Bioencapsulated nanoparticles with the control of particle's morphology and the surface modification

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Introduction

Nano-sized poly (D,L lactide-co-glycolide) (PLGA) particles, widely used as a biodegradable polymeric carrier, were prepared employing emulsification-diffusion method. One of the dosage forms as potential formulations for site-specific drug delivery system including drug targeting has been nanoparticles or nanocapsules. Especially interest has been focused on the use of particle formation prepared from polyesters such as poly (D,L lactide-co-glycolide) (PLGA), poly (D,L, lactide) (PLA), poly glycolide (PGA). This is largely due to their biocompatability and to their resorbability through natural pathways [1]. This is also related to the fact that polymers issued from glycolic acid and lactic acid polymers are approved by the FDA. Three techniques have been usually used for the preparation of nanoparticles based on preformed biodegradable polymers. A process of emulsification followed by solvent evaporation is the most widely used technique for preparing nanoparticles containing drugs. Nevertheless, several difficulties have been showed using these techniques when working with toxic solvents (solvent-evaporation), and salts that are incompatible with bioactive compounds (salting-out). To overcome the drawback of using toxic solvent investigated the emulsification-diffusion method using propylene carbonate (PC) as partially water-soluble solvent [2]. In this work, polymeric particles made of PLGA have been increasingly studied for therapeutic applications such as controlled release and drug targeting. Stealth nanoparticles were investigated to avoid rapid uptake of drug carriers by cell of the mononuclear phargocyte system (MPS) and reticuloendothelial system (RES). Poly(ethyleneglycol) (PEG) is attached onto stealth nanoparticle surface to avoid recognition by cell of the MPS and physical adsorption. Biodegradable hydrophobic PLGA nanoparticles were encapsulated by hydrophilic water soluble biodegradable tri-block copolymer using emulsification-diffusion method or self-assembly. Similarly, in this work, for the control of particle's core-shell morphology and the surface properties, reactive hydroxypropyl methylcellulose phthalate (HPMCP) resins from the pristine HPMCP resins synthesized by introducing HEMA for the encapsulation of polystyrene nanoparticles. The HPMCP fortified polystyrene particles showed core-shell structured morphology. HPMCP is one of the most useful natural polymers and has abundant carboxyl and hydroxyl groups. It can be dissolved in water to form aggregates, like micelles, under basic conditions like other alkali-soluble resins[3-8], For comparison, pristine and reactive HPMCPs were used in the emulsion polymerization under the same conditions. The rate of polymerization, the particle size and size distribution, the latex viscosity, the particle morphology, the glass transition temperature (T_g), and the gel content of the latex were measured to investigate the effects of reactivity of HPMCP.

Material and Methods

The PLGA nanocapsules with tri-block copolymer were prepared using the emulsification-diffusion method. Figure 1 shows the mechanism for the formation of PLGA nanoparticles with tri-block copolymer by this technique. After the mutual saturation of the two phases, the partially water-soluble solvent containing PLGA and water containing tri-block copolymer as a stabilizer, both liquids are in the state of thermodynamic equilibrium (a). Stirring causes the dispersion of the solvent solution as globules in equilibrium with the continuous phase: the stabilizing agent is then adsorbed on the large interfacial area created (b). The addition of water to the system destabilizes

the equilibrium (c). It causes the solvent to diffuse to the external phase. During this transport of solute, new globules of nanometer size are produced which gradually become poorer in solvent (d). As a result, the polymer of the globules aggregates because of the presence of a new, continuous non-solvent phase. Also, the reactive HPMCP was synthesized under N_2 atmosphere in a 300mL double-jacketed glass reactor fitted. The synthetic procedure was as follows: First, NCO-terminated HPMCP was obtained by a reaction between the primary NCO group of IPDI and the hydroxyl group of pristine HPMCP. Secondly, reactive HPMCP with carbon double bonds was obtained by reaction between the hydroxyl group of HEMA and the secondary NCO of the NCO-terminated HPMCP. Afterwards, HEMA and DBDTL were added to the reactant mixture, which was stirred for 24 hrs at 80 °C (2^{nd} step). The chemical structure of the dried reactive HPMCP resin was confirmed with ^{1}H-NMR. And also, batch-type emulsion polymerizations of styrene were carried out in a glass reactor equipped with a reflux condenser, a nitrogen gas inlet, an ingredient inlet, an initiator funnel, and a mechanical stirrer. The reaction temperature and the stirring rate were 50°C and at 400 rpm, respectively.

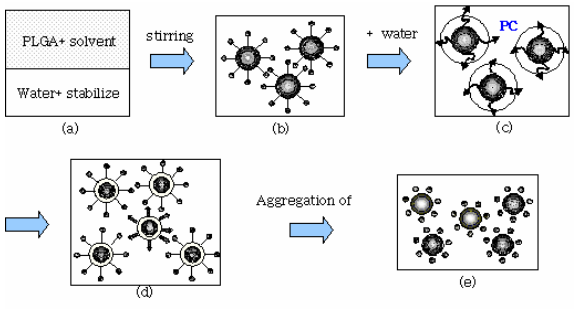

Figure 1. Schematic description of the proposed formation mechanism of PLGA nanoparticles with tri-block copolymer by emulsification-diffusion method.

Results and Discussion

Biodegradable hydrophobic PLGA nanoparticles were encapsulated by hydrophilic water soluble biodegradable tri-block copolymer using emulsification-diffusion method or self-assembly. Figure 2 shows the morphology of core-shell structures with nanoparticles. The particle size was found about 200nm. We also have studied the influence of adding water temperature of D.I. water. The important factor influencing on the particle size is diffusion. Stokes-Einstein equation shows that diffusion coefficient is proportional to temperature. So as temperature increased, the particle size decreased.

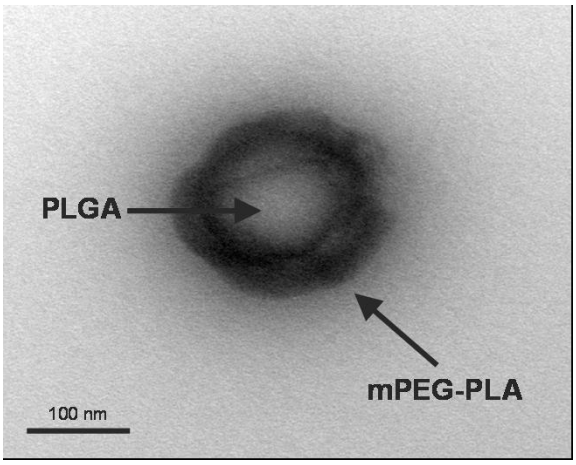

Figure 2. TEM image of core-shell structured PLGA nanocapsule with tri-block copolymer.

And also, the reactive HPMCPs were prepared by 2 step method. In order to confirm the reaction in each step, the intensity of NCO peak at 2274cm^{-1} was monitored every hour. The CH_2 peak in the 2880-2960 cm^{-1} range was used as internal standard. As the reaction progressed, the intensity of

NCO peak decreased, indicating that the OH groups of HPMCP resin had reacted with the primary NCO groups of IPDI, which was corroborated by the increased intensities of C=O stretching peaks at 1733cm^{-1}, N-H bending peak at 1532cm^{-1}, and C-N bending peak at 1419cm^{-1}[9]. After completion of the first-step reaction, the intensity of NCO peak at 2274cm^{-1} confirmed that the secondary NCO groups of IPDI still remained unreacted. Disappearance of the NCO absorption peak at 2274 cm^{-1} in FT-IR spectrum was taken as the end point of the urethane bonding reaction with HEMA and NCO-terminated IPDI-HPMCP intermediate. And resin-fortified emulsion polymerization of styrene with pristine and reactive HPMCP was carried out. In this resin-fortified emulsion system, high concentrations of the resins were often used as compared with conventional short-chain surfactant systems.[8] Linear rates of polymerization (i.e., $R_{p,max}$) were observed in all samples and the slope of conversion increased with increasing HPMCP concentrations from 6 to 24wt% based on the styrene monomer. There was an apparent difference in conversion profiles between the pristine and reactive HPMCPs. In the case of pristine HPMCP, its hydrophobicity was less than that of reactive HPMCP, so the rates of polymerization of pristine HPMCP were slower compared with those of reactive HPMCPs. Enhanced hydrophobicity of reactive HPMCP aggregates provides more polymerization loci (i.e., active micelles or aggregates) at a low concentration of resin and increases the $R_{p,max}$. The $R_{p,max}$ values, which were calculated from the slopes of fraction conversion at 0.43, are listed in Table 1.

Sample ID	$R_{p,max}$ /Ms^{-1}	$\overline{D_n}$ /nm	$\overline{D_w}$ /nm	PDI /-	Viscosity /cP	ζ-potential /mV
H-PS-6	0.6×10^{-4}	143	154	1.1	5.4	-67
H-PS-9	1.0×10^{-4}	106	153	1.4	6.0	-63
H-PS-12	1.2×10^{-4}	62	131	2.1	6.9	-58
H-PS-18	1.3×10^{-4}	56	130	2.3	8.4	-62
H-PS-24	1.4×10^{-4}	45	129	2.9	10.2	-62
RH-PS-6	0.9×10^{-4}	140	151	1.1	41.1	-57
RH-PS-9	1.5×10^{-4}	125	132	1.1	4.5	-47
RH-PS-12	1.6×10^{-4}	109	119	1.1	23.4	-53
RH-PS-18	1.6×10^{-4}	104	123	1.2	179.4	-52
RH-PS-24	1.6×10^{-4}	46	110	2.4	858.8	-52

Table 1. Characteristics of the core-shell poly (St/HPMCP) latex particles prepared with pristine and reactive HPMCPs.

Representative TEM images of core-shell poly (St/HPMCP) latexes are shown in Figure 3. It was expected that reactive HPMCP resin could produce more stable and smaller polystyrene latex particles than HPMCP resin at the same resin concentration. From TEM micrographs, the particle sizes at 12wt% of HPMCP were 120 and 90nm for pristine and reactive HPMCPs, respectively. Since reactive HPMCP has a higher molecular weight and longer hydrophobic chain in comparison with pristine HPMCP having more hydrophilic OH groups.[10] Subsequently reactive HPMCP resin produces more hydrophobic and a larger number of aggregates in the same condition. Furthermore, reactive HPMCP has vinyl groups that copolymerize with styrene monomers. As seen in Figure 3, both HPMCPs provide core-shell morphology and the vicinity of the polystyrene core part is clearly shown. In the case of the latexes prepared with pristine HPMCP (Figure 3(a)), the contrast between the core and shell part is discernible, indicating that HPMCP aggregates are favorably located in the outer periphery (surface) of a particle. However, as seen in Figure 3(b), reactive HPMCP shows somewhat difference morphology at different HPMCP concentrations.

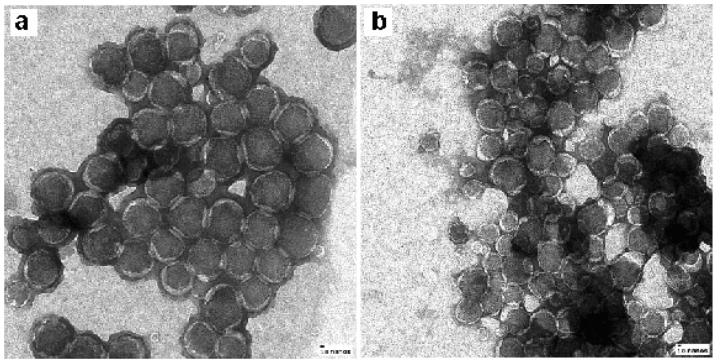

Figure 3. TEM micrographs of core-shell poly (St/HPMCP) latex particles prepared with pristine and reactive HPMCPs. (a) pristine HPMCP (×20K), (b) reactive HPMCP(×20K) at 12wt%.

Conclusions

The present work has shown that PLGA nanocapsule formation with tri-block copolymer by the emulsification-diffusion method as one part. It demonstrates the potential process to control the size of PLGA nanocapsules. The nanocapsule formation process was to be related to the reduction of globule size due to the rapid diffusion of solvent. Preparative variables such as the type and concentrations of stabilizer, homogenizer speed, polymer concentrations, could be the crucial factors for the formation of PLGA nanoparticles. Also diffusion coefficient is proportional to Kelvin temperature of system and the effect of viscosity of continuous phase is reverse. The obtained the reactive HPMCP showed more hydrophobic property and low CMC value compared with the pristine HPMCP. Due to the reactivity of reactive HPMCP, the latexes prepared by using the reactive HPMCP showed a different trend in particle size, viscosity, morphology, and physical properties compared with the pristine HPMCP. Core-shell structured poly (St/HPMCP) latexes with the reactive HPMCP showed a shear-thinning effect, which was corroborated by the grafted HPMCP shell structure, and which was also confirmed by TEM analysis.

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