Probing the effects of microwave irradiation on enzyme-catalysed organic transformations: the case of lipase-catalysed transesterification reactions†

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The lipase-catalysed transesterification reaction of methyl acetoacetate in toluene as a solvent has been studied using carefully controlled conditions. Results suggest that microwave heating does not have a noticeable effect on reaction rate or product conversion.

Introduction

Microwave heating is a valuable tool for synthetic chemists. It is possible to improve product yields and enhance the rate of reactions as well as being a safe and convenient method for heating reaction mixtures to elevated temperatures.[1,2] It is possible to perform reactions even at modest reaction temperatures and still see great improvements in rate and yield. Sealed reaction vessels are only one option, standard reflux and open vessel chemistry can benefit from microwave irradiation. As the range of techniques for microwave heating has expanded, so have the areas in which it can have a profound impact. One case is its application to biologically relevant processes such as the synthesis of peptides,[3] peptoids,[4] oligopeptides[5] and carbohydrates[6] and in the field of proteomics.[7] Also included in this broad area is the application of microwave heating to enzyme-catalyzed organic synthesis.[8] Over the last few years there have been a number of reports suggesting that enzyme activity can be enhanced by using controlled microwave irradiation and suggesting that these effects are non-thermal in origin. Much of this work has been focused around the use of lipases: researchers stating that reaction rates and product yields obtained in esterification and transesterification reactions catalysed by lipases are significantly higher when using microwave heating.[9–13] Also, enzymatic stability is reportedly higher under microwave heating than under conventional thermal heating.[14] Very recently, a paper by some of the authors of these previous reports[10,12] was published. It suggests that identical initial rate and conversion yield were obtained under microwave and conventional heating for the transesterification reaction between ethyl butyrate and butanol in a solvent-free system but that the kinetics of inactivation of the lipase are influenced differently depending on the heating method used.[15] This has prompted us to report recent findings from our laboratory directed around the investigation of microwave-promoted lipase-catalysed transesterification reactions.

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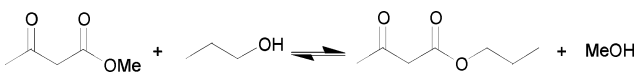
Results and discussion

Since the transesterification reaction between methyl acetoacetate and primary alcohols had been the subject of several previous investigations, we decided to start our study using this as a model.[13] As the enzyme, we used lipase from *Candida antartica.* Our initial objective was to compare the rate of reaction using microwave and conventional heating. To achieve this, we knew that it was key to perform the reactions under as closely comparable reaction conditions as possible. For microwave experiments we used a scientific monomode apparatus, equipped with a fiber-optic temperature measurement device. Reactions were performed in a round-bottomed flask with the temperature probe inserted into the reaction mixture by means of a sapphire thermal well. Conventional experiments were performed in the same vessel using the same temperature probe but were placed into a pre-heated oil bath. In both cases, the reaction mixture was stirred throughout. We focused on the reaction of methyl acetoacetate and *n*-propanol using toluene as a solvent. Toluene was chosen both because it has been used previously but also because it is not particularly microwave absorbent, a characteristic that would be useful in later experiments.



Reaction mixtures were heated to the desired temperature in the absence of the enzyme and only when this was stabilized did we add the lipase (the reaction did not occur in the absence of the enzyme). We performed the reaction at 50 °C and at 70 °C for 2.5 h. Plots of product conversion *vs.* time are shown in Figs. 1 and 2. The plots show that there is negligible difference in rate when using microwave and conventional heating.

When running the reaction at 70 °C, the microwave apparatus holds the reaction mixture at temperature using intermittent pulses of 25 W power. This is a relatively low irradiation power and thus we next wanted to determine the effect, if any, of varying microwave power delivered from the magnetron while keeping the bulk temperature of the reaction mixture the same. To achieve this we applied controlled cooling in conjunction with microwave heating. Data are shown in Table 1. By irradiating a reaction mixture with microwaves while simultaneously cooling the outer vessel walls with compressed air or cryogenic fluid, it is possible to irradiate the sample with significant microwave power while holding it at a set bulk temperature during the whole period of the

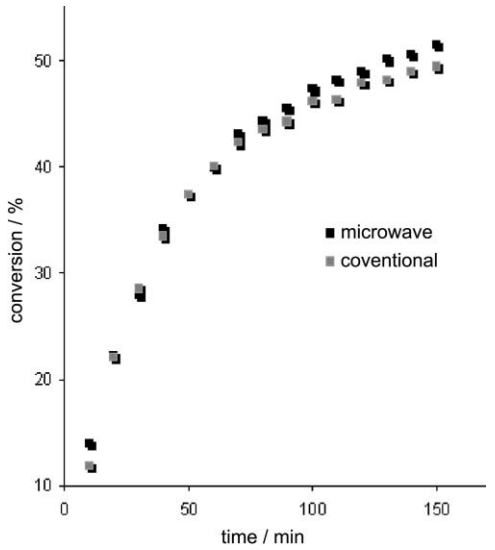


Fig. 1 Lipase catalysed transesterification between methyl acetoacetate and *n*-propanol at 50 °C.

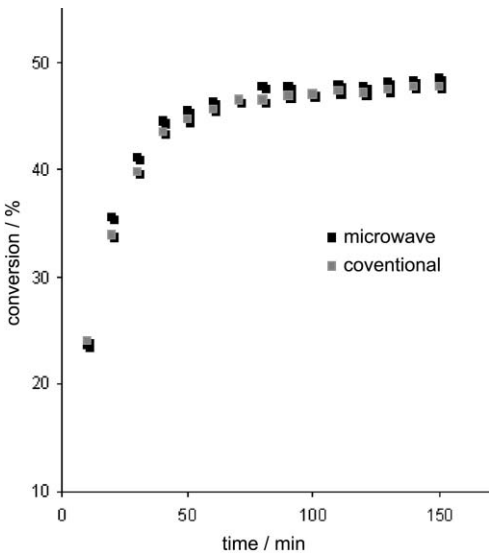


Fig. 2 Lipase catalysed transesterification between methyl acetoacetate and *n*-propanol at 70 °C.

Table 1 Effect of microwave power on the lipase-catalysed transesterification reaction of methyl acetoacetate in toluene as a solvent at 50 °C[a]

Entry	Reaction conditions	Product conversion/%
1	Conventional heating	31
2	Microwave heating, no cooling	32
3	Microwave heating, air cooling	28
4	Microwave heating, cryogenic cooling	30

[a] Microwave power defined as that delivered from the magnetron.

reaction.[16,17] We performed reactions using compressed air cooling and cryogenic fluid as coolant. In both cases it was necessary to move from working in a round-bottom flask to a glass tube. We foresaw the change in vessel as having an effect on the product conversion due to differences in scale, dimensions and stirring efficiency. Thus, both to address this and act as a control, we first performed the reaction conventionally using a glass tube. The reaction mixture was equilibrated at 50 °C in an oil bath before adding the enzyme and running the reaction for 1 h. A product conversion of 31% was obtained (Table 1, entry 1), compared to 49% when using a round-bottomed flask.

We next performed the reaction in the microwave with no external cooling. The reaction was performed using the CEM Coolmate apparatus. This comprises of the same monomode apparatus as used previously but equipped with a jacketed vessel around which microwave-transparent cryogenic fluid can be passed.[18,19] Due to the dimensions of the vessel (~0.6 cm i.d.), it was hard to ensure complete delivery of the enzyme while the equipment was running. As a result, rather than equilibrate the reagents at the desired temperature of 50 °C and then add the enzyme, we took a different strategy. We pre-equilibrated a toluene solution of propanol and the enzyme at 50 °C and then added the methyl acetoacetate. After 1 h microwave irradiation of the complete reaction mixture a product conversion of 32% was obtained (Table 1, entry 2); this being almost identical to that from the control reaction using oil-bath heating.

We performed the reaction using air cooling in conjunction with microwave heating. To do this, we used a 10 mL glass tube as the reaction vessel. Significantly more microwave power was applied during the course of the reaction as compared to the case with no cooling but a similar product conversion (28%) was obtained (Table 1, entry 3). We finally ran the reaction with cooling using cryogenic fluid. In this case it was possible to introduce the maximum power possible with the apparatus (300 W) but with no increase in product conversion (30%, Table 1, entry 4).

Our results suggest that the microwave power applied does not have a noticeable effect on product conversion in the lipase-catalysed transesterification reaction of methyl acetoacetate in toluene as a solvent. Also, because the enzyme was added before the equilibration stage, it is also apparent that microwave irradiation does not have a noticeable effect on the inactivation of the lipase during this period.

Kerep and Ritter recently reported the results of studies on the microwave-assisted lipase-catalysed ring-opening polymerization of ε-caprolactone in boiling solvents.[20] They found that in the case of boiling toluene or benzene, the microwave reaction proceeded slower compared to oil bath heating. On the other hand, using boiling diethyl ether as solvent, the product yield was about 10% higher when using microwave heating as opposed to oil bath heating. We believe that these results can be interpreted in terms simply of the temperature at which the reactions are performed. When refluxing toluene or benzene using significant microwave energy input, instantaneous local temperatures could be significantly higher than the boiling point and this could lead to rapid denaturing of the lipase. Using conventional heating, the lifetime could be somewhat extended and thus more product could be formed before the enzyme denatures. With diethyl ether, because the boiling temperature is lower, denaturing of the enzyme is less of a concern. The fact that the authors obtained a higher product yield in diethyl ether using microwave heating made us want to probe the effect of moving from toluene to diethyl ether on our reaction. Thus, we performed the lipase catalysed transesterification reaction between methyl acetoacetate and 1-propanol in boiling diethyl ether using both conventional and

microwave heating. When using microwave heating we obtained a 38% yield of the desired product after a reaction time of 1 h. Performing the identical experiment using conventional heating resulted in a 36% product yield.

Conclusions

While more studies are clearly required in order to be able to determine the effects of microwave irradiation across a broad range of enzyme-catalysed transformations, we find no differences between conventional and microwave heating in the reaction studied here. We have used carefully controlled comparisons to probe the effects both of reaction temperature and microwave power delivered by the magnetron.

Experimental

General

All reagents were obtained from commercial suppliers and used without further purification. Lipase acrylic resin from *Candida antartica* was used. The lipase was kept in a sealed dry environment and while it is hygroscopic no evidence of macroscopic water uptake was noted during the course of the study. ^{1}H- and ^{13}C-NMR spectra were recorded at 293 K on a 400 MHz spectrometer.

Description and use of the microwave apparatus

Microwave reactions were conducted using a commercially available monomode microwave unit (CEM Discover). The machine consists of a continuous focused microwave power delivery system with operator selectable power output from 0–300 W. Reactions were performed either in round-bottom flasks or glass tubes. The temperature of the contents of the vessel was monitored using a calibrated fiber-optic probe inserted into the reaction vessel by means of a sapphire immersion well. In all cases, the contents of the vessel were stirred by means of a rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stir bar in the vessel. Temperature, pressure and power profiles were monitored using commercially available software provided by the microwave manufacturer. For reactions performed using cryogenic cooling, a specially designed jacketed reaction vessel was used through which a microwave transparent fluid was passed continuously by means of a pump (CEM Coolmate).

General experimental procedures

Representative example of a transesterification reaction using a round-bottom flask. In a 50 mL round-bottom flask were placed propanol (1.5 mL, 1.202 g, 20 mmol), methyl acetoacetate (2.15 mL, 2.32 g, 10 mmol) and toluene (8 mL). The temperature probe was inserted into the reaction mixture and the vessel placed into the microwave cavity. Initial microwave irradiation of 35 W was used, the temperature being ramped from rt to 50 °C. Once this temperature was reached and held steadily, lipase (106 mg) was added and the reaction mixture held at 50 °C for 2.5 h.[21] Samples were removed over time, diluted in $CDCl_3$, the ^{1}H-NMR spectrum recorded and the product conversion determined.

Transesterification reaction with no cooling using a glass tube. In a dedicated jacketed glass tube were placed propanol (0.75 mL, 0.601 g, 10 mmol), lipase (53 mg) and toluene (4 mL). The temperature probe was inserted into the reaction mixture and the open vessel placed into the microwave cavity. Initial microwave irradiation of up to 300 W was used, the temperature being ramped from rt to 50 °C. Once this temperature was reached and held steadily, methyl acetoacetate (1.08 mL, 1.16 g, 10 mmol) was added and the reaction mixture held at 50 °C for 1 h. At the end of the reaction, the ^{1}H-NMR spectrum of the product mixture was recorded and the product conversion determined.

Transesterification reaction with air cooling using a glass tube. In a 10 mL glass tube were placed propanol (0.75 mL, 0.601 g, 10 mmol), lipase (53 mg) and toluene (4 mL). The temperature probe was inserted into the reaction mixture and the open vessel placed into the microwave cavity. Initial microwave irradiation of up to 300 W was used, the temperature being ramped from rt to 50 °C with compressed air (40 psi) being passed constantly over the outer vessel walls. Once this temperature was reached and held steadily, methyl acetoacetate (1.08 mL, 1.16 g, 10 mmol) was added and the reaction mixture held at 50 °C for 1 h. At the end of the reaction, the ^{1}H-NMR spectrum of the product mixture was recorded and the product conversion determined.

Transesterification reaction with cryogenic fluid cooling using a glass tube. In a dedicated jacketed glass tube were placed propanol (0.75 mL, 0.601 g, 10 mmol), lipase (53 mg) and toluene (4 mL). The temperature probe was inserted into the reaction mixture and the open vessel placed into the microwave cavity. Initial microwave irradiation of up to 300 W was used, the temperature being ramped from rt to 50 °C with cryogenic fluid being passed constantly over the outer vessel walls at a rate such as to maximise microwave input. Once the target temperature was reached and held steadily, methyl acetoacetate (1.08 mL, 1.16 g, 10 mmol) was added and the reaction mixture held at 50 °C for 1 h. At the end of the reaction, the ^{1}H-NMR spectrum of the product mixture was recorded and the product conversion determined.

Representative example of a transesterification reaction using a round-bottom flask using diethyl ether as a solvent. In a 50 mL round-bottom flask were placed propanol (1.5 mL, 1.202 g, 20 mmol), methyl acetoacetate (2.15 mL, 2.32 g, 10 mmol) and diethyl ether (16 mL). The temperature probe was inserted into the reaction mixture and the open vessel placed into the microwave cavity. Initial microwave irradiation of 100 W was used, the temperature being ramped from rt to 37 °C. Once this temperature was reached and held steadily, lipase (105 mg) was added and the reaction mixture held at 50 °C for 1 h. Samples were removed over time, diluted in $CDCl_3$, the ^{1}H-NMR spectrum recorded and the product conversion determined.

Acknowledgements

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