Hydrophilic Nanoreservoirs Embedded into Polymeric Micro/Nanoparticles: An Approach To Compatibilize Polar Molecules with Hydrophobic Matrixes

Hui Gao,[†] Marie-Christine Jones,[†] Jian Chen,[‡] Yongri Liang,[‡] Robert E. Prud'homme,[‡] and Jean-Christophe Leroux*,[†]

Canada Research Chair in Drug Delivery, Faculty of Pharmacy, and Department of Chemistry, University of Montreal, Montreal, Quebec, Canada H3C 3J7

Received May 10, 2008

Aliphatic polyesters such as poly(lactide) and its copolymers are the most widely studied artificial biomaterials owing to their biodegradability, biocompatibility, and relatively low cost.[1–4] They have been investigated for the preparation of medical devices, tissue scaffolds, colloidal drug carriers, and sustained release implants. These polymers are also found in a number of commercialized drug formulations.[5] Of particular interest is the use of aliphatic polyesters in the fabrication of biodegradable micro- and nanoparticles (NPs), especially in the fields of vaccines and drug delivery.[6] Several encapsulation techniques have already been described in the literature to load such particles with therapeutically relevant compounds, the selection of a suitable preparation method being primarily determined by the solubility of both the drug and the polymer.[7] While hydrophobic compounds can be simply entrapped by conventional oil-in-water (o/w) emulsion procedures, the incorporation of hydrophilic agents within hydrophobic nanosized matrices still remains a challenge. Hydrophilic molecules are generally encapsulated using a double emulsion (water-in-oil-in-water, w/o/w) method. However, given the lack of affinity of most polar guest molecules for the hydrophobic matrices, this procedure often results in low entrapment efficiencies (EE).[8] As a consequence, there remains a need for more efficient loading methods. To that end, Lambert et al. have reported a method based on the interfacial polymerization of poly(isobutylcyanoacrylate) to generate nanocapsules with a liquid aqeuous core which could encapsulate high levels of oligonucleotides.[9] Another method employed mixtures of phospholipid and surfactant to increase the loading of a peptide, insulin, in solid lipid NPs prepared by double emulsion method.[8] Alternatively, the affinity of water-soluble compounds for a hydrophobic surface can be improved through the formation of lipid derivatives.[10] However, such a method relies on the modification of the hydrophilic guest prior to encapsulation. In this work, we propose an original strategy to load hydrophilic molecules into polyester micro/and nanoparticles prepared by single emulsion. More specifically, reverse polymeric micelles (RMs) obtained from the self-assembly of partially alkylated star-shaped poly(glycerol methacrylate)s ($PG_{OH}MA$) were embedded into poly(D,L-lactide) (PDLLA) nanospheres to serve as hydrophilic reservoirs for polar guest molecules.

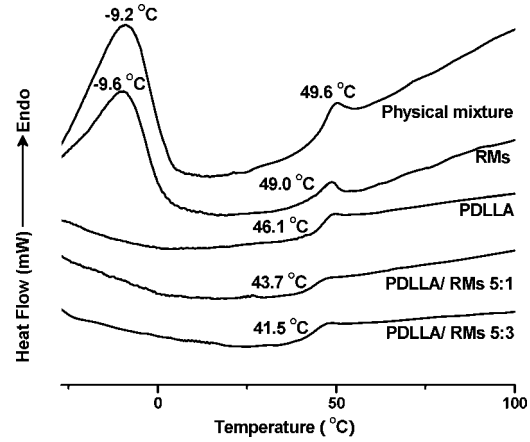

Figure 1. DSC thermograms of RMs, physical PDLLA/RM mixture, and PDLLA NPs fabricated in the presence and absence of RMs.

The NPs (~200−350 nm) employed here were prepared by emulsifying a dichloromethane (DCM) solution of PDLLA with or without RMs in water using poly(vinyl alcohol) as emulsifier, followed by the evaporation of the organic solvent in vacuo (Supporting Information). The RMs were obtained from a 6-arm $PG_{OH}MA$[11] partially esterified with myristoyl chloride (60 mol % of OH content).[12] ^{1}H NMR analysis of the dissolved NPs revealed that the RMs could be quantitatively recovered from the PDLLA matrix by filtration after three consecutive washing steps (Supporting Information).

Differential scanning calorimetry (DSC) and wide-angle X-Ray diffraction (WAXD) experiments were performed to characterize the RMs and PDLLA NPs. The RMs alone exhibited two endothermic transitions (Figure 1). The main transition at −9.6 °C could be attributed to the fusion of myristoyl chains.[13] The second minor peak at 49.0 °C might arise from the disruption of a liquid crystalline order[14] or from the melting of remaining crystals since WAXD confirmed the persistence of crystallinity at room temperature

[†] Canada Research Chair in Drug Delivery, Faculty of Pharmacy.
[‡] Department of Chemistry.

(1) Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. *Science* **1994**, *263*, 1600.
(2) Leroux, J.-C.; Cozens, R.; Roesel, J.-L.; Galli, B.; Kubel, F.; Doelker, E.; Gurny, R. *J. Pharm. Sci.* **1995**, *84*, 1387.
(3) Kalarickal, N. C.; Rimmer, S.; Sarker, P.; Leroux, J.-C. *Macromolecules* **2007**, *40*, 1874.
(4) Waris, E.; Ashammakhi, N.; Lehtimaki, M.; Tulamo, R.-M.; Kellomaki, M.; Tormala, P.; Konttinen, Y. T. *Biomaterials* **2008**, *29*, 683.
(5) Chaubal, M. *Drug Delivery Technol.* **2002**, *2*, 34.
(6) Oster, C. G.; Wittmar, M.; Unger, F.; Barbu-Tudoran, L.; Schaper, A. K.; Kissel, T. *Pharm. Res.* **2004**, *21*, 927.
(7) Lamprecht, A.; Ubrich, N.; Hombreiro Perez, M.; Lehr, C. M.; Hoffman, M.; Maincent, P. *Int. J. Pharm.* **1999**, *184*, 97.
(8) Liu, J.; Gong, T.; Wang, C.; Zhong, Z.; Zhang, Z. *Int. J. Pharm.* **2007**, *340*, 153.
(9) Lambert, G.; Fattal, E.; Pinto-Alphandary, H.; Gulik, A.; Couvreur, P. *Pharm. Res.* **2000**, *17*, 707.
(10) Cui, F.; Shi, K.; Zhang, L.; Tao, A.; Kawashim, Y. *J. Controlled Release* **2006**, *114*, 242.
(11) Sunder, A.; Krämer, M.; Hanselmann, R.; Mülhaupt, R.; Frey, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 3552.
(12) Jones, M. C.; Tewari, P.; Blei, C.; Hales, K.; Pochan, D. J.; Leroux, J.-C. *J. Am. Chem. Soc.* **2006**, *128*, 14599.
(13) Greenberg, S. A.; Alfrey, T. *J. Am. Chem. Soc.* **1954**, *76*, 6280.

10.1021/cm8012792 CCC: $40.75
Published on Web 06/06/2008

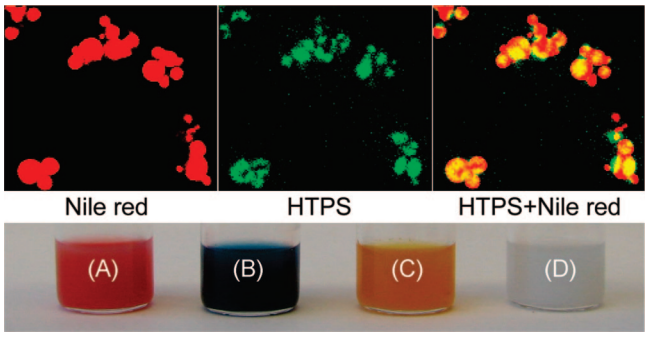

Figure 2. Upper panel: Confocal fluorescent microscopy of PDLLA/RMs microparticles. Nile Red was used to label the polymeric matrix while RMs were loaded with HPTS, a hydrophilic marker. The coexistence of both structures was seen as a yellow fluorescent signal. Lower panel: PDLLA/RMs NPs loaded with CR (A), MB (B), and MO (C) by the single emulsion method. In the absence of RMs, dye encapsulation was negligible (D). Even when a double w/o/w emulsification procedure was used the loading remained marginal, despite the extremely high feed ratios tested (Supporting Information). PDLLA/RMs NPs loaded with CR (A), MB (B), and MO (C) by the single emulsion method. In the absence of RMs, dye encapsulation was negligible (D). Even when a double w/o/w emulsification procedure was used the loading remained marginal, despite the extremely high feed ratios tested (Supporting Information).

(Supporting Information, Figure S3). In the absence of RMs, PDLLA NPs displayed a glass transition temperature (T_g) at 46.1 °C, as could be expected for moderately high molar mass PDLLA.[15] The thermogram of the physical mixture of PDLLA NPs and RMs NPs (5:1 weight ratio) resembled that of the micelles alone. The glass transition of PDLLA, however, could not be observed and was probably masked by the second endotherm of RMs. Interestingly, the incorporation of the micelles into PDLLA NPs suppressed all transitions related to RMs and lowered the T_g of PDLLA. This latter effect was more prominent at higher RM concentrations. These results demonstrated that RM ordering was hindered by their entrapment in PDLLA NPs. Similar conclusions were drawn from the analysis of the X-ray diffraction profiles (Supporting Information). Further evidence of the association of PDLLA and RMs was obtained with larger particles through confocal fluorescence microscopy where an overlap of the signals corresponding to PDLLA and RMs was observed (Figure 2, upper panel).

To observe the distribution of RMs in a PDLLA matrix, polymeric films with embedded RMs were prepared by casting a DCM solution of PDLLA/RMs on freshly cleaved mica. The RMs were then visualized by atomic force microscopy (AFM). It can be seen from Figure 3a that RMs are regularly distributed within the PDLLA film. The embedded dried RMs had a diameter of 41 ± 3 nm, which corroborated the micelle size measured in DCM (41 nm with a polydispersity index of 0.06 as determined by dynamic light scattering). This observation implies that the integrity of the core−shell structure was maintained in the PDLLA matrix.

As illustrated in Figure 2 (lower panel), the incorporation of RMs in PDLLA NPs resulted in a substantial improvement

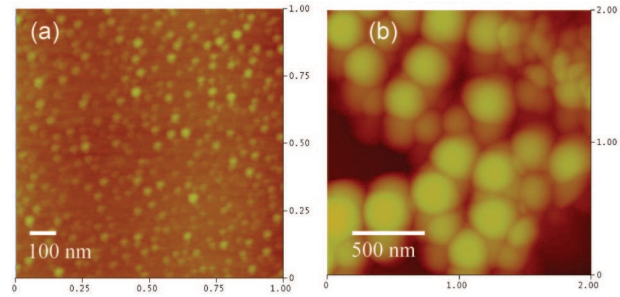

Figure 3. AFM images of (a) RMs embedded into a PDLLA film (d_{RM} = 41 ± 3 nm) and (b) CR-loaded PDLLA/RMs NPs (d_{NP} = 343 ± 44 nm).

Table 1. Influence of RMs Concentration and Encapsulated Guest on PDLLA NP Size and EE (%)[a]

C_{RMs}[b] (g/L)	guest	diameter (nm) [PI][c]	loading [d] (% w/w)	EE (%)
0		216 [0.15]		
5.4	CR	245 [0.19]	1.75 ± 0.36	22.8 ± 1.4
10.8	CR	277 [0.22]	3.05 ± 0.27	45.8 ± 1.0
16.2	CR	314 [0.25]	3.96 ± 0.85	67.3 ± 2.1
21.6	CR	326 [0.29]	4.19 ± 0.74	79.7 ± 2.9
27.0	CR	348 [0.29]	4.64 ± 0.82	97.4 ± 3.9
16.2	MB	286 [0.19]	3.12 ± 0.75	53.0 ± 2.8
16.2	MO	290 [0.19]	3.37 ± 0.93	57.3 ± 3.1
16.2	VP	304 [0.25]	3.68 ± 0.81	62.5 ± 2.1

[a] The organic/aqueous volume ratio was set at 1:6. The weight ratio of guest molecule/PDLLA was set at 1:10. [b] Concentration of RMs. [c] [PI] Polydispersity index. [d] Mean ± SD (n = 3).

of the loading capacity of hydrophilic guest molecules such as Congo red (CR), methylene blue (MB) and methyl orange (MO) (A−C). In these experiments, the NPs were prepared by the o/w emulsion method. The dye was dissolved in the aqueous phase and extracted from it by the RMs[16,17] upon the emulsification of the organic solvent. As expected, in the absence of RMs, no dye could be entrapped in PDLLA NPs (D).

Table 1 shows the characteristics of PDLLA NPs containing increasing concentrations of RMs and loaded with various polar guest molecules. These NPs displayed a spherical morphology (Figure 3b), similar to the dye-free blank PDLLA NPs (Supporting Information). Enhancing the concentration of RMs within the NPs was found to improve the EE of CR while also increasing the mean particle size (Table 1). This latter effect could be partly explained by the greater viscosity of the organic phase at higher RM concentrations, resulting in a less efficient emulsification of DCM in the aqueous phase.[18] The dye EE reached ~67% at a RM/PDLLA ratio of 3:5, which is relatively high considering the low affinity of small hydrophilic molecules for PDLLA. CR loading and EE could be further increased to 4.64% (w/w) and 97.4%, respectively, when the RM/PDLLA ratio was set at 1:1. However, this also led to a higher polydispersity. Increasing the initial CR concentration had little effect on dye loading but resulted in an enlarged particle size (Supporting Information), which could be attributed to the interfacial effect of water-dissolved CR on the emulsification

(14) Canilho, N.; Kasemi, E.; Schluter, A. D.; Mezzenga, R. *Macromolecules* **2007**, *40*, 2822.
(15) Pannu, R. K.; Tanodekaew, S.; Li, W.; Collett, J. H.; Attwood, D.; Booth, C. *Biomaterials* **1999**, *20*, 1381.
(16) Gao, H.; Jones, M. C.; Tewari, P.; Ranger, M.; Leroux, J.-C. *J. Polym. Sci., Polym. Chem.* **2007**, *45*, 2425.
(17) Gao, H.; Jones, M. C.; Chen, J.; Prud'homme, R. E.; Leroux, J.-C. *J. Chem. Mater.* **2008**, *20*, 3063.
(18) Kwon, H.-Y.; Lee, J.-Y.; Choi, S.-W.; Jang, Y.; Kim, J.-H. *Colloids Surf., A* **2001**, *182*, 123.

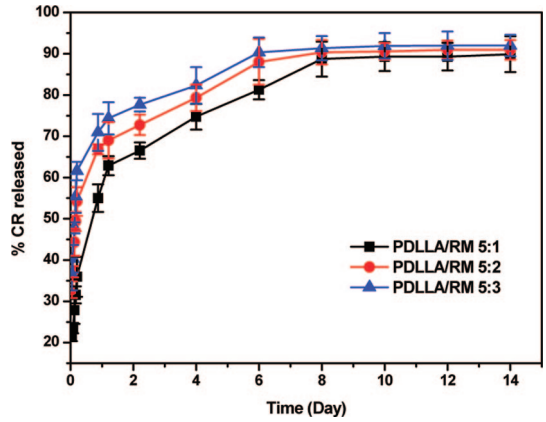

Figure 4. In vitro release kinetics of CR from PDLLA/RMs NPs. Mean ± SD (n = 3; CR loading, 1.7% w/w, 37 °C).

of DCM. MB, MO, and the antidiuretic peptide vasopressin (VP) were also successfully encapsulated in PDLLA NPs albeit with a slightly lower EE.

To verify that the encapsulated cargo could be readily released from the NPs, the in vitro release profiles of CR-loaded PDLLA NPs were examined at increasing RMs concentrations (Figure 4). For all formulations, ~90% of CR was released in a sustained fashion over 10 days. Between 20 and 35% of the total CR content was released during the first hour. The greatest burst was observed with the NPs containing the highest RM concentration. This could be explained by the reduced diffusion path of PDLLA NP matrices prepared at high RM/PDLLA ratios.

In conclusion, these findings clearly demonstrate that the RMs formed hydrophilic nanodomains within hydrophobic polymeric particles. This technique is efficient, versatile, and simple. It allows the incorporation of hydrophilic guest molecules into biodegradable polymeric micro- or nanoparticles with high efficiencies making this approach particularly interesting for drug delivery applications, especially in the field of vaccination.

Acknowledgment. Mistral Pharma Inc. (Laval, Qc, Canada), the Natural Sciences and Engineering Research Council of Canada, and the Canada Research Chair program are acknowledged for their financial support.

Supporting Information Available: Detailed experimental methods, encapsulation data, ^{1}H NMR, and CR release kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

CM8012792