SYNTHESIS AND CHARACTERIZATION OF NEW TETRAETHYLAMMONIUM-BASED CHIRAL IONIC LIQUIDS FOR BIOCATALYSIS APPLICATION

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By

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Chiral ionic liquids (CILs), molten salts at temperature below than 100 °C are unique liquids having different characteristics from ordinary liquids. Since these CILs are prepared by coupling various organic ions, there are many chances to create novel functionalities by changing structure of components ions. These liquids combine with their ability to perform a task with their “green” character, which makes them environmental friendly solvents. Lately, researchers focused to synthesize CILs for their potential applications in many reactions such as chiral discrimination.

In this study, eleven new CILs derived from chiral amino acids and plant acid have been synthesized and characterized. They are tetraethylammonium L-serinate, tetraethylammonium L-prolinate, tetraethylammonium L-threoninate, tetraethylammonium L-isoleucinate, tetraethylammonium L-asparaginate, tetraethylammonium L-glutaminate, tetraethylammonium L-glutamate, tetraethylammonium L-methioninate, tetraethylammonium L-histidinate, tetraethylammonium L-lysinate and
tetraethylammonium L-malate. Meanwhile, one compound derived from chiral plant acids (tetraethylammonium L-tartarate) can’t be classified as ILs due to high melting point above 100 °C.

All of these salts were prepared using simple neutralization reaction which gave good overall yield (>85 % yields for CILs derived from amino acids and 98 % for tetraethylammonium L-malate) at room temperature. \(^1\)H NMR and elemental analysis were carried out to identify the molecular structure and purity of CILs produced. Colour of each CILs’s produced depending on the anions used. All CILs were hygroscopic and easily dissolved in water and polar organic solvents. The new CILs synthesized from plant acid have higher melting point (\(T_m \ 87.7 ± 0.4 \ °C\)) compared to CILs derived from amino acids (\(T_m \ 54.3 ± 1.0 \ °C\) for tetraethylammonium L-histidinate and \(T_m \ 58.7 ± 1.0 \ °C\) for tetraethylammonium L-asparaginate).

The thermal properties were studied by using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC analysis showed that CILs in a liquid form had no melting point (\(T_m\)) and glass transition temperature (\(T_g\)). Meanwhile, in the TGA study, it was found that CILs derived from amino acids have a slightly low decomposition temperature (\(T_{onset} \ 168 \ to \ 210 \ °C\)) compared to tetraethylammonium L-malate (\(T_{onset} \ 210 \ °C\)), but all CILs were stable up to 160 °C.

Single crystal X-ray diffraction was used to solve the crystal structures of tetraethylammonium L-malate and tetraethylammonium L-tartarate. The crystal systems for both compounds were monoclinic. Analysis also carried out in order to reveal an extensive series of hydrogen bonds between H-atoms on the cation and the anion meanwhile each CILs’ optical polarity was measured using polarimeter. Their viscosity
and ionic conductivity for CILs in a liquid form were also determined and observed by using viscometer and conductivity meter. The strong correlation between viscosity and ionic conductivity was observed.

In biocatalysis application, chiral ionic liquid-coated enzyme (CILCE) was employed in the esterification of oleyl alcohol with various fatty acids. CILCE was prepared by simple method involving coating of *Candida rugosa* lipase with tetraethylammonium L-asparaginate and was found to give a better percentage of conversion of ester (60 – 81 %) compared to native enzyme (36 – 70 %) for all fatty acids from short, medium and long alkyl chains.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

**SINTESIS DAN PENCIRIAN CECAIR IONIK KIRAL YANG BAHARU BERASASKAN TETRAETILAMONIUM UNTUK APLIKASI BIOMANGKIN**

Oleh

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Cecair ionik kiral (CILs), garam lebur pada suhu di bawah 100 ºC adalah cecair unik yang mempunyai ciri yang berbeza daripada cecair biasa. Disebabkan cecair ini disediakan melalui pemadanan pelbagai ion organik, terdapat banyak kemungkinan untuk mencipta fungsi CILs yang baharu dengan menukar struktur komponen ion. Cecair ini digabungkan dengan kemampuannya untuk melakukan tugas dengan ciri “hijaunya” yang mana membuatkan cecair ini pelarut yang mesra alam. Kebelakangan ini, ramai penyelidik menumpukan perhatian terhadap sintesis CILs untuk melihat potensi kegunaannya di dalam banyak tindak balas seperti diskriminasi kiral.

Dalam kajian ini, sebelas cecair ionik kiral baharu yang berasal dari kiral asid amino dan asid tumbuhan telah disintesis dan dicirikan. Sebatian tersebut adalah tetraetilamonium L-serinat, tetraetilamonium L-prolinat, tetraetilamonium L-threoninat, tetraetilamonium L-isoleucinat, tetraetilamonium L-sasparaganat, tetraetilamonium L-glutaminat, tetraetilamonium L-glutamat, tetraetilamonium L-methioninat, tetraetilamonium L-histidinat, tetraetilamonium L-lysinat dan tetraetilamonium L-malat. Sementara itu, satu
sebatian lain yang disintesis daripada kiral asid tumbuhan (tetraetilamonium L-tartarate) tidak diklasifikasikan sebagai ceair ionic kiral kerana mempunyai takat lebur melebihi 100 °C.

Kesemua garam CILs ini telah disediakan menggunakan tindak balas penulenan mudah yang memberikan hasil keseluruhan yang baik (>85 % hasil untuk CILs yang berasal daripada kiral asid amino dan 98 % hasil untuk tetraetilamonium L-malat) pada suhu bilik. 

\(^1\)H resonan magnetic nuklear (RMN) dan analisis elemen dilakukan untuk mengenalpasti struktur molekul dan ketulenan CILs yang dihasilkan. Warna untuk setiap CILs yang terhasil adalah bergantung kepada anion yang digunakan. Semua CILs didapati bersifat higroskopik dan mudah larut di dalam air dan pelarut organik polar. Satu CILs baharu yang disintesis daripada kiral asid tumbuhan mempunyai takat lebur yang tinggi (\(T_m\) 87.7 ± 0.4 °C) berbanding CILs yang berasal daripada kiral asid amino (\(T_m\) 54.3 ± 1.0 °C untuk tetraetilamonium L-histidinat dan \(T_m\) 58.7 ± 1.0 °C untuk tetraetilamonium L-asparaginat).

Sifat termal telah dipelajari menggunakan kalorimeter pengimbasan kebezaan (DSC) dan analisis thermogravimetrik (TGA). Analisis DSC menunjukkan CILs dalam bentuk ceair tidak mempunyai takat lebur (\(T_m\)) dan suhu peralihan gelas (\(T_g\)). Sementara itu, dalam kajian TGA, didapati bahawa CILs yang berasal daripada kiral asid amino mempunyai suhu penguraian yang sedikit rendah (\(T_{onset}\) 168 hingga 210 °C) berbanding tetraethylammonium L-malate (\(T_{onset}\) 210 °C), tetapi kesemua CILs stabil sehingga suhu 160 °C.

Penyerakkan sinar-X kristal tunggal telah digunakan untuk membuktikan struktur sebatian tetraethylammonium L-malat dan tetraethylammonium L-tartarate. Didapati bahawa sistem kristal bagi kedua-dua sebatian ini adalah monoklinik. Analisis juga telah dilakukan untuk
menunjukkan rangkaian ikatan hidrogen di antara atom hidrogen kation dan anion manakala polariti optik bagi setiap CILs ditentukan dengan menggunakan polarimeter. Kepekatan dan konduktiviti ionik untuk CILs dalam bentuk cecair juga ditentukan dan diamati menggunakan alat kepekatan dan meter konduktiviti. Perhubungan yang kuat di antara kepekatan dan konduktiviti ionik telah diamati.

Dalam aplikasi biomangkin, enzim bersalut cecair ionic kiral (CILCE) telah digunakan dalam tindakbalas pengeksteran olil alkohol dengan pelbagai asid lemak. CILCE telah disediakan melalui kaedah yang mudah dengan menyalut lipase daripada *Candida rugosa* dengan tetraetilamonium L-asparaginat dan penggunaan CILCE telah dikaji dan memberikan peratus penukaran ester yang lebih baik (60 – 81 %) berbanding enzim asli (36 – 70 %) bagi semua asid lemak daripada asid lemak berantai pendek, sederhana dan panjang.
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I certify that a Thesis Examination Committee has met on 17th November 2009 to conduct the final examination of Khairulazhar Jumbri on his thesis entitled “Synthesis and Characterization of New Chiral Ionic Liquids for Biocatalysis Application” in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science Degree

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Date: 17 March 2010
DECLARATION

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

__________________________
KHAIRULAZHAR JUMBRI

Date: 18 January 2010
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LIST OF ABBREVIATIONS

ILs ionic liquids
RTILs room temperature ionic liquids
CILs chiral ionic liquids
MeOH methanol
\( T_m \) melting temperature
\( T_g \) glass transition
\( T_{\text{start}} \) start temperature
\( T_{\text{onset}} \) onset temperature
EtOH ethanol
dd doublet of doublets
t triplet
s singlet
q quartet
m multiplet
asn asparagine
gln glutamine
 glu glutamic acid
his histidine
ile isoleucine
lys lysine
mal malic acid
met methionine
pro proline
ser serine
thr threonine
\([\text{BF}_4^-]\) tetrafluoroborate
\([\text{PF}_6^-]\) hexafluorophosphate
\([\text{ Tf}_2\text{N}^-]\) bis(trifluoromethylsulfonyl)imide
[N\textsubscript{2222}] tetraethylammonium
VOCs volatile organic compounds
VOSs volatile organic solvents
CILCE chiral ionic liquid-coated enzyme
ILCE ionic liquid-coated enzyme
CRL \textit{Candida rugosa} lipase
CHNS/O carbon hydrogen nitrogen sulphur/oxygen
DSC Differential Scanning Calorimetry
TGA Thermogravimetric Analysis
OVERVIEW OF THE CHEMICAL STRUCTURES

1. \([\text{N}_2\text{H}_2\text{N}_2\text{H}_2\text{N}_2]\)[ser]: tetraethylammonium L-serinate

2. \([\text{N}_2\text{H}_2\text{N}_2\text{H}_2\text{N}_2]\)[pro]: tetraethylammonium L-prolinate

3. \([\text{N}_2\text{H}_2\text{N}_2\text{H}_2\text{N}_2]\)[thr]: tetraethylammonium L-threoninate

4. \([\text{N}_2\text{H}_2\text{N}_2\text{H}_2\text{N}_2]\)[ile]: tetraethylammonium L-isoleucinate

5. \([\text{N}_2\text{H}_2\text{N}_2\text{H}_2\text{N}_2]\)[asn]: tetraethylammonium L-asparagine

6. \([\text{N}_2\text{H}_2\text{N}_2\text{H}_2\text{N}_2]\)[gln]: tetraethylammonium L-glutamine
7. \([\text{N}_{2222}][\text{glu}]:\) tetraethylammonium L-glutamate

\[
\text{HO-}N^+ \text{NH} \quad \text{O-}
\]

8. \([\text{N}_{2222}][\text{met}]:\) tetraethylammonium L-methioninate

\[
\text{N}^+ \text{O-} \text{NH}_2 \quad \text{S} \quad \text{O-}
\]

9. \([\text{N}_{2222}][\text{his}]:\) tetraethylammonium L-histidinate

\[
\text{N}^+ \text{O-} \text{NH}_2 \quad \text{N}^+ \text{O-} \text{NH}_2 \quad \text{N}^+ \text{O-} \text{NH}_2
\]

10. \([\text{N}_{2222}][\text{lys}]:\) tetraethylammonium L-lysinate

\[
\text{N}^+ \text{O-} \text{NH}_2 \quad \text{N}^+ \text{O-} \text{NH}_2 \quad \text{N}^+ \text{O-} \text{NH}_2
\]

11. \([\text{N}_{2222}][\text{mal}]:\) tetraethylammonium L-malate

\[
\text{N}^+ \text{O-} \text{OH} \quad \text{O-}
\]
CHAPTER 1

INTRODUCTION

Lately, ionic liquids (ILs) are attracting a number of overwhelmed science and industrial communities as a reaction media, extraction solvents, electrolytes and in life sciences (Kagimoto et al., 2007). These liquids that contain ions show good and tunable solubility properties with negligible vapor pressure and excellent thermal stability have rapidly found as valuable substitutes for many volatile solvents (Welton, 1999 and van Rantwijk et al., 2003).

ILs are a new class of solvents that currently receiving much attention for their wide range of applications especially in catalysis and biocatalysis, dissolving polar to non-polar substrates and almost anything including coal, plastics, metal and even rock (Obliosca et al., 2007). They are not as flammable as the volatile organic solvents (VOSs) therefore, making process safety and environmental concerns less of an issue. The thermodynamics and kinetics of reactions carried out in ILs can possibly varied to those in traditional VOSs and therefore creating great interests amongst chemists in their potential as solvents, co-solvents and catalysts.

A wide range of ILs consisted of inorganic/organic cations and anions have been successfully synthesized. In view of the emerging importance of ILs as reaction media in organic synthesis, researchers have turn their attraction on the synthesis of chiral ionic liquids (CILs) for their particularly potential applications to chiral discrimination, including asymmetric synthesis and optical resolution of racemates (Wang et al., 2005). Several examples of CILs were mentioned in the literature and partial information can be
found in some reviews reported by Baudequin et al. (2005) and Tran et al. (2006). Due to their ease of synthesis and their particular chiral properties, these new CILs should play a central role in enantioselective research and expand the scope of chiral solvents. A significant transfer of chirality in these solvents can be expected due to their high degree of organization.

The specific properties of CILs should perform the classical chiral solvents for asymmetric induction. Studies about the application of CILs in asymmetric synthesis are not only an opportunity but also a challenge for researchers. It is interesting, meaningful and necessary to synthesize different kinds of CILs from different starting materials, especially from the chiral pool. Good chemical and configurational stability, are some of the most important criteria for synthesis of CILs and necessary properties for their application to chiral discrimination.

Another great property of ILs is their insolubility in most organic solvents which led us to envisage that they might be suitable as coating materials for immobilizing enzymes or cells. Interestingly, van Rantwijk et al. (2003) observed that ILs can enhance the selectivity of an enzyme. It was also demonstrated that they are useful as a media for the enzymatic reaction of polar substrates, which are difficult to dissolve in conventional organic solvents (Park and Kazlauskas, 2003). The very first ionic liquid coated enzyme (IL-CE) was reported by Lee and Kim (2002), which is easily prepared and exhibits markedly enhanced enantioselectivity and stability.

The aim of the study was to synthesis and characterizes a series of new CILs, and used the suitable CILs as biocatalysts in esterification reactions. To do this, the selected CILs, tetraethylammonium L-asparaginate was coated onto Candida rugosa lipase to form chiral
ionic liquid-coated enzyme (CILCE). CILCE was later tested for esterification reactions of oleyl alcohol with various fatty acids in order to figure out their amazing properties.

1.1 Objectives of the Research

This research embarks on the following objectives:

1) To synthesize twelve new tetraethylammonium-based chiral ionic liquids (CILs) derived from amino acids and chiral plant acids.

2) To characterize the physico-chemical properties of CILs.

3) To identify the suitable CILs for coating with Candida rugosa lipase (CRL) to form chiral ionic liquid-coated enzyme (CILCE).

4) To determine the enzymatic activity of CILCE in esterification reactions.