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ORIGINAL ARTICLE

Minocycline Hydrochloride Controlled-release Microsphere Preparation Process Optimization Based on the Robust Design Method

Sağlam Tasarım Yöntemine Dayalı Minosiklin Hidroklorür Kontrollü Salınımlı Mikroküreleri Hazırlanma Sürecinin Optimizasyonu

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ABSTRACT

Objectives: The objective of the present study is to establish a robust preparation method that could steadily produce minocycline hydrochloride (MCH) microspheres regardless of used polymer types.

Materials and Methods: Taguchi's Robust Experimental Design methodology was employed to optimize the process parameters for MCH-loaded poly(D,L-lactide-co-glycolide) (PLGA) microspheres. In the experimental design, seven controllable factors, i.e., preparation method, pH of the aqueous phase, volume of the aqueous phase, volume of dichloromethane, rotation speed, temperature, and amount of polyvinyl alcohol, were considered for the optimization of process parameters. PLGA types with different lactide/glycolide ratios were considered the uncontrollable (noise) factor. Based on the L18 orthogonal array, 18 experimental runs were conducted for each type of PLGA. The encapsulation efficiency (EE) and *in vitro* release rate were evaluated for all the prepared formulations.

Results: Regardless of the PLGA type with different lactic/glycolic acid ratios, microspheres prepared via the solid-in-oil-in-water (S/O/W) method, showed a much higher EE and faster drug release than the microspheres prepared via the co-solvent method. Preparation methods, pH of the aqueous phase, and volume of the aqueous phase were the most influencing parameters on the EE. The confirmation experiment results indicated that the signal-to-noise ratio increased by 5.76 db from that of an initial condition. The release of minocycline was fastest with the PLGA (50:50) microspheres, followed by PLGA (75:25) and PLGA (85:15).

Conclusion: Although the interaction between the selected factors in the evaluation was ignored, the orthogonal array design of the experiment based on Taguchi's robust experimental design methodology was sufficient to optimize the process parameters for the PLGA microspheres of MCH. The S/O/W was the main factor affecting the EE. Microspheres prepared via the S/O/W method exhibited a higher EE and faster drug release than the microspheres prepared via co-solvent method. The pH and volume of the aqueous phase were also effective parameters on the EE. A robust experimental design has been successfully applied to the optimization of the process parameters for microsphere preparation.

Key words: Design of experiment, minocycline hydrochloride, PLGA microspheres

ÖZ

Amaç: Bu çalışmanın amacı, kullanılan polimer türlerine bakılmaksızın sürekli olarak minosiklin hidroklorür mikroküreleri sağlayabilen sağlam bir hazırlama yöntemi oluşturmaktır.

Gereç ve Yöntemler: Minosiklin hidroklorürün poli (D, L-laktit-ko-glikolid) (PLGA) mikroküreleri için işlem parametrelerini optimize etmek için Taguchi'nin Güçlü Deneysel Tasarım metodolojisi kullanılmıştır. Deneysel tasarımda yedi faktör yani hazırlama yöntemi, sulu fazın pH'si, sulu fazın hacmi, diklorometan hacmi, dönüş hızı, sıcaklık ve polivinil alkol miktarı kontrol edilebilir faktörler olarak kabul edilmiştir ve laktit/glikolid oranını değiştiren PLGA türleri, işlem parametrelerinin optimizasyonu için kontrol edilemeyen (gürültü) bir faktör varsayılmıştır. L18 ortogonal dizisine (L18 OA) göre, her PLGA tipi için 18 deneysel çalışma gerçekleştirilmiştir.

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Bulgular: PLGA tipine bakılmaksızın, yağda-katı-suda (K/Y/S) çözücü buharlaştırma yöntemiyle hazırlanan mikroküreler, yardımcı çözücü yöntemine göre çok daha yüksek kapsülleme verimliliğine ve daha hızlı ilaç salımına sahiptir. Hazırlama yöntemleri, sulu fazın pH'si ve sulu fazın hacmi, kapsülleme verimliliği üzerinde en çok etkileyen parametreler olarak belirlenmiştir. Doğrulama deneyi, oranınınbaşlangıç oranına göre 5,76 db arttığını göstermiştir. Minosiklin salınımı en hızlı PLGA (50:50) mikroküreler, ardından PLGA (75:25) ve PLGA (85:15) ile olmuştur.

Sonuç: Değerlendirmede seçilen faktörler arasındaki etkileşimin göz ardı edilmesine rağmen, Taguchi'nin Güçlü Deneysel Tasarım metodolojisine dayalı deneyin ortogonal dizi tasarımı, minosiklin hidroklorürün PLGA mikroküreleri için proses parametrelerini optimize etmek için yeterli bulunmuştur. Mikroküreler hazırlama yönteminin yağda katı suda (K/Y/S) kapsülleme verimliliğini etkileyen ana faktör olduğu keşfedilmiştir. (K/Y/S) yöntemiyle hazırlanan mikroküreler, yardımcı çözücü yöntemiyle hazırlanan mikrokürelere göre daha yüksek kapsülleme etkinliği ve daha hızlı ilaç salımı göstermiştir. pH ve sulu faz hacmi de kapsülleme verimliliği üzerinde etkili parametreler olarak bulunmuştur. Minosiklin salınımı en hızlı PLGA (50:50) mikroküreler ile olmuştur; ardından bu sıralamayı PLGA (75:25) ve PLGA (85:15) izlenmiştir. Mikrokürelerin hazırlanması için proses parametrelerinin optimizasyonuna sağlam deneysel tasarım başarıyla uygulanmıştır.

Anahtar kelimeler: Deney tasarımı, minosiklin hidroklorür, PLGA mikroküreler

INTRODUCTION

Quality by design (QbD) is the main part of recent approaches to achieve pharmaceutical quality. It refers to designing and developing formulations and manufacturing processes to ensure predefined product quality specifications.[1] According to the ICH Q8 guideline, "product quality cannot be ensured by testing alone and should be built in by design".[2] Multiple variables are involved in the fabrication of pharmaceutical products with consistent quality specifications. Therefore, an important part of QbD implementation is the identification of critical quality attributes and understanding how these parameters affect the product quality and are optimized with respect to the final specifications.[3] The QbD method can be utilized to simultaneously investigate the effect of several variables on the quality of a product by performing a limited number of experiments.[4]

Design of experiment (DOE) is a systematic approach that designates the relation of variables influencing a method and responses generated by the process. Statistical DOE provides an organized and efficacious plan for experimentation to attain specific objectives and allows the simultaneous study of several control variables.[5] Statistically designed experiments consist of several stages: 1) Selection of predefined quality specifications; 2) identification of independent factors, which are influencing the procedure; and 3) estimating the different factor treatments (or levels) for an optimum response.[6]

The separate examination of several variables that affect product quality requires several experiments and is a time-consuming process. Moreover, a quantitative evaluation of these effects requires special statistical techniques.[7] In this regard, several DOE methods have been extensively used to minimize this problem. Particularly, Taguchi's design, developed by Dr. Genichi Taguchi, is a reliable DOE method.[8] This method was designed to improve the quality of final products based on the concepts of factorial/fractional designs and orthogonal arrays.[9]

According to Taguchi's suggestions, the design process contains three stages, i.e., system design, parameter design, and tolerance design. The first two stages are more significant to maximize product reliability and reducing cost, whereas the last stage has minimum effectiveness at reaching desirable products.[10] In Taguchi's method, the parameter design is the main step to achieve a product with high quality without incremental cost. In this method, the arrangement of variables in an orthogonal array can provide simultaneous studies on numerous parameter spaces by performing only a small number of experiments.[11] In an orthogonal array, all parameters and levels are designed equally. Hence, the independent evaluation of each parameter is attainable, and the effect of one parameter does not influence the estimation of other parameters.[12] According to Taguchi's design assertion, all parameters that cause variability are not controllable. This method evaluates and recognizes the controllable parameters that attenuate the effect of noise parameters.[13]

Minocycline hydrochloride (MCH), a semi-synthetic tetracycline derivative, was chosen as a model drug for the preparation of poly(D,L-lactide-co-glycolide) (PLGA) microspheres. MCH has a broad-spectrum antibacterial activity and is the main antibiotic in the treatment of acne vulgaris.[14]

In this study, Taguchi's robust experimental design methodology was applied to optimize the process parameters for MCH-loaded PLGA microspheres. The PLGA type was used as an uncontrollable factor. Seven controllable variables with three levels were selected. The L18 orthogonal array (L18 OA) was used to design the experiments. Eighteen runs were conducted for each type of PLGA to optimize the seven process parameters. The encapsulation efficiency (EE) and *in vitro* release rate were evaluated for all the formulations prepared.

MATERIALS AND METHODS

Materials

MCH, PLGA 50:50 (molecular weight: 38,000-54,000), PLGA 75:25 (molecular weight: 66,000-107,000), PLGA 85:15 (molecular weight: 190,000-240,000), and polyvinyalcohol (PVA) were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). All other chemicals, such as dichloromethane (DCM), which were used for microsphere preparation, were of analytical grade and used without further purification.

Preparation of microspheres

Two methods, i.e., solid-in-oil-in-water (S/O/W)[15] and co-solvent method, were utilized to investigate the EE of MCH microsphere. In the S/O/W method, 350 mg PLGA was dissolved

in DCM, and the mixture was further sonicated with occasional vortexing to ensure a complete dissolution of the polymer in DCM. Then, the S/O dispersion was produced by suspending 105 mg (30% w/w) of MCH into the PLGA DCM solution using T25 digital ULTRA-TURRAX® (13500 rpm). Subsequently, the PLGA/MCH mixture was slowly added to the PVA aqueous solution dropwise with continuous stirring. Mechanical stirring was performed for 3 h to completely evaporate the organic solvent. The resulting microspheres were collected via filtration and subsequently rinsed three times with deionized water to remove the non-loaded drug and remaining solvent. The washed microspheres were freeze-dried (FDU-2200, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) over night. In the co-solvent method, MCH was dissolved in methanol and then added to a polymer-containing DCM solution. The following procedure was the same as that of the S/O/W method. The investigated process parameters were preparation methods, pH and volume of the PVA solution, volume of DCM, rotation speed, temperature, and amount of PVA (Table 1).

In vitro drug release

The *in vitro* release tests were performed in triplicate in a phosphate-buffered solution (pH 7.4), and the temperature was kept constant (37°C). 10 mg of microspheres were suspended in a 5 mL phosphate-buffered solution containing 0.05 % (w/v) sodium azide and incubated at 37°C with 50 rpm stirring. 1 mL aliquot was withdrawn at each predetermined time point and replaced with fresh and preheated phosphate-buffered solution at each time point to maintain the sink condition. Then, the samples were analyzed via high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) in triplicate.

Encapsulation efficiency

3 mg of loaded microspheres were accurately weighed and completely dissolved in 1 mL acetonitrile, and then the organic solvent was evaporated under a nitrogen gas stream. The resultant was reconstituted with 1 mL distilled water and filtered through a 0.45 µm filter. The samples were analyzed via HPLC (Shimadzu, Kyoto, Japan). The HPLC apparatus equipped with a C8 (4.6×150 mm, Nacalai Tesque) reverse-phase chromatography column was flowed by the mobile phase consisting of acetonitrile and methanol, 0.01 M KH_2PO_4, 0.03 mM Na_2EDTA (5:20:72.1, v/v), and 60% $HClO_4$ (2.9 mL). The pH was adjusted to 2.5.[16] The EE was calculated as the amount of drug per unit weight of the microsphere (equation 1).

$$E.E.\% = \frac{Actual\ loading}{Theoretical\ loading} x100 \quad (1)$$

Experimental design

As represented in Table 1, seven variables, which significantly influence the microsphere EE, were investigated in the optimization study. Parameter A had two levels, and all the other parameters were examined at three levels. PLGA types with different lactide/glycolide ratios, which are an uncontrollable factor (noise), and EE as the response were considered to optimize the process parameters. Based on Taguchi's robust experimental design methodology, an orthogonal array L_{18} (Table 2) was employed to reduce the number of experiments for determining the optimal process parameters for (MCH) microspheres. Based on the layout of the orthogonal array (L_{18}), 18 experimental runs were performed. Under the same conditions, each run needs to be performed in triplicate, thus reducing the experimental error. The 18 experimental runs were accomplished for each type of PLGA.

Analysis of the signal-to-noise ratio

The signal-to-noise (S/N) ratio was calculated as a performance measure in a dynamic system to assess the robustness of a process, which showed the importance of the interaction between controllable parameters and noise factors. Primarily, to accomplish the S/N ratio analysis, the mean squared deviation (MSD) needs to be computed. The value of the MSD indicates the deviation from the target value. For the "larger-the-better" quality characteristic, the MSD and S/N ratio were calculated according to the following equations.[11]

$$MSD = \frac{1}{\mathrm{n}} \sum_{i=1}^{n} \frac{1}{Y_i^2} \quad (2)$$

$$S/N = 10\, Log_{10}(MSD) \quad (3)$$

Table 1. List of control factors and associated levels used in L_{18} design

Parameter	Name	Level-1	Level-2	Level-3
A	Preparation method	S/O/W	Co-solvent method	-
B	pH of PVA solution	3.5	4.5	5.5
C	Volume of PVA solution (mL)	150	250	350
D	Volume of DCM (mL)	2	3	4
E	Rotation speed (rpm)	350	450	550
F	Temperature (°C)	25	37	45
G	Amount of PVA (g)	1.5	2.5	3.5

PVA: Polyvinyalcohol, DCM: Dichloromethane, S/O/W: Solid-in-oil-in-water

Statistical analysis

To investigate and specify the relative significance of the different variables, ANOVA was performed. Statistical analyses were conducted with Prism 7 scientific software by GraphPad.

RESULTS AND DISCUSSION

Effect of the preparation method on the microsphere EE

Seven independent parameters, each having three levels (Table 1), were identified based on some preliminary experiments and related literature review. Figure 1 shows the EE of microspheres, which were made of different PLGA types. Regardless of the PLGA type, microspheres prepared by the S/O/W solvent evaporation method (conditions 1-9) had a much higher EE than that prepared by the co-solvent method (conditions 10-18). When the co-solvent, i.e., methanol, was added, the solution viscosity might be lowered in addition to an increase in water miscibility with the inner oil phase. Therefore, MCH might easily diffuse from the inner oil phase to the outer aqueous phase.[17]

Determination of optimal conditions

In this study, the S/O/W and co-solvent methods were used to encapsulate MCH in polymer matrices. The preparation method involves many parameters that impact the properties and quality of the final product. Optimizing all these parameters using the classic method is time-consuming and costly.[18] The S/N ratio was calculated for each of the formulation run with the larger-the-better EE. Table 3 summarizes the structure of Taguchi's L18 orthogonal array design, EE of the microspheres for each PLGA type, and S/N ratio for the EE. The average S/N ratio of each control factor at each level was also calculated. Figure 2 indicates that the S/N ratio increases as the pH of an aqueous phase (B) becomes higher. It would be due to the decreased solubility of MCH in the outer aqueous phase because MCH is

Table 2. Layout of orthogonal array L_{18} (2^1x3^7)

Run	A: Preparation method	B: pH of PVA solution	C: Volume of PVA solution (mL)	D: Volume of DCM (mL)	E: Rotation speed (rpm)	F: Temperature (°C)	G: PVA concentration (g)
1	S/O/W	3.5	150	2	350	25	1.5
2	S/O/W	3.5	250	3	450	37	2.5
3	S/O/W	3.5	350	4	550	45	3.5
4	S/O/W	4.5	150	2	450	37	3.5
5	S/O/W	4.5	250	3	550	45	1.5
6	S/O/W	4.5	350	4	350	25	2.5
7	S/O/W	5.5	150	3	350	45	2.5
8	S/O/W	5.5	250	4	450	25	3.5
9	S/O/W	5.5	350	2	550	37	1.5
10	Co-solvent method	3.5	150	4	550	37	2.5
11	Co-solvent method	3.5	250	2	350	45	3.5
12	Co-solvent method	3.5	350	3	450	25	1.5
13	Co-solvent method	4.5	150	3	550	25	3.5
14	Co-solvent method	4.5	250	4	350	37	1.5
15	Co-solvent method	4.5	350	2	450	45	2.5
16	Co-solvent method	5.5	150	4	450	45	1.5
17	Co-solvent method	5.5	250	1	550	25	2.5
18	Co-solvent method	5.5	350	3	350	37	3.5

PVA: Polyvinyalcohol, DCM: Dichloromethane, S/O/W: Solid-in-oil-in-water

a weak basic drug that is unionized at a higher pH.[19] Volumes of aqueous phase (C) and organic phase (D) are also important. As the volume of the aqueous phase was increased, the MCH of the aqueous solubility became approximately 2% (v/v), which can more readily distribute into the aqueous phase. Therefore, the drug would be more stably deposited in the polymer matrix. Because DCM reduced the viscosity of the organic phase, an initial state of dispersion would be better. The moderate rotation speed (E) and temperature (F) appeared to be optimal, which might be related to the stability of the S/O/W emulsion and evaporation rate of the organic phase. However, nonlinear interactions between factors might be present in the S/N ratios. The amount of PVA (G), which was used as a surfactant for stabilizing the emulsions, appeared to affect the EE of MCH microspheres, whereas the effect was saturated at an amount of 2.5 g. As shown in Figure 2, a greater S/N value corresponds to a better performance. Therefore, the optimized factor levels are the levels with the highest S/N value, which were predicted to be $A_1/B_3/C_2/D_2/E_2/F_2/G_3$, leading to a maximum EE.

Based on the S/N ratio, the optimal level of the control factors were determined, as shown in Table 4. The predicted mean of the S/N ratio (S_{mp}) was estimated to be 27.25±6.57 dB, using the following equation:[20]

$$S_{mp}=+(A_1-Y)+(B_3-Y)+(C_2-Y)+(D_2-Y)+(E_2-Y)+(F_2-Y)+(G_3-Y), \quad (4)$$

where *Y* is the total average of S/N ratio for the experimental test presented in Table 5. To validate the optimal process parameters, confirmation experiments were conducted. In equation 4, the S/N ratio of the EE for the optimum levels $A_1/B_3/C_2/D_2/E_2/F_2/G_3$ was 23.48 dB, which is within the range of 95% confidence intervals of the prediction. In addition, the S/N ratio increased by 5.76 dB from that of an initial condition. Table 4 shows the response table for the S/N ratio of the "the-bigger-is-better" EE, which was obtained for different parameter levels. The analysis of the S/N ratio of the EE reveals that the main factor that caused the EE increase was the preparation method (S/O/W). Other factors are the pH of the PVA solution, temperature (°C), rotation speed (rpm), volume of the PVA solution, amount of PVA (g), and volume of DCM (mL).

ANOVA

The main objective of ANOVA is to investigate and identify the factors that have statistically significant effects on the quality characteristics.[21] In Taguchi's method, after the analysis of

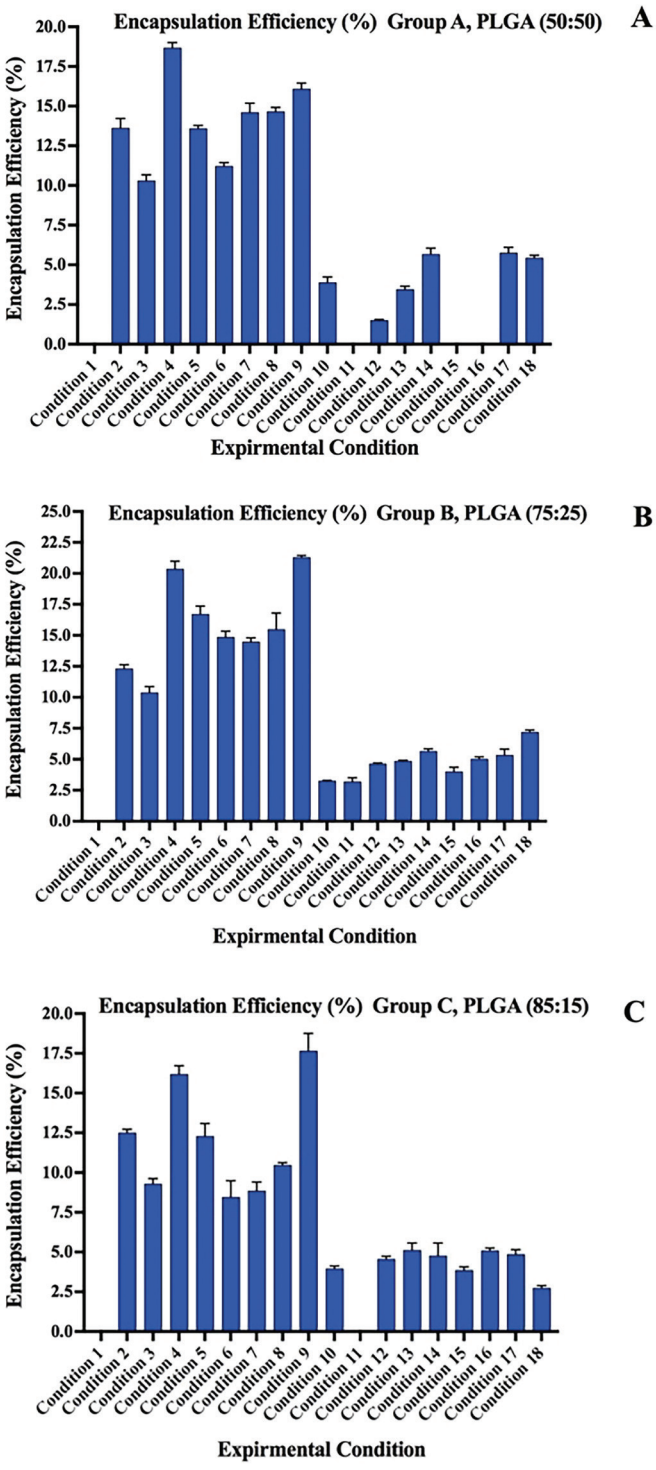

Figure 1. Encapsulation efficiency of minocycline microspheres (MCH) which was made PLGA with different ratio of lactide and glycolide acid (mean ± SD; n=3). A) PLGA (50:50), B) PLGA (75:25), and C) PLGA (85:25). PLGA: Poly(D,L-lactide-co-glycolide), MCH: Minocycline hydrochloride, SD: Standard deviation

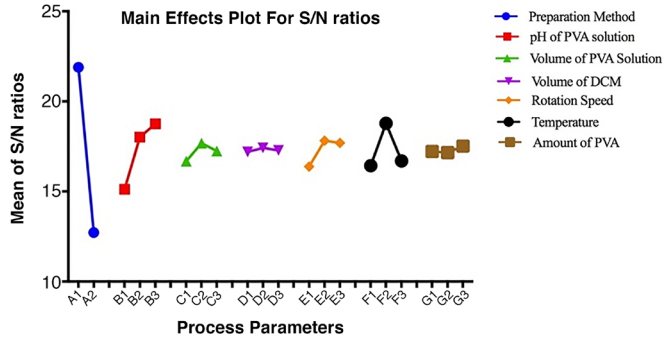

Figure 2. Variation of S/N ratios with factor levels for MCH microspheres encapsulation efficiency. A: Preparation method, B: pH of PVA solution, C: Volume of PVA solution, D: Volume of DCM, E: Rotation speed, F: Temperature, G: Amount of PVA. PVA: Polyvinyalcohol, MCH: Minocycline hydrochloride, DCM: Dichloromethane, S/N: Signal-to-noise

the S/N ratio, an ANOVA should be performed to estimate the variance and specify the relative significance of the different factors. As shown in Table 6, the preparation method and pH of the PVA solution are the most important factors that significantly affect the EE of MCH microspheres.

In vitro release of minocycline from PLGA microspheres

Figures 3A-C depict the release profile of MCH from microspheres, which were made of different PLGA types. The release of MCH from the microspheres was investigated up to 96 h. In the PLGA (50:50) and PLGA (75:25) microspheres, the release rate of MCH was higher in the initial 48 h and became

Table 3. Taguchi's L_{18} orthogonal array design, encapsulation efficiency of microspheres for each type of PLGA and S/N ratio for encapsulation efficiency (mean ± SD; n=3)

Exp. no	Controllable factors								Encapsulation efficiency %				S/N ratio
	A	B	C	D	E	F	G	H	PLGA (50:50)	PLGA (75:25)	PLGA (85:25)	Average	
1	1	1	1	1	1	1	1	1	#	#	#	#	#
2	1	1	2	2	2	2	2	2	13.60±0.62	12.3±0.36	12.5±0.23	12.8±0.70	22.1
3	1	1	3	3	3	3	3	3	10.30±0.39	10.4±0.33	9.27±0.35	9.98±0.61	19.9
4	1	2	1	1	2	2	3	3	18.70±0.35	20.3±0.49	16.2±0.53	18.4±2.09	25.2
5	1	2	2	2	3	3	1	1	13.60±0.22	16.7±0.64	12.27±0.34	14.18±2.27	22.8
6	1	2	3	3	1	1	2	2	11.20±0.24	14.8±0.66	8.98±1.05	11.5±3.31	20.5
7	1	3	1	2	1	3	2	3	14.60±0.58	14.5±0.50	8.84±0.57	12.6±3.28	21.3
8	1	3	2	3	2	1	3	1	14.60±0.29	15.5±0.34	10.5±0.16	13.5±2.68	22.2
9	1	3	3	1	3	2	1	2	16.10±0.37	21.3±1.33	17.6±1.12	18.3±2.67	25.1
10	2	1	1	3	3	2	2	1	3.78±0.35	3.24±0.07	3.93±0.19	3.66±0.38	11.2
11	2	1	2	1	1	3	3	2	#	3.19±0.06	#	3.19±0.06	5.65
12	2	1	3	2	2	1	1	3	1.50±0.05	4.61±0.33	4.54±0.20	3.55±1.77	15
13	2	2	1	2	3	1	3	2	3.44±0.21	4.85±0.10	5.11±0.46	4.47±0.89	12.6
14	2	2	2	3	1	2	1	3	5.65±0.41	5.64±0.06	4.76±0.81	5.35±0.51	14.5
15	2	2	3	1	2	3	2	1	#	3.98±0.20	3.84±0.22	3.91±2.26	11.8
16	2	3	1	3	2	3	1	2	#	5.01±0.38	5.07±0.18	5.04±2.91	14.1
17	2	3	2	1	3	1	2	3	5.74±0.37	5.32±0.18	4.84±0.31	5.3±0.45	14.4
18	2	3	3	2	1	2	3	1	5.42±0.17	7.19±0.50	2.72±0.17	5.11±2.24	12

#: Microsphere does not form, A: Preparation method, B: pH of PVA solution, C: Volume of PVA solution, D: Volume of DCM, E: Rotation speed, F: Temperature, G: Amount of PVA, PLGA: Poly(D,L-lactide-co-glycolide), S/N: Signal-to-noise, PVA: Polyvinyalcohol, DCM: Dichloromethane, SD: Standard deviation

Table 4. S/N ratio of initial and optimized preparation conditions

	Level	S/N ratio (db)
Initial	$A_2/B_2/C_2/D_2/E_2/F_2/G_2$	17.72
Predicted	$A_1/B_3/C_2/D_2/E_2/F_2/G_3$	27±6.57
Experimental	$A_1/B_3/C_2/D_2/E_2/F_2/G_3$	23.42

Improvement of S/N ratio: 5.76 dB, S/N: Signal-to-noise

Table 5. Response table for S/N ratio values for encapsulation efficiency by factor level

	Level	S/N Ratio (db)
Initial	$A_2/B_2/C_2/D_2/E_2/F_2/G_2$	17.72
Predicted	$A_1/B_3/C_2/D_2/E_2/F_2/G_3$	27±6.57
Experimental	$A_1/B_3/C_2/D_2/E_2/F_2/G_3$	23.42

Improvement of S/N ratio: 5.76 dB, S/N: Signal-to-noise

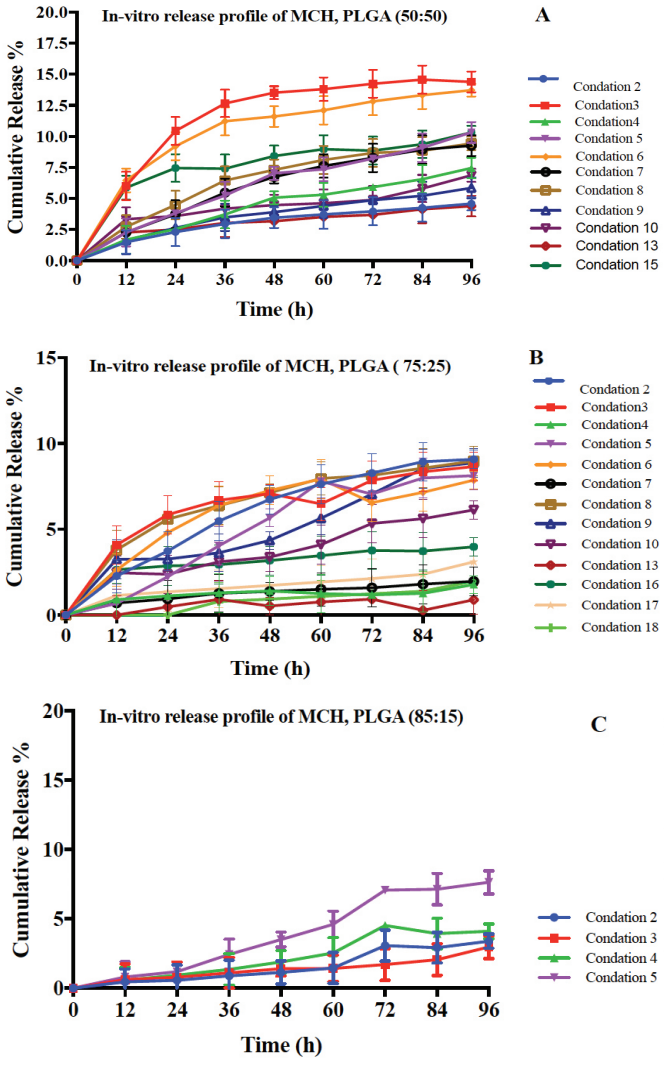

Figure 3. *In vitro* release profile of minocycline hydrochloride in PBS medium pH: 7.4 (mean ± SD; n=3). A: *In vitro* release profile of minocycline hydrochloride form microspheres made of PLGA (50:50), B: *In vitro* release profile of minocycline hydrochloride form microspheres made of PLGA (75:25), and C: *In vitro* release profile of minocycline hydrochloride form microspheres made of PLGA (85:15). MCH: Minocycline hydrochloride, PBS: Phosphate buffered saline, PLGA: Poly(D,L-lactide-co-glycolide), SD: Standard deviation

slower but steady thereafter. Furthermore, many PLGA (85:15) microspheres (Figure 3C) did not show any detectable drug release within 96 h. Release kinetics is well described by the Higuchi's equation, indicating that drug released from the microspheres is diffusion limited.

Optimizing all the parameters of the microsphere preparation methods require to achieve a maximum EE for MCH-loaded PLGA microspheres using the classic method, which is time-consuming and costly.[18] As such, Taguchi's orthogonal array design was employed to investigate the optimal conditions required for the production of microspheres with the highest percentage of EE and to establish a robust preparation method that could steadily provide MCH microspheres regardless of used polymer types. Taguchi's design is meant for investigating the influence of seven independent parameters, each having three-level values.

As shown in Table 3, the mean EE for PLGA (50:50), (75:25), and (85:15) over the 18 experimental runs were in the range of 18.70-1.50%, 21.3-3.1%, and 17.6-2.7%, respectively. Microspheres were not formed in experimental runs 1, 11, 15, and 16 for PLGA (50:50) and in experimental run 11 for PLGA (85:15). Microspheres with the highest EE (18.70%, 21.3%, and 17.6%) were obtained from experimental runs 4 and 9 for PLGA (50:50), (75:25), and (85:15), respectively.

The data obtained from the EE assessment revealed that the microspheres prepared by the S/O/W solvent evaporation method had a much higher EE in comparison to those by the co-solvent method. MCH is a hydrophilic and weak basic drug and shows high solubility in alcohols due to its ability to form hydrogen bonds with solute molecules.[22] Furthermore, the miscibility of methanol with water and increasing solubility of MCH are likely to have resulted in a diffusional loss of MCH in the aqueous phase.[17] Thus, the microspheres prepared via the co-solvent method had a low EE.

Furthermore, the Minitab software was used for the statistical analysis of the L18 OA design results.[23] The response table includes the mean S/N ratio for each level of the parameters and ranks based on the delta value, which shows the relative importance of effects (Table 5), and the parameters are

Table 6. ANOVA results for signal-to-noise ratio for encapsulation efficiency

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	f value	p value
Preparation method	1	383.219	84.66%	342.381	342.381	2116.71	0.001
Volume of PVA solution	2	17.885	3.95%	7.166	3.583	22.15	0.016
Amount of PVA (g)	2	1.541	0.34%	3.852	1.926	11.92	0.037
Temperature	2	17.135	3.79%	16.243	8.121	50.21	0.005
Volume of DCM (mL)	2	2.230	0.49%	0.701	0.351	2.17	0.261
pH of PVA solution	2	20.374	4.50%	18.310	9.155	56.60	0.004
Rotation speed (rpm)	2	9.814	2.17%	9.814	4.907	30.34	0.010
Error	3	0.485	0.11%	0.485	0.162	-	-
Total	16	452.683	100.00%	-	-	-	-

DF: Degrees of freedom, Seq SS: Sequential sums of squares, Adj SS: Adjusted sum of squares, Adj MS: Adjusted mean squares, PVA: Polyvinyalcohol, DCM: Dichloromethane

arranged according to their importance. In the main effect plot (Figure 2), the relatively horizontal lines indicate that the parameter has fewer effects on the response. Therefore, the factors with the highest gradient lines may have the greatest impact. As shown in Figure 2, parameter A (preparation method) is the most significant factor, whereas parameter D (volume of DCM) has no significant effect. The ANOVA results for the selected model outlined in Table 5 show that the preparation method with a p value of 0.001 and maximum contribution of 84.66% has the most significant effect on the EE.

The release profile of MCH-loaded into PLGA 50:50, 75:25, and 85:15 microspheres were investigated for 96 h, and the results are presented in Figure 3. The results indicate that only 14.5%, 8.9%, and 7.6% of the total drug load had been released from the microspheres prepared from PLGA 50:50, 75:25, and 85:15, respectively. This case could be associated with the polymer composition as the most important factor responsible for the hydrophobicity and rate of degradation of a delivery matrix.[24] The hydrolytic degradation of PLGA is related to the lactide/glycolide ratio, end group (ester or free carboxyl group), and molecular weight of the polymer.[25]

CONCLUSION

Although the interaction between the selected factors in the evaluation was ignored, the orthogonal array DOE on the basis of Taguchi's robust experimental design methodology was sufficient to optimize the process parameters for the PLGA microspheres of MCH. The microsphere preparation method (S/O/W) was the main factor affecting the EE. Microspheres prepared via the S/O/W method exhibited higher EEs and faster drug release than microspheres prepared via the co-solvent method. The pH and volume of the aqueous phase were also effective parameters on the EE. The release of minocycline was the fastest with PLGA (50:50) microspheres, followed by PLGA (75:25) and PLGA (85:15) microspheres in this order. A robust experimental design was successfully applied to the optimization of process parameters for MCH-loaded PLGA microsphere preparation.

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REFERENCES

1. Maltesen MJ, Bjerregaard S, Hovgaard L, Havelund S, van de Weert M. Quality by design - spray drying of insulin intended for inhalation. Eur J Pharm Biopharm. 2008;70:828-838.
2. Guideline IHT. Pharmaceutical development. Q8 (R2) Current Step. 2009;4. Available from: https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf
3. Shah P, Goodyear B, Haq A, Puri V, Michniak-Kohn B. Evaluations of quality by design (QbD) elements impact for developing niosomes as a promising topical drug delivery platform. Pharmaceutics. 2020;12:246-259.
4. Lawrence XY, Amidon G, Khan MA, Hoag SW, Polli J, Raju G. Understanding pharmaceutical quality by design. AAPS J. 2014;16:771-783.
5. Houng JY, Hsu HF, Liu YH, Wu JY. Applying the Taguchi robust design to the optimization of the asymmetric reduction of ethyl 4-chloro acetoacetate by bakers' yeast. J Biotechnol. 2003;100:239-250.
6. Varshosaz J, Tavakoli N, Minayian M, Rahdari N. Applying the Taguchi design for optimized formulation of sustained release gliclazide chitosan beads: An *in vitro/in vivo* study. AAPS PharmSciTech. 2009;10:158-161.
7. Jeon EC, Park JS, Kwon D. Statistical analysis of experimental parameters in continuous indentation tests using Taguchi method. J Eng Mater Technol. 2003;125:406-411.
8. Zhang J, Chen J, Kirby E. Surface roughness optimization in a end-milling operation using the Taguchi design method. J Mater Process. 2007;184:233-239.
9. Co H. Confirmation testing of the Taguchi methods by artificial neural-networks simulation. Int J Prod. 2008;46:4671-4685.
10. Li B, Nye T, Metzger D. Improving the reliability of the tube-hydroforming process by the Taguchi method. J Presssure Vesssel Technol. 2007;129:242-247.
11. Abdullah J, Khan Z, Khu SY. Optimizing flexible behaviour of bow prototype using Taguchi approach. Res J Appl Sci. 2006;6:622-630.
12. Huang ML, Hung YH, Yang ZS. Validation of a method using Taguchi, response surface, neural network, and genetic algorithm. Measurement. 2016;94:284-294.
13. Montgomery DC. Experimental design for product and process design and development. J R Stat Soc Ser D Stat. 1999;48:159-177.
14. Goulden V, Glass D, Cunliffe W. Safety of long-term high-dose minocycline in the treatment of acne. Br J Dermatol. 1996;134:693-695.
15. Marquette S, Peerboom C, Yates A, Denis L, Goole J, Amighi K. Encapsulation of immunoglobulin G by solid-in-oil-in-water: Effect of process parameters on microsphere properties. Eur J Pharm Biopharm. 2014;86:393-403.
16. Colovic M, Caccia S. Liquid chromatographic determination of minocycline in brain-to-plasma distribution studies in the rat. J Chromatogr B. 2003;791:337-343.
17. Rawat A, Burgess DJ. Effect of ethanol as a processing co-solvent on the PLGA microsphere characteristics. Int J Pharm. 2010;394:99-105.
18. Lai MK, Tsiang RCC. Microencapsulation of acetaminophen into poly(L-lactide) by three different emulsion solvent-evaporation methods. J Microencapsul. 2005;22:261-274.
19. Cha KH, Park J, Cho W, Gu DG, Jeong K, Hwang SJ. Design of pH-independent extended release matrix tablets of minocycline hydrochloride for the treatment of dementia. Arch Pharm Res. 2009;32:1593-1598.
20. Chaulia PK, Das R. Process parameter optimization for fly ash brick by Taguchi method. J Mater Res. 2008;11:159-164.
21. Asghar A, Raman A, Aziz A, Daud WMAW. A comparison of central composite design and Taguchi method for optimizing Fenton process. Sci World J. 2014;2014:869120.

22. Li R, Yin X, Jin Y, Chen X, Zhao B, Wang W, Zhong S, Han D. The solubility profile and dissolution thermodynamic properties of minocycline hydrochloride in some pure and mixed solvents at several temperatures. J Chem Thermodynamics. 2021;157:106399.

23. Manual MU. Making data analysis easier. Pennsylvania: MINITAB Inc; 2001.

24. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. J Polym. 2011;3:1377-1397.

25. Ansary RH, Awang MB, Rahman MM. Biodegradable poly (D, L-lactic-co-glycolic acid)-based micro/nanoparticles for sustained release of protein drugs-A review. Trop J Pharm Res. 2014;13:1179-1190.