Preparation of insulin-loaded PLA/PLGA microcapsules by a novel membrane emulsification method and its release in vitro

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Abstract
Uniform-sized biodegradable PLA/PLGA microcapsules loading recombinant human insulin (rhI) were successfully prepared by combining a Shirasu Porous Glass (SPG) membrane emulsification technique and a double emulsion-evaporation method. An aqueous phase containing rhI was used as the inner water phase (w1), and PLA/PLGA and Arlacel 83 were dissolved in a mixture solvent of dichloromethane (DCM) and toluene, which was used as the oil phase (o). These two solutions were emulsified by a homogenizer to form a w1/o primary emulsion. The primary emulsion was permeated through the uniform pores of a SPG membrane into an outer water phase by the pressure of nitrogen gas to form the uniform w1/o/w2 droplets. The solid polymer microcapsules were obtained by simply evaporating solvent from droplets. Various factors of the preparation process influencing the drug encapsulation efficiency and the drug cumulative release were investigated systemically. The results indicated that the drug encapsulation efficiency and the cumulative release were affected by the PLA/PLGA ratio, NaCl concentration in outer water phase, the inner water phase volume, rhI-loading amount, pH-value in outer water phase and the size of microcapsules. By optimizing the preparation process, the drug encapsulation efficiency was high up to 91.82%. The unique advantage of preparing drug-loaded microcapsules by membrane emulsification technique is that the size of microcapsules can be controlled accurately, and thus the drug cumulative release profile can be adjusted just by changing the size of microcapsules. Moreover, much higher encapsulation efficiency can be obtained when compared with the conventional mechanical stirring method.

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Keywords: SPG membrane emulsification; Double emulsion-solvent evaporation; Poly(lactide); Encapsulation efficiency

1. Introduction
Parenteral administration of insulin formulations has been the primary therapy utilized for the treatment of insulin-dependent diabetes mellitus (IDDM) since insulin’s discovery over 75 years ago [1]. Nevertheless, due to its poor oral bioavailability and short elimination half-life, patients always suffer from frequent injections daily. It is obvious that patient compliance and therapeutic effectiveness can be improved greatly if insulin could be released in a controlled fashion for longer periods. In order to achieve this purpose, encapsulation of insulin within microcapsules for drug delivery system, using biodegradable polymers, has been studied extensively over recent years [2–5]. Among different polymers, poly(D,L-lactide-co-glycolic acid) (PLGA) copolymers and poly(D,L-lactic acid) (PLA) have been extensively used as microparticle carriers in controlled-release delivery systems for many bioactive molecules due to their non-toxic, good bioavailability and biocompatibility, and approval by the Food and Drug Administration for human use [6].

Until now, various techniques are available to entrap water-soluble bioactive agents into biodegradable microcapsules, including double emulsion, organic phase separation, supercritical fluid and spray drying techniques [7,8]. Among them, the w1/o/w2 double emulsion-evaporation technique is the most commonly used method, which was patented by Vrancken and Claey’s [9] in USA in 1970 and by Jaeger and Tavernier [10] in UK in 1971, then developed by Ogawa et al. [11]. However, in the conventional process, the double emulsions are usually prepared by the mechanical stirring, homogenization or ultrasoundation method, the size is difficult to control, and the size distribution of microcapsules obtained is very broad, which will lead to many disadvantages, such as: (1) lower bioavailability of drug-loaded...
microcapsules in vitro because only the microcapsules within a certain size range can be absorbed [12]; (2) difficulty to control drugs’ release behavior precisely; (3) lower drug encapsulation efficiency; (4) poor reproducibility. Furthermore, the microcapsules with a narrow size distribution are necessary in the drug delivery system in order to decrease side effects of the drugs, especially anti-cancer agents, because the accumulated locations of the microcapsules containing anti-cancer agents also depend on the size of the microcapsules [13]. Therefore, it is very important to develop a method, which can provide the uniform-sized microcapsules composed of biodegradable polymers.

To obtain microspheres or microcapsules with a narrow size distribution, we found that the SPG (Shirasu Porous Glass) membrane emulsification technique is a promising technique. In addition to narrow size distribution, SPG membrane emulsification technique, when used to prepare PLA microcapsules, also offers following advantages: (1) the diameter of double emulsion and resultant microcapsules can be controlled easily by adopting the required pore size of the membrane; (2) break-up and coalescence between droplets rarely occur during the emulsification and solidification processes due to the narrow size distribution of the droplets, therefore, the drug will not escape out of the droplet by coalescence and break-up of the droplets, a much higher encapsulation efficiency can be expected; (3) emulsification is carried out with low shear, it is suitable for the encapsulation of shear-sensitive protein and peptide drugs. In a previous study [14,15], we have first prepared uniform-sized PLA microcapsules by combining the novel glass membrane emulsification technique and double emulsion-solvent evaporation method, where we have solved the difficulty that the w1/o primary emulsion was unstable during the emulsification process, and investigated the factors influencing the size distribution of microcapsules. However, the factors affecting the drug cumulative release profile have never been studied systematically when PLA/PLGA was used as the delivery biomaterials and the drug-loaded microcapsules were prepared by the novel membrane emulsification technique. In this study, the primary objective was to prepare uniform-sized protein-loaded PLA/PLGA microcapsules by combining a glass membrane emulsification technique and double emulsion-solvent evaporation method, using recombinant human insulin (rhI) as the model protein, and to realize a higher drug encapsulation efficiency. The second aim of this study was to investigate factors influencing the drug cumulative release profiles in vitro. Three mechanisms for controlling drug release from these polymer matrices have been confirmed [16]: Fickian diffusion through the polymer matrix, diffusion through water-filled pores (aqueous channels) formed by water penetration into the matrix and liberation by erosion of the polymer matrix. The actual release behavior from the polymer matrices has to be controlled by a combination of these three mechanisms and is affected by factors such as the copolymer composition, the NaCl concentration in outer water phase, the inner water phase volume, the drug loading amount, the pH-value in outer water phase, the microcapsules size, and all of these factors will be discussed in detail in the following sections.

2. Materials and methods

2.1. Materials

PLA (Mw = 300K) and PLGA (LAG ratio = 75:25, Mw = 8K) were purchased from Shandong Institute of Medical Instrument (Shandong, China). Recombinant human insulin (rhI) was provided by Gan&Lee Pharmaceutical Co. Ltd. (Beijing, China). Sorbitan Sesquioleate (Arlacel 83) of biochemical grade was purchased from Sigma (St. Louis, USA) and used as an emulsifier in oil phase. Acetic acid, dichloromethane (DCM) and tolune of analytical grade were purchased from Beijing Chemical Reagents Company (Beijing, China). Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%) was provided by Kuraray (Tokyo, Japan) and was used as a stabilizer in outer water phase. All other reagents were of reagent grade and used as received.

2.2. Apparatus

A miniature kit for emulsification with an MPG module (Microporous Glass, a brand name of the glass membrane) installed was purchased from Ise Chemical Company. A schematic diagram of this kit and the detailed emulsification process were given in a previous paper [15]. Membranes with pore size of 1.4, 2.8, 5.2 μm were used in this study, respectively.

2.3. Preparation of PLA/PLGA microcapsules

The PLA/PLGA microcapsules were prepared by combining a membrane emulsification technique and w1/o/w2 double emulsion-solvent evaporation method described previously [15]. A standard recipe is shown in Table 1. Unless specified, 2.5, 5.0, 7.5 or 10.0 mg of rhI was dissolved in 250 μl of 1.0 wt.% acetic acid (pH 2.8) and was used as the inner water phase (w1), 0.125 g PLA, 0.125 g PLGA and 0.25 wt.% Arlacel 83 were dissolved in DCM, a 2.5 ml mixture solvent. Poly(vinyl alcohol) (PVA) was dissolved as the outer water phase (w2). These two solutions were mixed and emulsified by a homogenizer at a rate of 9800 rpm for 50 s to form a w1/o/w2 primary emulsion. The 70 ml of aqueous phase where 0.7 g of poly(vinyl alcohol) (PVA) was dissolved was used as the outer water phase.

<table>
<thead>
<tr>
<th>Phase/Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner water phase (w1)</td>
<td>1.0 wt.% acetic acid (pH 2.8)</td>
</tr>
<tr>
<td></td>
<td>250 μl</td>
</tr>
<tr>
<td></td>
<td>2.5, 5.0, 7.5 or 1.0 mg</td>
</tr>
<tr>
<td>Oil phase (o)</td>
<td></td>
</tr>
<tr>
<td>Mixed solvent (DCM/toluene)</td>
<td>2.5 ml (DCM/toluene v/v = 21/79)</td>
</tr>
<tr>
<td>Oil-soluble emulsifier (Arlacel 83)</td>
<td>6.25 mg</td>
</tr>
<tr>
<td>PLA/PLGA</td>
<td>0.125 g/0.125 g</td>
</tr>
<tr>
<td>Outer water phase (w2)</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>70 ml</td>
</tr>
<tr>
<td>PVA</td>
<td>0.7 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0–5.0 (maximum 5.0 wt. % of water)</td>
</tr>
</tbody>
</table>
water phase (w2). The primary emulsion was permeated through the uniform pores of a SPG membrane (pore size: 2.8 μm) into the outer water phase by the pressure of nitrogen gas to form the uniform-sized droplets. Then, DCM and toluene were evaporated at room temperature for 24 h under a gentle stirring at a rate of 150 rpm. A pressure slightly above the critical pressure, which is defined as a minimum pressure at which the primary emulsion begins to permeate through the membrane into the outer water phase, was applied. After DCM and toluene were evaporated under vacuum, the hardened PLA/PLGA microcapsules were collected by centrifugation and washed with distilled water three times, and then freeze-dried for 48 h. After above purification process, no toluene was detected by gas chromatography. The procedure for preparing microcapsules by the stirring method was similar with the process mentioned above except that the w1/o/w2 double emulsion droplets were yielded by stirring method at a rate of 1000 rpm for 40 s.

2.4. SEM observation of microcapsules

The diameter and surface morphology of PLA/PLGA microcapsules were observed by a JSM-6700F (JEOL, Japan) scanning electron microscope (SEM). The specimens for SEM observation were prepared by mounting sample on metal stubs with double-sided conductive adhesive tape and coating a thin gold film (approximately 60 nm in thickness) on sample under a reduced pressure below 5 Pa with a JFC-1600 fine coater (JEOL, Japan).

2.5. Determination of microcapsules size and size distribution

The dried PLA/PLGA microcapsules were redispersed in distilled water and then measured by a Coulter Multisizer (Coulter LS230, USA). The particle size distribution was expressed by a CV (coefficient of variation) value, which is defined as:

\[
CV = \left( \frac{\sum (d_i - \bar{d})^2}{N} \right)^{1/2} / \bar{d}
\]

where \(d_i\) is the ith diameter, \(\bar{d}\) is the number-average diameter, and \(N\) is the total number of particles counted.

2.6. Measurement of the drug encapsulation efficiency

Total amount of rhI loaded was measured using Peterson-Lowry method [17], after disruption of the microcapsules with 2.0% SDS/0.1 M NaOH solution. Sodium hydroxide catalyzed the hydrolysis of PLA/PLGA, while SDS ensured the complete solubilization of rhI during polymer hydrolysis. The resulting solution was then neutralized by stepwise addition of 1 M HCl. The encapsulation efficiency was calculated from the following formula:

\[
\text{Encapsulation efficiency} (\%) = \frac{\text{Total amount of rhI loaded}}{\text{Total amount of rhI}} \times 100
\]

2.7. In vitro protein release studies

Twenty milligrams of freeze-dried microcapsules, accurately weighted, were placed in a test tube and incubated in 1.5 ml of PBS buffer, pH 7.4 (8 g NaCl, 0.2 g KCl, 0.24 g KH₂PO₄, 1.81 g Na₂HPO₄·H₂O, 0.5 g NaN₃, 0.1 g Tween 20 and 1000 ml distilled water). The samples were agitated in a 37°C incubator-shaker at 120 rpm. At defined time intervals, 1.0 ml of supernatant was collected by centrifugation at 8000 rpm for 5 min and 1.0 ml of fresh PBS buffer was added back. The amount of rhI released was determined by measuring protein concentration in the supernatant using Peterson-Lowry method. Each sample was assayed in triplicate.

3. Results and discussion

3.1. Surface morphology of microcapsules and its size distribution

The surface morphology of the typical PLA/PLGA microcapsules containing rhI was observed by scanning electron microscopy (SEM), and is shown in Fig. 1a. At the optimized

![Fig. 1. SEM photos of the original PLA/PLGA microcapsules (a) and the degraded PLA/PLGA microcapsules (b) prepared by SPG membrane emulsification.](image-url)
condition, all of the microcapsules were spherical in shape with a smooth surface, and its size distribution was rather narrow, the CV value was less than 14.0%. The change of some conditions may not affect the morphology of microcapsules, but had some influence on the size distribution, which will be discussed in detail in the following sections. Fig. 1b shows the SEM photograph of microcapsules after it was incubated in 1.5 ml of release medium for about 1 month. It indicated that the microcapsules were degraded into those with pores, which is favorable for the release of drugs encapsulated.

3.2. Effect of PLA/PLGA ratio

PLA and PLGA are the most investigated and advanced polymers with regard to available toxicological and clinical data. PLA and PLGA are non-toxic, biocompatible and biodegradable polymers approved by the Food and Drug Administration for human use. Here, due to the slow degradation rate of PLA, PLGA with lower molecular weight was added to increase the cumulative release rate of drugs encapsulated, and the effect of PLA/PLGA ratio on both drug encapsulation efficiency and cumulative release profile was investigated. The total amount of PLA and PLGA was 0.25 g. As shown in Fig. 2, the highest drug encapsulation efficiency was obtained when the PLA/PLGA ratio was equal to 1/1, and subsequently decreased with the increase of PLA or PLGA proportion, respectively. This may be related to the characteristics of PLA and PLGA. Because phase-separation between PLA and PLGA was easy to occur, it tended to form microcapsules with double-layer structure, in which PLGA formed the inner-layer wall which faced inner water phase and PLA formed the outer-layer wall [18]. As PLGA is more hydrophilic than PLA, hydrophilic drugs tended to locate in the inner-layer PLGA phase, while the outer-layer PLA phase formed a hydrophobic wall to retard the leakage of drugs, thus the drug encapsulation efficiency was enhanced. When the PLA/PLGA ratio was lower than 1/1, the PLGA inner-layer was thicker, and the distance between the drug located in inner-layer phase and the surface of microcapsules was shortened, which induced the leakage of the drugs into the outer water phase and led to the decrease of drug encapsulation efficiency. Interestingly, that when the PLA/PLGA ratio was higher than 1/1, the drug encapsulation efficiency was also decreased. The reason is still unclear. Usually, there are three main factors which are considered to affect the drug encapsulation efficiency during emulsification and solidification of double emulsion: (1) coalescence between inner water phase and outer water phase, leading to the leakage of the drug into the outer water phase. Therefore, the more stable the inner water phase, the higher drug encapsulation efficiency; (2) coalescence and break-up of double emulsions, also leading to the leakage of drug; (3) diffusion of drug from inner to outer water phase through oil phase. In the process of membrane emulsification, the probability for coalescence and break-up of the double emulsions was little because of their uniform size and stability. Therefore, the reason for the decrease of drug encapsulation efficiency with increase of PLA/PLGA ratio was mainly attributed to the coalescence between inner water phase and outer water phase. This was because the w/o primary emulsion became less stable with PLA/PLGA increased.

The release profiles of the microcapsules prepared with different PLA/PLGA ratio are shown in Fig. 3. The human insulin release profiles were biphasic, showing an initial burst followed by nearly a constant release. It was demonstrated that with the increase of PLGA proportion, the human insulin encapsulated released more rapidly with a higher burst release, mostly due to the rapid degradation of PLGA.

3.3. Effect of NaCl concentration in outer water phase

In the process of preparing microcapsules by double emulsion-solvent evaporation method, NaCl was usually added into the outer water phase to adjust the osmotic pressure difference between the inner and outer water phases of w/o/w emulsion. This different osmotic pressure affects microcapsules formation, the drug encapsulation efficiency and the cumulative release profile. As shown in Fig. 4, the rhI encapsulation efficiency increased from 65.61% to 92.21% when NaCl concentration in outer water phase increased from 0 to 5.0 wt.%.

This trend was similar to the results obtained by Meng et al. [19] on encapsulation of lysozyme. When the osmotic pressure of the outer water phase is greater than that of the inner water phase, it will lead to the microemulsion to be less stable, thus the microcapsules could be formed more easily and the drug encapsulation efficiency was increased.
phase, there would be an influx of water from inner water phase, as a result, the microcapsules are denser and have smaller particle sizes. This in turn depresses the drug’s leakage and is considered to be favorable for the enhancement of the drug encapsulation efficiency. Fig. 5 shows the SEM photographs of the microcapsules with different NaCl concentration in outer water phase. As can be seen, the mean size of microcapsules decreased with increasing NaCl concentration in outer water phase. The results agree well with above discussion.

Due to the influence of the osmotic pressure, NaCl concentration in outer water phase also affects the rhI cumulative release profile. As shown in Fig. 6, the initial release on the first day decreased from 34.43% to 6.33% when NaCl concentration in outer water phase was lower. When NaCl concentration in outer water phase was increased, the drug encapsulation efficiency was decreased. Moreover, increasing the volume of inner water phase also increased the number of internal cave volume of microcapsules, the distance between the drug encapsulated and the surface of microcapsules was shortened, and the drug cumulative release was fastened. In addition, from Fig. 8, it was obvious that increasing the volume of inner water phase led to the formation of a few larger microcapsules. This implied that the coalescence and break-up between the droplets occurred more frequently during the membrane emulsification and solidification process when a large volume of inner water phase was used. Therefore, the drugs escaped out of the droplets easily, and the drug encapsulation efficiency was decreased.

3.5. Effect of rhI-loading amount

Drug loading amount is a key factor influencing not only the drug encapsulation efficiency, but also the drug cumulative release profiles. In the process of membrane emulsification, we found an interesting phenomena that the primary emulsion cannot be pressed through the membrane pores successfully, i.e., the membrane pore was jammed, if the drug loading exceeded a certain amount when only PLA was used as the wall biomaterial, but a rhI released during the first 24 h. The limited loading amount for various proteins

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Limited amount (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>1.0</td>
</tr>
<tr>
<td>BSA</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>rhI</td>
<td>5.0</td>
</tr>
</tbody>
</table>
adding of drug caused the unstability of primary emulsion when only PLA was used, the droplets of primary emulsion coalesced to larger droplets, which can not pass through the pores of the membrane. On the other hand, PLGA was relatively hydrophilic, the primary emulsion was more stable due to the decrease of interfacial tension. Therefore, the coalescence between droplets of primary emulsion was prevented efficiently.

The effect of rhI-loading amount on the encapsulation efficiency and initial release is listed in Table 4. The results showed that when rhI-loading amount increased from 1.0 to 4.0 wt.%, the drug encapsulation decreased obviously from 86.93% to 43.89% and the initial release increased from 3.18% to 44.41%, respectively. A higher rhI-loading amount means higher rhI concentration in the inner water phase of the double emulsion droplets, which increased the concentration gradient of rhI between the inner and outer water phase, thus increasing the amount of rhI diffusing into the outer water phase and its accumulation near the surface of microcapsules.

Fig. 9 shows that the cumulative release profiles was also related to the rhI-loading amount. At higher loading amount, rhI followed a slow and pseudo-linear release after a larger initial burst. However, at lower loading amount, rhI released little by
Fig. 6. Effect of NaCl concentration in outer water phase on the drug cumulative release profile.

Table 4

<table>
<thead>
<tr>
<th>rhI-loading amount (mg)</th>
<th>Encapsulation efficiency (%)</th>
<th>Initial rhI burst* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>86.93</td>
<td>3.18</td>
</tr>
<tr>
<td>5.0</td>
<td>72.71</td>
<td>7.12</td>
</tr>
<tr>
<td>7.5</td>
<td>65.61</td>
<td>34.43</td>
</tr>
<tr>
<td>10.0</td>
<td>43.89</td>
<td>44.41</td>
</tr>
</tbody>
</table>

* rhI released during the first 24 h.

Fig. 7. Effect of the inner water phase volume on the drug cumulative release profile.

little and increased as a function of time following a smaller initial burst. The difference in release rate is due to the fact that there is a larger rhI concentration gradient between the microcapsules and the outer water phase when the rhI-loading amount is higher. Since the gradient is the driving force for rhI diffusion, 4.0 wt.% (10.0 mg of rhI) loading led to a higher initial burst and a more rapid release rate. Moreover, at a higher actual loading level, there was more rhI distributed near the surface area of microcapsules. This led to a greater initial release. As the rhI released, it left more pores and interconnecting channels for the release of

Fig. 8. SEM photos of the PLA/PLGA microcapsules with different inner water phase volume: (a) 250 μl; (b) 375 μl; (c) 500 μl; (d) 675 μl.
Fig. 9. Effect of the rhI-loading amount on the drug cumulative release profile.

Fig. 10. Effect of pH-value in outer water phase on the drug cumulative release profile.

3.6. Effect of pH-value in outer water phase

pH-value in outer water phase has been considered to be an important factor influencing the drug encapsulation efficiency and cumulative release. As shown in Table 5, a dramatic improvement of the drug encapsulation efficiency was observed when the pH-value of the outer water phase was 6.0, which was close to the iso-electric point of rhI encapsulated. At this iso-electric point, the solubility of the drug in the outer water phase was lowered, and it was easier for the drug to absorb onto the surface of the microcapsules, and both favored the improvement of the drug encapsulation efficiency. However, if the improvement of the drug encapsulation efficiency was just due to the adsorption of the drug on the surface of the microcapsules, it would inevitably lead to the high "burst effect". Fig. 10 shows that at pH 6.0, the initial release of rhI exceeded 34.0%, larger than those prepared in the acidic (pH 2.6) or basic (pH 10.0) outer water phase. This may suggest that at pH 6.0, a large amount of the drug were on the surface of the microcapsules.

3.7. Effect of microcapsules size

The size of drug-loaded microcapsules plays an important role in the drug encapsulation efficiency and affects the drug cumulative release. By using the membrane emulsification technique, the size of microcapsules can be controlled accurately by the choice of a membrane with proper pore size, because there exists a linear relationship between the microcapsules size and the pore size of membrane [20]. The effect of microcapsules size on the drug encapsulation efficiency is shown in Table 6. With increasing the size of microcapsules from 3.2 to 9.0 μm, the drug encapsulation efficiency increased significantly from 48.96% to 81.27%. To prepare drug-loaded microcapsules, w1/o/w2 double emulsion droplets were formed firstly, followed by the solvent removal process. However, during the process of the double emulsion droplets formation and solvent removal, the drug dissolved in the inner water phase was ready to diffuse into the outer water phase through the oil phase. This would lead to the loss of drug encapsulated, and thus affecting the drug encapsulation efficiency. When the size of drug-loaded microcapsules was smaller, the specific surface between the emulsion droplets and the outer water phase became larger, and the drug had more chance to diffuse into the outer water phase, as a result, the drug encapsulation efficiency decreased accordingly. At the same condition, drug-loaded PLA microcapsules were also prepared by stirring method, and the drug encapsulation efficiency was only 32.85% when microcapsules were prepared by stirring method, although the size of microcapsules was much larger than those prepared by membrane emulsification technique. The results confirmed that membrane emulsification technique was

| Table 6 | Effect of microcapsules size on the drug encapsulation efficiency and initial release |
| --- | --- | --- | --- |
| Emulsification technique | Mean size (μm) | Encapsulation efficiency (%) | Initial rhI burst (%) |
| Membrane | 5.8 | 65.61 | 34.43 |
| Stirring | 15.9 | 32.85 | – |

* rhI released during the first 24h.
more efficient for encapsulating drugs than stirring method. From Fig. 11, it was illustrated that the smaller the microcapsules size was, the faster the drug released from the microcapsules. This was largely due to the larger specific surface between the microcapsules and the outer water phase. As the size of microcapsules prepared can be controlled accurately and the repeatability of the experiment was rather good, it is convenient to control the drug cumulative release precisely.

4. Conclusion

Uniform-sized biodegradable PLA/PLGA microcapsules loading rhI were successfully prepared by combining a SPG membrane emulsification technique and a double emulsion–solvent evaporation method. Various factors of the preparation process influencing the drug encapsulation efficiency and the drug cumulative release were investigated systematically. The results indicated that the drug encapsulation efficiency and the cumulative release were affected by the PLA/PLGA ratio, NaCl concentration in outer water phase, inner water phase volume, rhI-loading amount, pH-value in outer water phase and the size of microcapsules. By optimizing the preparation process, the highest drug encapsulation efficiency of 91.82% was obtained. The unique advantage of preparing drug-loaded microcapsules by membrane emulsification technique is that the size of microcapsules could be controlled accurately, and thus the drug cumulative release profile can be adjusted just by changing the size of microcapsules. Moreover, much higher encapsulation efficiency can be obtained when compared with conventional mechanical stirring method.

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