FACTOR ANALYSIS OF DRUG LOADING OF NANOPARTICLES USING FLASH NANOPRECIPITATION

A Thesis submitted

By

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Dedication

This thesis is dedicated to my beloved parents – Dingqiang Lu and Lin Qiao. Your love, instruction, and support have always been an inspiration in my life.

ABSTRACT

Nanoparticles are an excellent drug delivery platform for cancer therapy. Flash nanoprecipitation (FNP) is a novel nanofabrication technique that enables high drug loading and narrow size distribution. Several factors such as drug amount, polymer amount, polymer molecular weight, solvent, and surfactant concentration play a critical role in determining the particle size, polydispersity index, and drug loading when producing nanoparticles. But how these factors affect the properties of nanoparticles prepared by FNP, and whether those properties can be predicted is not known. Design of experiment (DOE) method can help investigate the relationship between responses and factors (or interaction between factors) while significantly reducing the number of runs. We propose that applying DOE to nanoparticle preparation by FNP would allow us to predict the parameter values for an optimized nanoparticle formulation. In this study, paclitaxel is used as a model drug, and the goal of this study was to determine the effect of various formulation factors on properties of PLGA nanoparticles fabricated by FNP and draw the predict profile.

KEYWORDS: Flash nanoprecipitation; FNP; PLGA nanoparticles; paclitaxel; PTX; Design of experiment; DOE

OUTLINE

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Chapter I

Introduction

1.1 PTX-loaded PLGA NPs

Paclitaxel (PTX), a chemical isolated from the pacific yew tree, is one of the most effective chemotherapy drugs in use today. It targets a broad range of cancers, such as ovarian, breast, and lung cancers; however, it has poor water solubility, which has limited its clinical application. [1] Nanoparticle drug delivery is an excellent platform to address this problem, which provides a means to encapsulate hydrophobic drugs as well as enhance the permeability and retention (EPR) effect for tumor targeting in cancer therapy. [2] Biocompatible amphiphilic polymers, such as PEG, PLGA, and PCL, were introduced for the fabrication of nanoparticles to increase drug loading and enhance effectiveness and stability while minimizing its toxicity and side effects. [3] More specifically, paclitaxel has been loaded PLGA nanoparticles and may prove to be an effective treatment for cancer. [1, 4]

1.2 Flash Nanoprecipitation (FNP) and Confined Impingement Jet (CIJ) Mixer

The most widely used method to synthesize PLGA nanoparticles is emulsion-solvent evaporation; however, it is more suitable for water-soluble drugs. [5] Flash nanoprecipitation (FNP) is a process that has been used to load hydrophobic drugs into nanoparticles. It was developed by the Prud'homme group to realize the advantages of high drug loading and narrow particle size distribution. [6] Generally, an active pharmaceutical ingredient (API) and amphiphilic block polymer are dissolved in a water-miscible organic solvent and then rapidly mixed with an antisolvent (water) that contains a surfactant, which leads to the spontaneous formation of nanoparticles. [7] The fabrication of PLGA

nanoparticles via FNP goes through four stages: supersaturation, nucleation, growth by condensation, and growth by coagulation. [8] The Prud'homme group concluded that the nucleation rate constant, N, follows the Arrhenius equation [9]

$$N = Ae^{\frac{-16\pi\gamma^3 v^2}{3k^3T^3(lnS)^2}} \qquad (1)$$

where, γ is the surface tension, v is molar volume, T is absolute temperature, S is supersaturation ratio, which is defined as the ratio of solubility at the interface to the bulk solubility, A is the pre-exponential factor and k is Boltzmann's constant. The key parameter at room temperature of the drug nucleation kinetics is the degree of supersaturation. Figure 1.1 is a schematic illustration of the nanoprecipitation process.

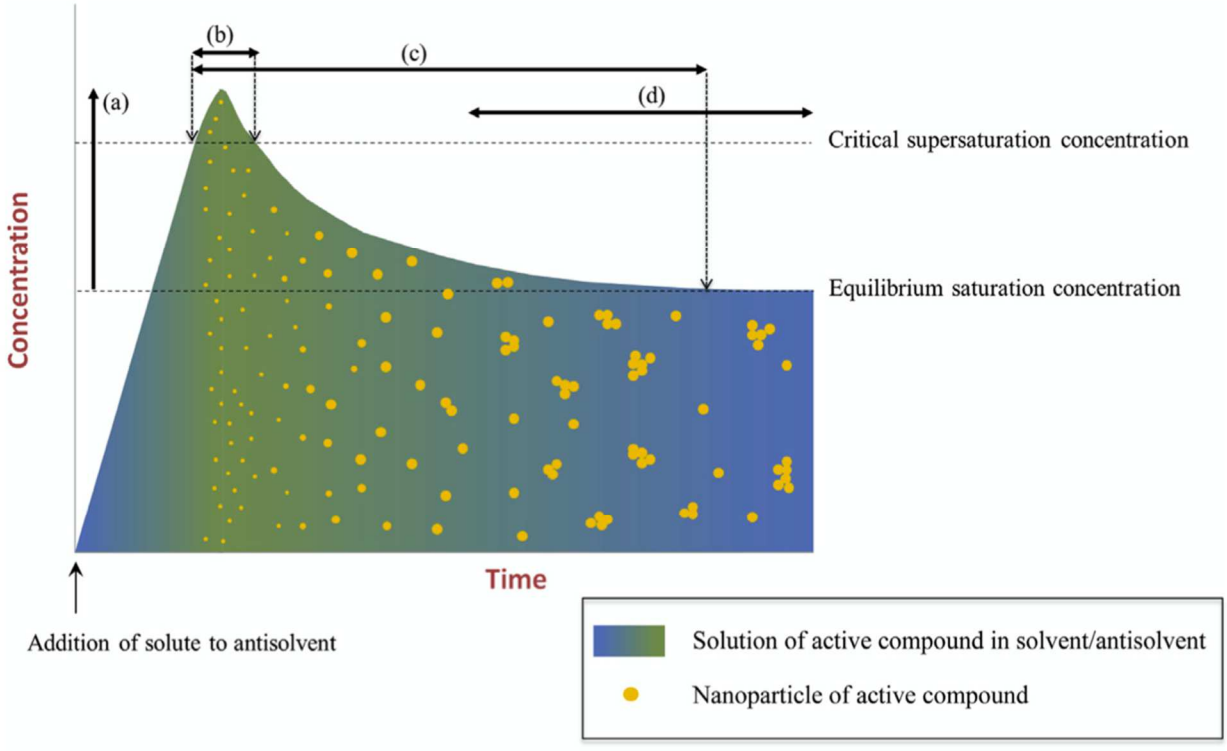


Figure 1.1 *Schematic illustration of polymer precipitation process, including supersaturation (a), nucleation (b), particle growth by condensation (c), and by coagulation (d). Adapted from Joye and McClements, 2013. [10]*

The drug is in solution at the initial state in the good solvent, and the graph depicts the concentration in the antisolvent, which rises with mixing. [10] When the amount of solute in the mixed solvent solution exceeds its solubility, the system is in a state of supersaturation, where the absolute Gibbs energy is relatively high. When solute aggregation reaches the size equal to the critical nuclei, which is a function of the critical supersaturation concentration, nucleation is initiated. This then causes the concentration of drug in solution to decrease, which continues until the concentration falls below the critical supersaturation concentration. The nuclei undergo continual growth by condensation or coagulation. As shown in the figure, growth by condensation occurs simultaneously with growth by nucleation and ends when the solute concentration decreases below the equilibrium saturation concentration. During this time, when attractive interactions between particles are stronger than their repulsive interactions, adhesion takes place, which promotes coagulation. The critical factor in coagulation is collision frequency, which is mainly affected by particle concentration, size distribution, and convective mixing. When controlling the particle size is a formulation requirement, it is necessary to devise a strategy to protect nanoparticles from growth by coagulation. Some surfactants or copolymers functioning as stabilizing agents can be added into the formulation. Those agents adsorb to the surface of nanoparticles and enhance the repulsive interaction. [10]

For example, block copolymers such as PLGA arrest nanoparticles at the growth stage, and thus limit the size growth and narrow size distribution. [11]

An essential requirement for successful FNP is rapid mixing in a confined volume, as this dictates the supersaturation conditions. For this purpose, Prud'homme et al. originally developed a confined impingement jet (CIJ) mixer. [12] Macosko et al. modified this CIJ with a dilution (CIJ-D) mixer that provides ease of use, requires less volume of solvent but maintains uniform particle size, narrow size distribution, and reproducibility.[13] Figure 1.2 is a schematic illustration of CIJ-D mixer (A) and mixing process (B). Instead of electrically powered input pumps, low-friction syringes with small volume allows simple and rapid hand operation. The addition of antisolvent dilution enables rapid quenching, immediate decrease in drug concentration and significantly enhanced nanoparticle stability. This CIJ-D mixer was made of high-density polyethylene and has a favorable design for quick screening of candidate formulations and easy operation in the laboratory.[13]

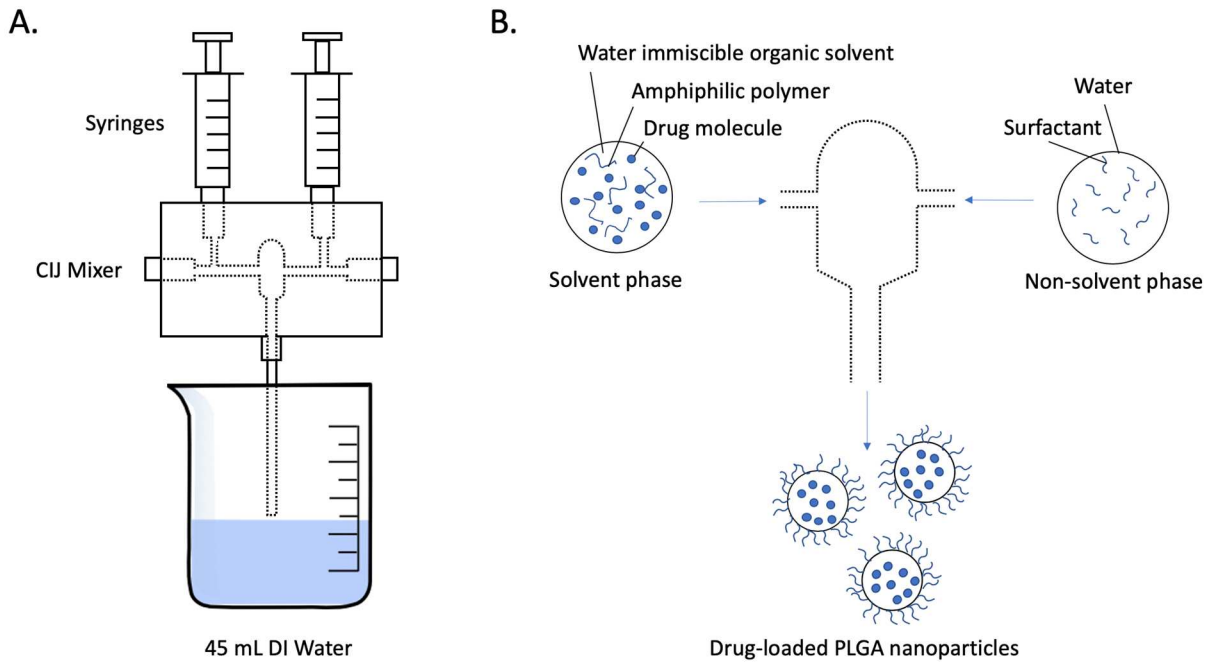


Figure 1.2 *Schematic illustration of experimental set up (A) and mixing process (B) of flash nanoprecipitation via modified CIJ-Dilution mixer.*

Advantages of FNP over other techniques are simplicity, scalability, reproducibility, and controllable narrow size distribution. However, the method has the drawback of being unsuitable for water-soluble drugs. [14, 15]

As expected from the governing equation, drug loading, size, and size distribution will vary with temperature, mixing time, and formulation (drugs, block polymers, solvent, and stabilizers). However, a specific outcome cannot be predicted at this time, but a number of investigators have examined several experimental conditions. Smaller particle sizes and narrower size distribution were achieved, if the operation temperature was decreased from 54 ^{o}C to room temperature to 4 ^{o}C. [16] As discussed earlier, rapid mixing is critical for

successful nanoparticle synthesis, as slow mixing will cause a large size with broad distribution.

Drug loading is defined as the ratio of drug amount to polymer amount; theoretically, increase drug loading can be achieved either by adding more drug or reducing the amount of polymer. However, this was not the case in Chorny et al. (2002), where there was no significant effect on particle size and drug loading with increasing amount of drug. In contrast, Govender et al. (1999) and Khayata et al. (2012) observed an increase in particle size with more added drug, although FNP was not used. In augmenting the polymer amount, Chorny et al. (2002) and Dong and Feng (2004) stated that particle size and drug loading increased at the same time, which differs from the earlier observations. This could be a consequence of interactions among drug molecule, polymer and surfactant.

A contribution from the water-miscible solvent could also be partly responsible for the size and drug loading. Commonly used FNP solvents are methylene chloride (boiling point, bp 39.8 ^{o}C), acetone (bp 56 ^{o}C), methanol (bp 64.6 ^{o}C), THF (bp 65 ^{o}C), hexanes (bp 69 ^{o}C), ethanol (bp 78.3 ^{o}C), and acetonitrile (bp 81 ^{o}C). These can provide different supersaturation conditions during rapid mixing leading to variable outcomes in particles size and drug loading. Moreover, the wide range of boiling points require different methods for solvent removal. [17]

The surfactant concentration in the solution will affect the particle size and polydispersity index (PDI) particularly during the coagulating growth stage. Contado et al. (2013)

revealed that as surfactant concentration increased, particle size initially increased, but with further increase in concentration the size was smaller. They suggested that at low surfactant concentration, the minimal adsorption on polymer was associated with a weak surface charge and small repulsive barrier, which resulted in particle aggregation. However, as the viscosity of aqueous phase was increased, there was a reorganization caused by polymer-surfactant interactions, and the net shear stress required for aggregates breakdown was reduced, leading to better dispersion of nanoparticles. As given in the review by Miladi et al. (2016), others did not observe any relationship among size, drug loading and surfactant concentration on the properties of the nanoparticles. As the effect remains ambiguous in the relevant literature, the surfactant concentration, which leads to optimized particle size in the formulation, needs further investigation.

Polymer molecular weight is an important factor as it directly affects the viscosity of the system as well as possible particle-particle interactions. Limaye Blouza et al. (2006) and Martin-Banderas et al. (2012) reported that as polymer molecular weight increased, the size decreased. This was different from the observation by Seremeta et al. (2013). In contrast, Budhian et al. (2007) claimed there is no significant influence of molecular weight on size. As such, the effect of polymer molecular weight on nanoparticle properties remains a complicated and unresolved area. Variable factors have been discussed separately; yet, the interaction between parameters could also play a role in forming nanoparticles. Thus, when screening for the optimized formulation for PTX-loaded PLGA nanoparticles, tools for simplification of research is important to reduce the experimental burden.

1.3 Design of Experiments

FNP is clearly a promising technique for nanoparticle preparation, but the complexity arising from the many parameters can influence the outcome poses a significant challenge. Historically, the approach to determine the effect of any given parameter on the experimental outcome is embodied in the concept of controlled trial. Here, a one-factor-at-a-time (OFAT) method is used to conduct the controlled trials, where the level of only one factor is changed in a given experiment and all other factors are kept constant. Although the approach will ultimately provide the needed information, the time and effort required increases with each parameter of interest. In addition, a major shortcoming arises, because the effect of interaction between multiple factors on the results is neglected. Moreover, when there are a large number of various factors, the experiment becomes unrealistic in terms of time and cost.

The design of experiment (DOE) is an effective approach to address the drawbacks of OFAT. The most commonly used method for screening critical factors among DOE methods is two-level factorial design. For a fractional screening design, it only requires a fraction of the total number of runs in the full factorial design. The fractional factorial design also provides statistical power to the estimation of effects, whereas OFAT needs extra replicate runs to provide the same level of power. For example, for a two-level and two-factorial study, OFAT requires six runs in total, whereas DOE only requires four runs. For a two-level and three-factorial study, OFAT requires sixteen runs, and DOE requires eight runs. [18] Given the complexity and the multiple parameters that affect the outcome of FNP, a fractional factorial design is the best choice of screening important factors.

Chapter II
Statement of the Problem

2.1 Statement of the Problem

When preparing nanoparticles for a certain drug at room temperature and maximizing the stirring rate, many factors could affect the outcome. These include drug amount, polymer amount, polymer molecular weight, solvent, and surfactant concentration. These factors can potentially impact the particle size, polydispersity index, and drug loading. Moreover, interaction between and among the factors may occur, which makes optimizing the levels of critical parameters a significant challenge. That is, a factorial experiment involving five factors given above at three levels carried out in triplicate would require 729 preparations, along with their characterizations. While the best formulation can be identified among those prepared, there is no means of identifying the optimal. However, a statistical approach using a design of experiment (DOE) can provide such information. Moreover, a preliminary study can also be carried out to constrain the number of factors as well as establish the appropriate range for the levels tested.

The objective of this thesis was to investigate the relationship between various parameters and responses for paclitaxel-loaded nanoparticles fabricated by FNP using design of experiment. We assumed that the nanoparticles prepared by FNP will be affected by drug amount, polymer amount, polymer molecular weight, solvent, and surfactant concentration. We proposed that applying DOE to nanoparticle preparation by FNP would allow prediction of the parameter values for an optimized nanoparticle formulation.

Thus, a simplified model was used to assess the effect of the five factors (drug amount, polymer amount, polymer molecular weight, solvent, and surfactant concentration) on

nanoparticle properties of (particle size distribution and drug loading) by using a 2-level fractional factorial design. Following this preliminary screen, the critical parameters and the appropriate range for testing were identified that formed the basis of a second DOE, which provided a prediction of the optimal formulation. Finally, this predicted optimized formulation was evaluated by preparation and characterization.

2.2 Specific Aims

Aim 1 – Develop a simplified model to predict the effect of various parameters on nanoparticle properties and screen critical parameters by using a 2-level fractional factorial design.

Aim 2 – Investigate the relationship between factors and responses using a full factorial design and validate the generated model.

Chapter III
Materials and Methods

3.1 Materials

Polyvinyl alcohol (PVA), sucrose, and methanol (HPLC grade) were purchased from Sigma-Aldrich, St Louis, MO. Acetonitrile (LC/MS grade), tetrahydrofuran (certified) were obtained from Fisher Chemical, Fair Lawn, NJ. Poly lactide-co-glycolide (PLGA, ratio 50/50, ester terminated, inherent viscosity range: 0.26-0.54; 0.55-0.74; 0.95-1.20 dL/g in HFIP) was obtained from LACTEL, Durect Corporation, Birmingham, AL. Paclitaxel was manufactured by Phytogen Life Sciences, Delta, B.C. Canada.

3.2. Methods

3.2.1. Screening critical factors design of experiments (DOE)

A two-level/five-factor fractional factorial design was applied to study the effect of the identified critical factors. The elements are shown in Table 1. The screening design was comprised of 16 formulations, and each formulation was prepared and tested in triplicate.

Table 3.1. List of the five identified factors and the two specific levels at which they were experimentally assessed for the design of experiment

Factor	Lower level/Negative	Higher-level/Positive
Polymer amount	5 mg	25 mg
Surfactant concentration	0.2 % PVA	2 % PVA
Drug amount	5 mg	25 mg
Solvent type *	Acetonitrile (ACN)	Tetrahydrofuran (THF)
Polymer molecular weight *	Low (L)	High (H)

*Note: Polymer amount, surfactant concentration, drug amount are numeric parameters, whereas solvent type and polymer molecular weight (marked with *) are categorical parameters. Since the LACTEL does not provide an accurate number of polymer molecular weight, this parameter is considered as categorical.*

3.2.2. Preparation of drug-loaded PLGA nanoparticles

Drug loaded PLGA nanoparticles were prepared using a custom-built, nano-precipitating device called Confined Impinging Jets (CIJ, schematically shown in Figure 1). [4] In this device, an aqueous solution containing polymeric surfactant is combined with an organic solution containing the nanoparticle matrix polymer, PLGA, and drug, paclitaxel. The two phases are introduced at equal flow rates and combined in a small volume, mixing chamber. In the study, the concentration of PVA, PLGA, and drug was varied according to the DOE. Acetonitrile (ACN) and tetrahydrofuran (THF) were used as organic solvents. In general, for the preparation of nanoparticles, PVA was dissolved in deionized/distilled (DI) water by stirring overnight at a temperature of 60 ^{o}C and then diluted to the desired concentration. PLGA and PTX were dissolved in the organic solvent by stirring for 30 min. A volume of 2.5 ml of each solution was drawn into separate 5 ml glass syringes, and air bubbles were carefully removed. The needles were attached to the mixer, and a bar was used to apply the same pressure to each syringe barrel to introduce the solutions at equal flow rates.

The outflow dispersion, containing the nanoparticles, was stirred for 14-16 hours under vacuum to allow the organic solvent to evaporate. The sample was centrifuged (Optima SPN-80 Ultracentrifuge, Beckman Coulter, Brea, CA) at 40,000 rpm for 1 hr to pellet the

nanoparticles. The pellet was suspended in 5 mL of DI water, transferred to a 50 ml centrifuge tube, and sonicated at 18 W for 30 seconds using a probe sonicator (QSONICA, Newtown, CT). This process was repeated once more. The final pellet of NPs was gently disrupted, with a small volume of water, and then centrifuged at 1,000 rpm for 5 minutes to remove microparticles. Finally, before the freezing sample at – 80 ^{o}C, 20 mg sucrose was added as a lyo-/cryo-protectant. [19]

3.2.3. Characterization of Nanoparticles

3.2.3.1. Dynamic Light Scattering

Nanoparticles were suspended in water, diluted to optimum concentration, and characterized by Delsa Nano C at room temperature (Beckman Coulter). The average of size and polydispersity index are reported to evaluate the nanoparticles.

3.2.3.2. Drug loading determination

The mass of paclitaxel per mass of nanoparticles was determined as follows. Approximately 5 mg of freeze-dried nanoparticles were weighed and dissolved in 5 ml methanol to form a 1 mg/ml solution by stirring overnight in a sealed vial. The nanoparticle suspension was centrifuged at 1,000 rpm for 5 min, and a 1.5 mL aliquot was taken, and the absorbance at 228 nm was measured with a spectrophotometer (Beckman Coulter, DU® 530 UV-Vis spectrophotometer; Beckman, Brea, CA). Samples were diluted, when needed. Blank and standard solutions at concentrations of 2, 4, 8, 12, 16, and 20 ug/ml were prepared in methanol. The concentration PTX in the NP was obtained by interpolation

of the concentration from the standard curve. With the known volume of the aliquot, the weight of PTX in the total value of NP was calculated.

$$Drug\ loading\ (w/w) = \frac{Amount\ of\ PTX\ loaded\ in\ ug}{Amount\ of\ PLGA\ NP\ in\ mg}$$

$$Weight\ of\ PLGA\ NP\ (mg) = Total\ weight\ of\ product\ (mg) - Weight\ of\ sucrose\ (mg)$$

3.2.4. Selection of Unrelated Factor Values via Rough Predict Formula

The main factors, polymer amount and drug amount, have been screened (section 4.1). A cursory predictive formula for drug loading was generated and is shown in section 4.2. Designing an experiment for deeper exploration is the next target. Since only two parameters were of the levels for the experiment were chosen to yield a higher drug loading outcome based on the predictive formula, while maintaining the other parameters constant. (Table 4.3)

3.2.5 3-Level Fractional Factorial DOE

In the previous study, 2-level fractional factorial (FF) DOE was applied for screening of the critical factors that affect drug loading, size and PDI for each formulation. The predictive formula was generated with each model and then used for selecting values of the unrelated factors in the next study. As the 2-level FFDOE model was inadequate to obtain a precise predict formula, a 3-level FFDOE study was required. The formulations are presented in Table 3.2 (Results from Table 4.3 are marked as * in Table 3.2).

Table 3.2 List of the two identified factors and the three specific levels at which they were experimentally assessed for the design of experiment

Factors	Lower Level	Middle Level	Higher Level
Polymer amount	5 mg	15 mg	25 mg
Drug amount	5 mg	15 mg	25 mg
Surfactant concentration*	0.2 % PVA	0.2 % PVA	0.2 % PVA
Solvent type*	Acetonitrile	Acetonitrile	Acetonitrile
Polymer molecular weight*	High	High	High

Note: * represents factor only have one level in the formulations.

Chapter IV

Results and Discussion

4.1 **Main factors selected by DOE**

In Table 4.1, the level of each factor for the 16 unique formulations reported in triplicate along with the characterization results are given, which includes size, polydispersity index (PDI) and drug loading. Given the complexity inherent in the number of levels and factors, it is difficult to detect trends in the data. However, by analyzing the data set as a full fraction factorial DOE, the statistical analysis revealed important factors. When the outcome changes with parameter linearly, the independent p-value is trustworthy, but the simultaneous p-value is reliable no matter the change occurs linearly or not. As can be seen, the drug amount and polymer amount are statistically significant factors in determining the drug loading as was the interaction between these (Table 4.2).

With closer inspection of the data in Table 4.1, unexplained high variability among the triplicate measurements was noted, particularly in drug loading data. To increase accuracy of the experimental observed mean, the drug loading of each batch was measured twice more. With the total of five observations, the high variability was clearly seen to arise from the first data point, i.e. the first batch prepared. These are highlighted in bold in Table 4.1. That is, in carrying out the experiment, all unique formulations were prepared and then tested, which was then repeated twice more (Figure 4.1).

Table 4.1 Experimental data of the 2-level fractional factorial design using DOE

Batch	polymer amount (mg)	drug amount (mg)	surfactant concentration (%)	Solvent type	MW	Size(nm)	PDI	DL (ug/mg)

1-1	25	5	2	ACN	H	257.6	0.173	48.29
2-1	25	5	2	ACN	H	290.2	0.132	66.41
3-1	25	5	2	ACN	H	255.8	0.243	37.50
1-2	25	5	2	THF	L	231.6	0.196	41.90
2-2	25	5	2	THF	L	242.2	0.17	30.46
3-2	25	5	2	THF	L	232.4	0.24	35.65
1-3	5	25	2	ACN	H	264.4	0.227	145.79
2-3	5	25	2	ACN	H	338.4	0.157	214.70
3-3	5	25	2	ACN	H	300.2	0.249	160.16
1-4	5	5	2	ACN	L	287	0.25	**70.88**
2-4	5	5	2	ACN	L	320.2	0.247	33.07
3-4	5	5	2	ACN	L	329.4	0.236	35.81
1-5	5	25	2	THF	L	323.5	0.19	**375.00**
2-5	5	25	2	THF	L	302.8	0.215	126.60
3-5	5	25	2	THF	L	358	0.291	127.47
1-6	25	25	2	THF	H	344	0.195	**275.78**
2-6	25	25	2	THF	H	411	0.181	125.09
3-6	25	25	2	THF	H	385.6	0.28	133.84
1-7	5	25	0.2	THF	H	240.2	0.231	**284.35**
2-7	5	25	0.2	THF	H	440.1	0.241	130.62
3-7	5	25	0.2	THF	H	417.8	0.233	189.54
1-8	5	5	0.2	ACN	H	245.8	0.331	53.46
2-8	5	5	0.2	ACN	H	261.1	0.127	56.02

3-8	5	5	0.2	ACN	H	304.6	0.143	46.85
1-9	25	25	0.2	ACN	H	271.3	0.297	**77.98**
2-9	25	25	0.2	ACN	H	472.1	0.215	206.92
3-9	25	25	0.2	ACN	H	406.3	0.191	217.94
1-10	25	5	0.2	THF	H	227.5	0.32	74.56
2-10	25	5	0.2	THF	H	302.7	0.182	56.51
3-10	25	5	0.2	THF	H	450.2	0.201	37.99
1-11	25	25	0.2	THF	L	266.4	0.319	59.08
2-11	25	25	0.2	THF	L	236.7	0.184	17.68
3-11	25	25	0.2	THF	L	349.4	0.157	34.40
1-12	5	5	0.2	THF	L	461.7	0.259	18.28
2-12	5	5	0.2	THF	L	272.6	0.219	15.74
3-12	5	5	0.2	THF	L	328.3	0.215	7.70
1-13	25	25	2	ACN	L	342.8	0.116	**173.44**
2-13	25	25	2	ACN	L	213.3	0.096	29.24
3-13	25	25	2	ACN	L	213.7	0.128	59.56
1-14	5	5	2	THF	H	349	0.264	**101.27**
2-14	5	5	2	THF	H	285.3	0.253	11.36
3-14	5	5	2	THF	H	233.2	0.266	29.60
1-15	25	5	0.2	ACN	L	229.5	0.55	50.72
2-15	25	5	0.2	ACN	L	433.7	0.196	31.24
3-15	25	5	0.2	ACN	L	477.1	0.21	9.45
1-16	5	25	0.2	ACN	L	265.5	0.336	**273.54**

2-16	5	25	0.2	ACN	L	415.3	0.261	36.09
3-16	5	25	0.2	ACN	L	465.6	0.3	61.29

Table 4.2 Results of 2-level fraction factorial design

A. Screening for Drug loading

Term	Contrast	Lenth t-Ratio	Individual p-Value	Simultaneous p-Value
Drug amount (mg)	150.275	8.12	<.0001*	0.0004*
Polymer amount (mg)	-124.428	-6.73	<.0001*	0.0013*
Polymer MW	56.126	3.03	0.0102*	0.1870
Surfactant concentration (%)	-43.579	-2.36	0.0326*	0.4394
Solvent	2.883	0.16	0.8832	1.0000
Drug amount (mg)*Polymer amount (mg)	-89.298	-4.83	0.0006*	0.0135*
Drug amount (mg)*Polymer MW	12.657	0.68	0.4851	1.0000
Polymer amount (mg)*Polymer MW	46.269	2.50	0.0255*	0.3577
Drug amount (mg)*Surfactant concentration (%)	-3.237	-0.17	0.8702	1.0000
Polymer amount (mg)*Surfactant concentration (%)	0.049	0.00	0.9981	1.0000
Polymer MW*Surfactant concentration (%)	-57.439	-3.10	0.0086*	0.1683
Drug amount (mg)*Solvent	33.264	1.80	0.0879	0.8222
Polymer amount (mg)*Solvent	3.669	0.20	0.8510	1.0000
Polymer MW*Solvent	-9.838	-0.53	0.6132	1.0000

Surfactant concentration (%)*Solvent	14.416	0.78	0.4270	1.0000

B. Screening for size

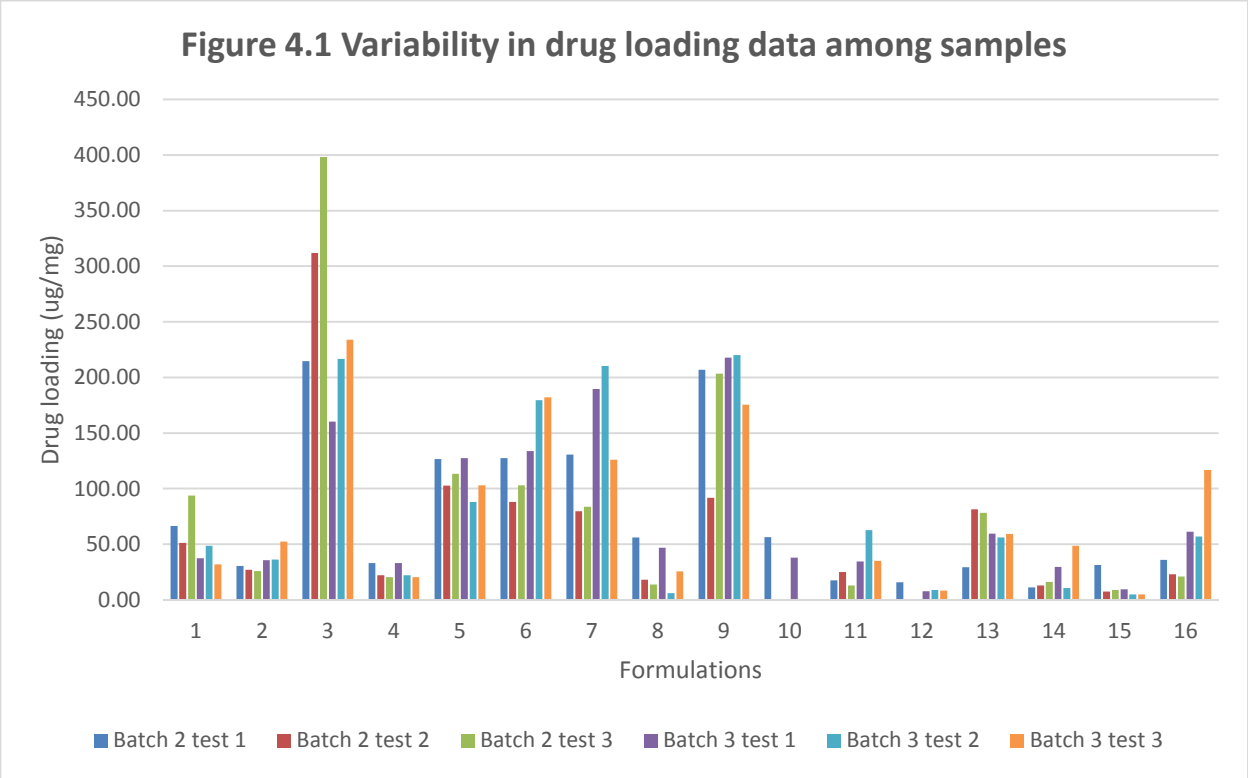
Term	Contrast	Lenth t-Ratio	Individual p-Value	Simultaneous p-Value
Polymer amount (mg)	-25.8219	-1.55	0.1270	0.9467
Polymer MW	-24.9594	-1.50	0.1403	0.9629
Solvent	-6.6469	-0.40	0.6915	1.0000
Drug amount (mg)	2.7531	0.17	0.8698	1.0000
Surfactant concentration (%)	0.9969	0.06	0.9518	1.0000
Polymer amount (mg)*Polymer MW	-13.6406	-0.82	0.4017	1.0000
Polymer amount (mg)*Solvent	5.9469	0.36	0.7237	1.0000
Polymer MW*Solvent	22.6969	1.36	0.1759	0.9880
Polymer amount (mg)*Drug amount (mg)	18.6344	1.12	0.2594	0.9995
Polymer MW*Drug amount (mg)	-24.0281	-1.44	0.1538	0.9757
Solvent*Drug amount (mg)	-18.2031	-1.09	0.2691	0.9997
Polymer amount (mg)*Surfactant concentration (%)	14.5156	0.87	0.3703	1.0000
Polymer MW*Surfactant concentration (%)	1.4906	0.09	0.9297	1.0000
Solvent*Surfactant concentration (%)	12.2906	0.74	0.4444	1.0000

Drug amount (mg)*Surfactant concentration (%)	-22.5469	-1.36	0.1788	0.9890

C. Screening for PDI

Term	Contrast	Lenth t-Ratio	Individual p-Value	Simultaneous p-Value
Polymer amount (mg)	-0.032875	-2.32	0.0335*	0.4534
Polymer MW	-0.026313	-1.86	0.0761	0.7841
Drug amount (mg)	0.024250	1.71	0.0968	0.8749
Surfactant concentration (%)	0.020250	1.43	0.1578	0.9779
Solvent	-0.001125	-0.08	0.9387	1.0000
Polymer amount (mg)*Polymer MW	0.007250	0.51	0.6234	1.0000
Polymer amount (mg)*Drug amount (mg)	-0.012688	-0.90	0.3590	1.0000
Polymer MW*Drug amount (mg)	-0.010125	-0.71	0.4565	1.0000
Polymer amount (mg)*Surfactant concentration (%)	-0.016438	-1.16	0.2432	0.9995
Polymer MW*Surfactant concentration (%)	-0.007750	-0.55	0.5998	1.0000
Drug amount (mg)*Surfactant concentration (%)	0.005687	0.40	0.7033	1.0000
Polymer amount (mg)*Solvent	-0.005438	-0.38	0.7151	1.0000
Polymer MW*Solvent	-0.016375	-1.16	0.2447	0.9996
Drug amount (mg)*Solvent	0.005187	0.37	0.7263	1.0000

Surfactant concentration (%)*Solvent	-0.014438	-1.02	0.2995	1.0000



Note: Samples of Batch 2 and Batch 3 of Formulation #10 lost by accident, thus, data of the extra measurements were not shown in this figure.

Insofar as this initial DOE was to serve as a guide for a subsequent, more focused DOE, the values obtained in the first data set were discarded, and the statistical analysis was performed on the four remaining values. With this approach, drug amount, polymer amount, and the interaction between drug amount and polymer amount were revealed as

the main factors that affect drug loading by JMP software (Figure 4.1). A predictive formula for drug loading generated by JMP software is given below:

Drug loading (ug/mg) = $226.07 + 28.82\ X_1 + 0.94\ X_2 - 43.11\ X_3 - 36.66\ X_4 + 70.11 X_5 -$

$0.89\ X_1\ X_2 - \quad 0.36\ X_1\ X_3 - 1.26\ X_1\ X_4 - 3.3\ X_1 X_5 + 0.0054\ X_2\ X_3 + 4.63\ \ X_2\ X_4 - 0.37$

$X_2\ X_5 + 63.18\ X_3\ X_4 + 15.86\ X_3 X_5 - 9.84\ X_4\ X_5$

X_1: Drug amount (mg)

X_2: Polymer amount (mg)

X_3: Surfactant concentration (%)

X_4: Polymer molecular weight (Low: -1, High: 1)

X_5: Solvent (THF: -1, ACN: 1)

As for particle size and PDI, the polymer amount may have a slight effect on the PDI slightly, whereas particle size was independent of all factors. Therefore, a second DOE study was focused on investigating the effects on drug loading.

4.2 **Values of Unrelated Factors Suggested via Rough Predict Formula**

In order to determine specific values of less relevant factors for high drug loading, the predictive formula generated in section 4.1 was used. Three values of factors were estimated, namely polymer MW, solvent and surfactant concentration. The latter one is a continuous numeric factor, whereas the other two are 2-level categorical factors. Thus, the drug amount and polymer amount were set at a medium level and the value of the less relevant factors was changed one by one. The predictive formula revealed clear guidance

of the specific value to choose based on previous data. The estimates of drug loading for each formulation are presented in Table 4.3.

Table 4.3 Estimation of drug loading value via predict formula

Groups	*Drug amount*	*Polymer amount*	*Surfactant concentration*	*Solvent type*	*Polymer MW*	*Drug Loading*
1	15 mg	15 mg	1 %	ACN	**H**	**494.25**
	15 mg	15 mg	1 %	ACN	**L**	349.56
2	15 mg	15 mg	1 %	**ACN**	H	**494.25**
	15 mg	15 mg	1 %	**THF**	H	477.14
3	15 mg	15 mg	**2 %**	ACN	H	365.99
	15 mg	15 mg	**1 %**	ACN	H	494.25
	15 mg	15 mg	**0.2 %**	ACN	H	**596.86**

According to the table, high polymer molecular weight, solvent acetonitrile and 0.2 % PVA are settled as fixed value in the next study (Section 3.2.5 and Table 3.2).

4.3 **Drug Loading Result of 3-level Full Factorial Design**

The 9 formulations which was randomly generated by JMP software had been investigated in triplicates, and the drug loading results are shown as Table 4.4. Applying these data to JMP analyzation system, predict formula was produced.

Drug loading (ug/mg) = $-119.95 - 5.72\ X_1 + 94.17\ X_2 + 0.35\ X_1^{\,2} - 3.06\ X_2^{\,2}$

X1: Drug amount (mg)

X2: Polymer amount (mg)

Table 4.4 Experimental data of the 3-level full factorial design using DOE

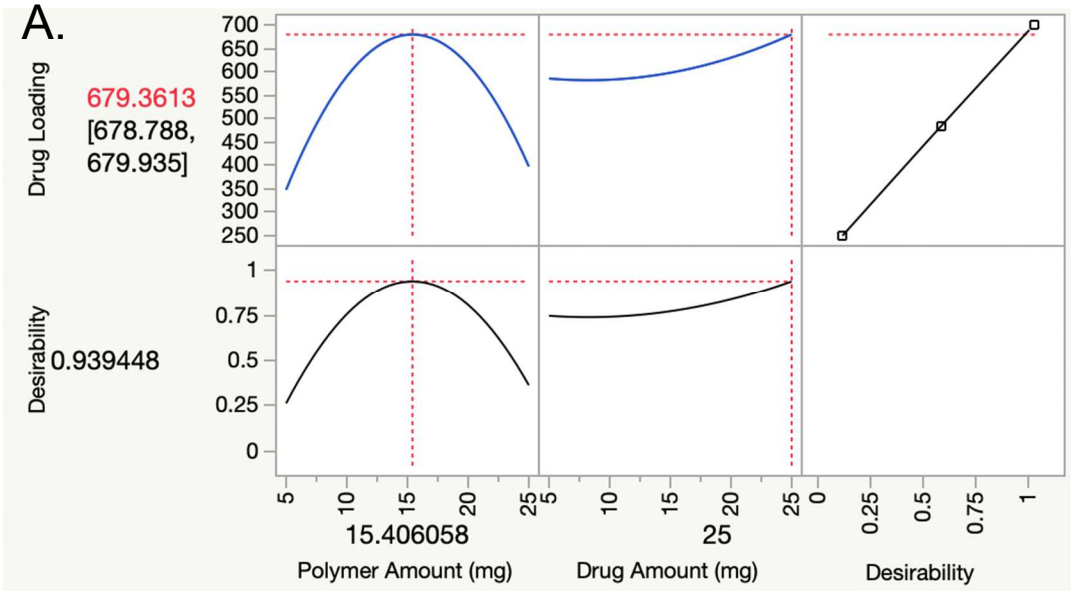
No	Solvent	Surfactant concentration	Polymer MW	**Drug amount (mg)**	**Polymer amount (mg)**	Drug Loading (ug/mg)
1	ACN	0.2 %	H	5	15	364.21
2	ACN	0.2 %	H	5	15	751.46
3	ACN	0.2 %	H	5	15	464.08
4	ACN	0.2 %	H	15	5	199.84
5	ACN	0.2 %	H	15	5	176.06
6	ACN	0.2 %	H	15	5	208.69
7	ACN	0.2 %	H	25	5	206.47
8	ACN	0.2 %	H	25	5	399.16
9	ACN	0.2 %	H	25	5	418.91
10	ACN	0.2 %	H	5	5	246.05
11	ACN	0.2 %	H	5	5	345.71
12	ACN	0.2 %	H	5	5	409.60
13	ACN	0.2 %	H	15	25	433.22
14	ACN	0.2 %	H	15	25	463.38
15	ACN	0.2 %	H	15	25	240.93
16	ACN	0.2 %	H	25	15	587.95
17	ACN	0.2 %	H	25	15	922.07
18	ACN	0.2 %	H	25	15	675.04
19	ACN	0.2 %	H	5	25	249.08
20	ACN	0.2 %	H	5	25	321.65
21	ACN	0.2 %	H	5	25	278.52
22	ACN	0.2 %	H	15	15	869.78
23	ACN	0.2 %	H	15	15	547.07
24	ACN	0.2 %	H	15	15	402.17
25	ACN	0.2 %	H	25	25	204.87
26	ACN	0.2 %	H	25	25	461.02
27	ACN	0.2 %	H	25	25	402.37

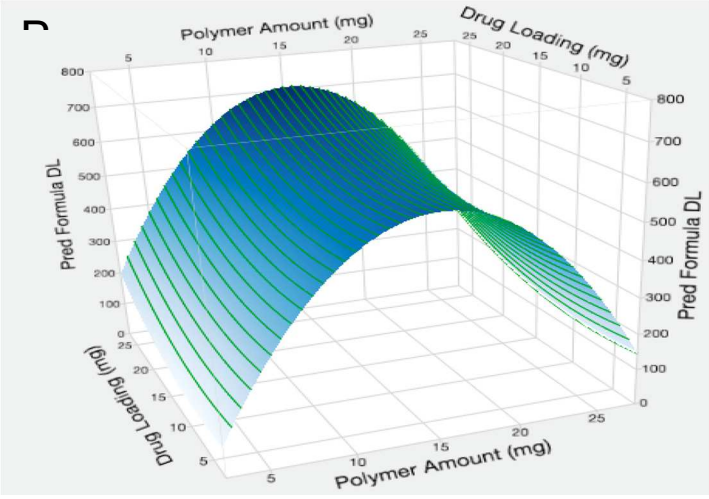
4.4 Prediction Profiler of Drug Loading

From the completion of the 3-level full factorial, a table of 2-factor Central Composite Design prepared. Response surface predict profiler and 3D surface plot of drug loading,

which was fit by drug amount and polymer amount are shown as Figure 4.2. Drug loading increased, as the drug amount was increased. Interestingly, as the polymer amount was increased, drug loading value in the prediction profiler did not rise or descend linearly. As we can see from Figure 4.2 (A), as the polymer amount initally increases, drug loading also increases; however, with a further increase, drug loading decreased.

Figure 4.2 Response Surface Predict Profiler (A) and 3D Surface Plot (B)





Chapter V
Conclusion and Future Studies

5.1 CONCLUSION

In this study, design of experiment was used to investigate the effects of various parameters on drug loading, particle size and PDI. According to 2-level fractional factorial design, polymer amount, drug amount and their interaction affects drug loading significantly among all 5 parameters and 20 interactions. This study shows that the effects of various categorical parameters can also be investigated and predicted via DOE. Although the predict formula is robust, high drug loading formulations can be determined by changing values of each parameter to obtain predictive results. In order to figure out how the two factors affects drug loading, those insignificant factors were arranged with fixed values in the next generation of formulations, and only polymer amount, drug amount and their interaction were considered as factors in the 3-level full factorial design and in reduced model equation. Therefore, the whole dataset can be filled into a 2-factor central composition design which generates surface predict profiler. At the beginning, as the polymer amount increases, drug loading also increases, however when the polymer amount increases to an extent, drug loading decreased. Drug loading increases together with the drug amount rises.

5.2 FUTURE STUDIES

Repeat the experiment more times to reduce the variability and confirm the findings. After that, a 30-day in vitro drug-release profile of the optimized formulation is the next target to be explored. Beside ACN and THF, more organic solvents can be taken into consideration to make new formulations. Also, effects of other categorical parameters like polymer type, surfactant type can be studied and then predicted in the future.

Chapter VI

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Chapter VII

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