Fabrication of core−shell PLGA/PLA−pNIPAM nanocomposites for improved entrapment and release kinetics of antihypertensive drugs

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A B S T R A C T

Polylactic acid (PLA) and poly(lactic-co-glycolic) acid (PLGA) are two commonly applied biodegradable polymers for the preparation of nanocomposites used in drug-delivery systems. However, these polymers lack desirable attributes such as resistance to aggregation during long-term storage due to lyophilisation. To improve their efficacy, in this work, PLA and PLGA were encapsulated within a shell of poly(N-isopropylacrylamide) (pNIPAM) using a single emulsion technique followed by an aqueous free radical precipitation polymerisation process, yielding core−shell PLA/PLGA−pNIPAM nanocomposites. The nanocomposites were characterised using zeta potential, dynamic light scattering, and transmission electron microscopy analyses and were further applied as a delivery system for ramipril, an antihypertensive drug. The drug-loaded PLGA−pNIPAM core−shell nanoparticles exhibited a higher drug content (91%) and entrapment efficiency (78%) than their PLA counterparts. An in vitro release study of the formulations at pH 7.3 in phosphate-buffered saline indicated that PLGA was more efficient than PLA with a sustained release of 86% of ramipril from the polymer matrix within 24 h. Furthermore, to determine the release kinetics, the data were fitted to Korsemeyer–Peppas and Higuchi models; the release of ramipril from the polymer matrix followed zero-order rate kinetics and an anomalous (non-Fickian) diffusion mechanism.

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Introduction

Biodegradable colloidal particles have received significant interest as a possible means of delivering drugs by several routes of administration (Lemoine et al., 1996). Polymeric nanoparticles (NPs) help to increase the stability of drugs/proteins and possess useful controlled release properties. Special interest has been focused on the use of particles prepared from polyesters such as poly(lactic-co-glycolic) acid (PLGA) and polyactic acid (PLA) because of their biocompatibility and biodegradability via natural pathways in the body (Basu, Pal, & Singh, 2016; Jain, 2000; Panyam, Zhou, Prabha, Sahoo, & Labhasetwar, 2002). PLGA copolymers degrade in the body via hydrolytic cleavage of ester linkage to lactic and glycolic acid. These two monomers are metabolised in the body through the Krebs cycle before finally being eliminated in the form of CO₂ and H₂O. Although PLGA and PLA NPs have been used commercially by many investigators because of their advantageous properties, certain issues remain that limit their use for drug delivery (Anderson & Shive, 1997; Hans & Lowman, 2002; Panyam & Labhasetwar, 2003; Wischke & Schwendeman, 2008). The long-term storage of aqueous suspensions of PLGA and PLA NP systems is difficult because of the hydrolytic degradation of the polymer and subsequent release of the encapsulated therapeutics. To overcome this issue, researchers have used lyophilisation to prepare therapeutics containing PLGA and PLA NPs for long-term storage. However, this simple solution introduces an additional difficulty, i.e., lyophilisation causes the NPs to aggregate into clumps that upon rehydration, readily fall out of the solution (Chacon, Molpeceres, Berges, Guzman, & Aburturas, 1999; Holzer et al., 2009).

In an attempt to overcome the limitations linked to PLGA and PLA NPs and to increase their functionality, in this work, these NPs were encapsulated within a shell of poly(N-isopropylacrylamide) (pNIPAM) because of its versatility and beneficial inherent properties. The pNIPAM shell can be synthesised as a homopolymer or as a copolymer with incorporation of various chemical moieties with defined concentrations that can be further modified with active targeting functional groups. In addition, it is one of the most stimuli-responsive polymers (Zhang & Zhuo, 2000). pNIPAM shows great potential for applications in biomedical fields (De Groot et al., 2001; Duracher, Elaissari, Mallet, & Pichot, 2000; Suzuki, Yumura, Tanaka, & Akashi, 2001) and for the immobilisa-
tion of enzymes (Zhang, Huang, Cheng, & Zhuo, 2004). Moreover, pNIPAM can tolerate lyophilisation (Cheng, Chu, Zhang, Wang, & Wei, 2008); therefore, it was hypothesised that a pNIPAM shell would protect the encapsulated PLGA and PLA NPs from aggregation during lyophilisation. The pNIPAM shell would enable the NPs to respond to environmental stimuli, such as temperature. This response could then further be used for loading and/or controlling the release of therapeutics from the pNIPAM layer (Afrassiabi, Hoffman, & Cadwell, 1987). The PLGA core would then act as a drug reservoir, and its release would depend on the thickness of the pNIPAM shell (Zeng, An, & Wu, 2011).

When administered orally, antihypertensive drugs exhibit low bioavailability and instability. One example is ramipril, a potent, long-acting angiotensin converting enzyme (ACE-II) inhibitor that reduces the level of aldosterone secretion, resulting in blood-pressure reduction (Atlas, 2007). Ramipril is used to treat high blood pressure (hypertension), congestive heart failure, and chronic renal failure in hypertensive patients (Ekambaram & Sathali, 2011; Ramu & Raghubabu, 2011). Peak plasma concentrations of the active metabolite of ramipril (ramiprilat) are reached within 2–4 h, and the absolute bioavailability of ramipril and ramiprilat are 28% and 44%, respectively. In addition, ramipril exhibits poor solubility in aqueous medium.

The performance of these poorly water-soluble antihypertensive drugs improves when administered in polymeric vehicles such as PLGA or PLA rather than conventionally, as these polymeric carriers prevent their degradation in the gastrointestinal tract (GIT) and boost their transmucosal transport in the body (Getin, Aktas, Vural, & Ozturk, 2012). In addition, the enhanced transport and prolonged release of the drug may improve its plasma half-life (Hsu, Cui, Mumper, & Jay, 2003). Hsu et al. (2003) reported the effect of particle size on the efficiency and absorption of drugs in the mucosal membrane. Particles with sizes of less than 500 nm can easily pass into M cells and the GIT, thereby transporting the drug to the systemic circulation system.

The aim of the current study was to assess the release of the antihypertensive drug ramipril from the biodegradable polymers PLA and PLGA modified with pNIPAM and to determine the release kinetics of ramipril. Enhanced efficiency and release of ramipril were observed upon modification of the surface of PLA and PLGA with pNIPAM. The shell of pNIPAM prevented the PLA and PLGA core from clustering and resulted in enhanced release efficiency compared with their bare counterparts. Nanoprecipitation resulted in physical adsorption of ramipril on the surface of the core–shell matrix, which was mainly due to hydrophobic interaction of the drug with the polymer.

Materials and methods

Materials

Poly(β, ε-lactide-co-glycolide) (50:50) (PLGA), poly lactic acid (PLA), Kolliphor P-188 (KP-188), poly(N-isopropylacrylamide) (pNIPAM), and sodium dodecyl sulphate (SDS) were purchased from Sigma–Aldrich. N,N′-methylenebisacrylamide (N,N′-MBA) and ammonium persulfate (APS) were purchased from Spectrochem India. A sample of ramipril was received from Mefro Pharmaceuticals (Mohali, Punjab, India). All the other reagents were obtained from Loba Chemie, India and used as received without further purification. Deionised water was obtained using an ultra filtration system (Milli-Q, Millipore).

Encapsulation of PLA/PLGA cores with pNIPAM shells

Synthesis of the core–shell structures was performed in the two steps illustrated in Scheme 1.

Preparation of PLA/PLGA core

The polymer core was prepared using a previously reported single emulsion technique (Xu et al., 2009). First, 0.25 g of PLA/PLGA was dissolved in 10 mL of dichloromethane (DCM) followed by the addition of 20 mL of 1% KP-188 and homogenized with a sonicator for 2 min to obtain an emulsion. The emulsion was then added to a continuously stirred aqueous phase (50 mL) and left overnight for complete evaporation of the DCM. The resulting core particles were

Scheme 1. Schematic representation of (a) synthesis of PLGA and PLA NP cores and (b) their encapsulation with pNIPAM shells to form core–shell NPs.
Fig. 1. UV–vis absorption spectra of (a) PLA/PLGA cores and pNIPAM shell, and (b) bare ramipril and ramipril loaded in core–shell NPs.

then washed with distilled water four times using centrifugation for 10 min at 10,000 rpm.

Encapsulation of PLA/PLGA cores with pNIPAM shell

The encapsulation was performed using an aqueous free radical precipitation polymerisation technique under nitrogen atmosphere (Berndt & Richtering, 2003; Jones & Lyon, 2000, 2003). pNIPAM (0.225 g), N,N′-MBA (0.017 g), SDS (0.01 g), and APS (0.012 g) were dissolved in distilled water, and the contents were purged with nitrogen. Then, 20 mL of the core suspension was added to a three-necked round-bottom flask and equilibrated for 20 min with continuous stirring at 70 °C under a nitrogen atmosphere. Next, 10 mL of the shell solution was added to the core and allowed to polymerise. An additional 5 mL of the shell solution was added after 30, 50, 70, and 90 min. After the final addition of the shell solution, polymerisation was allowed to continue for another 6 h. Further purification was performed by centrifugation.

Synthesis of ramipril-loaded NPs

The ramipril-loaded NPs were synthesised using the nanoprecipitation method shown in scheme S1 in the Supporting information (Zili, Sfar, & Fessi, 2005). The core–shell NPs were added to 25 mL of acetone, and ramipril (5 mg) was added and solubilised in this solution. The contents were added dropwise to 50 mL of distilled water containing (200 mg) KP-188. The suspension was stirred for 6 h. Acetone was evaporated under reduced pressure, and the volume of the suspension was adjusted to 10 mL. The nanosuspension was centrifuged, and the drug-loaded polymeric aggregates were collected.

Characterisation

The optical properties of the ramipril-loaded NPs were analysed using ultraviolet–visible (UV–vis) spectrophotometry (Specord 205, Analytikjena, Germany). Fourier-transform infrared (FT-IR) spectroscopy was performed using an Agilent Technologies Cary 600 (Agilent, USA) following the KBr pellet method. Dynamic light scattering (DLS) and zeta potential analyses were performed using a 90 Plus particle size analyser (Brookhaven, USA). The size and shape of the prepared NPs were determined using transmission electron microscopy (TEM; Hitachi 7500, Japan) with a resolution of 2 Å operating at a voltage of 120 kV. The in vitro release was determined by HPLC (Thermo Fisher, USA).

Drug content and entrapment efficiency of ramipril

The drug content was determined using the centrifugation method. The nanosuspension was centrifuged at 10,000 rpm for 15 min. The amount of free drug and the total drug content (TDC) were measured spectrophotometrically. The TDC was calculated (Jagdale, Dangat, & Kuchekar, 2012) using the formula:

\[
TDC(\%) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100.
\]

The entrapment efficiency (EE) was determined using the following equation:

\[
EE(\%) = \left(\frac{TDC - Df}{TDC}\right) \times 100,
\]

where \(Df\) was the amount of drug in the clear supernatant solution.

In vitro drug release of ramipril

Pre-weighed nanospheres (0.1 g) were suspended in 50 mL of phosphate-buffered saline (PBS, pH 7.3). At pre-determined intervals, 5 mL samples were withdrawn and replaced with fresh PBS. The withdrawn sample was centrifuged at 10,000 rpm for 15 min, and the amount of drug released was determined using high-performance liquid chromatography (HPLC), C18, 5 μm, 4.6 × 250 mm, reversed phase column with PBS:acetonitrile (ACN) (30:70 v/v) as the mobile phase. 20 μL of sample was injected with a flow rate of 2.0 mL/min at 210 nm wavelength.

In vitro release kinetics study

The data obtained from the in vitro release study were fitted to various drug-release kinetic models, and the resulting regression coefficient values were calculated. The data were also fitted to Korsmeyer–Peppas and Higuchi models to determine the release exponent ‘n’ value to describe the drug-release mechanism.

Results and discussion

Structural and morphological characterisation

The UV–vis absorption of PLA/PLGA revealed absorption bands at 202/240 nm, respectively; however, the pNIPAM shell was characterised by two absorption bands at 229 and 296 nm, as shown in Fig. 1(a). The absence of bands of PLA and PLGA in the spectrum of pNIPAM shell in Fig. 1(a) indicate encapsulation of the pNIPAM shell over the polymer core. In Figs. 1(b) and S1 (see the Supporting information), absorption bands for pure ramipril are observed at 237 and 257 nm; however, after entrapment of the drug in the core–shell and bare polymer matrix, the absorption bands appeared at 249/220 nm for PLGA/PLA, respectively.
The FT-IR spectra of ramipril, PLGA, PLA, and the formulations are presented in Fig. 2. The spectrum of ramipril contained an absorption band at 3277 cm\(^{-1}\) due to the \(-\text{NH}\) and \(-\text{OH}\) stretching of the acid group and bands at 2930 and 2860 cm\(^{-1}\) due to \(\text{C} - \text{H}\) aromatic and aliphatic stretching, respectively. The bands at 1748 and 1644 cm\(^{-1}\) are attributed to \(\text{C} = \text{O}\) of the acid and ester groups, respectively. \(\text{C} - \text{H}\) aliphatic vibrations are observed in the fingerprint region (1500 cm\(^{-1}\) to 500 cm\(^{-1}\)). The spectrum of PLGA showed signatures of \(\text{C} = \text{O}\) absorption at 1748 cm\(^{-1}\) and \(\text{C} - \text{H}\) deformation of the \(\text{O} - \text{CH}_2\) group at 1414 cm\(^{-1}\), whereas that of PLA contained bands at 1748 cm\(^{-1}\) due to stretching of the \(\text{C} = \text{O}\) group and at 1445 cm\(^{-1}\) due to asymmetric and symmetric \(\text{C} - \text{C} - (\text{O}) - \text{O}\) vibrations. The pNIPAM spectrum contained absorption bands at 3277 cm\(^{-1}\) due to \(\text{N} - \text{H}\) stretching, 2960 cm\(^{-1}\) due to asymmetric stretching vibrations of the \(\text{CH}_2\) group, and 1542 cm\(^{-1}\) due to amide linkages. In the formulations, the absorption bands at 1748 and 1644 cm\(^{-1}\) are due to carbonyl and ester groups of acid. Moreover, the band at 2860 cm\(^{-1}\) appeared due to \(\text{C} - \text{H}\) aliphatic stretching. An intense absorption band at 1088 cm\(^{-1}\) was observed in the formulation possibly as PLGA is characterised by a band at 1081 cm\(^{-1}\). The presence of bands at 2860, 1748, and 1088 cm\(^{-1}\) indicated that the drug was encapsulated within the polymer matrix and that any type of interaction that could change the properties of ramipril did not occur in the physical mixture.

DLS analyses revealed an increase in the size of the nanocomposite, as shown in Fig. S2 (see the Supporting information). It was observed from Fig. 3 that the particle size increased from 70 to 170 nm for PLA, whereas for PLGA, it increased from 68 to 155 nm after the successive addition of pNIPAM shell and drug loading. The low polydispersity index i.e. measure of the distribution of molecular mass in a given sample (0.141–0.389) indicated that the particles were nearly uniform in size. The zeta potential of the prepared formulations ranged from −28.4 to −32.7 mV (Table 1), indicating reasonable stability of the nanosuspensions. The shell thicknesses for PLGA/PLA were calculated to be 18.5/43.5 nm, respectively.

Lyophilisation is useful for the long-term storage of therapeutics loaded onto PLGA and PLA NPs but can cause aggregation of the NPs, preventing their resolubilization. The TEM images in Fig. 4 clearly reveal the aggregation of the core NPs after lyophilisation. However, encapsulation of the core NPs within the pNIPAM shells prevented this aggregation, confirming that the pNIPAM shell fully encapsulated the core NPs. In addition, lyophilisation of the core-shell NP system did not affect the size or zeta potential of the NPs (Table 1).

### Drug content and entrapment efficiency of ramipril

The TDC was measured using UV–Vis spectroscopy. The nanosuspension was centrifuged, and the free drug content was determined in clear supernatant (Fig. 5 & Table 2). Very low TDC and entrapment efficiency were observed for the drug loaded in PLGA/PLA only, which may be attributed to the initial burst of the polymer after resolubilization due to lyophilisation. However, the TDC increased to 73–91% for the ramipril-loaded PLGA/PLGA core–shell systems, as shown in Fig. 6. In addition, the entrapment efficiency increased for the ramipril-loaded core–shell NP systems. Higher drug content and entrapment efficiency (78%)

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polydispersity index (PDI)</th>
<th>Zeta potential (mV)</th>
<th>Shell thickness (nm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-lyophilisation</td>
<td>Post-lyophilisation</td>
<td></td>
</tr>
<tr>
<td>PLGA core</td>
<td>0.389</td>
<td>−28.4 ± 0.5</td>
<td>−28.4 ± 0.8</td>
</tr>
<tr>
<td>PLGA core–pNIPAM shell</td>
<td>0.272</td>
<td>−32.7 ± 0.7</td>
<td>−32.7 ± 0.6</td>
</tr>
<tr>
<td>PLA core</td>
<td>0.141</td>
<td>−22.7 ± 0.9</td>
<td>−22.7 ± 1.0</td>
</tr>
<tr>
<td>PLA core–pNIPAM shell</td>
<td>0.220</td>
<td>−30.3 ± 1.2</td>
<td>−30.3 ± 1.3</td>
</tr>
</tbody>
</table>

* Shell thickness was determined by subtracting corresponding core from core * shell sample and dividing by 2.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Free dissolved drug (mg)</th>
<th>Drug content (mg)</th>
<th>Total drug content (%)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA core</td>
<td>0.48</td>
<td>1.01</td>
<td>40</td>
<td>52.4</td>
</tr>
<tr>
<td>PLGA core–pNIPAM shell</td>
<td>0.35</td>
<td>2.3</td>
<td>91</td>
<td>78</td>
</tr>
<tr>
<td>PLA core</td>
<td>0.51</td>
<td>0.97</td>
<td>38.49</td>
<td>47.42</td>
</tr>
<tr>
<td>PLA core–pNIPAM shell</td>
<td>0.72</td>
<td>1.84</td>
<td>73</td>
<td>60</td>
</tr>
</tbody>
</table>
Fig. 4. TEM images of PLGA core, pNIPAM shell, and PLGA + pNIPAM before and after lyophilisation.

Fig. 5. Drug content in the ramipril-loaded PLGA/PLA cores and PLGA/PLA cores + pNIPAM shells.

Fig. 6. Percentage TDC, entrapment efficiency, and ramipril release in the ramipril-loaded PLGA/PLA cores and PLGA/PLA cores + pNIPAM shells.
were achieved for the drug loaded in PLGA coated with pNIPAM than for its PLA counterpart.

**In vitro release of ramipril**

The release profile of ramipril was determined using HPLC. First, appropriate aliquots of ramipril stock solution were placed in 10-mL volumetric flasks and diluted with the mobile phase to obtain the final concentration. A calibration curve for a standard solution of the drug was obtained, as shown in Fig. S3 (see the Supporting information). The release of ramipril from the PLGA/PLA matrix in PBS of pH 5.3 (Fig. 7 and the corresponding chromatographs in Fig. S4) (see the Supporting information) show the initial burst release followed by sustained release. After 24 h of incubation in PBS, the percentage release of drug was 67% and 45% for PLGA and PLA, respectively. The initial burst release was due to the presence of the free dissolved drug. However, an increase in the percentage release of drug was observed for the core–shell system. The highest drug release (96%) was observed for PLGA + pNIPAM, and the drug release for PLA + pNIPAM was 70%.

When placed in buffer solution of appropriate pH environment, the ramipril-loaded encapsulated NP initially allowed the penetration of water from the surface to the inside of the core–shell NP, which in turn activated hydrolytic cleavage within the polymer and diffusion of the encapsulated ramipril starts from the NP. As the degradation of the core–shell NP continues, the rate of ramipril diffusing from the matrix increases. A schematic representation of the release mechanism is shown in Fig. 8. This controlled release of ramipril from the core–shell matrix tends to increase the bioavailability of ramipril. Therefore, because greater effects can be achieved from a single dose, the dosage of the drug can be decreased.

**In vitro release kinetics study**

Fig. 9 shows the release exponent (n) values for PLGA and PLA obtained by fitting the drug-release profiles to Korsmeyer–Peppas and Higuchi models of drug release. The n values of 0.882 and 0.866 for PLGA and PLA, respectively, indicate that the drug release follows a zero-order rate (Fig. 9(c)–(f)) and an overall anomalous (non-Fickian) diffusion mechanism. Thus, the release of ramipril from the matrix of PLGA and PLA is diffusion controlled. The corresponding correlation coefficients of these kinetic plots are listed in Table 3.

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**Table 3**

Correlation coefficients of kinetic plots of various formulations.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Korsmeyer–Peppas equation</th>
<th>Higuchi equation</th>
<th>Zero order rate equation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>R²</td>
<td>n</td>
</tr>
<tr>
<td>Release from PLGA</td>
<td>0.882</td>
<td>0.708</td>
<td>1.958</td>
</tr>
<tr>
<td>Release from PLA</td>
<td>0.866</td>
<td>0.775</td>
<td>3.802</td>
</tr>
</tbody>
</table>

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**Fig. 7.** In vitro release profile of ramipril in PBS from PLGA/PLA cores and PLGA/PLA cores + pNIPAM shells.

**Fig. 8.** Schematic representation of release mechanism of ramipril from PLGA–pNIPAM core–shell NPs.
Conclusions

In summary, to improve the functionality of PLGA and PLA as therapeutic agents, these polymers were encapsulated within pNIPAM shells. pNIPAM was selected because of its ability to protect the core NPs from aggregation and external stimuli. In addition, these PLA/PLGA–pNIPAM core–shell NPs are non-toxic, suggesting their biocompatibility. The core–shell PLA/PLGA–pNIPAM NPs were used to study the release profile of ramipril, an antihypertensive drug. The size of the synthesised nanocomposites ranged from 68 to 170 nm. Higher percentage entrapment efficiency (78%) was achieved for the ramipril loaded in PLGA–pNIPAM NPs than in their PLA counterparts. In addition, a sustained release of up to 96% over 24 h in PBS medium was achieved for the core–shell PLGA–pNIPAM NPs as a matrix. This release pattern was attributed to the non-Fickian nature of the system, which suggested that the ramipril release was diffusion controlled. Overall, PLGA exhibited better characteristics than PLA as a drug carrier for ramipril adsorption over its matrix. Future in vivo studies will focus on probing the capability of these core–shell NPs to deliver the therapeutics.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.partic.2017.10.002.
References


