Loading Optimization of Mesoporous Silica Nanoparticle as Drug Delivery Agent

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Abstract. One of the potential candidates as a drug delivery agent that has been widely developed is mesoporous silica nanoparticle (MSN), which has several unique features. The high surface area and pore volume, tunable size particle, biocompatibility, and non-toxic are great features promising drug delivery carriers. The optimum condition to load the drug onto MSN is needed to maximize the loading of drugs. The drug loading is influenced by factors, such as silica to drug ratio, time, and pH condition. In this study, we had conducted the optimization of drug loading into MSN by implemented the Box-Behnken design of experiments. Also, the influence of each factor can be obtained through statistical calculation. The results showed that silica to drug ratio and pH condition significantly affect the loading capacity of MSN. The optimum condition obtained at silica to drug ratio, time, and pH conditions is 1, 48, and 3, respectively.

1. Introduction

Cancer cases become a significant concern worldwide due to the number of cases are speedily increase and the new cases are estimated at 1.8 million with 600 thousand cancer deaths in 2020 [1–3]. One of the most cancer therapy method used today is chemotherapy [4]. Despite the fact that this therapy has many fatal deficiencies such as killing normal cells together with cancer cells, chemo drugs that are not specific (un-targeted) become poisons in the body, chemo can damage the immune system [5–7]. Therefore, targeted drug delivery is the strategy to overcome the problem in cancer treatment.

The potential candidate as a drug delivery agent that has been widely developed is Mesoporous Silica Nanoparticle (MSN), which has several unique features [8–11]. Its high surface area and pore volume, tunable size particle, biocompatibility, and non-toxic are great features as promising drug delivery carriers [12,13]. Mesoporous material can describe as porous separation media without interparticular void [14]. Researchers had loaded several drugs for cancer therapy into some MSN types. Zheng and coworkers loaded several drug models, Doxorubicin (DOX), Methotrexate (MTX),
and Mitoxantrone (MX), onto MSN that has excellent release profile against pH condition [8]. Velusamy coworkers and Cheng coworkers have loaded DOX into MSN modified with disulfide linkages as super-pH-sensitive nanovalves [9,10]. Sarkar and coworkers reported that Quercetin (QUE) encapsulated MSN modified with folic acid exhibited high toxicity in breast cancer cells [11]. It can be concluded, MSN is promising material as drug carrier in cancer treatment.

In drug loading process, there are several conditions that have effect to drug loading efficiency, such as silica to drug, loading time and pH condition [15–17]. The optimization of loading condition is needed to carry out the effective condition for loading the drug model. The conventional optimization carried out by varying one variable while other variables were keeping constant. However, the experiment, which has many variables, will be taking a lot of time, cost, and uncontrolled-data to be carried out through blind experimental design [18,19]. Hence, introducing a design of experiment (DoE) is needed to eliminate the number of experiments and statistically optimize the experiment, one of DoE design is Box Behnken design (BBD) that had commonly applied in analytical chemistry [20]. In this study, we investigated the effect of loading condition through drug loading capacity using Quercetin as a drug model.

2. Experimental
2.1. Materials
Materials used in this research were Tryethanolamine (TEA), tetraethyl orthosilicate (TEOS), Cetyltrimethylammonium bromide (CTAB), and Quercetin (Que) purchased from Sigma-Aldrich. Hydrochloric acid (HCl), ethanol (Merck), and methanol (Merck) were purchased from Merck, and ultra-pure water (Integrated MIPA laboratory).

2.2. Synthesized of mesoporous silica nanoparticle (MSN)
A mixture solution of ultrapure water (20 mL) and 0.3 gram of TEA stirred under 700 rpm 50 °C. Then, 0.3 gram CTAB was introduced to the suspension and stirred for next 1 hour. Afterward, 1 mL TEOS was gradually added to the suspension at a speed of 1 mL/min. The SiO$_2$ nanoparticle formation was kept stirring for 1 hour, then cooled into room temperature and washed with ethanol and ultrapure water, and dried under vacuum.

2.3. Loading Optimization of MSN
Loading of QUE would be performed under room temperature. A specific amount of nanoparticles and 10 mg of QUE were dispersed in 5 mL of ethanol in specific pH condition and shaken for a specific time. The optimization used Box-Bhenken design of experiment to find out the loading runs. The resulted product was then called QUE/MSN and coded as listed in Table 1. The QUE/MSN was separated through centrifugation. The filtrate was collected and analyzed by UV-Vis spectrophotometer to determine the loaded concentration of QUE. The loading capacity was calculated by equation 1. The data obtained were analyzed using Minitab software by Pty Ltd.

| Code | Silica to Drug Ratio (w/w) | pH | Time (h) |
|------|----------------------------|----|----------|
| A1   | 1                          | 3  | 48       |
| A2   | 1                          | 4  | 24       |
| A3   | 2                          | 3  | 24       |
| A4   | 2                          | 3  | 72       |
| A5   | 2                          | 4  | 48       |
| A6   | 2                          | 5  | 24       |
| A7   | 2                          | 5  | 72       |
| A8   | 3                          | 4  | 24       |
| A9   | 3                          | 3  | 48       |
| A10  | 3                          | 5  | 48       |
\[ Q = \frac{C_0 - C_i}{m} \times V \]  

(1)

Where, \( C_0 \) (mg L\(^{-1}\)), \( C_i \) (mg L\(^{-1}\)), and \( V \) (L) are the initial and residual concentration of QUE in solution, respectively. Weight of MSN used for QUE loading is denoted as \( m \) (g). \( V \) (L) is the total volume of solution.

3. Result and Discussion

3.1. Synthesis of MSN
In this research, the synthesis of MSN have been carried out used sol-gel method. The synthesis were prepared using CTAB and under basic condition used TEA. Functional group of MSN have been characterized with FTIR spectroscopy in pellet form mixed with KBr powder. Analysis will be recorded in range 4000-400 cm\(^{-1}\) with resolution 2 cm\(^{-1}\) and 40 times of scans. Figure 1 is an infra-red spectra of MSN synthesis product. Based on that spectra, MSN has successfully formed which is characterized by the appearance vibrations of Si-O-Si at 1080 cm\(^{-1}\) for asymmetric stretching, 800 cm\(^{-1}\) for symmetric stretching, and 460 cm\(^{-1}\) for bending. Hydroxyl absorption (-OH) appear in the region of wave number 3421 cm\(^{-1}\). It correlate with the literature, MSN have been observed by FTIR analysis that exhibited infra-red peaks at 458 cm\(^{-1}\), 788 cm\(^{-1}\), and 1065 cm\(^{-1}\) attributed to Si-O-Si vibrations [9].

![FTIR Spectra of MSN](image)

3.2. Loading Optimization
The optimization has been carried out to obtain the best condition which has the highest loading capacity value. We used response surface methodology to optimize the loading process of QUE into MSN. This research used BBD design of experimental which has ten experimental runs were carried out to optimize three parameters. Three independent variables used in this optimization are silica to drug ratio, pH condition, and loading time with loading capacity (Q) as a response. The experimental
condition and the response results are summarized in Table 2. It can be seen from the table that the formulation which has the highest loading capacity is A1 with loading condition are 1 to 1 for silica to drug ratio, 3 for pH condition, and 48 hours for loading time.

### Table 2. Data summaries of loading capacity (Q)

| Code | Silica to Drug Ratio (w/w) | pH  | Time (h) | Q (mg·g⁻¹) |
|------|-----------------------------|-----|----------|------------|
| A1   | 1                           | 3   | 48       | 528.214    |
| A2   | 1                           | 4   | 24       | 217.214    |
| A3   | 2                           | 3   | 24       | 257.857    |
| A4   | 2                           | 3   | 72       | 257.357    |
| A5   | 2                           | 4   | 48       | 38.857     |
| A6   | 2                           | 5   | 24       | 36.357     |
| A7   | 2                           | 5   | 72       | 102.857    |
| A8   | 3                           | 4   | 24       | 27.905     |
| A9   | 3                           | 3   | 48       | 169.405    |
| A10  | 3                           | 5   | 48       | 32.571     |

In order to determine the best-fitted model, several factors in the analyses of variance play an important role. From the analyses of variance of the drug loading capacity (Q), the P values are equals to or less than a significance level (0.05) show the statistically significant association between the response of variable and the term [21,22]. It can be seen from the summary of the analysis of variance (table 3), the parameter with the strongest effect is silica to drug ratio and pH condition with the P values at 0.003 for both of those, followed by the F value at 39.58 and 45.04, respectively. However, the loading time does not affect the loading capacity as the P-value is more than 0.05.

### Table 3. The analysis of variance for loading capacity (Q) of MSN.

| Source                  | Mean Square | F Value | P Value |
|-------------------------|-------------|---------|---------|
| Model                   | 4.3864 x 10⁻⁴ | 34.23   | 0.002   |
| Silica to drug          | 5.0722 x 10⁻⁴ | 39.58   | 0.003   |
| pH                      | 5.7716 x 10⁻⁴ | 45.04   | 0.003   |
| Silica to drug*pH       | 1.0258 x 10⁻⁴ | 8.01    | 0.047   |
| pH*pH                   | 2.3565 x 10⁻⁴ | 18.39   | 0.013   |
| R²                      | 0.9772      |         |         |
| Adjusted R²             | 0.9486      |         |         |
| Predicted R²            | 0.8026      |         |         |

The coefficient correlation (R²) is measured by the degree of fit. The model shows that R² values are close to 1 which indicates a great fit. The response Q obtained an R² value of 0.9772 and an adjusted R² value of 0.9486 at a 95% confidence level. The predicted R² value of 0.8026 which has an inconsiderable difference with R², can be concluded that the regression is not over-fitting. Besides, the model is significant as proven by the high F values and low P values at 34.23 and 0.002, respectively.

The regression equation by applying multiple regression analysis on the experimental data was derived to represent drug loading as a function of tested variables and the equation is given in equation 3. From the equation can be seen that silica to drug ratio and pH condition have a negative effect on the loading capacity of MSN.

\[
Q = 3230 - 601 \text{Silica to Drug} - 1127 \text{pH} + 73.2 \text{Silica to Drug} \cdot \text{Silica to Drug} + 116.0 \text{pH} \cdot \text{pH} + 47.1 \text{Silica to Drug} \cdot \text{pH}
\]  

(2)
Figure 2. Main Effect Plot for Q of (a) silica to drug ratio, (b) pH condition, and (c) loading time.

Figure 2 shows the influence of variables towards the response by main effect plot. It can be seen that silica to drug ratio and pH condition of the loading process influence the loading capacity significantly. Whereas, the time of the loading process influences the loading capacity more slightly. The pH condition of the solution plays an important role which the influences the active site of adsorbent [22]. Also, the electrostatic interaction between negatively charge drug and negatively charge MSN active site causes to increase of QUE loading at low pH. The increasing drug loading capacity at low pH shows that the surface charge of QUE is negative. Additionally, it was observed that decreasing silica to drug ratio can increase the drug loading into MSN.

Figure 3 shows the surface and contour plot of loading time and pH condition on loading capacity at constant silica to drug ratio. In the strong acidic condition, the drug-loaded into MSN higher than in the higher pH value, whereas the time of loading did not influence significantly. The low pH condition can enhance the electrostatic interaction between the drug and MSN active site due to the high loading capacity. While the pH increase due to the more positive charge environment, it causes the weak interaction between drug and MSN which decrease the drug loading capacity.
Figure 4 shows the surface and contour plot of time and silica to drug ratio on loading capacity at a constant pH condition. The loading capacity increased insignificantly when the time of loading increased. Whereas, the drug-loaded into MSN is higher when silica to drug ratio in balance. When the silica amount is larger than drug amount, the loading of the drug is not at maximum condition due to the limited number of drugs with the large available site to load the drug. It causes the drug cannot be loaded into MSN maximally.

Figure 5 shows the surface and contour plot of pH condition and silica to drug ratio on Loading capacity at a constant loading time. The drug-loaded into MSN is higher when silica to drug ratio in balance. Additionally, the loading capacity increased when the pH condition decreased. The electrostatic interaction between the drug and MSN active site can enhance due to the positive charge of environment by decreasing pH condition of solution. Besides, the large amount of drug can cause the abundance that affect the effectiveness of drug loading [17,21,22].

The result showed that the optimization of drug loading process have been successfully conducted by applying response surface methodology. The experiment can be statistically analyze by linier regression analysis to find out the influence of each variable toward the response, loading capacity. In conclusion, the result will be usefully for conducting the drug loading with the high effectiveness for further application.
4. Conclusion
The optimization of Quercetin loaded into Mesoporous Silica Nanoparticle can be conducted using Box-Behnken Design. Silica to drug ratio, pH Condition, and loading time contribute toward loading capacity value. Silica to drug ratio and pH condition have significant effect through loading capacity value. Whereas, loading time slightly influenced the loading capacity.

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