Formation of Block Copolymer-Protected Nanoparticles via Reactive Impingement Mixing

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Reactive impingement mixing was employed to produce polymer-protected nanoparticles. Amphiphilic block copolymer was formed in situ by reactive coupling of hydrophobic and hydrophilic blocks. Simultaneously, a hydrophobic compound and the copolymer coprecipitated to form nanoparticles in the range of 100 nm. Specifically, β-carotene was stabilized by the amphiphilic diblock copolymer, formed from the reaction of an amino-terminated hydrophobic block, poly(ethylene glycol) (PEG-NH2), with an acid chloride-terminated hydrophobic block, either poly(ε-caprolactone) (PCL-COCl) or polystyrene (PS-COCl). Spherical particles were observed by scanning and cryogenic transmission electron microscopy. Process conditions, including feed concentration of β-carotene and feed concentrations of polymeric stabilizers, had little or no effect on average particle sizes over the range studied. Further, for Reynolds numbers greater than 500 the feed flow rates also had no effect. The effect of glass transition temperature (Tg) of the hydrophobic polymer on morphology and particle formation mechanism is discussed.

1. Introduction

Solubility and targeting are two of the most significant challenges to drug delivery systems.1–4 Lipinski at Pfizer estimated that 30–40% of new drug candidates have poor aqueous solubility,5 which leads to a low effective concentration in biofluids and therefore poor bioavailability.4–7 Increasing solubility of the drug by covalent modification requires new drug certification,8 whereas nanoscale carriers, such as dendrimers, micelles,9 liposomes,10 nanoemulsions,11 and nanoparticles12–19 do not alter the drug molecule. The large surface area of these tiny nanocarriers can greatly increase dissolution rate of hydrophobic drugs. Moreover, nanoscale carriers can inhibit uptake of drug particles by the reticuloendothelial system during circulation,20,21 permitting enough time for the particles to localize in the leaky vasculature of tumors (known as the enhanced permeability and retention or EPR effect).20–25 Among various potential carriers, nanoparticles show advantages in scale-up and drug loading.15,26

Nanoparticles are typically stabilized with amphiphilic copolymers. These have higher molecular weights and stronger cooperative adsorption than low-molecular weight surfactants, and thus impart greater stability to the nanoparticles. Moreover, in polymer–drug composite particles, polymers can act as antiplasticisers elevating the typically low glass transition temperature (Tg) of pure drugs.27–31 Two examples used in the pharmaceutical industry are water soluble polyvinylpyrrolidone

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media, long processing times, high pressures, particle agglomeration, and broad particle size distributions. Solution-based processes such as cryogenic spray and antisolvent precipitation in a stirred tank may overcome these problems, but these methods may not provide intense enough mixing and have had difficulty generating nanoparticles below 200 nm.

Higher-energy density mixing using confined impinging jets can minimize mass transfer limitations, enabling the preparation of even smaller particles via antisolvent precipitation. Horn et al. at BASF34,35 and Midler et al. at Merck36 tested the suitability of impingement mixers for rapid precipitation of pharmaceutical compounds. In their processes, a hydrophobic compound was dissolved in an organic solvent, which is miscible with an aqueous antisolvent. Then, a stream of the solution and one of water were forced toward each other at a high velocity to rapidly generate a high and relatively homogeneous supersaturation of the hydrophobic compound and consequently produce fine particles. Kirwan et al. quantified this rapid precipitation process.37–39 Johnson and Prud’homme improved it by using block copolymers to stabilize the nanoparticles formed during impingement.40–44 The advantage of BCP stabilizers is that they cannot bridge nanoparticles as can occur for random copolymers. Whereas Horn used stabilizing copolymers that were introduced in the aqueous stream, strongly anchoring BCPs must be introduced in the organic stream. With premade BCPs, concentration and molecular weight are limited by phase separation (i.e., the critical micelle concentration) when the BCP is dissolved in the organic phase. Also with premade BCPs during the rapid precipitation, the hydrophilic block, typically poly(ethylene glycol) (PEG), can be kinetically trapped inside the nanoparticle core rather than reach its desired location in the corona. The entrapped PEG with $T_g$ of $-60 \, ^\circ \text{C}$ could decrease the $T_g$ of the particle core, decreasing particle stability and facilitating crystallization in the hydrophobic core.

In order to overcome these disadvantages with preformed BCPs, in this paper we employ in situ coupling to create the amphiphilic block copolymer (BCP) during particle formation. A schematic is shown in Figure 1. In our process the PEG corona of the particles is anchored by fast reaction between amino-terminated poly(ethylene glycol) (PEG-NH$_2$) and acid chloride-terminated polystyrene (PS-COCl) or polycaprolactone (PCL-COCl). Highly hydrophobic $\beta$-carotene is used as a model for a drug. The effect of process conditions, including feed concentration of $\beta$-carotene, feed concentrations of polymers, and feed rate on particle size is discussed.

2. Experimental Section

2.1. Materials. $\varepsilon$-Caprolactone ($\varepsilon$-CL, $\geq$99%), octanoic acid ($n$-C$_8$H$_{17}$COOH, $\geq$99%), camphor-10-sulfonic acid (CSA, C$_{10}$H$_{16}$O$_4$S, $\geq$98.0%), oxalyl chloride ((COCl)$_2$, 98%), triethylamine (TEA, (PVP) and hydroxypropyl methylcellulose (HPMC), which have a $T_g$ of 168 °C (PVP-30) and 146 °C (HPMC-E5), respectively.32

Commercial mechanical processes to manufacture drug nanoparticles, such as media milling and high-pressure homogenization, have limitations including contamination with grinding

![Figure 1. Schematic of reactive impingement mixing to form block copolymer-protected nanoparticles. A stream of solvent containing a hydrophobic compound and a polymer with functional group Y are impinged against a stream of antisolvent, typically water, containing a hydrophilic polymer with functional group X. High-velocity impingement causes rapid mixing in the cylindrical chamber. The compound becomes supersaturated and precipitates into nanoparticles. The functional groups combine to form a diblock copolymer which precipitates with the compound, stopping particle growth and preventing aggregation.](image)

![Figure 2. GPC measurement of conversion. (a) The precursor PS-COCl and the PS-$b$-PEG block copolymer formed after impingement mixing PS-COCl and PEG-NH$_2$ (run 1 in Table 1). A UV-vis detector was used making the PEG-NH$_2$ invisible. (b) Reactive blocks PCL-COCl and PEG-NH$_2$ and the resulting PCL-$b$-PEG (run 10 in Table 1). An RI detector was used. The two dotted curves are the result of deconvolution. Their sum is the dashed curve in each figure.](image)

![Scheme 1. Synthesis of PCL-COCl](image)


Table 1. Effect of Feed Concentrations, Polymer Type, and Feed Rate on Particle Size

<table>
<thead>
<tr>
<th>run</th>
<th>[polymer-COCI] (mmolL⁻¹) in mixer chamber before coupling</th>
<th>[PEG-NH₂] (mmolL⁻¹) in mixer chamber before coupling</th>
<th>polymers wt % after dilution</th>
<th>β-carotene wt % after dilution</th>
<th>Re</th>
<th>(d_i) (nm)</th>
<th>(d_w) (nm)</th>
<th>mass average size distribution (d_10/d_50/d_90) (nm)</th>
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<tr>
<td>1</td>
<td>PS-COCI (2K)</td>
<td>PEG-NH₂ (5K)</td>
<td>0.42</td>
<td>0.13</td>
<td>1770</td>
<td>122</td>
<td>91</td>
<td>48/74/126</td>
<td>1.06</td>
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<td>2</td>
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<td>3.0</td>
<td>0.42</td>
<td>0.13</td>
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<td>355</td>
<td>128</td>
<td>58/79/272</td>
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<td>0.42</td>
<td>0.13</td>
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<td>143</td>
<td>124</td>
<td>76/105/156</td>
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<td>0.26</td>
<td>1770</td>
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<td>1770</td>
<td>130</td>
<td>108</td>
<td>64/90/139</td>
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</tbody>
</table>

* In runs 2, 10, and 11, not filtered before particle size measurement.

Scheme 2. Coupling Reaction of Amphiphilic BCP

2.2. Syntheses of Poly(ε-caprolactone) and Polystyrene with Acid Chloride End Functionality (PCL-COCI and PS-COCI).

PCL-COOH was synthesized by ring-opening polymerization of ε-CL with initiation by octanoic acid, \(n\)-CH₃COOH. The reaction was conducted at 230 °C with a strong acid, CSA, as a catalyst.⁴⁵ Then, crude PCL-COOH was fractionated by adding CH₃OH and precipitating from a THF solution. The molecular weight and molecular weight distribution of fractionated PCL-COOH were measured by GPC. Each sample was loaded in a Waters 717 Plus autosampler, connected to a Waters 590 programmable HPLC pump running at a flow rate of 1 mL/min at room temperature with THF as the mobile phase. Both ultraviolet--visible (UV-vis) and refractive index (RI) detectors were used.

Fractionated PCL-COOH \((M_s = 3.6 \times 10^3, M_d/M_s = 1.21)\) was converted into PCL-COCI by (COCl)₂ in CH₂Cl₂ with a catalytic amount of DMF (see Scheme 1).³³ PS-COCl was converted into PS-COCI using the same method. The PS-COCI had the same \(M_s\) and narrow distribution as the starting PS-COOH so fractionation was not needed. The functionality of PCL-COCI and PS-COCI were ≥90% by GPC measurement after coupling with a slight excess of PEG-NH₂ in the presence of TEA.

2.3. Preparation of Polymeric β-carotene Nanoparticles.

In previous work,³¹-³³ an impingement mixer was employed to prepare β-carotene-loaded nanoparticles with premade BCP PS-b-PEG. In this work, the same mixer³⁴-³⁶ (type 500A-Y2X with dimensions described in Figure 4 and Table 1 in ref 40) was used to perform reactive mixing. Each mixer inlet was connected to a 100-ml gastight glass syringe (SGE Inc.). Both syringes were loaded on an infusion syringe pump (Harvard Apparatus model 975). For typical mixing, β-carotene and PCL-COCI (or PS-COCI) were dissolved in 5 mL of THF. PEG-NH₂ and a 2-fold excess of TEA were dissolved in 5 mL of H₂O. The reactant solutions were loaded in separate syringes and were impinged at a high velocity inside the mixer chamber. In most cases the flow rate was 72 mL/min which produces a velocity of 6.1 m/s through the 0.500-mm diameter nozzle. With this velocity and nozzle diameter, a 50/50 THF/water mixture (density of 960 kg/m³ and viscosity of 1.66 mPa·s)³⁴ gives a Reynolds number \(Re\) of 1770. For all the runs in Table 1 the nozzle diameter and the fluid properties are the same. Thus \(Re\) changes in runs 4–6 are only due to a change in flow rate. The outlet of the mixer was connected via 12.7-cm of 1.59-mm diameter Teflon tubing to a beaker containing 40 mL of H₂O to further dilute the nanoparticles. The dilution diminishes Ostwald ripening and recrystallization.⁴⁶-⁴⁸ The residence time is about 110 ms from entering the chamber to falling into the beaker. Total injection time is about 5 s.

2.4. Coupling Reaction. Formation of the amphiphilic BCP by in situ coupling between the two homopolymers is shown in Scheme 2. TEA was added to remove HCl, which can deactivate PEG-NH₂. The experimental coupling conversion was estimated by GPC. Samples for GPC were capped with a 2-fold excess of phenyl isocyanate to prevent amine groups from interacting with the column.

2.5. Size and Size Distribution Measurements. Both scanning and transmission electron microscopy were employed to give direct images of particle shape. However, since neither is very useful for determining average particle size and distribution statistically,³⁹,⁴⁰ these were measured by dynamic light scattering (DLS). Since the intensity of scattered light is proportional to the sixth moment of diameter, aggregation of particles can significantly skew the average sizes.⁴¹ Except for runs 2, 10, and 11 (see Table 1), samples were passed through a 0.45 µm Milipore filter before DLS measurement.

The DLS apparatus consisted of a photometer equipped with an electrically heated silicon oil bath, Lexel 95-2 Ar⁺ laser operating at 488 nm. The scattered light was monitored using a 1-cm cylindrical detector at 90°. A Carlsbad model 6020 spectral analyzer was used to determine the size distribution of the nanoparticles. The Mie theory and convolutions of the standard DLS function were used to calculate the size distribution of the nanoparticles. The particle size was determined statistically from at least 2000 objects. The size distribution was calculated using the CONTIN program.³¹ The particle size was determined statistically from at least 2000 objects. The size distribution was calculated using the CONTIN program.³¹

at a wavelength of 488 nm, Brookhaven BI-DS photomultiplier, and Brookhaven BI-9000AT correlator. The intensity correlation function was collected at 25 °C and a scattering angle of 90°. Correlation functions were fit using the REPES model to determine average particle sizes and distribution. GENDIST was used for the REPES algorithm. 52,53 The intensity average particle size $d_I$ provided by DLS is defined as

$$d_I = \frac{\sum n_i d_i^6}{\sum n_i d_i^5}$$  \hspace{1cm} (1)

where $n_i$ is the number of particles with a diameter $d_i$.

The mass average particle size $d_W$ is defined as

$$d_W = \frac{\sum n_i m_i d_i^4}{\sum n_i m_i} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$  \hspace{1cm} (2)

where $m_i$ is the mass of particles.

$\text{span}^{54}$ is defined to describe a polydispersity in mass by

$$\text{span} = \frac{(d_{90} - d_{10})}{d_{50}}$$  \hspace{1cm} (3)

where $d_{50}, d_{90},$ and $d_{10}$ are diameters at which the cumulative mass of the particles is under 10, 50, and 90%, respectively. A monodisperse distribution will have a span of 0. A span of 1.0 means that 80% of the mass of all the particles lies within $d_{50}$ of the mean diameter, e.g., if $d_{50} = 75$ nm and the span $= 1.0$, then 80% of the particles could lie between 50 and 125 nm; if the span $= 2.0$ then 80% could be between 30 and 180 nm.

Each sample for cryogenic transmission electron microscopy (cryo-TEM) measurement was vitrified by liquid ethane in a controlled environment vitrification system, and transferred into a JEOL 1210 TEM by a Gatan 626 cryo-holder. Images were recorded below $-170$ °C, using liquid nitrogen cooling. 55,56


particles at room temperature, the sample was sputtered with a 30 Å layer made in this study. The effects of concentration of polymeric β-carotene nanoparticles made by reactive impingement mixing conditions used to make the samples. The experimental results for PS-b-PEG shown in Figure 2 are very close to these theoretical values. The lower value for PCL-b-PEG may come from trapping of functional groups inside particles before coupling, due to faster nucleation and condensation of PCL than reactive coupling.

3.2. Particle Morphology, Size, and Distribution. 3.2.1 PS-b-PEG Particles. Figure 3 shows an SEM image of the particles produced from impinging PEG-NH₂ with PS-COCl containing 1.3% β-carotene (run 2, Table 1). The particles are very spherical in contrast to the irregular, planar-surfaced shapes typically produced by milling. The particles are mostly nonaggregated but have a broad size distribution, span = 2.7. The intensity average diameter, 355 nm, is very sensitive to the few large particles as eq 1 indicates; \( \bar{d}_w \) is more than double the weight average diameter, \( \bar{d}_w = 128 \) nm. In run 3 particles were produced under the same conditions but were filtered before DLS measurement. Their distribution is much narrower, span = 0.76, but \( \bar{d}_s = 124 \) nm is very similar to that of run 2. Assuming that all the β-carotene and PS are trapped in the particles by the fast precipitation and that 17% of the PEG is attached to their surfaces, then the particles contain 43% β-carotene. Thus, we expect that the lower limit of drug-to-polymer concentration is the ratio of the value in column 5 to that in column 4 in Table 1. On the basis of the solubility of β-carotene in THF/water mixtures measured by Liu et al., we calculate that the nanoparticles contain 92 wt% of the β-carotene introduced in the feed.

Figure 4 shows DLS results and a cryo-TEM image of particles from run 9. The amounts of β-carotene and polymer are about double that of runs 2 and 3, yet the particles are still very spherical and relatively narrowly distributed (after filtration), span = 0.95. Their \( \bar{d}_w \) is only 36% larger than the most dilute case, run 7. In fact, as Table 1 and Figure 5 illustrate, over the range of variables studied diameter is little affected by \( Re \) or polymer or β-carotene concentration. The lack of dependence on flow rate indicates that \( Re \geq 500 \) is sufficient for good mixing, in agreement with simulations of Liu and Fox and experiments of Johnson and (57) Palling, D. J.; Jencks, W. P. J. Am. Chem. Soc. 1984, 106, 4869.


The lack of concentration dependence is in agreement with the results of Brick et al. for organic pigment particles formed by similar rapid nonsolvent precipitation via impingement mixing. They attributed this to the extremely high supersaturation leading to spinodal decomposition. Rieger and co-workers observed spinodal decomposition-like structures by cryo-TEM 10 ms after impingement of reactive streams, which formed boehmite AlOOH. They argued that high supersaturation at the interface of two solvents leads to spinodal decomposition and precipitation before mixing is complete. This mechanism can lead to particle size relatively independent of feed composition.

3.2.2. PCL-b-PEG Particles. Figure 6 shows unfiltered particles formed from impinging PEG-NH2 with PCL-COCl and β-carotene, run 11. Although the concentrations were close to those for the PS-containing particles in Figure 3, these particles formed with PCL were less spherical and more aggregated. PCL has a low Tg (about −60 °C, it solidifies by crystallization). This coupled with residual THF in the particle core may permit recrystallization of β-carotene and distortion of particle shape. Additionally, particles with a low Tg are sticky, and thus more flocculation of particles can occur in PCL-b-PEG cases than in those of PS-b-PEG.

Particles formed under the same conditions but filtered are shown in Figure 7 (run 12). These particles are composed of subunit particles of about 10 nm. This appearance implies that the preferred mechanism of particle formation in the PCL-b-PEG cases was condensation of nuclei. The possible reason for this preference is that nuclei with PCL are sticky, and condensed nuclei are not easily broken apart by the mechanical forces of turbulent flow. In contrast, PS has a high Tg, and thus the nuclei in the PS cases should be glassy and more easily separated by the turbulent flow. Thus, the preferred mechanism of particle formation could be growth of nuclei, rather than condensation of nuclei.

4. Conclusions

We have demonstrated creation of nanoparticles by reactive coupling of a hydrophilic polymer block with a hydrophobic one during impingement mixing. These particles form by rapid precipitation caused by impingement with a nonsolvent. They contain an insoluble compound, β-carotene, at high loading. We observed little effect of Reynolds number (for Re > 500) or concentration of β-carotene or polymer on particle size. We take this as evidence for particle formation by spinodal decomposition rather than nucleation and growth. While our measured conversion of block components to block copolymer is relatively low (~17%), this does not prevent efficient stabilization of the nanoparticles. Only a relatively sparse corona of PEG is required for stabilization. The unreacted hydrophobic block acts as a diluent for the compound in the core.

With polyethylene glycol as the corona block, polystyrene as the core block, and β-carotene as the model for a drug, smooth and spherical particles with the diameter of about 100 nm were formed. With simple filtration, aggregates could be removed, resulting in a relatively narrow particle size distribution. Substituting the biocompatible polymer polycaprolactone as the core block resulted in less spherical and more aggregated particles. This was attributed to the low Tg of PCL and perhaps residual THF in the core. High magnification images by SEM revealed that these particles were actually composites formed from 10-nm spheres, which may have been the result of primary nucleation.

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