Preparation, Optimization And Surface Modification Of Magnetic PLGA Nanoparticles Loaded With Model Drug

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INTRODUCTION AND OBJECTIVE

Nanomedicine research has revolutionized the field of drug delivery and this research is getting an even bigger scientific boost day by day. Poly(D,L-lactic-co-glycolic acid) (PLGA) is a biocompatible and biodegradable material being extensively used as one of the most popular drug carriers. Triblock copolymer poloxamers have generated considerable interest in medical applications because of their ability to reduce protein adsorption on hydrophobic surfaces like PLGA. For sustained systemic circulation of hydrophobic drug carriers, surfaces must be modified in order to avoid phagocytosis which can be successfully done by poloxamers.
This work involves the co-encapsulation of model drug human serum albumin (HSA) and superparamagnetic magnetite into PLGA to prepare nanoparticles (NPs) by double emulsion solvent evaporation method. Experimental design was carried out using STATISTICA® software and after performing all the experiments, the process was optimized using mathematical tools (GAMS™/MINOS software). Hydrophobic surfaces of PLGA NPs were modified by poloxamer (F68) due to avoid their quick recognition and engulfing by the macrophages.

MATERIALS AND METHODS

PLGA (50:50, M_w = 8,000, Resomer® RG 502H), model drug HSA solution (Trigon Biotechnological Ltd., Hungary), poly(vinyl alcohol) (PVA; M_w = 30,000-70,000, Sigma-Aldrich), phosphate-buffered saline (PBS, Sigma-Aldrich), dichloromethane (DCM, Spektrum-3D, Hungary), poloxamer F68 (M_w = 8350, BASF, Germany) and magnetite (which was synthesized by co-precipitation method).

Nanoparticles were prepared by double emulsion solvent evaporation method and detail method can be found in our previously published paper (Feczkó 2008). Size of the model drug loaded NPs was measured by Zetasizer (Malvern Instruments, UK) and model drug encapsulation efficiency (EE%) was determined using micro BCA protein determination method. Different concentrations of poloxamer (0.25, 0.5 and 1% wt/vol) were used to coat the PLGA NPs which were redispersed in phosphate buffered saline (PBS) solution before the addition of poloxamer. Attachment of poloxamer onto PLGA surface was checked by size and zeta potential measurement by Zetasizer.

Experimental design and mathematical optimization
A $3^{(p-1)}$ type fractional factorial experimental design was carried out using STATISTICA® software. From preliminary experiments, it was found that five process variables strongly influence the process: amount of iron oxide in the organic phase (F1) relative to the weight of PLGA used for encapsulation, concentration of PLGA in the organic phase (F2), concentration of HSA in the inner aqueous phase (F3), the outer aqueous/organic phase volume ratio (F4), and time of the ultrasonic treatment in the second emulsification (F5). Experimental design suggested 90 experiments for five variables ($3^{(5-1)}$ = 81 plus 9 repetition). The obtained experimental data were evaluated by statistical analysis, similarly to the method described by Feczkó et al. (Feczkó 2011). Due to high number of variables, optimization was carried out mathematically using GAMS™/MINOS Large Scale Nonlinear Solver for Windows.

RESULTS AND DISCUSSION

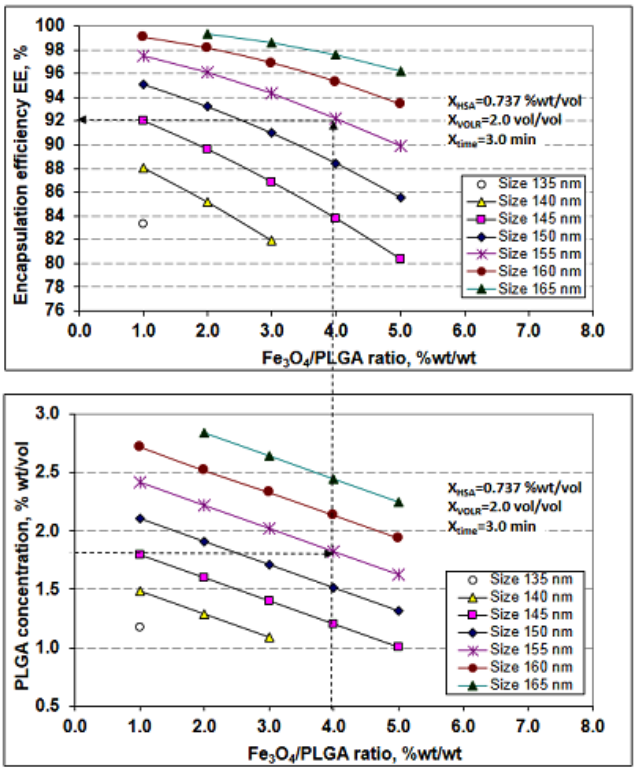

Figure 1: Optimum condition for different magnetite content (dotted line shows as an example)

In the production of drug-loaded NPs, the general goal is to achieve suitable small particle size and at the same time, high encapsulation efficiency. That's why

optimization was very important in our study. After performing all the 90 experiments, the results were analyzed statistically and process was optimized which is shown in Figure 1. The dotted lines in Figure 1 shows an example of optimization for PLGA concentration 1.83% (wt/vol) and magnetite concentration 4.0% (wt/wt), resulting in a mean diameter of about 155 nm (crossing point of the dotted lines) with 92.3% encapsulation efficiency.

The surface of PLGA NPs prepared using optimum condition was modified using poloxamer (F68). Volume mean size of control sample was 199.8 nm. It was found that for 0.25 and 0.5 % poloxamer concentrations, the size distribution of poloxamer covered PLGA NPs shifted towards higher particle size region and at the same time the volume mean particle size increased significantly. Average diameter of NPs enhanced by 7.6 and 56.5 nm for 0.25 and 0.5% poloxamer, respectively which is comparable to the result obtained by Greenwood et al. (Greenwood 1995). Figure 2 shows size distribution of control and modified PLGA NPs.

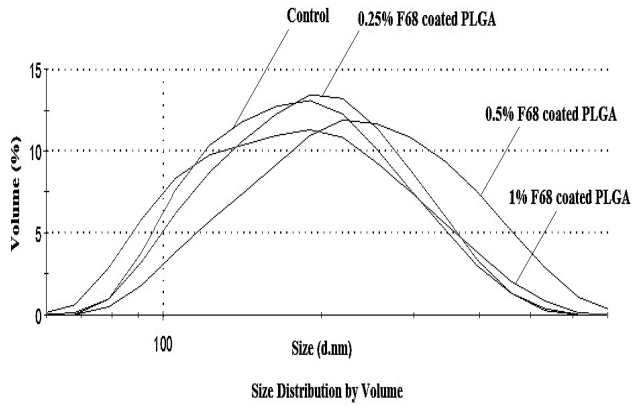

Figure 2: Size distribution of PLGA and poloxamer coated PLGA NPs

Ortega et al. (Ortega 2006) found a sharp increase in adsorption isotherm for F68 coating onto PLGA particles for low F68 concentration (up to 100 mg/L) and above that concentration, the increase was quite steady and reached a plateau. In our study, we got sharp increase in size up to the concentration of 0.5% (Table 1).

Table 1: Average size of PLGA NPs coated with poloxamer F68

Percentage of poloxamer	Size (nm)
0% (control)	199.8
0.25% F68	207.4
0.5% F68	256.3
1% F68	201.3

When the amounts of adsorbed poloxamer are higher, the surface will be more crowded, and the area, that one polymer molecule or PEO chain occupies, decreases, and the adsorbed layer thickness increases. Increase in poloxamer concentration from 0.5 to 1% resulted in both smaller and bigger particles than the control (Figure 2) indicating the formation of micelles, since the diameters of poloxamer micelles usually vary from ca. 10 nm to 200 nm (Li 2011). Interestingly, zeta potential didn't change significantly which is also comparable with the study of Gelperina et al. (Gelperina 2010).

CONCLUSION

Drug carriers are confronted with difficulties during the route to the target organ that need to overcome before they reach target site(s) within the body. The most important barrier is the adsorption of plasma proteins which make them more visible to phagocytic cells, and they are immediately engulfed by macrophages and removed quickly from the blood stream. Poloxamer coating makes hydrophobic PLGA carriers "stealth", hence they can reach the target site(s), where they can perform their biological roles. 0.5% poloxamer coating found suitable in this study since higher concentration will form micelle. Further analysis like protein adsorption study by X-ray photoelectron spectroscopy will be carried out.

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