Design of Experiments Approach to Identify Dominant Formulation Factors for Vancomycin-Loaded PLGA Microspheres

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Introduction: As one of the most common vehicles for delivering drugs, polymeric microspheres encapsulating biomolecules can release the drugs at the proper location, with a required dose, and in a desired pattern. Compared to systemic administration, such controlled release systems provide better efficacy, less systemic side effects, and improved patient compliance [1]. To accommodate different demands (drug loading, release profiles, etc.) for various applications, formulation of delivery systems must be optimized, which involves identifying ruling factors from many governing factors, such as microsphere preparation parameters, polymer type, polymer molecular weight, copolymer composition, etc. With many governing factors to be evaluated, using design of experiments (DOE) instead of one-factor-at-a-time (OFAT) methods can efficiently reduce the resources (time, materials, etc.) needed and find the interaction between factors [2].

This study evaluated the effect of three factors, including ratio of lactide to glycolide, ratio of volume of water phase 1 (W_1)to volume of oil phase, and surfactant concentration on drug loading, drug encapsulation, yield, and release profile for vancomycin loaded poly(lactic-co-glycolic acid) (PLGA) microspheres.

Materials and Methods: The experimental design was generated using software JMP Pro (version 10) as shown in the first 4 columns in Table 1.

Table 1: Experimental design and results

Setting #	lactide to glycolide	W1/O ratio	Surfactant conc %(wt/v)	Drug loading (%)	Encapsulation (%)	Yield (%)
1	50:50	2	0.35	10.2	59	87
1	50:50	2	0.35	10	57	86
2	100:0	1	0.35	7.8	32	62
3	100:0	1	0.175	5.5	28	75
4	75:25	2	0.175	6.3	38	91
5	50:50	1	0.175	7.5	47	95
6	100:0	2	0.175	8.3	45	81
7	100:0	2	0.35	1.6	7	64
8	75:25	1	0.35	8.5	47	84

Vancomycin-loaded PLGA microspheres were prepared using a double emulsion method. Briefly, 150 mg vancomycin was dissolved in phosphate buffered saline (PBS) as W_1; then 1 gram of PLGA was dissolved in 15 ml dichloromethane (DCM) as oil phase (O); the mixture of W_1 and oil phase was emulsified using sonicator and poured into second water phase containing methylcellulose and 2.5% NaCl; the $W_1/O/W_2$ mixture was homogenized at 4000 rpm for 5 minutes and then was stirred overnight to remove the DCM. The prepared microspheres were collected and freeze-dried.

To determine drug loading, microspheres were dissolved in dimethyl sulfoxide, polymer was precipitated with PBS, and UV absorbance of the supernatant was measured. For release experiments, microspheres were suspended in PBS and incubated at 37 ºC with shaking. The supernatant was collected and replaced with fresh PBS at predetermined times and the vancomycin concentration determined as described earlier.

Results and Discussion: The drug loading, encapsulation efficiency, microsphere yield are listed in Table 1. The model fit to these results suggested ratio of lactide to glycolide should be the main factor affecting the drug loading. In addition, the increased W_1/O ratio slightly lowered the drug loading, while the surfactant concentration had minimal effect. Based on the model, higher drug encapsulation can be achieved by using PLGA with lower lactide content instead of changing the other two factors. It also suggested using PLGA with lower lactide content and W_2 with less surfactant to achieve higher yield. From release studies of the prepared microspheres, the lactide content was the key factor determining the release patterns. Microspheres containing either low or high lactide content "burst" released most of the drug during the first 2 d, while microspheres made of 75:25 PLGA exhibited much less "burst" and sustained release through 6 wk (Figure 1).

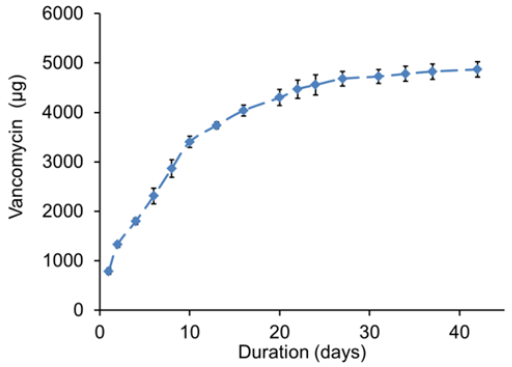

Figure 1. Cumulative release of vancomycin from PLGA microspheres.

Conclusions: Different applications are likely to have different formulation requirements, like drug loading, encapsulation efficiency, and release profile. Identifying the dominant factors from many governing factors and then making adjustment accordingly to achieve those specific goals can be complicated and time consuming if using the OFAT method. This study applied DOE to quickly identify the dominant factors for several properties of vancomycin-loaded microspheres. Compared to the OFAT method, DOE is a powerful and efficient method to develop suitable formulation of drug delivery system for different applications.

References:

1. Kim, K. and D. Pack, *Microspheres for Drug Delivery*, in *BioMEMS and Biomedical Nanotechnology*, M. Ferrari, A. Lee, and L.J. Lee, Editors. 2006, Springer US. p. 19-50.
2. Czitrom V. Am Stat.1999:53(2): 126-131.