KINETICS AND MODELLING FOR THE PRODUCTION OF (*S*)-IBUPROFEN ACID

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KINETICS AND MODELLING FOR THE PRODUCTION OF (*S*)-IBUPROFEN ACID

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS

		Unit
$[A]$	Concentration of substrate	mM
$[AE]$	Concentration of enzyme-substrate complex	mM
E	Enantioselectivitiy	-
$[E]$	Concentration of free enzyme	mM
$[E]_T$	Total concentration of enzyme	mM
ee_S	Enantiomeric excess of the substrate	%
ee_{So}	Initial enantiomeric excess of the substrate	%
ee_P	Enantiomeric excess of the product	%
$[EI]$	Concentration of enzyme-inhibitor complex	mM
$[ER]$	Concentration of enzyme-(*R*)-substrate complex	mM
$[ES]$	Concentration of enzyme-(*S*)-substrate complex	mM
$[ERR]$	Concentration of enzyme-(*R*)-substrate-(*R*)-substrate complex	mM
$[ESS]$	Concentration of enzyme-(*S*)-substrate-(*S*)-substrate complex	mM
$[ERI]$	Concentration of enzyme-(*R*)-substrate-inhibitor complex	mM
$[ESI]$	Concentration of enzyme-(*S*)-substrate-inhibitor complex	mM
H^+	Proton	-
$[I]$	Concentration of product inhibitor	mM
k	Rate constant	hr^{-1}

k_{2A}	Rate constant of enantiomer A	mM
k_{2B}	Rate constant of enantiomer B	mM
K_{cat}	Catalytic constant	mM
K_m^{org}	Michaelis constant in organic phase	mM
K_m^{aq}	Michaelis constant in aqueous phase	mM
K_{MA}	Michaelis constant of fast-reacting enantiomer	mM
K_{mA}	Michaelis constant of enantiomer A	mM
K_{MB}	Michaelis constant of slow-reacting enantiomer	mM
K_{mB}	Michaelis constant of enantiomer B	mM
$K_{MA/MB}$	Michaelis constant of fast- over slow-reacting enantiomer	-
k_{OH}	Racemization rate constant of hydroxide ion concentration	hr^{-1}
K_{IP}	Inhibition constant of non-competitive product inhibitor	mM
K_{IR}	Binding rate constant of slow-reacting enantiomer	mM
K_{IS}	Binding rate constant of fast-reacting enantiomer	mM
K_{IRR}	Inhibition constant of uncompetitive (*R*)-substrate inhibitor	mM
K_{ISS}	Inhibition constant of uncompetitive (*S*)-substrate inhibitor	mM
k_{rac}	Racemization rate constant	hr^{-1}
k_S	Racemization rate constant of substrate concentration	hr^{-1}
m	Mass of enzyme	g
m_s	Partition coefficient	-

$[OH]$	Concentration of base	mM
$[P]$	Concentration of product	mM
R	Standard of gas constant	kJ.mol^{-1}.K^{-1}
$[S]$	Concentration of (*S*)-substrate	mM
$[S_A]$	Concentration of fast-reacting enantiomer	mM
$[S_B]$	Concentration of slow-reacting enantiomer	mM
SSE	Sum of squared error	-
T	Temperature	°C
v	Reaction rate	mM.hr^{-1}
V_A	Reaction rate of fast-reacting enantiomer	mM.hr^{-1}
V_B	Reaction rate of slow-reacting enantiomer	mM.hr^{-1}
V_R	Reaction rate of (*R*)-enantiomer	mM.hr^{-1}
V_S	Reaction rate of (*S*)-enantiomer	mM.hr^{-1}
V_{Amax}	Maximum reaction rate of fast-reacting enantiomer	mM.hr^{-1}
V_{Bmax}	Maximum reaction rate of slow-reacting enantiomer	mM.hr^{-1}
y_{Exp}	Experimental data	-
y_{Sim}	Simulated data	-
$\Delta_{R-S}\Delta G^{\ddagger}$	Differential of free energy of activation between enantiomers	kJ
$\Delta_{R-S}\Delta H^{\ddagger}$	Differential of activation enthalpy between enantiomers	kJ
$\Delta_{R-S}\Delta S^{\ddagger}$	Differential of activation entropy between enantiomers	kJ

LIST OF ABBREVIATIONS

Amano PS-30	*Pseudomonas cepacia* lipase
BCC	Business Communications Company
b.p.	Boiling point
CAGR	Compound annual growth rate
CAL-B	*Candida antartica* lipase-B
CRL	*Candida rugosa* lipase
DKR	Dynamic kinetic resolution
DMSO	Dimethyl sulfoxide
Eqn.	Equation
eqiv	Equivalent
FTIR	Fourier transform infrared spectroscopy
H_2O	Water
H-NMR	1-Hydrogen nuclear magnetic resonance
HPLC	High performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
KOH	Potassium hydroxide
KR	Kinetic resolution
Lipase MY	*Candida cylindracea* lipase
NaOH	Sodium hydroxide
NSAIDs	Non-steroidal anti inflammatory drugs
Oct_3N	Trioctylamine
OH	Hydroxide
rpm	Rotations per minute

THF	Tetrahydrofuran
TOA	Trioctylamine
UK	United Kingdom
USA	United State of America
USD	United States Dolar
UV	Ultra violet
ρ-TBD	1,5,7-triazabicyclo[4,4,0]dec-5-ene

KINETIK DAN PERMODELAN UNTUK PENGHASILAN ASID (*S*)-IBUPROFEN

ABSTRAK

Kesan pelarut sampingan dan bes telah dikaji dalam lipase *Candida rugosa* (CRL) sebagai pemangkin hidrolisis (*R*,*S*)-2-etoksietil ibuprofen ester di dalam media dwi-fasa air-isooktana. Nilai log *P* yang tinggi dan nilai pKa yang rendah bagi pelarut sampingan telah mengurangkan kadar tindakbalas untuk penghasilan asid (*S*)-ibuprofen. Tambahan pula, proses hidrolisis menunjukkan prestasi optimum pada hanya 2% (v/v) dimetil sulfoksida (DMSO) dan pada suhu tindakbalas 30°C. Penambahan 2% (v/v) DMSO telah meningkatkan penukaran substrat dan peningkatan kepekatan DMSO seterusnya telah mengakibatkan penurunan pada kadar tindak balas awal dan penukaran substrat, bagaimanapun memberikan lebihan produuk enantiomerik (ee_P) yang lebih tinggi. Tambahan pula, perbezaan entalpi pengaktifan lebih tinggi di antara (*S*)- dan (*R*)-2-etoksietil ibuprofen ester di dalam media dengan 2% (v/v) DMSO telah mengakibatkan kepemilihan enantiomer yang lebih tinggi. Selain daripada itu, kehadiran DMSO telah meningkatkan kestabilan CRL pada kepekatan substrat yang lebih tinggi di dalam hidrolisis (*R*,*S*)-2-etoksietil ibuprofen ester.

Satu model kinetik menggambarkan tingkah laku CRL di dalam hidrolisis (*R*, *S*)-2-etoksietil ibuprofen ester telah berjaya dihasilkan dengan mengambil kira

perencat tanpa persaingan (2-etoksietanol), perencat substrat tiada tandingan ((*S*)-2-etoksietil ibuprofen ester) dan perencat substrat kompetitif ((*R*)-2-etoksietil ibuprofen ester). Model kinetik telah disahkan dan mampu menganggar pemalar kinetik. Didapati bahawa, DMSO telah mengurangkan pemalar Michaelis (K_{MA}=384 mM), kadar tindakbalas maksimum (V_{Amax}=4.5 mM/hr) dan pemalar perencat bagi perencat produk tanpa persaingan (K_{IP}=140 mM), sementara itu pemalar perencat bagi perencat substrat tiada tandingan (K_{ISS}=1950 mM) telah ditingkatkan.

Di dalam peraseman (*R*)-2-etoksietil ibuprofen ester, resin OH^- ialah pemangkin bes paling sesuai berbanding dengan natrium hidroksida (NaOH). Bagaimanapun, dengan menggunakan trioktilamina (TOA) sebagai pemangkin bes, tiada tindakbalas peraseman ditunjukkan. Pada umumnya, kadar peraseman bertambah dengan peningkatan kepekatan bes, suhu tindakbalas dan kepekatan DMSO, sementara dengan menggunakan kepekatan substrat yang rendah. Satu model kinetik bagi peraseman bermangkinkan bes telah dibangunkan dengan menggabungkan kesan kepekatan bes dan substrat awal. Nilai pemalar peraseman (k_{rac}) telah diperolehi dengan menggunakan dua set data pada keadaan kepekatan asas dan substrat awal yang tetap. Model kinetik peraseman bermangkinkan bes dengan nilai pemalar peraseman k_{rac}=0.48 hr^{-1} telah berjaya disahkan dengan nilai R^2=0.98.

KINETICS AND MODELLING FOR THE PRODUCTION OF (*S*)-IBUPROFEN ACID

ABSTRACT

The effect of co-solvents and bases have been investigated in *Candida rugosa* lipase (CRL)-catalyzed hydrolysis of (*R,S*)-2-ethoxyethyl ibuprofen ester in biphasic water-isooctane medium. A high log *P* and low pKa values of co-solvent has reduced the reaction rate for the production of (*S*)-ibuprofen acid. Moreover, the hydrolysis process shows an optimum performance at only 2% (v/v) dimethyl sulfoxide (DMSO) and at the reaction temperature of 30°C. The addition of 2% (v/v) DMSO has increased the conversion of substrate and further increase in the concentration of DMSO has resulted in a low initial reaction rate and conversion of substrate, however gives a high enantiomeric excess of the product (ee_P). Additionally, a higher differential activation enthalpy between (*S*)- and (*R*)-2-ethoxyethyl ibuprofen ester in the medium with 2% (v/v) DMSO has resulted in higher enantioselectivity. Moreover, the presence of DMSO has increased the CRL stability at higher substrate concentration in hydrolysis of (*R,S*)-2-ethoxyethyl ibuprofen ester.

A kinetic model to describe the behavior of the CRL in hydrolysis of (*R,S*)-2-etthoxyethyl ibuprofen ester was successfully developed by accounting the non-competitive inhibitor (2-ethoxyethanol), uncompetitive substrate inhibitor ((*S*)-2-ethoxyethyl ibuprofen ester) and competitive substrate inhibitor ((*R*)-2-ethoxyethyl

ibuprofen ester). The kinetic model was validated and able to estimate the kinetic constants. It has been found that, DMSO has reduced the Michaelis constant (K_{MA}=384 mM), the maximum reaction rate (V_{Amax}=4.5 mM/hr) and the inhibitors constant of non-competitive product inhibitor (K_{IP}=140 mM), meanwhile the inhibitor constant of an uncompetitive substrate inhibitor (K_{ISS}=1950 mM) was increased.

In the racemization of (*R*)-2-ethoxyethyl ibuprofen ester, OH^- resin is the most suitable base catalyst compared to sodium hydroxide (NaOH). However, by using triocthylamine (TOA) as base catalyst, racemization reaction was not indicated. Generally, the racemization rate is faster with increases base concentration, reaction temperature and concentration of DMSO, while using low substrate concentration. A kinetic model of base-catalyzed racemization was developed incorporating with the effect of base-concentration and initial substrate concentration. The racemization constant (k_{rac}) value was obtained by using two sets of experimental data at fixed base and initial substrate concentrations. The kinetic model of base-catalyzed racemization with a racemization constant of k_{rac}=0.48 hr^{-1} was successfully validated with a value of R^2=0.98.

CHAPTER 1

INTRODUCTION

1.1 Rationale For Single Enantiomer Drug

A racemic drug is an equimolar mixture with distinct pharmacokinetic and pharmacodynamic properties. Compared with the active enantiomer, the inactive enantiomer in a racemic mixture often has different rates of absorption, metabolism, and excretion, as well as different affinities for tissue receptor and protein receptor binding sites. It may be an agonist or antagonist, produce adverse effects, increase efficacy, or place an undue burden on clearance mechanisms. For example, (*S*)-ibuprofen is over 160-fold more potent, and inhibitor of cyclo-oxygenase I than (*R*)-ibuprofen (Adams et al., 1976). Meanwhile, (*S*)-citalopram is over 100-fold more potent as an inhibitor of the Serotonin Reuptake Transporter than (*R*)-citalopram (Somogyi et al., 2004).

Since the pharmacokinetics of both enantiomers differ, the contribution of each enantiomer in term of specific therapeutic effect and toxicity need to be considered before the products can be marketed. For example, the enantiomers of α-propoxyphene, the (+)-enantiomer of α-propoxyphene is a potent analgesic drug, whereas the (-)-enantiomer is a potent antitussive agent (an agent that suppresses coughing) devoid of analgesic activity (Eichelbaum et al., 1996). The different spectrum was documented on the pharmacological effects of the enantiomers of α-

dextropropoxyphene resulted in the development of two separate stereochemically pure drugs: α-dextropropoxyphene (Darvon), an analgesic (pain relieving), and α-levopropoxyphene (Novrad), an antitussive.

1.2 Market Survey

Single enantiomer drug sales show a continuous growth worldwide and many of the top selling drugs are marketed as single enantiomers (Shafaati, 2007). According to an annual report from Medicinal Chemistry, 754 new drugs (new chemical and biological entities) were launched in the world within 20 years covering between 1985 and 2004 (Murakami, 2007). The most prominent observations are the increase of single enantiomers lunched and the decrease of racemates, which at the beginning dominated two-thirds of the market in 1985. The increase of single enantiomers lunched from 24 to 44% of marketed drugs within that period from 1985 to 2004 showed an increase of interest towards single enantiomers in pharmaceutical industries rather than chirals.

Single enantiomer therapeutic had sales of USD 225 billion in 2005 (Table 1.1). This figure represents 37% of the total final formulation pharmaceutical market of USD 602 billion. The annual growth rate of a single enantiomer product for the past 5 years was 11%, which is at par with the pharmaceutical market as a whole (Erb, 2006). Figure 1.1 shows the global pharmaceutical market from 2006 to 2013. According to a report conducted by Business Communications Company (BCC) research, the global market for pharmaceuticals increased from USD 693.7 billion in

2007 to USD 737.6 billion by the end of 2008. It should reach over USD 1 trillion by 2013, a Compound Annual Growth Rate (CAGR) of 6.9%. The generic perception drugs segment is expected to experience the highest growth rate over the 2008 to 2013 period. The market is worth an estimated USD 88.7 billion in 2008 and it is expected to reach USD 151.4 billion by the end of 2013 with estimated CAGR of 11.3%. The future growth of single enantiomer products is expected to be strong based on the following key market drivers;

- increased evidence and awareness of improved therapeutic profiles.
- published policies and guidelines of regulatory agencies.
- advances in chiral technologies.
- racemic switches as a generic defense strategy.
- the need for new and better anticancer and antiviral drugs.

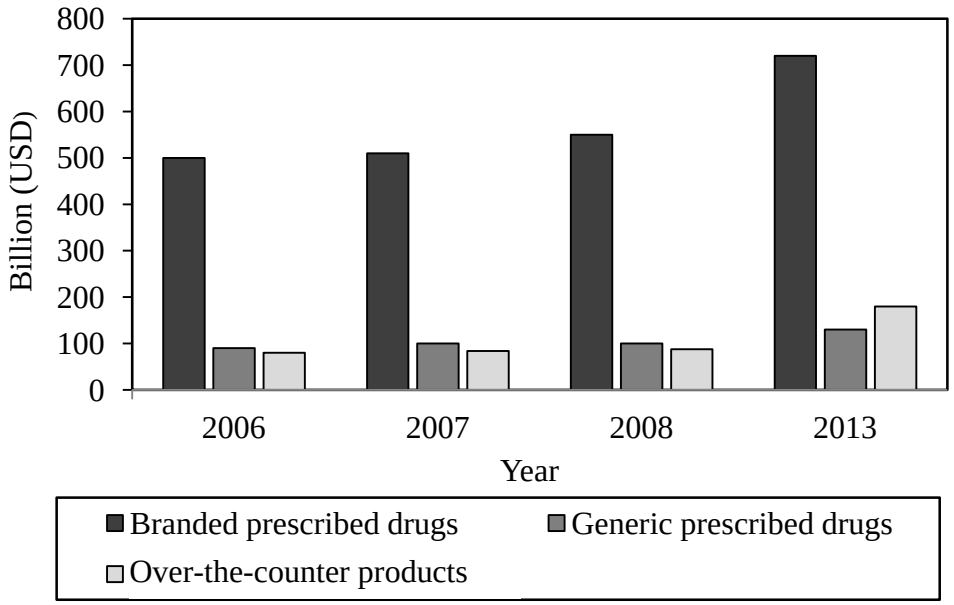

Figure 1.1: Global pharmaceutical market 2006 to 2013 (Krishan, 2008).

Table 1.1: Worldwide sales of single enantiomer pharmaceutical products final formulation (Erb, 2006).

Therapeutic category	2000 sales (USD billions)	2004 sales (USD billions)	2005 sales (USD billions)	CAGR (%) 2000-2005
Cardiovascular	27.650	34.033	36.196	6
Antibiotics and antifungals	25.420	32.305	34.298	6
Cancer therapies	12.201	21.358	27.172	17
Hematology	11.989	20.119	22.439	13
Hormone and endocrinology	15.228	20.608	22.355	8
Central nervous system	9.322	17.106	18.551	15
Respiratory	6.506	12.827	14.708	18
Antiviral	5.890	11.654	14.683	20
Gastrointestinal	4.171	11.647	13.476	26
Ophthalmic	2.265	3.063	3.416	9
Dermatological	1.272	1.486	1.561	4
Vaccines	1.427	2.450	3.100	17
Other	7.128	10.400	13.268	13
Total	130.991	199.056	225.223	11

1.3 Ibuprofen

Iso-butyl-propanoic-phenolic acid or commercially known as ibuprofen, belongs to a class of drugs called the non-steroidal anti inflammatory drugs (NSAIDs). NSAIDs are any of a large group of drugs that reduce pain and inflammation in muscles and joints. Other members of this class include aspirin, naproxen (Aleve), indomethacin (Indocin) and nabumetone (Relafen). These drugs are used for the management of mild to moderate pain, fever and inflammation. These symptoms are promoted by the release of chemicals in the body called prostaglandins. Ibuprofen blocks the enzyme that makes prostaglandins

(cyclooxygenase), resulting in lower levels of the molecules (Jensen, 2002). As a consequence, inflammation, pain and fever are reduced.

Ibuprofen contains a chiral carbon in the α-position of the propionate structure (Agatonovic-Kustrin et al., 2000). As such, there are two possible enantiomers of ibuprofen with different potential in terms of inversion and bioavailability (Cheng et al., 1994). Due to the high cost of purification process, for high enantiomeric ibuprofen in the most prescriptions, the drug is normally prescribed in the form of racemic ibuprofen (Figure 1.2).

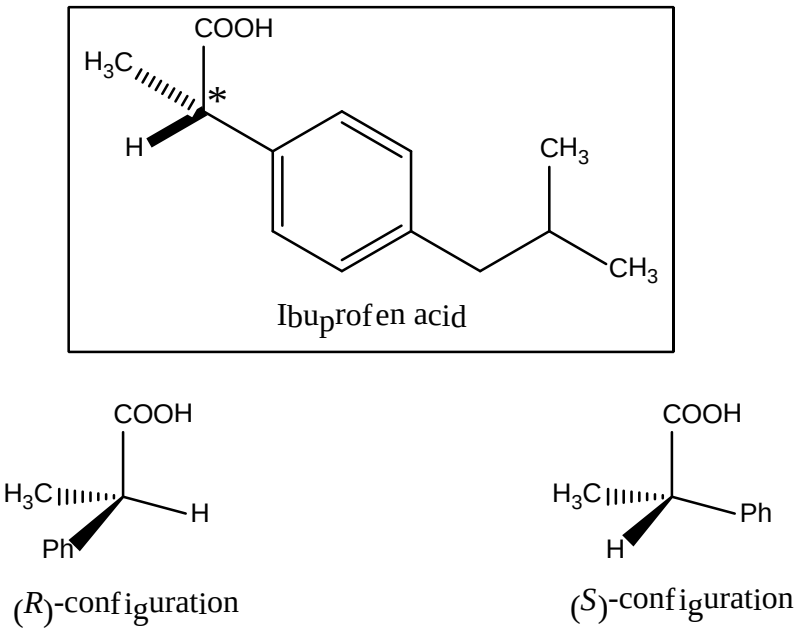

Figure 1.2: Racemic structure of (*R*)- and (*S*)-ibuprofen acid (* represent the position of the chiral carbon).

Currently the separation of an enantiomeric compound has been studied in order to produce optically pure enantiomers, instead of producing racemic mixtures. A multitude of methods and techniques for the separation of enantiomers exists such as asymmetric biotransformation, capillary electrophoresis, chromatography, liquid-

liquid extraction, membrane separation and crystallization (Maier et al., 2001; Carvalho et al., 2006). For each method, several advantages and disadvantages prevail, depending upon factors such as time, purity, chemical processing, and inherent side reactions. A large investment has been made in research to develop specific techniques, particularly for (*S*)-ibuprofen production.

Kinetic resolution for the production of (*S*)-ibuprofen acid has been intensely investigated. Various ibuprofen ester derivatives have been tested such as methyl ibuprofen ester, ibuprofen trifluroethyl ester, 2-ethoxyethyl ibuprofen ester and other types of esters. However, the reaction slows down after reaching 50% where the less reactive enantiomer remained. However, with the availability of racemization process, the conversion of substrate could reach 100%. Kinetic resolution and racemization are suitable to be applied in parallel for (*S*)-ibuprofen production. The racemization processs will be discussed further in the next chapter.

1.4 Problem Statement

Hydrolysis or kinetic resolution of ibuprofen ester by lipase-catalyzed reaction has become a method of interest to isolate pure (*S*)-ibuprofen acid from its racemic compound. The production of (*S*)-ibuprofen acid was tested in the enzymatic membrane reactor by giving 85% ee_p, 31% ee_s and E=13 of enantiomeric ratio (Long et al., 2005b). The performance of kinetic resolution of (*S*)-ibuprofen was further improved by integrating the resolution with racemization process or so called dynamic kinetic resolution (DKR). The DKR of (*S*)-ibuprofen acid was conducted in

a batch reactor giving 84% substrate conversion and 99.4% of enantiomerically pure (*S*)-ibuprofen (Fazlena et al., 2006).

In the kinetic resolution of ibuprofen ester in enzymatic membrane reactor, the product (*S*)-ibuprofen acid is simultaneously separated from the substrate solution. However, the maximum conversion is limited for only 50%. For the DKR in batch reactor, the conversion was exceeding 50% as its involved *in-situ* racemization (Fazlena et al., 2006). Hence, the DKR technique in an enzymatic reactor should be the answer for the production of (*S*)-ibuprofen acid. This configuration will give a comparatively high conversion, high optically pure single enantiomer and simplified downstream processing for the product purification. However, another matter that needs to be considered in the DKR is the compatibility of hydrolysis and racemization processes. *In-situ* racemization will occur when there was an untreated substrate or undesired enantiomer. The undesired enantiomer will be converted to the desired enantiomer in racemic form, until an equilibrium is reached. However, the rate of hydrolysis will be hindered by racemization process as this process occurs in a slow reaction rate. Hence it will be a limiting factor to the hydrolysis rate. In order to obtain a fast conversion of substrate, racemization rate should be increased, since the product from the racemization will be used as a substrate for the hydrolysis process. If the racemization rate could not satisfy hydrolysis substrate demand, the time taken for 100% conversion of (*R,S*)-ester to (*S*)-acid will be time consuming. The use of co-solvents or additives in DKR could substantially increase the reaction rate (Fazlena et al., 2006).

Additionally, in DKR, the compatibility of the hydrolysis and racemization reaction condition should be paid more attention. Since enzyme is very sensitive towards reaction environment, the choice of catalyst and co-solvent for racemization must not affect the optical purity of the product, enzyme selectivity and enzyme stability. Strong base such as sodium hydroxide already showed a great potential in DKR of ibuprofen ester (Fazlena et al., 2006). However, the presence of sodium hydroxide will change the reaction medium to a highly basic condition (>pH 9). It is rule of thumb that, the pH needs to be maintained according to the optimum condition for the enzyme (*Candida rugosa* lipase). An addition of the buffering agent could maintain the pH, and yet increase the ionic strength of the reaction medium. A minimal concentration of both buffering agent and sodium hydroxide should be used in order to avoid any additional effect caused by ionic compounds. Another alternative is to use an amine compound as a racemization catalyst. The study on the performance of amine in the racemization of ibuprofen ester is yet to be verified and the result is particularly important in order to determine the effectiveness of amine as base catalyst. Additionally, amine base such as triocthylamine is highly hydrophobic and suitable to be applied as base catalyst in the shell side of the enzymatic membrane reactor. This is advantageous, since the effect of base on enzyme immobilized in membrane porous could be minimized. However, it is a challenge to increase anion dissociation of triocthylamine with base dissociation constant value less than sodium hydroxide. The base anion in its free form is important to increase the base reactivity and the formation of carbanion for racemization process (Ebbers et al., 1997).

Hence, in this study, the performance of the base catalysts; sodium hydroxide and trioctylamine will be explored. Alternative catalyst such as OH^- resin consists of hydroxide ion shall also be tested. The reaction medium will be added with appropriate amount of co-solvents in order to investigate its implications on the racemization and hydrolysis processes. The co-solvents are expected to increase the conversion, enantiomeric excess and enzyme stability in the hydrolysis as well as to facilitate the racemization rate of (*R*,*S*)-2-ethoxyethyl ibuprofen ester.

1.5 Research Objective

The main objective of this research project is to investigate the feasibilities of enzyme and base catalysts as well as the co-solvent in the DKR of ibuprofen ester. This research project aims to achieve the following specific objectives:

i. To evaluate the effect of co-solvents in the enzymatic hydrolysis of (*R*,*S*)-2-ethoxyethyl ibuprofen ester.

ii. To evaluate the kinetic behavior of enzyme (*Candida rugosa* lipase) in the presence of dimethyl sulfoxide (DMSO).

iii. To compare the effectiveness of trioctylamine, sodium hydroxide and OH^- resin in the racemization of (*R*,*S*)-2-ethoxyethyl ibuprofen ester.

iv. To develop kinetic model, determine the kinetic parameters and validate the kinetic model based on the enzymatic mechanism for kinetic resolution and racemization of (*R*,*S*)-2-ethoxyethyl ibuprofen ester.

1.6 Organization of Thesis

This thesis is divided into six chapters as follows;

Chapter 1 gives the introductory of this research project. This chapter starts with the pharmaceutical market demand in single enantiomer drugs reported by market researcher. It also gives a brief overview of the single enantiomer production of (*S*)-ibuprofen through kinetic resolution. The problem statement and objectives of this research project are also clearly stated in this chapter.

Chapter 2 gives the review from the works of other researchers and methods applied in the present days for the hydrolysis and racemization of esters. This is followed by a discussion on the advantages of using co-solvents in the hydrolysis and racemization processes. Reviews on the kinetics, DMSO as co-solvent and base-catalyzed racemization are also covered in detail.

Chapter 3 describes the methods and analysis required for the hydrolysis and racemization processes. It also explains the chemical requirements and equipments used throughout the whole process of this study. The subsequent

topics describe clearly the methodology of this research project-synthesis using *Candida rugosa* lipase (CRL), co-solvents and base catalysts.

Chapter 4 presents the results obtained from experimental runs and discusses on the effect of parameters on the synthesis of (*S*)-ibuprofen acid. The discussion is focused on the co-solvents and bases employed in hydrolysis and racemization of ibuprofen ester respectively.

Chapter 5 presents the kinetic model proposed for hydrolysis and racemization of ibuprofen ester. Every kinetic constant was estimated using regression method available in MATLAB®. The variation of kinetic parameters obtained between previous studies was discussed, beside the validation of the model between estimated and experimental data.

Chapter 6 concludes the research project. Recommendations for future work related to this research project are also given.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of Enantiomerically Pure Drug Compounds

There are three possible strategies in the preparation of enantiomerically pure drug compounds (Martin-Matute et al., 2008b). One way is through performing a resolution of a racemate or known as kinetic resolution where the procedure depends on the difference of reaction rates of two enantiomers. Secondly, is to use naturally occurring starting materials of defined absolute configuration, provided by nature's chiral pool. The third approach is through asymmetric synthesis, where it involves the creation of an asymmetric (stereogenic) center, which in this case, the chiral discrimination of equivalent groups in an achiral starting material (prochiral).

For each of these techniques there are some pros and cons. In the first method, the maximum theoretical yield gives only 50%. In the second approach, chiral pool, suitable naturally occurring chiral compound as starting materials is needed. However, most of the commercially available chiral molecules consist of carbohydrates and amino acids. Looking for suitable precursor can be difficult besides, requiring the multi-step preparation leading to the overall low yields (Yazbeck et al., 2004). Meanwhile, asymmetric synthesis has been chosen as the best method to date (Martin-Matute et al., 2008b). This method is able to change many prochiral molecules via chiral multiplication with a single optically pure enantiomer

catalyst. A method for preparation of enantiopure compounds via asymmetric synthesis is still growing in numbers, and yet, the kinetic resolution is the most suitable method normally employed in the industry. Majority of the cases, enzymes are used as biocatalyst (Martin-Matute et al., 2008b).

2.2 Kinetic Resolution

Kinetic resolution (KR) can be defined as a partial separation of two enantiomers from a racemic mixture and it can transform one of the enantiomers of a racemic mixture into a product (enantiopure) rather than its mirror image. KR normally occurs based on the reaction rate of the enantiomers. In an ideal condition, both enantiomers show different reactivities, one of the enantiomers would react very fast to form the product, while its mirror image may react at a slower rate or even no reaction (Martin-Matute et al., 2008b). Figure 2.1 below shows the general mechanism of the KR;

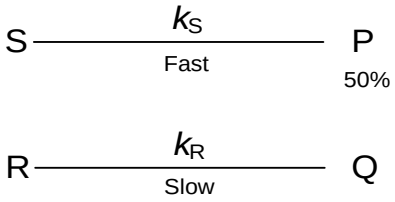

Figure 2.1: General mechanisms for kinetic resolution.

The symbols R and S represent the (*R*)- and (*S*)-enantiomer substrate whereas P and Q signify the product and by-product produced in the reaction, respectively. Meanwhile, the symbols k_S and k_R correspond to the rate constant for product P and

by-product Q, respectively. KR took place when $k_R \neq k_S$ and the reaction is stopped somewhere between 0 and 100% conversion. Usually the reaction is slowing down after 50% conversion is reached. During the reaction, the concentration of both enantiomers is not constant, therefore the conversion will affect reaction rate of the enantiomers (Martin-Matute et al., 2008b). It is advised that the reaction should be stopped when the conversion reached 50% in order to achieve high enantiomeric excess.

2.3 Racemization

Racemization is defined as the irreversible formation of a racemate from a pure enantiomer and linked with the total loss of optical activity (Ebbers et al., 1997). A racemate is defined as an equimolar mixture of two enantiomers regardless of their physical states (Ebbers et al., 1997). Racemization of the unwanted isomer is meaningful in resolution of chiral constituent to exceed the theoretical yield of 50% in an enantioselective synthesis. This would be an attractive option due to the current high cost of production of optically pure active pharmaceutical ingredients.

From the literature survey, a number of general chemical processes leading to racemization of asymmetric carbon atoms have been known. Most processes involved strong basic or acidic reaction conditions at elevated temperatures. The rate of racemization can be illustrated as the interconversion rate of enantiomers, or either as the rate of formation of the racemate (Ebbers et al., 1997). Racemization method has been classified into several methods as reported by Ebbers and co-workers

(1997). From all methods shown in Table 2.1, base-catalyzed racemization was the most employed technique.

Table 2.1: Racemization methods

Methods	Details	References
Base-catalyzed racemization	Applied for compounds bearing acidic hydrogen at the chiral center. For instant in the racemization of amino acid thioester, and profens ester.	(Chen et al., 2002a; D'Arrigo et al., 2011; Chavez-Flores et al., 2012)
Metal-catalyzed racemization	Feasible for wide range of compounds which include; ketones, esters, secondary alchohols. However the preparations of the metal catalysts are very complex, costly and require multi step synthesis.	(Kim et al., 2002; Mahn-Joo Kim et al., 2007)
Enzyme-catalyzed racemization	Applied to racemization of amino acids and derivatives and to α-hydroxy-carboxylic acids and derivatives. For instance, Mandelate racemase is employed to racemize trifluorolactate.	(Nagar et al., 2011)
Thermal racemization	Suitable for compound which racemize by rotation or deformation of bond (biaryls), phyramidal inversion and rearrangement of bonds. For instant, the (+)-10-exo-hydroxy-pentacyclo[6.2.1.13,6.02,7.05,9]dodeca-4-one was able to racemize at elevated temperature due to dyotropic rearrangement.	(Martins et al., 2003)
Acid-catalyzed racemization	Resulted from protonation of α-carbon at the chiral center. For instance, (*S*)-1-phenylethanol acetate was racemized in the presence of acidic resin.	(Cheng et al., 2010)

2.4 Base-Catalyzed Racemization

Up to this moment, base-catalyzed racemization is the most important method currently used for racemization of optically pure organic compound. It can be applied to almost all compounds bearing acidic hydrogen at the chiral center or α-carbon. In DKR of optically active organic compounds such as ibuprofen ester and naproxen ester, the substrates contain a proton at chiral center with low pKa value. In DKRs, base-catalyzed racemization are categorized according to the nature of the substrates, as being thioesters, α-activated esters, oxazolones, hydantoins or acyloins (Martin-Matute et al., 2008a). Fundamentally, base-catalyzed racemization usually involves the removal of a hydrogen ion from the chiral center to form a carbanion. The carbanion needs to be stabilized by adjacent groups such as keto, nitrile, nitro or other functionalities, or by a reversible elimination of a β-substituent (Figure 2.2) (Ebbers et al., 1997). Thus, preparation of a derivative is required to enhance the acidity of α-proton.

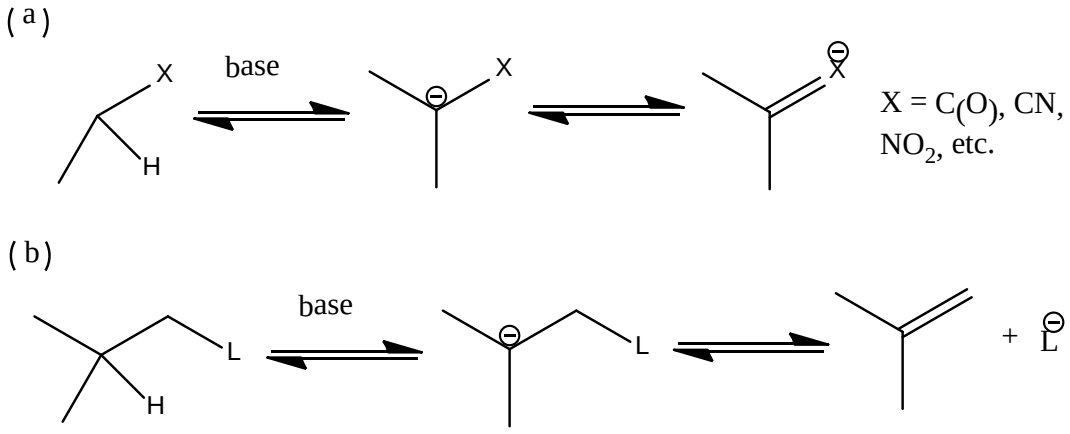

Figure 2.2: Mechanism for base-catalyzed racemization. (a) Carbanion stabilized by adjacent groups, and (b) carbanion stabilized by a reversible elimination of a β-substituent.

Substitution reactions of optically active molecule always involve breaking and reforming of bonds at the chiral center. These process sometimes occur stereospecifically with retention or inversion of configuration where optical activity is preserved (Wiberg, 2001). In other cases, they occur non-stereospecifically, with simultaneous inversion and retention of configuration, leading to racemization (Wiberg, 2001). Racemization depends on several parameters such as solvents, substituents and characteristic of the bases. The removal of hydrogen by a base in non polar solvents results in an intimate ion pair. In these solvents, the re-addition of the proton may lead to some preferences for retention and therefore, lead to a relatively slow racemization (Ebbers et al., 1997). For a polar solvent, separated ion pair is generated, rearrangement of proton (H^+) predominantly occurs from the opposite side, resulting in a net of inversion and fast racemization (Ebbers et al., 1997). In addition, bases tend to be more reactive in aprotic polar solvents than in protic polar solvents and results in faster racemization. This phenomenon occurs because the anions are less solvated in aprotic polar solvents than neutral species or cations due to lower acidities.

2.5 Dynamic Kinetic Resolution

The DKR is generally used to overcome the disadvantages encountered during KR. Previously, KR has been the main route in the enzymatic resolution procedure for the synthesis of optically pure compounds. However, with only 50% of maximum conversion, the method has driven researchers to switch into the method of DKR. The method of DKR, can take place by combining the standard KR and *in-*

situ racemization process of the chiral substrates (Figure 2.3). In the standard KR, the hydrolysis process took place by means of enantioselective biocatalyst to hydrolyze racemic substrate and left unreacted enantiomer. Once the KR is coupled with the *in-situ* racemization, the unreacted substrate will be recycled as a racemic mixture. Hence, further hydrolysis of substrate can take place and consequently, the conversion of 100% of the substrate to single enantiomeric product could be achieved. This method has been proven in the field of chiral drugs resolution such as naproxen, fenoprofen and ibuprofen (Chen et al., 2002b; Lin et al., 2003; Fazlena et al., 2006). DKR has provided highly valuable processes for production of enantiomerically pure drugs by converting achiral or racemic drugs compound. However, this process is influenced by the specific factor, the parameters which are also important include; reaction temperature, solvent type, concentration, base catalysts and enzymes (biocatalyst).

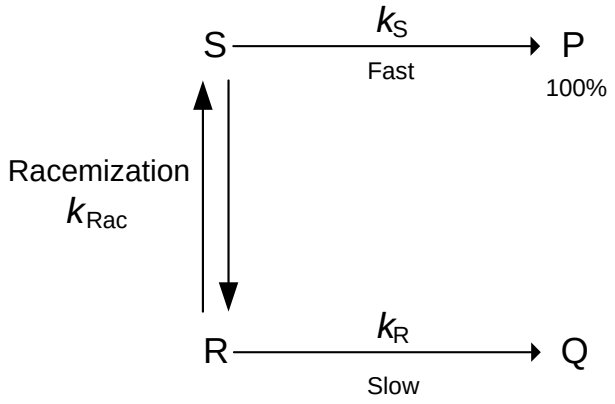

Figure 2.3: Dynamic kinetic resolution mechanism.

For efficient enzymatic DKR, the following necessities must be satisfied (Martin-Matute et al., 2008b);

i. The KR must be very selective (E > 20).

ii. The racemization must be fast, at least 10 times faster than the enzyme catalyzed transformation of the slow-reacting enantiomer (k_{rac} > 10 k_S).

iii. The racemization catalyst must not react with the product of the reaction.

iv. The KR and the racemization must be compatible under the same reaction conditions.

In an idyllic DKR, where the substrate stays racemic during the reaction, the optical purity depends only on the enantiomeric ratio (E), and is independent of the extent of conversion. The enantiomeric excess of the product formed under racemizing states is identical to the initial enantiomeric excess under non-racemizing states (Martin-Matute et al., 2008b).

2.6 DKR of *α*-substituted Carboxylic Acids or Esters

Considerable effort has been made to produce optically pure compound of non-steroidal anti-inflammatory drugs through DKR. However, the conversions of substrate, usually corresponding to ester derivatives are still in moderate level and hardly reaching more than 90%. The previous work on DKR of optically active drug productions is presented in Table 2.2. A variety of carboxylic acid derivatives have been employed for the resolution of optically active compounds such as thioester, methyl ester, ethyl ester and etc.

As for a substrate bound with thioesters as a leaving group, the feasibility of this approach was pioneered by Drueckhammer and co-workers (Tan et al., 1995). The DKR was performed with propionate thioesters bearing a phenylthio group, which also contribute to the acidity of α-proton (Tan et al., 1995). The α-protons of thioesters are acidic to allow the continuous racemization of the substrate by base-catalyzed deprotonation at the α-carbon. The enzymatic hydrolysis of the thioester was accompanied with a racemization catalyzed by trioctylamine (Figure 2.4). Because of the insolubility of the substrate and base catalyst in aqueous medium, they employed a biphasis system which is toluene-water mixture. By using *Pseudomonas cepacia* lipase (Amano PS-30) as the enzyme and a catalytic amount of trioctylamine, they obtained a quantitative yield of the corresponding carboxylic acid in 96.3% *ee*.

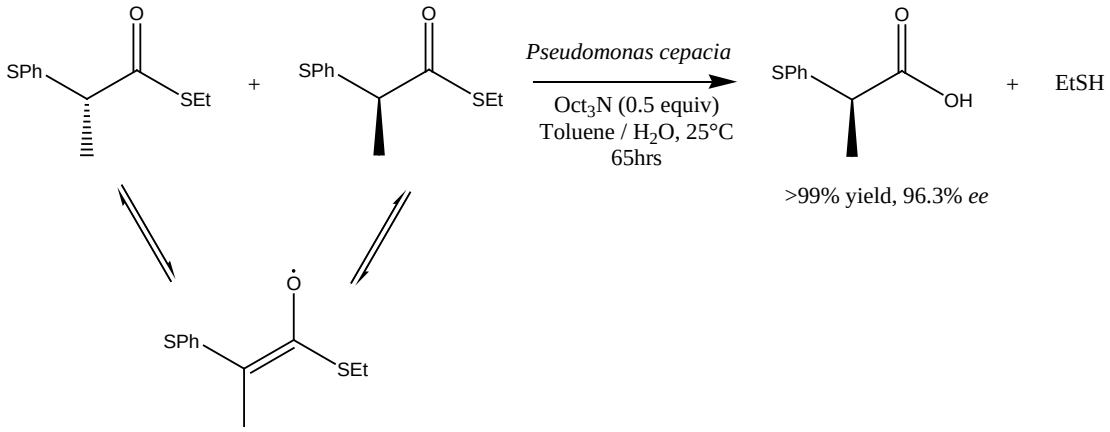

Figure 2.4: DKR of thioesters using a base for racemization performed by Drueckhammer et al. (Tan et al., 1995).

Has been reported that the DKR of naproxen, suprofen and fenoprofen thioesters were synthesized using trioctylamine as the base catalyst (Chang et al., 1999; Lin et al., 2000; Chen et al., 2002b). The corresponding thioesters substrate

was prepared with thiophenol or 2,2,2-trifluoroethanethiol. The base catalyst, trioctylamine was reported not only functioned for the formation of carbanion in racemization, but also helped to activate the lipase (Chang et al., 1999). However, in the DKR of suprofen thioesters, increasing the trioctylamine concentration could also result in decreasing the enzyme deactivation constant, which implies that trioctylamine could enhance the lipase stability (Lin et al., 2000). However, the data is not enough to elucidate the statement where trioctylamine can activate and increase the activity of enzyme. Therefore, a comprehensive study on the interaction between trioctylamine and lipase molecules is still needed.

Another DKR of esters which bears an electron-withdrawing group at the α-carbon has been reported on the DKR of (*R*,*S*)-2,2,2-trifluoroethyl α-chlorophenyl acetate in water-saturated isooctane (Wen et al., 2006). Lipase MY from *Candida rugosa* was used for the KR and an addition of trioctylamine which acts as a base for racemization (Figure 2.5). The product, (*R*)-chlorophenylacetic acid was obtained in 93% yield and 89.5% *ee*.

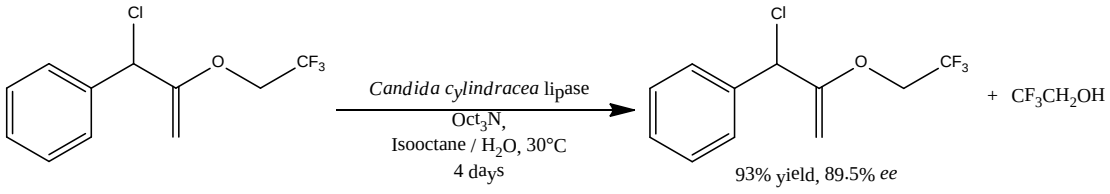

Figure 2.5: DKR of activated esters using a base for racemization.

The DKR carried out using strong base such as sodium hydroxide has also been conducted with an impressive conversion and enantiomeric excess. The lipase-catalyzed DKR of racemic naproxen methyl ester was carried out in a membrane

bioreactor (Xin et al., 2001). Tubular silicone rubber membrane was used inside the stirred tank reactor to separate the chemical catalytic racemization and biocatalytic resolution process in order to avoid incompatibility between *in-situ* chemical racemization with the presence of biocatalyst. Additionally, hydrophilic porous semipermeable membrane was used in the bioreactor to overcome product inhibition as well as to assist product recovery. The reaction gave 60% conversion and 96% enantiomeric excess (ee_P) of the desired (*S*)-naproxen acid. Similar work was also reported on the DKR of racemic ibuprofen ester (Fazlena et al., 2006). The 2-ethoxyethanol was chosen as nucleophiles to give 2-ethoxyethyl-ibuprofen ester as a substrate. The substrate was catalyzed by CRL and *in-situ* racemization was taken place with the addition of 0.5 M aqueous sodium hydroxide solution. The reaction took place in a biphasic batch reactor with isooctane as a solvent and dimethyl sulfoxide (DMSO) as co-solvent. The addition of 20% (v/v) of aprotic polar solvent (DMSO) finally gave 86% conversion and 99.4% optical purity of the desired (*S*)-ibuprofen acid.

The trend of base catalyst used for the resolution of carboxylic acid is closely depending on the substrate derivatives or the leaving group. For a strong nucleophiles leaving group such as thioester derivative, weak base such as trioctylamine was employed. In contrast for the weak nucleophiles leaving group, strong base such as sodium hydroxide was used to catalyze the racemization process. This trend might be related to the racemization process for a substrate with low acidity at the α-carbon position as reviewed previously.

Table 2.2: Dynamic kinetic resolution of optically active drugs.

Substrate	Solvent	Enzyme	Catalyst	Conversion	ee_p	References
(*R*,*S*)-suprofen 2,2,2-trifluoroethyl thioester	Isooctane	Lipase	Trioctylamine	>50%	95%	(Lin et al., 2000)
Tropic acid ethyl ester		Lipase PS	Ruthenium, Isopropyl acetate	60-88%	53-92%	(Atuu et al., 2007)
(*R*,*S*)-2-ethoxyethyl ibuprofen ester	Isooctane-water, DMSO 20% (v/v)	CRL	Sodium hydroxide	86%	99%	(Fazlena et al., 2006)
Naproxen methyl ester	Isooctane, Tris-HCl	CRL	Sodium hydroxide	60%	96%	(Xin et al., 2001)
(*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester	Isooctane	CRL	1,5,7-Triazabicyclo[4,4,0] dec-5-ene (ρ-TBD)	75.5%	58%	(Lin et al., 2003)
Naproxen 2,2,2-trifluoroethyl thioester	Isooctane	CRL	Trioctylamine	>70%	92%	(Chang et al., 1999)
1,2,3,4-Tetrahydroisoquinoline-1-carboxylic acid (ethyl ester)	Toluene-acetonitrile (4:1), 1eqiv H_2O	CAL-B	Dipropylamine	80%	96%	(Paal et al., 2007)
(*R*,*S*)-fenoprofen 2,2,2-trifluoroethyl thioester	Isooctane	Lipase MY	Trioctylamine	91%	91%	(Chen et al., 2002b)

2.7 Enzymes in Organic Solvents

An addition of enzyme in organic solution significantly changes its original natural environment as in an aqueous solution. It was found that enzyme, especially from the lipase group could retain their activities when placed in low-water media (Klibanov, 1990). This finding gives opportunity to substrate, which less soluble in aqueous medium such as chiral drugs. The use of organic medium in enzymatic catalysis has increased its application in industrial chemistry. The catalysis conducted in water medium always being accompanied by side reactions such as hydrolysis, racemization, polymerization and decomposition. Furthermore, the removal of water is energy consuming due to its large heat of vaporization and high boiling point. Biotransformation in organic solvents offers the following advantages (Ghanem, 2007);

i. Better overall yield and the recovery of the product is facilitated by the use of low-boiling point organic solvents.
ii. Non-polar substrates are converted at a faster rate, due to their increased solubility.
iii. Microbial contamination is negligible in the case of using living cells in biotransformations.
iv. Deactivation and substrate or product inhibition is minimized.
v. Side reactions such as unfavorable hydrolysis are largely suppressed.
vi. Immobilization of enzymes is not required; the enzyme can be recovered by simple filtration.
vii. Denaturation of enzymes is minimized in organic solvents.