P-029 **Effect of formulation variables on gentamicin-loaded PLGA microspheres**

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INTRODUCTION AND OBJECTIVE

Gentamicin sulfate (GS) has been loaded into PLGA microspheres by a water-in-oil-in-water (W/O/W) emulsification solvent evaporation technique (Prior 2000). However, because of its high hydrophilicity, the loading capacity of GS is generally low of about 1-10 µg GS/mg microspheres (Virtoa 2007), 3-6 µg GS/mg microspheres (Prior 2000). A high entrapment efficiency of hydrophilic drugs in microspheres of hydrophobic polymers (such as PLGA) is difficult to achieve because such drugs have a low compatibility with the polymer. The drug substance has the tendency to extract by the continuous aqueous phase during preparation of the microspheres using W/O/W emulsion technology. It has been shown that the encapsulation of macromolecular hydrophilic compounds (e.g. peptides, proteins, DNA) can be improved by the formulation parameters, such as type and polymer concentration (Ito 2007), type and concentration of surfactant (De RoSa 2000), volume of the internal phase of the primary emulsion and the volume of the external phase of the secondary emulsion (Parikh 2003). The aim of this study was to investigate the loading and release of GS in microspheres of PLGA by varying the formulation parameters.

MATERIALS AND METHODS

Preparation of GS microspheres Fifty mg of GS was dissolved in 0.2 M phosphate buffer (PBS) pH 6.0. Subsequently, the drug solution was added to 10% w/v PLGA in dichloromethane and emulsified using an ultrasonic probe at 40 % duty cycle under cooling for 3 min to yield a stable W1/O emulsion. Next, this W1/O emulsion was added to 1% PVA solution (W2) and further emulsified for 1 min at a stress-mixing speed of Polytron® at 5,000 rpm to obtain W1/O/W2 emulsion. The organic solvent was allowed to evaporate at 40 °C for 10 min.To investigate the role of surfactant on the primary emulsion, Pluronic F68 (PLU) was added in PLGA solution, and Benzalkonium chloride (BC) was added in drug-PBS solution. To investigate the effect of water soluble solvent in secondary emulsion, various ratio of ethanol was mixed with PVA aqueous solution. To investigate the effect of buffer strength of external water phase, different strength of buffer were used to dissolve PVA. Furthermore, effect of PLGA concentration on microsphere characteristic was also investigated.

Characterization of microspheres The particle size was determined by using particle sizing systems AccuSizer Model 780. The supernatant after microsphere formation was analyzed for drug loading by UV spectrophotometry. The morphology of the microspheres was investigated by scanning electron microscopy (SEM).

GS release Lyophilized microspheres were suspended in 1.5 ml PBS. The samples were placed horizontally with continuous shaking at 37 °C. At predetermined time points, the samples were centrifuged and 0.5 ml of supernatant was removed and replaced by the fresh buffer solution. The supernatant was analyzed for GS by UV spectrophotometry.

RESULTS AND DISCUSSION

GS-loaded PLGA microspheres: Effect of ethanol on loading and size. GS-loaded PLGA microspheres prepared without ethanol in the external aqueous phase showed a size of 24.7 µm and a low drug loading efficiency of 5.8 %. The addition of ethanol (10-30%) to the external aqueous phase resulted in a reduction microsphere size reduction. The entrapment efficiency of GS in PLGA prepared with ethanol in the external phase was shown in Table 1.

Table 1 : Physicochemical properties of GS microspheres prepared with ethanol in the external aqueous phase

Ethanol volume fraction (% v/v)	Volume weight mean diameter (µm)	*LC (µg GS/mg particles)	% LE**
0	24.7±1.9	9.7±3.6	5.8±1.6
10	20.4±2.0	0.8±0.4	0.5±0.3
20	14.5±0.4	0.8±0.4	0.5±0.2
30	8.9±2.8	0.6±0.3	0.4±0.2

* Loading capacity ** Loading efficiency

Effect of surfactant on GS-loaded microspheres Table 2 shows that the mean volume weight diameter of the particles was influenced by type and concentration of surfactants. Drug loading efficiency decreased when a surfactant was added to primary emulsion.

Effect of buffer concentration on GS-loaded microspheres As shown in Table 3, the size of the obtained particles was not affected by the buffer concentration (volume-weight mean diameter ranged from 24-34 µm). However, GS encapsulation efficiency increased signifi-

cantly from 5% to 18% with increasing buffer concentration.

Table 2 : Effects of surfactant on physicochemical properties of GS loaded PLGA microspheres.

Surfactant (%w/v)	Volume weight diameter (µm)	LC	% LE
0	24.7±1.9	9.7±3.6	5.8±1.6
PLU 2%	28.0±1.0	0.7±0.8	0.5 ±0.5
PLU 5%	32.8±2.6	0	0
BC 2%	20.2±5.6	8.1±6.0	4.9±2.6
BC 5%	15.9±1.8	3.7±2.2	2.2±1.3

PLU= pluronic F68; BC = benzalkonium chloride

Table 3 : Effect of buffer concentration on physicochemical properties of GS loaded PLGA microspheres

Buffer concentration (M)	Volume weight diameter (µm)	LC	% LE
0	24.7±1.9	9.7±3.6	5.8±1.6
0.1	32.5±6.3	25.5±2.0	14.1±2.1
0.2	29.5±2.0	27.6±10.5	17.0±4.0
0.3	33.9±13.6	27.5±6.3	18.5±4.5

Effect of polymer concentration and composition on GS-loaded microspheres The average particle size increases with PLGA concentration (Table 4). Importantly, the results shows that microspheres prepared with higher PLGA concentration indeed had a higher loading efficiency, in line with previous publications (Ito 2007). An increase concentration of PLGA from 10% to 15% resulted in approximately a 4-times increase of drug loading.

Table 4 : Effect of polymer concentration on physicochemical properties of GS loaded PLGA microspheres

concentration (% w/v)	Volume weight diameter	LC	% LE
10	29.5±2.0	27.6±10.5	17.0±4.0
15	32.7±2.0	75.7±5.3	64.0±4.1
20	45.1±0.7	62.8±5.4	68.6±5.8

Release of GS from PLGA microspheres Figure 1 shows that the PLGA microspheres showed a burst release of about 30, 20 and 10%, for the 10, 15% and 20% PLGA, respectively. After 35 day, however, the release of GS increased.

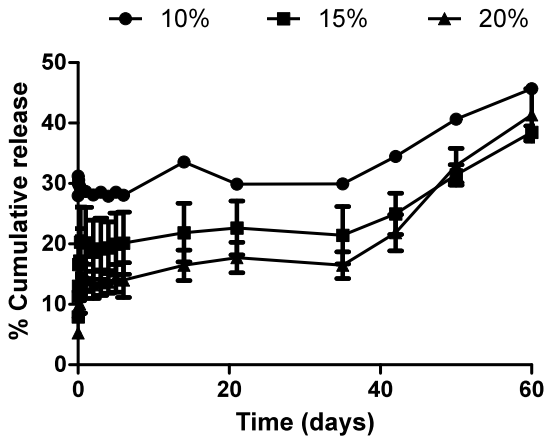

Figure 1 : Effect of polymer concentration on release behavior of GS from PLGA microparticles

CONCLUSIONS

This paper shows that GS can be entrapped with a high efficiency in PLGA microspheres. To obtain this high loading efficiency, it is important that the osmotic values of the inner and outer aqueous phases match. Further, the polymer concentration of the DCM solution is an important parameter to achieve a high loading. The PLGA particles showed a burst release of the drug depending on their porosity, followed by a phase of 35 days where hardly any release occurred. The drug was then slowly released for around 25 days likely governed by degradation of the microspheres. Taken the results together it can be concluded that PLGA microspheres probably gives a sustained release of the gentamicin for 60 days, which makes it an attractive system for antibiotic treatments.

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