CANDIDA ANTARTICA LIPASE B MEDIATED KINETIC RESOLUTION OF RACEMIC ACEBUTOLOL

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2015

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This thesis is submitted in fulfilment of the
requirement for the degree of
Doctor of Philosophy

October 2015

ACKNOWLEDGEMENT

Alhamdulillah. I am grateful towards Allah for giving me strength and guidance through my study.

First of all, I would like to convey my heartiest appreciation to both my supervisors, Prof. Dr. Azlina Harun @ Kamaruddin and Prof. Dr. Subhash Bhatia for their expert advice, prestigious guidance and supervision in the coordination of the research project till the completion of this thesis.

Thanks to all the lecturers, laboratory technicians, and support staff of School of Chemical Engineering USM for their assistance. On top of that, I would like to express my appreciation to all friends especially my laboratory-mate, for their support and encouragement.

Finally, would like to express my gratitude to my parents, Mr. Rajin Alip and Mrs. Mailin Duwat; my parents in law, Hj. Mat Midin Ya'ali and Mrs. Nursiah Satar; my supportive husband, Ustaz Mohd Norjefri Mat Midin; my beloved daughter, Khaulah Amsyar Mohd Norjefri, and all family members.

Only Allah can repay all of you. Thank you.

Mariani Rajin

October 2015

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LIST OF SYMBOLS

$[S_{ace}]$	Initial concentration of the acebutolol
$[S_{va}]$	Initial concentration of the vinyl acetate
C_o	Initial concentration of racemic acebutolol
C_R	Concentration of R-acebutolol
C_S	Concentration of S-acebutolol
C_t	Concentration of racemic acebutolol at *t*
E	Enantiomeric ratio
E_d	Deactivation energy
Ee_p	Enantiomeric excess of product
ee_s	Enantiomeric excess of substrate
h	Planck's constant
k_b	Boltzmann constant
k_d	Deactivation constant
K_{Iace}	Dissociation constant for acebutolol
K_{Iva}	Dissociation constant for vinyl acetate
K_{Mace}	Michaelis Constant for acebutolol
K_{Mva}	Michaelis Constant for vinyl acetate
R	Universal Gas Constant
v_i	Reaction velocity
V_{max}	Maximum reaction velocity
X	Conversion
ΔS	Entropy
ΔH	Entalphy

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CALB	*Candida antarctica* B lipase
CCD	Central composite design
CCL	*Candida cylindracea* lipase
CRL	*Candida rugosa* lipase
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EMR	Enzymatic membrane reactor
FCCD	Face centered central composite design
HPLC	High performance liquid chromatography
L	Liter
mg	Milligram
mM	Milimolar
PAN	Polyacrynitrile
PCL	*Pseudomonas cepacia* lipase
PFL	*Pseudomonas fluorescens lipase*
PSL	*Pseudomonas* species lipase
rpm	Rotation per minute
RSM	Response surface methodology
THF	Tetrahydrofuran
TPM	Transmembrane pressure
UF	Ultrafiltration

RESOLUSI KINETIK TERHADAP RASEMIK ACEBUTOLOL MENGGUNAKAN PERANTARAAN LIPASE *CANDIDA ANTARTICA* B

ABSTRAK

Asebutolol masih lagi dipasarkan dalam bentuk rasemik sehingga kini. Kecenderungan terhadap penghasilan enantiomer tunggal bagi ubat-ubatan berbentuk kiral adalah didorong oleh beberapa faktor antaranya kesan yang berbeza daripada enantiomer tersebut, permintaan dalam pasaran yang semakin meningkat, juga peraturan yang semakin ketat terhadap pengeluaran ubat-ubatan tersebut. Oleh itu, resolusi kinetik terhadap rasemik asebutolol dikaji menggunakan perantaraan lipase dalam reaktor kelompok dan reaktor membran berenzim. Kaedah sambutan permukaan dengan rekabentuk *central composite design* (CCD) digunakan untuk analisis data bagi reaktor kelompok. Faktor yang dikaji adalah merangkumi jumlah enzim, kepekatan substrat dan penderma asil dan suhu tindakbalas. Kajian mendapati bahawa tindabalas di dalam reaktor kelompok ini mencapai optimum dengan 320 mg enzim, 50 mM kepekatan Asebutolol, 140 mM kepekatan vinil asetat and suhu 40 ^{o}C, memberikan kadar pertukaran sebanyak 46% dengan nilai E dan ee_s masing-masing 15 and 73%. Tenaga pengaktifan dan penyahtabii bagi enzim di dalam reaktor kelompok dalam kajian ini dianggarkan masing-masing sebanyak 39.63 kJ/mol dan 54.90 kJ/mol. Pemalar pendeaktifan k_d meningkat sebanyak 0.012-0.031 per jam dengan peningkatan suhu daripada 45 ^{o}C ke 60 ^{o}C. Nilai entalpi adalah 52.12 kJ/mol.K dan entropi adalah -0.18 kJ/mol.K. Berdasarkan dapatan daripada tindak balas di dalam reaktor kelompok, resolusi kinetik Acebutolol telah berjaya dilakukan di dalam reaktor membran berenzim. Kesan jumlah enzim, kepekatan substrat dan

penderma asil, pH larutan penimbal, suhu tindakbalas, kadar aliran fasa organik dan tekanan transmembran. Tindakbalas di dalam reaktor membran berenzim mencapai nilai optimum pada pH 7, 40 ^{o}C dan TMP 6 psi, dengan kadar aliran fasa organik sebanyak 40 ml/min. Ia memberikan nilai pertukaran 40%, E sebanyak 23 dan ee_s sebanyak 84%. Tindakbalas enzim di dalam reaktor kelompok dan reaktor membran berenzim kedua-duanya mematuhi mekanisme Ping Pong Bi Bi. Parameter kinetik untuk enzim bebas di dalam reaktor kelompok adalah seperti berikut: K_{Mace} =8.53 mM, K_{Mva} =5.19 mM, dan V_{max} =1.18 mM/h. Manakala parameter kinetik untuk enzim tersekatgerak di dalam reaktor membran berenzim adalah seperti berikut; $K_{mace\ app}$ = 2.13 mM, K_{Mva}app = 1.23 mM dan $V_{max\ app}$ =2.33 mM/h. Nilai pemalar perencat pula adalah K_{Iace}=10.72 mM, $K_{Iva,}$= 3.71 mM, $K_{Iace\ app}$ = 11.56 mM dan $K_{Iva\ app}$=3.89 mM. Prestasi CALB di dalam kedua-dua jenis reaktor telah dibandingkan. Enzim tersekatgerak di dalam reaktor membran berenzim memberikan kapasiti tindak balas yang lebih tinggi, kestabilan terma yang lebih baik, afiniti yang lebih tinggi kepada substrat dan juga menunjukkan rintangan tinggi terhadap kesan perencat berbanding enzim bebas di dalam reaktor kelompok. Kelebihan enzim tersekatgerak dilihat amat berpotensi untuk diaplikasikan dalam industri penghasilan enantiomer tunggal, terutamanya penyekat beta dalam masa terdekat.

CANDIDA ANTARTICA LIPASE B MEDIATED KINETIC RESOLUTION OF RACEMIC ACEBUTOLOL

ABSTRACT

Acebutotol is still available in racemic form. The increasing preference for single or pure enantiomer of chiral drugs is driven mainly by the different effects of the enantiomers, the high market demand and the guidelines issued by regulatory authorities. Therefore, the kinetic resolution of racemic acebutolol is studied in batch and enzymatic membrane reactor. The response surface methodology based on central composite design (CCD) was employed for optimization and analysis of kinetic resolution of racemic acebutolol in a batch reactor. The process variables which were taken into account include; enzyme loading, substrate concentration, acyl donor concentration and temperature. The optimum conditions were found to be 320 mg of enzyme loading, with acebutolol concentration of 50 mM, vinyl acetate concentration of 140 mM and temperature at 40 ^{o}C, giving the overall conversion of 46.6%. The value of enantioselectivity *E* and enantiomeric excess of the substrate ee_s were found to be 15 and 73%, respectively. Lipase activation and deactivation energy was estimated to be 39.63 kJ/mol and 54.90 kJ/mol, respectively. Denaturation constant, k_d was increasing from 0.012-0.031 h^{-1} with the increasing temperature from 45 ^{0}C to 60 ^{0}C. The value of enthalpy and entropy for free *Candida antartica* lipase B were 52.12 kJ/mol.K and -0.18 kJ/mol.K, respectively. Based on the finding from the batch reaction, kinetic resolution of acebutolol has been successfully conducted in enzymatic membrane reactor (EMR). The effects of enzyme loading, substrate and acyl donor concentration, pH of buffer solution,

reaction temperature, and organic phase flow rates, and transmembrane pressure (TMP) were investigated. The optimum operating conditions for the lipase-catalyzed kinetic resolution in an EMR system were pH 7, 40 ^{o}C and TMP of 6 psi at organic flow rate of 40 ml/min. This condition gave 40% overall conversion, enentioselectivity of 23 and ee_s of 84%. The reaction kinetic was found to obey the Ping Pong Bi Bi mechanisms for both free and immobilized lipase from *Candida antartica* B . The kinetic parameters for the free lipase were: K_{Mace} =8.53 mM, K_{Mva} =5.19 mM, and V_{max} =1.18 mM/h. The apparent kinetic parameters for the immobilized lipase were: $K_{mace\ app}$ = 2.13 mM, K_{Mva}app = 1.23 mM and $V_{max\ app}$ =2.33 mM/h. The kinetics of kinetic resolution accounted for both substrates inhibitions. The inhibition constants were given by K_{Iace}=10.72 mM, $K_{Iva,}$= 3.71 mM, $K_{Iace\ app}$ = 11.56 mM and $K_{Iva\ app}$=3.89 mM. The performance of free and immobilized CALB were compared. The immobilized lipase in EMR gave higher reaction capacity, better thermal stability, higher affinity to the substrates and exhibited higher resistance towards the inhibition effect. The advantages of immobilized enzyme makes it possible for economical industrial production of chiral drugs, particularly beta blockers in the near future.

CHAPTER 1

INTRODUCTION

1.1 Chiral Drugs in Pharmaceutical Industries

The word chiral is derived from the greek word *cheir*, meaning hand, to describe the handedness of the molecule. An object is said to be chiral if its two mirror image forms are not superimposable in the three dimension. The opposite of chiral is achiral (Morris, 2002).

A pair of stereoisomers that are non-superimposable mirror images of one another is called enantiomer. They have different three dimension configurations. Whereas, the mixture of two enantiomers in equal portion is referred to as a racemate. Enantiomers can be named by different methods. For example, prefixes (R) and (S) can be used for right hand and left hand enantiomers, respectively. The other methods are by using (+) and (-) or D and L (Sheldon, 1993). This general prefix applies to all bioactive substances including pharmaceutical compounds (Sheldon, 1996).

In an achiral environment, the enantiomers of chiral drugs show similar physical and chemical properties. Both enantiomers have identical molecular weight, solubility and melting point. However, in a chiral environment, the chemical and pharmacologic behaviors of the enantiomers may be differ (Somogyi, 2004; Caldwell, 2001). Drugs work by binding to the specific biological sites (or drug binding sites), such as proteins (receptors, enzymes), nucleic acids (DNA and RNA) and biomembranes (phospholipids and glycolipids) present in the body.

Our body, which consists of numerous homochiral compound interacts with racemic drugs differently and metabolize each enantiomer by separate pathways, thus generate different pharmacological activity. One enantiomer may produce the desired healing effects, while the other may be inactive or produce undesired effects. In pharmacology, the active enantiomer is known as eutomer, while the inactive enantiomer is referred as distomer (Nguyen *et al*., 2006 and Shafaati, 2007).

Figure 1.1 illustrates the molecular mechanism of chiral pharmacology and toxicity, which is highly affected by the interaction between a chiral drug and its chiral binding site. As shown in the figure, the portion of the drug labeled as A, B and C. Meanwhile, a, b and c represent the region of the binding site. In order to have its desired therapeutic effect, A, B, and C must interact with the corresponding a, b, and c. The three dimensional structure of the eutomer can be aligned with the binding site in order to allow A to interact with a, B to interact with b, and C to interact with c. On the other hand, for the distomer, no matter how it is rotated in space, it failed to bind to its corresponding binding sites simultaneously. As a result, it fails to exert a desirable effect. In a few cases, the distomer interacts with an unusual binding site leading to undesirable biological effects (McConathy and Owens, 2003).

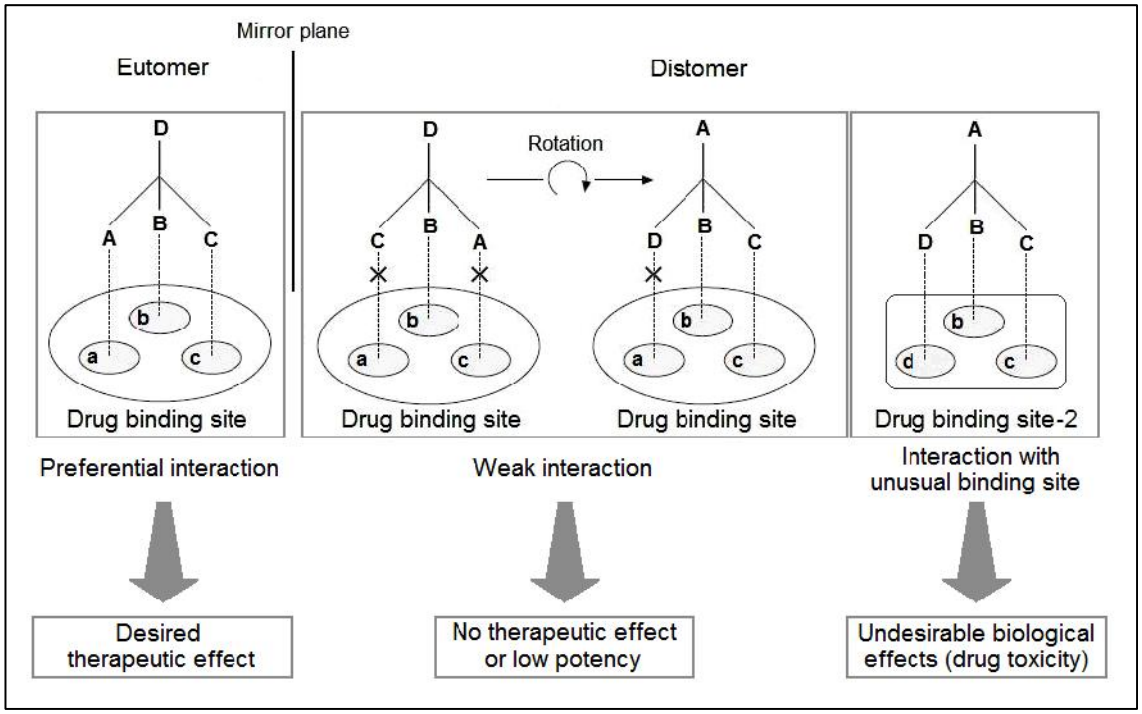

Figure 1.1 : The hypothetical interaction between the two enantiomers of a chiral drugs and its binding site (McConathy and Owens, 2003; and Vadya, 2011)

1.2 Market Trends of Chiral Drugs

Recently, much attention on the development of chiral drugs has been growing rapidly due to the increased awareness in stereochemistry of drugs. Strict regulation has been defined by the Food and Drug Administration (FDA). In order to patent a new racemic drug, full profiles for both enantiomers as well as the racemic mixture of the chiral drug has to be documented separately. The information in the document should provide pharmacological and pharmacokinetic properties of the new patented drug (Maier *et al*., 2001, Viegas *et al* ., 2007a, Cho *et al*., 2002).

From the economic point of view, chiral drugs continue to make a significant contribution to the global pharmaceutical markets. Earlier in 1997, about 50% of the top 500 drugs are marketed as single enantiomer (Stinson 1998; and Maier *et al*.,