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Synthesis Of Fatty Alkanolamides By Using Immobilized Lipases

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Abstract.: Enzyme catalyzed synthesis of fatty alkanolamides from different fatty acids and monoethanolamine was studied. The effect of using enzymes immobilised on to different matrices were investigated. Lipase was immobilised onto Amberlite XAD7, PMMA and Celite by adsorption. Maximum yield can be achieved within 96h. Generally, lipase immobilised to Amberlite XAD7 exhibited high activity, with slight preference for oleic acid. Amidation occurs only in selected non polar solvents.

Abstrak.: Sintesis alkanolamida..lemak dari berbagai asid lemak dan monoetanolamina dengan menggunakan lipase telah dikaji. Kesan penggunaan lipase yang diimobilisasikan kepada beberapa penyokong telah di selidiki. Lipase telah diimobilisasi kepada Ambelite XAD7, PMMA dan Celite dengan cara penjerapan. Hasil maksimum boleh didapati dalam masa 96 jam. Secara umumnya, lipase diimobilisasi kepada Amberlite XAD7 menunjukkan aktiviti yang tinggi, dengan sedikit pemilihan kepada asid oleik. Tindak balas pengamidan berlaku hanya dalam beberapa pelarut tidak polar.

Keywords: fatty alkanolamides, lipase, immobilised, synthesis

Introduction

Fatty alkanolamides are physically and chemically stable nitrogen containing compounds. It has a broad spectrum of uses due to their diversity of unique properties, economy and ease of preparation. Fatty alkanolamides can be used as anti-slip and anti-block additives for polyethlene films, water repellents for textiles, coating for paper, mold release agent, lubricant additives, printing ink additives, defoaming agents and flow improvers [1].

Industrially, fatty alkanolamides can be derived from N-acylation of alkanolamines with fatty acids (or methyl ester of fatty acid) at high temperature and pressure [2]. However, these methods give rise to a variety of undesirable side reactions and involve hazardous materials [3]. The applications of new biotechnology technique such as the use of lipase offer an improvement to the manufacture of fatty alkanolamides over these conventional methods. Lipase catalyzes reaction at mild temperature and atmospheric pressure. Therefore, the relatively pure product can be produced in a simple procedure with high efficiency.

Further improvement in the use of enzyme in chemical catalysis is the use of immobilised enzymes [4]. Immobilised enzymes offer several advantages in chemical processings. Among them, it offers recyclibility of the catalyst and simplify downstream processing. In particular, immobilised enzymes are specifically suited for use in reactions within low aqueous environment, a condition prevalent in synthetic reactions catalysed by lipases. Choice of suitable support matrices would allow the retention of the minimum water requirement for enzyme conformation and flexibility, while minimising any effect leading to the reverse reaction.

The present study described the enzyme catalyzed synthesis of fatty alkanolamides using monoethanolamine and different fatty acids. Lipases was immobilised to 3 different matrices and the activities of these derivitised enzymes were determined.

Experimental Procedures

Materials

Lipase was from *Candida rugosa* (Sigma, Type VII) The immobilisation matrices tested were Celite (Sigma), Amberlite XAD7 (Fluka) and PMMA (BDH). All fatty acids are from (Sigma, 95% purity), monoethanolamines (Fluka, 99% purity). All other reagents are of analytical grade.

Immobilization of lipase

Lipase (500 mg) was dissolved in 10 cm^3 distilled water. The suspension was centrifuged to remove any undissolved materials. The supernatant was mixed with 0.5 g of the specified matrix and shaken at 100 rpm for 1h at 30^{o}C. The mixture was filtered on Whatman No. 1 filter paper and the matrix was washed with 100 cm^3 distilled water, lyophilized and stored at 0^o prior to use.

Synthesis of alkanolamides
The reaction mixture consisted of a fatty acid (2.0 mmole), a monoethanolamine (2.0 mmole), enzyme and hexane (4 ml). The amount of enzyme used was 0.3 g for immobilized enzymes and 50.0 mg for the free form. The reaction mixture was incubated up to 120 h, 37^0C, 150 rpm in a horizontal shaker water bath. The reaction was terminated by diluting with 3.5 cm^3 ethanol-acetone mixture (1:1,v/v). The remaining fatty acid is titrated with 0.1M NaOH using an autotitrator (ABU 90, Radiometer, Copenhagen) to an end point of pH 12. All experiments
were done in triplicate and each experiment was repeated at least twice. The control experiments were carried out without enzymes.

Analysis of Products
Each of the reaction products of the synthesis was dissolved in 250 mL of hot hexane (about 45^0C). Enzyme was removed by filtration through Whatman No 1 filter paper. The mixture was then placed into 500 mL separation funnel and 25 mL of hot water (about 45^0C) was added, shaken and allowed to partition. The lower layer, which contained a mixture of aqueous solution and excess monoethanolamine, were separated from the upper hexane layer containing the alkanolamide. Addition of similar amount of hot water and separation of the lower layer was repeated at least three times. Alkanolamide was obtained from the hexane layer by crystallization at 4^0C. The crystals were collected by filtration using Whatman No 1 filter paper in a Buchner funnel. They were then washed with 25 mL hexane and dried in a vacuum desiccators until constant weight.

The products of the reactions were examined by thin layer chromatography (TLC) on precoated silica gels plate ($60F_{254}$, Merck, Darmstadt, Germany) and developed in chloroform/methanol (90:10, v/v).

Infrared (IR) analysis of the products was carried out using Fourier-transform Infrared spectrophotometer (Perkin-Elmer Corp., Model 1765, Norwalk, CT).

Determination of Percentage Yield of Products
The percentage yield (%) of alkanolamide synthesized in the optimization studies was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Mole of fatty acid used}}{\text{Initial mole of fatty acid}} \times 100\%$$

Effect of organic solvents
Effect of organic solvents was determined by replacing the hexane (in the synthesis of alkanolamides) with the selected solvents (Table 1).

Table 1: Selected solvents used and their log P values

Solvents	Log P
DMF	-1.00
Acetone	-0.23
Pyridine	0.71
Chloroform	2.00
Toluene	2.50
CCl_4	3.00
Hexane	3.50
Heptane	4.00
n-octane	4.50
Nonane	5.10
Decane	5.60
Undecane	6.10
Dodecane	6.60
Hexadecane	8.80

Results and Discussion

Time Course
The effect of reaction time on transamidation reaction by immobilised lipases on oleic acid and monoethanolmine substrates is shown in Figure 1. In the enzyme-catalyzed reaction, the reaction proceeds rapidly for 24 h, then slows down up to 72h. Maximum yield was obtained by 96h. Similar trends were observed when different fatty acids were used. However, there seems to be a preference for oleic acid as the substrate and the highest yield about (50%) is obtained with this substrate (Figure 2). It is known that *Candida rugosa* lipase has a preference for long chain fatty acids [5]. From Figure 2 it can be seen that oleic is the preferred substrate by all. The unsaturated oleic acid may have better solubility in the reaction system compared to palmitic and lauric. The substrate specificity does not seem to change with immobilisation.

Immobilisation lipases
Lipases immobilised to Amberllite XAD7 and Celite seems to exhibit the same level of activity. There is very little to compare as Amberlite is medium polar poly(acrylic) ester whereas celite in a relatively inorganic material. But Amberlite is apparently more porous than PMMA, also reported to be a medium polar poly (acrylic) ester. Our results showed that in term of protein adsorbed onto the matrices, Amberlite adsorbed most (65%) compared to PMMA (about 50%0 whereas celite adsorbed the least at about 46%. However the activity realised does not follow the amount of protein adsorbed. Furthermore activity may varies with the targeted reaction and its conditions. Previous reports on these matrices with other reaction systems also seemed to favour Amberllite XAD7 as the support matrix. [6].

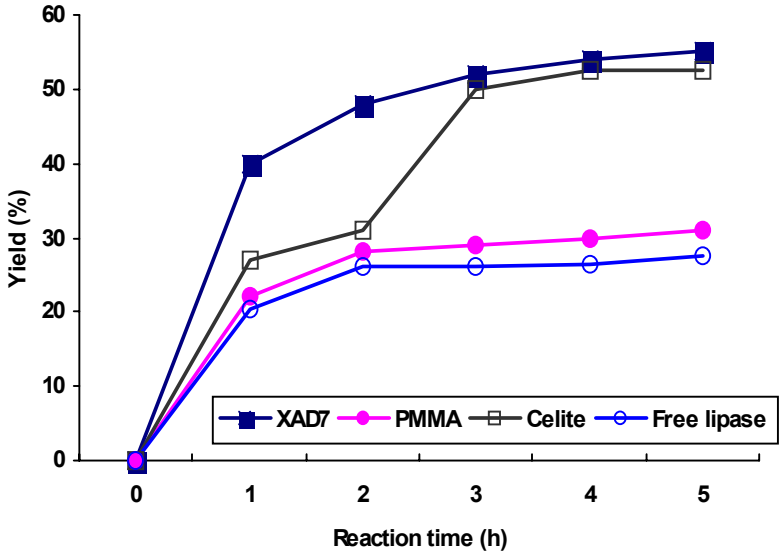

Figure 1: Progress curve for the enzymatic reaction of oleic acid and monoethanolamine

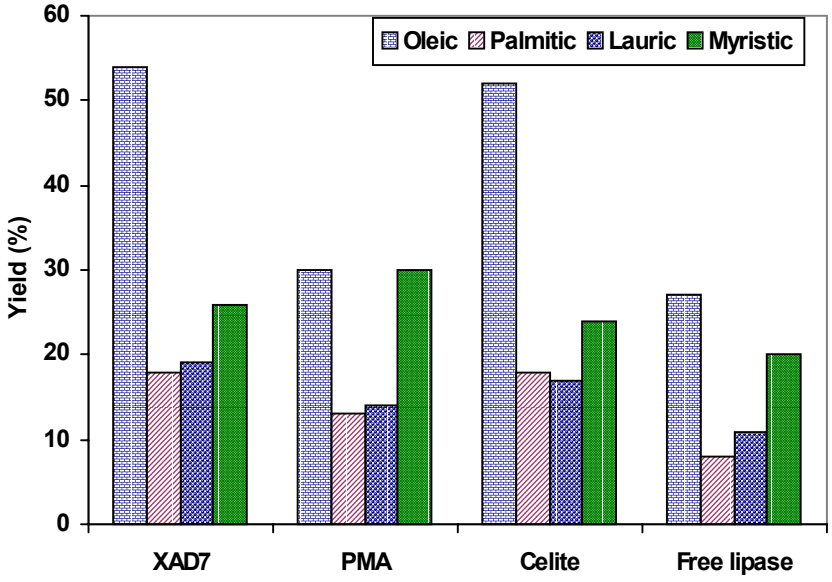

Figure 2: Yield of alknaolamides using different fatty acids and immobilized enzymes

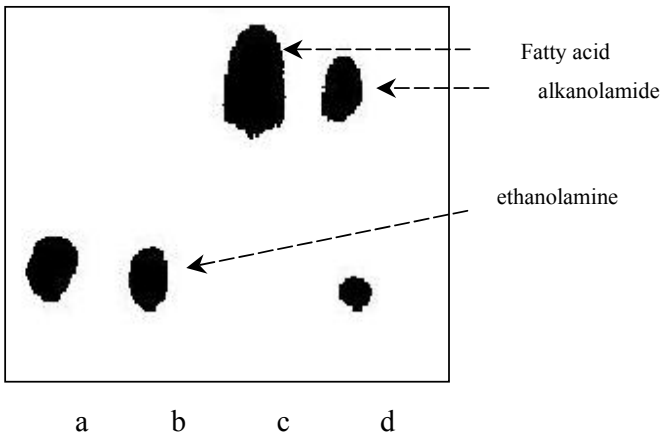

Figure 3: A Thin Layer Chromatogram showing the products formed by the enzymatic reaction using a fatty acid and a monoethanolamine as substrates

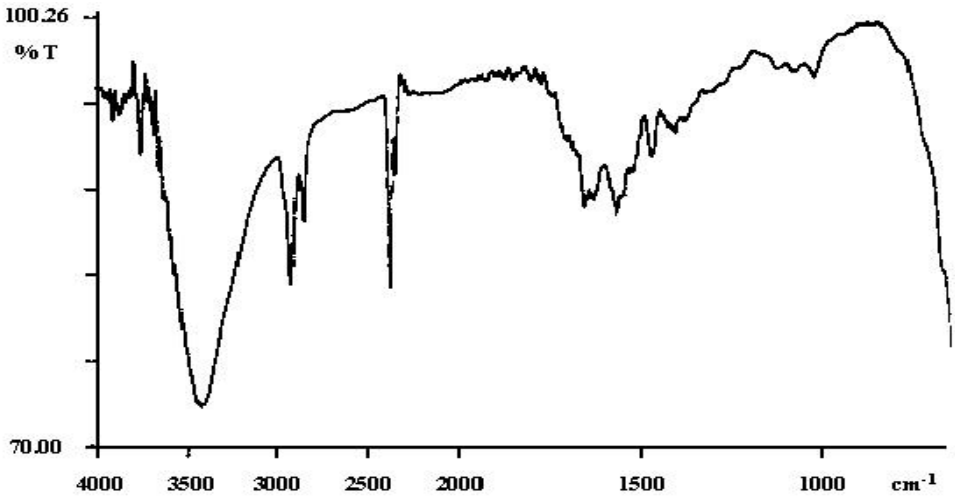

Figure 4: A typical IR spectrum for an alkanolamide (PKA) produced in the enzymatic reaction using a fatty acid and s monoethanolamine as substrates

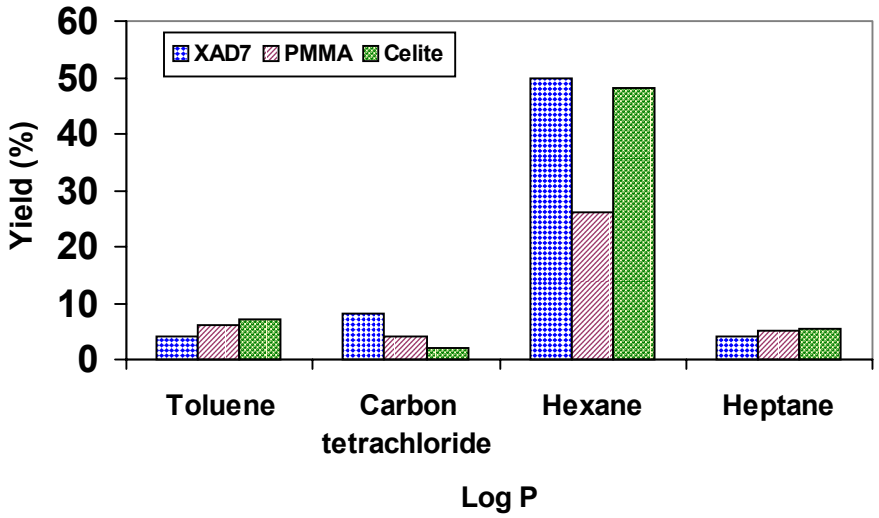

Figure 5: Effect of organic solvents on the amidation activity of different immobilised enzymes.

Analysis of product

Purity of amide formation was ascertained by TLC. The TLC result is presented in Figure 3. The product is shown in column d. All the alkaolamides moved within the same range of R_T values.

IR spectrum of the product is shown in Figure 4. All spectra exhibited the amide adsorption at 1638 cm^{-1} and 1548 cm^{-1} [7]. The N-H stretch at 3299 cm^{-1} could be observed clearly probably due to low concentration of sample.

Effect of organic solvents

Use of immobilized enzymes with in organic solvents environment is considered desirable, as the support matrices may retain the minimal water required for enzyme function. The results indicated that only toluene, carbon tetrachloride (CCl_4), hexane and heptane can be used (Figure 5). Hexane with a log P value of 3.5 seems to be the best solvent. Previous reports indicated that lipase reaction required high log P solvents [8]. However, solvents with log P higher than 4 seems to have solubility problem in the reaction mixture. As expected no activity was observed with solvent of low log P values. A certain amount of water is necessary for enzyme conformation and to maintain flexibility during catalysis. Solvent with low log P values (polar) may strip the water of the enzyme molecules, thereby rendering them inactive [9]. There might be some interaction between the immobilization matrices and different organic solvents, as the yield pattern is dissimilar for different solvents that exhibited activity. However, no conclusion can be made at this juncture as to the relationship between support matrices, solvents and enzyme activity.

Acknowledgment

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