Influences of the content of POA on the properties of poly(sebacic acid-octadecanoic diacid) copolyanhydrides

Yinglei Zhai, Shutao Guo, Anjie Dong, Fengmin Jin, Chaopeng Xie, Jinwei Zhang, Liandong Deng *

School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

**Abstract**

Poly(sebacic acid-octadecanoic diacid) copolyanhydrides (PSAOAs) were prepared by melt polycondensation of sebacic acid (SA) and octadecanoic diacid (OA). PSAOAs were characterized by Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and wide angle X-ray diffraction (XRD). In vitro degradation experiments and SEM micrographs show that the degradation rate of PSAOAs decrease with increasing the content of POA in copolyanhydrides and the erosion process of PSAOAs is neither bulk nor perfect surface erosion but rather has elements of both in phosphate buffer at 37°C. In vitro release experiments and SEM micrographs indicate that the release rate of drug from the drug-loaded PSAOAs discs decreases with increasing the content of POA in copolyanhydrides and the release rate of the hydrophilic drug is greater than that of the hydrophobic one. The results of the investigation suggest that POA can improve the properties of PSA and promote the applications of PSA in biomedicine.

**1. Introduction**

Polyanhydrides are novel biodegradable polymers and their surface-eroding property in aqueous medium makes them desirable for drug controlled release and functional soft tissue substitutes [1–4]. The first application of polyanhydrides as a bioerodible matrix for controlled drug delivery systems was reported by Rosen in 1983 [5]. Since then, polyanhydrides of aliphatic and aromatic diacids have been extensively investigated as useful biomaterials for controlled drug delivery systems [6,7]. For example, polyanhydrides are extensively used in controlled release systems of anticancer agents [8–11], antibiotics [12,13], DNA [14], peptides and proteins [15–18], etc.

Hundreds of polyanhydrides have been synthesized in the last recent 20 years, such as aliphatic polyanhydrides, aromatic polyanhydrides, crosslinked polyanhydrides [19,20], poly(ester anhydride) [21–23], poly(ether anhydride) [24–26], aliphatic-aromatic polyanhydrides [27] and poly(amide anhydride) [28,29], etc. But only poly(sebacic anhydride) and its derivations have been applied in controlled drug delivery systems [30–36]. For example, the Food and Drug Administration (FDA) has approved the use of poly[(1,3-bis(p-carboxy phenoxy)propane)-co-(sebacic anhydride) (P(CPP-SA)) to deliver the chemotherapeutic agent BCNU for the treatment of brain cancer [37].

As reported, the hydrolytic instability of polyanhydrides increases with decreasing the unit chain length. Therefore, the polyanhydrides with long unit chain length are more stable and beneficial to the application in biomedicine. According to the above analysis, we had synthesized POA by melt polycondensation of OA, which is refined from the edible plant lipid [38]. In this paper, we prepared PSAOAs by copolymerization of SA’s prepolyanhydride (PPSA) and OA’s prepolyanhydride (PPOA) to obtain a novel biomaterial with desirable properties. The copolymer was characterized through FTIR, DSC and XRD, and then in vitro degradation and release behaviors were studied at 37°C under phosphate buffer solution (PBS, pH 7.4).
2. Experimental

2.1. Materials

OA (99%) was provided by Kening (Shanghai, China) and recrystallized twice in ethanol. SA was obtained from Damao Chemical Reagent Manufactory (Tianjin, China). Paclitaxel was provided by Hengrui Co. (Shanghai, China). Salicylic acid was purchased from Tianjin First Chemical Reagent (Tianjin, China). Acetic anhydride (99%, Bodi, Tianjin, China), toluene (anhydrous, Kewei, Tianjin, China), ethyl ether (anhydrous, Damao, Tianjin, China), petroleum ether (anhydrous, Kewei, Tianjin, China), chloroform (anhydrous, Kewei, Tianjin, China), and tetrahydrofuran (anhydrous, Bodi, Tianjin, China) were the analytical grade and used as received.

2.2. Preparation of prepolyanhydride

Prepolyanhydride was prepared from the purified diacid monomer (10 g) by refluxing in the presence of excess acetic anhydride (100 ml) at 135 °C for 40 min under nitrogen protection. Acetic acid and excess acetic anhydride were removed under vacuum at 50–60 °C. The hot clear viscous residue was dissolved in 40 ml toluene, then cooled to 0 °C overnight and precipitated with 400 ml of a 1:1 mixture (v/v) of ethyl ether and petroleum ether. The white precipitate was collected by a LD5–2A centrifuge (Beijing Medical Centrifuge Factory, Beijing, China), dried in vacuo at room temperature for 48 h, and stored at −20 °C until used. PPOA or PPSA was prepared by the above method, respectively.

2.3. Preparation of PSAOAs

The desired quantity of PPSA and PPOA was placed into the reactor in an oil bath at 180 °C, under vacuum for 90 min, to perform melt polycondensation [24,39]. The final product was dissolved into chloroform (Beijing Medical Centrifuge Factory, Beijing, China), dried for 48 h in vacuum at room temperature. The drug-load PSAOAs discs were immersed in a culture medium (100 mL PBS (pH 7.4) in incubator shaker (SHZ-88, Jintan Medical Treatment Instruments manufactory, Jiangsu, China)) at 130 r/min and 37 °C for 5 min. The degradation of PSAOA discs was performed in 100 mL PBS (pH 7.4) in incubator shaker (SHZ-88, Jintan Medical Treatment Instruments manufactory, Jiangsu, China) at 130 r/min and 37 °C. The degradation media was replaced by the fresh PBS everyday to maintain the degradation conditions. The erosion rate of PSAOAs was measured by the change of dry weight of the polymer samples as shown in formula (2).

\[
\text{wt} \% = \frac{W_0 - W}{W_0} \times 100\%
\]

(2)

where \(W_0\) is the initial weight of PSAOAs discs, \(W\) is the weight of PSAOAs discs after degradation.

2.4. Characterization of PSAOAs

FTIR spectroscopy (Nicolet MAGNA-IR 560, Bio-Rad, United States) was used to confirm the structure of PSAOAs and their degradation products. The polymer samples were pressed into KBr pellets (1:100 copolymer/KBr ratio) and analyzed with IR data manager software.

DSC measurements were carried out with Diamond DSC (Perkin–Elmer Co., USA). All the measurements were carried out at a heating (cooling) rate of 10 °C/min from −50 °C to 200 °C.

XRD patterns were recorded with graphite-filtered Cu Kα radiation produced with a X’Pert PRO diffractometer (PANalytical Co., Holland). All the samples were measured under the voltage of 20 KV, the electrical current of 20 mA, the scan range of 3–60° and the scan interval of 0.0334°.

The microstructure of PSAOAs samples was investigated by scanning electron microscopy (SEM). For the SEM studies, the dried samples were fixed in a split specimen mount. Due to brittle enough, the upper part of the sample could be broken off with tweezers. Then, the samples were sputter-coated for several minutes with gold at room temperature, then placed on a stage and observed using a XL30 ESEM (PHILIPS).

2.5. In vitro degradation

The PSAOAs discs (200 mg in weight, 13 mm in diameter and 1.0 mm in thickness) were prepared by compression molding from PSAOAs powder with a press (769YP-24B, Tianjin KeQi New Technology Co., China) at 40 MPa and room temperature for 5 min.

The degradation of PSAOA discs was performed in 100 mL PBS (pH 7.4) in incubator shaker (SHZ-88, Jintan Medical Treatment Instruments manufactory, Jiangsu, China) at 130 r/min and 37 °C. The degradation media was replaced by the fresh PBS everyday to maintain the degradation conditions. The erosion rate of PSAOAs was measured by the change of dry weight of the polymer samples as shown in formula (2).

2.6. In vitro release of drug-loaded PSAOAs discs

The drug and PSAOAs were dissolved in chloroform and dried for 48 h in vacuum at room temperature. The drug-loaded discs (200 mg in mass, 13 mm in diameter and 1.0 mm in thickness) were prepared by the method mentioned in Section 2.5.

The drug-loaded PSAOAs discs were immersed in a conical flask containing 50 ml PBS (pH 7.4). In vitro release
was preformed in an incubator shaker at 130 r/min and 37 °C. At the appropriate time intervals, 50 ml PBS was removed to measure the amount of drug released from the drug-loaded PSAOAs discs. After the removal of each 50 ml PBS, a fresh PBS of 50 ml was supplemented. The standard curve of salicylic acid was calibrated by WFZ-26A UV–vis spectrophotometer (Tianjin Science Instrument Plant, China) at 296 nm. The standard curve of paclitaxel was calibrated by HPLC (Agilent 1100, USA).

Salicylic acid and paclitaxel were used as the model drugs. The amount of salicylic acid in the release medium was measured by UV.

The amount of paclitaxel in the release medium was determined by HPLC. The release medium was injected into a HPLC with a GEM ODS-2 (250 mm x 4.6 mm) C18 column. The mobile phase, composed of acetonitrile and water (60:40, v/v), was performed at a temperature of 30 °C and at a flow rate of 1.0 ml/min. The paclitaxel peak was detected at 254 nm.

The cumulative release was calculated as follows:

\[ R = \frac{\sum n C_i m_{\text{drug}}}{m_{\text{drug}}} \]  

where \( R \) is the cumulative release (%), \( V \) is the sampling or initial volume (50 ml), \( C_i \) is the drug concentration (\( \mu \)g/ml), \( i \) and \( n \) are the sampling times, and \( m_{\text{drug}} \) is the mass of drug in the drug-loaded copolyanhydride discs (\( \mu \)g).

3. Results and discussion

3.1. Characterization of PSAOAs

The FTIR spectra of PSAOAs are illustrated in Fig. 1 and Table 2. The spectra of PSAOAs present the characteristic peaks of PSA or POA. The peaks at 2935–2915 cm\(^{-1}\) and 2854–2840 cm\(^{-1}\) correspond to the methyl and methylene vibrations. The peaks at 1826–1817 cm\(^{-1}\) and 1748–1738 cm\(^{-1}\) are the characteristic peaks of anhydride bonds. The C–O–C stretching band appears at 1074 cm\(^{-1}\). Disappearance of carboxylic hydroxyl band between 3500 cm\(^{-1}\) and 3000 cm\(^{-1}\) and carboxylic carbonyl band at 1704 cm\(^{-1}\) shows that all of carboxylic groups change into anhydride linkages.

The thermal properties of PSAOAs, such as the melting point \( T_m \), crystallizing point \( T_c \), enthalpy of melting \( \Delta H_m \) and enthalpy of crystallizing \( \Delta H_c \), were determined by DSC, and the results are shown in Table 3. There are a melting point and a crystallizing point, \( T_m \) during second run is slightly greater than that during the first but \( \Delta H_m \) during second run is lower than that during the first. It also can be seen from Table 3 that \( T_c, T_m, \Delta H_c \) and \( \Delta H_m \) of PSAOAs increase with increasing the content of POA, which is due to that the crystallization degree of POA is higher than that of PSA [38].

XRD method was used to investigate the crystallizability of PSAOAs. As shown in Fig. 2, PSA has four middle-intensity characteristic crystalline peaks at 2\( \theta \) values of 19.4°, 20.9°, 23.2° and 25.5°. With increasing the content of POA, there is not any variety in the angle of the crystalline peaks, but the intensity of the crystalline peak at 2\( \theta \) value of 20.9° is enhanced and the intensity of the other crystalline peaks are reduced, which suggests that PSA is of better compatible with POA in the crystalline domain. However, the chain length of OA is longer than that of SA, so the chain of POA is more flexible and regular. Flexibility and regulation give rise to the change in the crystallizability of POA. Therefore, with the increment of the content of POA, the crystallization degree of PSAOAs increases, which makes \( T_c, T_m, \Delta H_c \) and \( \Delta H_m \) increase.

**Table 2**

<table>
<thead>
<tr>
<th>Copolymers</th>
<th>Peaks</th>
<th>C–H</th>
<th>C=O</th>
<th>C–O–C</th>
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</thead>
<tbody>
<tr>
<td>PSA</td>
<td></td>
<td>2935</td>
<td>2854</td>
<td>1823</td>
</tr>
<tr>
<td>PSAOA1</td>
<td></td>
<td>2931</td>
<td>2840</td>
<td>1826</td>
</tr>
<tr>
<td>PSAOA2</td>
<td></td>
<td>2920</td>
<td>2850</td>
<td>1820</td>
</tr>
<tr>
<td>PSAOA3</td>
<td></td>
<td>2915</td>
<td>2815</td>
<td>1817</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Process</th>
<th>PSA</th>
<th>PSAOA1</th>
<th>PSAOA3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_m (°C) )</td>
<td>( T_c (°C) )</td>
<td>( \Delta H_m (J/g) )</td>
</tr>
<tr>
<td>First run</td>
<td>68.4</td>
<td>54</td>
<td>117</td>
</tr>
<tr>
<td>Cooled</td>
<td>-</td>
<td>-101</td>
<td>-</td>
</tr>
<tr>
<td>Second run</td>
<td>71.4</td>
<td>-</td>
<td>-101</td>
</tr>
</tbody>
</table>
3.2. In vitro degradation of PSAOAs

The degradation is an important character for biomaterials. In this paper, the *in vitro* degradation of PSAOAs in 100 mL PBS (pH 7.4) at 37 °C was evaluated. In the degradation process, polyanhydride can degrade to diacids, which changes the pH of the degradation media. We have systematically investigated the change of degradation media’s pH in the process of the degradation in previous paper [38]. Therefore, the degradation media was completely replaced with fresh PBS (pH 7.4) every day to make sure the pH of PBS unchanged [40].

FTIR spectra of PSAOA2 before and after degradation are shown in Fig. 3. After degradation, the wavenumbers of the characteristic bands of PSAOA2, such as the methylene-characteristic bands, the anhydride bond-characteristic bands, the C–O–C stretching band, hardly vary. However, the strong carboxylic hydroxyl band appears between 3500 cm⁻¹ and 2500 cm⁻¹ and the strong carboxylic carbonyl band appears at 1701 cm⁻¹ and their intensities become stronger with prolonging the degradation time. The intensities of the anhydride bond-characteristic bands at 1815 cm⁻¹ and 1742 cm⁻¹ and the C–O–C stretching band at 1101 cm⁻¹ obviously become weaker. The above phenomena illuminate that the content of carboxylic groups in PSAOA2 sample gradually increases and the number of anhydride bonds in PSAOA2 sample gradually decreases in the degradation process.

The degradation rate was determined by weight loss of the polymers in the degradation process. As shown in Fig. 4, with increasing the content of POA, the degradation rate of PSAOAs is reduced. After degradation for 13 days, the weight loss of PSAOA3 is only 68.9%. However, the weight loss of PSA is greater than 97.6% after degradation for 5 days. With increasing the content of POA, the crystallization degree and hydrophobicity of PSAOAs are enhanced, which makes the degradation rate slow. It can be also seen from Fig. 4 that PSAOAs undergo surface front erosion due to the linearity of weight loss to hydrolytic degradation time. For example, the degradation profile of PSAOA3 can be fitted to formula (4).

\[
Y = 5.5134X
\]

Of which R is 0.987.

In order to see how the microstructure changes during erosion, the outer surface and cross section of PSAOA2 samples before and after 3 days erosion were observed by SEM as shown in Fig. 5. After erosion for 3 days, the smooth surface (Fig. 5A) of the PSAOA2 sample becomes very rough as shown in Fig. 5B. Similar to the investigation of Langer [41], there are three different layers as sandwich of the cross section of eroded PSAOA2 samples in Fig. 5C: a middle layer where the polymer is non-eroded as shown in Fig. 5D and two outer layers where the buffer has already eroded the polymer as shown in Fig. 5E. The outer eroded layers consist of loosely associated polymer plates separated by large pores. It is obvious that the polymer has changed from non-porous bulk to highly porous material due to the faster erosion in amorphous domains than that...
in crystalline domains. These phenomena are consonant with the polyanhydride degradation model mentioned by Kipper and Narasimhan [42]. When the surface of polymers is eroded, the amorphous domains are first dissolved in water, which makes the crystalline domains emerge in water. The erosion process is restrained until the crystalline domains are decomposed and dissolved in water. According to the Kipper’s degradation model and the results of degradation experiments, we think that the erosion process of PSAOAs is neither bulk nor perfect surface erosion but rather a combination of both. The moving erosion front is characteristic of surface erosion whereas the remaining porous shell stems from bulk erosion.

3.3. In vitro release of drug

In order to investigate the influences of the content of POA on in vitro drugs release from the drug-loaded PSAOAs discs, salicylic acid and paclitaxel were respectively used as model drugs. We have noticed that the HPLC retention times of free and released paclitaxel are consistent, and the total amount of paclitaxel in residual carrier and delivered media equals to that of fed in. Moreover, Domb has also used model drug with similar structure to investigate the drug-loaded efficiency of polyanhydride [40]. Therefore, the two model drugs used here are feasible. The release results are shown in Figs. 6 and 7.

With increasing the content of POA, either paclitaxel or salicylic acid, the release rates of drug from the drug-loaded PSAOAs discs decrease. This result is consistent with the relationship between the degradation rate and the content of POA. The enhancement of the crystallization degree and hydrophobicity of PSAOAs makes the release rate of drug reduce.

It can be also seen from Figs. 6 and 7 that the release rate of salicylic acid is higher than that of paclitaxel when
The carrier is a kind of polyanhydride. For example, when PSAOA2 was used, the cumulative release of salicylic acid is 76.0% after 9 days, but for paclitaxel, it is only 60.7%. Salicylic acid is a kind of hydrophilic drug but paclitaxel is a lipophilic one. The strong hydrophilicity of salicylic acid makes itself dissolve rapidly in water, which makes the crystalline domains of copolyanhydrides emerge in water. The increment of the degradation rate is beneficial to the release of salicylic acid. However, the strong hydrophobicity of paclitaxel further restrains the degradation of copolyanhydrides. Therefore, the release rate of paclitaxel is lower than that of salicylic acid.

In order to further investigate the microstructure of PSAOA2 during in vitro release, the outer surface and cross section of PSAOA2 discs after in vitro release of 3 days were characterized by SEM and the results are shown in Fig. 8. After in vitro release of 3 days, the outer surface of PSAOA2 discs loading salicylic acid is much rougher than that of PSAOA2 discs loading paclitaxel. As can be seen, the outer layers of the cross section of PSAOA2 discs loading salicylic...
acid contain a lot of large pores. However, the outer layers of the cross section of PSAOA2 discs loading paclitaxel are relatively smooth. The results of SEM indicate that the above explanations on in vitro release of drug are reasonable.

4. Conclusion

PSAOAs were prepared by melt polycondensation of SA and OA. The results of FTIR show that all the carboxyl groups change into anhydride bonds. Compared to PSA, $T_m$, $T_c$, $D_{Hm}$ and $D_{Hc}$ of PSAOAs become higher with increasing the content of POA. The degradation rate of PSAOAs depends on the content of POA. With increasing the content of POA, the degradation of PSAOAs becomes slow. The erosion process of PSAOAs is neither bulk nor perfect surface erosion but rather has elements of both. The in vitro release rate of drug decreases with increasing the content of POA and the release rate of salicylic acid is greater than that of paclitaxel from drug-loaded PSAOAs discs. The above results suggest that PSAOAs can be applied in biomedicine.

Acknowledgements

This work had been supported by Tianjin Municipal Natural Science Foundation Key Project (08JCZDJC17200), Tianjin Municipal Natural Science Foundation (08CYFJOC1800), National Nature Science Foundation of China (30672554) and Programme of Introducing Talents of Discipline to Universities (B06006).

References