Optimization of Liposome Production via Microfluidic Method: A Comparative Study of Design of Experiments Approaches

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ABSTRACT

This study presents an optimized approach for liposome production using the microfluidic method by integrating Design of Experiments (DoE) and machine learning. Three DoE methodologies—Box-Behnken Design (BBD), Central Composite Design (CCD), and Full Factorial Design—were systematically compared to identify the most efficient strategy for process optimization while minimizing the number of experimental runs. Process modeling was performed using the Gradient Boosting Regressor algorithm, with model performance assessed based on R^2, MAE, and RMSE metrics. The findings demonstrated that the CCD approach achieved the highest predictive accuracy for liposome size ($R^2 = 0.9870$) with a reduced number of experiments. Conversely, the full factorial design yielded comparable accuracy but proved inefficient in terms of time and resource allocation due to the extensive number of required experiments. The BBD method was deemed unsuitable due to its lower predictive accuracy. This study underscores the potential of leveraging DoE in conjunction with machine learning to enhance liposome production efficiency and reduce experimental costs.

Keywords: *Design of Experiments, Liposome Production, Microfluidic Method, Optimization, Machine Learning*

1. *INTRODUCTION*

Liposomes are nanometric structures composed of bilayer phospholipids that, due to their unique characteristics, are widely utilized in the pharmaceutical, biological, and therapeutic industries[1,2]. These nanocarriers play a crucial role in enhancing drug delivery efficiency by encapsulating and transporting drugs, proteins, and other bioactive compounds[3,4]. Due to their biocompatibility and ability to reduce drug side effects, liposomes are used in the treatment of diseases such as cancer, microbial infections, and genetic disorders. Recent advancements in nanomedicine have led to increased focus on optimizing liposome production processes[5,6].

Various methods for liposome production have been introduced, including traditional techniques such as sonication, solvent evaporation, ethanol injection, and thin-film methods, as well as more advanced approaches like microfluidics, electrohydrodynamic methods, and nanotechnology-based technologies[7,8]. *The microfluidic method, which is examined in this study, is considered one of the innovative techniques for liposome production. It has garnered significant attention due to its precise control over operational parameters, the production of uniform structures, and its high scalability. However, optimizing this method requires a comprehensive understanding of the effects of process variables on the final liposome properties, which can be facilitated through the use of Design of Experiments (DoE) methods[9,10].*

Design of Experiments (DoE) is a powerful statistical tool for planning and analyzing experiments that allows researchers to systematically assess the influence of various factors on a desired outcome[11,12]. This approach helps reduce laboratory costs, enhance accuracy, and minimize the number of required experiments. In the fields of biotechnology and pharmaceuticals, DoE is a key tool for optimizing production processes, improving yield, and enhancing the quality of pharmaceutical products[13,14].

Among the various DoE methods, Central Composite Design (CCD), Box-Behnken Design (BBD), and Factorial Design are some of the most commonly used techniques for optimizing biological processes. These three methods have been employed to determine the optimal set of process conditions for the production of stable and uniform liposomes. CCD and BBD are frequently used for nonlinear modeling in pharmaceutical and biological studies, while Full Factorial Design is particularly effective in analyzing the interaction effects between variables.

Machine Learning (ML) is an emerging approach in the analysis of complex data and the optimization of industrial and biological processes[15,16]. By using intelligent algorithms, such as Gradient Boosting Regressor, Random Forest, and Support Vector Machines (SVM), ML enables high-accuracy prediction and analysis of experimental data. The integration of machine learning with DoE methods forms a powerful combined approach for identifying complex patterns in data and optimizing production processes.

In recent years, numerous studies have examined the impact of DoE and machine learning methods on the production and optimization of biological processes[17,18]. Some studies have shown that combining DoE with machine learning can improve model prediction accuracy and reduce the number of required experiments. For instance, researchers demonstrated that using the CCD method alongside the Gradient Boosting algorithm improves the prediction of liposome size. Other studies have emphasized the effectiveness of Factorial Design and BBD methods in optimizing production conditions.

In this study, various DoE methods, including CCD, BBD, and Factorial Design, are compared for liposome production using the microfluidic method. Additionally, machine learning algorithms are employed to evaluate the performance of each of these methods. The aim of this research is to determine the optimal DoE method in terms of reducing the number of experiments, enhancing model accuracy, and optimizing liposome production conditions. The findings of this study could serve as a practical guide for selecting the most suitable DoE method in pharmaceutical and biological processes.

2. *METHOD AND MATERIALS*

2.1 Materials

In this study, experimental data were collected from various studies conducted on liposome production using the microfluidic method. In all of these studies, phospholipids, cholesterol, and polyethylene glycol (PEG) were used as the main components in the formation of the liposomes. Ethanol as a solvent and an aqueous NaCl solution were used as the primary phases for liposome preparation. The quality of the raw materials and the precise conditions for each study were adjusted based on the standards established in those studies.

2.2 Microfluidic System

The liposome production process in this study was based on a standard microfluidic system, which includes a microfluidic chip, a tubing system, a temperature control unit, and syringe pumps. This system allows for precise control of influencing parameters, including Flow Rate Ratio (FRR) and Total Flow Rate (TFR). In this method, the lipid solution and aqueous solution enter the microfluidic chip through separate paths and are mixed in micron-sized channels.

2.3 Liposome Preparation Method

Liposomes were produced by combining the lipid solution and aqueous phase within the microfluidic system. Initially, phospholipids, cholesterol, and PEG were dissolved in ethanol to prepare a lipid solution with a specific concentration. The aqueous solution consisted of NaCl at various concentrations, which were adjusted according to the experimental design. Both solutions were placed in polypropylene syringes and precisely adjusted within syringe pumps.

The mixing process occurred in the microfluidic mixer, where parameters such as Total Flow Rate (TFR) and Flow Rate Ratio (FRR) were set before each experiment. To ensure the accuracy of the process, the system was cleaned with the corresponding solutions and degassed before each load. After the liposomes were formed, the resulting solution was collected in special tubes, and ethanol was removed by performing a vacuum evaporation process. Finally, the lost volume was replaced with pure water.

2.4 Design of Experiments and Modeling

In this study, three experimental design methods, including Central Composite Design (CCD), Box-Behnken Design (BBD), and Full Factorial Design, were used to optimize the liposome production process. Each of these methods suggested a specific number of experiments to determine the impact of various variables on liposome size. After collecting data from the different designs, modeling was performed using machine learning algorithms, including Gradient Boosting, to evaluate the prediction accuracy and efficiency of each design. The variables under investigation were adjusted within the ranges specified in Table 1 below.

Table 1. Range of variables under investigation

Factor	Experimental values	Unit
Cholesterol concentration	0, 41, 19	% in molarity
NaCl concentration	0, 9, 18	mg/mL
Total flow rate	0.2, 0.5, 1, 1.5, 2	mL/min
Flow rate ratio	3, 9, 15, 19, 39	-
PEG concentration	1, 2, 3, 4, 5	% in molarity
Temperature	15, 25, 35	C

3. *Results and discussion*

In this section, the results obtained from modeling liposome production using various Design of Experiments (DoE) methods, including Box-Behnken Design (BBD), Central Composite Design (CCD), and Factorial Design, are analyzed and compared. The primary objective of this section is to evaluate the efficiency and accuracy of each method in modeling the liposome production process and to determine the best method based on reducing the number of experiments and improving prediction accuracy through machine learning.

After collecting data related to liposome production using the microfluidic method and applying the three experimental design methods to this data, the results obtained for modeling the process using the Gradient Boosting algorithm are presented in Table 2.

Table 2. Accuracy results of different design of experiment (DoE) methods

Methods	R^2	MAE	RMSE
BBD (Box-Behnken Design)	0.2583	9.5606	12.5859
CCD (Central Composite Design)	0.9870	0.6628	2.4422
Factorial Design	0.9860	1.0436	2.2464

These results show that the CCD method has the best performance among the three methods studied. The Factorial Design method also has a very close performance to CCD, but BBD could not provide the desired accuracy. These results are discussed in detail below.

3.1 Analysis of the Results from the BBD Method

The Box-Behnken Design (BBD) method is a commonly used experimental design approach for investigating the effect of multiple variables on a response. However, the results of this study demonstrated that the use of this method in modeling liposome production via microfluidics did not provide an acceptable level of accuracy. The value R^2=0.2583 indicates a weak correlation between the predicted and actual values.

Possible reasons for this poor performance may include:

- *Insufficient distribution of experimental points in the parameter space: The BBD method covers central points but tends to consider boundary points less frequently.*
- *Lack of sufficient training data for the machine learning model: This method generates fewer data points compared to other experimental designs, which may reduce the accuracy of the machine learning model.*
- *Inadequate coverage of interactions between variables: In complex processes such as liposome production, interactions between factors like temperature, flow rate, salt concentration, and cholesterol can play a significant role. The BBD method may not adequately address these interactions.*

Due to these limitations, the BBD method is not an optimal choice for experimental design in microfluidic liposome production, and alternative methods should be considered.

3.2 Analysis of the Results from the CCD Method

The Central Composite Design (CCD) method showed significantly higher accuracy than BBD. The value R^2=0.9870 indicates that the model can predict the actual data very well. Additionally, the values of MAE = 0.6628 and RMSE = 2.4422 reflect the low error of this method.

Reasons for the superior performance of CCD include:

- *Better coverage of the parameter space**:** This method uses star points, which allow for the examination of extreme values and have a greater impact on training the machine learning model.*
- *Better optimization of interactions between variables**:** Compared to BBD, CCD more effectively investigates the interactions between variables, leading to better performance in the machine learning model.*
- *Increased data points without a significant increase in experimental costs**:** Unlike the factorial method, which exponentially increases the number of experiments, CCD provides a balance between the number of experiments and model accuracy.*

Thus, CCD is an ideal experimental design method for microfluidic liposome production, as it increases prediction accuracy and reduces the number of required experiments.

3.3 Analysis of the Results from the Factorial Design Method

The Factorial Design method also demonstrated excellent performance. The value R^2=0.9860 indicates that the accuracy of this method is nearly as high as that of CCD. Additionally, the value of RMSE = 2.2464 shows the low error of the model.

This method provides a comprehensive view of the effects of different parameters by considering all possible combinations of variables. However, its main challenge lies in the significant increase in the number of required experiments, which may not be practical or cost-effective.

4. *Conclusion*

This study demonstrates the effectiveness of integrating Design of Experiments (DoE) with machine learning techniques to optimize liposome production via the microfluidic method. Among the three DoE methods—Box-Behnken Design (BBD), Central Composite Design (CCD), and Full Factorial Design—CCD was found to provide the highest predictive accuracy (R^2 = 0.9870) while minimizing the number of experimental runs. The CCD approach offers a balanced optimization of experimental efficiency and prediction accuracy, making it the most suitable choice for microfluidic liposome production. Although the Factorial Design also achieved high accuracy, its impracticality due to the large number of required experiments makes it less optimal. Conversely, the BBD method did not provide satisfactory results, primarily due to its inability to sufficiently cover the parameter space and account for variable interactions.

The integration of machine learning, specifically the Gradient Boosting Regressor, further enhanced the model's predictive power, underscoring the potential of this combined approach in process optimization. This study not only highlights the advantages of using CCD in the production of liposomes but also provides valuable insights into the efficiency of experimental design methodologies in reducing resource consumption and improving the quality of outcomes. The findings are significant for both pharmaceutical and biotechnological industries, where the optimization of production processes is critical to ensuring high product quality while minimizing costs. Future research could focus on refining these models and extending them to other biotechnological processes.

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