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Biocatalytic Synthesis of Diethanolamide Surfactants Under Mild Reaction Conditions

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Synthesis of fatty acid diethanolamide was carried out by the condensation of diethanolamine with lauric acid, decanoic acid and octanoic acid in the presence of Novozym 435. The influence of solvent, time, temperature and agitation on the reaction rate was studied. Formation of lauroyl diethanolamide was complete in 6h in acetonitrile at 50° C whereas in toluene the reaction was not complete in 6h. Increase of reaction temperature from 50° C to 70° C increased the reaction rate. Proper agitation of the reaction mixture enhanced the reaction rate. Reaction in solution appeared to be more efficient than the reaction in solvent free system. The surface active property of the fatty acid amides was studied by determining their critical micelles concentration. The critical micelle concentration of lauroyl diethanolamide, decanoyl diethanolamide and octanoyl diethanolamide were found to be 0.63mM, 1.10mM, 1.45mM respectively in deionized water. Micelles of lauroyl diethanolamide were less than 1µm in size.

Key Words: Diethanolamide, Novozym 435, Biocatalysis

INTRODUCTION

Fatty acid amides are the surfactants generally derived from the condensation of fatty acids and amines. They are considered as nonionic surfactants of considerable interest and economical importance. The surface active properties of fatty acid amides makes them an essential ingredient of several formulations such as lubricants, dispersants, detergents, shampoos, antistatic agents, antimicrobial agents, dye corrosion inhibitors, antifoaming agents, and pulping aids (Schmitt 2001; Gunstone 1996; Visek 1990). Fatty acid amides are favored in these applications because of their emollient and lubricating properties, ability to stabilize emulsions, and low reactivity (Johansson 2003). Various approaches for the synthesis of fatty acid amide synthesis have been cited in literature. One route involves the reaction of fatty acid and ammonia under pressure at elevated temperature (Roe et al. 1952). There is also a report of producing fatty acid amide by reacting fatty acid with amino alcohols, catalyzed by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline in ethanol reflux (Baek 2006). Another industrial synthesis procedure involves reaction of excess diethanol amine with long chain monocarboxylic acid ester in the presence of sodium methoxide under a slight vacuum (US patent 1994). Apart from utilization of toxic chemical catalysts, these techniques also suffer from the drawback that the product contains several impurities. As an example, in the process of synthesis of diethanolamide from diethanolamine contaminants in the form of N,N'-bis(2-hydroxyethyl) piperazine formed by self-condensation of diethanolamine at elevated temperatures, other impurities include monoesteramines,

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diesteramines, monoesteramides and diesteramides (O'Connell 1977). Moreover, these high temperature reactions cannot be considered to be highly energy efficient.

To overcome these drawbacks there is a need for the development of an innovative technique to produce fatty acid amides wherein these problems are addressed and eliminated. Replacement of traditional chemical catalyst by biocatalysts can be looked upon as a solution to such problems. The feature of enzyme catalysis is its superior catalytic power and high selectivity under mild reaction conditions. Also, the use of environmental friendly catalyst is conducive for the pharmaceutical or cosmetic applications (Gross et al. 2001).

Some of the recent studies have focused on the use of enzymes to formulate an energy-efficient and mild pathway to produce fatty acid amides. Fernández-Pérez and co-workers. performed selective synthesis of diethanolamide using Novozym 435 in dioxane and hexane (Fernández-Pérez and Otero 2003). Lipases have successfully catalyzed the synthesis of oleamide from fatty acid and ammonium salts in organic solvent (Litjens et al. 1999). Lipases have also been used to catalyze the reaction of hydroxyl fatty acids and ammonia to produce novel fatty acid amides at low temperatures (Levison et al. 2005). Solvent-free synthesis of lauroyl monoethanolamide using lipase has been documented (Infvesson et al. 2006). The enzymes are capable of catalyzing the reaction of methyl laurate and N-methylethanol amine to produce N-methyl lauroylethanolamide under mild conditions (Sharma et al. 2005). Liu et al. demonstrated the feasibility of enzymatic amidation of diethanolamine using various lipases sources under several reaction conditions (Liu et al. 2001).

Though various substrates and enzymes have been explored for the biocatalytic synthesis of fatty acid amides, in particular diethanolamides, the study of their surface active behavior has not yet been established. Also, the effect of various parameters such as the rate of agitation, feasibility of the reaction in solvent free medium has not been studied systematically.

The objective of our work was to investigate the amidation of diethanolamine by long chain fatty acids catalyzed by Novozym 435, under various reaction conditions. It also aimed at studying the surface active property of synthesized diethanolamides by measuring the critical micelle concentration (CMC) values and determining the size of the micelles.

MATERIALS AND METHODS

Chemicals

Diethanolamine (DEA), lauric acid (LA), octanoic acid (OA), decanoic acid (DA) and chloroform were purchased from Merck Chemical Co. (Darmstadt, Germany). Acetonitrile and toluene were from J.T. Baker (Phillipsburg, USA), ninhydrin from Carlo Erba (Milan, Italy) and deuterated chloroform from Cambridge Isotope Labs. Inc. (Andover, USA). Novozym 435 was received from Novozymes (Novozyme, Bagsvaerd, Denmark) as a gift. Novozym 435 beads were dried over P_2O_5 in vacuum (16 h, 25° C) to remove the moisture, from the catalyst. Activity of the dry enzyme was determined by following the procedure reported by Petkar et al (Petkar et al. 2006). The activity was found to be 65.7 μmoles/min/g enzyme. The solvents were used as received.

^{1}H NMR Spectroscopy

^{1}H NMR spectra were recorded at 25 °C on a JEOL Lambda 500 MHz spectrometer. The proton (1H) NMR chemical shifts in parts per million (ppm) were referenced relative to tetramethylsilane (TMS). To perform the 1H NMR experiments, 8.0 wt % of samples were dissolved in deuterated chloroform. The rate of disappearance of the fatty acids was calculated based on the relative intensity of peaks from reacted and unreacted fatty acids.

Determination of Critical Micelle Concentration (CMC)

CMC of the products was determined by conductivity measurement. In order to determine CMC, purified product was dissolved in deionized water in a 25 mL volumetric flask. Duplicate measurements of the conductance of the solution were obtained using Oakton portable conductivity wand. Serial dilution was performed until a breakpoint in the graph of conductance vs. concentration was observed.

Microscopy

One drop of approximately 0.01 M aqueous dispersion of the product was viewed under an Olympus Tokyo 239788 microscope with an A4Tech Web Camera mounted on top of the body tube. The total magnification was x1000. Micelles were observed as randomly moving spheres on the surface of the droplet.

Synthesis of lauroyl diethanolamide in the presence of Novozym 435

In a typical synthesis, 0.01 g of Novozym 435 was added to test tube containing a mixture of 1:4 ratio (mol/mol) of LA (0.04 g, 180 mmol) and DEA (0.07 g, 720 mmol)

in 0.2 mL solvent. The test tube was covered by a rubber septum. The reaction mixture was purged with nitrogen gas for 2 minute to remove atmospheric oxygen, and then incubated in a thermostat bath with constant magnetic stirring at desired temperature. After a certain period of time, aliquot was drawn out of the reaction vessel for analysis. Reaction was finally quenched after certain period of time, Novozym 435 was removed by gravity filtration and the excess solvent was removed under reduced pressure by using a rotary evaporator. Reaction solvent, time and temperature were varied according to the . Effect of chain length of the fatty acids was studied by replacing LA with OA and DA for the synthesis.

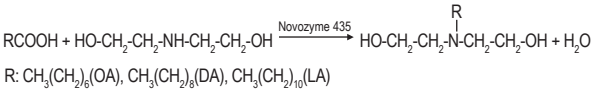
To understand the influence of agitation on the progress of reaction lauroyl diethanolamide was synthesized by replacing the mechanical stirrer with a Julabo SW23 shaking water bath at 140 rpm.

Lauroyl diethanolamide was also synthesized using the chemical catalyst sodium methoxide. LA was refluxed with DEA for 6h at 100-110° C in the presence of freshly prepared sodium methoxide. Control reactions were performed as above but in the absence of Novozym 435 at 70° C for 6h in acetonitrile and toluene. All these reaction conditions are tabulated in Table 1.

The products were purified by column chromatography. A 30 cm column packed with silica gel of size less than 0.08 mm as the stationary phase. The mobile phase was chloroform and methanol in a 10:1 ratio (v/v). The product was detected by the development of purple spot on the TLC plate in the presence of ninhydrin.

RESULTS AND DISCUSSION

DEA was allowed to react with LA, OA and DA in the presence of Novozym 435 to form diethanolamide.



Scheme 1: Synthesis of diethanolamide from fatty acid and diethanol amine using Novozym 435

The extent of product formation was monitored by the appearance of the peaks in the 1H NMR spectroscopy. The 1H NMR spectrum of II is as follows (Figure 1): 1H NMR in CDCl3 (δ in ppm): 1.54 (LA-H, t, 2H), 2.15 (LA-H, t, 2H), 2.90 (DEA-H, t, 2H), 3.75 (DEA-H, t, 2H).

The rate of disappearance of the fatty acid was calculated based on the relative intensities of the signals at 1.54ppm (reacted fatty acid) and 1.65 ppm (residual fatty acid). The amount of residual fatty acid for all the reactions has been summarized in Table 1. Completion of reaction is indicated by the complete disappearance of the fatty acid peak at 1.65 ppm.

The ^{1}H NMR spectrum of the control reactions(XI, XII) performed revealed that the reaction did not proceed indicating that the reaction of LA with DEA is not feasible in nature and the presence of catalyst is essential to trigger the reaction.

It was observed that the reaction temperature influences the rate of the reaction. Increased reaction temperature

Table 1. Effects of various conditions on reaction of fatty acids with diethanolamine

Fatty Acid	Solvent	Temperature (°C)	Reaction Time (h)	Sample ID	Residual Fatty Acid (%)
LA	Acetonitrile	50	2	I	10
LA	Acetonitrile	50	6	II	0
LA	Acetonitrile	70	2	III	0
LA	Toluene	50	6	IV	12
LA	Toluene	70	2	V	0
LA	-	50	6	VI	17
DA	Acetonitrile	50	2	VII	2
OA	Acetonitrile	50	2	VIII	0
LA	Acetonitrile	50	6	IX[a]	36
LA	-	100-110	6	X[b]	21
LA	Acetonitrile	70	6	XI[c]	100
LA	Toluene	70	6	XII[c]	100

a: reaction mixture was agitated in the shaking water bath, for other reactions mechanical sturrer was used for agitation;
b: reaction was catalyzed by sodium methoxide, rest of the reactions were catalyzed by Novozym 435;
c: control reactions

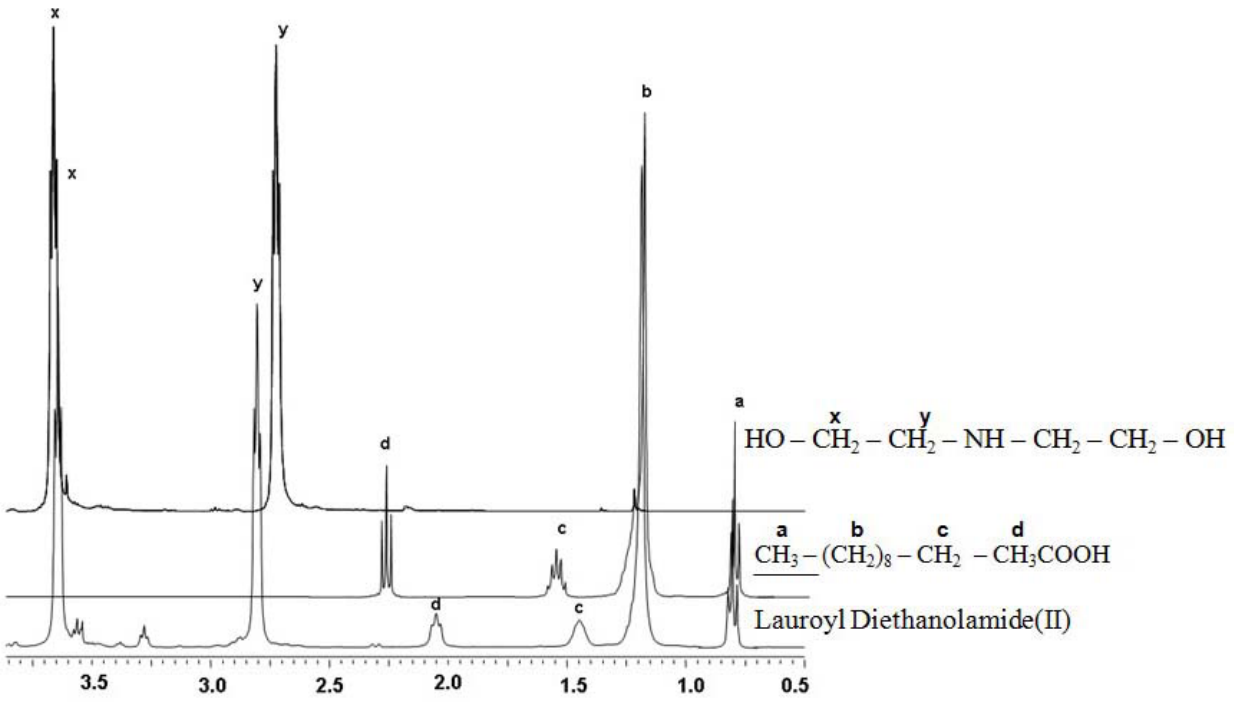


Figure 1. ^{1}H NMR spectrum of diethanolamide (DEA0, lauric acid (LA) and lauroyl diethanolamide(II)

from 50° C to 70° C enhanced the reaction rate in acetonitrile and toluene.

To investigate the effect of solvent, acetonitrile was replaced by toluene. As seen in Table 1, the progress of the reaction rate slowed down in toluene. Hence acetonitrile can be considered as most suitable solvent medium for this type of amidation. However, the presence of residual LA was not detected in the ^{1}H NMR spectrum of (V), similar to that observed for (III) indicating that at higher temperature the impact of solvent polarity is minimized.

Since solvent free reactions are in general preferred over solution synthesis to avoid contamination from the solvent and the tedious solvent removal procedure, attempt was made to explore the efficacy of the amidation in bulk. As shown in Table 1 reaction rate was slower in solvent free system(VI) as compared to that in the solution(II). For the reaction to take place in the presence of the Novozym 435 beads, it is required that the substrate should diffuse into the porous Novozym 435 beads to come in contact with the catalytic enzyme residing in the outer core of the beads (Mei et al. 2003), the lack of solvent might have hindered the diffusion of the substrates into the porous beads and thus slowed down the rate of fusion of LA with DEA.

Effect of agitation on the rate of reaction gets reflected by comparing the amount of residual LA for the reaction performed using the mechanical stirrer(II) and the shaker(IX). In the shaker bath reaction could not go to completion in 6h due to the poor contact between the substrate and the catalytic enzymes.

The point to be noted here is that the trend of our observations for the impact of temperature, time and solvent agreed with the observations made by Liu et al. (Liu et al. 2001), however it took them much longer time to perform the reactions as their reactions were performed using the shaker.

Influence of the chain length of fatty acid on synthesis was investigated by replacing LA by DA and OA acid for the formation of decanoyl diethanolamide(VII) and octanoyl diethanolamide(VIII). Table 1 shows that condensation of fatty acids of shorter chains with diethanolamine is more. Decreasing the chain length of the substrate might have facilitated their diffusion into the porous beads of Novozym 435 to come in contact with the catalytic enzyme.

Synthesis of lauroyl diethanolamide was also performed using the chemical catalyst. The ^{1}H NMR spectrum of the product(X) showed the presence of 21% residual LA after it was refluxed with DEA for 6h in the presence of sodium methoxide at 100-110° C, whereas similar reaction went to completion in 6h when it was performed in the presence of Novozym 435 at 50° C. This observation reinstates the fact that biocatalytic synthesis

of diethanolamides takes place under milder conditions as compared to the chemical synthesis.

In order to get insight into the ability of the obtained diethanolamides to form micelles, CMC of (I), (VII) and (VIII) was determined in aqueous medium. The CMC of the purified products was determined from the slope break in a conductivity concentration diagram. For the calculation of CMC value, tangents were drawn on the two portions of the plots. Point of intersection of these tangents gave the CMC. For each product a drop in the molar conductivity was observed at a particular concentration, the drop was relatively sharp for (I), however the sharpness of transition diminished with the decrease in the length of the fatty acid chain. The CMC values as determined from the point of intersection of the tangents were 0.63 mM, 1.10mM and 1.45mM for (I), (VII) and (VIII) respectively (Figure 2). Low CMC value for (I) can be attributed to the fact that the nonpolar long chain fatty acid diethanolamide tends to minimize the interaction with the polar aqueous environment by undergoing aggregation to form micelles. The tendency of minimum interaction with the aqueous medium by forming micelles decreases with the reduction in the length of the fatty acid, thus they form micelles at higher concentration.

The size of the micelles formed by (I) was determined by viewing them under a microscope. As seen in Figure 3, the spherical micelles, formed in deionized water were lower than 1µm in size. The size distribution of the micelles appeared to be almost uniform.

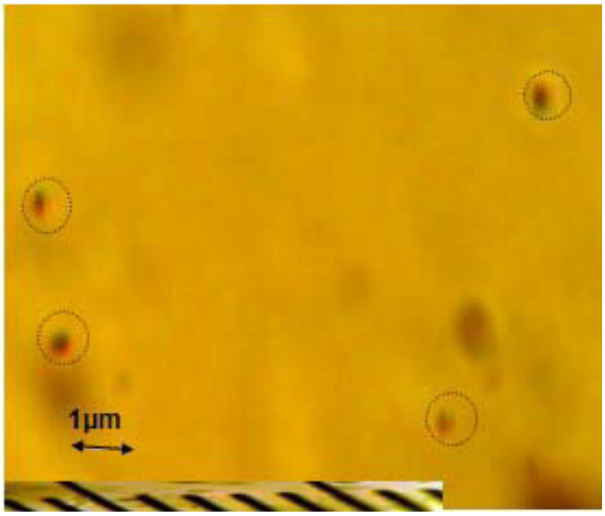


Figure 3. Micelles of lauroyl diethanolamide(I) in deionized water as viewed under an optical microscope

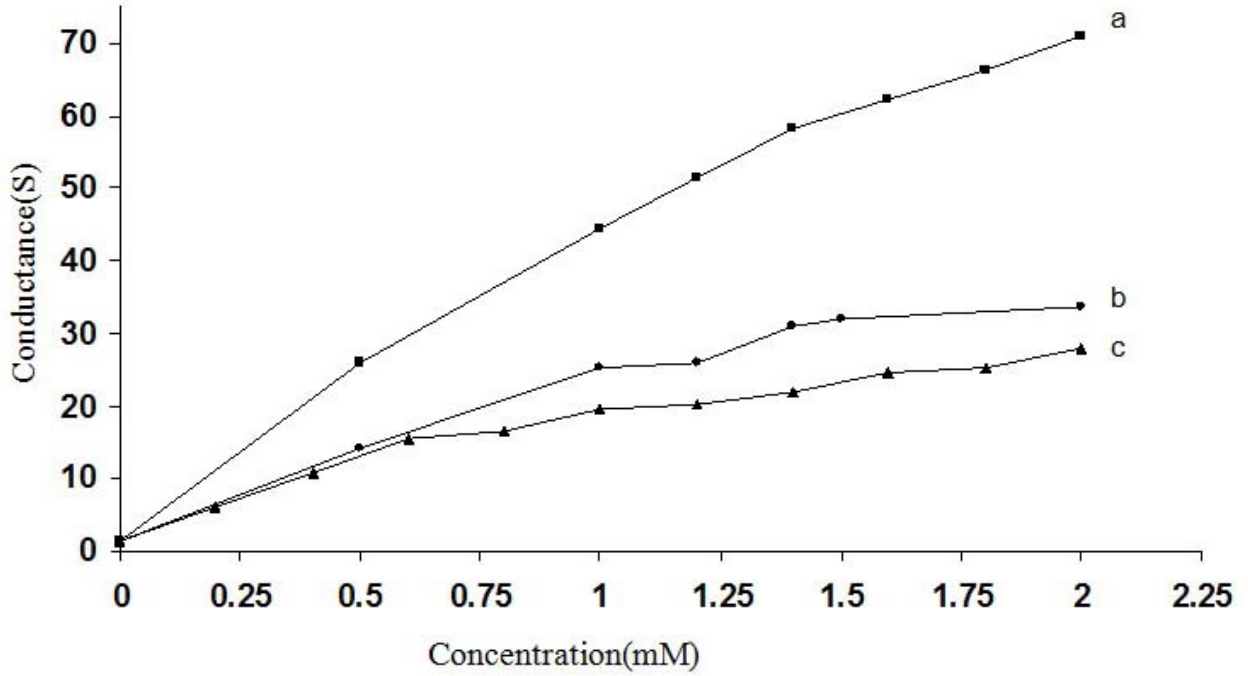


Figure 2. Conductance vs. concentration of fatty acid amides; a:octanoyl diethanolamide(VIII), b: decanoyl diethanolamide(VII), c: lauroyl diethanolamide(I)

CONCLUSION

This study reveals that the Novozym 435 is efficient towards amidation when employed as the biocatalyst for the condensation of DEA and LA under mild conditions. Diethanolamides of lauric acid, decanoic acid and octanoic acid behave as surfactants and form small size micelles. These micelles can be looked upon as the scaffold for the delivery of pharmaceutical agents, cosmetics, and other attributes. Since coconut is one of the major cash crops in Philippines, the country can harness its benefit. Fatty acids extracted from coconut oil can be converted to benign surfactant and can be tapped for the above applications.

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