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To cite this article: Taína D. Matos , Nacole King , Lauren Simmons , Charmaine Walker , Aliecia R. McClain , Anil Mahapatro , Fred J. Rispoli , Kevin T. McDonnell & Vishal Shah (2011) Microwave assisted lipase catalyzed solvent-free poly- ϵ -caprolactone synthesis, Green Chemistry Letters and Reviews, 4:1, 73-79, DOI: [10.1080/17518253.2010.501429](https://doi.org/10.1080/17518253.2010.501429)

To link to this article: <https://doi.org/10.1080/17518253.2010.501429>



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Published online: 23 Feb 2011.



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RESEARCH LETTER

Microwave assisted lipase catalyzed solvent-free poly- ϵ -caprolactone synthesis

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(Received 28 March 2010; final version received 11 June 2010)

Microwave (MW) assisted enzymatic polymerizations is an area that is largely unexplored. In the current study, the effect of MW reaction parameters on poly- ϵ -caprolactone (PCL) properties has been investigated using a statistical design. A {3,5} modified mixture experimental design was used to identify the parameter values that gave the desired properties of PCL. The three process parameters that were tested are temperature, MW intensity, and the reaction time. Experimental results showed that in the range of values tested, temperature had the highest positive influence on the properties of PCL, whereas high MW irradiation is not desirable. A cubic regression model was developed and optimal process parameters were obtained using this model. Conducting the polymerization reaction under optimal conditions (90°C, 240 min, 50 W), PCL with M_n of 20,624 and polydispersity index of 1.2 were obtained. The regression model was validated by carrying out validation experiments and by 3D visualization.

Keywords: microwave assisted reactions; lipase catalysis; poly- ϵ -caprolactone; polyester synthesis; mixture design

Introduction

Microwave (MW) heating has been gaining increased popularity in green chemistry as an alternative heat source. Not only does it provide a safe, clean, and convenient way to heat reactions to elevated temperatures, but it also accelerates many syntheses providing selective activation and allows fast optimization of reactions (1). As the range of techniques for MW heating has expanded, so have the areas in which it can have a significant effect (2,3). Apart from traditional organic chemistry applications, MW has been used in biological sciences for the synthesis of peptides, oligopeptides, carbohydrates, and in the field of proteomics (3,4). Comprehensive reviews cover the use of MW in organic chemistry (5) and more recently in polymer chemistry (6).

Only few reports exist on the applications of MW assisted enzymatic reactions, focusing predominantly on organic small molecule transformations (7–12). An understanding of this area is poor and often controversial. Researchers have reported an enhancement in the initial rate of reaction (4,13), product yields (11,14), and enantioselectivity (9) when using

MW heating as compared to conventional heating. However, Leadbeater (8) and coworkers studied the effect of MW irradiation on lipase catalyzed transesterification of methyl acetoacetate in toluene. They found no differences between conventional and MW heating. Rejasse et al. (15) investigated the influence of MW heating on the stability of *Candida antarctica* Lipase B (CALB) and the kinetics of butyl butyrate synthesis. They reported an increase in enzymatic stability under MW field in organic medium suggesting this as a possible explanation for an increase in conversion rates observed for some enzymatic syntheses carried out under MW heating (15).

We could find few reports exploring MW assisted enzymatic polymerizations (16,17). Kerep and Ritter investigated the influence of MW irradiation on lipase catalyzed ring opening polymerization of ϵ -caprolactone (17). They concluded that MW assisted enzymatic polymerizations had accelerating, as well as decelerating, effects depending on the kind of boiling solvent. Toluene and benzene showed decelerating effects while diethyl ether showed accelerating effects. The area of MW assisted enzymatic polymerizations

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remains largely unexplored. Enzymatic polymerizations offer unique advantages such as unique selectivity, avoidance of toxic metal catalyst, and the use of mild reaction conditions (18,19). However, due to the higher cost of the enzymes these processes are not commercially viable. Combination of MW irradiation with lipase catalysis offers a potentially alternative green chemistry synthetic route for enhancement of reaction rates and shorter reaction time, while the ability of enzyme recyclability could make these biotransformations more viable. Our goal was to systematically investigate the effects of MW process parameters (power, intensity, time, and temperature) on lipase catalyzed polymerizations. Poly- ϵ -caprolactone (PCL) synthesis is the most widely used reaction as a model for enzymatic ring opening studies (18,20). PCL synthesis has also been studied extensively using chemical catalysis in various conditions including bulk (solvent less) conditions (6,20–22). A schematic representation of ring opening polymerization of ϵ -caprolactone is shown in Scheme 1. We have used the same reaction, catalyzed by CALB under solvent-free conditions to obtain the fundamental understanding of the reaction in presence of MW.

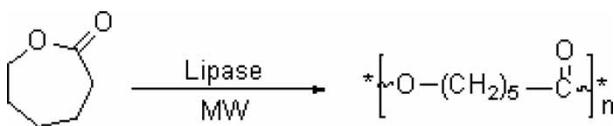
Materials and methods

Design of experiment (DOE)

A {3,5} modified mixture design was designed similar to the one used in our earlier study (23). The experimental design and the range for each of the variables are described in Table 1.

Microwave (MW) assisted synthesis of polycaprolactones

Novozyme-435 (0.057 g, 10% by weight relative to the total weight of monomer), was transferred into a 10 ml MW reaction glass tube containing 0.57 g of ϵ -caprolactone. The reaction tube was then placed into a CEM MW synthesizer (Discover) at a preset temperature, time, and MW power settings. The contents of the reaction vessel were stirred by means of a rotating magnetic plate located below the floor of the MW cavity and a Teflon coated magnetic stir bar in the vessel. Temperature, pressure, and power were monitored using commercially available software provided by the MW manufacture. Power MAX,



Scheme 1. Schematic representation of microwave assisted ring opening polymerization of ϵ -caprolactone.

developed by CEM was used to allow continuous cooling of MW vessel while MW heating. Compressed air at 30 psi was introduced into the cavity while simultaneously applying MW irradiation which allowed controlling bulk temperature rise and minimizing variations of temperature from the preset value. The reactions were terminated by adding excess cold Tetrahydrofuran (THF), and removing the enzyme by filtration (glass-fritted filter, medium porosity). The preliminary characterization of the polymers was carried out by gel permeation chromatography (GPC) to determine the number average molecular weights (M_n) and polydispersity index (PDI) of the polymer formed.

Gel permeation chromatography (GPC)

The M_n was determined by GPC using a Viscotek GPCMAX VE 2001 system, with triple detector array (TDA) 302 multi-detector system and using a conventional calibration method with six calibration points. Omnisc GPC software version 4.2 was used for molecular weight calculations. THF was used as the eluent at a flow rate of 1.0 ml per minute. Sample concentrations of 1–5 mg/ml and injections of 0.1 ml were used.

Statistical analysis

Two dependent variables (process output variables) were considered in the present study: M_n and PDI. This was carried out to optimize the independent variables such that high M_n and low PDI product could be obtained. Regression analysis was first carried out and linear, quadratic, and cubic were obtained from the data. These models were used to understand the interactions amongst the ingredients and illustrate the primary and secondary effects of each variable. Two-dimensional (2D) ternary plots were then obtained to visualize the interactions.

Three-dimensional (3D) visualization of data

The experimental data were also visualized with three-dimensional (3D) plots in which the coordinate axis corresponds to three variables. Using one of the mixture models, we calculate the predicted product at each point in 3D so as to define a scalar field. By computing isosurfaces at different product levels we are then able to explore the space of possible combinations of parameters to find those combinations which maximize the M_n or minimize the PDI. Hence, we can exploit visualization not only to understand the data but also to guide parameter selection for future experiments.

Table 1. {3,5} mixture design experiments and the obtained values for M_n and PDI of the poly- ϵ -caprolactone using lipase catalyzed microwave assisted reaction.

Run no.	Temperature (X1)	Power (X2)	Time (X3)	M_n	PDI
Min (0)	20	50	30		
Max (1)	90	250	240		
1	90	50	30	7518	1.3
2	20	250	30	6822	1.3
3	20	50	240	8518	1.4
4	20	210	72	2256	1.4
5	20	170	114	6271	1.3
6	20	130	156	2065	1.5
7	20	90	198	5894	1.1
8	76	50	72	10367	1.5
9	62	50	114	8032	1.4
10	48	50	156	4102	1.8
11	34	50	198	8457	1.3
12	76	90	30	8485	1.2
13	62	130	30	3489	1.7
14	48	170	30	4465	1.4
15	34	210	30	4893	1.4
16	62	90	72	4167	1.6
17	48	130	72	3855	1.6
18	48	90	114	3825	1.6
19	34	170	72	3239	1.6
20	34	130	114	2885	1.6
21	34	90	156	3081	1.7

Results and discussion

Modified Mixture design was chosen over other types of statistical design of mixtures because of its proven ability to capture interaction amongst the media ingredients and the uniform distribution of the design points over the design space (23). The three independent variables chosen for the study were MW power (W), temperature ($^{\circ}$ C), and reaction time (min) under the hypothesis that any reaction enhancement due to MW irradiation would be because of these variables. M_n and PDI were selected as the dependent variables with the polymer formed desired to have the highest M_n with lowest PDI. We have used PowerMAX (an option available with CEM Discover Microwave Systems) to explore the technique of simultaneous cooling while MW heating. This allows for higher levels of MW energy to be introduced to the reaction while maintaining constant power and temperature, and resulting in higher product yields in conventional chemical reaction (24). The design of experiment (DOE) and the obtained results are described in Table 1.

The first step in analyzing the experimental data is to confirm the presence of interactions amongst the process variables. Ternary plots predicting the M_n and PDI of the PCL polymer across the design space

were prepared based on a quadratic model and are shown in Figure 1. The plots clearly indicate that the M_n of the polymer increases as we move toward the boundary connecting the temperature and time vertices. The PDI of the polymer, however, increases as we move toward the center of the design triangle. This provides a good indication that the M_n and PDI of the polymer are dependent on the proportions of the three variables. Figure 1 also indicates that in the region where higher M_n chains of PCL are synthesized, the PDI of the polymer is low. This is in agreement with enzymatic ring opening mechanisms where the lipase tends to first ring open the monomer (lactone) to its corresponding hydroxy acid which leads to low M_n , however, high PDI (18). Subsequent chain propagation and build up of oligomers lead to higher M_n of the polymer while decreasing its PDI (18). Reported literature of lipase catalysis of caprolactones showed that at higher conversions the PDI of the poly-caprolactone formed are about 2 (25,26), however, comparison of use of MW heating and thermal heating of caprolactone polymerization using chemical catalysts showed that the use of MW heating favors chain growth in the initial stage thus limiting the number of polymer chains, whereas thermal heating favors the formation of growing centers (22). We hypothesize that the ability of MW

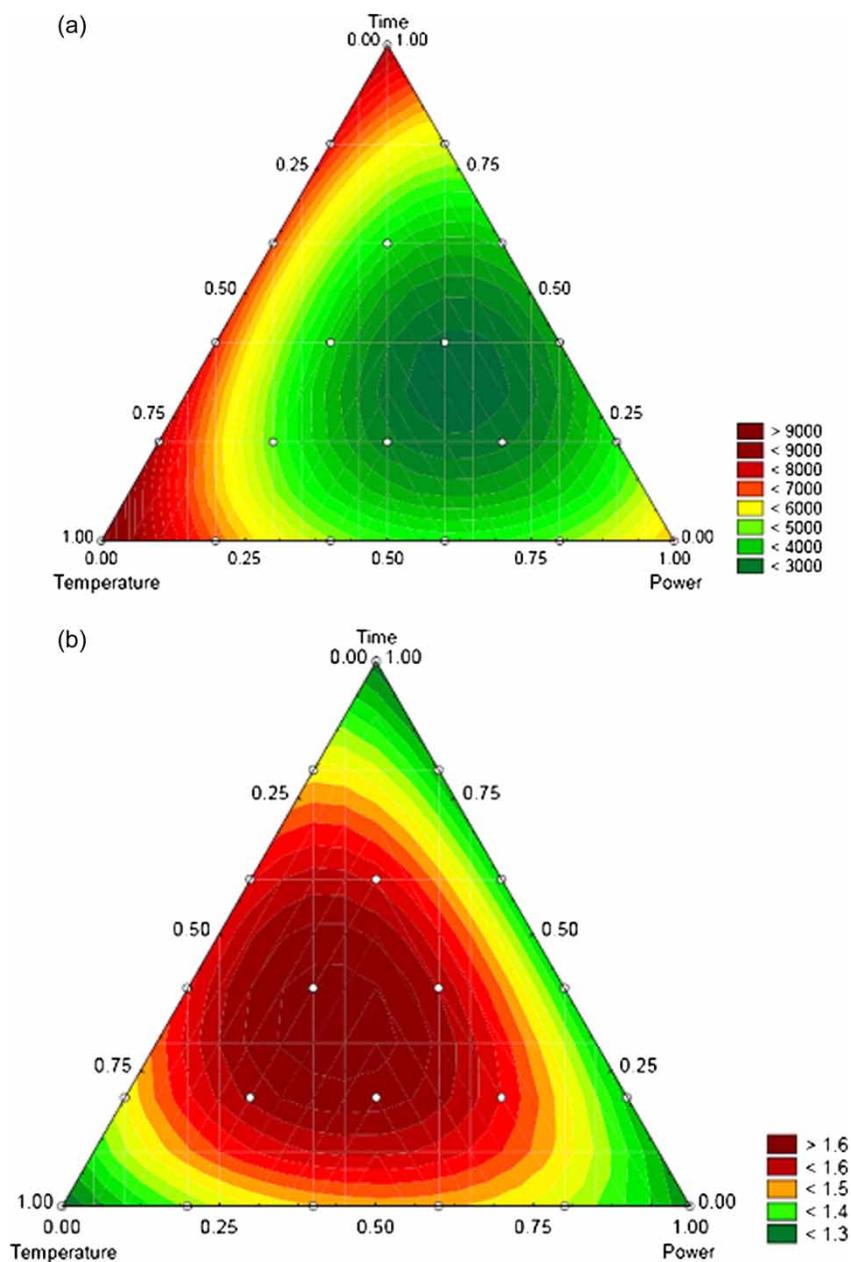


Figure 1. Ternary plots of a quadratic model developed to predict the (1) average molecular weight (M_n) and (2) polydispersity index (PDI) of the poly- ϵ -caprolactone produced using lipase catalyzed microwave assisted reaction. Predicted values represented through color scheme.

to limit growing centers and the ability of lipase for chain length selectivity would result in lower PDI polymers. This is also supported by the fact that in the center of the design triangle, the chains grow slowly due to low enzymatic activity, and thus there is high variability from chain to chain. On the other hand, along the temperature and power vertices where the M_n is higher, the enzyme activity could be presumed to be high, leading to lower variability amongst the synthesized chains.

The next step was to develop a mathematical model that can be used to predict the behavior of the polymerization process under various experimental conditions. The results described in Table 1 were used to obtain a cubic model using multiple regression analysis. The cubic regression model obtained using regression analysis had a R^2 value of 0.94, an adjusted R^2 value of 0.84 and is shown in Table 2. The model has the significance F -value of 3.49×10^{-7} . This low value indeed indicates that the model can be used to

Table 2. Coefficients and statistically significant values for the various terms related to the cubic model developed to predict the M_n of the poly- ϵ -caprolactone produced using lipase catalyzed microwave assisted reaction.

Variable	Coefficients	P-value
X1	9438.5	0.0000137
X2	6359.5	0.000617
X3	8232.5	0.0000565
X1.X2	-13216.8	0.070498
X1.X3	-7522.42	0.283931
X2.X3	-15285.8	0.039967
X1.X2.X3	-30155.1	0.456556

make reliable predictions. The standard error of estimate was 1750. This number reflects the standard deviation of the differences between the observed values and the values predicted by the model. Based on the coefficients of the main effect variables, all of the three variables have a positive influence on the polymerization reaction. Temperature has the highest influence on the reaction, whereas the power of MW irradiation has the least positive influence. The positive influence of the variables is further supported by very low P values of the variables. The secondary effect of the variables via interaction with each other is always negative (because of the negative coefficients). This also supports our earlier conclusion that there is a high level of interaction among the process variables. Of all the interactions of statistical significance is the interaction between power and time with a P -value < 0.05 . Other two way interaction and the three way interaction are not statistically significant indicated by a P -value > 0.05 .

Using the mathematical model described in Table 2, optimal values for each of the process variable were calculated for maximum M_n using Solver within

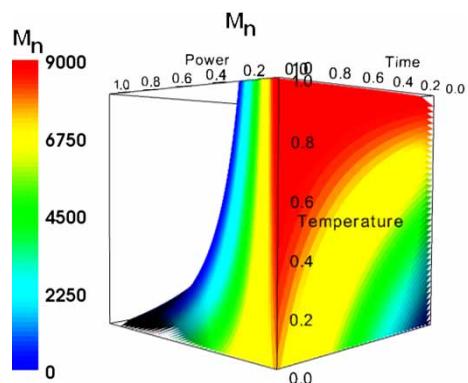


Figure 2. A 2D screen shot of the 3D visualization system used to verify the cubic model. Highest values of M_n (shown in red) are produced when time and temperature are maximized and power is minimized (color online).

Microsoft Excel[®]. The constraint that was used to solve the problem was $0 \leq X_i \leq 1$. The output of the Solver indicated that when temperature and time have the values of 1, and power has the value of 0, the maximum M_n of the polymer chain could be obtained. By decoding the values based on the minimum and maximum values for each variable described in Table 1, the optimal process conditions for carrying out the CALB catalyzed MW assisted reaction are a temperature of 90°C for 240 min under MW irradiation of 50 W.

The validation of the optimized conditions was obtained using 3D visualization and carrying out an experiment under optimal conditions. A 3D plot (Figure 2) predicting the M_n of the PCL was developed based on the cubic model. The plot shows high M_n along the region where the value for power is low and when time and temperature are high. If one rotates the 3D view so that the temperature axis is aligned in the positive x direction and time in the positive y direction, a wide region colored red that indicates high M_n is around the region with high time and temperature values. Additionally, if we follow the z axis (power) in the positive direction, we find that the color changes quickly to yellow, green, and blue, which correspond with very low M_n (color online). Thus, the visualization validated the cubic model, which indicates higher temperatures, longer times, and lower power are required to produce high M_n PCL. Conducting the polymerization reaction under the optimal conditions, PCL with M_n of 20,624 and PDI of 1.2 were obtained. The obtained results are twice the maximum M_n obtained in the experiments mentioned in Table 1. The low PDI value validates our initial observation that when the M_n of the PCL is high, PDI values are low.

Based on the Solver output, one could doubt the positive influence of MW irradiation on the polymerization reaction because of the lowest possible value (0) in the output. PCL synthesis was carried out under the optimal temperature and time (90°C and 240 min), but with the MW power of only 10 W. Under these conditions, PCL with M_n of 7644 and PDI of 1.6 were obtained. This not only confirms the positive influence of the MW irradiation on the enzyme catalyzed polymerization reaction, but also supports the optimal values obtained. Kerep and Ritter carried out PCL synthesis using CALB in the presence of various solvents and varying the MW irradiation for 90 min (17). They obtained a maximum M_n of 5800 when the reaction was carried out in diethyl ether, at 38°C and MW power of 200 W. The results described in the current study not only show a four-fold improvement in the M_n of the

polymer, but also provide the advantage of using low MW power and no solvent.

We observe the trend that M_n increases with temperature and/or time. This trend is similar to that observed under conventional heating. The enhanced rates of the polymerization are in agreement with results of Yu and Liu (22) who in their study of benzoic acid initiated polymerization of caprolactone, reported enhanced rates using MW heating. They state that MW heating favors chain growth in the initial stage by limiting the number of polymer chains, whereas thermal heating favors formation of growing centers in the initial stage. As a result the MW assisted polymerization procedure resulted in higher molecular weight polymers (22). A detailed investigation would be needed to see if MW activation limits formation of the acyl enzyme intermediate and favors chain propagation for similar reaction enhancements. However, it needs to be emphasized that MW effect on the polymerization reaction is not linear. After an optimal point, MW has a negative influence on the reaction. In lipase catalyzed reactions, high temperatures could lead to the loss of catalytically active conformation of the enzyme. Also Lipase catalysis requires a minimal water content which acts as a nucleophile for ring opening of caprolactone and a certain optimal water level in the local environment of the enzyme for it to function efficiently. Previous studies (27) on effect of application of vacuum of lipase catalyzed polymerization of hydroxyl acid showed that the polymer molecular weight of hydroxyl acid including 6-hydroxyhexanoic acid was greater when vacuum was not applied for time period of about 4 hrs or less. 6-Hydroxyhexanoic acid would be the hydroxyl acid monomer formed after ring opening of caprolactone. Since all our reactions were carried out at 4 hrs or less we had opted not to apply vacuum. However, the stability of the catalytically active conformation of the enzyme is closely dependent on the interaction between an enzyme and its microenvironment. In an organic medium, the hydrophobic interactions are strongest and the structural rigidity of the enzyme increases resulting in greatly enhanced thermal stability. Caprolactone being in liquid form could provide some thermal stability too. However, caprolactone can absorb MW energy effectively and thus increase in power would result in higher instantaneous microenvironment temperature in the reaction as the bulk temperature increases. This could drastically affect the enzyme and its microenvironment leading to a loss in enzyme activity/stability. This hypothesis is in agreement with results of Rejasse et al. (28) state that MW activity affects the enzyme stability based on its microenvironment. Further research is warranted on

the thermal/kinetic origin of the MW radiation and the effect of the local microenvironment such as reaction water content.

Summary and conclusions

The current study provides the first demonstration of the ability of MW irradiation to positively influence the enzyme catalyzed reaction in the absence of solvents.

Acknowledgements

Authors at NSU would also like to acknowledge the NIH center for Biotechnology and Biomedical Science and the Center for Materials Research at Norfolk State University for the financial support for the work. Vishal Shah, Fred Rispoli, and Kevin McDonnell acknowledge Dowling College for providing funds to carry out the research.

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