, between 296 and 276 eV.
2. Oxygen, O1s, between 545 and 525 eV, using a pass energy of 23 eV.

Curve fitting was performed using PHI-Multipack v.5.0 software, and Gaussian functions were applied to the data. Each carbon C1s spectrum was decomposed into three or four peaks with the following chemical assignments: 285.0 eV for methylene carbon and adventitious hydrocarbon, 286.4–287.0 for ether carbon and alpha ether carbon to carboxyl, 288.4 for amide carbon, and 289.0 for carboxyl carbon [17–19], as expressed in Table 2.

Quantification of microsphere surface composition was by evaluating the contribution of each component to the corresponding areas at various binding energies. This composition is expressed as the ratio between the constituent polymer and the adsorbed surfactant, as expressed in Table 2.

2.5. Labelling procedure

Microspheres were labelled with ^{99m}Tc according to the procedure previously described [20]. Microspheres (13.33 mg) were mixed with 2 mg $SnCl_2$. 2 H_2O and 0.33 mg poloxamer 188, in a vial under nitrogen atmosphere. Labelling was carried out adding 1–1.5 ml of saline solution containing 2.5–3 mCi of ^{99m}Tc derived from a commercial molybdenum generator. The suspension was sonicated for 30 s to disaggregate, then vigorously shaken for 3 min and allowed to react for a further 10 min, shaking from time to time. Labelled microspheres were then recovered by centrifugation and washed once with water to eliminate any free pertechnetate. For injection, the final suspension was made in 0.9% saline solution (4–5 ml).

The commercial kit, SFEROTEC (Sorin Biomedica), was used for the preparations of ^{99m}Tc-HAM.

The labelling efficiency was obtained following the

Table 1
Microsphere preparation

Formulation	Polymer	DCM (ml)	Polymer (mg/ml)	External aqueous phase	Screen type[a]	Emulsification	
						(rev/min)	*t* (min)
PLGA (PVA)	PLGA	2.5	100	1% PVA	1	1000	30
PEG–PLGA (PVA)	MeO-PEG–PLGA	2.5	120	1% PVA	1	1300	30
PEG–PLGA (PEG)	MeO-PEG–PLGA	1.5	200	4% PEG	2	6000	5
PLGA (BSA)	PLGA	2.5	100	1% Albumin	1	2500	30
PLGA(Poloxamer 1%)-20	PLGA	2.5	100	1% Poloxamer	1	6000	20
PLGA (Poloxamer 4%)-7	PLGA	6	100	4% Poloxamer	1	8000	15
PLGA(Poloxamer 4%)-20	PLGA	2.5	120	4% Poloxamer	2	6000	5
PLGA(Poloxamer 4%)-40	PLGA	4	200	4% Poloxamer	1	6000	15
PLGA(Poloxamer 8%)-20	PLGA	3	100	8% Poloxamer	2	7000	7

[a] (1) 3/4" tip with a square hole high-shear screen. (2) 3/4" tip with emulsor screen.

European Pharmacopeia method. Briefly, 200 μl of microsphere suspension were filtered (0.45 μm Millipore), washing the filter with 20 ml of saline solution. The remaining radioactivity in the filter and in solution was measured using a dose calibrator (Capintel). To test the labelling stability, the procedure was repeated after 24 h.

2.6. Biodistribution study

The local committee for animal studies of the University of La Laguna had previously approved animal experiments.

For each formulation, six Wistar rats (225 ± 25 g) were injected with 40–50 μCi contained in 100 μl of microsphere suspension, via the jugular vein. They were sacrificed 15 min after injection and 1 ml of blood (by cardiac puncture), the liver, kidneys, spleen, lung, heart, thyroid, one femur and a sample of muscle were removed. The organ and blood associated activity was counted using a gamma counter (Packard, Cobra II). Total blood volume, bone and muscle were estimated at 5.4, 10 and 40% of total body weight, respectively [21].

The results are expressed as a percentage of the total activity measured and are a mean of the six rats ± standard deviation.

3. Results and discussion

^{99m}Tc-HAM is one of the radiopharmaceuticals most frequently used for lung imaging. These microspheres must be in the diameter range of 10–50 μm [1]. As particles larger than 10 μm are trapped in the lung capillaries, the HAM action mechanism is based mainly on particle size. Considering this, we hypothesized that any particle in this size range could be useful for lung targeting.

Biodegradable PLGA and PEG–PLGA microspheres were prepared by the solvent evaporation method, using different compounds as surfactant in the external aqueous phase, then characterized in terms of size distribution,

Table 2
XPS carbon C1s spectra decomposition and surface chemical composition of the microspheres

Product	Bond				Polymer: surfactant ratio	Surfactant (%)
	Methylene (area (%))	Ether (area (%))	Carboxyl (area (%))	Amide (area (%))		
BSA	49.56	27.84	–	22.60		
PEG-10000	–	100.00	–	–		
Poloxamer	10.84	89.16	–	–		
PVA	60.51	39.49	–	–		
Methoxy-PEG-PLGA	55.03	23.65	21.32	–		
PLGA	30.08	37.87	32.05	–		
Microspheres						
PLGA (BSA)	58.45	22.71	10.81	8.03	1:0.77	43.50
PLGA (PVA)	45.99	32.70	21.30	–	1:0.30	23.08
PEG–PLGA (PEG)	30.49	40.81	28.70	–	1:0.28	21.88
PLGA (Poloxamer 1%)-20	49.28	30.51	20.20	–	1:0.28	21.88
PLGA (Poloxamer 4%)-20	36.81	37.80	25.39	–	1:0.26	20.63
PLGA (Poloxamer 8%)-20	31.58	42.03	26.39	–	1:0.35	25.93

Table 3
Summary of microsphere size distribution

Formulation	Mean diameter	Particles (%)	
	(μm)	<10 μm	>50 μm
HAM (SFEROTEC)	31	7.3	4.9
PLGA (PVA)	27	7.6	0.0
PEG–PLGA (PVA)	24	13.2	4.5
PEG–PLGA (PEG)	21	18.0	2.0
PLGA (BSA)	22	10.1	2.5
PLGA(Poloxamer 1%)-20	21	20.6	3.0
PLGA(Poloxamer 4%)-7	7	81.2	0.0
PLGA(Poloxamer 4%)-20	18	11.2	0.0
PLGA(Poloxamer 4%)-40	43	6.6	37.9
PLGA(Poloxamer 8%)-20	18	22.5	1.5

morphological characteristics, surface chemical composition and biodistribution in rats.

To simplify the nomenclature, formulations were designated by acronyms reflecting the type of polymer used in the microsphere preparation (PLGA or PEG-PLGA), the compound used as surfactant in brackets and the mean size; e.g. PLGA (Poloxamer 4%)-7 was prepared with PLGA as polymer, 4% Poloxamer 188 in the continuous phase and a mean microsphere size of 7 μm.

3.1. Microsphere size distribution

Bearing in mind that ^{99m}Tc-HAM accumulate in lung after intravenous administration because their size is between 10–50 μm, the prepared biodegradable particles were measured. Table 3 shows the size distributions compared to HAM. Particles made with PLGA and PVA as surfactant presented a size distribution similar to the HAM distribution curve (Fig. 1), while 12% of HAM were outside the recommended size range versus 7.6% of PLGA(PVA) smaller than 10 μm. The other formulations presented a wider size range with a significant percentage of particles in the range 10–50 μm In fact, PLGA (Poloxamer 4%)-7 and PLGA (Poloxamer 4%)-40 were intentionally made, respectively, smaller and larger than the recommended size.

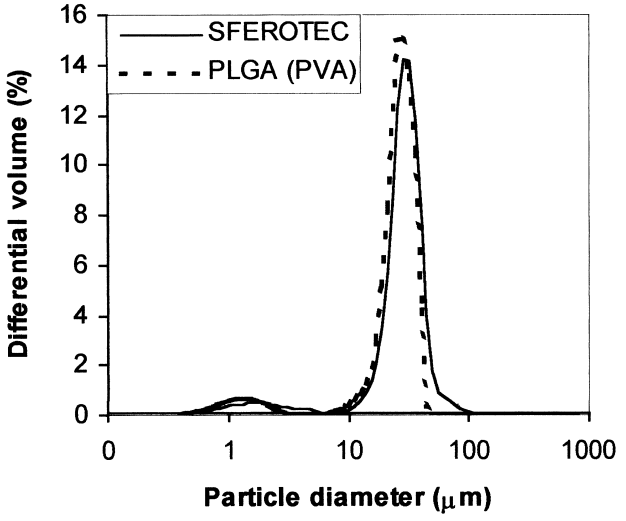

Fig. 1. Size distribution curves of ^{99m}Tc-HAM (SFEROTEC®) and ^{99m}Tc-PLGA(PVA) microspheres.

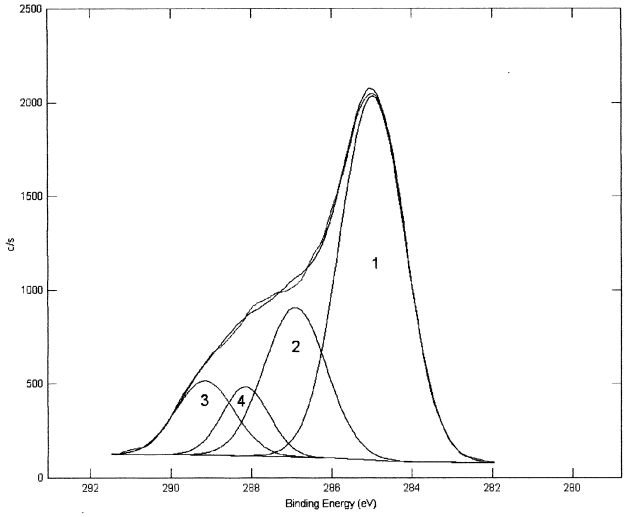

Fig. 2. C1s regions of XPS data for PLGA(BSA) microspheres. Curve fitting using peak 1 (methylene carbon at 285.0 eV); peak 2 (ether carbon at 286.4–287 eV); peak 3 (carboxyl carbon at 289.0 eV) and peak 4 (amide carbon at 288.4 eV).

3.2. Labelled microspheres

The labelling efficacy was in all cases >95%. Labelling

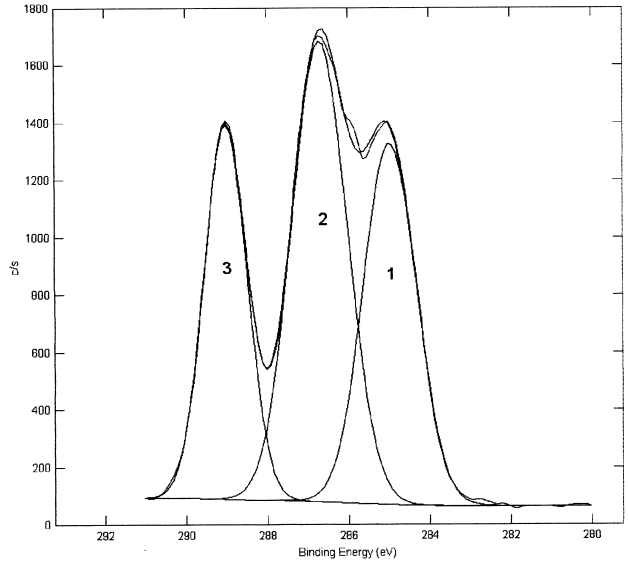

Fig. 3. C1s regions of XPS data for PLGA(poloxamer 8%)-20 microspheres. Curve fitting using peak 1 (methylene carbon at 285.0 eV); peak 2 (ether carbon at 286.4–287 eV) and peak 3 (carboxyl carbon at 289.0 eV).

stability was demonstrated, since after 24 h incubation in saline solution, labelling efficacy remained >95%.

3.3. Microsphere surface chemistry

To study the effect of surfactants on the surface of microparticles, XPS analyses were carried out. Table 2 gives the surface chemical composition obtained by XPS analysis for the different batches of microspheres.

Fig. 2 shows the C1s regions of the PLGA(BSA) microspheres. Peaks 1 and 2 reflect the contribution of methylene carbon at a binding energy of 285.0 eV and ether carbon at 286.9 eV from PLGA and BSA, whereas peak 3 at 289.0 eV corresponds to carboxyl carbon present in the PLGA monomer units and peak 4 at 288.4 eV to amide carbon present only in the BSA. The respective contributions of PLGA and BSA to the ether carbon area in the microsphere spectrum can be calculated from the area ratio of ether carbon to amide carbon in the BSA. The ratio PLGA:BSA on the microsphere surface was 1:0.77, therefore the contributions of PLGA and BSA to the surface composition were 56.5 and 43.5% respectively.

The XPS results of PLGA (Poloxamer 8%)-20 microspheres are shown in Fig. 3. As before, peaks 1 and 2 reflect the methylene and ether carbon, respectively, in both PLGA and Poloxamer, and peak 3 corresponds to carboxylic carbon present only in the PLGA monomer units. Therefore, in this case the area ratio of ether carbon to carboxylic carbon from PLGA was used to calculate the chemical composition. The contributions to the surface composition were 78.12% for PLGA and 21.88% for poloxamer, thus poloxamer partially masks the PLGA.

3.4. In vivo biodistribution studies

To carry out the distribution assay, 100 μl (40–50 μCi) of 2–3 mg/ml ^{99m}Tc-microsphere suspension was injected as described previously. ^{99m}Tc-HAM was taken as a reference.

3.4.1. Microspheres prepared with PVA as surfactant

The first experiment entailed injecting ^{99m}Tc-HAM and ^{99m}Tc-PLGA(PVA). The results were totally unexpected. ^{99m}Tc-PLGA(PVA) accumulated preferentially in the liver, while ^{99m}Tc-HAM accumulated in the lung (Fig. 4) according to the corresponding European Pharmacopeia monograph. However, negligible differences between HAM and PLGA (PVA) microspheres were observed in terms of size distribution (Table 3). SEM images showed that PLGA(PVA) microspheres presented a spherical shape and smooth surface whereas HAM had a rough surface (Fig. 5).

Although the effectiveness of ^{99m}Tc-HAM for lung perfusion imaging seems normally to be based on particle size, the above results indicate that other factors must be involved in the PLGA microsphere biodistribution. The contribution of PVA to the surface chemical composition of PLGA(PVA) microspheres is known [17,22,23] and XPS results confirmed the presence of PVA (23%) on the microsphere surface (Table 2). On the other hand, some authors have successfully changed the PLA and PLGA nanoparticle biodistributions, by modifying their surface characteristics. Therefore, the

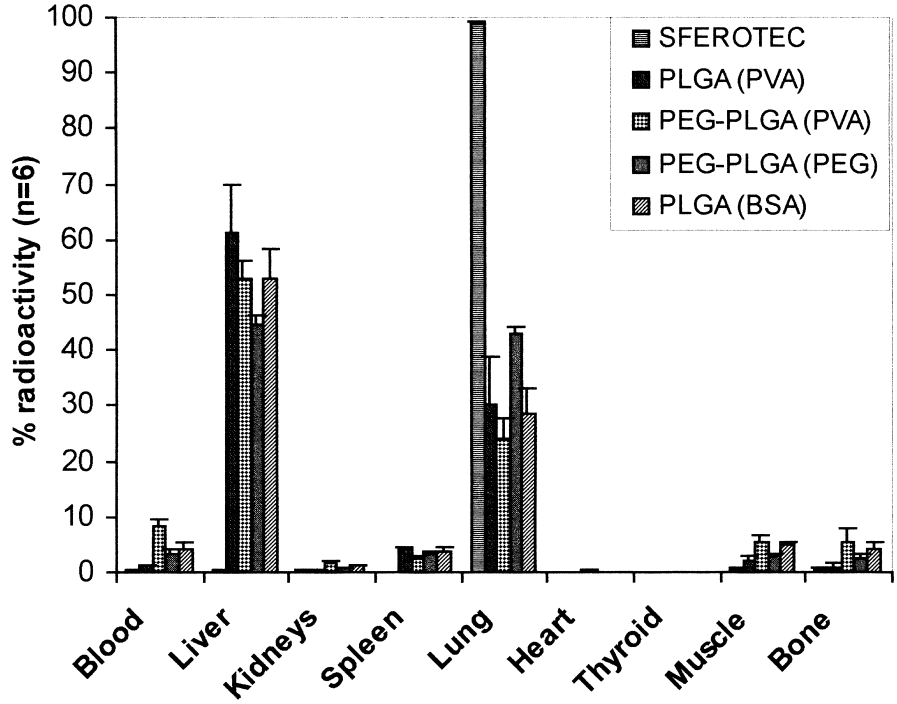

Fig. 4. Tissue biodistribution of ^{99m}Tc-HAM (SFEROTEC®), ^{99m}Tc-PLGA(PVA), ^{99m}Tc-PEG–PLGA(PVA), ^{99m}Tc-PEG–PLGA(PEG) and ^{99m}Tc-PLGA(BSA) microspheres.

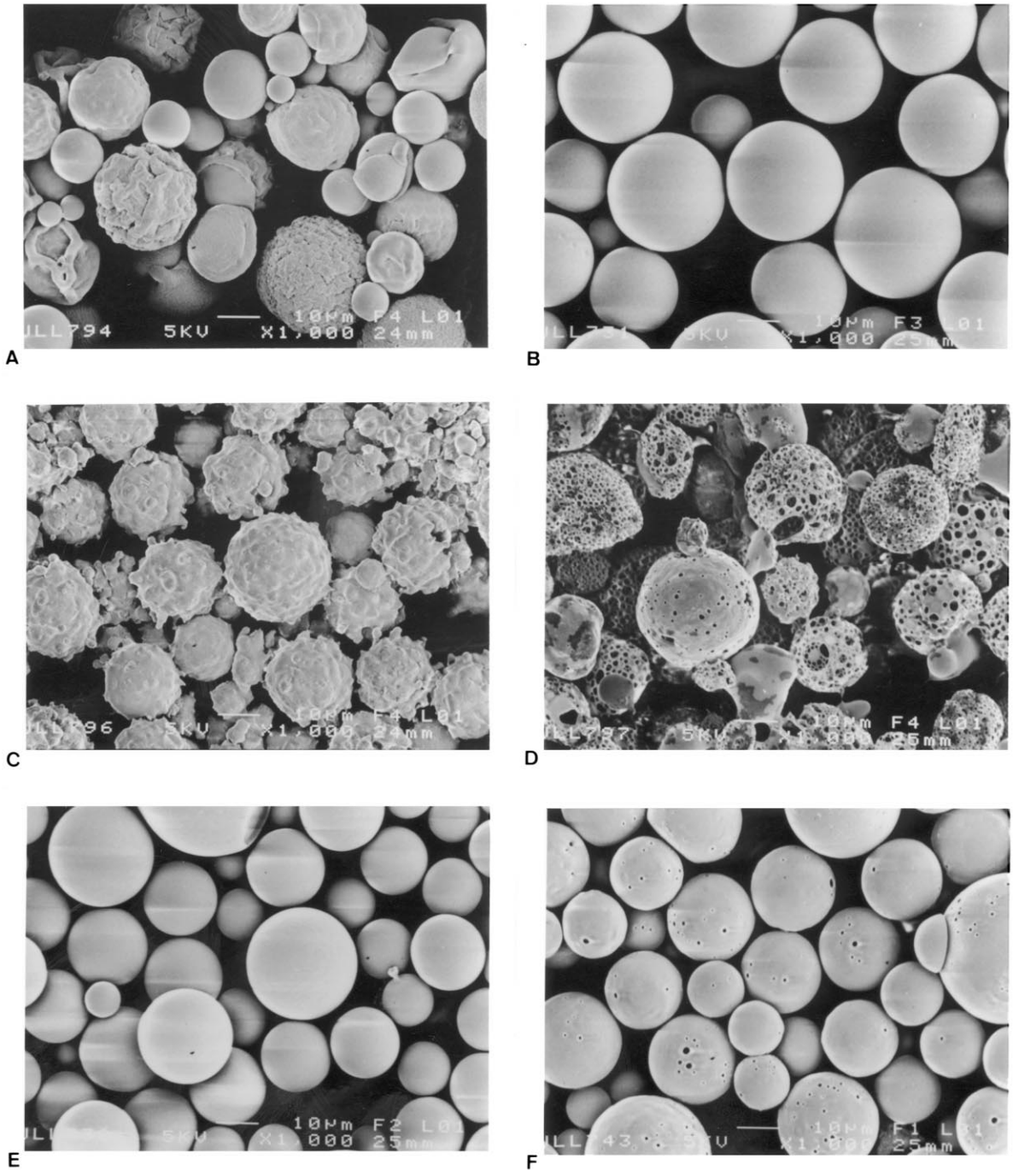

Fig. 5. SEM micrography of the different microsphere formulations: (A) HAM (SFEROTEC®); (B) PLGA(PVA); (C) PEG–PLGA(PVA); (D) PEG–PLGA (PEG); (E) PLGA(BSA); (F) PLGA(Poloxamer 8%).

effect of surface composition on the biodistribution of PLGA microspheres was then investigated. Since Gref et al. [8] have reported that PEG present on the particle surface dramatically decreases the nanosphere liver uptake, microspheres were made using PVA in the external aqueous phase as before but using a new polymer, PEG–PLGA. The size and morphology of formulation PEG–PLGA(PVA) (Table 3 and Fig. 5, respectively) show that PEG–PLGA(PVA) microspheres are different to PLGA(PVA). Their shape is not well defined, they are certainly not perfect spheres and their surface is not as smooth as those made with PLGA(PVA). Biodistribution results (Fig. 4) are very similar for both types of microspheres, PLGA(PVA) and PEG-PLGA(PVA). In the latter case the XPS analysis was very similar to that of PLGA, so it was not possible to distinguish the characteristic peaks of the PEG.

As PVA did not confer the appropriate surface characteristics for lung targeting microspheres it was decided to test other stabilizers that might provide the desired biodistribution.

3.4.2. Microspheres prepared with other surfactants

PEG chain length and PEG density at the surface of the particles have been reported [10,24] to be very important parameters in determining nanoparticle biodistribution. PEG–PLGA(PEG) microspheres were obtained using 1% PEG (Mw 10 kDa) as the external phase. These particles kept a spherical shape but were very porous (Fig. 5). Besides this, XPS showed the presence of PEG (22%) on the surface (Table 2). However, these changes slightly improved its biodistribution (Fig. 4), reducing liver accumulation to 45% (lung was 39%). As with the other two formulations: PLGA(PVA) and PLGA–PEG(PVA), the radioactivity located in the liver was still higher than in the lung.

At this point it was thought that the presence of protein could be necessary to reach an adequate lung accumulation for pulmonary trapping, therefore an aqueous solution of 1% bovine serum albumin (BSA) was used as microsphere stabilizer. The microsphere characteristics, size and spherical shape are shown in Table 3 and Fig. 5. According to XPS analysis (Fig. 2), microspheres were partially coated with BSA, approximately 43% (Table 2), but this was not enough to change the biodistribution or might not be its determining factor since the results (Fig. 4) were the same as before with PVA.

In summary, the four types of prepared microspheres are similar in size distribution to the reference sample, HAM, and differ in shape and surface characteristics. Biodistribution studies showed no great difference between them but the results are significantly different from the reference. The four biodistributions obtained were not affected by the change in surface composition, as radioactivity accumulated preferentially in the liver with only 30–39% being located in the lung versus 95% for HAM. However, this shows that particle accumulation in the lung does not depend only on size distribution or morphological surface characteristics. The surface composition could be involved but the optimum composition has not yet been achieved for lung accumulation.

Finally, as has been reported [25,26], poloxamer and poloxamine can significantly alter the biodistribution of polystyrene nanospheres. Poloxamer 188 was tested in the continuous phase to prepare PLGA microspheres, at concentrations of 1, 4 and 8%.

In principle, the results obtained with PLGA(Poloxamer 1%)-20 were very promising, as lung accumulated a higher proportion than liver (Fig. 6). It must be pointed out that the main difference between all the previous microspheres and PLGA(Poloxamer 1%)-20 is the presence of poloxamer (21%) on their surface (Table 2).

For this reason, we increased the poloxamer concentration to 4% and prepared 3 lots of microspheres with different mean sizes (Table 3): PLGA(Poloxamer 4%)-7, PLGA(Poloxamer 4%)-20 and PLGA(Poloxamer 4%)-40. The SEM images showed that these microspheres were spherical and slightly porous. Biodistribution results are shown in Fig. 6.

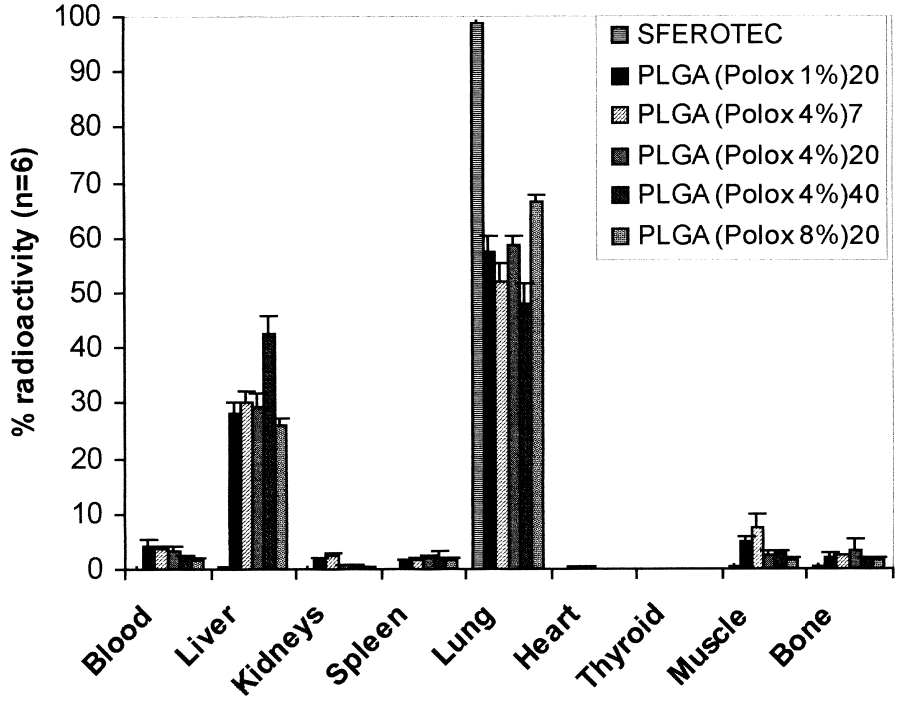

Fig. 6. Tissue biodistribution of ^{99m}Tc-PLGA microspheres prepared with Poloxamer F-68 in the external aqueous phase: ^{99m}Tc-PLGA(Poloxamer1%)-20; ^{99m}Tc-PLGA(Poloxamer4%)-7; ^{99m}Tc-PLGA(Poloxamer4%)-20; ^{99m}Tc-PLGA(Poloxamer4%)-40 and ^{99m}Tc-PLGA(Poloxamer8%)-20.

Approximately 30% of both PLGA(Poloxamer 4%)-7 and PLGA(Poloxamer 4%)-20 accumulated in liver versus 42.5% using PLGA(Poloxamer 4%)-40. The highest lung accumulation, approximately 60%, was obtained with PLGA(Poloxamer 4%)-20. For the same composition, the best lung accumulation was reached with 20 μm mean-diameter microspheres, which presented a very similar size distribution to HAM, with around 11% of particles outside the recommended range (Table 3).

Comparing PLGA(Poloxamer 1%)-20 and PLGA(Poloxamer 4%)-20 (Fig. 6), the results indicate that the increase in poloxamer 188 concentration in the external aqueous phase was not reflected on the microsphere surface (Table 2) nor in the biodistribution. When PLGA microspheres with 8% of poloxamer 188 were prepared and analyzed by XPS (Fig. 3), the surface was made up of 26% poloxamer 188. These microspheres did not differ significantly in size or morphological characteristics from other poloxamer microspheres (Table 3 and Fig. 5). However, lung accumulation slightly increased and radioactivity located in liver and blood fell a little (Fig. 6). Since 67% of particles were accumulated in lung and 25% in liver, more than 90% of the injected radioactivity was located in these two organs, the lung retaining 2.68 times more than the liver.

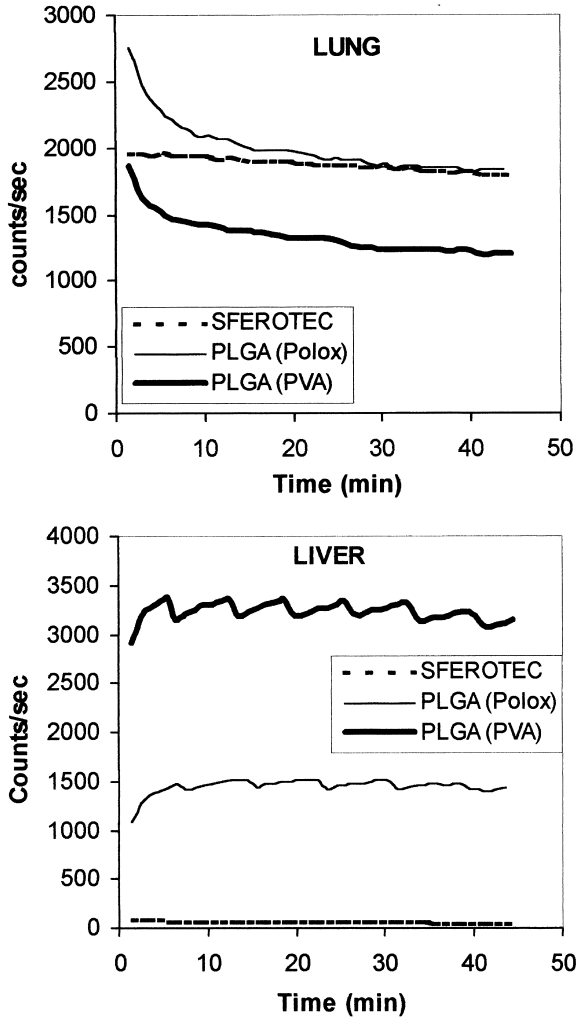

Fig. 7. Lung and liver internal age-distribution curves after intravenous administration of ^{99m}Tc-HAM, ^{99m}Tc-PLGA(PVA) and ^{99m}Tc-PLGA(Poloxamer8%)-20 in rats.

The last step was to test the quality of the images using a gamma camera. Three microsphere formulations: PLGA(PVA) (from the first group of experiments), PLGA (Poloxamer 8%)-20 and the reference, HAM, were labelled to obtain a microsphere suspension with 2–3 mCi/100 μl to be directly injected as before but under the gamma camera. Lung and liver internal age-distribution curves are shown in Fig. 7. The ^{99m}Tc-HAM were instantaneously distributed within the lung (the liver curve corresponds to the background), whereas ^{99m}Tc-PLGA(PVA) and ^{99m}Tc-PLGA (poloxamer 8%)-20 lung curves showed a biphasic profile, an initial rapid distribution phase followed by an equilibrium phase. As illustrated, the liver curves also reached a plateau. The difference between the three lung curves appears in the first phase, the plateau is then reached and the slopes of the second phases are similar, showing very slow elimination. At this point, the radioactivity located in lung after 15 min. was 99% for ^{99m}Tc-HAM, 67% for ^{99m}Tc-PLGA(Poloxamer 8%)-20 and 30% for ^{99m}Tc-PLGA(PVA). The effect of these radioactivity differences is illustrated in Fig. 8, where ^{99m}Tc-HAM gives a sequence of perfect lung images, while ^{99m}Tc-PLGA(Poloxamer 8%)-20 shows a good initial image but after some minutes the radioactivity accumulated in the liver overlaps the lung image, reducing its definition. For ^{99m}Tc-PLGA(PVA), liver is more visible than lung since a significant amount of radioactivity is located in the liver immediately after the injection.

4. Conclusion

We have shown that to trap PLGA-particles in the capillary bed of the lung, several factors besides particle size must be taken into account. The particle surface is important because its composition can modify properties such as smoothness or density that could be involved in the affinity of the particles to the lung. Chemical composition changes in 20 μm PLGA microspheres modify their biodistribution. It is possible to produce varying degrees of PLGA-microsphere lung accumulations by changing the surfactant used in the external aqueous phase. The highest lung uptake was achieved with PLGA(Poloxamer 8%)-20, with 67% of the injected radioactivity.

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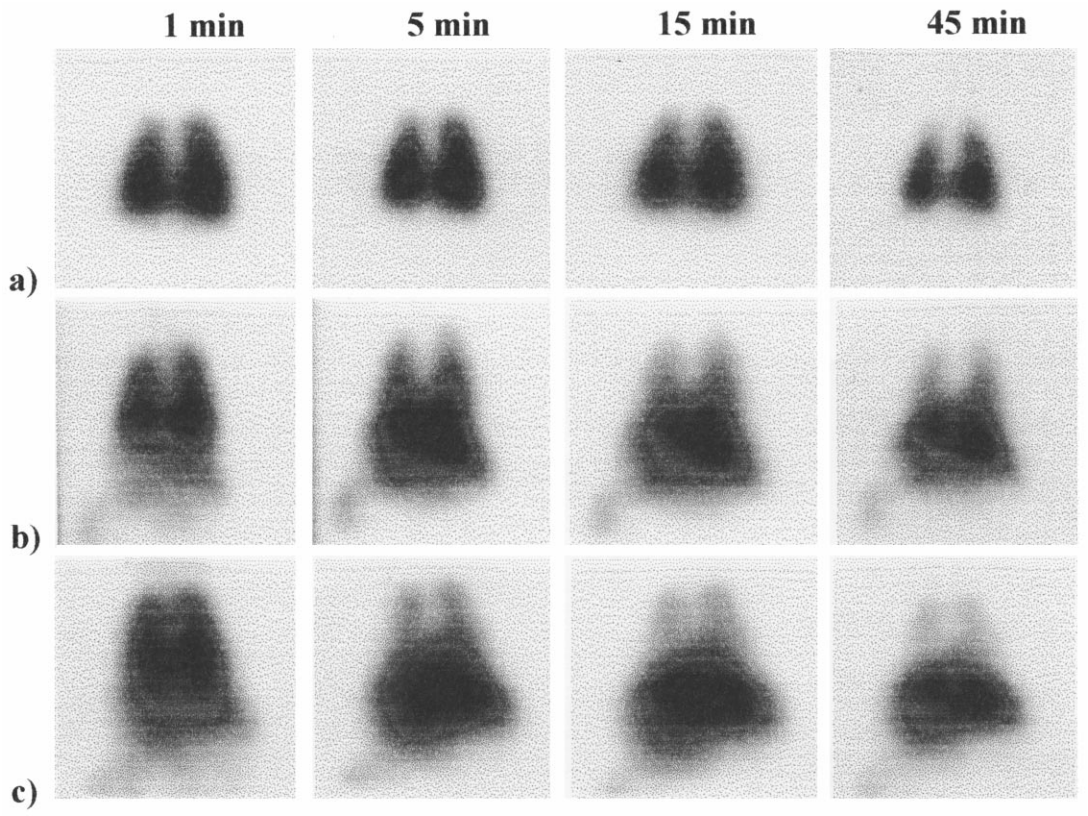

Fig. 8. Serial gamma camera images showing lung and liver accumulation of (a) ^{99m}Tc-HAM, (b) ^{99m}Tc-PLGA(Poloxamer 8%)-20 and (c) ^{99m}Tc-PLGA(PVA), in rats.

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