Journal of Applied Polymer Science

PLA/Pluronic® nanoparticles as potential oral delivery systems: Preparation, colloidal and chemical stability, and loading capacity

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ABSTRACT: Mixed nanoparticles of PLA and Pluronic® were developed to overcome the low chemical and colloidal stability in the gastrointestinal track for PLA nanoparticles. The aim of present study was to improve the stability of coating the PLA nanoparticle surface with hydrophilic polymers, such as poly(ethylene oxide). The Pluronic® covered PLA nanoparticles were prepared through nanoprecipitation method. The method was optimized in terms of addition order, type, and concentration of Pluronic® and stirring velocity to obtain particles with sizes around 100 nm. The ζ potential measurements and thermal analysis confirmed the formation of PEO layers around the nanoparticles. Dynamic Light Scattering studies of mixed PLA/Pluronic® nanoparticles showed colloidal and chemical stability during storage and in simulated gastrointestinal fluids, suggesting this system as an alternative for the delivery of therapeutics via oral administration. The nanoparticles ability of encapsulating hydrophobic drugs was evaluated using Nile Red as a probe molecule. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43828.

KEYWORDS: adsorption; colloids; surfactants; thermal properties

Received 15 February 2016; accepted 24 April 2016
DOI: 10.1002/app.43828

INTRODUCTION

Among the different routes of drug administration, the oral route is the most convenient, since it is the easiest and the least invasive.[1] The low permeability of the active ingredients through the gastrointestinal (GI) mucosa, and/or their low water solubility restricts this route of administration. The encapsulation of active ingredients inside polymeric nanoparticles (Nps) has been reported as an alternative to increase the drugs bioactivity, overcome poor solubility and/or permeability, and enhance metabolic stability.[2] Nonetheless, common Nps[3] present two major disadvantages: (i) they are prone to be trapped by mucus via steric or adhesive forces, and rapidly eliminated via mucociliary clearance and (ii) are unstable in GI fluids. Consequently, there are some requirements that the Nps surface need to satisfy to improve the Nps translocation through GI mucus: possessing surfaces without mucoadhesive hydrophobic areas and densely coating exposed surfaces with net-neutral, highly hydrophilic surface.[4,5] In addition, they must be small enough to avoid steric obstruction in the mucus.[5]

Although poly(lactic acid) nanoparticles (PLA Nps) are one of the most extensively studied delivery carriers, their use is limited in oral administration due to their hydrophobicity, low colloidal stability, and susceptibility to be hydrolyzed in the harsh conditions of the GI tract.[6] Previous studies have demonstrated that PLA Nps may undergo a fast degradation in simulated intestinal fluids, producing water-soluble oligomers.[7] PLA hydrolysis can be slowed by coating the surface of the Nps with hydrophilic segments such as poly(ethylene oxide) (PEO).[1,7,8] PEO is a hydrophilic, nonionic, and nontoxic polymer, and it does not possess strong bioadhesive properties compared with chitosan or carbomers.[4] It has been reported that a dense PEO coating (PEGylation) allows by a near-neutral surface charge and negligible protein adsorption; which improve the transport properties of the Nps through mucus.[4] PEGylation is known to enhance the Nps stability in mucus, preventing enzymatic attack and reducing the interactions with food or mucus components.

PEGylation of PLA Nps has been achieved through different approaches, including covalently binding of PEO to PLA (block copolymer approach) and adsorption of PEO to previously formed PLA Nps. It has been reported that the PEGylated effect on the mucus-permeating is more effective when the PEG is covalently bound (PLA–PEO copolymers) than when is adsorbed on Nps. However, this approach requires additional purification steps and coverage-density is limited by steric hindrance. On the contrary, the adsorbed PEO is effective, easy to prepare and cheaper, but can display anchorage instability.[9]

In order to improve the compatibility between hydrophobic and hydrophilic polymers, amphiphilic copolymers such as Pluronic®

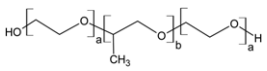

Figure 1. Pluronic® structure.

(poloxamer) have been used. Pluronic® is the trade name of a family of amphiphilic triblock copolymers consisting of a central (lipophilic) PPO block, length *b*, flanked by two symmetrical hydrophilic PEO blocks of length *a* (Figure 1). They are semi-crystalline, where PEO units confer crystallinity to the polymer.[10] Pluronics® are biocompatible, non-toxic, and are commercially available. These properties make them suitable for drug delivery applications.[11–13]

The HLB can be adjusted by varying the block length *a* and *b*, respectively. The HLB have an effect on thermal and colloidal stability of the Nps, which are important parameters that control adhesion and delivery properties and it can be tuner by varying the molar ration of PEO to PPO in the surfactant.[9,14]

Here in we report a methodology for the preparation of mixed PLA/Pluronic® Nps as a mean to get PEO-covered PLA Nps in a cheap an easy way, where the hydrophobic interactions between PLA and the PPO middle block of the Pluronic® copolymer providing a stable anchor point. Three different Pluronic® were used (F127, F108, and F88) and optimization of the method is presented in which the variables such as solutions addition and stirring velocity where statistically studied to obtained the smallest Nps. The obtained particles were characterized by particle size measurements, ζ potential, and analysis of thermal transition. The colloidal stability of PLA and mixed PLA/Pluronic® Nps during storage and in simulated GI fluids was evaluated in terms of their aggregation. The stability of the mixed Nps under simulated GI fluids suggest this system as a good candidate for oral delivery. Nile Red (NR) was used as a model hydrophobic drug to evaluate the loading capability of the mixed PLA/Pluronic® Nps.

EXPERIMENTAL

Materials

Poly(L-lactic acid) "NatureWorks® PLA Polymer 3051D," 96% L-Lactide (PLLA) was obtained from Natureworks Minneapolis Minnesota (The United States), hydrochloric acid (HCl, 37%), sodium hydroxide pellets (NaOH), sodium chloride (NaCl), acetone, and ethanol were supplied by Merck. Pancreatin from porcine pancreas, pepsin from porcine gastric mucosa, acetonitrile (ACN), and Nile Red (NR) were purchased from Sigma-Aldrich. Pluronic® copolymers (F127, F88, and F108) were donated by BASF Corp.

Mixed PLA/Pluronic® Nanoparticles Preparation

Mixed PLA/Pluronic® Nps were prepared by a modified nanoprecipitation method.[15] Two protocols for the Nps preparation were used: (i) addition of polymer solution (0.5% w/v PLA in acetone) into the non-solvent [water/ethanol (47% v/v ethanol[16]) and Pluronic®] (M1) and (ii) addition of the non-solvent in to the polymer solution (M2) (Figure 2). The mixture was stirred at room temperature until the organic phase was fully evaporated.[17]

In addition, a systematic study of the effects of the stirring velocity [fast (F) and slow (S)] and addition velocity [dripping (D) and fast (F)], the composition of the used Pluronic® copolymer (F127, F88, and F108) and concentration (0.10, 0.25, and 0.50%w/v) on the particle size and ζ potential was done. PLA Nps were also prepared as a control.

Storage Stability of Mixed PLA/Pluronic® Nanoparticles

Freshly prepared mixed PLA/Pluronic® Nps using M1 protocol and containing 1% Pluronic® F127 were stored at 4 °C, during 4 weeks the samples were analyzed by DLS. At predetermined time intervals, the changes of particle size were monitored.

Stability of Mixed PLA/Pluronic® Nanoparticles in Simulated Gastrointestinal Fluids

The mixed PLA/Pluronic® Nps were incubated at 37 °C in simulated gastric (USP XXIII, pH 1.2, pepsin 0.32% w/v) and intestinal fluid (USP XXIII, pH 7.5, pancreatin 1% w/v). The samples were analyzed by DLS at predetermined time intervals.

In addition, the study of chemical stability of PLA and mixed PLA/Pluronic® Nps at pH = 1.2 (gastric fluids) and pH= 7.5 (intestinal fluids) was carried out through FTIR analysis (Perkin Elmer Spectrum One). The dispersions were stored at 4 °C during 4 weeks and were freeze-dried using a Labconco (Kansas City) Freeze Dryer over 24 h. The solid obtained was dispersed in KBr and the spectra were recorded between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1} using 16 cumulative scans. These results were compared with the spectrums for the particles in the zero hours.

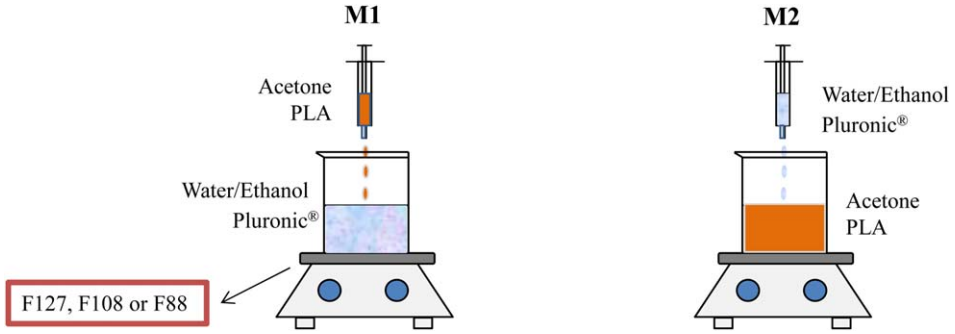

Figure 2. Nanoprecipitation method using two protocols: M1 and M2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Size of PLA and Mixed PLA/Pluronic® Particles Produced by M1 and M2 Protocols

Name	Formulation	Stirring	Addition	Particle size ± SD (nm)	PDI ± SD[a]
M1-FF/F127	PLA and F127	Fast	Fast	107.3 ± 3.7	0.10 ± 0.01
M1-FD/F127	PLA and F127	Fast	Dripped	109.5 ± 3.4	0.11 ± 0.01
M1-SF/F127	PLA and F127	Slow	Fast	116.1 ± 8.5	0.13 ± 0.08
M1-SD/F127	PLA and F127	Slow	Dripped	133.7 ± 4.8	0.10 ± 0.02
M1-FF	PLA	Fast	Fast	101.5 ± 2.8	0.12 ± 0.00
M1-FD	PLA	Fast	Dripped	101.1 ± 2.5	0.11 ± 0.01
M1-SF	PLA	Slow	Fast	128.2 ± 3.9	0.10 ± 0.01
M1-SD	PLA	Slow	Dripped	121.3 ± 14.6	0.10 ± 0.01
M2-FF/F127	PLA and F127	Fast	Fast	177.3 ± 12.9	0.09 ± 0.00
M2-FD/F127	PLA and F127	Fast	Dripped	177.9 ± 7.3	0.10 ± 0.01
M2-SF/F127	PLA and F127	Slow	Fast	Aggregates	Aggregates
M2-SD/F127	PLA and F127	Slow	Dripped	Aggregates	Aggregates
M2-FF	PLA	Fast	Fast	142.9 ± 15.9	0.10 ± 0.01
M2-FD	PLA	Fast	Dripped	167.7 ± 12.3	0.09 ± 0.02
M2-SF	PLA	Slow	Fast	164.4 ± 16.0	0.09 ± 0.01
M2-SD	PLA	Slow	Dripped	228.8 ± 13.3	0.09 ± 0.00

Were used two stirring velocity: fast (F) and slow (S) and addition velocity was fast (F) and dripped (D).
Mean ± S.D., $n = 3$.
[a] PDI, polydispersity index.

Formulation of Nile Red Loaded Mixed PLA/F127 Nps

The nanoprecipitation method was employed for encapsulation of Nile red inside mixed PLA/F127 Nps. A Sotck solution of NR in ACN (1 mg/mL) was added to the PLA solution in acetone to obtain different amounts of NR (0.02, 0.06, 0.10, and 0.5 mg). Nps were formed by the addition of this solution into the aqueous phase (water, ethanol, and Pluronic®). The resulting suspension was stirred until the organic phase was evaporated at room temperature. Nps were purified by centrifugation (5 min, 8965*g*).

Methods

Particle size and size distribution based on intensity were measured by Dynamic Light Scattering (DLS) in Horiba LB 550 equipment. The measurements were carried out at 25 °C in aqueous dispersion of the samples. The reported values correspond to the average of five measurements.

The ζ potential measurements were performed in a Malver zetasizer (nano series) at room temperature. Each value corresponds to the average of three measurements.

PLA and mixed PLA/Pluronic® Nps were freeze-dried using a Labconco (Kansas City) Freeze Dryer over 24 h and the morphology was observed with a Field Emission Scanning Electron Microscopy (FE-SEM, Jeol Model F-7100). To prepare the sample for FE-SEM measurement, the powder obtained from the freeze-dryer was placed onto a strip of double-sided carbon tape and sputter-coated with gold.

Thermal properties of the Pluronics®, PLA and mixed PLA/Pluronic® frozen-dried particles were studied by Differential scanning calorimetry (DSC) using a TA Instruments Q100 equipment. The samples were heated from room temperature up to 200 °C at 20 °C/min, and then cooled at 20 °C/min to −50 °C, to erase their thermal history. After that, the thermograms were collected by heating from −50 to 200 °C at 10 °C/min under nitrogen flow.

Fourier transform infrared spectra (FTIR) of PLA and mixed PLA/Pluronic® Nps frozen-dried were obtained using a Perkin Elmer, model Spectrum one. KBr tablets were prepared by grinding the polymer sample with KBr and compressing the whole into a transparent tablet.

Molecular weights were determined by gel permeation chromatography (GPC) instrument, Viscotek GPCmax Autosampler system, consisting of a pump, a columns type Waters Styragel HR 4E and a Waters 410 differential refractive index (RI) detector with a tetrahydrofuran (THF) flow rate of 1.0 mL/min at 30 °C. The molecular weights were determined with a calibration based on linear polystyrene standards using Viscotek OmniSEC Omni-01 software.

The quantification of the amount of encapsulated NR was performed by UV/vis spectrophotometry in ACN, using a calibration curve of NR solutions in ACN. The samples were measured in triplicates on a UV/vis Perkin Elmer Lambda 35 UV/vis spectrophotometer with UV Winlab software at 537 nm. NR incorporation efficiency was expressed as loading efficiency (%) and loading capacity according with the eqs. (1) and (2), respectively.

$$\text{Loading efficiency } (\%) = \frac{\text{mass of Nile red in Nps}}{\text{mass of Nile red used in formulation}} \times 100 \quad (1)$$

$$\text{Loading capacity} = \frac{\text{mass of Nile red in Nps}}{\text{mass of Nps}} \quad (2)$$

Table II. Physicochemical Characteristics of the Pluronic® Block Copolymers Used

Copolymer	M_w	Average no. of PO[a] units (N_{PO})	Average no. of EO[b] units (N_{EO})	HLB[c]	CMC[d]	
					%w/v	Temperature (°C)
F88	11400	39.3	207.8	>24	—	25
F108	14600	50.3	265.4	>24	4.500	25
F127	12600	65.2	200.4	18-23	0.700	25

[a] PO, propylene oxide.
[b] EO, ethylene oxide.
[c] From Ref. 50.
[d] CMC, for pluronic aqueous solutions at different 25 °C (Ref. 51).

Release Profiles of Nile Red from PLA/F127 Particles

The release profile of Nile Red from PLA/F127 particles was evaluated by dialysis method. Nile Red loaded mixed PLA/F127 Nps were prepared and characterized as was mentioned previously. A volume of 1 mL of the prepared sample was loaded into a dialysis device with a MWCO of 20,000 g/mol (Float-A-lyzer® G2, Spectrum Labs.). The device were placed in 2.0 L of phosphate-buffered saline[18] which was changed every 3 h to ensure sink conditions for NR and polymer. An aliquot of 100 μL was drawn from each cassette at various sampling time intervals and then replaced with 100 μL of fresh buffer. The amount of NR in each sample was quantified by UV/vis.[19]

Analysis of Release Data

The resulting release data were treated according to zero-order (cumulative percentage of released drug vs. time), first-order (log cumulative percentage of remaining drug vs. time), Higuchi (cumulative percentage of released drug vs. square root of time) and Korsmeyer–Peppas (log cumulative percentage of released drug vs. log time) equation models. The criterion employed to select the "best model" was the one with the highest coefficient correlation (R^2).[20]

Statistics

The optimization of the preparation process for obtaining mixed PLA/Pluronic® Nps was carried out in two steps, according to the Factorial Experimental Design (FED). The preparation method, solutions addition and stirring velocity were studied in the first step by using 2^3 FED to evaluate their influence on the particle size. Afterward, a 3^2 FED was used to determine the adequate concentration and type of Pluronic® surfactant and the particle size and ζ potential were the response variable.[21,22]

Statistical analysis of the data was performed via one way analysis for the variance (ANOVA) using statgraphics software; a value of $P < 0.05$ was performed to determine the statistical significance of data. In addition, the assumptions of normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene test) were tested.

RESULTS AND DISCUSSION

Preparation of the Nanoparticles

Particle size and surface properties need to be optimized to enhance the Nps colloidal and chemical stability as well as their transport across the intestinal epithelium.[23,24] In the present study, the effect of different variables during the Nps preparation on size and ζ potential of obtained Nps was investigated. Particles with mean particle sizes smaller than 200 nm, low PDI values and monomodal distributions, were obtained when M1 was employed in presence of Pluronic® F127 (Table I).

Table III. Characterization of PLA and Mixed PLA/Pluronic® Nanoparticles

Name	Concentration Pluronic® (w/v)%	Average particle size (nm)	PDI[a]	ζ potential (mV)
PLA/F88	0.10	96.2 ± 2.3	0.11 ± 0.03	−8.94 ± 3.63
	0.25	97.8 ± 3.4	0.10 ± 0.02	−9.10 ± 4.67
	0.50	99.0 ± 1.1	0.12 ± 0.02	−7.17 ± 1.97
PLA/F108	0.10	106.2 ± 9.7	0.10 ± 0.01	−8.23 ± 0.15
	0.25	107.6 ± 3.5	0.11 ± 0.01	−10.00 ± 0.85
	0.50	104.9 ± 6.2	0.11 ± 0.01	−6.83 ± 0.46
PLA/F127	0.10	90.1 ± 2.9	0.09 ± 0.01	−7.79 ± 0.27
	0.25	85.4 ± 3.7	0.11 ± 0.02	−9.24 ± 0.62
	0.50	87.9 ± 2.2	0.09 ± 0.01	−7.45 ± 1.34
PLA	—	101.4 ± 4.8	0.12 ± 0.03	−32.93 ± 1.24

Mean ± S.D., $n = 3$.
[a] PDI, polydispersity index.

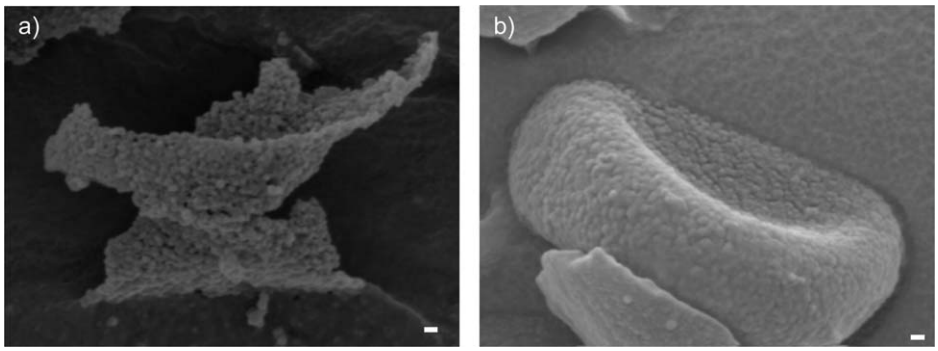

Figure 3. FE-SEM images of (a) PLA and (b) mixed PLA/F127 nanoparticles (bar = 100 nm) prepared by M1 with fast addition and stirring.

Polydispersity index (PDI) values, which are between 0.09 and 0.11, indicate that the size of the particles in all the systems, exhibits a narrow normal distribution. The monomodal and narrow particle size distribution indicate appropriate choice of the components of the systems.

When employing M2, bigger particles were obtained. This could be explained based on the effect of relative concentration of surfactant and PLA during the Nps preparation (Table I). In both protocols, the surfactant concentration is the same in aqueous solution, but the conformation of PEO chains changes when it gets in contact with the organic phase. These chains are more extended for M1 method, where the aqueous phase has a higher proportion during the preparation process. The aqueous phase is a better solvent for PEO segments, which enables the steric stabilization of the system.[17] In addition, for M2 the relative concentration of PLA is high, compared with M1 during the addition time. As a consequence, in protocol M1, PLA precipitates faster in presence of an excess of water, decreasing the particles size.[16]

Effect of the Addition and Stirring Velocity

For the M2 protocol, the stirring velocity has a significant effect ($P < 0.05$) on the particle size, while the solutions addition velocity does not present an effect on this parameter. It is observed that the particles prepared with fast stirring, exhibit a smaller mean particle size (Table I). This result can be explained since there is a short time period to form the Nps, due to the diffusion of the organic solvent is faster to the aqueous phase.[16] The particle aggregation in samples M2-SF/F127 and M2-SD/F127 (Table I) with slow stirring velocity corroborates the important effect of the diffusion of the organic solvent velocity and relative PLA concentration. In contrast, when using M1 method, there is no significant effect for the stirring and addition velocity on the size of the particles ($P < 0.05$).

Effect of the Pluronic® Type and Concentration

Fast stirring and addition into water were selected for studying the effect of the Pluronic® type and concentration, on the particle stabilization against aggregation. This protocol allows the preparation of Nps with tailored size for oral administration[25,26] in a short time.

The steric stabilization achieved by coating PLA Nps with polymer that containing PEO segments, is influenced by the molecular weight of the PEO chain, the surface PEO density, and the method used to attach the PEO segments to the Nps surface.[8] In the present study, the stabilization effect of PLA based on Nps using three Pluronic® copolymers (F127, F88, and F108) with different hydrophilic/lipophilic balance (HLB) values and chain length, was assessed through measurements of particle size and ζ potential. The effect of the Pluronic® concentration in the aqueous non-solvent phase on these two parameters was also evaluated. The most important characteristics for used Pluronic® copolymers are showed in Table II.

For the three evaluated surfactants, there is no significant effect of their concentration on the Nps size and ζ potential ($P < 0.05$). This behavior could indicate that due to the amphiphilic nature of Pluronic® copolymers, some of their chains are reversibly adsorbed on the particles surface maintaining an equilibrium condition. Probably, at the concentration range evaluated in this work, the surface density of Pluronic® chains, did not change significantly.

Particle size and ζ potential values of PLA and mixed PLA/Pluronic® Nps are summarized in Table III. The smallest particle sizes were obtained for mixed PLA/F127 system ($P < 0.05$). It could be due to the low HLB value of Pluronic® F127 compared with the Pluronic® F108 and F88, which is in agreement with early reports.[27,28] Therefore, the length of hydrophobic

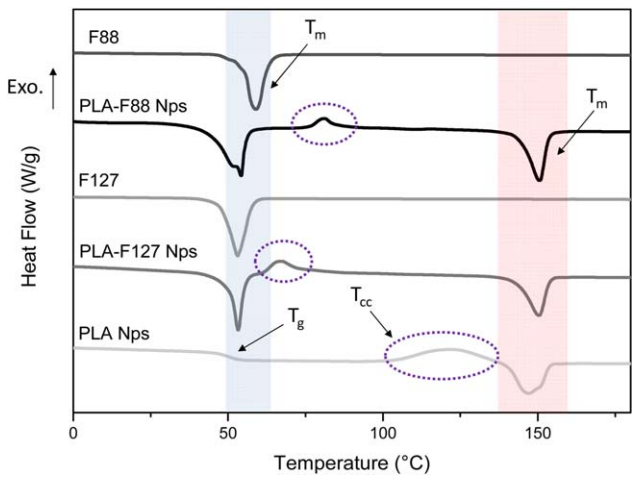

Figure 4. Heating DSC curves of PLA, mixed PLA/F88 and mixed PLA/F127 Nps, and Pluronic® F88 and F127. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table IV. Results from DSC for the PLA, Mixed PLA/F88, and Mixed PLA/127 Mixed Nps

Name	T_m PLA (°C)	T_{cc} PLA (°C)	ΔH_m PLA (J/g)	Crystallinity (%)[a]
PLA Nps	146.9	121.5	21.3	22.9
PLA/F88 Nps	150.6	81.2	21.5	23.1
PLA/F127 Nps	150.4	66.9	21.7	23.3

[a] The crystallinity was calculated from the DSC melting enthalpies, using a melting enthalpy value of 93 J/g for 100% crystalline PLA (Ref. 52).

segment of Pluronic® promotes the interaction with PLA chains, enhancing the coating of Nps and diminishing the size of the particle. This assumption is corroborated by the fact that the size of particles obtained using surfactants with similar HLB value, (Pluronic® F108 and F88) did not change significantly. These results could be related with the occurrence of two competitive phenomena: solvation of PEO chains, and binding to the Nps through the PPO blocks.[28]

The higher ζ potential of the PLA Nps compared with the mixed ones, may be attributed to the presence of carboxylic end groups on the Nps surface. The fact that mixed PLA/Pluronic® Nps became less negative than PLA Nps, suggests that the presence of a hydrophilic PEO steric barrier shifts the "slipping plane" away from the surface of the PLA core resulting in a reduced ζ potential.[29] These close-to-neutral ζ potentials, would prevent the GI mucus for forming polyvalent adhesive interactions, via anionic forces with the Nps.[4,5,30]

Morphology of Nanoparticles. The FE-SEM images of the PLA and mixed PLA/F127 Nps revealed their regular spherical (Figure 3) after the freeze-dry process. For both systems a good correlation between the particle size measured by DLS (PLA Nps: 101.4 nm and mixed PLA/F127 Nps: 90.1) and SEM (PLA Nps: 99 nm and mixed PLA/F127 Nps: 81 nm) was observed.

Thermal Behavior of Mixed PLA/Pluronic® Nanoparticles. Thermal properties such as crystallinity and glass transition temperature (T_g) of the polymers in the Nps are closely related to their mechanical stability and degradation rate. Visualizing the mixed Nps as a blend the thermal properties of the polymers inside them can be used to monitor compatibility between the Nps components.[31]

In this study, the thermal properties of the mixed Nps PLA/F127 and PLA/F88 were compared. According to the precedent results and given that only F127 and F88 have differences in HLB, these two Pluronic® were selected to study these properties. DSC profiles are depicted in Figure 4, and the values of melting temperature (T_m) and normalized melting enthalpy (ΔH_m) for PLA and mixed Nps are listed in Table IV.

Glass transition analysis. In the case of amorphous and semicrystalline polymer blends, the glass transition can be used to monitor miscibility. However, an analysis of the T_g of PLA (50.3 °C) in the mixed PLA/Pluronic® Nps is hampered with the peak due to the melting of Pluronic®, as seen in Figure 4.

Crystallinity analysis of PLA on mixed nanoparticles. The DSC thermogram for PLA Nps exhibits a peak at 147.0 °C, associated with its melting. This peak is broad and superimposed to an exothermic event assigned to its cold crystallization (121.5 °C). Besides, the presence of a shoulder near 151 °C can be related with the melting of crystals with larger size formed during the heating.[32–34]

For the mixed systems, (Figure 4) the value of ΔH_m and crystallinity percentage for Nps are similar to those of PLA Nps (Table IV). This behavior is associated with the Pluronic® which is located mainly in the particle surface and therefore the effect on the PLA crystallinity is low.

Even though, there is not an appreciable change in the crystallinity percentage for binary systems, the shift of T_m to higher values than PLA Nps indicates that the formed domains are more stable, which can be explained by a major interaction between PLA chains. Specifically, this major interaction and also

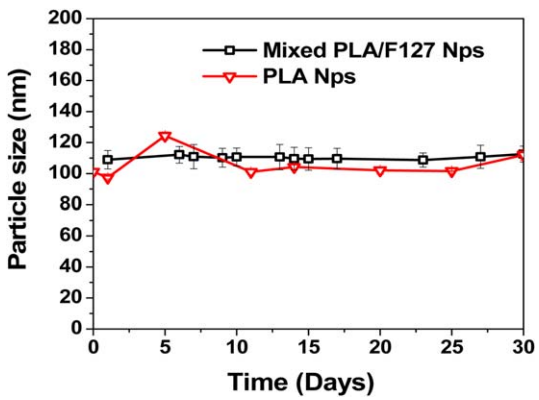

Figure 5. Storage stability of PLA and mixed PLA/F127 Nps in water. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

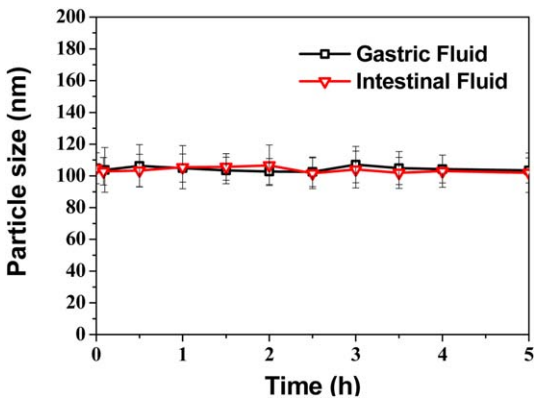

Figure 6. Colloidal stability of mixed PLA/F127 Nps in simulated gastric and intestinal fluids (USP XXIII) (mean ± SD, $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table V. ζ Potential of Mixed PLA/F127 Nps in Simulated Gastric and Intestinal Fluids for Fresh Nanoparticles (Day 1) and After Incubation (Day 30)

	ζ potential	
Time (days)	Gastric	Intestinal
1	−2.66 ± 0.37	−14.45 ± 1.20
30	−2.70 ± 0.30	−11.60 ± 0.14

the compatibility between PLA chains and the hydrophobic PPO chains of Pluronic® can be observed for the mixed PLA/F127 system, which showed less cold crystallization temperature (T_{cc}) value.

For polymer blends where one of the components is a surfactant, it has been reported that the differences in thermal behavior are related with HLB value of the surfactant. A previous study points out that the HLB of Pluronic® seems to be related to its effect on the nucleation and growth of spherulitic structures by changing the interfacial free energy between the crystalline and amorphous phase in the PLA.[35] Nevertheless, from DSC analysis is possible to infer that the HLB value have not an influence in the thermal properties, which can confirm that Pluronic® chains are localized mainly on the PLA surface and its effect on the PLA crystallinity is low.

Stability of the PLA and Mixed PLA/F127 Nanoparticles. The major challenge in designing particles for oral delivery is maintaining their chemical and colloidal stability during storage and in the gastrointestinal fluid after oral administration,[36] where the homogeneity of the administration depends on the homogeneity of the initial product. If irreversible flocculation and posterior sedimentation or creaming occurs during storage, the quantity of drug delivered for each administration is unknown.[37]

To evaluate the storage stability in water of mixed PLA/F127 and PLA Nps, all systems were kept at 4 °C during a month and the particle size was measured at predetermined times. As shown in Figure 5, the particles size of PLA and mixed PLA/F127 Nps remained constant under these storage conditions. This show the stability of the particles against, aggregation among particles.[38]

For PLA Nps, the stabilization is through an electrostatic repulsion of negative charges of deprotonated carboxylic acid groups.[15,39] On the other hand, in mixed PLA/F127 Nps, the stabilization is given by PEO chains on the particles surface, through steric repulsion as indicated by the low ζ potential values.

These coating are essential for designing Nps capable of penetrating the mucus barrier, since it possessing surfaces without mucoadhesive hydrophobic areas, surfaces with net-neutral charge and a highly hydrophilic surface.[1,2,40] Also, coating the Nps surface with PEO chains improves stability through mucus and, can decrease the interaction between the Nps and the enzymes of digestive fluids.[4,5,7,41]

Colloidal and chemical stability in the gastrointestinal medium were determined by incubating the Nps in simulated gastric and intestinal fluids. The PLA Nps precipitated immediately in gastric fluid (pH = 1.2), due to the low pH, which induces the protonation of the COO^- groups, removing the electrostatic stabilization favoring aggregation. In intestinal fluids (pH = 7.5) the PLA Nps were stable (data do not show).

The mixed PLA/F127 Nps (Figure 6) remained stable in both fluids due to the steric effect of the Pluronic®, as consequence of the extensive coverage of the surface with PEO chains. Have been reported that the presence of free PEO chains on Nps surface have an antifouling effect and prevent their interaction with pepsin and pancreatin and aggregation process observed in other systems.[6,7]

This colloidal stability can be confirmed by ζ potential measures. The Table V shows the comparison of the ζ potential values of mixed PLA/F127 Nps in simulated gastric and intestinal fluids for fresh Nps (day 1) and after incubation at 4 °C (day 30). These results confirm the colloidal stability, without changes of ζ potential values during the incubation time.[15] The chemical stability of these particles was corroborating by FTIR and GPC analysis.

The possible degradation of PLA during incubation in gastric and intestinal fluids was followed by FTIR spectroscopy. Figure

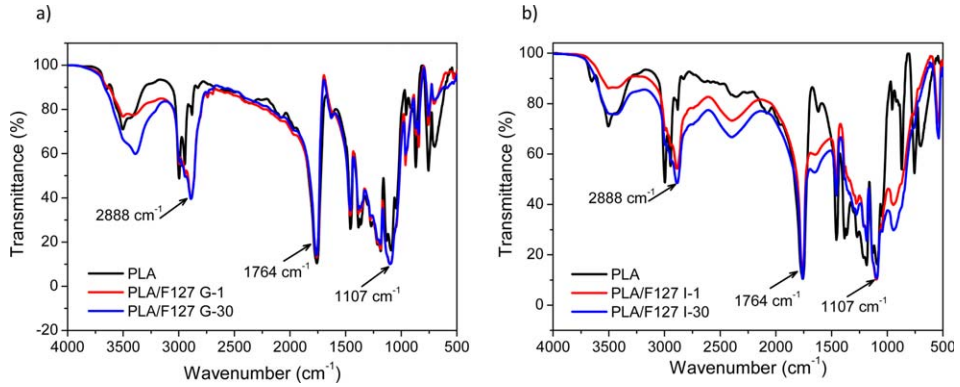

Figure 7. FTIR of PLA and mixed PLA/F127 Nps in (a) gastric (G) and (b) intestinal (I) fluids for fresh (1), and incubated (30) Nps. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

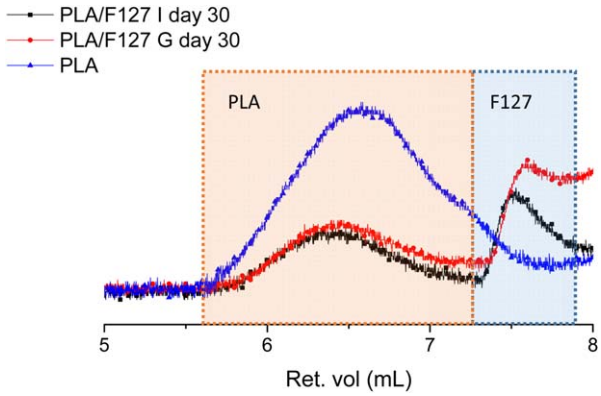

Figure 8. Molecular weight distribution of PLA polymer and mixed PLA/F127 Nps after incubation (day 30) in gastric (G) and intestinal (I) fluids. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

7 presents the infrared spectra for the PLA polymer (to compare the characteristic signals) and for mixed PLA/F127 Nps fresh (day 1) and after the incubation (day 30) in simulated gastrointestinal fluids. In all spectra are possible to observe the C—H stretching vibrations of PEO segments in F127 and PLA around 2888 cm^{-1}. The change in intensity for the peak at 1107 cm^{-1} for mixed Nps respect PLA polymer, is attributed to the characteristic C—O—C stretching vibration in F127.[42] For PLA, a strong band at 1764 cm^{-1} is associated at C═O asymmetric stretching.[43] The presence of these signals—and absence of new ones—after incubation indicate that there are not apparent degradation of PLA. However, the possible chemical degradation that can occur during the incubation time, was not significant for causing differences at a molecular level[44] and be evidenced by this technique.

In addition, GPC analysis was done for studying if any changes in the molecular weight of PLA occurred by degradation process in the gastric and intestinal fluids of mixed Nps. The molecular weight distributions are showed in the Figure 8 and it is observed that all distributions are overlapped. For the mixed system there are two peaks, one at around 117 kDa associated at PLA chains, and the second at 12 kDa that correspond at the molecular weight of F127. The differences in the molecular weight distribution between the PLA and mixed systems can be associated with the conformation of PLA chains in presence of the surfactant. Therefore, no reduction in molecular weight of the mixed system after incubation allow conclude that there is no degradation of PLA chains during evaluated time.

These results suggest that the mixed Nps PLA/Pluronic® are promising therapeutic delivery vehicle for oral administration due to their high chemical and colloidal stability in GI fluids.[4,5,7,41] These systems with excellent properties for oral administration were obtained under an easy and fast experimental process, and using commercially available polymers.

Nile Red Encapsulation

Nile Red (NR) is a hydrophobic molecule with fluorescence property which is strongly influenced by the media conditions. This property of NR, allows to use it as a molecular probe to determine the polarity of organic solvents and to test the environment of zeolites, synthetic polymers, and liquid crystals. Also, NR has been used as a probe in fluorescence microscopy and flow cytometry.[45] For those reasons, NR was chose as a model hydrophobic drug to study its encapsulation in mixed PLA/Pluronic® Nps.

Table VI summarizes the particle size, loading efficiency (%) and loading capacity of mixed PLA/F127 Nps, loaded with different NR amounts. The size of the particles was not affected with the amount of NR added ($P < 0.05$) except to the sample PLA/F127/$NR_{0.5}$. However, the PDI values for all mixed PLA/F127/NR Nps indicate a monomodal and narrow size distribution.

The results in the Table VI showed that when the added amount of NR is increased to 0.1 mg, the loading efficiency decreases near to 46% ($P < 0.05$). However, when the amount of NR is increased to 0.5 mg, there is not a significance difference in the loading efficiency. This implies that this increase in amount of NR leads to a greater loss of this reagent during the preparation of different formulations. Nonetheless, the loading capacity increase with the amount of NR added.

Figure 9 displays a photograph of NR in acetone, in a mixture ACN:water (1:3) and in a dispersion in water of mixed PLA/F127/NR Nps. The differences in color between the NR in acetone [Figure 9(a)] and water [Figure 9(b)] show the changes in fluorescence for this molecule in hydrophobic and hydrophilic media, respectively. The color observed for the dispersion in water of mixed PLA/F127/NR Nps [Figure 9(c)] show that although the system is an aqueous media, the fluorescence

Table VI. Particle Size and Loading Efficiency with Different NR Amount

Name	Initial NR amount (mg)	Particle size (nm)	PDI	Loading efficiency (%)	Loading capacity (g/100g Nps)
PLA/F127	0.00	107.3 ± 3.7	0.10 ± 0.01	—	—
PLA/F127/$NR_{0.02}$	0.02	113.7 ± 5.3	0.16 ± 0.036	94.3 ± 8.6	0.18 ± 0.04
PLA/F127/$NR_{0.06}$	0.06	118.0 ± 1.3	0.18 ± 0.040	68.1 ± 7.7	0.41 ± 0.03
PLA/F127/$NR_{0.1}$	0.10	111.2 ± 6.5	0.40 ± 0.050	46.9 ± 9.9	0.44 ± 0.12
PLA/F127/$NR_{0.5}$	0.50	132.8 ± 1.5	0.27 ± 0.160	39.1 ± 4.7	1.57 ± 0.19

Mean ± S.D., $n = 3$.

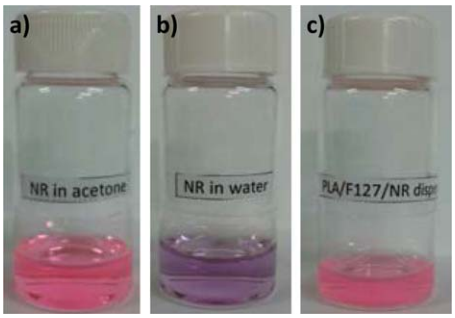

Figure 9. Photograph of (a) NR in acetone, (b) NR in a mixture ACN:water, and (c) dispersion in water of PLA/F127/$NR_{0.06}$ Nps. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

displays that the NR is in hydrophobic media, indicating that the molecules are inside the PLA Nps core.[46]

Nile Red Release. The release profiles of NR from the mixed PLA/F127 Nps are shown in Figure 10. It showed a biphasic release with a burst effect in the first 3 h of the experiment and a successive sustained release. The burst effect could be principally due to desorption of NR superficially adsorbed onto the Nps and/or the rapid diffusion of the encapsulated probe molecule near the surface of the Nps. After the burst effect, sustained release was obtained and later than 50 h the release of NR from mixed PLA/F127 Nps was nearly 80%.[20]

The release data were treated according to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell equation models in order to determine the release rates and mechanisms of NR release from the mixed PLA/F127 Nps. The model that best fitted the release data was evaluated by correlation coefficient (R^2). Table VII, shows the release parameters.

The correlation coefficient (R^2) values were used as a criteria for choose the best model to describe NR release from the mixed PLA/F127 Nps. The "best model" was the one with the highest coefficient correlation ($R^2 = 0.960$), obtained for fitting the NR release data to the Korsmeyer–Peppas equation. The n values obtained was 0.714, this value is characteristic of anomalous kinetics (non-Fickian), implying that a combination of polymer erosion, swelling and dissolution were all involved in the release process contributes to the control of NR release.[20,47,48] However, the polymer erosion is not important for these systems, as was showed by FTIR and GPC, the degradation of PLA chains does not occurs during the analysis.[49]

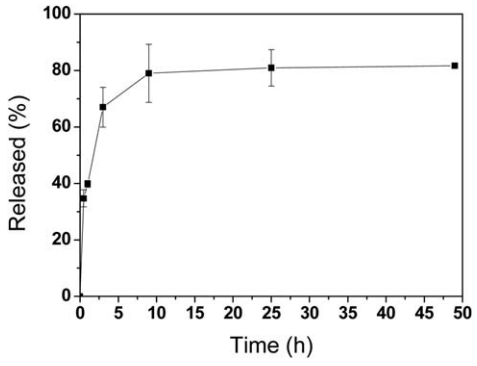

Figure 10. Nile Red release kinetics ($n = 3 \pm$ SD).

Table VII. Diffusion Exponent (n) of Korsmeyer–Peppas Model, Regression Coefficient (R^2), and Rate Constant (k) of NR Release Data from Mixed PLA/F12 Nps According to Different Kinetic Models

Mathematical models	k	R^2	n
Zero order kinetics	0.271	0.515	—
First order kinetics	0.441	0.813	—
Higuchi model	0.437	0.950	—
Korsmeyer–Peppas model	0.306	0.960	0.714
Hixson–Crowell model	0.123	0.725	—

CONCLUSIONS

Mixed Nps from PLA and nonionic surfactant Pluronic® were prepared by nanoprecipitation method and the experimental conditions were modulated to obtain the particle sizes close to 100 nm. Presence of hydrophilic moiety (PEO) on PLA surface, provides efficient colloidal and chemical stability under the storage conditions and also in GI fluids. Furthermore, the presence of surfactant on the Nps surface does not have an effect on the PLA crystallinity. The encapsulation of NR was confirmed by changes in the fluorescence of this molecule. An increase in amount of NR leads to a decrease in loading efficiency, but an increase in the loading capacity. The presence of NR encapsulated does not have an important effect on the particle size. Kinetic studies confirmed that the release profile showed two release stages and the NR release from these mixed PLA/F127 Nps might be controlled by an anomalous mechanism. The particle size around 100 nm, close-to-neutral ζ potential and high stability for the mixed PLA/Pluronic® Nps make these materials suitable candidates as delivery systems for oral administration.

ACKNOWLEDGMENTS

The given work was supported by Colciencias for the financial support to the project code 111556933575, corresponding to the convocation number 569 of 2012. Juliana Palacio and Natalia Agudelo thank Colciencias Colombia for the program "Francisco José de Caldas Convocatoria Nacional para Estudios de Doctorado en Colombia" N° 511, 2010 and N° 528, 2011, respectively.

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