The polymer-alloys method as a new preparation method of biodegradable microspheres: principle and application to cisplatin-loaded microspheres

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Abstract

The new preparation method for multi-reservoir type microspheres was investigated on the basis of the phase separation of poly(ε-lactic acid) (PLA) and poly(ε-lactic-co-glycolic acid) (PLGA). Phase diagram study suggested that, in the PLA-PLGA-methylene chloride ternary system, the distinctive phase separation occurred when the total polymer concentration exceeded a critical level. The critical polymer concentration inducing this phase separation was found independent of the PLA/PLGA ratio but highly dependent on the solvent species, molecular weight, or lactide/glycolide ratio of PLGA. When various amino acid powders were added to the resultant biphasal polymeric solution, each powder was distributed either to the PLGA-rich phase or to the PLA-rich phase depending on its solubility parameter. It was also found that cisplatin powder was completely distributed in the PLGA-rich phase. Utilizing these findings, the preparation of the multi-reservoir type microspheres of cisplatin was tried. The PLGA-PLA biphasal polymeric solution dispersing the drug powder was emulsified, and then solidified by the solvent evaporation method. Microscopic observation proved that the obtained microspheres have the unique ‘polymer alloys’ structure and the drug was distributed in the internal phase (PLGA-rich phase). The encapsulation efficiency was almost 100% at 10% loading. The in vitro dissolution study revealed that the release of cisplatin lasted 45 days without initial burst. © 1997 Elsevier Science B.V.

Keywords: Poly(ε-lactic acid); Poly(ε-lactic-co-glycolic acid); Multi-reservoir type microspheres; Polymer alloys; Cisplatin

1. Introduction

Over the past decade, various studies have been made on the development of controlled-release systems for the delivery of a variety of biologically active agents. Microspheres of biodegradable polymers, such as poly(DL-lactic acid) (PLA), and poly(ε-lactic-co-glycolic acid) (PLGA), have received much interest for the development of injectable or implantable systems, because they have the potential...
to achieve a suitable duration of drug release and to provide a good patient acceptability.

Various technologies have been proposed so far for fabricating microspheres, which include solvent evaporation [1,2], phase-separation [3], and spray-drying [4,5]. Of those, the solvent evaporation of oil-in-water (O/W) emulsion is the method most widely used because of its relative ease in processing. However, this conventional method sometimes brings about the low encapsulation efficiency of a water-soluble drug and the fast release occurring at an early time (often referred to as the ‘initial burst’). These problems usually depend on the solubility of the drug incorporated, the size of the microsphere and the distribution of drugs in the microsphere.

To overcome these problems, the preparation of reservoir-type microspheres has been tried. Iwata et al. reported the preparation of multi-phase microspheres, in which the drugs were incorporated in a lipid emulsion of internal phase [6]. Pekarek et al. also prepared the double-walled microspheres utilizing the phase separation of a polymer solution in which two immiscible polymers were dissolved in a mutual solvent [7].

It was already found by Dobry and Boyerkawenoki that two polymers in an organic solvent spontaneously separated into two solution phases [8]. Presumably, this phenomenon can be applied to PLA and PLGA, and the polymer mixture could be a PLA-rich phase and a PLGA-rich phase under the specified condition. In this system, if drug is predominantly distributed to the internal polymer phase, a new multi-reservoir type of microspheres with ‘polymer alloys’ structure is realized, by which the drug release characteristic can be modified. The emulsifying of this ‘biphasal polymeric solution containing powder’ and subsequent solvent evaporation could provide the microspheres with powder localized within [9].

The final goal of the present study is to develop a new preparation method for multi-reservoir type microspheres based on the in situ phase-separation of PLA and PLGA. In this paper, using cisplatin as the model drug, the preparation method and release characteristics of the microspheres are demonstrated. Also the factors affecting the incorporation of cisplatin and in vitro release behavior characteristics are discussed.

2. Materials and methods

2.1. Materials

PLA (molecular weight 10 000, 15 000, 20 000) and PLGA (molecular weight 10 000, 15 000, 20 000; lactide/glycolide ratio, 50/50) were purchased from Wako Pure Chemical Industries Ltd., Japan. Cis-dichlorodiammineplatinum (II) (cisplatin) was obtained from Aldrich Chemical Company Inc., Germany. Poly(vinyl alcohol) (EG-40) was obtained from Nihon Synthetic Chemical Industries Ltd., Japan. Amino acids were obtained from Tanabe Seiyaku Co. Ltd., Japan. All other materials or solvents were of reagent grade.

2.2. Phase diagram

The mixture of PLA and PLGA with various ratios was dissolved in methylene chloride, acetonitrile, or ethyl acetate. The solvent was slowly evaporated at room temperature, and the polymers’ concentration was calculated when the phase separation was observed.

2.3. Identification of polymer composition

One hundred mg of the polymer mixture (PLA/PLGA=4/1) were dissolved in 200 mg of methylene chloride to make a polymeric solution containing fine coacervate droplets. A few drops of the solution were cast on a potassium bromide tablet with a diameter of 10 mm and a flat surface and dried at room temperature. The polymer composition of the internal phase and the external phase of the film were determined using a Fourier transformed infrared spectrometer (FTIR) installed with a microscope (FT-153, HORIBA Ltd. Japan), with the focus on each phase.

2.4. Localization of drug in the PLA-PLGA biphasal polymeric solution

Polymers (PLA/PLGA=4/1) were dissolved in methylene chloride, acetonitrile, or ethyl acetate to the concentration of 50% (v/v), which is the concentration when the phase separation can be observed in all these solvents. Glycine, alanine, valine,
or leucine powders were then dispersed in this solution. The localization of these drugs was observed with an optical microscope (DIAPHOT, Nihon Kogaku Ltd., Japan). The solubility parameters of solvents reported by Hansen [10] were used. The solubility parameters of amino-acids were calculated by Fedor’s method [11], the solubility parameters of PLA and PLGA were measured by the turbidimetric titration [12], and the solubility parameter of cisplatin was estimated by the solubility in the mixtures which varied the ratio of acetonitrile and ethyl alcohol.

2.5. Preparation of microspheres

Cisplatin microspheres were prepared basically according to the O/W emulsion solvent evaporation method. Briefly, 600 mg of PLA were dissolved in 1.0 g of methylene chloride to make ‘solution A’; 100 mg of pulverized cisplatin crystals (mean diameter: 1 μm) were dispersed in 800 mg of the solution consisting of PLGA (300 mg) and methylene chloride (500 mg) to make ‘solution B’. The ‘solution A’ and ‘solution B’ were then mixed and the mixture was stirred by a homogenizer (Polytron, Kinematica Ag Littau, Switzerland) for 30 s at 12 000 rev./min. The resultant was added into 400 ml of the water containing poly(vinyl alcohol) at a concentration of 0.5% (w/v) and emulsified using the Polytron homogenizer, at 12 000 rev./min for 5 min at 15°C, then the temperature was gradually raised up to 30°C to remove the solvent. The hardened microspheres were washed with water and filtered. The obtained microspheres were dried under vacuum overnight. A total of 3–4 batches were prepared for each prescription.

2.6. Determination of size distribution of cisplatin and microspheres size

The size distribution of pulverized cisplatin crystals and microspheres were determined using a laser diffraction particle size analyzer (SALD-1100, Shimadzu Co. Ltd., Japan). Ethanol and water were used as the dispersing medium for cisplatin and microspheres, respectively.

2.7. Microscopic observation

The localization of cisplatin in the PLGA-PLA- methylene chloride biphasal polymeric solution was observed using an optical microscope (DIAPHOT, Nihon Kogaku Ltd., Japan). The cross-sections of microspheres were prepared as follows: the microspheres were suspended in a 10% (w/v) poly(vinyl alcohol) solution; the suspension was then dried under vacuum; and the obtained PVA block was sliced. A piece of the cross-section, containing the slice of microspheres, was observed with a scanning electron microscope (S-2250N, Hitachi Ltd., Japan).

2.8. Determination of cisplatin content

Microspheres (5–10 mg) were extracted with a mixture of methylene chloride (2 ml) and 0.01 N HCl (5 ml) for 30 min. The aqueous layer was assayed for the platinum concentration at 265.9 nm using a flameless atomic absorption spectrophotometer (Z-9000, Hitachi Ltd.). The cisplatin content in microspheres was calculated from the platinum concentration.

2.9. In-vitro release study

About 10 mg of microspheres were weighed out in 8 test tubes. The test tubes were filled with 10 ml of phosphate buffered saline (PBS, 0.15 M, pH 7.4) and then stirred at 25 rev./min in an air chamber thermostatted at 37±1°C. Each test tube was taken out at the predetermined day interval. The microspheres were collected by filtration, washed with 0.1 N HCl and 0.01 N HCl, and analyzed for residual cisplatin by the method described above.

3. Results and discussion

3.1. Formation of PLA-PLGA polymer-alloys

In the field of polymer science, ‘polymer-alloys’ is defined as the polymer mixture where one polymer phase is dispersed in another polymer phase [13]. Mixing of PLA and PLGA organic solutions induced a phase separation under a certain condition, and finally formed a polymer-alloys structure. The typi-
Fig. 1. Optical micrograph of a typical phase separation observed in the PLGA-PLA-methylene chloride ternary system. The molecular weights of both polymers were 20 000.

A typical example of PLA-PLGA phase separation is demonstrated in Fig. 1. When the PLGA-methylene chloride solution was added into the PLA-methylene chloride solution, a large number of small droplets (PLGA-rich phase) were formed in a continuous phase (PLA-rich phase).

It was found that the phase separation always occurred when the total concentration of both polymers exceed a critical level, independent of the mixing ratio of the polymers. This phase separation could be influenced by various physicochemical factors, including solvent species, polymer concentration, molecular weight, and lactide/glycolide ratio in a PLGA polymer chain. In fact, as is shown in Table 1, the critical concentration of polymers inducing phase separation (CCp) obtained in different polymer-solvent systems largely varied in accordance with the solvent species. It can be assumed that the CCp value depends upon the solubility parameter of the solvent. Acetonitrile, for instance, which has a higher solubility parameter than methylene chloride, required a higher concentration of

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Solubility parameter of solvents</th>
<th>CCp % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>11.75</td>
<td>51.1</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>9.93</td>
<td>25.5</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>9.10</td>
<td>19.2</td>
</tr>
</tbody>
</table>

The molecular weights of PLA and PLGA used in this experiment were 20 000.

*The values were reported by Hansen [10].
polymers than methylene chloride to induce the phase separation. Meanwhile, ethyl acetate, which has a lower solubility parameter than methylene chloride, required lower concentration of polymers than methylene chloride.

Fig. 2 shows the effect of molecular weight ($M_w$) of polymer on CCp. In this experiment, $M_w$ of either polymer of PLA or PLGA was fixed at 20 000, and $M_w$ of the counterpart was varied from 5000 to 20 000. In both cases, larger $M_w$ resulted in lower CCp, meaning that the phase separation more easily occurs. This tendency is presumably due to the fact that the polymer with higher $M_w$ generally shows a higher mixing entropy.

3.2. Identification of polymer composition in the internal phase and the external phase

Each phase in the PLA-PLGA-methylene chloride biphasal polymeric solution system was analyzed for polymer composition using the FTIR method (Fig. 3). Notable differences in the IR spectrum between PLGA and PLA standard samples were observed in the range from 1350–1500 cm$^{-1}$; PLA showed a C-H bending vibration of methyl group at 1450 cm$^{-1}$ and 1380 cm$^{-1}$, while PLGA showed a C-H bending vibration of methyl group at 1460 cm$^{-1}$ and 1400 cm$^{-1}$, and a C-H vibration of methylene group at 1430 cm$^{-1}$. The IR spectrum of the internal phase in the PLA-PLGA-methylene chloride ternary mixture exhibited a strong absorbance at 1430 cm$^{-1}$, whereas that of the external phase exhibits only a very small peak at the same position. The transmittance ratio of two bands at 1460 cm$^{-1}$ and 1430 cm$^{-1}$ of the internal phase gave a higher value than that of PLGA alone. From these results, it can be said that the external phase mainly consisted of PLA but containing a very small portion of PLGA (PLA-rich phase). On the contrary, the internal phase mainly consisted of PLGA but containing a very small portion of PLA (PLGA-rich phase).

3.3. Distribution of drug in the PLGA-PLA biphasal polymeric solution

When various drug powders were dispersed into the PLGA-PLA biphasal polymeric solution, the drug powder was found to be distributed predominantly to either polymer phase. Such an interesting phenomenon could be explained along the distribution theory [14], in which the distribution of a drug substance between two phases is represented as follows:

![Fig. 2. Effect of molecular weight of polymers on CCp.](image)

![Fig. 3. FTIR spectrum of PLGA-PLA-solvent ternary system.](image)
\[ \frac{[X_a]}{[X_b]} = V_s \left( \frac{(\delta_a - \delta) - (\delta - \delta_b)}{2.3RT} \right)^2 \]  

where \([X_a]\) and \([X_b]\) are concentrations of drug substance S in A phase and B phase, respectively; \(\delta_a\), \(\delta_b\) and \(\delta\) are solubility parameters of A phase, B phase and substance S, respectively; \(V_s\) is the molecular volume of substance S; \(R\) is the gas constant; and \(T\) is absolute temperature. This equation means that the distribution of substance S varies depending on the solubility parameters of A phase and B phase. In the present situation, however, since substance S always exists as a solid state, \(V_s\) is considerably large so that the distribution of substance S leans to one side.

To examine the applicability of Eq. (1) to predict the distribution of drug substance in the PLA-PLGA biphasal polymeric solution, 5 amino acids with different solubility parameters are selected as the model compound. If PLGA and PLA separated completely, solubility parameters of the PLGA phase \(\delta_{PLGA/p}\) and PLA phase \(\delta_{PLA/p}\) were calculated by the following equations:

\[
\delta_{PLGA/p} = \alpha \delta_{PLGA} + (1 - \alpha) \delta_{solv} \]  

\[
\delta_{PLA/p} = \beta \delta_{PLA} + (1 - \beta) \delta_{solv} \]

where \(\alpha\) and \(\beta\) are volume fractions of PLGA and PLA, respectively. \(\delta_{PLGA}\), \(\delta_{PLA}\) and \(\delta_{solv}\) are solubility parameters of PLGA (11.00), PLA (10.69) and solvent.

Table 2 summarizes the calculation results of the solubility parameter difference \(\left[ (\delta - \delta_{PLA/p})^2 - (\delta_a - \delta_{PLGA/p})^2 \right]\) and the location where the drug was actually distributed in each solvent system. As is shown, the distribution of drugs was dependent on the solubility parameter difference; that is, in case of a positive value, drugs had a tendency to be distributed on the PLGA phase; in case of a negative value, drugs had a tendency to be distributed on the PLA phase; and when the value was close to zero, drugs had a tendency to be distributed on the interface between PLGA and PLA phase. In this experiment, however, some drugs with small positive values (0.09–0.3) still distributed into the PLGA phase. Probably, this is caused by the fact that the calculated values of \(\delta_{PLGA/p}\) and \(\delta_{PLA/p}\) can deviate to some extent from the real values because PLGA and PLA do not seem to separate completely in this system. Referring to the values of drugs which distributed on the interface between PLGA and PLA phase, this deviation was estimated as around 0.2–0.3. Nevertheless, those results suggest that we can

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### Table 2

Distribution of amino-acids in PLGA-PLA-solvent biphasal solution systems

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Drugs</th>
<th>Distribution</th>
<th>Solubility parameter difference$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>Histidine (14.12)$^a$</td>
<td>PLGA phase</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>Glycine (13.02)</td>
<td>PLGA phase</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Alanine (12.01)</td>
<td>PLGA phase</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Valine (10.95)</td>
<td>Interface</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Leucine (10.68)</td>
<td>Interface</td>
<td>0.22</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>Histidine</td>
<td>PLGA phase</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>PLGA phase</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Alanine</td>
<td>PLGA phase</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>PLGA phase</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>PLGA phase</td>
<td>0.09</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Histidine</td>
<td>PLGA phase</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>PLGA phase</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Alanine</td>
<td>Interface</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>PLGA phase</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>PLGA phase</td>
<td>-0.19</td>
</tr>
</tbody>
</table>

$^a$Solubility parameter of drugs.

$^b\gamma(\delta_{PLA/p} - \delta) - (\delta - \delta_{PLGA/p})^2$. 

predict which phase the drug can be distributed to by Eq. (1).

3.4. Application of polymer-alloys method to the cisplatin-loaded microspheres

Cisplatin is known to be one of the most effective anticancer drugs in current chemotherapy. However, due to various side effects (renal disturbances, nausea, vomiting etc.), a lot of care has been paid in its clinical use. To minimize the side effects and to increase the therapeutic effects, it must be possible to control the blood level of the drug to the therapeutic level for a long period of time.

Cisplatin was predicted to be distributed in the PLGA phase from its solubility parameter (≈12.2). Actually, this was proven by the fact that the drug was merely incorporated in the PLGA-rich phase when cisplatin powder was added to the PLGA-PLA biphasal polymeric solution. Fig. 4 demonstrates the distribution of cisplatin fine crystals in various PLGA-PLA-methylene chloride ternary systems. The distribution of the drug was highly dependent on the mixing ratio of polymers. When the PLGA/PLA ratio was given as 20/80 or 33/67, cisplatin was partitioned to the inner phase (PLGA-rich phase). On the other hand, when the PLGA ratio increased up to 67 or 80%, due to accompanying the phase inversion, the drug powder was distributed in the continuous phase.

This unique configuration suggested the possibility of development of a new type of microsphere with a ‘polymer-alloys’ structure. Thus, we tried to prepare a multi-reservoir type cisplatin-loaded microsphere based on this principle. The preparation procedure includes a phase-separation, an O/W type emulsification, and a solvent evaporation process.

The morphological feature of the cisplatin-loaded microspheres obtained is shown in Fig. 5. Microscopic observation revealed that the structure of the microspheres is the multi-reservoir type, in which the cisplatin crystals are distributed exclusively in the inner small particles. The microspheres had smooth surfaces and no pit-holes were found thereon. The mean size of microspheres was about 30 μm and ~90% were in the range of 10–100 μm. Aggregation was not found through the whole preparation process.

The in vitro drug release behaviors of three microsphere preparations are compared in Fig. 6. About 90% cisplatin were released from the PLGA microspheres for 30 days. The PLA microspheres

![Fig. 4. Optical micrographs showing the distribution of cisplatin powder in the biphasal polymeric solution. (A) The PLGA/PLA ratio was 33/67% (w/w). (B) The PLGA/PLA ratio was 67/33% (w/w). The molecular weights of both polymers were 20 000.](image-url)
also released about 90% drug for 30 days, but it provided a 4-day lag phase. The microspheres prepared by the polymer-alloys method released the drug over 45 days without initial burst. Encapsulation efficiencies (mean±S.D.) of PLGA, PLA, and polymer-alloys microspheres were 68.6±9.9%, 68.6±3.8%, and 103.8±3.1%, respectively.

Encapsulation efficiency and drug release behavior could be intimately related to the drug distribution in microspheres. When the conventional O/W emulsion method was applied, drugs were uniformly distributed in the microspheres. In this case, however, the drugs existing near the surface of emulsion droplets easily leak into the external water phase during emulsification or the solvent evaporation process, causing a low encapsulation efficiency. Meanwhile, when applying the polymer-alloys method, most of the drugs charged are well retained in the microspheres during emulsification and the subsequent solvent evaporation process, resulting in high encapsulation efficiency. This is because the drug powder does not distribute in the continuous phase but in the inner polymer phase. Furthermore, this unique drug distribution can realize a long-term sustained release of the drug without initial burst.

4. Conclusions

A new technology for the preparation of PLA-PLGA microspheres, the 'polymer alloys method', was established through the present study. The principle of this method involves two physicochemical phenomena; the phase separation of the PLA-PLGA-methylene chloride ternary system and the localization of drug particles in the resultant biphasal polymeric solution. When applying this technology, a multi-reservoir type microsphere, which contains
drug crystals in the internal phase, can be obtained at an excellent encapsulation efficiency. Also, due to such unique drug distribution, this type of microsphere can provide a long-term sustained release without initial burst.

References