

SOLVENT EXTRACTION OF OLEORESINS FROM VANILLA PLANT  
(VANILLA PLANIFOLIA ANDREWS) BY USING SOXHLET EXTRACTOR

MOHD AMIRUL HAFIZA BIN CHE ABDUL AZIZ

A thesis in fulfillment of the  
requirements for the award of degree of  
Bachelor engineering in Chemical Engineering

Faculty of Chemical Engineering and Natural Resources  
Kolej Universiti Kejuruteraan & Teknologi Malaysia

NOVEMBER 2006

“I declare that this thesis is the result of my own research expect as cited references.  
The thesis has not been accepted for any degree and is concurrently submitted in  
candidature of any degree.”

Signature :

Name of Candidate: MOHD AMIRUL HAFIZA BIN CHE ABDUL  
AZIZ

Date : 22 / 11 / 2006

Dedicated to my beloved mother, father and family.....

## ACKNOWLEDGEMENT

First of all, I would like to express my humble and gratitude to ALLAH S.W.T for the strength, encouragement and inspiration given to me while the thesis is running and lastly I can finish the research without any difficult obstacles. A lot of experiences and thought were gained through out the completing the thesis.

During preparing this thesis, I was met with many people, practitioners and academicians that were encouraging me to finish this research. They have contributed me towards my understanding and knowledge about the thesis which I have research about two semesters until the project have finished. In particularly, I wish to express my sincere appreciate to my supervisor, Mr Ahmad Ziad B Sulaiman for his guidance, ideas and encouragement regardless place and time. Without his continued support and interest, this thesis would not be finished.

I am also would like to express my thanks to all lecturer and teaching engineers in the Faculty of Chemical Engineering & Natural Resources (FKKSA) for their support and motivation during this project development. For Miss Sumaiyah Bt Zainal Abidin as a coordinator for Industrial Project 4BKC and helping to make me stay on the right task concerning the presentation and the thesis progress.

## ABSTRACT

In this study, Soxhlet Extraction method is one of the extraction technique can be used to extract a soluble fraction from solid medium into an organic solvent such n-hexane and ethanol. In this research, the soxhlet extraction method used to extract the vanilla plant ( *Vanilla planifolia Andrews* ) and the product is oleoresin of vanilla. To make sure a good quality of oleoresin, parameters that affect the performance of the soxhlet extraction method were investigated. The examined parameters were vanilla surface area and the solvent ratio. The yield of vanillin was observed. Ground vanilla produced higher yield compared to cut vanilla. In addition, ethanol was found to be better than hexane. Both extraction using ethanol and hexane consisted vanilla component such as vanillin.

## ABSTRAK

Dalam kajian ini, proses pengekstrakkan menggunakan soxhlet merupakan teknik pengekstrakkan untuk mengekstrak pecahan bahan terlarut dari medium pepejal menggunakan bahan pelarut seperti N-heksana dan etanol. Dalam menjalankan kajian ini, proses pengekstrakkan menggunakan soxhlet untuk mengekstrak pokok vanilla (*Vanilla Planifolia Andrews*) dan hasilnya ialah minyak vanilla. Untuk memastikan sama ada hasil pengekstakkan mempunyai kualiti yang baik, parameter- parameter yang mempengaruhi produktiviti menggunakan soxhlet sebagai alat pengekstrakkan dikaji. Antara parameter- parameter yang dikaji ialah luas permukaan vanilla yang digunakan dan nisbah bahan terhadap pelarut. Penghasilan minyak vanilla dikaji. Vanilla yang dikisar menghasilkan lebih banyak minyak daripada vanilla yang dipotong. Tambahan pula, penggunaan etanol sebagai pelarut adalah lebih baik daripada heksana. Dalam proses pengekstrakan vanilla ini, kedua- dua pelarut iaitu etanol dan heksana digunakan.

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Introduction

Vanilla is one of the plants that come from Orchid plant. The Orchid plant is the second largest flower in the world. The orchid family (Orchidaceae) is the second largest family of flowering plants with approximately 20,000 species. Vanilla comes from several species of perennial vines of the genus vanilla native to Mexico and tropical America. The vines commonly climb on the trunks of trees or poles by means of adventitious (aerial) roots. The primary source of commercial vanilla comes from *Vanilla Planifolia* also listed in many references as *Vanilla Fragrans*. Another lesser known species is West Indian vanilla (*Vanilla Pompona*).

Vanilla extract is obtained from the unopened seed capsules (called vanilla beans) which superficially resemble bean pods. The unopened pods are picked when the color changes from green to yellow. The pods are placed in the sun for up to five hours, then they are tightly wrapped in blankets and placed in airtight boxes to sweat. This process is repeated for up to 36 days. During this curing process the pods undergo fermentation and turn black in color. After the laborious curing process, the pods are thoroughly dried. The long fermentation process causes several glucosides (including glucovanillin) to decompose into glucose and vanillin.

## 1.2 The Properties of Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla, which is one of the most widely used and important flavoring materials worldwide. The source of vanilla is the bean, or pod, of the tropical Vanilla orchid (principally *Vanilla planifolia* Andrews). Although more than 12,000 tons of vanillins are produced each year, less than one percent of this is natural vanillin from Vanilla plant; the remainder is synthesized much more cheaply via chemical processes. The value of vanillin extracted from Vanilla pods is variously calculated as being between \$1200 per kg and \$4000 per kg, in contrast to the price of synthetic vanillin, less than \$15 per kg (Lomascolo *et al.*, 1999; Muheim and Lerch, 1999).

Vanillin is a component of vanilla oleoresin responsible for ginger's medicinal effects. Specifically, vanillin has been identified as an anti-pyretic for the pharmacologist effects, food flavor, cosmetics agent and etc. There are two main products from vanilla, vanilla oleoresin and essential oil. For the active components, there are nine (9) main components from the vanilla's extracted; ethyl hexanoate, *p*-methoxybenzaldehyde, 5-propenyl-1,3-benzodioxole, ethyl nonanoate, one unidentified component, *p*-methoxybenzoic acid methyl ester, 3-phenyl- 2-propenoic acid methyl ester, ethyl decanoate and vanillin.

The largest single use for vanillin as a starting material is for the manufacture of the antihypertensive drug called Aldomet (1-3-(3,4-dihydroxyl phenyl)-2-methylalanine). L-dopa is another drug made from vanilla and is used for the treatment of several neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Kumar *et al.*, 2004). Like many polyphenols found in plants, vanillin has antioxidant and anti-tumor activity and has been reported to show antimutagen, anticlastogen and anticarcinogen (Durant *et al.*, 2003).

### 1.3 Research Background / Problem Statement

There are many methods for the extraction process. Most of them use steam distillation to extract the component or plant. But, a few of the extraction process use the soxhlet extraction method. For this research, soxhlet extraction is used. The advantages by using soxhlet extraction are only clean warm solvent used to extract the sample that is vanilla beans. So, the efficiency of the extraction is increased. Besides, the sample in the extraction thimble continuously re-exposed to fresh. It is because once contaminant and vanilla bring into the solution, siphoned back to the flask. Vanillas that already extract stay in the round bottom flask and it re-exposed to fresh. Due to that, the extraction rate for the extraction also increases.

The main aim is to check if the soxhlet extraction method for extracting the vanillin from vanilla plant will be the best extraction method compare to the other extraction methods. Also, the work is aim to determine the different parameters such as vanilla surface area, the solvent to material ratio and sonication time (exposure time), associated with extraction process in order to obtain the maximum yield of the vanillin from vanilla plant. The other goal is to choose the best extracting solvent (from hexane and ethanol) to ensuring the highest yield of vanillin. The selectivity and the yield of the vanilla product analyzed using HPLC (High Performance Liquid Chromatography).

### 1.4 Objective

The objective of this research is to obtain an oleoresin from *Vanilla Planifolia* plant source using soxhlet extraction technique and to study the effect of raw material size and ratio of raw material and solvent for vanilla extraction.

## 1.5 Research Scope

In order to achieve the objectives, a few scopes were set. A dried vanilla was divided into two types, ground into 0.01 mm and cut into 2 cm long. In addition, two types of solvent, ethanol and hexane were prepared. The purity of those solvent were more than 95 %.

The prepared vanilla was then put inside a sample thimble before soxhlet was run. Further more, solvent was poured into round bottom flask and was heated until the solvent condensed into a rate of 1 drop/ 2 seconds. The experiments were done with each experiment was run based on study operating time.

The mixture of solvent and oleoresins of vanilla were separated using rotary evaporator. An oleoresin, a black high viscosity wax, was analyzed using High Performance Liquid Chromatography (HPLC). A neoclosil C18 (meta-chem) 150 x 4.6 mm column was used.



## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Vanilla Properties**

##### **2.1.1 The history of Vanilla**

Vanilla is the world's next most expensive spice. Growers are known to "brand" their beans with pin pricks before they can be harvested, to identify the owner and prevent theft. Vanilla is native to Mexico, where it is still grown commercially. Vanilla was used by the Aztecs for flavoring their royal drink xocolatl - a mixture of cocoa beans, vanilla and honey

For ancient Romans, vagina meant sheath or scabbard. The Spanish adopted the word as vaina, which developed a diminutive form, vainilla, and meaning "little sheath". The Spanish made this diminutive the name of the plant because its pods resemble sheaths. . Figure 2.1 showed the picture of vanilla plants.



**Figure 2.1** Vanilla plant

Cortez brought vanilla back to Europe in the sixteenth century, after having observed Montezuma drinking the cocoa concoction. It has many non-culinary uses, including aromatizing perfumes, cigars and liqueurs. Europeans prefer to use the bean, while North Americans usually use the extract. Substances called “vanilla flavour” don’t contain vanilla at all, being synthesized from eugenol (clove oil), waste paper pulp, coal tar or ‘coumarin’, found in the tonka bean, whose use is forbidden in several countries. Ice cream producers are unlikely to point out that their most popular flavour derives its name from the Latin word vagina.

### **2.1.2 The Types of Vanilla**

The most differences of the Vanilla are they are come from difference country. Vanilla first left Mexico in the early 1500s on ships bound for Spain. It was originally believed only to have value as a perfume. Until the late 19th century, Mexico had the monopoly on growing vanilla, but now Madagascar and Indonesia grow the majority of the world's crop. These are the types of vanilla:

- Mexico - Mexican beans also come in a variety of grades. Beans tend to be overly moist and mold easily. Chocolate brown to black in color, their aroma is clean and delicate.
- Madagascar - Cultivated by the French on the Island of Reunion, which is east of Madagascar, and was known at that time as the Island of Bourbon.

The beans processed here are superior, with flavor and aromatic qualities not found in any other vanilla-growing region in the world. Vanilla extracts made from these beans have a smooth, rich flavor and a deep brown color, plus a wonderful aroma.

- Tahitian - These are good beans, medium to dark brown in color, with very high aromatics. Tahitian beans contain only half the vanillin of Madagascar beans. Thus, even though the beans emit strong overtones of heliotropine (a vanilla aroma), their flavor is less than half as strong as Madagascar beans.
- Guadeloupe - They tend to be reddish in color with pods that are not uniform in appearance. These beans also tend to be more fragrant than those of Madagascar.
- Indonesian - Produced in Indonesia and Bali, these beans are picked early and are underdeveloped. Curing is done over wood fires for only two or three weeks. Smoky aroma and bitter taste can be easily detected.

### **2.1.3 The Categories of Vanilla**

The standards of Vanilla have been classified by FDA and there are three categories of vanilla. There are:

- Category 1 - Pure Vanilla only. Product must contain extractives directly from the vanilla bean.
- Category 2 - Vanilla-Vanillin. Products that contain pure vanilla extractives and vanillin. Vanillin is a product derived from certain coniferous trees.
- Category 3 - Imitation Vanilla. The flavoring ingredients in these products are Vanilla and/or Ethyl Vanillin, an artificial product 2 to 3 times stronger than Vanillin

#### **2.1.4 The Grades of Vanilla**

The grades of vanilla can be divided into six grades. Beans are separated according to length, plumpness, color, shape, appearance, and moistness. Better quality beans demand a higher market value. The grades are:

- Prime - Whole, uniform, fine, fatty, oily pods. Deep, dark, chocolate brown in color. No defects, blemishes, or galls. Pleasant aroma, pliable with good moisture content, about 8 inches in length.
- 1<sup>st</sup> - Similar in quality and characteristics to Prime grade beans, but slightly less fatty.
- 2<sup>nd</sup> - Slightly smaller than Prime and 1st grade, and not as fatty. Dark brown in color with a good aroma. About 6-7 inches in length.
- 3<sup>rd</sup> - The pods are not uniform; they are thinner, twisted, and not as pliable as higher grades. Reddish-brown in color with numerous galls. Fair aroma; about 5-6 inches in length.
- 4<sup>th</sup> - Ordinary pods may have numerous galls, are reddish in color, and have no distinguishing characteristics.
- Inferior - The lowest category which contains all pods that cannot be classified into one of the above categories. Pods are of varied lengths, are very reddish in color, and are hard and dry with low moisture content.

#### **2.1.5 Vanilla Description and Cultivation**

Vanilla is a tropical climbing orchid, with a long green fleshy stem that sprouts roots that cling to trees parasitically. Its yellow or orange orchidaceous flowers grow in bunches, which bloom one flower each day, opening one by one during the two month season. Vanilla is a tropical crop and cannot grow naturally in temperate climates. When cultivated the vines are trained using posts and support trees.

In nature they are only pollinated by Mexican bees and hummingbirds that are capable of penetrating a tough membrane that separates the plant's pistil and stamen. European entrepreneurs had transplanted vanilla to grow in other tropical locations but could not get them to produce the pods. It was not until 1836 that a botanist from Belgium, Charles Morren, recognized that the flowers were not being pollinated and would require some human assistance.

In 1841, Edmond Albius, a former slave, on the French Island of Réunion, perfected a method to artificially fertilize the short-lived vanilla flower using a thin bamboo skewer to lift the membrane and use his thumb to smear the pollen. This gave great impetus to vanilla bean husbandry, and the method is still used today.

The pods take about nine months to mature and are harvested when the tips begin to turn from yellow. Until recently, the curing process was long and complicated. The beans are first wrapped and subjected to high temperature and humidity to 'kill' the vegetative life. The next process involves alternate drying in the sun by day and sweating by night for several days. At this point the beans are dark, oily and pliable and are then slowly dried in the shade for up to two months. They are then sorted and graded and placed in chests for a further conditioning period of one or two months. Figure 2.2 shows the picture of vanilla beans after a pre treatment process.



**Figure 2.2** Vanilla beans after pre treatment process

### **2.1.6 Spice Description**

The flavouring comes from the seed pod, or the 'bean' of the vanilla plant. The prepared beans are very dark brown, slender, pleated and about 20 cm (8 in) long. The bean is tough and pliable, quality vanilla having a frosting of crystal called givre. The crystals contain the active ingredient 'vanillin' that produces the characteristic fragrance and is produced during the process of induced fermentation. These pods are called 'fine vanilla'. 'Woody vanilla' is shorter, lighter coloured, uncrystallized, stronger and slightly bitter. All beans contain thousands of tiny black seeds. Vanilla extract is also available and, if of good quality, is identical in flavour to the pods.

### **2.1.7 Preparation and Storage**

Vanilla extract is made by percolating alcohol and water through chopped, cured beans, somewhat like making coffee. Vanilla extract is very powerful, a few drops sufficing for most uses. Vanilla bean is a bit more time consuming to use than the extract, but imparts the strongest vanilla flavor without the alcohol of extract.

To flavor a liquid base for creme sauces, puddings and ice creams, allow one bean per pint to steep in the liquid by boiling and allowing cooling for an hour before removing the bean. This can be repeated a few times if the bean is washed after use, dried and kept airtight. Ground vanilla can also be used, but use half as much and leave in the liquid. Many recipes call for slitting the bean lengthwise and scraping out the tiny black seeds. Airtight storage is necessary, otherwise the aroma will dissipate.

A good way to store whole vanilla is to bury it in sugar. Use a jar with a tight-fitting lid that will hold about a pound of sugar, burying the bean so that no light can reach it. After 2 -3 weeks the sugar tastes of vanilla and can be used in coffee or in other recipes and the bean can be removed for other uses and returned to the sugar after cleaning. Keep topping up the sugar.

### 2.1.8 Vanilla Culinary Uses

Vanilla's mellow fragrance enhances a variety of sweet dishes: puddings, cakes, custards, creams, soufflés and, of course, ice cream. Classic examples include crème caramel, peach Melba and apple Charlotte. Vanilla flavor is detectable in many chocolate and confectionery items and several liqueurs such as Crème de Cacao and Galliano.

### 2.1.9 Other Names of vanilla

- French: vanille
- German: Vanille
- Italian: vaniglia
- Spanish: vainilla

## 2.2 Introduction of Separations

In chemistry and chemical engineering, a separation process is a process that transforms a mixture of substances into two or more compositionally-distinct products. Much processing is devoted to separate one phase or material from another. (*McCabe et al, 2001*). Separation can divide into two classes that are:

- ✚ Mechanical separation- Appropriate to heterogeneous mixtures. This technique is based on physical differences between the particles like size and shape of particles. They are applicable to separate solids from gases, liquid from gases, solids from solids and solids from liquids. Two general methods are the use of septum or membrane.
- ✚ Diffusion operation- The transfer of material between two phases. Diffusion is the movement under the influence of physical stimulus of an individual component through a mixture. The most common cause

of diffusion is a concentration gradient of the diffusing component. The unsteady state of diffusion operation takes place in leaching or solid liquid extraction and adsorption.

### **2.3 Introduction of Extraction**

Extraction is a process where two immiscible liquid phases come in contact through mixing. An extraction procedure is one that separate one constituent phase from another (*Geankoplis, 2003*). During an extraction process one or more compounds or solutes are transferred from one liquid phase to another immiscible liquid phase.

### **2.4 Types of Extraction**

There are many types of extraction process. The example of extraction processes are liquid-liquid extraction, solid-liquid extraction, solid-liquid and soxhlet extraction.

#### **2.4.1 Liquid-liquid extraction**

Liquid-liquid extraction (or more briefly, solvent extraction) is a useful method to separate components (compounds) of a mixture. The success of this method depends upon the difference in solubility of a compound in various solvents.

It is based on the transfer of a solute from one liquid phase into another liquid phase. Extraction becomes a very useful tool if you choose a suitable extraction solvent. You can use extraction to separate a substance selectively from a mixture, or to remove unwanted impurities from a solution.



Usually one phase is water or water-based (aqueous) solution and the other an organic solvent (i.e. vegoil) which is immiscible with water. Solvent extraction is used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes and other industries. It is interesting to note that liquid-liquid extraction is possible in non aqueous systems, for instance in a system consisting of a molten metal in contact with molten salt, metals can be extracted from one phase to the other.

This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam which modifies the electrochemistry greatly. For example it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations will not be reduced, instead water is reduced to hydrogen). It is important to note that if a detergent or fine solid can stabilize an emulsion which in the solvent extraction community is known as a third phase.

#### **2.4.2 Solid- liquid extraction**

Solid-liquid extraction or leaching is one process that separates the desired solute constituent or to remove an unwanted from the solid phase in the process. There are many substances used in modern processing industries occur in a mixture of components dispersed through a solid material.

Extraction always involves two steps. The first step is contact of the solvent with the solid to be treated so as to transfer the soluble constituent or solute to the solvent. Then, the separation or washing of the solution from the residual solid.

These two steps may be conducted in separate equipment or in the same piece of equipment. Liquid always adheres to the solid which must be washed to prevent either the loss of solution if the soluble constituent is the desired material or the contamination loss of the solids if these are the desired material. The complete process also includes the separate recovery of the solute and solvent. This is done by another operation such as evaporation or distillation.

### 2.4.3 Soxhlet Extraction

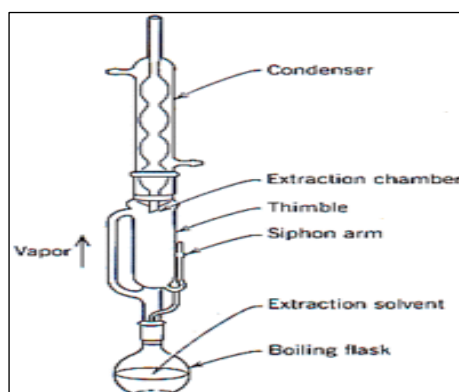
A Soxhlet extractor is a type of laboratory glassware invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of lipid from a solid test material, but can be used whenever it is difficult to extract any compound from a solid.

The key advantage of this type of extraction; only clean warm solvent is used to extract the solid in the thimble. This increases the efficiency of the extraction when compared with simply heating up the solid in a flask with the solvent.

In the soxhlet extractor, there are five main components. The components are condenser, extraction chamber, thimble, siphon arm and round boiling flask. Figure 2.3 and figure 2.4. showed the picture and schematic of soxhlet extractor.



**Figure 2.3** Soxhlet extractor



**Figure 2.4** The schematic of soxhlet extractor

- ✚ Condenser- A condenser is the apparatus in which some type of condensation occurs. Chemists may conduct either a condensation reaction or a vapor-to-liquid condensation in their work.
- ✚ Extraction chamber- It allows the sample of solvent that used during the extraction process. The solvent which condense at the condenser drip down through the extraction chamber.
- ✚ Extraction thimble- cellulose and glass microfiber extraction thimbles are known for their purity and consistent high quality. The thimbles are widely used in Soxhlet extraction units providing a safe, convenient and efficient method of solvent extraction of solids and semi-solids. Cellulose extraction thimbles are produced from high-quality alpha cellulose cotton linter and have excellent mechanical strength and retention.
- ✚ Condenser- it is placed at the top of the soxhlet extractor body. It is converted a vapor into a liquid that trickles into the extraction chamber containing the sample.
- ✚ Round Bottom flask- it containing a solvent that used in this extraction. The capacity is 1000 ml.

## 2.5 Rotary Evaporator

A rotary evaporator is a device used in chemical and biochemical laboratories for the efficient and gentle evaporation of solvents. The main components of a rotary evaporator are a vacuum system, consisting of a vacuum pump and a controller, a rotating evaporation flask which can be heated in a heated fluid bath, and a condenser with a condensate collecting flask.

The solvent is removed under vacuum, is trapped by a condenser and is collected for easy reuse or disposal (John *et al*, 2000). Most labs use a simple water aspirator vacuum on their rotary evaporators, so rotary evaporators can not be used for air and water-sensitive materials unless special precautions are taken.

The system works because lowering the pressure lowers the boiling point of liquids and thus the solvent. This allows the solvent to be removed without excessive. Figure 2.5 showed the picture of rotary evaporator.



**Figure 2.5** Rotary evaporator

### 2.5.1 Procedure to use Rotary Evaporator

Firstly, empty and then replace the solvent collection flask on the unit. Place flask on the rotary evaporator. Used a bump bulb to prevent the material from accidentally splashing into the condenser. Make sure that the bump bulb must be clean before start the experiment.

Use the speed control to rotate the flask. A typical rotavap uses a variable speed sparkless induction motor that spins at 0- 220 rpm and provides high constant torque. Turn on the aspirator vacuum. Lower the flask into the water bath. A convenient handle moves the entire condenser/motor/flask assembly up and down. Be sure not to put the flask into a water bath that exceeds the boiling point of the solvent.

The solvent should start collecting on the condenser and drip into the receiving flask. Some solvents (such as ether or methylene chloride) are so volatile that they will also evaporate from the receiving flask and be discharged down the drain. To prevent this, place a cooling bath on the receiver or (on some models) use a dry ice condenser.

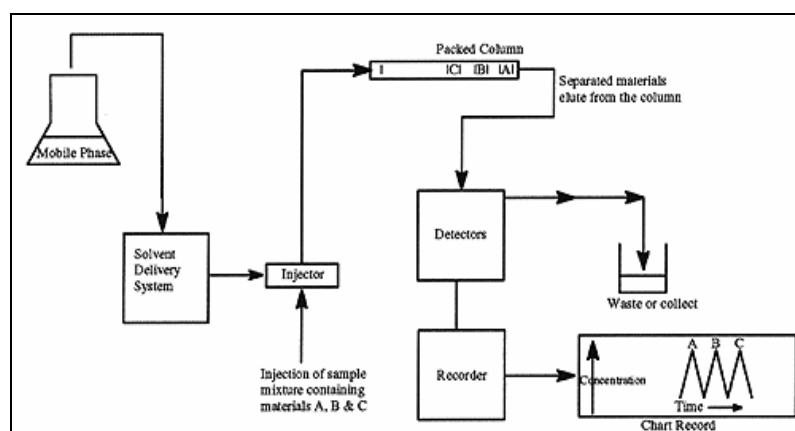
Once all the solvent has evaporated, release the vacuum, raise the flask out of the water bath and turn off the rotation and remove it. Lastly, clean the bump bulb and empty the receiving flask when the experiment is done.

## **2.6 High Performance Liquid Chromatography (HPLC)**

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase. Figure 2.6 and 2.7 showed the picture and schematic high performance liquid chromatography (HPLC).



**Figure 2.6** High Performance Liquid Chromatography



**Figure 2.7** The schematic of High Performance Liquid Chromatography

## 2.6.1 Instrumental Components

### 2.6.1.1 Mobile Phase

The mobile phase in HPLC refers to the solvent being continuously applied to the column, or stationary phase. The mobile phase acts as a carrier for the sample solution. A sample solution is injected into the mobile phase of an assay through the injector port. As a sample solution flows through a column with the mobile phase, the components of that solution migrate according to the non-covalent interactions of the compound with the column. The chemical interactions of the mobile phase and sample, with the column, determine the degree of migration and separation of

components contained in the sample. There are several types of mobile phases, these include: Isocratic, gradient, and polytypic.

In isocratic elution compounds are eluted using constant mobile phase composition. This type of elution is both simple and inexpensive, but resolution of some compounds is questionable and elution may not be obtained in a reasonable amount of time (Snyder, 1983).

In gradient elution different compounds are eluted by increasing the strength of the organic solvent. The sample is injected while a weaker mobile phase is being applied to the system. The strength of the mobile phase is later increased in increments by raising the organic solvent fraction, which subsequently results in elution of retained components.. Compared with isocratic elution, resolution and separation are improved, and bandwidths are nearly equal: (Snyder, 1983)

Polytypic Mobile Phase, sometimes referred to as mixed-mode chromatography, is a versatile method in which several types of chromatographic techniques, or modes, can be employed using the same column. These columns contain rigid macroporous hydrophobic resins covalently bonded to a hydrophilic organic layer.

#### **2.6.1.2 Detector**

The detector for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column. There are many types of detectors that can be used with HPLC. Some of the more common detectors include: Refractive Index (RI), Ultra-Violet (UV), Fluorescent, Radiochemical, Electrochemical, Near-Infrared (Near-IR), Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR), and Light Scattering (LS).

- Refractive Index (RI) - Detectors measure the ability of sample molecules to bend or refract light. This property for each molecule or compound is called its refractive index. For most RI detectors, light proceeds through a bi-modular flow-cell to a photodetector.
- Ultra-Violet (UV) - Detectors measure the ability of a sample to absorb light.
- Fluorescent- detectors measure the ability of a compound to absorb then re-emit light at given wavelengths.
- Radiochemical- Detection involves the use of radiolabeled material, usually tritium ( $^3\text{H}$ ) or carbon-14 ( $^{14}\text{C}$ ).
- Electrochemical- Detectors measure compounds that undergo oxidation or reduction reactions.
- Mass Spectroscopy (MS) - The sample compound or molecule is ionized, it is passed through a mass analyzer, and the ion current is detected.
- Nuclear Magnetic Resonance (NMR) - Detect the certain nuclei with odd-numbered masses, including H and  $^{13}\text{C}$ , spin about an axis in a random fashion.
- Light Scattering (LS) - When a source emits a parallel beam of light which strikes particles in solution, some light is reflected, absorbed, transmitted, or scattered.
- Near-Infra Red (Near-IR) - Operates by scanning compounds in a spectrum from 700 to 1100 nm. Stretching and bending vibrations of particular chemical bonds in each molecule are detected at certain wavelengths.

### 2.6.1.3 Column

There are various columns that are secondary to the separating column or stationary phase. They are Guard, Derivatizing, Capillary, Fast, and Preparatory Columns.

- Guard Column- This Columns are placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column.



- Derivatizing- Pre- or post-primary column derivatization can be an important aspect of the sample analysis. The derivatization step can serve to cause data to become questionable, (*Brown, 1990*).
- Capillary Column- This column has a diameter much less than a millimeter and there are three types: open-tubular, partially packed, and tightly packed.
- Fast Column- It is designed to decrease time of the chromatographic analysis without forsaking significant deviations in results. Advantages include increased sensitivity, decreased analysis time, decreased mobile phase usage, and increased reproducibility (*DiCesare, 1987*).
- Preparatory Column- It is used is to prepare bulk (milligrams) of sample for laboratory preparatory applications. A preparatory column usually has a large column diameter which is designed to facilitate large volume injections into the HPLC system.

## 2.7 Solvents

### 2.7.1 N-hexane

#### 2.7.1.1 Description

N-hexane  $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$  is a chemical made from crude oil. It is used in laboratories, primarily when it is mixed with similar chemicals to produce solvents (Cunha et al, 1999). Common names for these solvents are commercial hexane, mixed hexanes, petroleum ether, and petroleum naphtha. The major use for solvents containing n-hexane is to extract vegetable oils from crops such as soybeans, flax, peanuts, and safflower seed.

N-hexane are also used as cleaning agents in the textile, furniture, shoemaking, and printing industries, particularly rotogravure printing. N-hexane is

also an ingredient of special glues that are used in the roofing, shoe, and leather industries. N-hexane is used in binding books, working leather, shaping pills and tablets, canning, manufacturing tires, and making baseballs.

### **2.7.1.2 Chemical properties**

N-hexane is a colorless liquid with a slightly disagreeable odor. It is an alkane hydrocarbon with the chemical formula  $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$ . The "hex" prefix refers to its six carbons, while the "ane" ending indicates that its carbons are connected by single bonds. Hexane isomers are largely unreactive, and are frequently used as an inert solvent in organic reactions because they are very non-polar.

They are also common constituents of gasoline. It evaporates very easily into the air and dissolves only slightly in water. It is highly flammable, and its can be explosive. It also is ignited by heat, sparks and flames. It can react vigorously materials such as liquid chlorine, concentrated oxygen ad sodium hypochlorite. It will attack some forms of plastics, rubber, and coatings. It is incompatible with strong oxidisers.

### **2.7.1.3 Health Effects**

The most probable route of human exposure to hexane is by inhalation. Individuals are most likely to be exposed to hexane in the workplace. Hexane evaporates very quickly and so the most common exposure is from breathing air containing hexane. It can also enter via the skin.

Chronic intoxication from hexane has been observed in recreational solvent abusers and in workers in the shoe manufacture, furniture restoration and automobile construction. The initial symptoms are tingling and cramps in the arms and legs, followed by general muscular weakness.

## **2.7.2 Ethanol**

### **2.7.2.1 Description**

Ethanol,  $C_2H_5OH$ , (also called Ethyl Alcohol) is the second member of the aliphatic alcohol series. It is a clear colorless liquid with a pleasant smell. Except for alcoholic beverages, nearly all the ethanol used industrially is a mixture of 95% ethanol and 5% water, which is known simply as 95% alcohol. Although pure ethyl alcohol (known as absolute alcohol) is available, it is much more expensive and is used only when definitely required.

Ethanol is prepared as 95% alcohol (i.e. a 95% solution of ethanol in water) by distillation of the solution which results from the fermentation of sugars. (*Yinhong et al, 2003*). Ethanol is a colorless liquid with a pleasant smell. It is completely miscible with water and organic solvents and is very hygroscopic.

### **2.7.2.2 Uses of Ethanol**

Ethanol is used in the manufacturing of alcoholic beverages like Vodka. It also used as a solvent for paint, varnish and drugs. In the manufacturing of ethanal and ethanoic acid, ethanol also used. In a laboratory, ethanol is used in preserving biological specimen and as a fluid in the thermometer. The last of ethanol used is a fuel, that is a gasoline.

## **CHAPTER 3**

### **METHODOLOGY**

#### **3.1 Introduction**

In the extraction of Vanilla extraction by using a soxhlet extraction method, there are two processes that involved. Those are drying of vanilla beans and the extraction process.

#### **3.2 Drying the Vanilla (Pre Treatment)**

Before the extraction was done, the vanilla beans must be treated. First the beans were `killed` by heating (e.g. 20 seconds in boiling water or 48 hours in an oven) or freezing. Then they were wrapped in blankets, heated in the sun and allowed to sweat followed by drying and conditioning. During this process enzymes naturally present in the beans (glycosidases, proteases and oxidases) fermented the beans, which made the vanilla beans shrunk and turned their characteristic into brown colour.