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Surface and morphology of spray-dried pegylated PLA microspheres

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Abstract

The morphology, the surface structure, and the mean diameter of spray-dried biodegradable pegylated microspheres were studied by X-ray photoelectron spectroscopy (XPS) technique, scanning electron microscopy (SEM) and photo correlation spectroscopy. PEG 400-distearate (PEG-400(C_{18})$_2$) was incorporated into poly(D,L-lactic acid) (PLA) by spray-drying using different concentrations of PLA and polyethylene glycol-distearate (PEG-distearate). The use of these different concentrations resulted in systems with different sizes, morphologies and surfaces. Microsphere characteristics such as size distribution, morphology, and PEG distribution were investigated and proven to be highly dependent on the concentrations of PLA and PEG in the solutions to be spray-dried. Scanning electron microscopy showed that the PLA concentration in the polymeric solution rise to microparticles rather than microspheres. Red blood cell-like structures were observed for a high PLA concentration. Photocorrelation spectroscopy proved that the size distribution depended on the initial viscosity of the polymeric solution. The more viscous was the solution, the bigger the microspheres (and vice versa). X-ray photoelectron spectroscopy confirmed the assumption that greater is the amount of PEG-distearate in the formulation, the more it is found on the surface. These results have allowed us to predict pegylated biodegradable microspheres to be the best microencapsulation process.

Keywords: Poly(D,L-lactide); Microspheres; Spray-drying; PEG; XPS

1. Introduction

Biodegradable microspheres made of poly(D,L-lactic acid) (PLA), and poly(D,L-lactic-co-glycolic acid) (PLGA) have become very popular delivery systems for parenteral delivery because of their potential and availability to optimize therapy (Lewis, 1990). The double emulsion (Celebi et al., 1996; Yeh et al., 1995), and the spray-drying methods (Gander et al., 1995, 1996; Lacasse et al., 1997) are the techniques most often used to mi-

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croencapsulate different kinds of drugs. Spray-drying is a single step method which can lead to injectable microspheres (less than 5 μm), with residual solvent less than the 50 p.p.m. limit (Bitz and Doelker, 1996). Few studies however, have considered the physico-chemical considerations of spray-dried microspheres. Gander et al. (Gander et al., 1995, 1996) proposed an interesting thermodynamic approach to protein microencapsulation into PLA by spray-drying. They demonstrated that microencapsulation by spray-drying generally involves the three components present in the initial formulation: the polymer, the solvent, and the drug. Native spray-dried microspheres greatly depend on the molecular interaction between the components.

The use of polyethylene glycol (PEG) in many drug particulate carriers is not only very useful to decrease biomolecule adsorption and consequently to inhibit the uptake by the cells from the reticulo endothelial system; but it has also been widely studied in liposomes (Senior et al., 1991; Filion and Phillips, 1997), nanoparticles (Allemann et al., 1995) and recently in microspheres (Lacasse et al., 1997, 1998). In the manufacturing of microspheres and/or liposomes by a double emulsion technique, the PEG is inevitably adsorbed or covalently bound on the surface of these particulate carriers. For instance, we proposed in a recent study (Lacasse et al., 1997) the use of polyethylene glycol-400-distearate (PEG-400(C_{18})$_2$) in spray-dried microspheres to improve their dispersion in the dissolution medium, confirming the fact that there was some PEG on the surface of the microspheres. We also demonstrated recently, by confocal laser microscopy, that PEG was homogeneously dispersed on the microspheres at 1 and 10% levels and decreased by phagocytosis to a 0.1% level (Lacasse et al., 1998). X-ray photoelectron spectroscopy (XPS) is a powerful technique to determine the chemical elements and the chemical bonds on the surface of a material. Shakesheff et al. (1997) studied the adsorption of poly(vinyl alcohol) (PVA) to biodegradable microparticles with this technique. They demonstrated that an amount of PVA was adsorbed on microspheres made by the double emulsion technique, even after several washes.

The aim of this study was to evaluate the morphology and the localization of different amounts of PEG-400 distearate on spray-dried microspheres by XPS. Moreover the prediction of the concentration of PLA and PEG-400-distearate in order to manufacture spray-dried pegylated-PLA microspheres was evaluated. Thus injectable microspheres should present no aggregation, stealth behavior and a mean particle size near one micron.

2. Materials and methods

2.1. Materials

The synthesis of D,L-PLA was carried out as previously described (Ratcliffe et al., 1984) with slight modification. Briefly, PLA was synthesized by a ring opening method using D,L-dilactide from Aldrich, (Milwaukee, WI) and tetraphenyltin as a catalyst under anhydrous and high vacuum conditions. The crude reaction product was purified by precipitation of PLA in water after dissolution in acetone. The PLA was then dried under vacuum over phosphorous pentoxide for 2–3 days. PEG 400-disterate was obtained from Aldrich (Milwaukee, WI).

2.2. Characterization of PLA

The molecular weights of the PLA samples was determined by gel permeation chromatography (GPC) using a Waters 717 Autosampler coupled to a Waters™ 600E System Controller and a Waters 410 Differential Refractometer. The columns used were Ultrastyragel 10^3 Å and 10^4 Å. Chloroform from Fischer (Neapan, Ont., Canada) was used as the mobile phase at a flow rate of 1 ml/min. The molecular weight (M_w) of PLA used in this study was 82000.

2.3. Preparation of microspheres

Microspheres of PLA were prepared in the following manner: (1) a methylene chloride solution containing 0.1, 0.5, 1, 3, 5, 7 and 10% (w/v) of polymer and; (2) 0.1, 1 or 10% w/w (of PLA)

PEG 400-distearate was added to the methylene chloride solution for the samples containing PEG-distearate. The sample was then spray-dried with a Büchi Mini Spray Dryer-Model 190 (Büchi, Flawil, Switzerland) using a 0.5 mm nozzle. The process parameters were as follows: 43–44°C inlet air temperature; 36–38°C outlet air temperature; aspirator control 10; pump control 10 (245 ml/h); 600 nl/h airflow.

2.4. Particle morphology

Morphological evaluation of the microspheres were conducted by scanning electron microscopy (SEM) (JSM 820 JEOL). The microspheres were attached to the specimen holder with a double-coated adhesive tape and coated for 3 min at 40 mA with a layer of gold (Coating unit: Polaron E5100).

2.5. Particle size measurement

The mean particle size of each sample was determined using photon correlation spectroscopy (N4 Plus, Coulter Electronics, Hialeah, FL). Microspheres were ultrasonicated (Branson 3210 Ultrasonic bath) for 30 s, and were diluted with phosphate buffer (pH 7.4) to give a particle count rate between 5×10^4 and 1×10^6 counts/s. The mean particle diameter was calculated, in size distribution processor mode (SDP), using the following conditions: 1.33 fluid refractive index; 20°C temperature; 0.93 centipoise viscosity; 90.0° angle of measurement; 10.5 ms sample time, and sample run time of 60 s.

2.6. Surface analysis by XPS

PLA (1% w/v in dichloromethane) microspheres with either 0, 0.1, 1 and 10% (w/w in PLA) of PEG-400$(C_{18})_2$ and bulk PEG-$(C_{18})_2$ were analyzed by XPS on a double Mg and Al anode system (Escalab MKII of VG). The radiation of Mg, which had energy of 1253.6 eV, was used because it gave a better peak resolution. The source was adjusted at a powerful 280 W (power of emission of 20 mA and a tension of 14 kV). A pressure of about 1.10^{-9} mbar was maintained in the analysis unit. The use of a slit of 15×6 mm at the end of the hemispherical analyzer, allowed the surface analysis of 3×2 mm of sample. Photoelectrons exited at an angle of 90° thus allowing probe close to 50 Å on the material. Only the microspheres made of 1% w/v solution were analyzed by XPS considering that they present the best shape.

3. Results and discussion

3.1. Size distribution

Spray-dried pegylated microspheres were analyzed by photocorrelation spectroscopy for their size distribution. Table 1 shows that the largest diameter observed was 3 μm for the microspheres made with an PLA solution of 5% (w/v), irrespective of the PEG-distearate concentration. The only factor that had an effect on the diameter, was the concentration of the initial PLA solution, also shown in Table 1. There did not seem to be a difference between the 0.5 and 1% concentrations in which the microspheres displayed a diameter near 1.5 μm. Increasing (or decreasing) the concentration of the initial polymer solution

Table 1
Size distribution of PLA microspheres versus concentration (% w/v) of PEG 400$(C_{18})_2$ and PLA in the initial formulation

Percentage (w/v) PLA sol.	Percentage (w/w) PEG 400$(C_{18})_2$	Size (nm) $\pm$ S.D.[a]
0.1	0	963.2 $\pm$ 201.3
	10	945.7 $\pm$ 173.1
0.5	0	1584.3 $\pm$ 299.5
	10	1665.2 $\pm$ 329.1
1	0	1409.8 $\pm$ 259.2
	0.1	1473.7 $\pm$ 329.9
	1	1337.2 $\pm$ 314.8
	10	1452.3 $\pm$ 215.2
3	0	1804.7 $\pm$ 325.9
	10	1780.5 $\pm$ 259.2
5	0	3001.0 $\pm$ 1743.7
	10	2967.0 $\pm$ 856.3

[a] S.D. is for standard deviation.

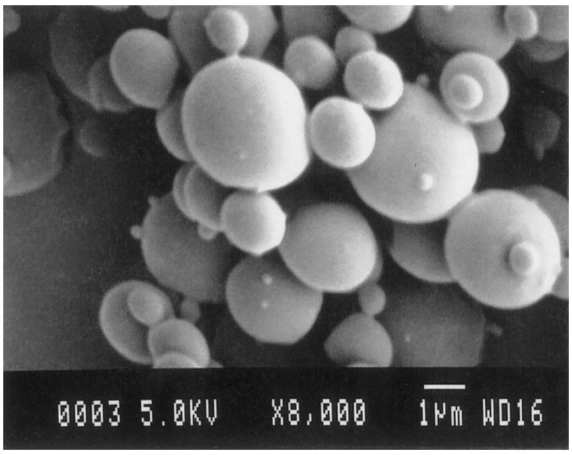

Fig. 1. PLA (1% w/v) microspheres included 10% w/w (PLA) of PEG-distearate obtained by the spray drying technique.

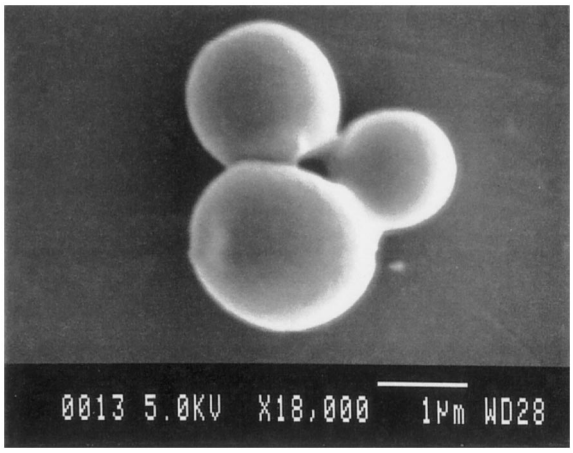

Fig. 3. PLA (3% w/v) microspheres included 1% w/w (PLA) of PEG-distearate obtained by the spray drying technique.

resulted unavoidably in an increase (or decrease) in viscosity of the same initial solution. This phenomenon will induce bigger (or smaller) droplets at the tip of the nozzle, thus resulting in bigger (or smaller) particles.

3.2. Particle morphology

Microencapsulation by spray-drying resulted in spherical, smooth particles, as shown in Figs. 1 and 2. It was difficult to observe pores on the surface of the microspheres because of the gold coating necessary for the SEM technique. Hence, we are not able to conclude whether the use of PEG-distearate decreased the roughness of the surface, or whether the gold coating was responsible for this smoothness. Figs. 3–5 display microspheres that are coalesced, that is having pearl necklace structure. Fig. 6 shows a red blood cell shape. This could be attributed to the initial viscosity of the polymer solution. On the one hand, if the initial solution has been made with a low molecular weight polymer or if there is too much solvent, the solution will not be viscous enough so

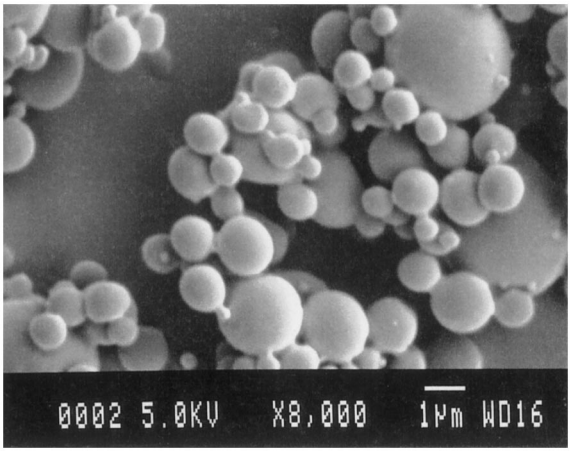

Fig. 2. PLA (1% w/v) microspheres included 1% w/w (PLA) of PEG-distearate obtained by the spray drying technique.

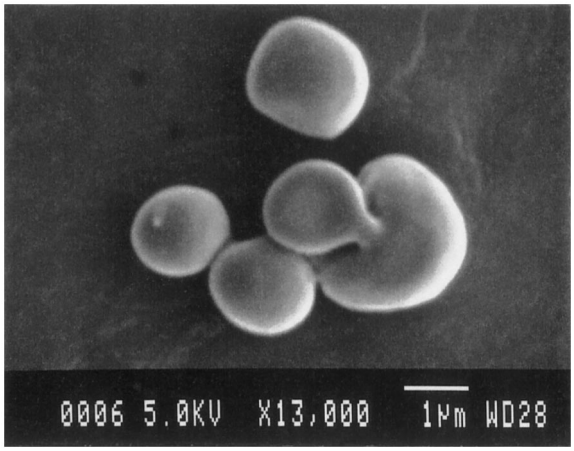

Fig. 4. PLA (5% w/v) microspheres included 1% w/w (PLA) of PEG-distearate obtained by the spray drying technique.

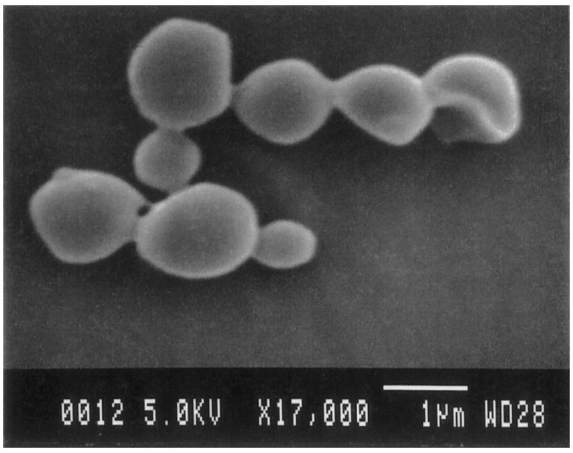

Fig. 5. PLA (7% w/v) microspheres included 1% w/w (PLA) of PEG-distearate obtained by the spray drying technique.

the native droplet which leaves the nozzle will 'collapse' during the drying process. This would produce a microparticle rather than a microsphere. On the other hand, if the viscosity is too high, an extrusion process will occur, resulting in 'spaghettis', because the droplets would not separate from each other during the drying process. With these microphotographs, it is now possible to view the significant difference between microparticles and microspheres. This difference which was not observed with the particle diameter

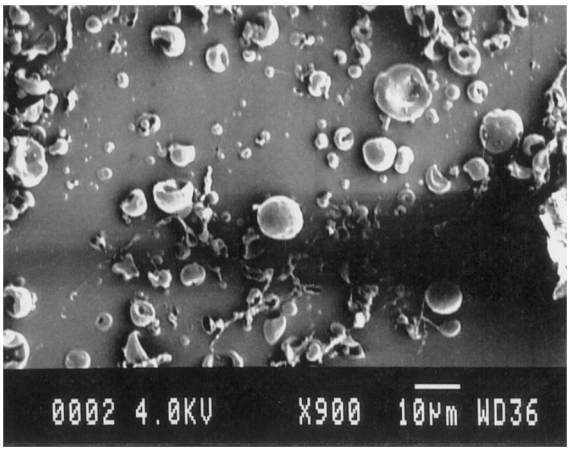

Fig. 6. PLA (0.1% w/v) microspheres and microparticles included 1% w/w (PLA) of PEG-distearate obtained by the spray drying technique.

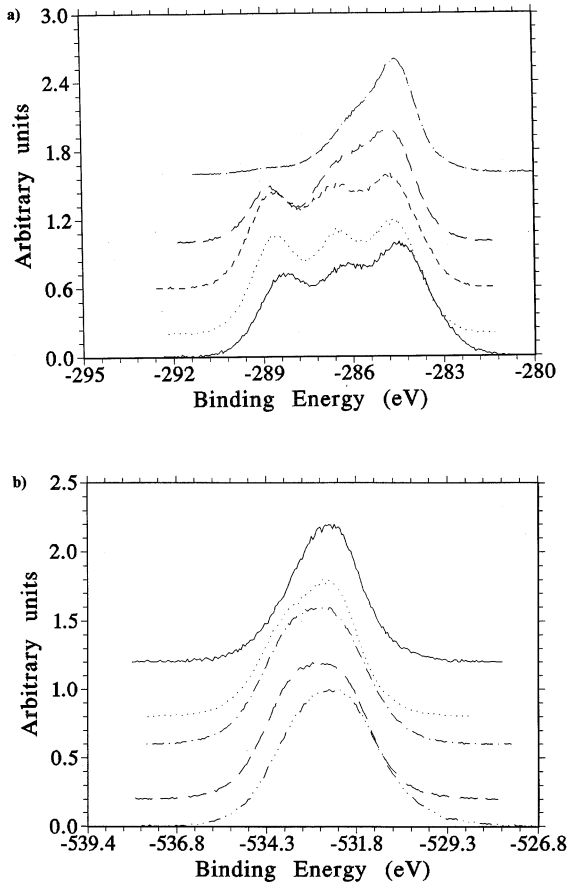

Fig. 7. XPS spectrum of (a) Carbon bond C_{1s}, PEG content from top to bottom 100, 10, 1, 0.1 and 0%. (b) Oxygen bond O_{1s}, PEG content from top to bottom 100, 10, 1, 0.1 and 0%.

(Table 1) of the 0.5 and 1% polymer (w/v) in the initial formulation, but the difference in shape can be seen under SEM. Indeed, the spray-dried powder is made of microparticles rather than microspheres.

3.3. XPS analysis-PEG disposition

The XPS technique allowed the elucidation of the surface structure of the pegylated microspheres. Fig. 7a and b illustrate the binding energies for carbon and oxygen, respectively. (It should be kept in mind that with this technique, an analysis of the surface with a depth close to 50 Å, can be achieved.) As shown, the figure with the

carbon displays more information than the figure with the oxygen because the XPS analyzes only two oxygen bonds in the PLA/PEG-400(C_{18})$_2$ mix corresponding to C–O*–C + O–C=O* and O*–C=O (the analyzer does not distinguish between the oxygen environment in the ether and carbonyl bonds of the ester). There are however four different carbon bonds: C–C, C*–O–C=O, C–O–C and O–C=O (carbon or oxygen atoms denoted with an asterisk correspond to the precise atom being evaluated in the bond). In Fig. 7a, the reference (first spectrum at the bottom of the graph), made of pure PLA, shows three different peaks at 285.0, 286.8 and 288.8. It can be observed that the more the percentage of the PEG-400(C_{18})$_2$ is increased, the more the carbon spectrum shifts to the right. Moreover, when the level of PEG-400(C_{18})$_2$ is 10%, the second PLA peak has almost disappeared. Hence, it can be assessed that the greater the level of PEG-400(C_{18})$_2$ in the initial mix, the more PEG-400(C_{18})$_2$ segregation will occur on the surface of the spray-dried microspheres.

Table 2
Characteristics of carbon and oxygen bonds implicated in the PLA-PEG-400(C_{18})$_2$ mix

Percentage of PEG-400(C_{18})$_2$	B.E.[a]	Surface percentage	Identification	at.%	B.E.[a]	Surface percentage	Identification	at.%
Reference 1				**61.47**				**37.53**
(pure PLA)	285.0	33.27	C–C	20.45	532.1	51.45	C–O–C + O–C=O*	19.31
	286.8	28.00	C*–O–C=O	17.21	533.5	43.78	O*–C=O	16.43
	288.8	28.37	O–C=O	17.44				
0.1%				**61.64**				**37.94**
	285.0	25.34	C–C	15.62	532.4	51.88	C–O–C + O–C=O*	19.68
	285.8	16.36	C–CO_2	10.08	533.9	48.12	O*–C=O	18.26
	287.3	28.33	C*–O–C=O	17.46				
	289.3	29.97	O–C=O	18.47				
1%				**61.97**				**37.84**
	285.0	30.15	C–C	18.68	532.3	52.24	C–O–C + O–C=O*	19.77
	286.0	12.11	C–CO_2	7.50	533.7	47.76	O*–C=O	18.07
	287.2	26.98	C*–O–C=O	16.72				
	289.2	30.76	O–C=O	19.06				
10%				**68.14**				**31.00**
	285.0	35.65	C–C	24.29	532.5	61.92	C–O–C + O–C=O*	19.20
	286.0	20.81	C–CO_2	14.18	533.9	38.08	O*–C=O	11.80
	287.2	22.84	C*–O–C=O	15.56				
	289.3	20.7	O–C=O	14.10				
Reference 2				**77.68**				**16.45**
Pure PEG-400(C_{18})$_2$	285.0	66.44	C–C	51.61	532.5	70.58	C–O–C + O–C=O*	11.61
	286.4	24.14	C–CO_2	18.75	533.5	29.42	O*–C=O	4.84
	287.4	6.10	C*–O–C=O	4.74				
	289.2	3.31	O–C=O	2.57				

[a] B.E. is the binding energy.

Table 3
Full width half max (Fwhm) of XPS spectrums

Samples	Carbon (Fwhm)	Oxygen (Fwhm)
Pure PLA	1.85	2.05
PLA + 0.1% PEG-400(C_{18})$_2$	1.80	2.00
PLA + 1% PEG-400(C_{18})$_2$	1.80	1.80
PLA + 10% PEG-400(C_{18})$_2$	1.60	1.60
Pure PEG-400(C_{18})$_2$	1.60	1.60

The identification and values of the carbon (on the left) and oxygen (on the right) bonds analyzed by XPS are listed in Table 2. It is interesting to note that the ether bond (C–O–C) only appears at 1% of PEG-400(C_{18})$_2$, and that the atomic percentages converge to the pure PEG-400(C_{18})$_2$ percentages. Another possible method to determine whether PEG-400(C_{18})$_2$ is localized on the surface is to evaluate the peak area sensitivity, that is the full width half maximum (Fwhm). Table 3 shows these results. It may be observed that the more the PEG-400(C_{18})$_2$ is added to the mix, the more the Fwhm decreases to reach the PEG-400(C_{18})$_2$ values, thus increasing the presence of this excipient on the surface. There is no difference in the Fwhm between the 0.1 and 1% formulations, which could be attributed to a similar dispersion in the microsphere surfaces.

By assessing the photomicrographs, the XPS analysis and results from our previous work (Lacasse et al., 1998), it is now possible to predict the best area to have pegylated microspheres. First of all, the morphology of the spray-dried microspheres was not influenced by the PEG-400(C_{18})$_2$ concentrations. Secondly, by considering the percentage of PLA in the initial solution, the best spray-dried microspheres were obtained from a range between 1 and 3% of PLA. Microparticles presenting irregular structures (red-blood cell) were obtained at percentages lower than 1% (Fig. 6). The pearl necklace structures were observed between 7 and 3% (Figs. 3–5). Levels above 7% displayed the extrusion or 'spaghetti' structures.

Numerous strategies, which include injectable microsphere preparation, can be applied for different therapeutic purposes. The tendency is to make stealth microspheres in order to avoid rapid clearance from the systemic circulation. However, Löbenberg et al. (1996) have demonstrated that a promising strategy for AIDS therapy involved a macrophage targeting. In a previous work (Lacasse et al., 1998), we demonstrated that spray-dried pegylated microspheres were associated within the macrophages if the PEG-400(C_{18})$_2$ concentration was greater than 1% (w/w), despite the presence of the phagocytosis inhibitor cytochalasin D. At 0.1%, this association was inhibited, demonstrating the stealth protection of the spray-dried PEG-400(C_{18})$_2$ coated microspheres. PEG-coated microspheres and PEG-coated colloidal vectors are already well known and manufactured. However, with the double emulsion technique, one of the most useful manufacturing processes, the PEG will inevitably be adsorbed on the surface of the microspheres due to its hydrophilic and surfacting behavior at the microspheres interface. The same phenomenon will occur in the stealth liposome preparations. Whereby, in this case, the PEG is conjugated with phospholipids (Filion and Phillips, 1997). This kind of PEG was found to be at the surface of the liposomes. With a solvent evaporation technique, the conjugated PEG will not be mixed within the phospholipid bilayer because of its hydrophilicity and its phospholipid anchor. This interfacial phenomenon, due to a liquid–liquid system is nonexistent with the spray-drying technique. The characteristics of these spray-dried pegylated microspheres will depend on the spray-drying conditions, the solubility parameters, the partition coefficient of the PLA, the PEG-400(C_{18})$_2$ in the solvent and its vapor pressure. A hypothesis for the migration of the PEG-400(C_{18})$_2$ to the surface of the spray-dried microspheres can now be proposed based on the substantial results obtained by XPS analysis. In order to do so however, one must look at the chemical nature and behavior of the substituent reagents, that is PLA and PEG, comprising the microspheres. PLA in solution is known to act like a polar molecule and a Lewis base (Gander et al., 1996). PEG has a molecular weight of 400 and because it is conjugated with two stearate molecules, the excipient displays a

high affinity for dichloromethane (the solvent used in this work). Thus, the elevated concentration found on the surface of the microspheres could be attributed to the PEG-400(C_{18})$_2$ being transported by the dichloromethane during the drying process. This hypothesis is plausible because XPS analysis (Table 2) showed that there is not a significant difference in the amount adsorbed on the surface between the 0.1 and 1% PEG-400(C_{18})$_2$ levels. The fact that the surface is not the same at these two specific concentrations however was confirmed from our previous work (Lacasse et al., 1998) whereby evaluation of phagocytosis at these levels was observed to be quite different. Hence, the partition coefficient is not the only phenomenon that may explain the PEG-400(C_{18})$_2$ segregation on the surface of the microspheres during the spray-drying process.

The conformation and transport properties of polymer chains in a selected solvent depend on several factors such as the type of chains (molecular weight, charged or neutral) and the concentration of the solute and solvent (Ferry, 1980). The dynamic of a single chain in polymer melts has been described by de Gennes' reptation model (de Gennes, 1979). Deegan et al. (Deegan et al., 1997) have recently demonstrated that capillary flow of solvent during a droplet drying process, explains the deposition of small solid particles at the periphery of the liquid. The PEG-400(C_{18})$_2$ segregation at the surface of the spray-dried microspheres may be interpreted in the same way. With a 10% PEG-400(C_{18})$_2$ level, the droplets at the end of the nozzle are made of both high (PLA) and low (PEG-400(C_{18})$_2$) molecular weight polymers. The PLA, due to its high molecular weight, is not able to move easily in the droplet. PLA fibers act as a network where PEG-400(C_{18})$_2$ solid particles could be trapped after the evaporation of the solvent surface. Because of this phenomenon, PEG-400(C_{18})$_2$ particles could adopt movements through the PLA chains, and consequently be extracted from the PLA matrix with solvent migration during the drying process. This phenomenon is not only attributed to a 10% concentration, or the presence of a small solute molecule. We demonstrated in a previous study by confocal laser microscopy (Lacasse et al., 1997) that a 10% (w/w) level of drug in spray-dried microspheres, was homogeneously dispersed on the microspheres. Moreover, the burst was not big, confirming the absence of segregation of the drug on the surface of the microspheres. This may be explained by specific chemical interactions (ionic interactions, covalent interactions, etc.) between the drug and the polymer resulting in the drug not being extracted with the solvent during the drying process. Hence, all the physico-chemical characteristics of the reagents must be taken into account.

In this work, we have demonstrated that it is possible to manufacture several kinds of spray-dried pegylated microspheres having different levels of PEG-400(C_{18})$_2$ on their surfaces. The XPS technique has allowed us to demonstrate that the more PEG-400(C_{18})$_2$ is concentrated in the initial formulation, the more it will be on the surface of the spray-dried microspheres.

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