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Effect of Polymer Characteristics on the Thermal Stability of Retinol Encapsulated in Aliphatic Polyester Nanoparticles

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The present study investigates how the thermal stability of retinol (vitamin A) encapsulated in polyester nanoparticles is influenced by the types of polyester used for the nanoparticles. A variety of polyester-retinol nanoparticles were prepared with various polyesters like: poly(ethylene adipate), PEA; poly(butylene adipate), PBA; poly(hexamethylene adipate), PHMA; and three polycaprolactones, PCL, of different molecular weights (M_n ~10, 40, and 80K). The chemical stability of retinol in these nanoparticles, monitored in an aqueous solution at 25 ºC and 40 ºC for 4 weeks, was high in the following order of the nanoparticles prepared with PHMA > PCL 40K > PCL 10K > PCL 80K > PBA~PEA at 25 ºC and PCL 10K > PCL 40K > PHMA > PCL 80K > PEA > PBA at 40 ºC. More importantly, this study has also found that the thermal stability of the retinol in the nanoparticles was closely connected with the melting temperatures of polyesters and polyester nanoparticles. The results were further discussed with possible factors - such as sample preparation condition (or history) and miscibility between the polyesters and retinol - affecting T_m of the polyesters and the nanoparticles.

Key Words : Polyester-retinol nanoparticles, Thermal stability, Melting temperature, Preparation condition, Miscibility

Introduction

Aliphatic polyesters have received considerable attention to many biomedical fields such as drug delivery system (DDS),[1-3] tissue engineering,[4,5] and biomedical device.[6-8] Their versatile applications are attributable to the biodegradability and biocompatibility:[7,9-11] their degradation rates are controllable by properly tailoring the chemical structure in the main chain; the chemicals after their degradation are mostly safe to our body; and the chemicals are eventually cleared out through physiological pathways. In DDS, many dosage forms are made from the aliphatic polyesters, and nanoparticles are one of the most popular types to deliver bioactive drugs efficiently to a target site through an enhanced permeation and retention effect.[12] To maximize the performance of the nanoparticles, most researches have been focused on improving their target specificity to a disease site[13-18] and on controlling a release behavior of the drugs in the nanoparticles in a stimuli-responsive fashion.[17-21] However, some bioactive drugs are easily denatured or degraded during storage or circulation in our body.[22-27] As such, before reaching the target site, the drugs are no longer effective to cure a disease. Under this situation, in addition to above-mentioned functions, the nanoparticles may sometimes be required to prevent the bioactive drugs from degradation caused by heat, oxygen, pH, radicals, or combination of these factors. For this reason, it is necessary to extensively study on the role of polyesters in the chemical stability of bioactive drugs encapsulated in polyester nanoparticles.

This research presents the effect of polymer types on the thermal stability of a bioactive drug encapsulated in polyester nanoparticles. Figure 1 shows a schematic depicting the outline of this study. Retinol (vitamin A or (2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol) was selected as a model drug in this work. Retinol and retinoids have been extensively used in biomedicines and cosmetics due to its outstanding functions to suppress infection, aging, and generation of

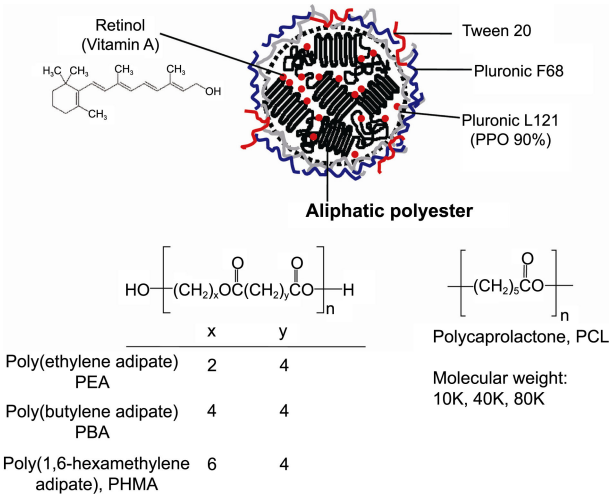

Figure 1. A schematic depicting the polyester-retinol nanoparticles prepared in the present study. Various types of polyesters were used for the nanoparticles to investigate the effect of polymer types on the thermal stability of the retionol.

tumor in animal body.[28-32] However, from literatures and the researcher's experience, their chemical stability against heat or light is very poor.[33,34] Previous researches have suggested some ways to prevent the degradation of retinoids.[33-36] These works include the encapsulation of retinol in liquid crystalline lipids,[33] the formation of a complex between retinol and γ-cyclodextrin,[34] the solubilization of the drug in oily compounds,[35] and the storage of retinol in a dosage form containing anti-oxidants.[36] In addition, studies on the use of polymeric particles were also recently conducted. Cirpanli *et al.* reported that the thermal degradation of retinoic acid could be prevented from its encapsulation in polyester microparticles.[37] Very recently, Beck and co-workers reported that the photostability of retinoic acid could be improved through an encapsulation of retinoic acid in polycaprolactone nanoparticles.[38,39] However, essentially no work has been conducted to seek factors affecting the degradation of retinol encapsulated in the polyester nanoparticles, and this is the motivation of the current research. Specifically, the study was concerned to investigate how the thermal stability of retinol in the polyester nanoparticles is influenced by the types or characteristics of polyesters used for the nanoparticles. To attain this end, this study prepared polyester nanoparticles containing retinol by using various types of polyesters, and the thermal stability of retinol in the nanoparticles was monitored. The thermal stability was correlated with the polyesters' melting temperatures which could be affected by both preparation condition and the miscibility of the polyesters with retinol.

Experimental

Materials. Poly(ethylene adipate), PEA and $M_n \approx 10{,}000$, Poly(butylene adipate), PBA and $M_n \approx 12{,}000$, poly(1,6-hexamethylene adipate), PHMA and $M_n \approx 3{,}800$, and polycaproplactone (PCL) having $M_n \approx$ 10K, 40K, and 80K were purchased from Aldrich (Yongin, Korea). Pluronic L121 (100%) and Pluronic F68 (99.99%), and were purchased from BASF (New Jersey, USA). Retinol was purchased as a form of mixture with Tween 20 (1:1 by weight) from BASF (Retinol 50C; Ludwigshafen, Germany). Acetone was obtained from Fisher Scientific (> 99%, New Jersey, USA).

Preparation of Polyester Nanoparticles Containing Retinol. As a typical method, 0.33 g of an aliphatic polyester, 0.33 g of the retinol/Tween 20 mixture, and 0.33 g of Pluronic L121 were solubilized in 67 mL of Acetone. Then, the mixture was transferred into 100 mL of deionized water containing 0.17 g of Pluronic F68. During and after the transfer, the mixture was kept stirring with an agitator (Matsushita Electric Industrial Co. Ltd, Tokyo, Japan). In 5 min, acetone in the mixture was removed by using a rotary evaporator at 40 °C for 30 min. After the removal of acetone and some water, the concentration of retinol for all the samples was 1.6 mg/mL.

Size Measurement of the Polyester-Retinol Nanoparticles. The hydrodynamic diameters of polyester-retinol nanoparticles were determined by using a photocorrelation spectroscopy (PCS, Malvern Instruments 3000HS, Malvern Instruments, Worcestershire, UK). The aqueous dispersions of polyester-retinol nanoparticles were diluted to 1 mg/mL. The samples were irradiated with a 633-nm light from a He-Ne laser, and an intensity of 90°-scattered light was measured. For each specimen, 10 autocorrelation functions were analyzed using the scattered intensity. The mean diameter of the nanoparticles was calculated using the Stokes-Einstein equation.

Thermal Stability Tests of the Retinol. Thermal stability of retinol encapsulated in the polyester nanoparticles was monitored at two temperatures for 4 weeks. Plastic bottles containing the as-prepared polyester-retinol nanoparticles dispersed in aqueous solution were stored in thermostatic chambers (JISICO, Seoul, Korea) at 25 °C and 40 °C whose relative humidity were 60 and 75%, respectively. At every week, 1 mL of aqueous dispersions of the polyester-retinol nanoparticles was taken and added to 100 mL of methanol. The mixture was sonicated for 30 min to completely disrupt the nanoparticles and thus dissolve the retinol in methanol. After filtering the mixture with a 0.45 μm filter made of poly(vinylidene fluoride), a high performance liquid chromatography (Hewlett-Packard Agilent 1100, Waldbronn, Germany) was used to determine the concentration of retinol. The mobile phase was a mixture of acetonitrile and water (97:3 by v/v), and its flow rate was 1 mL/min. A Nova-Pak C18 (3.9 × 150 mm, Waters, Milford Massachusetts, USA) was used as the column, and the concentration of retinol was determined by the area of the peak detected at 325 nm. The injection volume of the sample was 10 μL. The stability of the retinol at a certain time of storage, was expressed as the following equation: stability (%) = $C_t/C_0 \times 100$, where C_t is the concentration of the retinol at a certain storage time in the chamber, C_0 is the initial concentration of retinol before storage (concentration of retinol of the as-prepared samples). For each data point, the average value was shown from 3 parallel experiments.

Thermal Analysis of the Polyesters. A differential scanning calorimetry (DSC, DSC Q1000, TA Instrument, New Castle, USA) was introduced to record thermograms of polyesters used for the polyester-retinol nanoparticles. 5-10 mg of a polyester was sampled in a hermetic pan, and the pan was heated from 0 to 100 °C with a heating rate of 5 °C/min under the flow of N_2 gas (50 mL/min). After the first run, the sample was cooled to 0 °C with a cooling rate of 10 °C/min and the sample was heated again with a heating rate of 5 °C/min to 100 °C (second scan). In addition, DSC thermograms for the polyester-retinol nanoparticles were also recorded after the nanoparticles being freeze-dried. The heating rate of the nanoparticles samples were 5 °C/min.

Results and Discussion

Preparation and Characterization of the Polyester-retinol Nanoparticles. It is well known a solvent evaporation (or oil-in-water emulsion) and a solvent displacement (or precipitation) method are the most common for the encapsulation of bioactive drugs.[40-43] Both methods have

Table 1. Hydrodynamic diameters of the polyester-retinol nanoparticles

Polymer type	Hydrodynamic diameter (nm)
PEA	196 ± 9
PBA	168 ± 3
PHMA	181 ± 2
PCL, 10K	201 ± 2
PCL, 40K	209 ± 6
PCL, 80K	219 ± 15

been introduced to prepare polyester nanoparticles or microparticles containing retinol and retinoic acid for improving the thermal/photostability or controlling the release of the drugs.[37-39,44,45] Between the two methods, the solvent displacement method is more convenient for the preparation of nanoparticles. In this method, a water-miscible organic solvent solubilizing a polymer and a hydrophobic drug are mixed with water. At this moment, the polymer and bioactive drugs are precipitated to form nanoparticles due to a phase-separation of the polymer and the hydrophobic drug from the co-solvent.

In the present study, acetone was selected as a water-miscible organic solvent because the polyesters and retinol were soluble in this solvent. To prevent aggregation and caking of the polyester-retinol nanoparticles for a long period of time, it was essential to introduce proper stabilizers (Figure 1). This study used Pluronic L121 (hydrophilic to liphophilic balance, HLB, of 1-7) and Pluronic F68 (HLB of > 24) as a hydrophobic and hydrophilic stabilizer, respectively. The two polymers were triblock copolymers of poly(ethylene glycol)-*b*-poly(propylene glycol)-*b*-poly(ethylene glycol). In addition, due to its hydrophilic character, it is expected that Tween 20 (from the retinol/Tween 20 mixture; HLB of 16.7) would mostly reside in the outside of the nanoparticles. For all the preparations, the amount of the polyesters, retinol, and stabilizers were kept constant. As such, it is possible to exclude the concentration of nanoparticle constituents and their ratios as factors affecting the stability of the retinol. As shown in Table 1, the sizes of the polyester-retinol nanoparticles ranged from 150 to 220 nm, depending on the type of polyesters.

Thermal Stability of Retinol in the Polyester-Retinol Nanoparticles. Figure 2 shows the thermal stability of retinol encapsulated in the polyester-retinol nanoparticles for the two temperatures. The stability of the retinol in the nanoparticles was expressed as % of the retinol concentration at a certain storage time (C_t) relative to the retinol concentration of the as-prepared nanoparticles (C_0). For the two storage temperatures, it was found that the concentration of retinol decreased more steeply at 40 °C than that at 25 °C. As for the effect of the polymer type, the thermal stability of the retinol in the nanoparticles was quite influenced by this factor. At 25 °C, after 4 weeks, the stability of the retinol was high with the following order: PHMA > PCL 40K > PCL 10K > PCL 80K > PBA~PEA. In the meantime, at 40 °C, the stability of retinol was high in the following order: PCL 10K > PCL 40K> PHMA > PCL 80K > PEA > PBA. In general, the polyester-retinol nanoparticles made with PCL or PHMA ensured a better thermal stability of retinol than the nanoparticles with PEA and PBA for both the temperatures. In addition, it is noticeable that the concentration of retinol in the nanoparticles made of PBA dropped very steeply at 40 °C. As for the effect of molecular weight of PCL in the nanoparticles, PCL 10 K and 40 K showed a little better thermal stability of retinol molecules than the nanoparticles made of PCL 80 K.

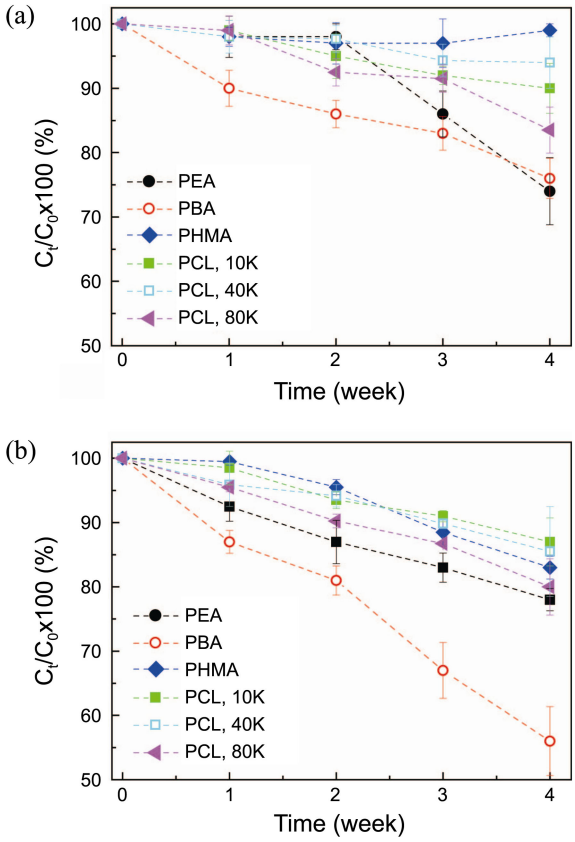

Figure 2. Stability of retinol in the polyester-retinol nanoparticles dispersed in an aqueous solution at (a) 25 °C and (b) 40 °C. The stability of the retinol in the nanoparticles was expressed as % of the retinol concentration at a certain storage time (C_t) relative to the retinol concentration of the as-prepared nanoparticles (C_0). Error bars are the standard deviation.

Thermal Analysis of the Polyesters and Polyester-Retinol Nanoparticles. Next, thermal behaviors of the polyesters and the polyester-retinol nanoparticles were investigated to find a relationship between the thermal stability of retinol and the properties of polyesters or polyester-retinol nanoparticles. Figure 3(a) and (b) shows differential scanning calorimetry (DSC) thermograms of the polyesters. As-received polyester samples were scanned from 0 to 100 °C (first scan), as shown in Figure 3(a). In addition, after cooling the sample to 0 °C, the polyesters were scanned again to 100 °C with the same scanning rate (second scan, Figure 3(b)). The results were summarized in Table 2. It was found that there was a large difference between the thermograms

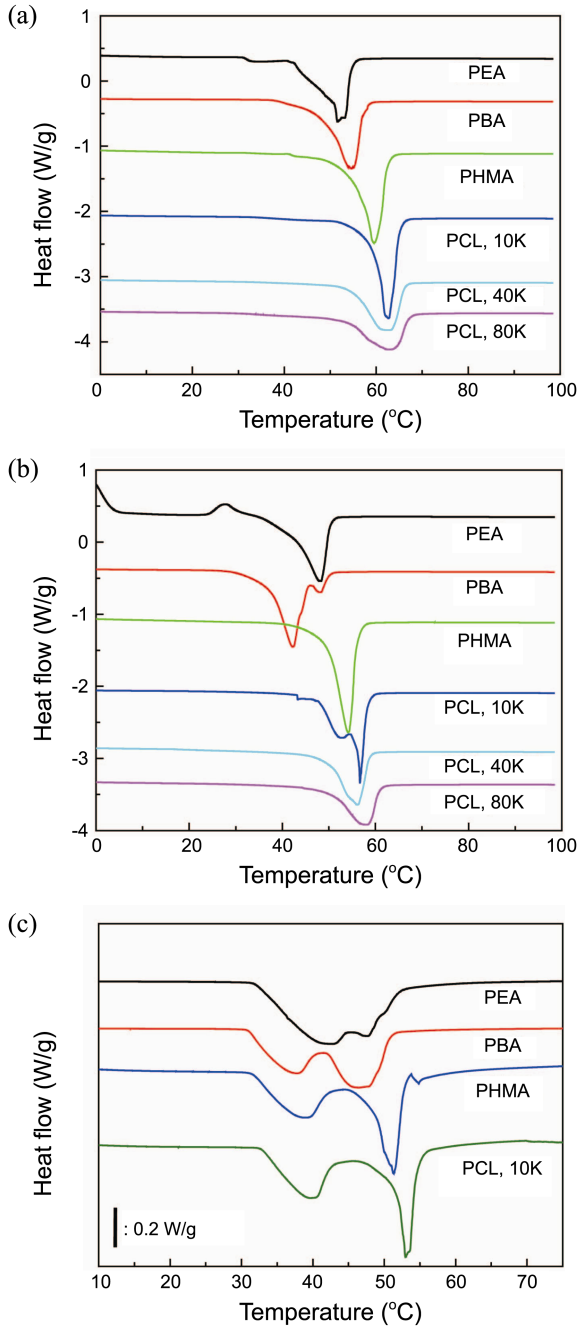

Figure 3. DSC thermograms obtained from (a) first scans (with as-received polyesters) and (b) second scans (with polyesters after heating to 100 °C and cooling to 0 °C). The scanning rate for the all the scans were 5 °C/min. (c) DSC thermograms for the polyester-retinol nanoparticles after being freeze-dried. The scanning rate was 5 °C/min.

obtained from the first and the second scans. Most samples showed that the T_m from the second scan was lower by 2-6 °C than those obtained from the first scan. Exceptionally, the T_m of PBA from the second scan was decreased by 12 °C from 54.6 °C to 42.2 °C and it was even lower than that of PEA. Interestingly, PBA-retinol nanoparticles had the lowest thermal stability of retinol at 40 °C. Generally, it seemed that the thermal stability of retinol was increased with increasing the polyesters' T_m obtained from the second scan. In addition, enthalpies of fusion, H, obtained from the second scan was also lower than those obtained from the first scan.

Table 2. Melting peak temperatures (T_m) and heat of fusion (ΔH) of the peaks for the polyesters used in the preparation of polyester-retinol nanoparticles

Polymer type	T_m (°C)		ΔH (J/g)	
	1st scan	2nd scan	1st scan	2nd scan
PEA	51.6	48.2	88.2	71.6
PBA	54.6	42.2	89.7	88.2
PHMA	56.0	54.1	102	92.0
PCL,10K	62.7	56.7	101	79.1
PCL, 40K	62.8	56.0	78.0	64.6
PCL, 80K	62.8	58.0	77.9	58.1

DSC experiments were further conducted to obtain the thermograms of the polyester-retinol nanoparticles made with PCL10K, PEA, PBA, and PHMA after the nanoparticles being freeze-dried (Figure 3(c)). Since the nanoparticles dispersions were containing Pluronic F68, Pluronic L121, and Tween 20, the thermograms were actually reflected by the mixture of nanoparticles and these constituents. Therefore, in the thermograms, it was rather difficult to accurately assign the origin of the two peaks. However, a careful look at the thermograms revealed that the first peaks were more or less similar for all the samples while the second peak was different from sample to sample. Therefore, it was suggested that one peak at low temperature was largely reflected by Pluronic F68 and the other peak at high temperature was mostly reflected by the polyester (Pluronic L121 and Tween 20 are the liquids at room temperature and they might not have any peak). When regarding the second peak as the T_m of the polyesters in the polyester-retinol nanoparticles, the peak temperature was increased in the order of the following polymers: PCL 10K (52.9 °C) > PHMA (51.4 °C) > PEA (47.6 °C) > PBA (46.3 °C). Even considering the first peak, the PBA polyester-retinol nanoparticles also had the lowest melting peak temperature.

Clearly seeking the influence of thermal behavior of polyesters on the thermal stability of retinol in the nanoparticles, thermal staibility of retinol at 40 °C was plotted with T_m or enthalpy of fusion (H), as shown in Figure 4. The results showed that the thermal stability had a stronger correlation with the T_m obtained from the second scan than the T_m from the first scan. The dataset with the T_m from the second DSC scan *vs* thermal stability had the coefficient of determination (R^2) of 0.89 (data not shown) for a first-order line fit. On the other hand, the dataset with T_m from the first DSC scan *vs* thermal stability had much lower R^2 (0.53) for a first-order line fit (Figure 4(a)). Furthermore, it was also found that an exponential line fit showed an improved reliability for the dataset with the T_m from the second DSC scan *vs* thermal stability (R^2 = 0.95), as shown in Figure 4(b). This implied that the thermal stability of retinol decreased significantly when the T_m of the polyester was close to the test temperature (40 °C). This was futher confirmed from the result that the dataset with the T_m of the nanoparticles *vs* thermal

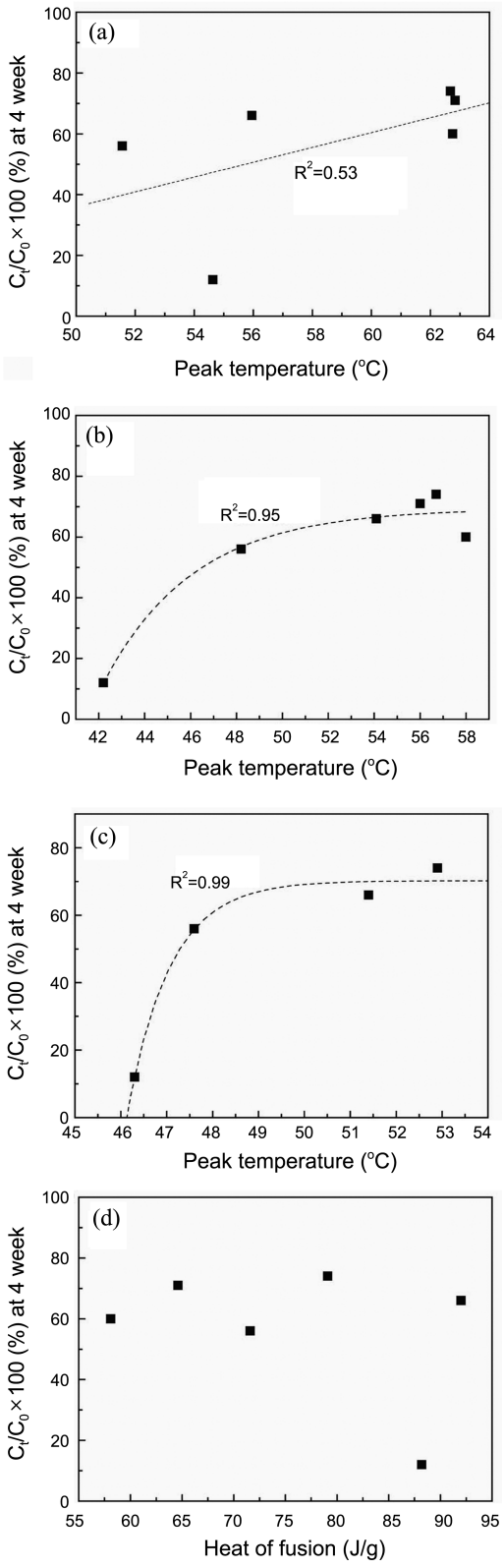

Figure 4. Plots for the thermal stability of retinol at 40 °C with respect to various parameters: (a) the thermal stability *vs* T_m obtained from the first DSC scan of polyesters; (b) the thermal stability *vs* T_m obtained from the second DSC scan of polyesters; (c) the thermal stability *vs* T_m of the nanoparticles (right side peaks in the thermograms shown in Figure 3c of the nanoparticles); and (d) stability *vs* heat of fusion of polyesters (ΔH) during the second scan. The dashed lines were regression fits for the experimental data.

stability of retinol showed a good relationship (R^2 = 0.99) with an exponential fitting line (Figure 4(c)). Meanwhile, H did not seem to have a good correlation with the retinol (Figure 4(d)).

Discussion on the Factors Affecting the Thermal Stability of Retinol. The retinol molecules are degraded by oxygen, hydroxyl group, or their radicals, light, and heat.[33,34] Therefore, finding factors affecting the stability of retinol in the polyester nanoparticles is of primary importance: this enables us to tailor the nanoparticles for an effective protection of the retinol from these degradation factors. Several factors may influence the thermal stability of the retinol in the polyester-retinol nanoparticles. Firstly, it is possible to suspect that the nanoparticle sizes could influence thermal stability of retinol because the sizes of the nanoparticles were different from sample to sample (Table 1). However, it was hard to find a certain correlation or trend between the stability of retinol with the nanoparticles size. This indicates that the thermal stability of retinol was not much influenced by such differences in sizes. Secondly, degradation (or hydrolysis) of the polyesters could destruct the structure of the nanoparticles, thereby influencing the thermal stabilities of the retinol. Based on literatures, the aerobic degradation of the PCL and PEA was faster than that of PBA in river water and seawater.[46,47] Furthermore, the purified deionized water (18 mΩ) was used to prepare the polyester-retinol nanoparticles. Under such condition, this factor could not significantly influence the thermal stability of retinol.

The nanoparticles' cores in the polyester-retinol nanoparticles here were mostly composed of the semi-crystalline polyesters. As such, the crystalline structure of the nanoparticles could rely on T_m and/or ΔH of the polyesters. The present results suggested that the stability of retinol was closely connected with the T_m of polyesters (obtained from the second scan) and polyester nanoparticles. It was reported that the crystalline structure of the polyesters microparticles determined the interior structure of particles, and this affected the release behavior of a drug from the particles.[41] Similarly, it was speculated that the different crystalline structure of the nanoparticles could alter the diffusion pathways of the chemical species causing degradation into the particles. The T_m of the polyesters can be influenced by sample preparation condition.[48] Figure 4 and Table 2 showed that the T_m of the polyesters were greatly influenced by the process/preparation history of the polyesters, and the thermal stability of retinol was more correlated with the T_m being reflected by the history rather than the T_m of the as-prepared polyesters. Regarding this effect, the study prepared the polyester-retinol nanoparticles from the polyesters and retinol being solubilized in acetone followed by their precipitation (or crystallization) to form the nanoparticles in acetone/water mixture. The condition is likely close to the polyesters experienced by melting and cooling (crystallization). For this reason, it is suggested that the preparation condition of the polyester-retinol nanoparticles in part affects the T_m of the polyester in the nanoparticles, thereby influencing the thermal stability of retinol in the polyester-

Table 3. Solubility parameters of the polyesters and retinol

Polymer type	Density (g/cm^3)	G	δ^a $(cal/cm^3)^{1/2}$	$\delta_{polyester} - \delta_{retinol}$
PEA	1.18	1418	9.75	1.89
PBA	1.02	1684	8.58	0.71
PHMA	1.13	1950	9.66	1.80
PCL, 10K	1.15	975	9.79	1.93
PCL, 40K	1.15	975	9.79	1.93
PCL, 80K	1.15	975	9.79	1.93
Retinol			7.87	

[a]The solubility parameters of the polyesters were obtained from the Group Contribution Method shown in Equation (1) in the main text. The solubility parameter of the retinol was obtained from the Equation (2) in the main text. In equation (2), ΔH_v and υ of the retinol were obtained from the Chemspider supplied by Royal Society of Chemistry (http://www.chemspider.com/Chemical-Structure.393012.html).

retinol nanoparticles.

Note that the T_m of polyesters from the second scan (Figure 4(b) and Table 2) was only reflected by the process effect (or preparation condition). In addition to the preparation condition, the T_m of the polyester-nanoparticles can be also influenced by the miscibility of polyesters with retinol. When a polyester and retinol showed a good miscibility, the existence of retinol would affect the crystalline structure of the polyester nanoparticles, thereby influencing T_m and ΔH.[49] Conversely, the bad miscibility between the two molecules will less influence the structure of the polyester in the nanoparticles due to their phase separation. Thermodynamically, the miscibility between the polyesters and retinol is predictable by calculating the solubility parameters (δ) of the polyesters and retinol and their differences. The solubility parameters of the polyesters were estimated by using the equation suggested by Small,[50]

$$\delta = \frac{\rho \Sigma G}{M} \quad (1)$$

where ρ is the density of the polymer, M is the molecular weight of the repeating unit in the polymer, and G is the group molar attraction constant derived by Small.[50] δ of the retinol was obtained by using the following equation:[51]

$$\delta = (\Delta H_v / \upsilon)^{1/2} \quad (2)$$

where ΔH_v ($\approx \Delta E_v$) and υ are the enthalpy (or internal energy) of vaporization and the molar volume of the retinol molecules, respectively. Table 3 shows δ of the polyesters and retinol, and their differences ($\delta_{polyester} - \delta_{retinol}$). PBA and the retinol had a small $\delta_{polyester} - \delta_{retinol}$ (< 1), indicating that they were likely to be miscible in the nanoparticle.[51] In contrast, all the other polymers showed a large $\delta_{polyester} - \delta_{retinol}$ (> 1) and hence these polymers were probably phase-separated from retinol in the nanoparticles.

Putting the discussion together, it was suggested that the chemical stability of retinol, being exposed to a high temperature (*e.g.*, 40 °C), in the polyester nanoparticles was exponentially decreased when the T_m of polyesters and polyester nanoparticles approached the test temperature. Especially, the extraordinary low stability of retinol in the PBA-retinol nanoparticles could be explained by a large decreased in the T_m compared with T_m of as-received PBA, and the T_m might be much influenced by both the preparation condition (preparation history) and the good miscibility of PBA with the retinol molecules.

Conclusions

The current study presents the effect of polymer characteristics or types on the thermal stability of the retinol in the polyester-retinol nanoparticles. Results showed that a potential parameter to influence the thermal stability of retinol could be melting temperatures of the polyesters and polyester nanoparticles. The closer T_m of the polyesters approached a test temperature, the lower thermal stability of retinol was observed. From fitting the data with an exponential line, the thermal stability of retinol was expected to decrease significantly with decreasing T_m of polyesters and polyester nanoparticles. It was further suggested that the nanoparticle preparation conditions (or the preparation history) of polyesters and the miscibility of polyester with retinol affected the T_m. In fact, however, due to insufficient analytical tool characterizing the interior structure of the nanoparticles in an aqueous system, much extensive studies were not possible to fully demonstrate the suggestion. Nevertheless, the study provides fundamental and practical information to those who work in pharmaceutics and cosmetics to select and screen an encapsulating material suitable for bioactive drugs.

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