Nanosparticles of Biodegradable Polymers
Applied for Clinical Administration of Anticancer Drugs

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ABSTRACT

Paclitaxel (Taxol®) is an excellent antineoplastic drug approved by US FDA for chemotherapy of various cancers. Due to its high hydrophobicity, however, adjuvant such as Cremophor EL has to be used in its current clinical administration, which causes serious side effects such as hypersensitivity reactions, nephrotoxicity, neurotoxicity and cardiotoxicity. Nanoparticles of biodegradable polymers could be an alternative administration system to have better interaction with cancer cells with lower side effect. Hence, the main aim of this project was to explore the various fabrication factors and identification/application of natural emulsifiers for nanoparticle manufacture. The solvent precipitation technique was applied to prepare nanoparticles of poly(DL-lactide-co-glycolide) (PLGA). Such a FDA approved PLGA is chosen due to its biocompatibility and biodegradability. Suitability of alternative organic solvent and stabilizer (Vitamin E TPGS and gelatin) other than the well-established PVA for preparing nanoparticles was explored. The effect of using binary organic solvent phase instead of single solvent phase was investigated as well.

METHODOLOGY
Solvent Precipitation

100 mg of PLGA were dissolved in 10 ml organic solvent. This organic solution was added dropwise into an aqueous phase under moderate stirring, prepared by dissolving 90 mg stabilizer in 30 ml milipore water. The organic solvents were evaporated by gentle stirring at room temperature by a magnetic stirrer for 4 hours to evaporate the organic solvent. The resultant sample was collected by centrifugation (10000 rpm, 15 min, 5°C, unless stated otherwise) and washed three times to remove the emulsifier. The produced suspension was freeze-dried to obtain the fine powder of nanoparticles, which was placed and kept in vacuum dessicator.

Particle Size and Particle Morphology

The sizes of the nanoparticles are measured by laser light scattering at a scattering angle of 90° at 25°C. Scanning Electron Microscope (SEM) is used to determine the morphology of the nanoparticles.

EXPERIMENTAL DESIGN AND RESULT

Speed of Centrifugation

Nanoparticles are prepared with PVA as stabilizer and Acetone as the organic solvent. Samples of nanoparticles are collected before centrifugation and after centrifugation at 5000 rpm, 8000 rpm and 10000 rpm respectively with period of centrifugation fixed at 15 minutes.

<table>
<thead>
<tr>
<th>Centrifugation Speed</th>
<th>Before Centrifugation</th>
<th>5000 rpm</th>
<th>8000 rpm</th>
<th>10000 rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size / nm</td>
<td>570.9</td>
<td>355.5</td>
<td>273.5</td>
<td>407.1</td>
</tr>
<tr>
<td>Polydispersity</td>
<td>0.355</td>
<td>0.165</td>
<td>0.201</td>
<td>0.302</td>
</tr>
</tbody>
</table>
Variation of Organic Solvent and Stabilizer

THF and Gelatin are being used instead of Acetone and PVA.

Table 2. Diameter and polydispersity of PLGA nanoparticles prepared by varying organic solvent and stabilizer

<table>
<thead>
<tr>
<th>Organic Solvent / Stabilizer</th>
<th>THF / PVA</th>
<th>Acetone / Gelatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size / nm</td>
<td>536.1</td>
<td>496.1</td>
</tr>
<tr>
<td>Polydispersity</td>
<td>0.299</td>
<td>0.199</td>
</tr>
</tbody>
</table>

Binary Solvents in Organic Solvent Phase

PLGA is dissolved in the solvent mixture consists of acetone (ACN) and ethanol (ETH).

Table 3. Diameter and polydispersity of PLGA nanoparticles prepared in binary organic solvent phase

<table>
<thead>
<tr>
<th>Organic phase</th>
<th>8.5ml ACN + 1.5ml ETH</th>
<th>7.0ml ACN + 3.0ml ETH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size / nm</td>
<td>248.3</td>
<td>424.2</td>
</tr>
<tr>
<td>Polydispersity</td>
<td>0.273</td>
<td>0.315</td>
</tr>
</tbody>
</table>

Image produced by SEM shows that the nanoparticles are spherical.

DISCUSSION

The variation in size of nanoparticles collected at different centrifugation speed is consistent with the working principle of centrifugation, except for that of 10000rpm which the nanoparticles size increases instead of decreases. This could be due to the agglomeration of the nanoparticles. Polydispersity is observed to decrease after centrifugation as the result of differential centrifugation. The result of the second experimental setup shows that THF and
gelatin are possible alternative organic solvent and stabilizer in producing nanoparticles. This is crucial as residual PVA is carcinogenic. In the final experimental setup two water miscible organic solvents are used where sufficiently small nanoparticles with satisfactory polydispersity are formed. Interestingly the size of nanoparticles in the supernatant is found to be 117.5nm (polydispersity 0.106) and 104.3nm (polydispersity 0.136) for the samples with 1.5ml ETH and 3.0ml ETH respectively. Therefore smaller nanoparticles could be collected at a higher centrifugation speed.

CONCLUSION

It is important to explore other suitable stabilizer like TPGS and gelatin as PVA is reported to be potentially carcinogenic. Water miscible organic solvents are used here over water immiscible organic solvent as this eliminates the step of solvent evaporation. Binary solvents (especially water miscible solvent) in organic solvent phase is a potentially good method in preparing or even manufacturing nanoparticles.

REFERENCES
