MOLECULAR INTERACTION OF PATCHOULI EXTRACTION PROCESS USING MOLECULAR DYNAMIC SIMULATION APPROACH

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Thesis submitted in fulfilment of the requirements
for the award of the degree of
Master of Engineering (by research) in Chemical Engineering

Faculty of Chemical and Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

JULY 2014

ABSTRACT

Patchouli is one of the native commercial crops in Malaysia which has various applications in the fragrances and cosmetics industries as well as in alternative medicine area. These are due to the interesting pharmacological properties and aroma of its essential oil. Currently, patchoulol, a marker compound is still not produced synthetically. The demand for patchouli essential oil will increase as patchoulol has been found to possess antiviral property specifically towards H1N1 virus. This study aims to recognise the intermolecular interaction during the patchouli extraction process through the application of molecular dynamics simulation and explaining the process at molecular scale with the extraction yield as a correlation. Therefore this study is focussed on the solvent extraction experiment and molecular dynamic simulation. Simulation works comprise comparison between hydro-distillation and solvent extraction techniques with three types of solvents; the polar protic, polar aprotic and non-polar solvents. The temperature variation effect on solvent extraction is also simulated. Results suggest that a polar protic solvent of ethanol can establish a higher degree of hydrogen bonding and produce the highest extraction yield (30.99% wt/wt) which suggested a good correlation. Meanwhile an interesting finding is that hexane as non-polar solvent managed to shift the O1P---H1P interaction up to 5.75Å from 1.75Å in pure patchoulol system and extracted patchouli oil in higher yields compared to polar aprotic solvent of acetone. The molecular dynamics simulation work revealed that there is repulsion between O1A---O1P atom which may retard the solute solubility and produced the lowest extraction yield. The molecular dynamics simulation enable visualisation of the hydrophobic character of the patchoulol compared to well distribute solute in the ethanol solvent extraction binary system. The temperature variation effect study found that the small temperature increment of 5K may be the reason for a slight perturbation in the interaction structure which was captured by the molecular dynamics simulation.

ABSTRAK

Nilam adalah salah satu tanaman komersial asli di Malaysia yang mempunyai pelbagai aplikasi dalam industri wangian dan kosmetik serta sebagai perubatan alternatif. Ini adalah disebabkan oleh sifat-sifat farmakologi yang menarik dan aroma minyak patinya. Pada masa ini patchoulol sebagai sebatian penanda masih tidak boleh dihasilkan secara sintetik. Permintaan untuk minyak pati nilam dijangka akan meningkat disebabkan penemuan sifat farmakologi menarik patchoulol sebagai anti virus terutamanya terhadap virus H1N1. Kajian ini bertujuan untuk mengenal pasti interaksi molekul semasa proses pengekstrakan nilam menerusi penggunaan simulasi dinamik molekul dan digunakan untuk menjelaskan proses pada skala molekul dengan hasil pengeluaran sebagai petanda korelasi antara dua perkara ini. Terdapat dua aktiviti utama dalam kajian ini iaitu eksperimen pengekstrakan pelarut dan kerja-kerja simulasi. Kerja-kerja simulasi terdiri daripada perbandingan antara teknik penyulingan hidrodan teknik pengekstrakan menggunakan pelarut dengan tiga jenis pelarut iaitu pelarut kutub protik, kutub aprotik dan bukan polar. Kesan perubahan suhu pada pengekstrakan pelarut juga turut disimulasi. Keputusan simulasi menunjukkan bahawa pelarut etanol sebagai pelarut polar protik boleh menghasilkan ikatan hidrogen yang lebih banyak serta kadar pengeluaran ekstraksi yang tinggi (30% berat/berat) yang menunjukkan pertalian yang baik antara kerja simulasi dan eksperimen pengekstrakan. Sementara itu satu penemuan menarik ialah heksana sebagai pelarut bukan kutub berjaya menjarakkan interaksi O1P---H1P sehingga 5.75Å daripada 1.75Å dalam sistem patchoulol tulen dan mengekstrak minyak nilam yang lebih tinggi berbanding dengan pelarut kutub aprotik, seperti aseton. Simulasi molekul dinamik menunjukkan bahawa terdapat penolakan O1A---O1P atom yang mungkin mengurangkan keterlarutan bahan larut dan menghasilkan hasil pengeluaran yang rendah. Simulasi molekul dinamik dapat menggambarkan sifat hidrofobik bahan larut patchoulol berbanding dengan penyerakkan bahan larut yang baik di dalam sistem binari pelarut etanol-patchoulol bagi teknik ektraksi menggunakan pelarut. Kajian kesan perubahan suhu mendapati bahawa kenaikan suhu yang kecil iaitu hanya 5K menghasilkan perubahan yang kecil didalam struktur interaksi, namun masih dapat diukur oleh simulasi molekul dinamik.

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LIST OF SYMBOLS

a	Acceleration
ρ_y	Density of y type atom
$E_{stretch}$	Energy bond stretch term
$E_{qoulumbic}$	Energy Coulombic interaction
E_{bend}	Energy angle term
$E_{out\ of\ plane}$	Energy improper dihedral term
$E_{torsion}$	Energy torsion term
$E_{van\ der\ waal}$	Energy van der waals interaction
h	Plank's constant (6.626×10^{-34} m^2kgs^{-1})
υ	Orbiting frequencies of electrons
ε	Well depth
N_y	Number of atoms of the y type
q	Partial charges
α	Polarisability
σ	Collision diameter
Å	Angstrom
Q	Fictitious mass
V	Potential energy
r	Radius
l	Bond length
ω	Dihedral angle
θ	Angle
φ	Torsion
m	Mass

μ	Dipole moment
$\pi\varepsilon_o$	Permeability of vacuum

LIST OF ABBREVIATION

2^{nd}	Second
3^{rd}	Third
μm	Micrometer
atm	Atmospheric pressure
CCRC	Caritas Czech Republic Corporation
COMPASS	Condensed-phase Optimized Molecular Potentials for Atomistic Simulation Studies
DPPH	1,1-diphenyl-2-picylhydrazyl
fs	Femtosecond
GCMS	Gas Chromatography Mass Spectrometer
g(r)	Probability valueof how frequently the nearest neighboring atoms move along the spherical radius
kg	Kilogram
LJ	Lennard-Jones
MD	Molecular dynamics
N	Number of molecules
NIST	National Institute of Standards Technology
NVE	Constant number of moles, volume and energy ensemble
NPT	Constant number of moles, pressure and temperature ensemble
NVT	Constant number of moles, volume and temperature ensemble
ns	Nano second
P	Pressure
ps	Pico second
OPLS	Optimized Potential for Liquid Simulations

R, r	Radial
rdf	Radial distribution function
T	Temperature
TraPPE	Transferable Potentials for Phase Equilibria
TIP3P	Transferable Intermolecular Potential, Three-Position model
vdw	Van der waals
wt	Weight
wt/wt %	Weight / weight percent

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

This chapter discusses the general research background, motivation, objectives, scope and the summary of thesis presentation. This study will investigate the suitability of molecular dynamic simulation as a prediction tool in chemical engineering to enhance the understanding of the extraction mechanism at the molecular level. The general background will describe the materials of study and the molecular dynamic simulation technique.

1.1 BACKGROUND OF THE STUDY

Malaysia has an abundance of native aromatic plants such as patchouli (*nilam*), aloeswood (*gaharu*), jasmine (*melur*) and some other with its own natural scent compounds which can be extracted as essential oils. These essential oils are incorporated into cosmetic, perfumery and toiletries products. During January to April 2012, Malaysia imports about RM 1268 million worth of these essential oils and related products (Malaysia Monthly External Statistic, 2012). Beside its natural scent, the essential oil, also function as the natural antioxidant, aromatherapy agent and additive/preservative agents (Burt, 2004; Celiktas et al., 2007; Dastmalchi et al.,2008).

The demands for natural products particularly essential oils continue to increase as more customers believe that natural products are safer compared to artificial products. In addition, the demand and awareness for green and eco-friendly products today have further promoted the usage of natural scent compounds in cosmetics and toiletries.

Patchouli essential oil extracted from dried patchouli leaves, is well known for its woody and earthy scent (Frater et al., 1998; Deguerry et al., 2006). The patchouli scent has been incorporated in many cosmetic and perfumery products especially in products for men due to their earthy and long lasting aroma characteristic (Raghu, 2006). An important marker constituent of the patchouli essential oil is patchoulol ($C_{15}H_{26}O$). It is also the major compound in patchouli essential oil and has been used to quantify the patchouli essential oil quality which is reflected in the essential oil market price. The global demand for patchouli essential oil is estimated to be around 1600 tonnes annually (Raghu, 2006). Aside from the application of its scent, the patchouli essential oil has also been investigated for various applications such as insect repellent and fungicide application (Hybertson, 2007). Furthermore, the patchouli essential oil has also shown valuable pharmacology potential (Miyazawa et al., 2000; Wei and Shibamoto, 2007; Lu et al., 2011). In addition, the major compound of patchouli oil (patchoulol) has also been explored for its application in the medical field such as anticancer, antioxidant and antiviral properties (Kiyohara et al., 2012; Jeong et al., 2013). The detail discussion on the application of patchouli essential oil is further discussed in the next chapter.

The vast potential of patchoulol and patchouli essential oil in various applications has indeed risen up the demand for patchouli essential oil production. The price for patchouli essential oil in 2011 is estimated around RM600/kg (Fahlevi, 2011). In addition, according to Nilam Aceh: A Study of the Patchouli Oil Industry in Aceh, Indonesia; a book on marketing research on the patchouli plant in 2011 prepared by the Caritas Czech Republic Corporation (CCRC); has reported that there is still a 600 to 800 tonnes gap between the supply and the world demand each year for patchouli essential oil. Hence, it is expected that patchouli related industries in Malaysia such as patchouli plant cultivation and essential oil production will probably expand especially due to the suitability of patchouli to be cultivated in Malaysia with lower catastrophe

risk. Furthermore, up to this point, there is no synthetic technique that has been established to produce patchouli essential oil or its marker compound (Deguerry et al., 2006). Mulder-Krieger et al. (1988) reported that there were no free volatile sesquiterpene compounds accumulated in callus and suspension cultures of the patchouli plant from their study. This indicates that the expansion of patchouli related industries will require continuous research activities mainly in the patchouli extraction field to support the growth of demand.

In the patchouli essential oil production, the optimum condition which contributes to the increased yield of patchouli essential oil during the extraction process is gaining a lot of interest from researchers (Hybertson et al., 2007; Donelian et al., 2009; Yahya, 2012). One of the possible exploration tools is through the computer simulation which focuses on the molecular level interaction. This molecular level simulation technique can reduce the amount of time on the research experimental work, is more economical and environmentally friendly compared to the experimental study. It also promotes a better understanding on interaction between molecules that can be used to explain the mechanism of the extraction process and can be seen as a virtual laboratory (Rapaport, 2004) at the molecular scale.

In this study, the computer simulation technique applied is the molecular dynamics (MD) simulation. It is a powerful tool to help comprehend and reveal intermolecular interaction behaviour between the solute and solvent molecules during the extraction process (Maginn and Elliot, 2010). The solute in this study is patchoulol as the major and marker compound in the patchouli essential oil. While the solvent used is based on the chosen extraction technique, for example the water for hydrodistillation technique. By applying Newton's second law, the simulation will simulate the dynamic aspect of the system. The coordinate system will be used to calculate the radial distribution function (rdf), and diffusion coefficient which can be used to describe the solvation strength towards patchoulol mass transfer behaviour during the extraction. It is expected that the modelling results will be able to give an insight into how crucial parameters can be controlled during the extraction process of patchouli oil, such as solvent type and temperature.

1.2 MOTIVATION

The expansion of various industries such as food and cosmetic have amplified the demand for value added natural products in particular patchouli essential oil. In Malaysia, the market value for the essential oil industry is estimated to be around RM2 billion (Berita IDS, 2000) with the demand for it continuing to increase since the usage has become wider. At present, the focus for most of the researches in the patchouli oil extraction is on how to increase the production yield. For example it was done by utilizing a different extraction technique such as hydro-distillation, solvent extraction and other extraction techniques. However, there is a limitation of present studies which is the inability to explain the interaction at the molecular level during the extraction process, which is an important fundamental aspect to be recognized for optimum process parameter such as solvent, temperature and others.

Thus, the core of this study focuses on understanding the molecular level interaction and providing some molecular information such as solute solubility during the patchouli oil extraction process. The molecular dynamic simulation was used to simulate the molecular interaction of the extraction process. The exploration of the interaction between the solute (patchoulol) molecules and the solvent molecules will enlighten the interpretation of which atoms play a significant role in the interaction of both solute (patchoulol) and solvent molecule. The interaction of solute (patchoulol) and the solvent molecular interaction in the system will help to describe the extraction process through the calculation of solubility parameter. Later, this data can be used to predict the extraction behaviour of solute (patchoulol) using solvent extraction and hydro-distillation method. This tool has been applied by Gunther et al. (2005) to predict the extractability of *Aesculus hippocastanum* and *Harpagophytum procumbent* utilizing super critical carbon dioxide extraction technique. They predicted the extracted yield through calculation of solutes: (-)–α-Bisabolol, Aescin, Harpagoside and Stachyose solubility in the pure and modified supercritical carbon dioxide solvent and have concluded that molecular dynamic simulation can be used to predict the minimum requirement for the supercritical carbon dioxide modifier to extract both lipophilic and hydrophilic compounds.

In addition to fascinating molecular environment exploration, the limited studies (Kongkathip et al., 2009; Fan et al., 2011) that used solvent extraction technique to extract the patchouli oil had given further an encouragement for this study to apply this extraction technique. In addition, both of the study publications applied the solvent extraction to extract the patchouli essential oil which did not discuss gas chromatography results in details. The GCMS is an important characterization because it will determine the patchoulol composition and confirm the patchouli essential oil. Therefore, this study will link GCMS results and estimate the quality of extracted patchouli essential oil using the solvent extraction technique.

Solvent extraction offers various types of solvent with different properties and characteristic which can be applied to have optimum production. One of the appealing concepts is the ability of the solute (patchoulol) to establish the hydrogen bonding with the solvent molecules through its hydroxyl functional group. The hydrogen bond is the strongest intermolecular interaction that is stable and has given the unique properties of water. It is expected the intermolecular interaction will affect the solubility solute in the solvent and then influence the extraction yield which is dependent on the solution chemistry (Adam, 2012). The solution chemistry can be characterised through intermolecular interaction which rely on the solvent properties.

1.3 OBJECTIVES

The main motivation for this study is to gain a fundamental understanding of the molecular science during the patchouli essential oil extraction process as there is avoid in literation on reported work on the usage of this simulation technique to visualize the solute solubility during patchouli extraction process. The key objective of this study is to recognize the intermolecular interaction which is a significant factor influencing the patchouli essential oil extraction process and yield. Furthermore it is interesting to figure out how the simulation work and extraction experiment results can complement each other. Good simulation results will support the extraction experiment results and would be able to describe the process. Therefore this study was embarked upon with the following objectives;

a. To recognise the type of intermolecular interaction has significant effect during the patchouli extraction which was produced via molecular dynamic simulation technique. The simulation will measure the intensity of intermolecular interaction between the solute and the solvent which reflect the solubility of the solute molecules in the solvents.
b. To examine the linkage between simulation work and extraction experiment results to figure out the suitability of the molecular dynamic simulation technique as a prediction tool. Simulation results which in accordance with the extraction experiment results could be used to describe the intermolecular interaction during the extraction process. Furthermore, it is interesting to discover how well the simulation of the chosen solute would be able to describe intermolecular interaction compared to combination of solutes.
c. To determine which solvent can extract the highest patchouli essential oil yield and support with the simulation results as molecular evidence.
d. To examine the prediction capability through simulation at different temperatures since the molecular environment is sensitive to temperature variation.

1.4 SCOPE OF THE STUDY

This study is divided into two main tasks in addressing the objectives. The tasks are the simulation study and the experimental works. The simulation study will determine the molecular interaction during the experimental process. Both of the task results should be parallel and supporting each other. Therefore, the experimental results can be used as validation of the simulation works which would help to determine the suitability of the simulation technique as a prediction tool.

Generally, the simulation study will investigate two main factors which are the solvent types and temperature effect. While the extraction experiments with different type of organic solvents will produce the extraction yield and then will be correlated with the simulation result. The schematic illustration of the scope of the study is summarised in Figure 1.1.

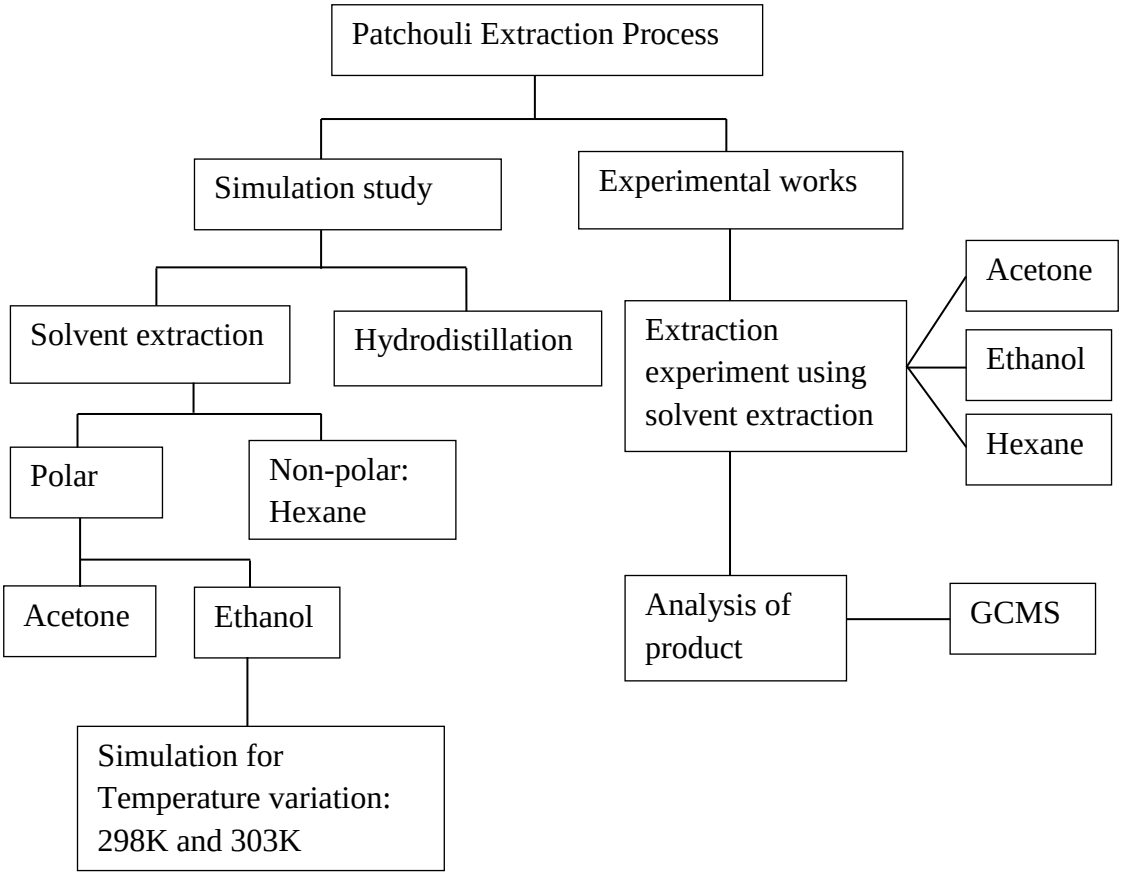

Figure 1.1: Schematic of the scope of study.

1.5 THESIS LAYOUT

This chapter discusses the background information and the significance of the research motivating this study. It also describes the study limitation through the research scope. Chapter Two, further elaboration on the literature review of this study is detailed out. The focus material (patchouli essential oil) chemistry, extraction technique used and the detailed on simulation technique is described in this chapter. In Chapter Three a detailed description of the methodology of simulation study and extraction experiments are defined. The results and discussions are presented in the Chapter Four. It discussed solvent extraction experiment and simulation study results, the comparisons between simulation for hydrodistillation and solvent extraction with ethanol as the solvent and the simulation results for simulation at different temperature (298K and 303K) in ethanol as the solvent. The conclusion of the study and some suggestions with recommendations for future works is described in Chapter Five.

CHAPTER 2

LITERATURE REVIEW

2.0 INTRODUCTION

The aim of this chapter is to review the fundamental science of the extraction process and the simulation technique. It begins with the description of the patchouli essential oil as the study material. This includes the properties of the patchouli essential oil and the compound in the patchouli essential oil that will be used as the target molecule in the simulation. This is followed by the discussion on the extraction process that was used to produce the patchouli essential oil. This chapter also defines the underlying principle of molecular mechanics which is the main concept behind the molecular dynamics (MD) simulation. In addition, the properties such as radial distribution function (rdf) and hydrogen bond formation is explained as they are relevant to the experimental work and simulation study output.

2.1 PATCHOULI ESSENTIAL OIL

Patchouli essential oil comprises of mostly terpenoids. It is extracted from the patchouli plant or *Pogostemon cablin* Benth (Figure 2.1). The patchouli plant belongs to Laminacaeae family originating from the Philippines, Malaysia and India (Raghu, 2006; Hu et al., 2006). The oil gland which containing terpenoids is located on the underside

of the patchouli leaves (Rhind, 2012) and has been identified by Holmes in 1986 (Raghu, 2006). The current top world producer of patchouli essential oil is Indonesia with production estimated around 80 to 90% of global demand which is around 1200 to1400 metric tonnes per year (CCRC, 2011). The patchouli essential oil is being appreciated for its pleasant odour which is contributed by its major compound which is patchoulol or patchouli alcohol (Deguerry et al., 2006). Researchers in the fragrance field describe the odour as woody and earthy odorants and one of the natural sources with this characteristic (Frater et al., 1998).It is normally incorporated into heavy perfumes due to its longer lasting properties besides its application in cosmetic and toiletries (Raghu, 2006).

Figure 2.1: The patchouli plant

Source: Rukmana (2003)

Other than its attractive woody scent (Naf et al., 1981), the patchouli essential oil possesses various types of pharmacological benefits such as antidepressant, antioxidant, anti-inflammatory, antiseptic, antifungal (Hybertson, 2007; Miyazawa et al., 2000; Ichikawa et al., 1989). In Chinese traditional medicine, the patchouli essential oil is used to treat dyspepsia, vomiting, diarrhoea and poor appetite (Yang et al., 1999) as well as to “remove dampness” (Fan et al., 2011). An earlier study by Lapin and