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Optimization of Photooxygenation of Dihydroartemisinic Acid in a Continuous Micro-reactor utilizing Physically Immobilized Methylene Blue on Polystyrene Micro-particles

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Extensive studies reported upon the application of microfluidic devices for photooxygenation reactions. Only a few researches, however, have studied the optimization of experimentally controllable parameters towards achieving the best reaction conversion. Accordingly, in the current study, the optimum values of operating parameters towards achieving the highest dihydroartemisinic acid (DHAA) conversion were determined through the response surface methodology (RSM) technique. In order to do that, first, heterogenized micro-photosensitizers were synthesized during a super facile procedure not requiring any chemical reaction or modifications. Then, these micro-photosensitizers were utilized to perform continuous photooxygenation of the DHAA by means of a glass micro-reactor at room temperature and atmospheric pressure. Finally, the impacts of experimental parameters such as the flow ratios of solution to oxygen as well as DHAA and micro-photosensitizers' concentrations upon the DHAA conversions were examined by using Design of Experiments (DOE) software in order to optimize and statistically model the DHAA conversion. The optimum value for this conversion was predicted by the RSM technique to be 70 % which was in a very good agreement with that of the experimental value of 69 % obtained in this study. The optimal values for the flow rates ratio, DHAA initial concentration, and micro-photosensitizer's concentration were obtained to be 0.5, 0.5 mg/ml, as well as 1.5 mg/ml, respectively.

1. Introduction

Continuous synthesis has been widely employed in order to perform photooxygenation of the dihydroartemisinic acid (DHAA). For example, Lévesque et al. used Tetraphenylporphyrin (TPP) as a photosensitizer and Polytetrafluoroethylene (PTFE) tubes as a coil-based micro-reactor to continuously perform photooxygenation reaction upon the DHAA at 8 bars followed by one acid-catalysed reaction for the production of Artemisinin (Lévesque and Seeberger 2012). Then, Seeberger et al. investigated the temperature impact upon the aforementioned process (Kopetzki et al. 2013). Based on their findings, enhancement of the temperature did not produce any noticeable effect upon the DHAA conversion, but significantly lowered selectivity of hydroperoxide 3, shown through Figure 1. Lee et al. used vortex reactor to continuously perform photooxygenation of several organic materials (Lee et al. 2017). Burgard et al. investigated effects of the light source upon the continuous photooxygenation of the DHAA (Burgard et al. 2016). In spite of extensive studies reported upon applications of microfluidic devices for the photooxygenation reactions, only a few have determined the optimization of operating parameters towards obtaining the highest reaction conversion. Such optimization tasks might have helped obtaining the highest performances with already-constructed microfluidic devices undertaken. Accordingly, in another study, the optimum values of operating parameters towards achieving the highest DHAA conversion were determined through the response surface methodology (RSM) technique (Kim et al. 2020).

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In the present study, a glass micro-reactor was utilized as a continuous medium for the photooxygenation of the DHAA at room temperature and atmospheric pressure without a need for refrigeration and back pressure regulation. These were done utilizing one novel micro-photosensitizer towards heterogenizations of photosensitizers. Moreover, such materials were easily separated from the solution through using centrifugation or a simple filtration step at the end of the process. Figure 1 schematically displays the DHAA photooxygenation toward producing hydroperoxides 3, 4, and 5 (Amara et al. 2015). As depicted in this schematic, the singlet oxygen ($^1O_2$) from micro-photosensitizers is produced in the presence of light then reacted with the DHAA to produce hydroperoxides. It is reiterated that, amongst the produced materials only the hydroperoxide 3 is the desired product toward producing Artemisinin (i.e., the ultimate pharmaceutical material goal of this research) through an acid-catalysed reaction (Feng et al. 2019). Artemisinin drug is a natural peroxide with high antiparasite, antibacterial, and antivirus properties which is the first line and only effective treatment for Malaria and also a potential treatment for COVID-19.

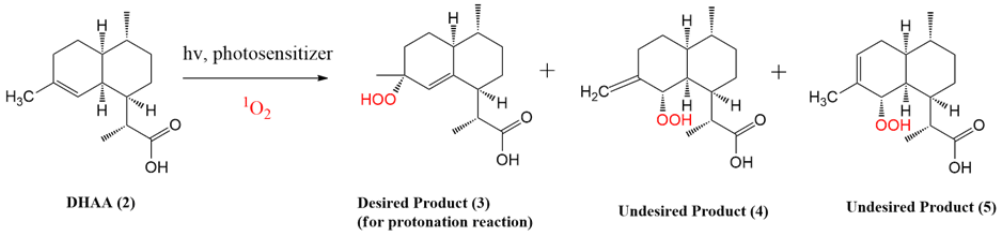

Figure 1: Schematic of photooxygenation of the DHAA to produce hydroperoxides 3, 4, and 5. Hydroperoxide 3 is the desired product while hydroperoxides 4 and 5 are undesired products.

2. Experiments: Materials, setup, and procedures

2.1 Materials

Chemical materials used in this research included the dichloromethane (≥ 99.9%, Merck), toluene (99.5%, Rci Labscan Co.), ethanol (99.9%, Merck), dihydroartemisinic acid (DHAA, 98%, Xi'an Quanao Biotech Co.), methylene blue (MB, ≥ 82%, Sigma Co.), styrene (99%, Sigma Co.), divinylbenzene (80%, Sigma Co.), potassium persulfate (≥ 99.0%, Sigma Co.), and methacrylic acid (99%, Sigma Co.). All these materials were utilized as received without any further treatment.

2.2 Fabrication of micro-reactor

The micro-reactor was fabricated using one glass slide as the cover glass and another one as the substrate glass. Micro-reactor construction composed of the following steps (Tamtaji and Mohammadi 2019; Zeibi Shirejini and Mohammadi 2017): i) the micro-reactor patterns were engraved upon the substrate glass slide by a CO_2 laser machine, ii) the outlet as well as inlet fluidic access holes (2.1 mm) were mechanically drilled on the cover glass slide, iii) the cover glass slide was thermally bonded at 655 °C at a 120 min duration and iv) as shown in Figure 2a, the micro-reactor was placed inside a plexiglass frame, being assembled by means of screws at the edges.

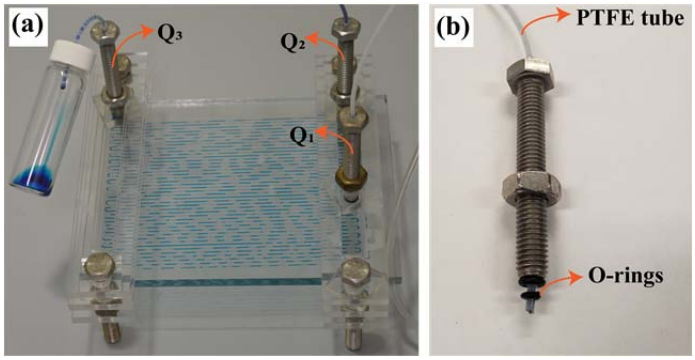

Figure 2: (a) Fabricated glass micro-reactor assembled with plexiglass holder, consists of two inlets as well as one outlet ports, and a region for performing photoreaction. (b) Punctured steel screws sealed using O-rings and PTFE tube.

In this venue, the PTFE tubes (inner and outer diameters=1.6 and 2 mm) were placed into the punctured steel screws being placed at outlet and inlet sections and sealed using appropriate O-rings (Figure 2b). The Q_1, Q_2, and Q_3 will be explained in the section 2.4.

2.3 Preparation of the Micro-photosensitizers

First, polystyrene microspheres (PSt) were synthesized using emulsion polymerization (Tamtaji and Kazemeini 2020). In this venue, 0.12 g of potassium persulfate and 70 mL DI water were added into two-neck round-bottom 250 ml flask. This solution was degassed for 5 minutes by using nitrogen. Then, it was stirred until its temperature reached to 75 °C. Next, 0.5 ml of the methacrylic acid and 2 ml of styrene were added to this mixture. After 1 h, 0.6 ml of the divinylbenzene was slowly added to it for 3 h (i.e., 0.1 ml at each 30 min interval) and the mixture was kept being stirred for another 10 h. Afterwards, the PSt were washed with ethanol and DI water for 3 and 2 times, respectively. Ultimately, the PSt's were separated through centrifuging at 15000 rpm for 15 min, and dried in oven at 50 °C overnight.
After synthesizing the PSt, a 200 mg portion of them were dispersed into 20 ml of Toluene by using ultrasonic bath for 30 min to make sure that all the micro-particles were dispersed ideally in Toluene and subsequently the solution was vigorously stirred for another 5 h. In order to embed the methylene blue (MB) into the polystyrene structure, 10 mg of MB in 1 ml of the dichloromethane was slowly added to 10 ml of the aforementioned mixture while it was vigorously stirred for 12 h. Afterwards, the temperature was enhanced to and maintained at 50 °C for 6 h to totally remove the Toluene and dichloromethane. At this stage, all of the solvents were evaporated and the polymer structure came back to the primary state during which a lot of MB molecules were captured inside the polymer structure. After drying, the micro-photosensitizers (MB-PSt) were washed 10 times with ethanol to make sure that all the uncaptured MB molecules were removed. Afterwards the micro-photosensitizers were dried at 40 °C overnight before being used for characterization as well as photooxygenation reaction. Figure 3a displays the SEM images of the prepared PSt at different magnifications while Figure 3b demonstrates the difference in colour and texture between the PSt and MB-PSt.

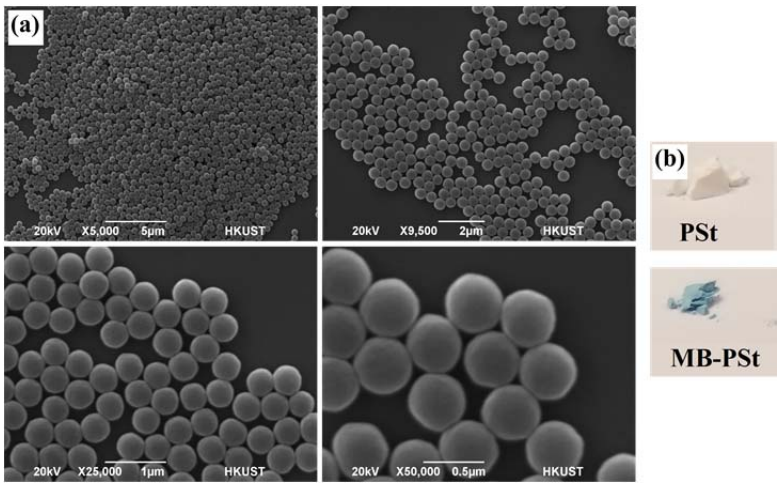

Figure 3: (a) SEM images of synthesized polystyrene microspheres (PSt), (b) colour and texture difference between the PSt and MB-PSt.

2.4 Operating procedures for continuous photooxygenation reactions

Taking into account the advantages of micro-reactors, several continuous photooxygenation reactions were attempted within them using micro-photosensitizers coated onto their walls. Figure 4 shows the utilized micro-reactor setup composed of a glass micro-reactor, a white LED lamp (20 W), a peristaltic pump for the injection of oxygen from the oxygen balloon into the microreactor as well as one glass syringe and a syringe pump for the injection of solution into the micro-reactor. To perform photooxygenation reaction, a mixture of micro-photosensitizers, DHAA and solvent was injected with the flow rate of Q_2, oxygen was injected with the flow rate of Q_1, and product was collected with the flow rate of $Q_3=Q_1+Q_2$ (Figure 2a). The Central Composite Design (CCD) for designing experiments through the RSM was utilized to achieve a suitable synergistic equation for the conversion of the DHAA as a function of operating parameters including the ratio of solution flow rate to oxygen flow rate (Q_2/Q_1), initial concentration of the DHAA (C_{DHAA0}) and concentration of micro-photosensitizer (C_{Mphtz}).

In each experiment, about 1.5 ml of the product suspension was collected and was centrifuged at 10000 rpm for 10 min. The collected supernatant liquid from the centrifuged solution was injected into the HPLC for analysing.

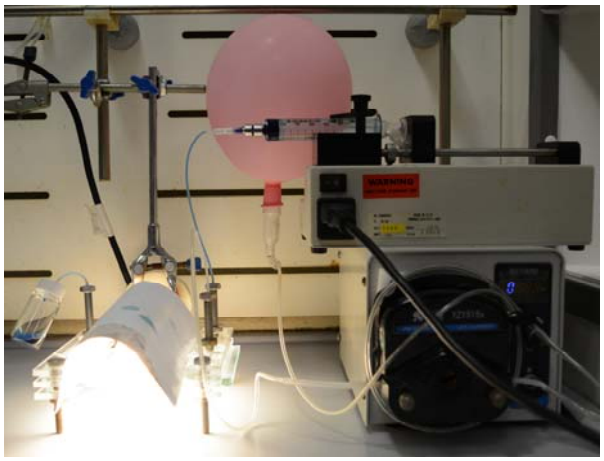

Figure 4: The utilized micro-reactor setup for the photooxygenation of the DHAA.

3. Results and discussion

Results of continuous photooxygenation of the DHAA using the MB-PSt are reported in this section. In addition, effects of various experimentally controllable parameters such as the ratio of the solution flow rate to that of the oxygen (Q_2/Q_1), initial concentration of the DHAA (C_{DHAA0}) and the micro-photosensitizer's concentration (C_{Mphtz}) upon the reaction conversion using the Design of Experiments (DOE) software (version 7.0) were investigated. Ultimately, an optimized set of such conditions was determined toward obtaining the highest DHAA conversion.

3.1 The surface response plot for the continuous photooxygenation of the DHAA

The operating parameters were coded at the levels of -1, 0, and +1 with the Q_2/Q_1, C_{DHAA0}, and C_{Mphtz} being set in the ranges of 0.5-1.5, 0.5-1.5 mg/ml, and 0.5-1.5 mg/ml, respectively, through using the DOE software. The oxygen flow rate (Q_1) was set equal to 1.62 ml/h. Fifteen experiments were thus suggested by the Design-Expert (DOE) software (indicated by numbers in the 2^{nd}-4^{th} columns). The corresponding measured vs. predicted DHAA conversions were also tabulated in Table 1.

Table 1: Experiments suggested through the Design-Expert® software and the measured responses.

Run number	Q_2/Q_1	C_{DHAA0} (mg/ml)	C_{Mphtz} (mg/ml)	Measured Conversion (%)	Predicted Conversion (%)
1	1.5	0.5	1.5	44	44
2	1	0.5	1	53	52
3	1.5	1.5	0.5	24	24
4	1	1	1.5	53	52
5	0.5	1.5	1.5	52	52
6	1	1	1	46	46
7	1	1	1	44	46
8	0.5	0.5	0.5	53	53
9	1	1	1	46	46
10	1	1	0.5	35	34
11	1	1	1	45	46
12	1	1.5	1	42	41
13	0.5	1	1	55	54
14	1.5	1	1	37	36
15	1	1	1	45	46

The experiments were performed utilizing the fabricated glass micro-reactor and the corresponding responses for each experiment were measured. Subsequently, the appropriate quadratic polynomial equation for the conversion of the DHAA, which accounts for the main influences (Q_2/Q_1, C_{DHAA0}, and C_{Mphtz}) as well as their synergistic effects ($Q_2/Q_1 \times C_{DHAA0}$, $Q_2/Q_1 \times C_{Mphtz}$, and $C_{DHAA0} \times C_{Mphtz}$) in addition, considers their curvature influences $(Q_2/Q_1)^2$, $(C_{DHAA0})^2$, and $(C_{Mphtz})^2$ was determined by the DOE and displayed in Eq. (1):

$$\begin{aligned} \text{Conversion}^{0.2} = {} & 2.323 - 0.2455\,Q_2/Q_1 - 0.3281\,C_{DHAA0} + 0.3180\,C_{Mphtz} + 0.1174\,Q_2/Q_1 \times C_{DHAA0} \\ & + 0.05062\,Q_2/Q_1 \times C_{Mphtz} + 0.05683\,C_{DHAA0} \times C_{Mphtz} - 0.04602\,(Q_2/Q_1)^2 \\ & + 0.02662\,C_{DHAA0}^2 - 0.1246\,C_{Mphtz}^2 \end{aligned} \quad (1)$$

R^2 value of this obtained equation is 0.993, confirming that the model accurately predicted the conversion of the DHAA. The predicted values of the DHAA conversion were also presented in Table 1 which, when compared with the corresponding experimentally determined values a very satisfactory agreement is revealed. Now that a statistically sound model has been developed for the conversion of the DHAA, one may examine the DHAA conversions due to the surface responses. Such results are shown in Figure 5. Based upon outcomes displayed in Figures 5a and 5b one notices that, upon incrementing of the flow rates' ratio (Q_2/Q_1), the conversion of the DHAA was lowered. This observation is attributed to a decrease of the residence time and oxygen concentration in the reactor upon such an increment. Besides the flow rates ratio, the DHAA conversion was decreased with an increase in the initial concentration of the DHAA (C_{DHAA0}). This was rationalized upon the fact that more residence time was needed for more DHAA to get converted through the reactor. Also, Figures 5a and 5c show that with increase in micro-photosensitizer's concentration, regardless of the flow rates ratio or concentration of the DHAA, the DHAA conversion was raised. The reason for such a behavior is that, the more micro-photosensitizers inside the reaction medium, the more produced $^1O_2$ and as a result the more reaction between the $^1O_2$ and DHAA.

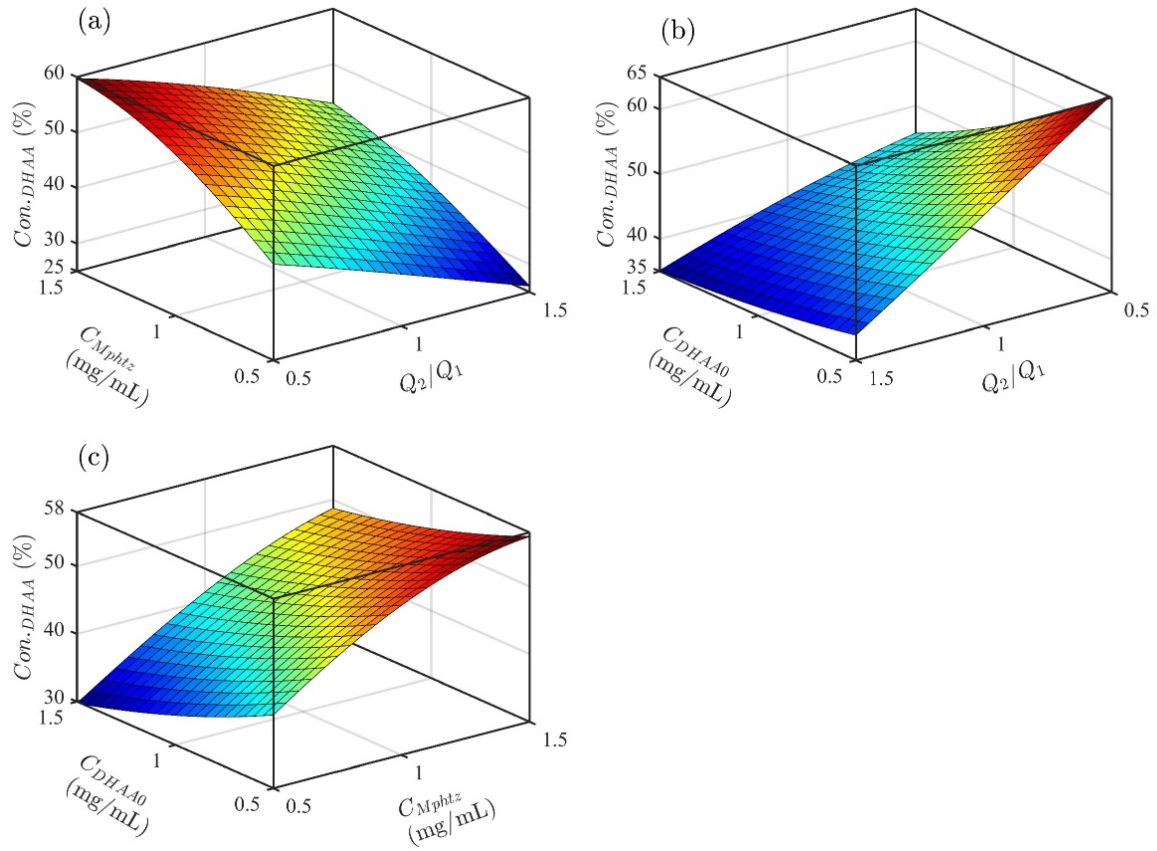

Figure 5: Effects of (a) Q_2/Q_1 vs. C_{Mphtz} (C_{DHAA0} is 1 mg/mL), (b) Q_2/Q_1 vs. C_{DHAA0} (C_{Mphtz} is 1 mg/mL), and (c) C_{Mphtz} vs. C_{DHAA0} (Q_2/Q_1 is 1) upon the conversion of the DHAA all predicted by Eq. (1).

3.2 Optimization of operating parameters for DHAA photooxygenation

The optimum values of operating parameters were determined to achieve the highest DHAA conversion, using the Design-Expert version 8.0 software and through the numerical optimization method. The determined optimum values for the flow rates ratio, and DHAA as well as micro-photosensitizer's concentrations were 0.5, 0.5 mg/ml, and 1.5 mg/ml, respectively. The corresponding conversion suggested for this optimum point was revealed to be 70 %. To evaluate reliability of the optimization results, one experiment under these optimum conditions was conducted, and the conversion of the DHAA was measured to be 69 %. This demonstrated a successful verification of the RSM analysis.

4. Conclusions

In this contribution, the photooxygenation reaction of the DHAA inside a micro-reactor utilizing micro-photosensitizers (MB-PSt) was optimized using the Design of Experiments (DOE) software while effects of the experimentally controllable parameters were investigated through the RSM technique. Micro-photosensitizers were prepared through one super facile method enabling photooxygenation reaction inside a micro-reactor. The DHAA conversion suggested under optimum conditions (i.e., the flow rates ratio=0.5, DHAA concentration=0.5 mg/ml, as well as the micro-photosensitizer's concentration=1.5 mg/ml) revealed to be 70% compared to that of the actual conversion experimentally determined to be 69%. Based on results suggested by the RSM technique, when the micro-photosensitizer's concentration was increased and the flow rate ratio as well as the DHAA concentration were lowered, the DHAA conversion was enhanced. This latter result was the goal sought through this research.

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