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Effects of Biodegradable Triblock Copolymers on the Microencapsulation of Ascorbic Acid-2-Glucoside in $W_1/O/W_2$ Multi-Emulsions

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Summary: A skin-active functional material, AA2G (ascorbic acid-2-glucoside, W1 phase), was encapsulated via a stepwise emulsification method using PEO-PLGA-PEO and PEO-PCL-PEO biodegradable triblock copolymers as wall materials. The emulsion stability of the $W_1/O/W_2$ multi-emulsions was observed using a ferroxyl test method and on-line turbidity analysis for a short period of time. The morphology of the multi-emulsions was observed using the laser confocal and optical microscopes. The use of copolymers in W_1 phase was found to be more effective. The copolymers decreased the size of the multi-emulsions and enhanced emulsion stability.

Keywords: ascorbic acid-2-glucoside; biodegradable; emulsion stability; microencapsulation; triblock copolymers

Introduction

Until recently, various research works concerning the effective and stable delivery of functional materials had been carried out in a number of potential fields. A variety of method to carry active materials have been reported such as, micelles,[1,2] nanocapsules,[3,4] lipid and polymeric vesicles.[5] In addition, the representative techniques, like spray drying, double emulsion method and solvent evaporation have been established for nanocapsules.[4,6,7] Among the techniques, water in oil in water ($W_1/O/W_2$) has been regarded as a multi-emulsion system that contains water-soluble species in the inner aqueous phase (W_1 phase). This system can protect a variety of vulnerable functional materials from the surroundings, such as heat, oxygen, light, and pH levels. In addition colloidal stability could be enhanced. The $W_1/O/W_2$ multi-emulsions, however, are thermodynamically unstable and they often show phase separation as time goes on. To overcome this instability, a lot of works have been accomplished in the application field such as food, cosmetics, and pharmaceutics.[8–13] In this study, ascorbic acid-2-glucoside (AA2G), which is relatively more stable than pure Vitamin C, was dissolved in a W_1 phase and encapsulated using biodegradable and amphiphilic triblock copolymers, i.e., poly (ethylene oxide)-poly(lactic acid-co-glycolic acid)-poly(ethylene oxide) (PEO-PLGA-PEO) and poly(ethylene oxide)-poly(ε-caprolactone)-poly(ethylene oxide) (PEO-PCL-PEO). Concerning biodegradable copolymers in the encapsulation process, there has been a vast amount of published research. Michaout et al. suggested that the stability of multiple emulsions can be enhanced by using amphiphilic polyelectrolyte in the outer aqueous phase in multiple emulsions.[14] Ozer et al. studied stability by increasing viscosity induced by

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adding cellulose derivative or cabomer in outer aqueous of the multi-emulsion system.[15] Shima et al. reported on the effect of a hydrophilic stabilizer regarding the surfactant location, however, most published results have been limited to nano- or microspheres, i.e. solid particles.[4,16–18] Thus, we focused on multi-phase liquid droplets. The effects of biodegradable triblock copolymers on emulsion stability, particle size, and morphology of the $W_1/O/W_2$ multi-emulsions were investigated by using two kinds of triblock copolymers having with the similar molecular weight and composition (i.e., lengths of PEO and PCL (or PLGA) blocks).

Experimental

Synthesis of Biodegradable Triblock Copolymers

Synthetic methods for PEO-PCL-PEO and PEO-PLGA-PEO triblock copolymers are well known and have been described in other papers.[19] 8.06 g of ε-CL (ε-caprolactone, Aldrich Co.) was purified under a vacuum for 3 hrs. Water-free m-PEG (monomethoxy polyethylene glycol, 2,000 $gmol^{-1}$, Aldrich Co.) was completely dissolved in 80 ml of anhydrous toluene for 5 hrs and then ε-CL as a monomer and $SnOct_2$ as a catalyst were added to the mixture. The reaction was conducted for 12 hrs at 120 °C. HMDI (hexamethylene diisocyanate) was added to the reaction mixture and it was reacted for 7 hrs at 60 °C. The product was isolated in diethyl ether and the residual solvent was removed under a vacuum, and then PEO-PCL-PEO tri-block copolymers were obtained.[20–22] All reactions were conducted under a nitrogen atmosphere. The PEO-PLGA-PEO copolymer, which was kindly donated by Seoul National University, was synthesized by a ring opening polymerization of lactide and glycolide, and followed by a condensation reaction with m-PEG.[23] The $SnOct_2$ was used as a catalyst.

Preparation of $W_1/O/W_2$ Multi-Emulsions Containing AA2G

$W_1/O/W_2$ multi-emulsions were prepared by the following stepwise emulsification method: First, W_1/O emulsions were prepared with a selected surfactant system and the most suitable conditions determined by a trial-and-error method. Emulsification was conducted using a horn-type ultrasonicator (VCX-750, Vibracell). Temperature was maintained in an icy water bath. W_1/O emulsions prepared in the first step were poured into the W_2 phase and the mixture was stirred with a homogenizer for 2 min at 13,500 rpm. The recipe for the preparation of $W_1/O/W_2$ multi-emulsions is shown in Table 1. It is common to use triblock copolymers which having hydrophilic-hydrophobic-hydrophilic structure during the stabilization of oil droplets in water phase. In this research, however, the copolymers were added when the W_1/O emulsions were prepared, since phase separation occurred in the $W_1/O/W_2$ multi-emulsions within 24 hrs after the addition of copolymers into the W_2 phase.

Characterizations

Chemical Structures

The chemical structures of PEO-PCL-PEO and PEO-PLGA-PEO copolymers were confirmed by FT-IR and ^{1}H-NMR analyses. The peaks from FT-IR spectra and ^{1}H-NMR were concisely assigned as the following;

Table 1.
The basic recipe for the preparation of $W_1/O/W_2$ multi-emulsion.

Phases	Ingredients	Composition (%, w/w)
W_1 Phase	Pure water	3.73
	Ethanol	0.62
	AA2G	2.17
	Copolymer[a)]	0.08
O Phase	Mineral oil	12.43
	Surfactant[b)]	1.96
	Rheopearl 450	0.07
W_1/O in W_2		21.06
W_2 phase	Pure water	73.68
	Surfactant[b)]	5.26

[a)] PEO-PLGA-PEO or PEO-PCL-PEO.
[b)] Span80:Tween60 = 65:35 (HLB = 8.01).

For PEO-PCL-PEO:

- FT-IR (KBr): 2885 cm^{-1} (s, CH_2) by the PEO block, 2945 cm^{-1} (s, CH_2) and 1726 cm^{-1}(C=O) by the PCL block.[1,22]
- ^{1}H-NMR ($CDCl_3$): $\delta = 3.6$ ppm (PEO), $\delta = 2.2$ ppm (PCL), $\delta = 1.35$ ppm (PCL).[20]

For PEO-PLGA-PEO:

- FT-IR (KBr): 2885cm^{-1} (s, CH_2) by the PEO block, 1758 cm^{-1} (s, COO) by the glycolic acid and lactic acid units, 1641 cm^{-1}(s, C=O) by glycolic acid unit.
- ^{1}H-NMR ($CDCl_3$): $\delta = 3.38$ ppm (CH_3), $\delta = 3.65$ ppm (PEO), $\delta = 4.8$ ppm (GA), $\delta = 5.2$ ppm (LA). $\delta = 1.55$ ppm (LA).[23]

Molecular Weights

The molecular weights (MW) and molecular weight distributions (MWD) of the copolymers were determined using a GPC. Analyses were carried out using a GPC (Viscotec) system which was fitted with a series of Waters columns (HR4, HR3, and HR2) at a temperature of 35 °C. The eluent was tetrahydrofuran (THF) with a rate of 1.0 $mlmin^{-1}$ and raw data were calibrated using universal calibration from polystyrene standards (M.W. 580–7,500,000 $gmol^{-1}$, Polymer Laboratories). The MWs and MWDs of the copolymers are listed in Table 2.

Results and Discussion

Critical Aggregation Concentration and Hydrodynamic Diameter

As shown in Figure 1-(A), critical aggregation concentrations (CAC) of the copolymers were measured by pyrene absorption (at 372 nm) analysis with various concentration between 0.001 $mgml^{-1}$ and 2 $mgml^{-1}$. Due to the poor water solubility of PEO-PLGA-PEO, the small amount of ethanol was used (refer to Table 1). The CACs of the PEO-PLGA-PEO and PEO-PCL-PEO triblock copolymers were measured as 0.31 $mgml^{-1}$ and 0.14 $mgml^{-1}$, respectively. The pyrene absorption intensity of PEO-PCL-PEO was very strong compared with that of PEO-PLGA-PEO. This indicates that the solubilization ability for the hydrophobic probes of PEO-PCL-PEO is superior to that of PEO-PLGA-PEO, which might be due to the strong interaction of the hydrophobic blocks, i.e., PCL units. It is well known that the crystallinity and degradation rate of PLGA depend on the ratio between lactic acid (LA) and glycolic acid (GA). The ratio between LA and GA of the PEO-PLGA-PEO used in this work is 9:l. This indicates that the PLGA-based copolymer would have a high level of crystallinity.[7] The hydrodynamic size of the aggregates as a function of copolymer concentration were shown in Figure 1-(B), The hydrodynamic size of both aggregates decreased as the concentration of copolymer as in other polymeric surfactants increased.[24] At the same low concentration, the hydrodynamic size of PEO-PCL-PEO is smaller than that of PEO-PLGA-PEO. As the concentration of the copolymer increased, however, the size of PEO-PLGA-PEO and PEO-PCL-PEO gradually decreased to 676 and 744 nm, respectively.[25] This result indicates that the PEO-PCL-PEO copolymer can effectively form smaller aggregates, as

Table 2.
Molecular weights, CAC, and size of micelle of the triblock copolymers.

Copolymers	M_{nmr} a) ($gmol^{-1}$)	M_n b) ($gmol^{-1}$)	PDI c) (–)	CAC ($mgml^{-1}$)	Size d) (nm)
PEO-PLGA-PEO	10105	13005	1.44	0.31	676
PEO-PCL-PEO	7438	7939	1.34	0.14	744

a) Determined by ^{1}H-NMR ($CDCl_3$).
b) The number-average molecular weight, M_n, was determined by GPC (THF).
c) Polydispersity index, PDI = M_w/M_n and the weight-average molecular weight, M_w, was determined by GPC (THF).
d) Measured at 5 mg/ml concentration by a DLS after dissolving in ethanol water mixture (ethanol:pure water = 1:5).

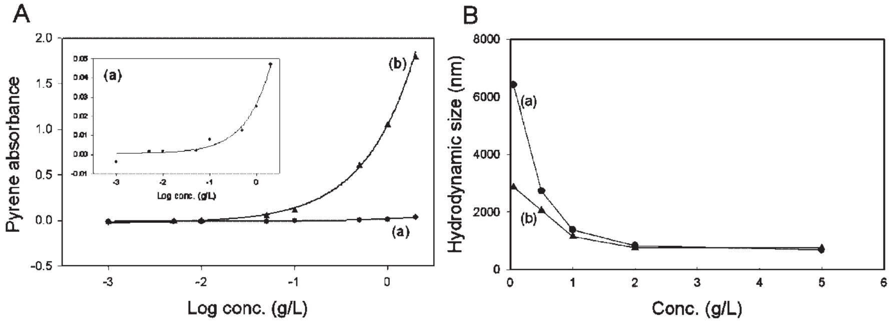

Figure 1.

A: critical aggregation concentration of (a) PEO-PLGA-PEO and (b) PEO-PCL-PEO copolymers in ethanol-water mixture (1:5) at room temperature. B: hydrodynamic sizes of (a) PEO-PLGA-PEO and (b) PEO-PCL-PEO copolymers in ethanol-water mixture (1:5) at room temperature.

compared with PEO-PLGA-PEO, at low concentration.

Ferroxyl Test

The short-term stability of the $W_1/O/W_2$ multi-emulsions was observed through a ferroxyl test for 3 days, as shown in Figure 2. In preparing the $W_1/O/W_2$ multi-emulsions, traces of $FeSO_4$ and $K_3Fe(CN)_6$ were dissolved in inner- and outer- aqueous phases, respectively. If the Fe^{2+} of $FeSO_4$ and $KFe(CN)_6^{2-}$ of $K_3Fe(CN)_6$ meet and react, Prussian blue color will results. As might be anticipated, a phase separation occurred in a sample of the multi-emulsions without copolymers. The layer of Prussian blue became clearer, darker and thicker. Probably, Fe^{2+} from the inner aqueous phase diffuses out into the outer aqueous phase and then settles down because of gravity. Both samples with copolymers showed a slower color development; however, the sample with PEO-PGLA-PEO showed a better stability at the same concentration. This result can be explained by the hydrophobicity change of the copolymers during emulsification of the W_1/O phase, where by the temperature increased to 60 °C by ultrasonication. It was reported that the CAC of PEO-PLGA-PEO decreased by half when the temperature increased to 50 °C.[23]

On-line Turbidity Analysis

The time-evolution stability of each multi-emulsion was measured by the on-line turbidity method. As shown in Figure 3, (a), (b) and (c), plots show raw flux profiles obtained from a Turbiscan at the interval of 10 min over a 6 hr-period. The x- and y-axes represent the height of the sample from bottom to top and the flux of transmission (%, T) or backscattering (%, BS) scanned, respectively. According to the Turbiscan principle, the three important factors

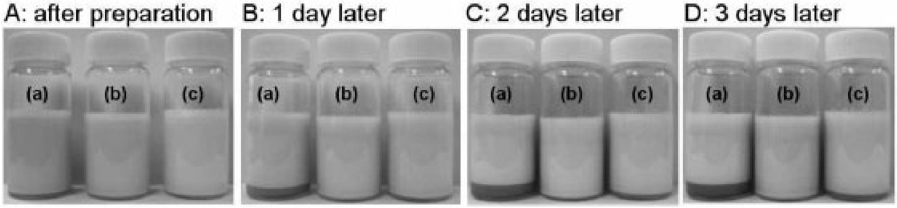

Figure 2.

Stability of $W_1/O/W_2$ multi-emulsions by ferroxyl test; (a) the multi-emulsion without copolymer (control), (b) the multi-emulsion with PEO-PLGA-PEO, and (c) the multi-emulsion with PEO-PCL-PEO.

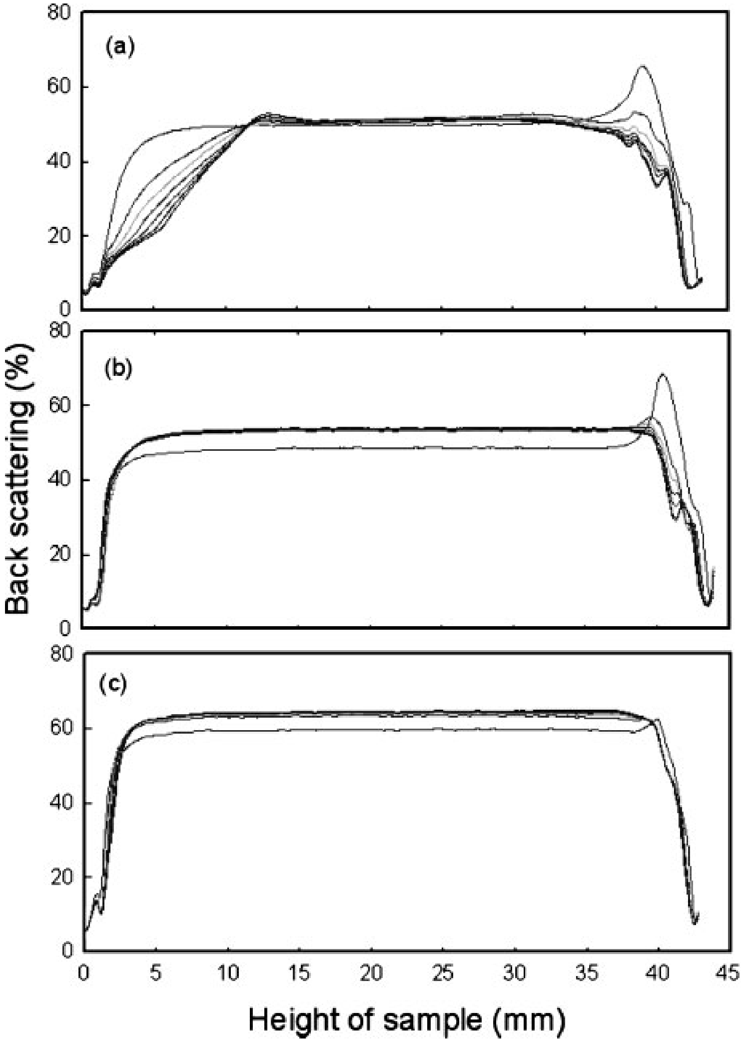

Figure 3.
A: time-evolution variations of the backscattering flux (%) of the three multi-emulsions measured by using an on-line turbidity analysis; (a) the multi-emulsion without copolymer (control), (b) the multi-emulsion with PEO-PLGA-PEO, and (c) the multi-emulsion with PEO-PCL-PEO.

governing the fluxes are concentration, size, and the reflective index of the dispersed droplets. In this analysis, the T variation was indiscernible because of the large amount of W_1/O in the W_2 phase. On the other hand, as shown in Figure 3, the BS variation of each sample could be observed clearly. For multi-emulsion without copolymers (a), the BS value at the bottom of the sample bottle decreased and the value at the top increased, respectively. This implies that creaming occurs as time goes on. In the cases of (b) and (c), both samples showed slight changes in the BS variation upon measuring, but the BS value gradually became a constant value. Neither creaming nor sedimentation was observed; however, the BS value slightly increased throughout the sample height. This suggests that coalescence among the droplets is predominant as compared with creaming or sedimentation. In addition, backscattering

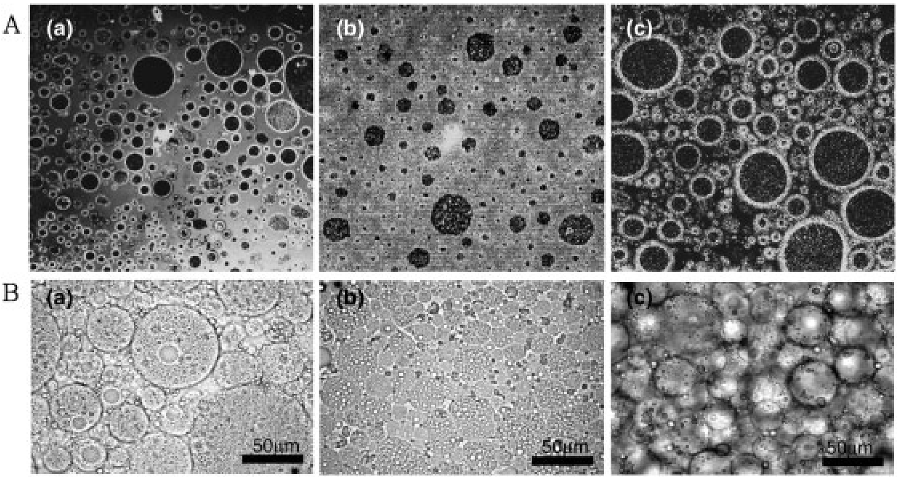

Figure 4.
Laser confocal images of the $W_1/O/W_2$ multi-emulsions at ×400 magnification; A (a) multi-emulsion without copolymer (control), (b) multi-emulsion with PEO-PLGA-PEO, (c) multi-emulsion with PEO-PCL-PEO. B: morphology of $W_1/O/W_2$ multi-emulsions at ×500 by optical microscopy; (a) multi-emulsion without copolymer (control), (b) multi-emulsion with PEO-PLGA-PEO (c) multi-emulsion with PEO-PCL-PEO (scale bar = 50 μm).

flux rate calculated at a height of 5 mm was $0.072\%\ \mathrm{min}^{-1}$ for sample (a). For the (b) and (c) samples, the values were less than $0.01\%\ \mathrm{min}^{-1}$ due to the barrier effect of the polymer rich, water-oil interface. Concerning multi-emulsions without copolymers, it was concluded that the inner aqueous phase easily became diffused from unstable droplets and that phase separation occurred within a short period of time.

Laser Confocal and Optical Microscope Analyses
The morphology of the multi-emulsions was examined by a laser confocal imaging system (MRC 1024/ES, Bio-Rad) and an optical microscope (Axioplan 2, Carl Zeiss). A trace of FITC (fluorescein isothiocyanate-albumin) was added to the inner aqueous phase (W_1) for the confocal imaging analysis. As shown in Figure 4-(A), the W_1 phases are fine and well dispersed in the oil phase. A considerable amount of FITC, however, seems to be concentrated at the periphery of the oil droplets, which might be due to its amphiphilic nature. Both sizes of droplets and the distribution of multi-emulsions with copolymers were relatively smaller than that without copolymers. PLGA- and PCL-based copolymers effectively protect the W_1 phase contains AA2G by reducing Ostwald ripening[26,27] that cause coalescence and phase separation, since the coalescence of the droplets increases the size of droplets. As shown in Figure 4-(B), the optical images are in good agreement with confocal images.

Conclusion

$W_1/O/W_2$ multi-emulsions containing PEO-PLGA-PEO or PEO-PCL-PEO triblock copolymers were prepared and compared with the $W_1/O/W_2$ multi-emulsion without copolymers in terms of stability and morphology. Addition of the copolymers in W_1 phase was found to be more effective than W_2 phase for emulsion stability. At the same concentration of copolymers, PEO-PLGA-PEO shows better stability and smaller droplet size as compared with PEO-PCL-PEO, which might be due to the longer chain length of PLGA block in PEO-PLGA-PEO copolymer.

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