



UNIVERSITI PUTRA MALAYSIA

**SYNTHESIS OF ADIPATE ESTERS USING *CANDIDA RUGOSA* LIPASE
IMMOBILIZED ONTO LAYERED DOUBLE HYDROXIDES**

USWATUN HASANAH Z Aidan

FS 2007 20



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**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirement for the Degree of Master of
Science**

January 2007



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
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PhD**

Faculty : Science

The increasing demand for specially formulated adipate esters make it necessary to develop highly specific catalysts. For this reason, the application of immobilized enzyme as industrial biocatalyst for the synthesis of adipate ester via environmental benign process is undergoing a rapid development. Because of the unique and reproducible properties, layered double hydroxides (LDHs) were used as supports for enzyme immobilization.

In this study, layered double hydroxides (LDHs) of Mg/Al-NO_3^- , Zn/Al-NO_3^- and Ni/Al-NO_3^- of molar ratio of $\text{M}^{2+}/\text{M}^{3+}$ (4:1) was prepared by co-precipitation method through continuous agitation. Their structures and characteristics were determined using X-ray Diffractometer (XRD),



Accelerated of Surface Area and Porosimeter (ASAP), Fourier Transform Infra-red (FTIR) and Scanning Electron Microscopy (SEM). From the analysis results, the supports were found to be successfully synthesized.

Lipase from *Candida rugosa* was immobilized onto these newly synthesized supports by a simple and inexpensive method of physical adsorption. Immobilization of lipase onto these support was confirmed using Energy Dispersion X-ray (EDX). EDX spectra of immobilized LDHs-lipase proved that immobilization process occurred based on the presence of both the metal compounds of the supports and protein components. The percentages of protein loading on LDHs were from 58 - 71%, due to the larger surface area ($24.0 - 52.6 \text{ m}^2\text{g}^{-1}$), porosity ($8.7 \times 10^{-3} - 22.8 \times 10^{-3} \text{ cm}^3\text{g}^{-1}$) and basal spacing ($8.2 - 9.3 \text{ \AA}$) of the supports.

The synthesis of dimethyl adipate (DMA) and dibutyl adipate (DBA) esters catalyzed by immobilized lipases were carried out via esterification of adipic acid and methanol as well as butanol as substrates and hexane as reaction medium. The effects of various reaction parameters such as reaction time, temperature, organic solvent, water activity and mole ratio of substrates were studied to determine optimal conditions for the production of adipate esters. The optimal conditions for both DMA and DBA esters syntheses using immobilized lipases were obtained at reaction time; 2.5 hours, temperature; $50 \text{ }^\circ\text{C}$ and molar ratio of substrates; 2. High yields (>65%) of



the products were obtained in hexane ($\log P = 3.5$) as the reaction medium and optimal percent conversion (up to 85%) was found to be dependant on the water activity (A_w) of 0.53 for DMA and 0.75 for DBA.

The stability studies on the effects of thermal stability, leaching study, stability in organic solvent, storage study and reusability were investigated for their influence on the enzymatic esterification for production of adipates. The immobilized lipases retained high catalytic activity and showed increased stability compared to the native lipase in all cases. In thermal stability study, immobilized lipases were less affected and showed high activity even upon incubation at 70°C. When stored at different temperatures for 60 days, all immobilized lipases performed much better in activity.

Furthermore, the commercial immobilized lipases and the LDHs-lipases showed an efficient reuse which makes this system attractive and applicable to various reactions.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SINTESIS ESTER ADIPATE MENGGUNAKAN LIPASE *CANDIDA RUGOSA* TERSEKATGERAK KE ATAS LAPISAN GANDA DUA HIDROKSIDA

Oleh

USWATUN HASANAH ZAIDAN

Pengerusi : Profesor Madya Mohd. Basyaruddin Abd. Rahman, PhD

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Permintaan yang tinggi terhadap rumusan istimewa ester adipat membuatkan ianya penting untuk memajukan katalis berspesifik tinggi. Oleh kerana ini, penggunaan enzim tersekatgerak sebagai biokatalis industri untuk sintesis ester adipat melalui proses persekitaran yang selamat sedang menerajui pembangunan yang pesat. Berdasarkan ciri-ciri unik dan reproduktif, lapisan ganda dua hidroksida (LDHs) telah digunakan sebagai penyokong untuk proses sekatgerak enzim.

Dalam kajian ini, lapisan ganda dua hidroksida (LDHs) dengan $Mg/Al-NO_3^-$, $Zn/Al-NO_3^-$ dan $Ni/Al-NO_3^-$ pada nisbah M^{2+}/M^{3+} (4:1) telah disediakan melalui kaedah ko-pemendakan bersama melalui penggoncangan berterusan. Struktur dan ciri-ciri bahan tersebut telah ditentukan



menggunakan Pembelau Sinar-X (XRD), Penguji Keporosan dan Luas Permukaan (ASAP), Spektroskopi Infra-merah (FTIR) dan Mikroskopi Pengimbas Elektron (SEM). Berdasarkan keputusan analisis, penyokong-penyokong ini didapati telah berjaya disintesis. Lipase dari *Candida rugosa* disekatgerakkan kepada bahan penyokong ini melalui kaedah penjerapan fizikal yang mudah dan murah. Proses sekatgerak enzim kepada penyokong ini telah dipastikan menggunakan X-Ray Penyebaran Tenaga (EDX). Spektrum EDX bagi LDHs-lipase tersekatgerak telah membuktikan bahawa proses sekatgerak telah berlaku berdasarkan kepada kehadiran kedua-dua komponen logam penyokong dan komponen protein. Peratus penjerapan protein bagi LDHs adalah dari 58 - 71% berdasarkan kepada luas permukaan ($24.0 - 52.6 \text{ m}^2\text{g}^{-1}$), keporosan ($8.7 \times 10^{-3} - 22.8 \times 10^{-3} \text{ cm}^3\text{g}^{-1}$) dan ruang lapisan ($8.2 - 9.3 \text{ \AA}$) penyokong tersebut.

Sintesis ester dimetil adipat (DMA) dan dibutil adipat (DBA) bermungkinan lipase tersekatgerak ini telah dijalankan melalui tindakbalas esterifikasi menggunakan asid adipik dan metanol serta butanol sebagai substrat dan heksana sebagai medium tindakbalas. Kesan-kesan pelbagai parameter tindakbalas seperti masa tindakbalas, suhu, pelarut organik, aktiviti air dan nisbah kepekatan substrat telah dikaji untuk menentukan keadaan-keadaan optima bagi penghasilan ester adipat. Keadaan-keadaan optimum untuk sintesis ester DMA dan DBA menggunakan lipase tersekatgerak telah didapati pada masa tindakbalas; 2.5 jam, suhu; 50°C dan nisbah kepekatan

substrat; 2. Hasil produk yang tinggi (>65%) telah didapati dalam heksana (log P=3.5) sebagai medium tindakbalas dan peratusan pertukaran optimum (mencapai 85%) telah didapati bergantung kepada aktiviti air (A_w) pada 0.53 bagi DMA dan 0.75 bagi DBA.

Kajian-kajian kestabilan terhadap kestabilan terma, kajian basuhan, kestabilan dalam pelarut organik, keadaan penyimpanan dan kebolehan lipase untuk dikitar semula telah diselidik bagi menentukan pengaruhnya terhadap tindakbalas esterifikasi berenzim pembentukan adipat. Lipase tersekatgerak telah mengekalkan aktiviti pemangkinan yang tinggi dan menunjukkan peningkatan kestabilan berbanding lipase asli dalam semua kes. Dalam kajian kestabilan terma, lipase tersekatgerak kurang dipengaruhi dan mempamerkan aktiviti yang tinggi walaupun setelah pengeraman pada 70°C. Apabila disimpan pada suhu berlainan selama 60 hari, semua lipase tersekatgerak mempamerkan aktiviti jauh lebih baik.

Tambahan pula, lipase tersekatgerak komersial dan lipase tersekatgerak pada LDH telah menunjukkan kebolehan guna semula yang berkesan seterusnya menjadikan sistem ini menarik dan mampu diaplikasikan dalam pelbagai tindakbalas.

ACKNOWLEDGEMENTS

Alhamdulillah, praises to Allah s.w.t. for giving me the strength to endure all problems and complete this study.

I wish to express my sincere appreciation and gratitude to Assoc. Prof. Dr. Mohd. Basyaruddin Abd. Rahman, Prof. Dr. Mahiran Basri, Prof. Dr. Abu Bakar Salleh, Prof. Dr. Mohd. Zobir Hussein and Assoc. Prof. Dr. Raja Noor Zaliha Abd. Rahman of the Enzyme and Microbial Technology Research group for their great concern, advices, patience, persistence encouragement and invaluable assistance from the beginning till the end of this study.

A special thanks to the staff members of the Microscopy and Microanalysis Unit, Institute of Bioscience and Department of Chemistry, who were so helpful and cooperation in many ways during the course of the study.

Love and thanks to my parents, Umi and Abah, for their never-ending moral and constant support during my education. Not forgetting, my special thanks to my beloved husband, Mohd. Nasir Othman for his encouragement and patience, and our son, Muhammad Auza'ie, for simply being there and loving me with all heart.



Finally, I wish to thank to Siti Salhah, Salina, Rozainita, Norhayati, Nor Mona, Nora, Shie Ling, Rafidah and other members of Lab 401, for the bond of friendship and for making my stay in UPM a bearable one with many sweet memories and experiences. Thank you for being friends in need.



APPROVAL

I certify that an Examination Committee has met on **date of viva** to conduct the final examination of name of **name of student** on his degree thesis entitled “**title of thesis**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

USWATUN HASANAH Z Aidan

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi
LIST OF FIGURES	xv
LIST OF TABLES	xviii
LIST OF ABBREVIATIONS	xix
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	
Enzymes as Catalysts	6
Mechanism of Enzyme Action	8
Lock and Key Model	9
Induced Fit Theory	10
Lipases	13
Sources of Lipases	15
Lipase-Catalyzed Reactions	17
Immobilization of Enzymes	20
Advantages of Immobilized Enzymes	23
Applications of Immobilized Enzymes	25
Novozyme 435 and Lipozyme IM	26
Supports for Enzymes Immobilization	28
Layered Double Hydroxides	29
Adipic acid	35
Dimethyl adipate (DMA)	38
Dibutyl adipate (DBA)	40
Enzymatic Esterification of DMA and DBA	43
III MATERIALS AND METHODOLOGY	
Materials	
Sources of Enzymes and Protein	44
Chemicals for Synthesis of LDHs	44
Substrates	45



Chemicals for Protein Assay	45
Solvents	45
Hydrated Salts for Water Activity	46
Instrumentations	46
Methodology	
Water Extraction of Lipase	47
Synthesis of LDHs	47
Characterization of LDHs	
Fourier Transform Infra-red (FTIR)	48
X-ray Diffractometer (XRD)	48
Surface Area and Porosimeter (ASAP)	48
Scanning Electron Microscopy (SEM)	49
Energy Dispersive X-ray (EDX)	49
Immobilization of Lipase onto LDHs	49
Protein Assay	50
Synthesis of Adipate Esters	
Esterification Reaction	52
Optimization of Esterification Reaction	
Effect of Time Course	53
Effect of Temperature	53
Effect of Mole Ratio of Substrates	53
Effect of Organic Solvents	54
Effect of Initial Water Activity	54
Stability Study of Immobilized Enzymes	
Effect of Thermostability	55
Effect of Storage Stability	55
Effect of Leaching Study	55
Effect of Stability in Organic Solvent	56
Effect of Reusability	56
Product Identification	
Thin Layer Chromatography (TLC)	57
Fourier Transform Infra-red Spectroscopy (FTIR)	57
Gas Chromatography (GC)	57
Gas Chromatography - Mass Spectrometer (GCMS)	58

IV RESULTS AND DISCUSSION

Characterization of LDHs	
Fourier Transform Infra-red (FTIR)	59
X-ray Diffractometer (XRD)	59
Surface Area and Porosimeter (ASAP)	64
Scanning Electron Microscopy (SEM)	67



Energy Dispersive X-ray (EDX)	72
Immobilization of Lipase	76
Esterification Reaction	
Optimization of Esterification Reaction	
Effect of Time Course	80
Effect of Temperature	83
Effect of Mole Ratio of Substrates	87
Effect of Organic Solvents	91
Effect of Initial Water Activity	95
Stability Study of Immobilized Enzymes	
Effect of Thermostability	100
Effect of Storage Stability	103
Effect of Stability in Organic Solvent	106
Effect of Leaching Study	107
Effect of Reusability	110
Optimal conditions for DMA and DBA syntheses	113
Product Identification	
Thin Layer Chromatography (TLC)	114
Fourier Transform Infra-red Spectroscopy (FTIR)	114
Gas Chromatography (GC)	119
Gas Chromatography – Mass Spectrometer (GCMS)	124
V CONCLUSION	128
RECOMMENDATION	131
REFERENCES	132
APPENDICES	144
BIODATA OF THE AUTHOR	150



LIST OF FIGURES

Figure		Page
1	Schematic representation of an enzyme with one active site binding a substrate molecule to convert to products	8
2	The Lock and Key Analogy	10
3	The Induced Fit Theory Analogy	11
4	The Lock and Key Model and Induced Fit Model	12
5	Transesterification reaction catalyzed by lipase	20
6	Schematic top view of a brucite-like layer of LDH	32
7	Schematic side view of a brucite-like layer of LDH	32
8	Expanded schematic view of the layer of LDH	33
9	Structural formula of adipic acid	36
10	Structural formula of dimethyl adipate (DMA)	39
11	Structural formula of dibutyl adipate (DBA)	40
12	Some examples of DMA and DBA products	42
13	Schematic diagram of lipase-catalyzed esterification of adipic acid and monohydric alcohols	43
14	FTIR spectrums of layered double hydroxides (LDHs)	60
15	XRD patterns of layered double hydroxides (LDHs)	61
16	N ₂ isotherms of layered double hydroxides (LDHs)	66
17	SEM image of (a) native lipase at magnification of x 110 and (b) immobilized lipase at of x 8000	68
18	SEM images of Mg/Al-LDH at magnification of (a) x 110 and (b) x 2,700	69
19	SEM images of Zn/Al-LDH at magnification of (a) x 110 and (b) x 2,700	70



20	SEM images of Ni/Al-LDH at magnification of (a) x 110 and (b) x 2,700	71
21	EDX spectrums of native lipase, Mg/Al-LDH and Mg/Al-lipase	73
22	EDX spectrums of native lipase, Zn/Al-LDH and Zn/Al-lipase	74
23	EDX spectrums of native lipase, Ni/Al-LDH and Ni/Al-lipase	75
24	Effect of Time Course in DMA syntheses	81
25	Effect of Time Course in DBA syntheses	82
26	Effect of Temperature in DMA syntheses	85
27	Effect of Temperature in DBA syntheses	86
28	Effect of Mole Ratio of Substrates in DMA syntheses	89
29	Effect of Mole Ratio of Substrates in DBA syntheses	90
30	Effect of Organic Solvents in DMA syntheses	93
31	Effect of Organic Solvents in DBA syntheses	94
32	Effect of Water Activity in DMA syntheses	98
33	Effect of Water Activity in DBA syntheses	99
34	Thermostability of Immobilized Lipases on Esterification Activity	101
35	Storage Study of Immobilized Lipases on Esterification Activity	105
36	Stability of Immobilized Lipases in Organic Solvent	108
37	Leaching Study of Immobilized Lipases on Esterification Activity	109
38	Reusability of Immobilized Lipases on Esterification Activity	112
39	TLC diagrams of substrates, standard and DMA ester product	115
40	TLC diagrams of substrates, standard and DBA ester product	116



41	FTIR spectra of standard and DMA ester product	117
42	FTIR spectra of standard DBA ester product	118
43	GC chromatograms of standard and DMA ester product	120
44	GC chromatograms of standard and DBA ester product	121
45	GC-MS chromatograms of (a) DMA ester product and (b) DBA ester product	125
46	GC-MS spectra library of DMA ester product	126
47	GC-MS spectra library of DBA ester product	127



LIST OF TABLES

Table		Page
1	Identification of adipic acid	37
2	Physical and chemical properties of adipic acid	37
3	Physical and chemical properties of dimethyl adipate (DMA)	39
4	Physical and chemical properties of dibutyl adipate (DBA)	41
5	Amount of enzymes used in order to reach the equivalent protein concentration as in 0.15g of native lipase from <i>Candida rugosa</i>	51
6	XRD analysis data of LDHs prepared at ratio 4 and pH 10	63
7	BET surface area, micropore volume and BJH desorption pore size distribution of LDHs	65
8	Amount of protein adsorbed on LDHs and percentages of immobilization (%)	79
9	Optimal conditions for DMA syntheses	113
10	Optimal conditions for DBA syntheses	113
11	Composition and retention time of DMA product	123
12	Composition and retention time of DBA product	123



LIST OF ABBREVIATIONS

Å	Angstrom
Al	aluminium
ASAP	Accelerated Surface Area and Porosimetry
A_w	water activity
BET	Brunauer, Emmett and Teller
BJH	Barrett, Joyner and Halenda
° C	degree Celcius
Ca^{2+}	calcium
Cl	chloride
cm	centimeter
CO_3^{2-}	carbonate
Co	cobalt
Cr	chromium
Cys	cystein
DBA	dibutyl adipate
DMA	dimethyl adipate
EDX	Energy Dispersive X-ray
<i>et al.</i>	et alia
FTIR	Fourier Transform Infra-Red
g	gram
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometer



I	Intensity
K	Kelvin
kDa	kilo Dalton
LDH	layered double hydroxide
Log P	logarithma of partition coefficient of octanol-water system
M	molarity
Mg	magnesium
µm	micrometer
µL	microliter
mg	milligram
mL	milliliter
Na	sodium
NL	native lipase
Ni	nickel
nm	nanometer
NO ₃ ⁻	nitrate
O	oxide
OH	hydroxide
%	percentage
PDB	Protein Data Bank
S	sulphur
SEM	Scanning Electron Microscopy
XRD	X-ray Diffractometer



Zn	zinc
ν	stretching vibration mode
δ	bending vibration mode
*	impurity peaks/phase



CHAPTER I

INTRODUCTION

Petro-based adipate esters which are derived from compounds of C6 straight-chain dicarboxylic adipic acid and alcohol are one of the most important classes of valuable raw material especially in petrochemical industries. The excellent properties of adipic esters such as its low toxicity, good thermal stability, low volatility and high biodegradability (Rudnick and Shubkin, 2000), make it a very useful compound and significant to many industrial applications especially in domestic and lubricant industries. The specially formulated esters such as methyl adipates and butyl adipates are widely synthesized due to their relatively low cost and good balance of properties (Gryglewicz, 2001) using C6 straight-chain dicarboxylic acid, particularly adipic acid. Adipic esters which are produced with alcohol of 1 - 10 carbon are called adipates and they are most commonly used in manufacturing of plasticizers, lubricants, adhesives, paint stripper and coating industry (Kirk and Othmer, 1985). Adipate esters can be formed by reacting adipic acid with monohydric alcohol namely methanol or butanol via esterification reaction under mild conditions.

The green synthesis of esters in organic medium catalyzed by using immobilized enzyme has greatly expanded the enzyme potential for its use as industrial biocatalyst (Deng *et al.*, 2004; Klibanov, 1986; Dordick, 1992). The

