Solvent Effect in PLA-PEG Based Nanoparticles Synthesis through Surfactant Free Polymerization

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Summary: In this work one-pot synthesis of PEGylated PLA-based nanoparticles (NPs) without using any surfactant has been performed. Adopting ring opening polymerization of L,L–Lactide and 2-hydroxyethyl methacrylate (HEMA), vinyl end functionalized poly(lactic acid) macromonomers (HEMA-LA_n) have been produced with tunable number of lactic acid units (larger than 5) and a low molecular weight distribution. Macromonomers have been further copolymerized with modified PEG chains (HEMA-PEG_m) through a monomer starved semi-batch emulsion polymerization performed without using any surfactant. In these conditions, small and monodispersed NPs of around 150 nm are obtained. Since macromonomers with *n* larger than 5 are highly viscous at room temperature, they have to be dissolved in a solvent before their injection in the reactor. In this work the effects in changing the solvent adopted in the starved process (water miscible or non-miscible) and its amount have been investigated. Moreover, the effect of both PEG chains concentration and MW on the final NPs properties has been elucidated. The colloidal stability of the NPs produced using different solvents has been verified in phosphate buffered saline (PBS) solution via dynamic light scattering measurements; in addition the critical coagulation concentration of these PEGylated NPs has been determined.

Keywords: drug delivery systems; emulsion polymerization; nanoparticles; PLA; PEG

Introduction

Degradable NPs based of polyesters such as poly(lactic acid) (PLA) are widely selected as vectors for targeted drug delivery because of their good biocompatibility and biodegradability due to the hydrolytic degradation of esters bonds.[1,2] Among all the different processes adopted to produce polymeric NPs, emulsion polymerization allows a good control of the final NPs features. Recently, adopting this synthetic procedure and starting from macromonomers obtained through ring opening polymerization (ROP), degradable NPs have been produced.[3] However, the synthesis of small NPs (below 100 nm) requires the use of a significant amount of surfactant that has to be removed to limit biological side effects.[4] A surfactant free process allows to produce NPs useful for drug delivery applications without any further modification, even if it is difficult to obtain monodispersed NPs with a controlled particle size without surfactants added.[5] Moreover, the use of NPs for drug delivery applications is limited by their recognition as foreign bodies by the reticuloendothelial system (RES).[6,7] This immune response is triggered by the fast adsorption of proteins on the NPs surface. To eliminate or at least substantially reduce the protein adsorption on the NPs, the surface properties have to be modified. As reported in the literature to avoid the protein adsorption, the NPs outer corona should be a hydrophilic, neutral layer with hydrogen-bond acceptors instead of donors.[8] Consequently poly(ethylene glycol) (PEG) has been selected as one of

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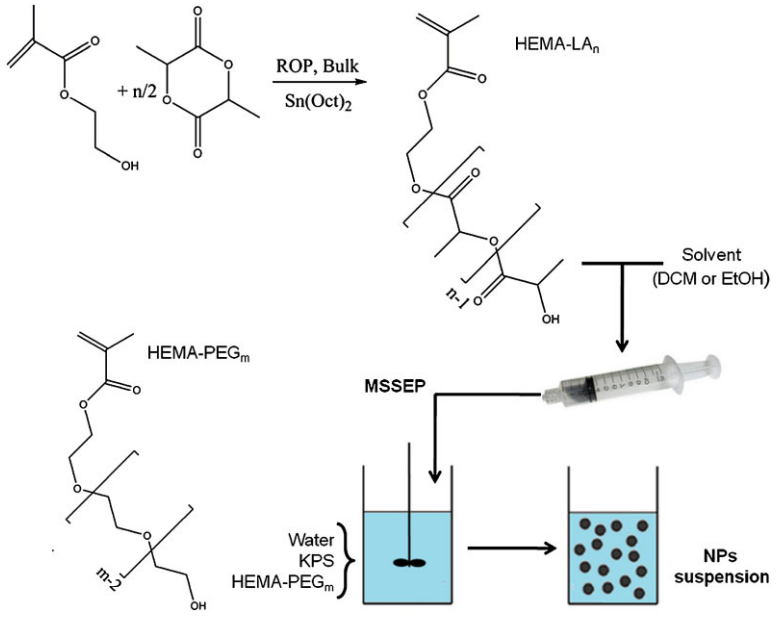
the most effective synthetic biomaterials to counteract the adsorption of proteins over NPs surface.[9,10] Recently, the possibility to copolymerize through a monomer starved semi-batch emulsion polymerization (MSSEP) PLA-based macromonomers and polymerizable PEG chains in order to obtain PEGylated NPs avoiding the use of surfactants has been reported.[11] In particular a methacrylic group functionalized with a controlled number of PLA units (HEMA-LA_n) has been copolymerized with PEG-based macromonomers characterized by different chain lengths *m* (HEMA-PEG_m) as reported in Scheme 1. The starved process performed with HEMA-LA_n with *n* higher than 5 requires the use of a suitable solvent since macromonomers cannot be directly injected in the reactor due to their high viscosity.

In this work the effects of different solvents on the final NPs characteristics have been investigated. In particular a water nonmiscible (dichloromethane, DCM) and miscible (ethanol, EtOH) solvents have been selected and their effect on the final particle size evaluated. A semi-batch procedure in which HEMA-PEG_m is introduced in the reactor and HEMA-LA_n added continuously during the polymerization (see Scheme 1) has been adopted since in the case of a starved procedure performed feeding simultaneously both HEMA-LA_n and HEMA-PEG_m no stable latexes are produced.[11,12] Finally, the latexes stability has been tested through dynamic laser light scattering (DLLS) measurements conducted for 30 days at 37 °C in PBS solution along with the critical coagulation concentration (CCC) determination.

Experimental Part

L,L–Lactide (PURAC, 99.5%), 2-ethylhexanoic acid tin(II) salt ($Sn(Oct)_2$; Sigma, ~ 95%), 2-hydroxyethyl methacrylate (HEMA; Sigma, ≥ 99%), poly(ethylene glycol) methyl ether methacrylate (HEMA-PEG, Aldrich-Fine Chemicals, Molecular weight: ca. 300, 475, 950, and 2080 Da), potassium persulfate (KPS, VWR, ≥ 99%), tetrahydrofuran (THF, Sigma, ≥ 99.7%), dichloromethane (DCM, Sigma, ≥ 99.8%) and ethanol



Scheme 1.
Semi-batch process adopted for the synthesis of surfactant-free PEGylated PLA-based NPs.

(EtOH, Sigma, $\geq$ 99.5%) were used as received.

L,L–Lactide was polymerized through ROP in bulk at 130 °C using $Sn(Oct)_2$ as a catalyst and HEMA as co-catalyst in order to obtain HEMA-LA_n macromonomers with n controlled by the molar ratio of L,L–Lactide and HEMA. HEMA-LA_n macromonomers with n equal to 5 and 8 were produced and then characterized using both ^{1}H-NMR (500 MHz Ultrashield NMR spectrometer, Bruker, Switzerland), by dissolving samples in $CDCl_3$ and size exclusion chromatography (SEC), using THF as eluent with a flow rate of 0.6 mL/min and a temperature of 35 °C. The instrument (Agilent, 1100 series, Germany) was equipped with two detectors in series (ultraviolet (UV) and differential refractive index (RI)), three PLgel columns (Polymer laboratories Ltd., UK; two with pore sizes of MXC type and one oligopore; length of 300 mm and 7.5 mm ID) and a precolumn. Universal calibration was applied, based on polystyrene standards from 580 Da to 3,250,000 Da (Polymer Laboratories). For PEGylated macromonomers, the chain length m was confirmed by ^{1}H-NMR measurements.

NPs were produced using a semi-batch procedure. In detail, HEMA-PEG_m macromonomer was dissolved in 100 mL of distilled water and the obtained solution was heated at 70 °C following nitrogen purge for 5 minutes. KPS 1.6 wt% with respect to monomer was added into the purged solution. HEMA-LA_n was dissolved in DCM or EtOH (the amount of solvent was properly selected as described in the Results and Discussion section) and the mixture was subsequently introduced into the reactor with a syringe pump (Model NE-300, New Era Pump System, US) at 3 ml/h. The two macromonomers, for a total of 5 g, have been fed in the reactor with a mass ratio w equal to 3 calculated adopting the Equation (1):

$$w = w_{\text{HEMA-LAn}}/w_{\text{HEMA-PEGm}} \quad (1)$$

Reactions were run for three hours. When the lipophilic solvent was used, NPs were stirred overnight to remove DCM from the suspension. Particle size were characterized by dynamic laser light scattering (DLLS, Malvern, Zetasizer Nano) and each measurement was repeated 2 times (reported data are the average of the intensities values). DLLS provides accurate measurements of monodispersed samples with low PDI while the accuracy is diminished in the case of samples with a large PDI.''

To test the latexes stability, 0.1 mL of NPs were added to 10 mL of PBS solutions and the resulting suspensions was heated up to 37 °C in an incubator. Particle size of the samples was measured at different times through DLLS measurements performed at 37 °C. Finally, the CCC of the NPs has been determined by adding the latexes to NaCl solutions with different salt concentration.

Results and Discussion

Synthesis of PLA Macromonomer and Their Characterization

HEMA-LA_5 and HEMA-LA_8, with PLA chain length equal to 5 and 8, have been synthesized adopting the ROP.[13,14] The MW of the obtained macromonomer has been determined through ^{1}H-NMR adopting the Equation (2).

$$M_n = M_{HEMA} + \frac{M_{lactide}}{2} \times \left(\frac{\mathrm{D}\,(methylene\,proton\,signal)}{\mathrm{F}\,(\alpha\,methylene\,proton\,signal)} + 1 \right) \quad (2)$$

Where the term into brackets represents the average number of lactic acid unit added to the HEMA molecule. By analyzing ^{1}H-NMRspectra, the structure of the macromonomers has been confirmed after the designation of all protons, as reported in Figure 1.

Lactide conversion has been calculated from ^{1}H-NMR data as higher as 99.5%. In parallel, macromonomers have been characterized through SEC (see Figure 2) using the universal calibration of PLA.[15]

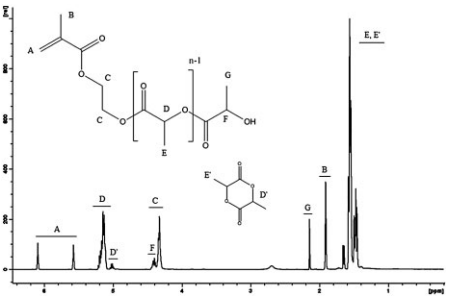


Figure 1.
^{1}H-NMR spectrum of the HEMA-LA$_8$macromonomer.

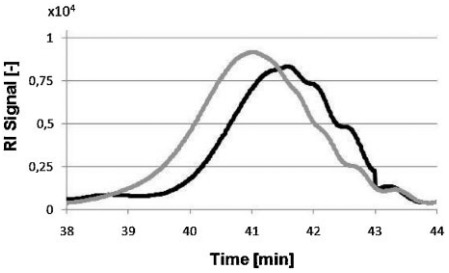


Figure 2.
SEC chromatograms of HEMA-LA$_5$ (black line) and HEMA-LA$_8$ (grey line).

A comparison between the M_n values determined adopting ^{1}H-NMR and SEC is given in Table 1, in which a good agreement between the two techniques can be observed. In particular, SEC data give information about polydispersity of the produced macromonomer, validating the ROP living characteristic of the synthetic procedure.

Commercially HEMA-PEG$_m$macromonomers have been also analyzed by ^{1}H-NMR and the so determined PEG chain lengths m is reported in Table 1.

PEGylated Particles Production and Characterization

As a first step of this work, the effect of the solvent polarity on the final particle size was investigated. DCM has been selected since it has been already used in the polymerization of HEMA-CL$_n$ macromonomers since it is a not miscible with water and it can easily stabilize viscous or solid macromonomers droplets in aqueous phase during the reaction.[3] While EtOH has been selected as a water miscible solvent with a relative good biocompatibility (at low concentrations). The polymerizations are performed adopting a solvent-to-macromonomer weight ratio equal to 0.4 for HEMA-LA$_{5.2}$ and 1.2 for HEMA-LA$_{7.9}$, respectively. Dimensions and polydisperisity index (PDI) of NPs characterized by a PEG chain length m equal to 8.6 and obtained adopting these two solvents to inject the PLA-based macromonomers are collected in Table 2. By a close inspection of data reported in Table 2 it is possible to observe that the final particle size is comprised between 160 and 118 nm and that all the NPs produced are monodispersed. Obtained results prove that EtOH, which has a low toxicity at the adopted concentrations, can be fruitfully adopted as a substitute of the more toxic DCM. In particular it is worth mentioning that the use of EtOH does not lead to the formation of a solid in the reactor as a result of the macromonomer precipitation.

The same semi-batch procedure has been applied with different PEGylated macromonomers (m equal to 19.3 and 45.6) and all the obtained results are reported in Table 2. Clearly, it possible to observe that the PEG chain length strongly

Table 1.
Characteristics of the adopted macromonomers.

Recipe		^{1}H NMR		SEC		
Chain length (n or m)	M_n (Da)	(n or m)	M_n (Da)	(n or m)	M_n (Da)	M_w/M_n
HEMA-LA$_n$ n = 5	490	5.2	505	5.8	548	1.31
HEMA-LA$_n$ n = 8	707	7.9	700	8.9	770	1.35
HEMA-PEG$_m$ m = 8.5	475	8.6	479	–	–	–
HEMA-PEG$_m$ m = 19.2	950	19.3	953	–	–	–
HEMA-PEG$_m$ m = 44.9	2080	45.6	2153	–	–	–

Table 2.
Size and PDI of surfactant free NPs produced using different solvents to dissolve HEMA-LA_n macromonomers.

Solvent	n^1H NMR	m^1H NMR	Diameter [nm]	PDI
DCM	5.2	8.6	153	0.18
DCM	5.2	19.3	122	0.10
DCM	5.2	45.6	88	0.05
EtOH	5.2	8.6	160	0.03
EtOH	5.2	19.3	127	0.08
EtOH	5.2	45.6	63	0.15
DCM	7.9	8.6	127	0.05
DCM	7.9	19.3	97	0.10
DCM	7.9	45.6	91	0.05
EtOH	7.9	8.6	118	0.10
EtOH	7.9	19.3	135	0.21
EtOH	7.9	45.6	61	0.12

influences the final particle size. In fact, a reduction of the NPs diameter down to 60 nm is observed when a PEGylated macromonomer with *m* equal to 45.6 is adopted, independently upon the selected solvent. Moreover it is possible to conclude that the solvent choice has not a univocal effect on the final NPs features since in some cases the use of EtOH leads to smaller NPs while in others the effect is the opposite. Finally, obtained results show that the PLA chain length slightly affects the final particle and, in general, a decrease of about 20 nm can be observed when the macromonomer with longer PLA chain is used. The decrease of the particle size can be explained by the increase of the macromonomer hydrophobicity, which, for PLA oligomers, is related to the increase of their degree of polymerization.[16] In fact, in starved conditions the polymerization rate in aqueous phase is strictly dependent upon the macromonomer concentration. Thus, macromonomers with high hydrophobicity show a slow reaction rate in aqueous phase. As a consequence, there is an increase in the nucleation time leading to a higher number of NPs and consequently to a lower final NPs size.[17]

As a second part of this work, the effect of the solvent-to-macromonomer ratio on the final NPs characteristics has been studied. Therefore the two selected PLA-based macromonomers have been dissolved in different volumes of DCM and EtOH and then polymerized with the same PEG-based macromonomer (i.e. HEMA-$PEG_{8.6}$). The total amount of PLA-based macromonomer and solvent has been injected keeping constant the macromonomer feeding rate since it is known that the feeding mode influences the final NPs properties.[18] The obtained results are reported in Figure 3(a–b). For the polymerization of HEMA-$LA_{5.2}$ the use of DCM leads to smaller NPs rather than EtOH and, even maintaining the same feed ratio of the macromonomer, a slightly decreasing trend in the particle size is observed by increasing the solvent-to-macromonomer ratio. At the contrary, EtOH has minor effect on the final particle size. In the case of NPs based on HEMA-$LA_{7.9}$, a strong decreasing in particle size, as reported in Figure 3(b) is

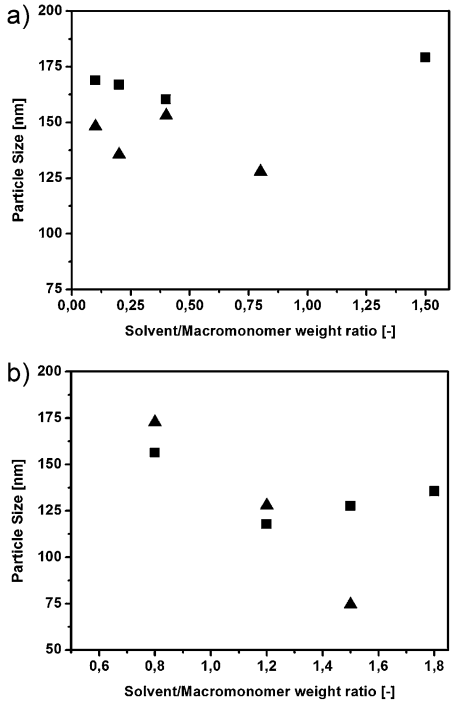


Figure 3.
Particle size as a function of the solvent-to-macromonomer ratio for NPs produced adopting: (a) HEMA-$LA_{5.2}$and (b) HEMA-$LA_{7.9}$. (▲) and (■) represent NPs produced using DCM and EtOH, respectively.

observed by increasing the amount of DCM, while EtOH has not significant effects. These evidences can be correlated with the macromonomer droplets viscosity. In fact, when the macromonomer is dissolved into DCM droplets a decrease in particle size is observed by increasing the solvent amount. On the contrary, using the water miscible EtOH, macromonomer droplets viscosity is not diminished and consequently no relevant effects on particle size has been detected. Finally, it is worth noticing that the minimum amount of solvent used to feed the HEMA-$LA_{7.9}$ macromonomer into the reactor (solvent-to-macromonomer ratio of 0.8) leads to higher NPs size for both DCM and EtOH; this result can be explained by the relatively high viscosity of the mixture fed into the reactor.

Stability of PEGylated Nanoparticles

The test performed in isotonic solution (PBS) at 37 °C has the purpose to verify the stability of the obtained NPs synthesized adopting DCM and EtOH. As reported in Figure 4, for the polymerizations of HEMA-$LA_{5.2}$ and HEMA-$PEG_{8.6}$ adopting DCM and EtOH, the NPs dimension does not change significantly after one month. Similar results are obtained for all the materials reported in Table 2, confirming the good stability of the latexes obtained using both DCM and EtOH.

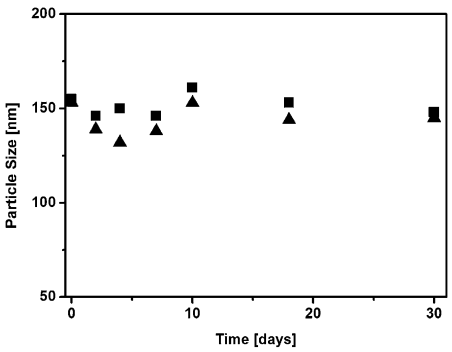


Figure 4.
Particle size for HEMA-$LA_{5.2}$ based NPs during stability test performed in PBS. NPs produced using (▲) DCM and (■) EtOH.

Finally, in order to quantify the relative stability of the latexes, the aggregation rates in different salt (NaCl) concentrations have been measured by DLLS. Results obtained for all the materials reported in Table 2 prove the high stability of these PEGylated NPs that show a CCC comprised between 2 and 4 mol L^{-1}.

Conclusion

PLA-based NPs with different degree of PEGylation have been produced in this work through a free radical polymerization process performed without the use of any surfactant. Brushed polymers made by poly-HEMA backbone with PEG and PLA pendants both with controlled chain length constitute the final polymeric NPs. A semi-batch procedure in which HEMA-PEG_m is introduced in the reactor and HEMA-LA_n added continuously during the polymerization has been adopted. PEG chains act as surfactant leading to monodispersed nanosized particles with final diameter ranging from 60 to 160 nm. It was found that macromonomer with longer PEG chains leads to smaller NPs. The use of a solvent which is required to dissolve the relatively high molecular weight macromonomers has been elucidated. Water non-miscible (DCM) and water miscible (EtOH) solvents have been tested and it was found that both of them allow obtaining small and monodispersed NPs. Therefore the influence of the solvent amount used during the polymerization has been investigated. It was found that by increasing the DCM-to-macromonomer weight ratio it is possible reduce the final NPs diameter while the use of different volumes of EtOH has minor effect on the final NPs size. In addition, the produced NPs latexes are stable in relevant biological conditions (PBS and 37 °C) and at relatively high salt concentration, up to 4M NaCl.

[1] V. Lassalle, M. L. Ferreira, *Macromol. Biosci.* **2007**, *7*, 767.

[2] K. A. Athanasiou, G. G. Niederauer, C. M. Agrawal, *Biomaterials* **1996**, *17*, 93.
[3] R. Ferrari, Y. Yu, M. Morbidelli, R. A. Hutchinson, D. Moscatelli, *Macromolecules* **2011**, *44*, 9205.
[4] D. Qiu, T. Cosgrove, A. M. Howe, *Macromol. Chem. Phys.* **2005**, *206*, 2233.
[5] G. Z. Zhang, A. Z. Niu, S. F. Peng, M. Jiang, Y. F. Tu, M. Li, C. Wu, *Acc. Chem. Res.* **2001**, *34*, 249.
[6] E. Ostuni, R. G. Chapman, R. E. Holmlin, S. Takayama, G. M. Whitesides, *Langmuir* **2001**, *17*, 5605.
[7] J. J. Shi, A. R. Votruba, O. C. Farokhzad, R. Langer, *Nano Lett.* **2010**, *10*, 3223.
[8] S. Kommareddy, S. B. Tiwari, M. M. Amiji, *Tec. Canc. Res. Treat.* **2005**, *4*, 615.
[9] Y. Tabata, Y. Ikada, *Pharm. Res.* **1989**, *6*, 296.
[10] T. Betancourt, J. D. Byrne, N. Sunaryo, S. W. Crowder, M. Kadapakkam, S. Patel, S. Casciato, L. Brannon-Peppas, *J. Biomed. Mater. Res., Part A*, **2009**, *91A*, 263.
[11] R. Ferrari, Y. Yu, M. Lattuada, G. Storti, M. Morbidelli, D. Moscatelli, *Macromol. Chem. Phys.*, **2012**, DOI: 10.1002/macp.201200368.
[12] S. Sajjadi, *Langmuir* **2007**, *23*, 1018.
[13] A. Duda, A. Kowalski, J. Libiszowski, S. Penczek, *Macromol. Symp.* **2005**, *224*, 71.
[14] R. Ferrari, C. Colombo, M. Dossi, D. Moscatelli, *Macromol. Mater. Eng.*, **2012**, DOI: 10.1002/mame.201200069.
[15] J. R. Dorgan, J. Janzen, D. M. Knauss, S. B. Hait, B. R. Limoges, M. H. Hutchinson, *J. Polym. Sci., Part B: Polym. Phys.* **2005**, *43*, 3100.
[16] F. Codari, D. Moscatelli, G. Storti, M. Morbidelli, *Macromol Mater Eng.* **2010**, *295*, 58.
[17] Y. Chen, S. Sajjadi, *Polymer* **2009**, *50*, 357.
[18] M. Dossi, R. Ferrari, L. Dragoni, C. Martignoni, P. Gaetani, M. D'Incalci, M. Morbidelli, D. Moscatelli, *Macromol. Mater. Eng.*, **2012**, DOI: 10.1002/mame.201200122.

