European Journal of Pharmaceutical Sciences 102 (2017) 196–202

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Process optimization by use of design of experiments: Application for liposomalization of FK506

Hiroyasu Toyota [a,b], Tomohiro Asai [a], Naoto Oku [a,*]

[a] Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[b] Pharmaceutical Research and Technology Labs., Technology, Astellas Pharma Inc., 180 Ozumi, Yaizu, Shizuoka 425-0072, Japan

ARTICLE INFO

Article history:
Received 14 December 2016
Received in revised form 28 February 2017
Accepted 5 March 2017
Available online 7 March 2017

Keywords:
Liposomes
Design of experiments
Box-Behnken design
Process optimization

ABSTRACT

Design of experiments (DoE) can accelerate the optimization of drug formulations, especially complexed formulas such as those of drugs, using delivery systems. Administration of FK506 encapsulated in liposomes (FK506 liposomes) is an effective approach to treat acute stroke in animal studies. To provide FK506 liposomes as a brain protective agent, it is necessary to manufacture these liposomes with good reproducibility. The objective of this study was to confirm the usefulness of DoE for the process-optimization study of FK506 liposomes. The Box-Behnken design was used to evaluate the effect of the process parameters on the properties of FK506 liposomes. The results of multiple regression analysis showed that there was interaction between the hydration temperature and the freeze-thaw cycle on both the particle size and encapsulation efficiency. An increase in the PBS hydration volume resulted in an increase in encapsulation efficiency. Process parameters had no effect on the ζ-potential. The multiple regression equation showed good predictability of the particle size and the encapsulation efficiency. These results indicated that manufacturing conditions must be taken into consideration to prepare liposomes with desirable properties. DoE would thus be promising approach to optimize the conditions for the manufacturing of liposomes.

1. Introduction

Liposomes are biodegradable vesicular structures composed of a lipid bilayer and are used as a drug delivery system (Bangham and Horne, 1964; Gregoriadis and Florence, 1993). Liposomes have been used to improve therapeutic efficiency and to decrease the adverse side effects of drugs. These effects are manifested because liposomalization changes the pharmacokinetics of drugs (Samad et al., 2007). Polyethylene glycol (PEG) modification and conjugation of targeting ligands to the surface of liposomes are used for further improvement of therapeutic efficacy (Blume and Cevc, 1990; Blume et al., 1993).

FK506 is an immunosuppressant and mainly used to prevent rejection of organ transplants. FK506 binds to FK506-binding protein, and this complex interacts with calcineurin to inhibit the production of inflammatory cytokines (Liu et al., 1991). It was reported that treatment with FK506 has positive effect on acute stroke in animal studies. Even though a high dose of FK506 induces adverse side effects, polyethylene glycol-modified liposomes encapsulating FK506 (FK506 liposomes) show therapeutic efficacy at a lower dose compared with free FK506 (Ishii et al., 2013).

The thin-film method is used for manufacturing FK506 liposomes. This method consists of the following processes: dissolution, evaporation, hydration, and extrusion (Samad et al., 2007). The International Conference on Harmonization Q8 guideline has recommended gaining information and knowledge of drug products from pharmaceutical development studies to support manufacturing controls (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009). One of the disadvantages of the usage of liposomes is the batch-to-batch variation (Muthu et al., 2014). In order to manufacture FK506 liposomes with desirable properties, it is necessary to confirm the relationship between manufacturing conditions and drug product quality and then establish the optimal manufacturing conditions.

In order to optimize manufacturing conditions, design of experiments (DoE) is used in many areas (Singh et al., 2005b). In DoE, at first a study design is prepared to collect appropriate data and then the data obtained from the experiments is analyzed by using a statistical method to extract the maximum amount of information (Singh et al.,

Abbreviations: ANN, artificial neural networks; ANOVA, analysis of variance; DoE, design of experiments; DPPC, dipalmitoylphosphatidylcholine; DSPE, distearoylphosphatidylethanolamine; FK506 liposomes, polyethylene glycol-modified liposomes encapsulating FK506; LUV, large unilamellar vesicles; MLR, multiple linear regression; MLV, multilamellar vesicles; ODS, octadecylsilane; PCR, principal component regression; PEG, polyethylene glycol; PLSR, partial least square regression; s.e.m, standard error of mean; SUV, small unilamellar vesicles.

* Corresponding author.
E-mail address: oku@u-shizuoka-ken.ac.jp (N. Oku).

http://dx.doi.org/10.1016/j.ejps.2017.03.007
0928-0987/

Table 1
Experimental design and observed results of the optimization study.

No.	Hydration process			Particle size (nm)	ζ-potential (mV)	Percent entrapped FK506 (%)
	PBS volume (ml)	Hydration temperature (°C)	Number of freeze-thaw cycles			
1	1	45	3	107.1	−4.32	44.2
2	1	55	3	102.9	−4.04	45.3
3	5	45	3	104.7	−4.26	57.8
4	5	55	3	107.9	−3.20	55.7
5	1	50	1	113.1	−3.86	41.1
6	1	50	5	102.0	−7.46	36.0
7	5	50	1	107.9	−4.51	53.6
8	5	50	5	108.0	−4.48	51.9
9	3	45	1	100.1	−6.29	53.4
10	3	45	5	106.1	−3.89	55.0
11	3	55	1	113.3	−3.04	58.3
12	3	55	5	101.0	−4.58	50.7
13	3	50	3	104.3	−3.40	57.0
14	3	50	3	112.0	−3.17	56.7
15	3	50	3	104.1	−4.23	54.5

2005a). The most common experimental design is the full-factorial design. In this design, all combinations of variables are examined. When a two-level full-factorial design is selected, the number of runs is given by 2^k, where k is the number of the variables. This full-factorial design is not useful when the number of the variables is increased because the number of runs goes on increasing exponentially (Adenso-Diaz and Laguna, 2006). Instead of the full-factorial design, the fractional-factorial design or Box-Behnken design is used in DoE. The fractional-factorial design is able to examine the effects of a large number of variables with relatively few experimental runs by ignoring interaction effects (Gunst and Mason, 2009), whereas the Box-Behnken design is used for optimization studies. This design is suitable to create a quadratic model. A three-factor Box-Behnken design is almost rotatable, which means that all design points are at the same distance from the center of the design. This property is preferable to create a response surface plot, because the prediction error is the same for all design points (Box and Behnken, 1960). The number of runs required for this Box-Behnken design is given by $2k(k-1) + C_0$, where k is the number of variables and C_0 is the number of center points. This design is more efficient compared with the three-level full-factorial design (Ferreira et al., 2007). Even though DoE is a useful tool for process optimization, few studies using DoE have been reported regarding process optimization for liposomes (Ćurić et al., 2013; Singh et al., 2005b).

In this present study, DoE was used to confirm the relationship between the process parameters of the preparation process and the properties of FK506 liposomes. Prediction models for the properties of FK506 liposomes were generated by DoE and were validated to confirm the usefulness of DoE. This study should help to establish optimal conditions for the manufacturing of liposomes.

2. Materials and methods

2.1. Materials

Dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylethanolamine (DSPE)-PEG (molecular weight of PEG: 2000) were gifts from Nippon Fine Chemical Co., Ltd. (Hyogo, Japan). FK506 was obtained from Astellas Pharma Inc. (Tokyo, Japan). Chloroform, methanol, and *tert*-butanol were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

2.2. Design of experiment

The three-factor Box-Behnken design was used to generate a quadratic model. In this design, all factors have 3 levels: low, center, and high. Three center samples were included in this design and used as a source for error estimation. The quadratic model is calculated by using multiple regression analysis, and the model is described by the following formula:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1X_2 + a_5X_1X_3 + a_6X_2X_3 + a_7X_1^2 + a_8X_2^2 + a_9X_3^2$$

where Y is the response, X is a variable, and a is a regression coefficient. Analysis of variance (ANOVA) was conducted to identify the statistically

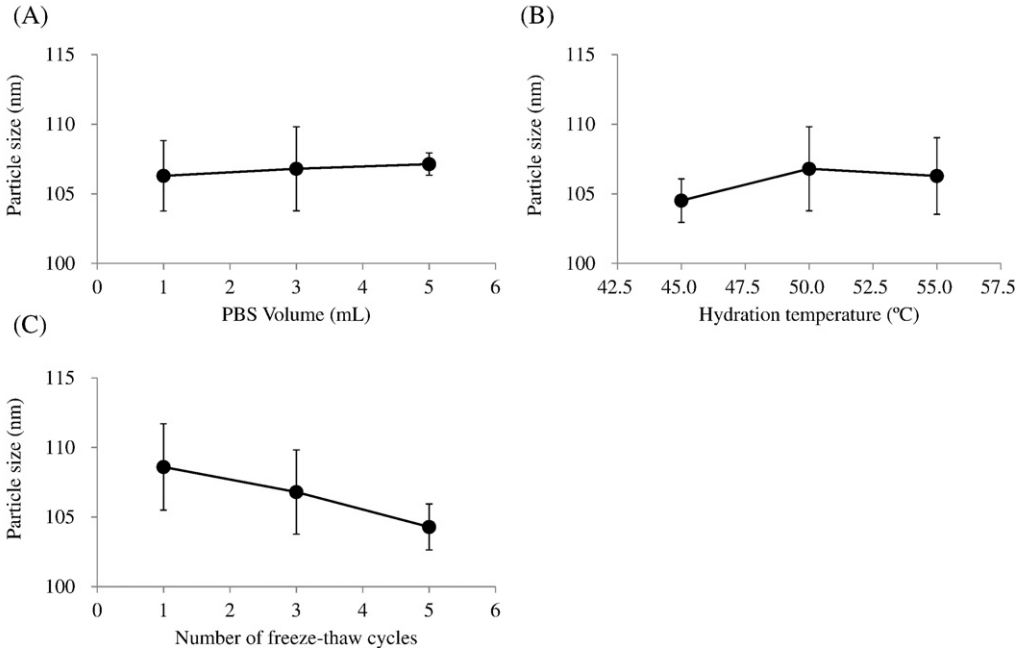

Fig. 1. Factorial effects on the particle size of FK506 liposomes. (A) Effects of the PBS volume at 1 ml ($n = 4$), 3 ml ($n = 3$) and 5 ml ($n = 4$). (B) Effects of the hydration temperature at 45 °C ($n = 4$), 50 °C ($n = 3$), and 55 °C ($n = 4$). (C) Effects of the number of freeze-thaw cycles at 1 cycle ($n = 4$), 3 cycles ($n = 3$), and 5 cycles ($n = 4$). Each plot shows the average value ± s.e.m.