Development and Optimization of PLGA Nanoparticles as a Carrier System for Oral Delivery of Gemcitabine

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ABSTRACT SUMMARY
The oral delivery of anticancer drugs represents a significant challenge for global scientist. The aim of this study was to develop and optimize drug-loaded PLGA based nanoparticle as a potential technology to achieve sustained release profiles and later to improve the oral bioavailability of gemcitabine. PLGA nanoparticles loaded with gemcitabine were prepared by using water-in-oil-in-water (W/O/W) double emulsion solvent evaporation method. Optimization of the delivery system using 2-level full factorial design by evaluating various concentrations of PLGA and outer phase stabilizer as well as ultrasonication time. Particle size, size distribution, zeta potential, drug entrapment efficiency, drug release profile were investigated. As a result, the optimal delivery system showed promising particle size and morphology, acceptable entrapment efficiency, as well as to provide a sustained drug release profile, thus it demonstrated gemcitabine can be delivered using this nanoparticulate delivery system via oral route.

INTRODUCTION
Gemcitabine is a difluorinated analog of the naturally occurring nucleoside deoxycytidine, and has shown significant clinical activity in a variety of solid tumors. However, gemcitabine is currently administered intravenously because the drug has poor oral bioavailability which due to its high polarity and low intestinal permeability. A solid lipid nanoparticle (SLN) is typically spherical with an average diameter between 10 to 1000 nm. Due to its improved stability, sustained and targeted release properties, as well as enzymatic degradation prevention characteristic. Thus a PLGA based nanoparticles loading gemcitabine formulated by solvent evaporation method of W/O/W double emulsion was investigated in this study.

EXPERIMENTAL METHODS
Preparation of nanoparticles by solvent evaporation method. The formulations were prepared by using indicated amount of PLGA dissolved in 3 ml of Ethyl acetate. 300 mg of gemcitabine was dissolved in 12 ml of milli-Q water forming 25 mg/ml drug. 0.6 ml of drug added dropwise into 4 ml of PLGA organic solvent forming w/o emulsion after ultra-sonicated for 60 sec by ultrasonicator. Then added dropwise into 12 ml of PVA solution, and ultra-sonicated for the indicated time forming w/o/w emulsion. Solvent evaporated by rotator evaporator and centrifugated for 40 mins, and washed the precipitated nanoparticles thoroughly and freeze-dried for 24 hours at -50°C. Additionally, two-level full factorial design with three factors (e.g. different concentration of PLGA and PVA, and various ultra-sonication time), and two responses (e.g. particle size and entrapment efficiency) was applied to predict the optimal formulation.

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Average Particle size (d.nm)</th>
<th>Zeta Potential (mV)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The total 27 factorial design formulations</td>
<td>435.1± 18.4</td>
<td>From +4.7 to −2.1</td>
<td>From 53.1 to 78.3</td>
</tr>
<tr>
<td>The predicted optimal formulation</td>
<td>272.0± 14.5</td>
<td>+3.2 ± 0.4</td>
<td>76.4 ± 0.2</td>
</tr>
</tbody>
</table>

Table 1. The particle size, zeta potential and EE of all the formulations and the optimal formulation of 7 % w/v PLGA + 2 % w/v PVA and 300 sec sonication time. (n=3).

Figure 1. Size distribution output of the optimal formulation 7 % w/v PLGA + 2 % w/v PVA with 300 sec sonication time. (PdI: 0.178).

Characteristic studies.
The particle size, size distribution, and zeta potential of nanoparticles were measured by ZetaSizer (Nano ZS, Malvern, UK).

Entrapment Efficiency of nanoparticles, amount of gemcitabine entrapped in the nanocapsules was determined by substracting the non-entrapped gemcitabine from the amount of gemcitabine added to the nanocapsules and expressed as a percentage.

Optimal formulation prediction by using 2-level full factorial design from DesignExpert 9.0 software.

In vitro release of gemcitabine entrapped in the nanoparticles. The Franz diffusion cells (VTC 200, Logan) were used for in vitro drug release studies. 10 mg of Drug was added directly into the receptor chamber filled with PBS at pH 6.5 with pepsin, tripsin and chymotripsin digestive enzymes and the temperature was maintained at 37°C to simulated gastric environment. 0.5 ml of sample was withdrawn at various time intervals and filtered through 45 µm Acrodisc® syringe filters, the drug concentration was then evaluated by High Performance Liquid Chromatography (HPLC).

Morphology and surface characteristics of the nanoparticles were studied by using Scanning Electronic Microscopy (SEM) (XL30S FEG model, Philips. USA).
For particle size of nanoparticles, the range of the average diameter of the particles was around 100 nm up to 700 nm with an average of (435.1 ± 18.4) nm. The optimal formulation has particle size of (235.1 ± 18.4) nm. From the results, we can see that higher the concentration of PLGA, longer ultra-sonication time lead to smaller particle size. The formulation has shown a good size distribution due to less dispersed and the higher intensity of the peak, which demonstrated the good uniformity of the nanoparticle. In addition, The Polydispersity Index (PdI) describes variation in sizes. The higher the PdI value, means the wider the particle size distribution. The formulation has shown a low PdI value which indicating the acceptability of the particle uniformity. The zeta potential of all the formulations are from +4.7 mV to -2.1 mV, and the optimal formulation has (+3.2 ± 0.4) mV. However, the zeta potential of all the formulations are within the range of +15 mV and -15mV indicates the extent of repulsion between nanoparticles was not significant, thus the stability of the colloidal dispersion shall be further improved toward this characteristic. It was found that an increase in PLGA concentration resulted in an increase in EE. There was about 10% increase in EE when PLGA concentration was increase from 3% to 7%. This was due to the fact that higher PLGA concentration serves as a greater barrier to prevent the diffusion of the drug out of the polymeric shell. Additionally, ultrasonication time also influences EE. In general, longer ultrasonication time leads to breakage of the particles and drug leakage.

Figure 2. The response surface from factorial design to predict the optimal formulation.

From the result of factorial design prediction, the optimal formulation was obtained as 7 %w/v PLGA + 2 %w/v PVA with 300 sec ultra-sonication time. The statistic analysis of variance by ANOVA for quadratic factorial model has been evaluated, and the p-value “Prob > F” was less than 0.050 indicate model terms was significant. The desirability of the predicted optimal formulation was 0.832. This formulation was then carried out the drug release studies.

In vitro release studies. From figure 3, we can see all the optimal formulation shown steady sustained release compared to the release of control drug without the delivery system. We can see 80% of the gemcitabine (control) was released in buffer within 15 minutes, which demonstrate the over rapid release profile, thus gemcitabine should be formulated into the nanoparticulate delivery system that allows to generate better steady release as well as elevation in drug retention during the release. From the result, we can see the nanoparticulate delivery system gave rise to a moderate initial drug release rate follow by a greater sustain release and about 76% of the total drug released within 1400 mins. It demonstrated the formulation allows to persist longer in blood and avoid liver and spleen for many hours. Thereby it is better suited for carrying drugs in vivo.

Figure 3. In vitro release profile of GSH with different nanoparticle formulations. (Mean ± SD, n=3)

SEM morphology studies. The drug-loaded nanoparticles appeared to be spherical uniform, and well defined in shape with smooth surfaces from the SEM pictures (Figure 4). However, some of the particles were aggregated together due to the centrifugation with high energy that forces the particles back together to form nuggets.

Figure 4. The SEM micrograph of optimal drug-loaded nanoparticles (Magnification: 5000x).

CONCLUSION
The drug loaded PLGA based nanoparticles was developed and optimized, results were shown to be promising. Thus this delivery system has built a great platform for oral delivery of gemcitabine.

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REFERENCES