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Synergism between microwave and enzyme catalysis in intensification of reactions and selectivities: transesterification of methyl acetoacetate with alcohols

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Abstract

β-Keto esters serve as important synthons since they can be easily transformed into chiral building blocks via enzymatic transformation. As a consequence, their inter-conversion into different esters has gained a great deal of attention. This work focuses on the transesterification of methyl acetoacetate, a β-keto ester, with various alcohols in the presence of immobilized lipases the influence of microwave irradiation. A number of commercially available lipases such as Novozym 435, Lipozyme RM IM and Lipozyme TL IM were screened and Novozym 435 was found to be the most active. The effect of chain length of alcohol was also studied.

There is a synergism between the enzyme catalysis and microwaves. The reaction follows the ping pong bi–bi mechanism. The theoretical predictions and experimental data match very well.

Keywords: Microwave catalysis; Lipase catalysis; β-Keto esters; Methyl acetoacetate; Synergism; Intensification of rates; Immobilized lipases; Selectivities

1. Introduction

Under the ambit of green chemistry, biocatalysis using renewable resources is very attractive to produce chemicals which are also safer. Enzymes are the most sought after catalysts due to their specificity, selectivity and high effectiveness under mild reaction conditions including their benign nature [1]. In recent years, enzymes have gained pivotal importance in the production of fine chemicals, pharmaceuticals, fuels and numerous other manufactured goods [2,3]. Although a majority of enzymatic reactions are conducted in aqueous media, non-aqueous enzymology finds a few applications in synthetic chemistry [4]. Lipases (triacyl glycerol hydrolases EC 3.1.1.3) are one of the important enzymes, which are used in organic synthesis mainly for hydrolysis [5], esterification [6,7], transesterification [7,8], interesterification, thioesterification [9], amidation [10], epoxidation [11,12], etc. Lipases are the most widely used enzymes because they are cheap, easily available, cofactor free and have broader substrate specificity. The use of immobilized lipases in non-aqueous media is on the rise [5–12].

One of the novel approaches towards clean and green chemistry is the application of microwaves, which is relatively a very convenient, safe and rapid methodology [13–15]. Since lipase-catalyzed reactions are rather sluggish in nature, the synergism with microwave can be expected to enhance the rates of reactions. Such an exploration has earlier been tried out to witness rate enhancement in microwave irradiated lipase-catalyzed reactions [16–20]. Thus, it was thought worthwhile to study some reactions of industrial importance, particularly in non-aqueous media, under both microwave and enzyme catalysis including kinetic modeling. Thus, β-Keto esters were chosen for the study.

β-Keto esters serve as important synthons for they can be easily transformed to chiral building blocks by enzymatic transformation [21–25]. Consequently, their inter-conversion

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to different esters has received considerable attention. Several synthetic routes are available for transesterification but most of them are dirty and produce enormous wastes. Recently, Taber et al. [25] reported 4-(dimethyl-amino)-pyridine (DMAP) catalyzed transesterification of β-keto esters with good to excellent yields. However, their methods make use of toxic and expensive DMAP in relatively large amount (30 mol%), in addition to excess use of β-keto esters, under stringent reaction conditions. Solid acids have been employed in a number of esterification reactions and several such systems have been studied in our laboratory by using ion exchange resins, heteropoly acid supported on clays [26], mesoporus solid acids [27], sulfated zirconia [28], etc. However, a majority of methods reported with solid acids as catalysts require higher temperatures over longer reaction times.

This paper investigates the synergism between enzyme catalysis and microwaves on transesterification of methyl acetoacetate, a β-keto ester, with different alcohols.

2. Materials and methods

2.1. Enzymes

The enzymes Novozym 435, Lipozyme RM IM and Lipozyme TL IM were procured as gift samples from Novo Nordisk, Denmark. Novozym 435 is *Candida antarctica* lipase immobilized on a macroporous polyacrylic resin; Lipozyme RM IM is *Mucor miehei* immobilized on an anionic resin while Lipozyme TL IM is *Thermomyces lanuginosus* immobilized on silica.

2.2. Chemicals

All chemicals were AR grade, procured from firms of repute and used without any further purification: methyl acetoacetate (Merck, India), toluene, *n*-propanol, *n*-pentanol, *n*-hexanol, *n*-octanol, *n*-butanol, *n*-decanol, *iso*-butanaol, *iso*-amyl alcohol, *iso*-propanol (all from s.d. Fine Chemicals Pvt. Ltd., Mumbai, India).

3. Experimental setup

3.1. Conventional heating

The experimental setup consisted of a 3 cm i.d. fully baffled mechanically agitated glass reactor of 50 cm^3 capacity, which was equipped with four baffles and a six-bladed pitched-turbine impeller. The entire reactor assembly was immersed in a thermostatic water bath maintained at the desired temperature with an accuracy of ±1 °C.

In a typical experiment, the reaction mixture contained 0.01 mol methyl acetoacetate and 0.01 mol alcohol, diluted to 10 mL with toluene as a solvent. It was agitated at 50 °C for 15 min at a speed of 300 rpm and a known quantity of the enzyme was then added to initiate the reaction (typically 3% (w/w) of reactants). Samples were withdrawn periodically, filtered to remove fine particles, if any, and analyzed by GC.

3.2. Microwave reactor

The studies were carried out in a microwave reactor [Discover, CEM-SP1245 model]. The reactor was a 120 mL capacity fully baffled, 4.5 cm i.d. cylindrical glass vessel with provision for mechanical stirring. A standard four-blade-pitched turbine impeller of 1.5 cm diameter was used for agitation. However, the actual reactor volume exposed to the microwave irradiation was 45 mL with 5.5 cm height.

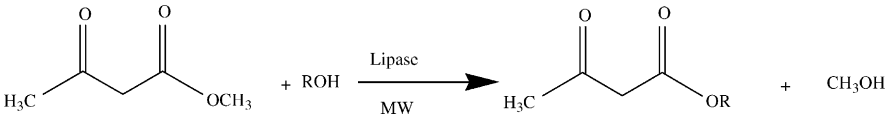
The quantities of reactant and catalyst and reaction procedure were identical to those used for the conventional heating.

3.3. Analysis

The concentration of the reactants and products were determined on Chemito Gas chromatograph (Model 8510) equipped with a flame ionization detector. A 3 m × 3.8 mm stainless steel column packed with OV-101 was used for analysis. Synthetic mixtures were prepared of pure samples and calibration was done to quantify the collected data for conversions and rates of reactions.

4. Results and discussion

The enzyme catalyzed transesterification of methyl acetoacetate with an alcohol ROH is depicted as follows.



4.1. Screening of catalysts

Control experiments were done to establish the efficacy of various enzymes in the absence of microwaves. Transesterification of methyl acetoacetate with *n*-butanol chosen as the model reaction and different supported lipases were evaluated. The conversion varied markedly with the type of lipase (Fig. 1). The conversions with Novozym 435 and Lipozyme RM IM were 72 and 45%, respectively. Lipozyme TL IM showed very little activity which was far less than those of Novozym 435 and Lipozyme RM IM. Lipozyme TL IM is mainly intended for interesterification of bulk fats and production of frying fats. Novozym 435 is a thermostable lipase and mainly useful for the synthesis of esters and amides. The

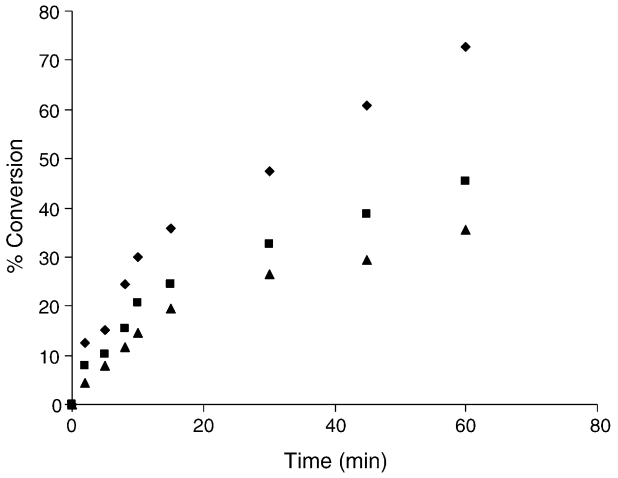


Fig. 1. Screening of catalysts for the transesterification of methyl acetoacetate with *n*-butanol. Reaction conditions: methyl acetoacetate, 0.01 mol; *n*-butanol, 0.01 mol; solvent toluene up to 10 mL: speed of agitation, 300 rpm; temperature, 50 °C; (♦) novozym 435; (■) lipozyme RM IM; (▲) lipozyme TL IM.

purpose of using these enzymes was to find out if significant activation could be achieved through microwave irradiation, despite their known use. Since Novozym 435 was found to be the best catalyst, it was used in all further studies.

4.2. Conventional heating versus microwave irradiation

Enzymatic transesterification were performed in the presence of controlled, microwave irradiation. It was found that the overall conversion as well as rate of reaction was higher under microwave irradiation vis-à-vis the conventional heating. This shows that the effect may not be purely thermal (Table 1). Control experiments in the absence of Novozym 435 did not show any conversion. Further, only microwave irradiation without the enzyme also did not initiate the reaction. Thus, there is a definite synergism between enzyme catalysis and microwave irradiation.

4.3. Effect of different alcohols

4.3.1. Linear alcohols

Transesterification of methyl acetoacetate with various *n*-alcohols, such as *n*-propanol, *n*-butanol, *n*-pentanol, *n*-hexanol, *n*-octanol and *n*-decanol was studied under otherwise similar conditions with Novozym 435. The effect of microwave irradiation was significant in enhancing the rates in all the alcohols in comparison with conventional heating. The rates in both types of excitation also decreased with increasing chain length of the linear alcohol (Table 1).

4.3.2. Secondary alcohols

A similar observation as regards the chain length was made in the case of secondary alcohols (Table 1). Thus, it can be concluded that the activity of enzyme depends on the type of excitation without any change in mechanism, which will be discussed later.

4.4. Effect of mole ratio

The effect of mole ratio of alcohol to methyl acetoacetate was studied by using *n*-butanol with Novozym 435. The reactions were conducted with and without microwave irradiation.

In one set of experiments, different moles of *n*-butanol were used in the range of 0.01–0.025 mol, whereas the amount of methyl acetoacetate were kept constant at 0.01 mol. When the mole ratio of *n*-butanol to methyl acetoacetate was increased from 1:1 to 2.5:1, it was found that the rate of reaction as well as the overall conversion increased from 47 to 65% under microwave irradiation. By comparison, in conventional heating, the rate as well as conversion increased from 36 to 48% (Fig. 2). In another set of experiments, the mole ratio was changed by keeping the moles of *n*-butanol constant at 0.01 mol, whereas the moles of methyl acetoacetate were varied from 0.01 to 0.025 mol. When the mole ratio of methyl acetoacetate to *n*-butanol was increased from 1:1 to 2.5:1, it was found that the rate as well as overall conversion increased from 47 to 69% under microwave irra-

Table 1
Transesterification of methyl acetoacetate with different alcohols with Novozym 435: synergism of microwave and enzyme catalysis

ROH	Time (min)	Catalyst loading (w/w)	Temperature (°C)	Conversion (%)	
				Microwave	Conventional
1-Propanol	60	3	50	74	57
1-Butanol	60	3	50	72	53
1-Pentanol	60	3	50	66	42
1-Hexanol	60	3	50	61	34
1-Octanol	60	3	50	55	17
1-Decanol	60	3	50	33	9
2-Propanol	60	3	50	68	48
2-Butanol	60	3	50	64	43
2-Pentanol	60	3	50	57	38

Experimental conditions: 0.01 mol methyl acetoacetate, 0.01 mol alcohol, solvent toluene to make 10 mL, speed 300 rpm.

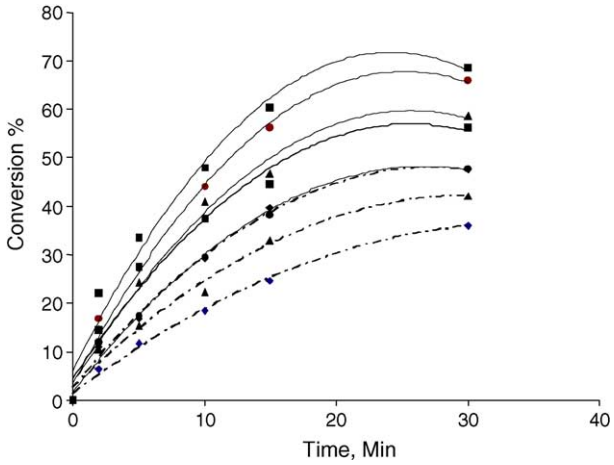


Fig. 2. Effect of mole ratio of *n*-butanol to methyl acetoacetate. Reaction conditions: methyl acetoacetate, 0.01 mol; solvent toluene up to 10 mL; speed of agitation, 300 rpm; temperature, 50 °C; (♦) 0.01; (▲) 0.015; (●) 0.02; (■) 0.025; (—) microwave irradiation; (- - -) conventional heating.

diation in comparison with an increase from 36 to 56% the absence of microwave irradiation (Fig. 3). These experiments demonstrated that there was no poisoning by the reactants at higher concentrations and that there was a synergism between the enzyme and microwave irradiation.

4.5. Reusability

The catalyst reusability studies were carried out to determine the stability of the enzyme under microwave irradiation. After each experiment, the enzyme was filtered and washed with toluene three to four times and dried in air at room temperature. There was a marginal decrease in activity after three reuses, which might be due to loss of enzyme during handling (Fig. 4). Thus, it can be concluded that the enzyme does not get deactivated or denatured due to microwave irradiation.

4.6. Kinetic model based on initial rates

The effect of concentrations of both the reactants on the rate of reaction was studied systematically over a wide range. For determination of the initial rates, two set of experiments were conducted by using a fixed loading of Novozym 435 (57 mg) with appropriate quantities of *n*-butanol and methyl acetoacetate and the total volume was made upto 10 mL with toluene. In one set of experiment, *n*-butanol amount was varied from 0.01 to 0.025 mol at a fixed quantity of methyl acetoacetate (0.01 mol) and in another set, the amount of methyl acetoacetate was varied from 0.01 to 0.025 mol at fixed quantity of *n*-butanol (0.01 mol). These experiments were repeated both in the presence and absence of microwave irradiation. The initial rates were determined from the quantified data.

These experiments indicated that that the initial rate (r_0) increased with increasing concentrations of methyl acetoacetate (A) and *n*-butanol (B) in both the cases of microwave and conventional heating. There was no evidence of inhibition by any of the substrates. The Lineweaver–Burk plot $1/r_0$ versus $1/[A_0]$ for both conventional as well as microwave irradiation are shown in Fig. 5. The Lineweaver–Burk double reciprocal plot is based on the rearrangement of the Michaelis–Menten equation into a linear form and the inverse of initial rate (r_0) is plotted against inverse of initial concentration of the reacting species.

In the case of lipase catalyzed reactions, it has been established that the lipase first forms an acyl–enzyme complex with the acyl donor, ruling out the random mechanism. The data obtained by microwave and conventional heating were fitted with two different mechanisms: ping–pong bi–bi and ternary complex mechanism.

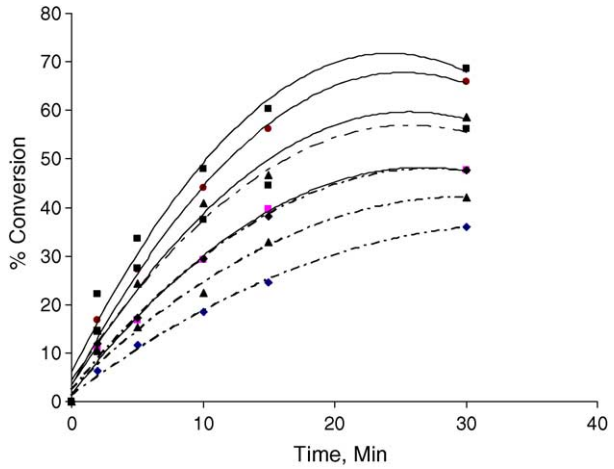


Fig. 3. Effect of mole ratio of methyl acetoacetate to *n*-butanol. Reaction conditions: *n*-butanol, 0.01 mol; solvent toluene up to 10 mL; speed of agitation, 300 rpm; temperature, 50 °C; (♦) 0.01; (▲) 0.015; (●) 0.02; (■) 0.025; (—) microwave irradiation; (- - -) conventional heating.

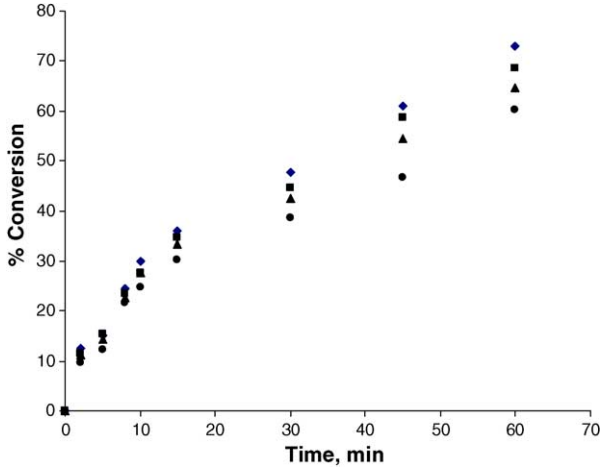


Fig. 4. Reusability of catalysts under microwave irradiation. Reaction conditions: methyl acetoacetate, 0.01 mol; *n*-butanol, 0.01 mol; solvent toluene up to 10 mL; speed of agitation, 300 rpm; temperature, 50 °C; (♦) fresh; (■) first reuse; (▲) second reuse; (●) third reuse.

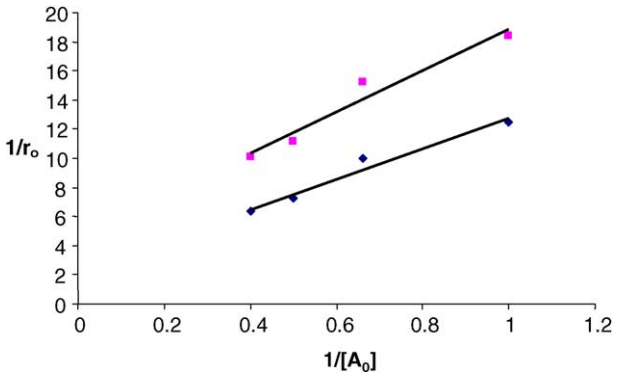


Fig. 5. Lineweaver–Burk double inversion plots for methyl acetoacetate concentration showing synergism between enzyme catalysis and microwave irradiation: (■) conventional; (♦) microwave irradiation.

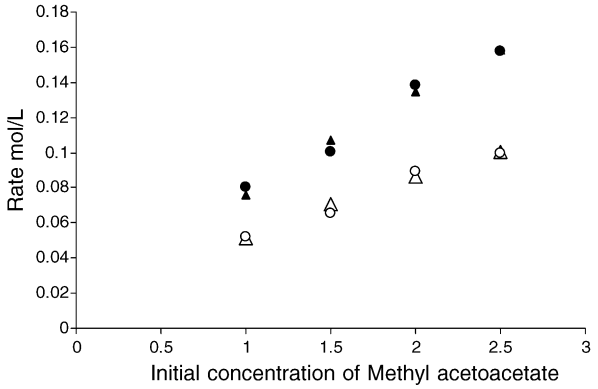


Fig. 6. Comparison of simulated vs. experimental initial rates as a function of initial concentration of methyl acetoacetate for conventional heating and microwave irradiation: (●) r_{exp} microwave irradiation; (▲) r_{sim} microwave irradiation; (◯) r_{exp} conventional; (△) r_{sim} conventional.

The rate equation for the ternary complex mechanism, for initial conditions is as follows:

$$r = \frac{r_{max}[A_0][B_0]}{K_{M-B}[A_0] + K_{M-A}[B_0] + [A_0][B_0] + K} \quad (1)$$

The rate equation for the Ping–Pong Bi–Bi mechanism, for initial conditions is as follows

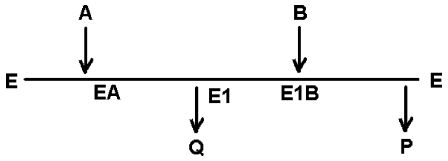
$$r = \frac{r_{max}[A_0][B_0]}{K_{M-B}[A_0] + K_{M-A}[B_0] + [A_0][B_0]} \quad (2)$$

where r = rate of reaction, r_{max} = maximum rate of reaction, $[A_0]$ = initial concentration of methyl acetoacetate, $[B_0]$ = initial concentration of *n*-butanol, K_{M-A} = Michaelis constant for methyl acetoacetate, K_{M-B} = Michaelis constant for *n*-butanol; $K = K_{M-A}K_{M-B}$.

The initial rates were calculated from the linear portion of the concentration–time profiles and the kinetic constants were obtained by non-linear regression analysis for the above models (Table 2). It was observed that the sum of the squared residuals was minimum in the case of the ping–pong bi–bi model.

According to the ping–pong bi–bi mechanism, methyl acetoacetate (*A*) first binds with the lipase (*E*) and gives the methyl acetoacetate–enzyme complex (EA). This intermediate then transfers to carboxylic-lipase (E1) and methanol (*Q*) is released. This is followed by the interaction of carboxylic–lipase (E1) with *n*-butanol (*B*) to form another binary complex (E1B), which then yields butyl acetoacetate (*P*) and free lipase (*E*)

The reaction mechanism is shown by the Cleland plot as follows:



The comparison of experimental rate versus simulated rate showed an excellent fit in the both the cases—that is—with microwave and without microwave irradiation. It proves the validity of the model (Fig. 6). Thus, based on the effects of various parameters on the transesterification reaction and the model-fitting, it is proved that there is a synergism between microwave irradiation and enzymatic catalysis whereby the enzyme gets better activated in the presence of microwaves vis-à-vis pure thermal energy. However, the mechanism does not change during microwave irradiation. The Michaelis constants are smaller in the presence of microwaves without any change in the form of the rate equation. Further, the rate enhancement is due to a combined effect of the microwave absorption properties of some liquid and solid materials, due to their polar and ionic characteristics, as suggested in, "dipolar polarization mechanism" [15], and also enzyme seems to behave slightly differently when heated with microwaves and it gets energized. The enzyme is in the immobilized form and there could be better accessibility of the enzyme to the reacting molecules. This altogether enhances the rate of chemical

Table 2
Model fitting for transesterification of methyl acetoacetate with *n*-butanol with Novozym 435

Kinetic parameters	Conventional heating		Microwave heating	
	Ternary complex model	Ping–pong bi–bi model	Ternary complex model	Ping–pong bi–bi model
r_{max} (mol L^{-1} min^{-1})	0.06506	0.5761	0.6967	0.6322
K_{M-A} (mol L^{-1})	4.985	9.11	3.71	7.20
K_{M-B} (mol L^{-1})	1.3681	1.096	0.21900	0.1061
Sum of squares	7.7328×10^{-5}	4.15×10^{-5}	8.452×10^{-5}	5.5134×10^{-5}

r_{max} = maximum rate of reaction, K_{M-A} = Michaelis constant for methyl acetoacetate, K_{M-B} = Michaelis constant for *n*-butanol, $K = K_{M-A}K_{M-B}$.

reactions significantly when compared to traditional energy application (conventional heating) techniques.

5. Conclusion

The reactions of methyl acetoacetate with different alcohols were studied under microwave as well as conventional heating. Transesterification of methyl acetoacetate with *n*-butanol was chosen as a model reaction. Novozym 435 was the best catalyst among others such as Lipozyme RM IM and Lipozyme TL IM. There is a synergism between enzyme catalysis and microwave irradiation. Linear and secondary alcohols were used to study the effect of chain length. It was found that as the chain length of the alcohol increases, the conversion increases in the presence of microwave as compared to conventional heating. Enhancement of initial activity was in the range of 2.2–4.6 for microwave irradiated reactions over conventional reactions. A kinetic model was proposed by collecting both initial rate data as well as concentration–time profiles for transesterification of methyl acetoacetate with *n*-butanol. A ping–pong bi–bi mechanism was found to fit the data well for enzyme catalysis alone and also for microwave irradiated enzyme catalysis.

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