Polyol Induced Partitioning of Essential Oils in Aqueous Organic Solvent Mixtures

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Polyol Induced Partitioning of Essential Oils in Aqueous Organic Solvent Mixtures

BY

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DISSERTATION

Submitted to the Department of Chemistry and Biochemistry at Seton Hall University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 2016
We certify that we have read this dissertation and that in our opinion it is adequate to scientific scope and quality as a dissertation for the degree of Doctor of Philosophy.

APPROVED

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ABSTRACT

Polyol Induced Extraction (PIE) was developed and patented at Seton Hall University by Drs. John R. Sowa Jr., Wyatt R. Murphy, and Mithilesh Deshpande. It was originally discovered and implemented as a method to recycle and reuse waste acetonitrile during the production shortage in 2008. Through the use of PIE, a solvent mixture containing acetonitrile and water can be separated by employing a polyol mass separating agent, which induces a phase separation. The system is separated into its corresponding aqueous and organic phases, with the organic phase being a highly purified organic liquid. Based on the successful experimental results that were obtained, it was decided to assess the potential of PIE as a sample extraction technique. The goal of this work was to demonstrate that PIE can be applied for the extraction of essential oils which can then be analyzed using gas chromatography-mass spectrometry (GC/MS). The research is broken down into a fundamental application, two comparison applications and a final optimization application of PIE.

Chapter 1 provides background information on essential oils and their importance and significance, as well as basic theory of GC/MS. Essentials oils are generally complex mixtures of compounds extracted from plants with the most abundant compound present said to be the “essence” of the plants fragrance. Many different techniques for the extraction of essentials oil from their corresponding botanicals exist with four major techniques dominating: steam distillation, solvent extraction, cold-pressing, and enfleurage. The applications and uses of essential oils are widespread, with flavors and fragrances and as therapeutic agents being the most common.
Since essential oils contain volatile organic compounds, they are excellent candidates for analysis using GC/MS. Gas chromatography is an analytical separation technique that separates compounds based on their vapor pressure and intermolecular interactions with the stationary phase. Following separation of the mixture on a column, quantitation and identification of the components is carried out through the use of detectors that are coupled to the column. Quantitation for organic compounds is most commonly assessed using a flame ionization detector (FID) with identification of these compounds being established through the use of a mass selective detector (MSD). Accurate quantitation at or near the limit of detection (LOD) of the MSD is achieved through the use of analytical standards and operating in specialized modes such as full scan and selected ion monitoring (SIM) simultaneously.

The first application study involves the partitioning of essential oils in acetonitrile and water solvent systems using glycerol as a mass separating agent. The six essential oils that were investigated were subjected to PIE and then analyzed via GC/FID. Method validation was performed which included extraction efficiency, percent recovery, and partition coefficient calculations. The thermodynamic properties of PIE were also addressed, which included Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS). Finally, the GC/MS compositional profiles of the extracted essential oils were compared to essential oils extracted by traditional extraction techniques.

The second application is a comparison study in which PIE was compared to QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe). QuEChERS was discovered to be
particularly adept for polar and basic compounds, which is why it is considered the “gold standard” of extraction techniques for the analysis of pesticides from a variety of different matrices. PIE is similar to QuEChERS in the sense it uses an organic solvent and a mass separating agent to generate a phase separation, with the analytes of interest being extracted into the organic phase for analysis. Based on this, it was decided to compare and contrast these two techniques. The same six essential oils that were analyzed previously were subjected to both extraction techniques and then analyzed via GC/MS. Method validation was carried out in terms of extraction efficiency (where percent recovery and partition coefficients were compared), limit of detection (LOD) and limit of quantitation (LOQ). Finally, the compositional profiles of the essential oils for both techniques were evaluated using GC/MS in order to determine matrix suppression ability as well as the abundance of the main component present in each essential oil.

The third application concentrates on the organic solvent that is used in the PIE process. Acetonitrile is considered to be a safer, green, less toxic solvent than halogenated solvents such as dichloromethane, which are often used for solvent extractions. However, acetonitrile is not considered to be generally recognized as safe (GRAS), so the idea to investigate PIE with GRAS solvents was initiated. The Flavor and Extract Manufactures Association (FEMA) and their Expert Panel make determinations on the toxicology of compounds and there recommended use level that are destined to be used in flavor and fragrance applications. The only GRAS solvents that are fully miscible with water in all concentrations are acetone and Isopropyl Alcohol, so these solvents were investigated for use with PIE. The same six essential oils were subjected to PIE using acetone and isopropyl alcohol as solvents and then analyzed
via GC/MS. Method validation was evaluated in terms of extraction efficiency, where percent recovery and partition coefficients were compared. Limit of detection (LOD) and limit of quantitation (LOQ) were also compared. Finally, the compositional profiles of the essential oils and the abundance of the main components in each oil were assessed using GC/MS in order to determine matrix suppression ability for both techniques.

The last application focuses on the optimization of the PIE process through the use of pH adjustment. Essentials oils are generally made up of compounds that are categorized as phenolic terpenoids or propanoids. These compounds contain a phenol moiety, which acts as a weak acid, so therefore is ionisable at pH values greater than the molecule’s pKa. Based on this concept, it was believed that by adjusting the pH of the extraction solvent system, a highly purified essential oil could be obtained. This was taken a step further by applying the idea to highly purified commercial steam-distilled essential oils that were to be sold to consumers. For this study, three essential oils that contain compounds belonging to the phenylpropanoid class of compounds were subjected to pH optimized PIE and then analyzed via GC/MS. The experiments compared the abundance of constituents present in the organic phase for the initial essential oil before extraction and to the essential oil components after pH optimized extraction.

The final portion of this work includes a brief look at future work and applications that can be performed using PIE. There are many possible uses of PIE and are truly unlimited as different solvent combinations with different polyols can be explored and tailored to fit the application
at hand. Method automation for the extraction of essentials oils on an industrial scale is certainly an excellent next step as this would be a more cost effective alternative to current essential oil extraction techniques. Other areas of interest for PIE include the extraction and purification of biochemical and inorganic analytes such as proteins and metal complexes.
CHAPTER 1 - INTRODUCTION TO ESSENTIAL OILS: BACKGROUND, APPLICATIONS AND ANALYSIS

1.1 Essential Oils

Essential oils are liquid mixtures composed of volatile organic compounds that are obtained from different parts of aromatic plants, (i.e. leaves, peels, barks, flowers, buds, and seeds)\(^1\) with various odiferous plants shown in Table 1-1. They represent what is called the “essence” of the plant \(^2\), which is why different essential oils vary in odor and flavor. These differences can be attributed to the different types and amounts of constituents present in that particular oil.

Essential oils are complex mixtures of natural aromatic compounds that are comprised of both non-polar and polar chemical compounds. In general, there are two major classes of constituents present in essential oils. They are terpene hydrocarbons and oxygenated aromatic compounds or terpenoids. Some major constituents of various essential oils are shown in Table 1-2.

1.1.1 Aromatic Compounds: Terpenes and Terpenoids

Terpenes are the most common class of chemical compounds found in essential oils. Terpene hydrocarbons are compounds consisting of carbon and hydrogen molecules arranged in linear
Table 1-1: Parts of Plants Containing Essential Oils. Reprinted with permission from P. Tongnuanchan; S. Benjakul; Essentials Oils: Extraction, Bioactivities, and Their Uses for Food Preservation, *Journal of Food Science*. 2014, 79(7), 1231-1249. Copyright 2014 John Wiley and Sons

<table>
<thead>
<tr>
<th>Parts</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Basil, bay leaf, cinnamon, common sage, eucalyptus, lemon grass, citronella, melaleuca, mint, oregano, patchouli, peppermint, pine, rosemary, spearmint, tea, rosemary, spearmint, tea tree, thyme, wintergreen, kaffir lime, laurel, savory, tarragon, cajuput, lantana, lemon myrtle, lemon teatree, niaouli, may chang, petitgrain, laurel, cypress</td>
</tr>
<tr>
<td>Seeds</td>
<td>Almond, anise, cardamom, caraway, carrot celery, coriander, cumin, nutmeg, parsley, fennel</td>
</tr>
<tr>
<td>Wood</td>
<td>Amyris, atlas cedarwood, himalayan cedarwood, camphor, rosewood, sandalwood, myrtle, guaiac wood</td>
</tr>
<tr>
<td>Bark</td>
<td>Cassia, cinnamon, sassafras, katrafray</td>
</tr>
<tr>
<td>Berries</td>
<td>Allspice, juniper</td>
</tr>
<tr>
<td>Resin</td>
<td>Frankincense, myrrh</td>
</tr>
<tr>
<td>Flowers</td>
<td>Blue tansy, chamomile, clary sage, clove, cumin, geranium, helichrysum hyssop, jasmine, lavender, manuka, marjoram, orange, rose, baccharises, palmarosa, patchouli, rhododendron anthopogon, rosalina, ajowan, ylang-ylang, marjoram sylvestris, tarragon, immortelle, neroli</td>
</tr>
<tr>
<td>Peel</td>
<td>Bergamot, grapefruit, kaffir lime, lemon, lime, orange, tangerine, mandarin</td>
</tr>
<tr>
<td>Root</td>
<td>Ginger, plai, turmeric, valerian, vetiver, spikenard, angelica</td>
</tr>
<tr>
<td>Fruits</td>
<td>Xanthoxylum, nutmeg, black pepper</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Botanical Name</th>
<th>Major Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajowan oil</td>
<td><em>Trachyspermum ammi</em></td>
<td>Thymol, carvacrol, <em>p-</em></td>
</tr>
<tr>
<td>Basil oil</td>
<td><em>Ocimum sanctum</em> O. basilicum O kilmanandaschricum*</td>
<td>Eugenol, Linalool, Methyl chavicol, Camphor, 1,8-cineole</td>
</tr>
<tr>
<td>Camphor oil</td>
<td><em>Cinnamomum camphora</em></td>
<td>Camphor</td>
</tr>
<tr>
<td>Caraway oil</td>
<td><em>Carum carvi</em></td>
<td>Carvone, Limonene</td>
</tr>
<tr>
<td>Cardamomum oil</td>
<td><em>Elettaria cardamomum</em></td>
<td>1,8-cineole, Terpinyl acetate</td>
</tr>
<tr>
<td>Celery seed oil</td>
<td><em>Apium graveolens</em></td>
<td>Limonene</td>
</tr>
<tr>
<td>Chamomile oil (German)</td>
<td><em>Matricaria chamomila</em></td>
<td>Azulenenes</td>
</tr>
<tr>
<td>Cinnamon oil</td>
<td><em>Cinnamomum zeylanicum</em></td>
<td>Cinnamaldehyde</td>
</tr>
<tr>
<td>Clary sage oil</td>
<td><em>Salvia sclarea</em></td>
<td>Linalool, Linalyl acetate</td>
</tr>
<tr>
<td>Coriander oil</td>
<td><em>Corindrum sativum</em></td>
<td>Linalool, Linalyl acetate</td>
</tr>
<tr>
<td>Clave oil</td>
<td><em>Syzygium aromaticum</em></td>
<td>Eugenol</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td><em>Eucalyptus globulus</em></td>
<td>1,8-cineole, Citronellol, Citronellol</td>
</tr>
<tr>
<td>Geranium oil</td>
<td><em>Pelargonium graveolens</em></td>
<td>Geraniol, Citronellol</td>
</tr>
<tr>
<td>Ginger oil</td>
<td><em>Zingiber officinale</em></td>
<td>Sesquiterpenes</td>
</tr>
<tr>
<td>Holiday leaves</td>
<td><em>Curcuma longa</em></td>
<td>Terpinoline, Virdifloral</td>
</tr>
<tr>
<td>Lavender oil</td>
<td><em>Lavandula officinalis</em></td>
<td>Linalool, Linalyl acetate</td>
</tr>
<tr>
<td>Lemongrass oil</td>
<td><em>Cymbopogon flexiosus</em></td>
<td>Citral</td>
</tr>
<tr>
<td>Patchouli oil</td>
<td><em>Pogostemone patachauli</em></td>
<td>Patchouli alcohol</td>
</tr>
<tr>
<td>Palmarosa oil</td>
<td><em>Cymbopogon martini</em></td>
<td>Geraniol, Geranyl acetate</td>
</tr>
<tr>
<td>Rose oil</td>
<td><em>Rosa damecena</em></td>
<td>Citronellol, Geraniol, Nerol</td>
</tr>
<tr>
<td>Rosemary</td>
<td><em>Rosmarinus officinalis</em></td>
<td>1,8-cineole</td>
</tr>
<tr>
<td>Valarian root oil</td>
<td><em>Valeriana wallichii</em></td>
<td>Patchouli alcohol, Virdifloral</td>
</tr>
<tr>
<td>Jatamansi oil</td>
<td><em>Nardostachys jatamansi</em></td>
<td>Patchouli alcohol</td>
</tr>
<tr>
<td>Mint oils</td>
<td><em>Mentha arvensis</em></td>
<td>Menthol, Menthone, Menthyl Acetate, Carvone, Linalool, Linalyl Acetate</td>
</tr>
<tr>
<td></td>
<td><em>Mentha piperita</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mentha spicata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mentha cardiac</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mentha citrata</em></td>
<td></td>
</tr>
</tbody>
</table>
or branched chains and can be acyclic or alicyclic (monocyclic, bicyclic, or tricyclic). The terpene chemical backbone structure is made up of several five carbon (C₅) base units, or a combination of two isoprene units, which is commonly referred to as a terpene unit. Essential oils consist of terpenes with the general empirical formula (C₅H₈)ₙ, which are further categorized into monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes, (C₂₀), triterpenes (C₃₀), and tetraterpenes (C₄₀). Essential oils mostly contain monoterpenes (C₁₀H₁₆) and sesquiterpenes (C₁₅H₂₄), with the higher terpene units being present less frequently and at lower concentrations.

Aromatic oxygenated constituents present in essential oils are comprised of chemical compounds that consist of carbon, hydrogen and oxygen molecules. They are often oxygenated analogs of terpenes, which is why they are commonly referred to as terpenoids. These oxygenated compounds are mainly responsible for the varying flavor and smell of aromatic botanicals with the most predominant classes of compounds being phenols, alcohols (mono- or sesquiterpene alcohols), aldehydes, ketones, esters, oxides, lactones, and ethers.

1.1.2 Extraction Techniques

Essential oils can be extracted from different parts of botanicals by several different methods which are highly dependent on the type of botanical. The extraction method used to obtain the essential oil is critical and is one of the major factors that determines the quality of the essential oil produced. Improper extraction techniques can lead to the decomposition of the chemical
constituents present in the essential oil which can lead to changes in the oil that include discoloration and alteration of the odor and flavor profile.

1.1.2.1 Distillation

Steam distillation is the most widely used method for essential oil extraction from plants. A basic schematic of a steam distillation process is shown in Figure 1-1. Steam distillation begins by generating hot steam via heating water. The steam is then directed into a vessel that contains the botanical matter to be extracted. The heat causes the breakdown of the cell structure present in the material which in turn causes the release of vaporized aromatic compounds or essential oils that are present. The temperature being used plays a crucial role and is critical as it must be hot enough to break down the plant material to release the aromatic constituents present. The vaporized essential oils are then passed through a cooling column which condenses the vapors back into liquid form. The essential oil and water mixture is then separated using an oil separator which separates the mixture based on immiscibility of the components present.

Hydrodistillation is an essential oil extraction technique that is used to isolate non-water soluble components that have high boiling points. A basic schematic of the hydrodistillation technique is show in Figure 1-2. The process involves submerging the botanicals in water which is then followed by boiling. The water is used to act as a protective barrier in order to prevent the overheating of the material which serves as an advantage as the material can be
Figure 1-1: Steam Distillation Method. Reprinted with permission from P. Tongnuanchan; S. Benjakul; Essentials Oils: Extraction, Bioactivities, and Their Uses for Food Preservation, *Journal of Food Science*. 2014, 79(7), 1231-1249. Copyright 2014 John Wiley and Sons
Figure 1-2: Hydrodistillation Method. Reprinted with permission from P. Tongnuanchan; S. Benjakul; Essentials Oils: Extraction, Bioactivities, and Their Uses for Food Preservation, *Journal of Food Science*. 2014, 79(7), 1231-1249. Copyright 2014 John Wiley and Sons
distilled at temperatures of less than 100°C. Once the water boils, the steam and the vaporized essential oils are condensed to liquid form and then separated by an oil separator.

1.1.2.2 Solvent Extraction

Solvent extraction is employed when the labile compounds present in the botanical for extraction are very delicate or fragile and cannot tolerate the intense heat of distillation methods. Many different solvents can be used that exhibit a range of different polarities with the most common being acetone, hexane, petroleum ether, methanol, and ethanol. The process begins with the solvent of choice being mixed with the botanical matter, heated slightly to extract the essential oil and then filtered to remove the residual solid matter. The filtrate is subsequently concentrated via solvent evaporation and then it is mixed with pure ethanol to extract the essential oil and distilled at very low temperatures. Once the alcohol is evaporated, you are left with your essential oil. This method is relatively time consuming, which is why essentials oils extracted by this method are generally more expensive.

1.1.2.3 Cold Pressing

Cold pressing or expression is a method of essential oil extraction that is specific to citrus fruits such as tangerines, oranges, lemons and limes. The process encompasses a mechanical separation that involves prodding, pricking, or sticking of the fruit to release the essential oils present. The rind of the fruit is placed in a vessel with spikes that rotates in order to puncture the peel. The puncturing releases the essential oils that are present, which collect in a container
below the vessel. The resulting mixture is then subjected to centrifugal force in order to separate the oil from the fruit juice. These expressed essential oils have odor and flavors that are identical to fresh citrus fruits and also contain a small amount of non-volatile waxes that are natural occurring in the fruit.\(^2\)

### 1.1.2.4 Enfluerage

Enfluerage is the oldest method of essential oil extraction and is very rarely used today because of the high costs associated with it. The technique involves placing the botanical matter on a sheet of glass that is spread with a very thin layer of fat called “chassis”. The volatile components present in the plant dissolve and diffuse into the layer of fat. The fat is subsequently collected and then the oil is extracted using pure ethanol. Once the ethanol evaporates, it leaves being the essential oil, which is termed the absolute. This technique is mainly used for very delicate flowers, such as jasmine,\(^4\) as other techniques would decompose and compromise the chemical constituents present in the oil.

### 1.2 Essential Oil Applications

The essential oil market is a multi-billion-dollar industry that is continuing to grow at a rapid pace. The growing consumer preference for natural products has led to an increase in demand for essential oils and the natural products that are made from them, with countries such as the USA, China, and India being the major producers.\(^5\) There are six broad sectors in which essential oils are used and they are shown in Figure 1-3. These include the areas of the flavor
Figure 1-3: Essential Oil Market Application. Adapted from Essentials Oil Trade Information Brief
and fragrance industry, aromatherapy, pharmaceuticals, industrial use, intermediates, and cosmetic applications. Of all the applications, the flavor and fragrance industry, aromatherapy and pharmaceuticals make up the majority of the sectors accounting for more than 75% of the industry total.  

1.2.1 Flavor and Fragrance Industry

The flavor and fragrance industry is the most significant to the essential oil market. It is a worldwide industry that had revenue of $27 billion in 2013 and it is expected to reach close to $45 billion by 2020, which is an estimated compound annual growth rate of 6.4%. Significant companies that contribute to this exponential growth are Givaudan, International Flavors and Fragrances (IFF), Syrprise, Takasago, and Firmenich, who market aroma chemicals and flavor and fragrance blends. Their products are used in many varieties of consumer products that include foods, beverages, bakery & dairy products, oral care, cosmetics and toiletries, soap and detergent, and household cleaners.

Essential oils are a crucial commodity to the flavor and fragrance companies whether they use the oil in its natural state or isolate specific chemical constituents from the oil in order to make other natural chemicals. An example of this is the process flowchart diagram for Cassia Oil as seen in Figure 1-4. The raw botanical, \textit{Cinnamomum Cassia}, is steam distilled or solvent extracted to yield crude cassia oil. The oil is then fractionally distilled to separate the main components, with cinnamic aldehyde being the most abundant, which has a characteristic
**Figure 1-4: Cassia Oil Process Diagram**

**Cinnamomum Cassia**

1. **Steam Distillation/Solvent Extraction**
2. **Cassia Oil**
3. **Fractional Distillation**

- **Cinnamic Aldehyde**
  - Odor/Flavor: Cinnamon
  - Oxidation

- **Benzaldehyde**
  - Odor/Flavor: Cherry
  - Reduction

- **Benzyl Alcohol**
  - Odor/Flavor: Fruity, Balsamic
  - Esterification

  - **Benzyl Formate**
    - Odor/Flavor: Cranberry

  - **Benzyl Acetate**
    - Odor/Flavor: Jasmine

  - **Benzyl Propionate**
    - Odor/Flavor: Apricot
cinnamon odor and flavor. Cinnamic aldehyde is then be oxidized using natural processing methods to yield benzaldehyde which has cherry odor and flavor. Benzaldehyde then undergoes a reduction reaction to its corresponding alcohol, benzyl alcohol, which has a floral and sweet aroma. In the final step, benzyl alcohol is subjected to an esterification reaction with the corresponding acid to form the corresponding esters, which all exhibit different odor and flavor profiles.

1.2.2 Aromatherapy

Aromatherapy is the use of essential oils as therapeutic agents to promote psychological and physical well-being. This type of therapy dates back to ancient civilizations in Egypt, China, and India where essential oils were used as treatments for ailments and diseases. Aromatherapy is classified into five major classes that utilize different types of essential oils for different parts of the body. These classes are cosmetic, massage, olfactory, psycho, and medical aromatherapy. Figure 1-5 shows the volatile chemicals present in essential oils. These compounds are very potent and are used by plants to make their surroundings free of bacteria, viruses, and fungi. Based on this, many essential oils exhibit antimicrobial, antibacterial, antifungal, antiviral, antioxidant, anticancer, antinociceptive, and antiphlogistic properties, with antioxidant and anticancer being the most widely studied and researched.
Figure 1-5: Properties of Chemicals Present in Essential Oils. Reprinted with permission from http://veriditasbotanicals.com/wp-content/uploads/2013/02/veriditas_functional_groups.pdf
1.2.2.1 Antioxidant Properties

The antioxidant properties of essential oils are mainly attributed to the presence of phenolic compounds. Phenolic compounds act as antioxidants because of their high reactivity with peroxy radicals, which are disposed of by formal hydrogen transfer.\(^9\) Due to its stability, the product phenoxy radical will not propagate the radical chain, but rather “wait” for a second peroxy radical and quench it in a very fast radical-radical reaction\(^9\) as shown in Figure 1-6. In research performed by Riccardo and colleagues,\(^9\) eugenol, which is the main component in clove bud oil, was shown to have antioxidant activity comparable to butylated hydroxyl toluene (BHT), which is a synthetic antioxidant compound.

In terms of phenol-free compounds, sulfur compounds, like those present in *Allium* species such as garlic, onions, shallots, leeks, and scallions, are shown to exhibit antioxidant activity. Figure 1-7 shows that allylsulfenic acid is a chain breaking antioxidant that is able to react with reactive oxygen species (ROS) to form non-radical products. Sulfides and disulfides are also able to react with ROS through the oxidation of sulfur to form sulfoxides, which reduces peroxides and hyperperoxides to water and alcohols.\(^8\)

1.2.2.2 Anticancer Properties

Essential oils are most widely used for treatment of oxidative and inflammatory diseases. In these types of diseases, there in an increase in the production of ROS which is the same effect that can lead to cancer. Based on this similarity, essential oils were thought to have anti-cancer
Figure 1-6: Phenol Antioxidant Mechanism. Reprinted with permission from Riccardo Amorati; Mario C. Foti; Luca Valgimigli; Antioxidant Activity of Essential Oils, *J. Agric. Food Chem.* 2013, 61, 10835-10847. Copyright 2013 American Chemical Society.

\[
\text{PhOH} + \text{ROO}^\cdot \xrightleftharpoons{\text{fast}} \text{PhO}^\cdot + \text{ROOH} \\
\text{PhO}^\cdot + \text{ROO}^\cdot \xrightarrow{k_{\text{inh}}} \text{nonradical products}
\]
Figure 1-7: Formation of the Sulfides and Polysulfides present in Garlic Oil. Reprinted with permission from Riccardo Amorati; Mario C. Foti; Luca Valgimigli; Antioxidant Activity of Essential Oils, *J. Agric. Food Chem.* 2013, 61, 10835-10847. Copyright 2013 American Chemical Society.
effects as well. During the recent years, phytomedicine has become more widespread which has sparked interest in cancer research scientists. Several studies have been performed and have shown that essential oils and their individual components can have detrimental effects on cancer cells.

Frankincense oil is a steam distilled essential oil from the *Boswellia serrata* plant, which is comprised of approximately 30% boswellic acid and its derivatives. Boswellic acid and its isomers belong to a class of organic compounds called pentacyclic triterpenes, which have been shown to exhibit anticancer properties. Their chemical structures are shown in Figure 1-8. In research done by Ni et. al, they demonstrated that frankincense oils with greater concentrations of boswellic acids were able to induce cell apoptosis. Also, visual shrinkage of tumor growth was observed compared to that of a control sample when treated with boswellic acid over the course of a week and half.

1.3 Basic Theory of Gas Chromatography

1.3.1 Mobile and Stationary Phases

Chromatography is a technique used to separate a mixture of analytes into their individual components via partitioning of the individual analytes between the mobile and stationary phases. The stationary phase in GC is contained within a column, which can either be a packed column (i.e. column containing solid particles) or a capillary column (i.e. column that has a liquid coating on the
Figure 1-8: Boswellic Acid and its Isomers
walls of the capillary tube). There are numerous different stationary phases available for use in GC and the choice of stationary phase depends greatly on the nature of the analyte mixture.

Separation is based on the same principle as solvent extraction where “like dissolves like”. If using a non-polar stationary phase, polar compounds will not be attracted to the stationary phase and therefore not be retained. The non-retained components will elute the column quicker than non-polar compounds. Vapor pressure of the compounds also play a crucial role in separation as GC methods can use a variety of different oven ramp rate programs. For example, if using a non-polar capillary column, a mixture of non-polar analytes will separate based on their vapor pressures and intermolecular interactions with the stationary phase, with the least non-polar (most polar) compounds eluting the column first, followed by other non-polar compounds in increasing order relative to polarities. The more non-polar a compound is, the longer it will be retained on the non-polar column. The same idea is true for polar compounds being separated on a polar stationary phase.

The mobile phase used in GC is an inert gas with the most common being helium, hydrogen, and nitrogen. The effects of separation efficiency using different carrier gases have been studied and the results are plotted in what is known as the van Deemter plot. This plot associates the kinetic and mass transfer effects in chromatography through the use of the van Deemter equation (Equation 3-1) which explains the broadening of peaks in a chromatogram and can further be simplified into Equation 3-2.
\[ H = 2\lambda d_p + \frac{2\gamma D_G}{\bar{\mu}} + \frac{8kd_f^2\bar{\mu}}{\pi^2(1+k)^2D_S} + \frac{\omega d_p^2\bar{\mu}}{D_G} \]  
(Equation 3-1)

\[ H = A + \frac{B}{\bar{\mu}} + (C_s + C_m) \bar{\mu} \]  
(Equation 3-2)

In Equation 3-2, the term \( A \) refers to the eddy diffusion, \( B \) refers to the longitudinal diffusion and \( C_s \) and \( C_m \) refer to the mass transfer effects in both the mobile and stationary phases. As shown in Figure 1-9, eddy diffusion is not much concern in GC using capillary columns. It is of more importance in liquid chromatography (LC) and packed GC columns as the analyte molecules can take multiple paths through the column as depicted in Figure 1-9A. Linear velocity of the carrier gas is denoted by \( \bar{\mu} \) and this is directly proportional to terms for eddy diffusion, longitudinal diffusion, and mass transfer effects for the mobile and stationary phases.

For example, if we increase the flow of the carrier gas, longitudinal diffusion will decrease and mass transfer effects will increase. The importance of the equation is demonstrated by looking at Figure 1-10, where we can see that the type of carrier gas used is also very important in addition to the linear velocity. The optimal linear velocity providing the best column efficiently significantly varies based on the type of carrier gas used. In this research, helium was used as the carrier gas as it is safer to use than hydrogen, provides faster analysis times than nitrogen and also has a wider range of flow rates as depicted in Figure 1-10.\textsuperscript{11,12,13}
Figure 1-9: The three terms in the van Deemter equation. Reprinted with permission Schmidt, Michelle L., "QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) Extraction – Gas Chromatography for the Analysis of Drugs" (2015), Seton Hall University Dissertations and Theses (ETDs). Paper 2060. http://scholarship.shu.edu/dissertations/2060
Figure 1-10: The three terms in the Van Deemter plot (left) and carrier gases in the Van Deemter plot (right). Reprinted with permission Schmidt, Michelle L., "QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) Extraction – Gas Chromatography for the Analysis of Drugs" (2015). Seton Hall University Dissertations and Theses (ETDs). Paper 2060. http://scholarship.shu.edu/dissertations/2060
1.3.2 Analyte Retention

The retention of analytes on the column is best understood by looking at the partitioning of the analyte between the mobile phase (MP) and stationary phase (SP), as shown in Equation 3-3, which results in a partition coefficient (K_C) calculation.

\[ [A]_{MP} \leftrightarrow [A]_{SP} \quad \text{where} \quad K_C = \frac{[A]_{MP}}{[A]_{SP}} \quad \text{(Equation 3-3)} \]

The partition coefficient is directly related to the retention factor of the analytes (k) and the phase ratio (β), which is determined using the dimensions of the capillary column being used (Equation 3-4), where r is equal to capillary the column’s radius and \( d_f \) is equal to the film thickness of the stationary phase.

\[ K_C = k \beta \quad \text{where} \quad \beta = \frac{r}{2d_f} \quad \text{(Equation 3-4)} \]

In addition to the stationary phase selected, analyte retention is also based on the analyte’s chemical properties, such as boiling point, vapor pressure and volatility.\(^{12}\) Of the mentioned properties, volatility of the analyte is the most important.

1.3.3 Sample Introduction

In GC, the sample to be analyzed is introduced to the capillary column via the injection port. The injection port is heated to a certain temperature, so it is imperative that the analyte be
volatile or semi-volatile so that it can be vaporized and introduced into the carrier gas. There are several different types of GC inlets with the most common being a split/splitless inlet, which was employed in this research. A schematic of a split and splitless injection port is shown in Figure 1-11.

Sample introduction is accomplished through a variety of techniques such as liquid injection, headspace injection, and solid phase micro extraction (SPME) into a liner suited for that specific injection type. In this work, a liquid injection via syringe into a split or splitless liner was utilized. The major difference between these two types of inlets is the opening of the purge valve. During a split injection, the purge valve remains open which only allows a set amount of sample (split ratio) to be injected and analyzed. Since the sample is being “split”, typically neat or dirty sample samples can be analyzed without further dilution. During a splitless injection, the purge valve remains closed, which allows the entire sample to be injected. This type of injection increases the sensitivity and reproducibility of the method, but unwanted interactions occurring in the inlet and well as column and detector overloading must be taken into consideration as well.

1.4 GC Detectors

There are many types of different detectors that can be coupled to gas chromatography. The most commonly used detectors are shown in Table 1-3. The primary detectors used in this research were a flame ionization detector (FID) and a mass selective detector (MSD).
Figure 1-11: Split and Splitless Inlets. Adapted from Y. Kazakevich GC Injectors Class Lecture Slides 2013
Seton Hall University
<table>
<thead>
<tr>
<th>Name</th>
<th>Acronym</th>
<th>Type of Response</th>
<th>Detected Species</th>
<th>Response Characteristic</th>
<th>Destructive</th>
<th>LOD</th>
<th>Dynamic Range</th>
<th>Linear Range</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame Ionization Detector</td>
<td>FID</td>
<td>universal to C</td>
<td>carbon</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-12} , g$ C/sec</td>
<td>$10^6$</td>
<td>$10^7$</td>
<td>na</td>
</tr>
<tr>
<td>Thermal Conductivity Detector</td>
<td>TCD</td>
<td>selective</td>
<td>thermal conductivity</td>
<td>Concentration</td>
<td>No</td>
<td>$10^{-6}$ g/mL</td>
<td>$10^5$</td>
<td>$&lt; 10^5$</td>
<td>na</td>
</tr>
<tr>
<td>Nitrogen/Phosphorous or Thermionic Detector</td>
<td>NPD</td>
<td>selective</td>
<td>N or P</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-12} , g$ N/sec</td>
<td>$10^5$</td>
<td>$10^6$</td>
<td>25000 N vs C, 75000 P vs C</td>
</tr>
<tr>
<td>Flame Photometric Detector</td>
<td>FPD</td>
<td>selective</td>
<td>P or S</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-12} , g$ P/sec</td>
<td>$10^5$ for P and non-linear for S</td>
<td>$10^5$ for P vs C, $10^6$ S vs C</td>
<td></td>
</tr>
<tr>
<td>Electron Capture Detector</td>
<td>ECD</td>
<td>selective</td>
<td>Halogens and oxygen containing groups</td>
<td>Concentration</td>
<td>No</td>
<td>$10^{-14}$ g/mL</td>
<td>$10^5$</td>
<td>$10^4$</td>
<td>up to $10^9$ vs C depending on type and number of halogens</td>
</tr>
<tr>
<td>Chemiluminescence Detector</td>
<td>SCD or NCD</td>
<td>selective</td>
<td>S and N</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-12} , g$ S/sec</td>
<td>$10^5$ for S and $10^7$ for N</td>
<td>$&gt; 10^8$ for S and N</td>
<td>$10^5$ S vs C</td>
</tr>
<tr>
<td>Photoionization Detector</td>
<td>PID</td>
<td>selective</td>
<td>Ions of photo dissociated compounds</td>
<td>Mass</td>
<td>No</td>
<td>$10^{-12}$ g/sec</td>
<td>$10^7$</td>
<td>$10^6$</td>
<td>$\approx$ against compounds with ionization potentials higher than source energy</td>
</tr>
<tr>
<td>Atomic Emission Detector</td>
<td>AED</td>
<td>both</td>
<td>Atomic emission</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-12}$ to $10^2 , g$/sec</td>
<td>$10^{-14}$ to $10^5$</td>
<td>$10^2$ - $10^4$ vs C</td>
<td></td>
</tr>
<tr>
<td>Mass Selective Detector or Mass Spectrometer</td>
<td>MSD</td>
<td>both</td>
<td>Ionized molecular fragments</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-13}$ g</td>
<td>$10^6$</td>
<td>$10^6$</td>
<td>$\approx$ for ions outside mass resolution window</td>
</tr>
<tr>
<td>Inductively Coupled Plasma Mass Spectrometer</td>
<td>ICP-MS</td>
<td>both (universal if measuring C)</td>
<td>Ionized atoms</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-14}$ g/sec</td>
<td>$10^6$</td>
<td>$10^6$</td>
<td>$\approx$ for ions outside mass resolution window</td>
</tr>
<tr>
<td>Electrolytic Conductivity Detector</td>
<td>ELCD</td>
<td>selective</td>
<td>Halogens</td>
<td>Mass</td>
<td>Yes</td>
<td>$10^{-15}$ g/sec</td>
<td>$10^6$</td>
<td>$10^7$</td>
<td>$10^6 - 10^8$ vs C</td>
</tr>
<tr>
<td>Infrared Detector</td>
<td>IR</td>
<td>both</td>
<td>Molecular vibrations</td>
<td>Concentration</td>
<td>No</td>
<td>$10^{-19}$ g/sec</td>
<td>$10^5$</td>
<td>$10^4$</td>
<td>$10^6 - 10^7$ depending on functional group</td>
</tr>
</tbody>
</table>

Table 1-3: Common GC Detectors. Adapted from Separation Science GC Solutions #4: GC Detectors. http://www.sepscience.com/Techniques/GC/Articles/831/-GC-Solutions-4-GC-Detectors
1.4.1 Flame Ionization Detectors

The flame ionization detector (FID) is the most widely used detector in GC because of its unique properties and performance. These properties include unit carbon response and a wide linear operating range as well as low cost, ease of use, ruggedness, and speed of response.\textsuperscript{14} The FID is a mass selective detector which means its response is proportional to the number of carbon atoms present in the compound that passes through the detector. The FID response is stated in terms of picograms carbon per second and the limit of detection is in the low pg C / sec range.\textsuperscript{14}

Unit carbon response means the FID responds linearly to the mass of carbon flowing through it regardless of the chemical structure of the compound. This attribute allows quantitation of all analytes present in a sample based on the relative peak areas without having use calibration standards. The FID gives a unit of response within a couple of percent error so the area percent report will very closely reflect the mass percent of each component present in the sample.

A basic schematic of a FID detector is shown in Figure 1-12. FID’s were first described by two independent research groups\textsuperscript{15,16} and then shortly became commercially available later on in the early 1960’s. Since then, no major modifications have been made in terms of functionality, but rather modifications in adaption for capillary columns instead of packed columns and improvements in the electronics associated with signal processing.
Figure 1-12: Schematic of a Flame Ionization Detector. Reprinted with permission from LCGC North America; Flame Ionization: GC’s Workhorse Detector, *LCGC North America*, 2015, 33(7), 470-477. Copyright 2015 LCGC North America.
In order to understand how a signal is produced, an explanation of FID flame chemistry is necessary. Organic compounds are reduced into their saturated counterparts in the initial portion of the flame, where temperatures are lower. As they continue up the flame, these saturated compounds react with hydrogen atoms to form methane as shown in Equation 4-1.

\[
\text{CH}_3\text{(CH}_2\text{)}_n\text{-CH}_3 + \text{H}^+ \rightarrow \text{CH}_4 + \text{CH}_3\text{(CH}_2\text{)}_{n-1}\text{-C}^\bullet\text{H}_2 + \text{C}_2\text{H}_4 + \text{C}_2\text{H}_3 \rightarrow \ldots \text{(n+2)CH}_4
\]  
(Equation 4-1)

Methane is further combusted to form the formylum ion (CHO\(^+\)), which is the primary FID signal producing ion. Other reactions also occur to form the positive ions, such as hydronium ions. All of the positive ions are collected by the negatively biased collector causing a current to flow, which is then amplified and digitized. The current generated is proportional to the number of ions collected, which allows quantitation of the individual analytes present in the sample.

1.4.2 Mass Selective Detector

A mass selective detector (MSD) is composed of three main components: an ion source, a mass analyzer, and a detector. In the ion source, the sample is bombarded with a beam of electrons at 70 eV that is obtained from a tungsten filament. This impact causes the excitation and ionization of the analyte molecules, which causes molecular fragmentation based on the analyte structure. In this research, an electron impact (EI) ionization source was used and a schematic is shown in Figure 1-13. EI is deemed a “hard” ionization technique, meaning it
produces more fragmentation than “soft” ionization techniques, such as chemical ionization (CI) or electrospray ionization (ESI).

After the ions are generated, they are pulled into the mass analyzer where the ions are separated based on their mass-to-charge (m/z) ratio in the quadrupole mass analyzer. A schematic of a quadrupole, which was used for this research, is shown in Figure 1-14. The quadrupole is comprised of four parallel rods at right angles to each other with alternating magnetic fields and electrostatic charges that are formed by a radio frequency surrounding the poles. The ions travel through the center of the parallel rods and reach the detector only if they are within the selected mass range. There are two different modes that can be used to acquire mass data. In full scan mode, the entire range of masses are scanned and in Selected Ion Monitoring (SIM) only selected mass ions are analyzed, which increases the sensitivity of the MS.

The detector used in this research is an electron multiplier for which a schematic is shown in Figure 1-15. An electron multiple uses dynodes to significantly amplify the signal in response to the original signal through a process called secondary electron emission. When a charged particle strikes a surface, it causes secondary electrons to be released from atoms in the surface layer. The process continues to strike the surface generated more and more secondary electrons, until they all ultimately reach the end of the dynode and the signal is processed into usable data.
**Figure 1-14: Schematic of a Quadrupole Mass Analyzer.** Adapted from P. Gates. Gas Chromatography Mass Spectrometry (GC/MS): Figure 2. University of Bristol. http://www.bris.ac.uk/herclsmsf/techniques/gcms.html (accessed February 2016).
1.5 Conclusions

Essential oils are a significant and important commodity as their uses have many applications. The main components present in the essential oils can be extracted through numerous different techniques for use in a variety of different functions. In today’s society, the flavor and fragrance industry is the dominant market for essential oils with therapeutic uses following close behind. The chemical properties associated with the compounds present in these oils give them great biochemical applications as well as being used as flavoring agents and fragrance enhancers in an assortment of different consumer products. Volatility of the analytes present in these oils make them great candidates for analysis using gas chromatography. Through the use of gas chromatography coupled with detection methods, the main components as well as impurity compounds present in these essential oils can be properly identified and quantified.
CHAPTER 2 - POLYOL INDUCED EXTRACTION OF ESSENTIAL OILS IN ACETONITRILE/WATER SOLVENT SYSTEMS

2.1. Introduction

2.1.1. Polyol Induced Extraction (PIE): Background and Methodology

Traditional extraction of essential oils is performed by steam/hydro distillation (the most common methods), solvent extraction (liquid-liquid extraction), expression (cold-pressing) and CO₂ (super-critical fluid) extraction, which are followed by multiple purification steps. These processes are labor-intensive and time-consuming. Also, the organic solvents used in some of these techniques (e.g. dichloromethane (DCM)) are known to be more toxic and hazardous to the environment based on predicted no-effect concentration (PNEC) values. Based on this, a partitioning effect can be induced via polyol induced extraction (PIE) and it can be seen as an alternative method that is potentially more cost effective, scalable, and leads to high product integrity and purity of the oil extracted.

Previously, it has been demonstrated that aqueous two-phase systems (ATPS) are applicable in the recovery and purification of proteins, enzymes, nucleic acids, antioxidants, alkaloids, antibiotics, and flavor compounds. ATPS have been demonstrated by employing the use of two polymers, and by combinations of a polymer-salt, alcohol-salt, ionic liquid-salt, ionic liquid-polymer, ionic liquid-carbohydrate, sugars, and most recently polyols.
Acetonitrile (CH$_3$CN or ACN) is an organic solvent that is used widely in the chemical industry. It is most notably used as a mobile phase coupled with water in reversed-phase high performance liquid chromatography (HPLC). ACN is a polar, aprotic solvent that is miscible with water in all proportions. The intra-molecular bonding between ACN molecules is very weak, leaving a hydrogen bonding network formed by water.$^{40}$

PIE was developed in response to the ACN shortage in 2008 when there was quest to find new ways to recycle and separate it from water for further use. Some techniques exist to remove water from organic solvents, such as “salting out”,$^{41-44}$ “sugaring out”,$^{45, 46}$ and extractive and azeotropic distillation,$^{47-51}$ but these often cannot achieve the desired purity of the solvent needed for further use. By using a polyol as a MSA, it was determined that ACN/water mixtures can be separated with an upper organic-rich layer with high enough purity for further use.$^{22}$

Polyols are a hydrogenated form of carbohydrates in which the carbonyl group has been reduced to a primary or secondary hydroxyl group.$^{52}$ Since polyols have multiple hydroxyl groups, they are able to form an extensive hydrogen bond network with water.$^{40}$ Additionally, the majority of polyols are non-toxic (with the exception of ethylene glycol), widely available, inexpensive, biodegradable and recyclable. Their high boiling points facilitate their recovery by distillation and make them ideal MSA’s.
The objective of the work presented in this chapter is to demonstrate that phase partitioning through PIE can be effective as an alternative method for extraction of essential oils. Figure 2-1 depicts the basic theory behind PIE. When you have an essential oil suspended in a miscible aqueous/organic solvent mixture, addition of a polyol induces a phase separation which creates two phases: an organic phase that contains your essential oil of interest and an aqueous phase that contains the polyol, water, and unwanted matrix impurities. By not using heat (as required by steam and hydrodistillation), the integrity of heat-sensitive compounds is maintained, which is advantageous when trying to isolate particular components of an essential oil associated with a specific taste, smell, or function.

2.2. Materials and Methods

2.2.1. Chemicals, Reagents and Samples

The essential oils investigated in this work were isolated from dried clove buds [main component: eugenol (4-allyl-2-methoxy phenol)], cinnamon bark [main component: cinnamaldehyde (3-phenyl-2-propenal)], caraway seeds [main component: d-carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexenone)], spearmint leaves [main component: l-carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexenone)], peppermint leaves [main component:
Figure 2-1: Polyol Induced Extraction (PIE) of Analytes
menthol (2-isopropyl-5-methyl cyclohexanol), and anise seeds [main component: anethole (1-methoxy-(4-propenyl) benzene)], all of which were purchased from internet suppliers.

The reagents used in this experiment were all purchased from Sigma Aldrich (St. Louis, MO): acetonitrile (HPLC-grade with a purity of 99.9%), glycerol (purity of > 99.5%), and methanol (anhydrous, HPLC-grade with a purity of 99.9%). Distilled and deionized water was used in all experiments. Chemical structures of the main components present in each essential oil can be found in Tables 2-1 through 2-6.

2.2.2. Sample Preparation

The procedure outlined below was used for extraction of the main compounds present in six essential oils. Dried plant matter was homogenized and approximately 0.5 g was added to five separate 15 mL, high-density polyethylene (HDPE) conical tubes with screw caps. Then, 10 mL of an ACN/water mixture (1:1 v/v) was added to each tube and the tube was vortexed for 15 min. The solid matter was filtered using a syringe and polytetrafluoroethylene (PTFE) filter. Glycerol was added (20% w/v) to each tube and the tube was shaken thoroughly. Each tube was then equilibrated for 30 min at the following temperatures: -20, -10, 0, 10 and 20 °C. After the two phases separated, the volume of each phase was recorded and the upper and lower phases were withdrawn via pipette into separate vials.
Both phases were then subjected to the same preparation in order to quantitate the amount of the main component present. One milliliter (1 mL) aliquots of both the upper and lower phases were separately removed via pipet, weighed, and added to separate 10 mL volumetric flasks. The internal standard (1.00 mL, ethyl caproate) was added to each flask and the volumetric was diluted to the 10 mL mark with methanol. At least four independent replicates were made and the average partition coefficients and associated standard deviations were determined.

2.2.3. Instrumental Parameters

2.2.3.1 Partition Coefficients

The main compounds present in each essential oil were quantified using gas chromatography (GC) on an Agilent 6890N gas chromatograph (Santa Clara, CA), coupled with a flame ionization detector (FID), according to the mentioned conditions. The GC analysis was performed on an a Restek-Rtx®-Wax capillary column (60 m, 0.25 mm id, 0.50 µm) with the following parameters: He (g) constant flow, 1.0 mL/min; inlet temperature, 250°C; injection volume 3µL (split 50:1); initial oven temperature, 50°C, held for 7 min, then a 10°C/min ramp to 250°C and held for 15 min.; The FID temperature was set at 280°C with a He (g) flow rate of 45.0 mL/min.

2.2.3.2 Essential Oil Compositional Profiles
GC/MS was used to analyze the chemical composition of six essential oils that were extracted using the PIE process. The analysis was performed on an Agilent 6890N gas chromatograph (GC) (Santa Clara, CA) equipped with a CTC Analytics CombiPAL Autosampler (Zwingen, Switzerland) and an Agilent 5973 Inert Mass Selective Detector (MSD) (Santa Clara, CA) according to the mentioned GC/MS conditions. The data was interpreted using Agilent’s Chemstation software. The identities of the compounds were determined by the similarity of their mass spectra with those from the Wiley Flavors and Fragrances of Natural and Synthetic Compounds 3 (3rd edition) and the NIST 11-MS mass spectral databases.

The GC/MS analysis was performed with a Restek-Rxi®-5ms capillary column (60 m, 0.25 mm id, 0.25 μm) with the following parameters: He (g) constant flow, 1.2 mL/min; inlet temperature, 250°C; injection volume 3μL (split 50:1); initial oven temperature, 100°C, held for 7 min, then a 10°C/min ramp to 300°C and held for 20 min. The quadrupole mass analyzer (electron impact ionization type, 70 eV) was operated in full scan mode at a rate of 3.89 scans/sec and a range of 35-400 amu with the source temperature set at 230°C and the transfer line temperature set at 280°C.

2.2.4 Experimental Parameters

2.2.4.1 Partitioning of Essential Oils

The response factor (R_F) for each compound of interest was determined according to Equation 2-1,
\[
RF = \frac{C_{IS} \times PA_{EO}}{PA_{IS} \times C_{EO}}
\]  
(Equation 2-1)

where C is the concentration, PA is the peak area, the subscript IS denoting the internal standard and the subscript EO denoting the main compound of interest in the essential oil.

The partition coefficient (\(K_{PC}\)) for each of the main components present in the essential oils was determined by taking into account the concentration of the component in each phase according to Equation 2-2.

\[
K_{PC} = \frac{C_{EO(UP)}}{C_{EO(LP)}}
\]  
(Equation 2-2)

\(K_{PC}\) is the partition coefficient for the phase separation, \(C_{EO}\) represents the concentration of the compound of interest in each essential oil and the subscripts UP and LP denotes the upper (acetonitrile-rich) and lower (glycerol/aqueous-rich) phases.

The percent recovery (\(R_T\)) of the main compound in each essential oil in the organic rich top phase was calculated using Equation 2-3,
\[
R_T = \frac{100}{1 + \frac{1}{K_{PC} \times R_v}} \quad \text{(Equation 2-3)}
\]

where \( R_v \) is the ratio between the volumes of the upper and lower phases.

### 2.2.4.2 Thermodynamics

The standard molar Gibbs free energy (\( \Delta G^\circ \)), enthalpy (\( H^\circ \)), and entropy (\( \Delta S^\circ \)) were determined via the van’t Hoff method by measuring partition coefficients at different temperatures in the range of -20 to 20°C. The thermodynamic parameters of the system were determined using the van’t Hoff equation.\(^{53-57}\)

Enthalpy and entropy contributions can be obtained from the van’t Hoff plot. When the van’t Hoff plot gives a straight line, a similar mechanism is likely and the slope is \( \frac{-\Delta H^\circ}{R} \) and the y-intercept is \( \frac{\Delta S^\circ}{R} \). The standard free energy (\( \Delta G^\circ \)) is determined from the equation

\[
\Delta G^\circ = RT \ln K_{PC}
\]

### 2.3. Results and Discussion

#### 2.3.1 Extraction Efficiency
To determine the potential of PIE as an alternative extraction process, six essential oils were evaluated. The experimental data obtained for extraction of the main components present in the six essential oils are shown in Tables 2-1 through 2-6. Figure 2-2 shows the phase separation that was induced in all six essential oil samples through the addition of glycerol. Based on this phase separation, data was obtained and used to calculate the phase ratio (recording the volumes on the upper and lower phases after equilibration at different temperatures), partition coefficient ($K_{PC}$) (Equation 2-2) and percent recovery (Equation 2-3) for all six essential oils.

The results for the extraction of eugenol from clove buds are shown in Table 2-1. The data obtained indicated that as temperature decreased, the phase ratio increased from 0.24 to 0.53. The partition coefficients are > 1 at all temperatures, which is consistent with the partitioning of the main analyte into the upper organic phase. As temperature decreased in the range of 20 °C to -10 °C, the partition coefficients increased linearly from 12 to 87. However, at -20°C, there was a break in the linear trend ($K_{PC} = 43$) because the sample began to freeze. The percent recovery of eugenol is depicted in Figure 2-3. Percent recovery is directly proportional to the partition coefficient thus, the same trend as a function of temperature was observed. As temperature decreased, the percent recovery increased from 74% to 97%.

There was an interesting contrast in the $K_{PC}$ values and percent recoveries for eugenol at -10 and -20°C. Even though the sample was partially frozen at -20°C, the percent recovery (96%) was within experimental error the same as that at -10°C (97%). However, there was a
Figure 2-2: Partitioning of Essential Oils via PIE

Essentials oils suspended in ACN / aqueous solvent mixture

Addition of glycerol with temperature equilibration

Phase Separation
(Organic upper phase and aqueous lower phase)
Table 2-1: Clove Bud Oil Experimental Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Temperature</th>
<th>-20°C</th>
<th>-10°C</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove Bud Oil (Eugenol)</td>
<td>* Volume of Upper Phase (mL)</td>
<td>4.5</td>
<td>4.0</td>
<td>3.5</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>* Volume of Lower Phase (mL)</td>
<td>8.5</td>
<td>9.0</td>
<td>9.5</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>a/b Phase Ratio</td>
<td>0.53</td>
<td>0.44</td>
<td>0.37</td>
<td>0.30</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Partition Coefficient (K_{PC})</td>
<td>43 ± 1.3</td>
<td>87 ± 2.4</td>
<td>61 ± 0.3</td>
<td>30 ± 0.9</td>
<td>12 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>% Recovery</td>
<td>96 ± 0.1</td>
<td>97 ± 0.2</td>
<td>96 ± 0.1</td>
<td>90 ± 0.4</td>
<td>74 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>ΔG (kJ)</td>
<td>-8.0</td>
<td>-9.3</td>
<td>-9.4</td>
<td>-8.3</td>
<td>-6.0</td>
</tr>
<tr>
<td></td>
<td>ΔH (kJ)</td>
<td>40</td>
<td>9.2</td>
<td>-23.0</td>
<td>-56.4</td>
<td>-91.0</td>
</tr>
<tr>
<td></td>
<td>ΔS (J/K)</td>
<td>190</td>
<td>70</td>
<td>-50</td>
<td>-170</td>
<td>-290</td>
</tr>
<tr>
<td></td>
<td>TAS (kJ)</td>
<td>48</td>
<td>18.4</td>
<td>-13.7</td>
<td>-48.1</td>
<td>-85.0</td>
</tr>
</tbody>
</table>
Figure 2-3: Percent Recovery of Essential Oils
significant drop in the $K_{PC}$ at -20°C (43) compared to $K_{PC}$ at -10°C (87). According to Equation 2-2, this indicated that when the sample freezes, the concentration of the essential oil in the liquid portion of the lower aqueous phase ($C_{LP}$) slightly increased due to the liquid-solid phase transition. Since percent recovery does not change within experimental error, this indicated that the analyte was not trapped in the solid matrix.

The results for extraction of $d$-carvone from caraway seeds are shown in Table 2-2. The trends are similar to eugenol: as temperature is decreased, phase ratios increased from 0.19 to 0.47; partition coefficients are >1; as temperatures is decreased, $K_{PC}$ increased from 9.9 to 69, with the exception of -20°C, where $K_{PC}$ decreased to 42 due to freezing. The percent recovery was 65% at 20°C and it increased to 96% when the temperature was lowered to -10°C. The percent recovery was the same within experimental error (96%) at -20°C, which indicated that the analyte was not trapped in the solid matrix.

The results for extraction of menthol from peppermint leaves are shown in Table 2-3. As the temperature decreased, the phase ratios increased from 0.18 to 0.44. The partition coefficients are >1 at all temperatures and as the temperature was decreased, $K_{PC}$ increased from 7.5 to 54, with the exception of -20°C, where $K_{PC}$ decreased to 36 due to freezing. The percent recovery was 60% at 20°C and was increased to 96% when the temperature was lowered to -10°C. The percent recovery was the same within experimental error (94%) at -20°C, which indicated that the analyte was not trapped in the solid matrix.
<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Temperature</th>
<th>-20°C</th>
<th>-10°C</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caraway Seed Oil ((d)-carvone)</td>
<td>a Volume of Upper Phase (mL)</td>
<td>4.0</td>
<td>3.5</td>
<td>3.5</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>b Volume of Lower Phase (mL)</td>
<td>8.5</td>
<td>9.0</td>
<td>9.5</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>a/b Phase Ratio</td>
<td>0.47</td>
<td>0.39</td>
<td>0.37</td>
<td>0.25</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Partition Coefficient ((K_{PC}))</td>
<td>42 ± 0.4</td>
<td>69 ± 0.7</td>
<td>51 ± 0.7</td>
<td>29 ± 0.8</td>
<td>10 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>% Recovery</td>
<td>96 ± 0.1</td>
<td>96 ± 0.1</td>
<td>94 ± 0.1</td>
<td>88 ± 0.3</td>
<td>65 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(\Delta G) (kJ)</td>
<td>-5.1</td>
<td>-6.1</td>
<td>-5.9</td>
<td>-4.5</td>
<td>-1.9</td>
</tr>
<tr>
<td></td>
<td>(\Delta H) (kJ)</td>
<td>34.5</td>
<td>4.1</td>
<td>-27.6</td>
<td>-60.4</td>
<td>-94.4</td>
</tr>
<tr>
<td></td>
<td>(\Delta S) (J/K)</td>
<td>157</td>
<td>39</td>
<td>-79</td>
<td>-197</td>
<td>-315</td>
</tr>
<tr>
<td></td>
<td>TAS (kJ)</td>
<td>39.7</td>
<td>10.2</td>
<td>-21.7</td>
<td>-55.9</td>
<td>-92.5</td>
</tr>
</tbody>
</table>
Table 2-3: Peppermint Leaf Oil Experimental Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Temperature</th>
<th>-20°C</th>
<th>-10°C</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peppermint Leaf Oil (Menthol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a Volume of Upper Phase (mL)</td>
<td>4.0</td>
<td>3.5</td>
<td>3.0</td>
<td>2.5</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>b Volume of Lower Phase (mL)</td>
<td>9.0</td>
<td>9.5</td>
<td>10.0</td>
<td>10.5</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>a/b Phase Ratio</td>
<td>0.44</td>
<td>0.37</td>
<td>0.30</td>
<td>0.24</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Partition Coefficient (Kpc)</td>
<td>36 ± 1.7</td>
<td>54 ± 1.3</td>
<td>34 ± 1.7</td>
<td>22 ± 0.1</td>
<td>7.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>% Recovery</td>
<td>94 ± 0.3</td>
<td>95 ± 0.1</td>
<td>91 ± 0.4</td>
<td>84 ± 0.1</td>
<td>58 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>ΔG (kJ)</td>
<td>-9.5</td>
<td>-10.5</td>
<td>-10.4</td>
<td>-9.4</td>
<td>-7.4</td>
<td></td>
</tr>
<tr>
<td>ΔH (kJ)</td>
<td>26.6</td>
<td>1.3</td>
<td>-25.0</td>
<td>-52.3</td>
<td>-80.1</td>
<td></td>
</tr>
<tr>
<td>ΔS (kJ/K)</td>
<td>0.14</td>
<td>0.04</td>
<td>-0.05</td>
<td>-0.15</td>
<td>-0.25</td>
<td></td>
</tr>
<tr>
<td>TAS (kJ)</td>
<td>36.1</td>
<td>11.7</td>
<td>-14.6</td>
<td>-42.9</td>
<td>-73.1</td>
<td></td>
</tr>
</tbody>
</table>
The results for extraction of l-carvone from spearmint leaves are shown in Table 2-4. The trends are very similar to d-carvone as the molecules orientation should affect the extraction technique. As temperature is decreased, the phase ratios increased from 0.24 to 0.53. The partition coefficients are >1 at all temperatures and as the temperature was decreased, $K_{PC}$ increased from 8.9 to 69, with the exception of -20°C, where $K_{PC}$ decreased to 45 due to freezing. The percent recovery was 68% at 20°C and it increased to 97% when the temperature was lowered to -10°C. The percent recovery was the same within experimental error (96%) at -20°C, which indicated that the analyte was not trapped in the solid matrix.

The results for extraction of anethole from anise seeds are shown in Table 2-5. As the temperature decreased, the phase ratios increased from 0.19 to 0.47. The partition coefficients are >1 at all temperatures and as the temperature was decreased, $K_{PC}$ increased from 56 to 255, with the exception of -20°C, where $K_{PC}$ decreased to 128 due to freezing. The percent recovery was 92% at 20°C and was increased to 99% when the temperature was lowered to -10°C. The percent recovery was the same within experimental error (98%) at -20°C, which indicated that the analyte was not trapped in the solid matrix.

The results for extraction of cinnamic aldehyde from cinnamon bark are shown in Table 2-6. As the temperature decreased, the phase ratios increased from 0.20 to 0.50. The partition coefficients are >1 at all temperatures and as the temperature was decreased, $K_{PC}$ increased from 7.7 to 44, with the exception of -20°C, where $K_{PC}$ decreased to 31 due to freezing. The percent recovery was 60% at 20°C and was increased to 95% when the temperature was
Table 2-4: Spearmint Leaf Oil Experimental Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Temperature</th>
<th>-20°C</th>
<th>-10°C</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearmint Leaf Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(l-Carvone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a Volume of Upper Phase</td>
<td>4.5</td>
<td>4.0</td>
<td>3.5</td>
<td>3.0</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>(mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Volume of Lower Phase</td>
<td>8.5</td>
<td>9.0</td>
<td>9.5</td>
<td>10.0</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>(mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/b Phase Ratio</td>
<td>0.53</td>
<td>0.44</td>
<td>0.37</td>
<td>0.30</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Partition Coefficient (K_{PC})</td>
<td>45 ± 0.73</td>
<td>69 ± 1.6</td>
<td>53 ± 1.8</td>
<td>26 ± 1.3</td>
<td>8.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>% Recovery</td>
<td>96 ± 0.3</td>
<td>97 ± 0.1</td>
<td>95 ± 0.2</td>
<td>89 ± 0.5</td>
<td>68 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>ΔG (kJ)</td>
<td>-8.0</td>
<td>-9.2</td>
<td>-9.1</td>
<td>-7.8</td>
<td>-5.4</td>
<td></td>
</tr>
<tr>
<td>ΔH (kJ)</td>
<td>36.0</td>
<td>5.1</td>
<td>-27.1</td>
<td>-60.5</td>
<td>-95.1</td>
<td></td>
</tr>
<tr>
<td>ΔS (kJ/K)</td>
<td>0.17</td>
<td>0.05</td>
<td>-0.07</td>
<td>-0.19</td>
<td>-0.31</td>
<td></td>
</tr>
<tr>
<td>TAS (kJ)</td>
<td>44.0</td>
<td>14.2</td>
<td>-18.0</td>
<td>-52.7</td>
<td>-89.7</td>
<td></td>
</tr>
</tbody>
</table>
Table 2-5: Anise Seed Oil Experimental Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Temperature</th>
<th>-20°C</th>
<th>-10°C</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anise Seed Oil</td>
<td>a Volume of Upper Phase (mL)</td>
<td>4.0</td>
<td>3.5</td>
<td>3.5</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>(Anethole)</td>
<td>b Volume of Lower Phase (mL)</td>
<td>8.5</td>
<td>9.0</td>
<td>9.5</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>a/b Phase Ratio</td>
<td>0.47</td>
<td>0.39</td>
<td>0.37</td>
<td>0.25</td>
<td>0.19</td>
</tr>
<tr>
<td>Partition Coefficient (Kpc)</td>
<td>128 ± 1.4</td>
<td>255 ± 1.6</td>
<td>183 ± 1.0</td>
<td>110 ± 0.5</td>
<td>56 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>% Recovery</td>
<td>98 ± 0.1</td>
<td>99 ± 0.1</td>
<td>99 ± 0.1</td>
<td>97 ± 0.1</td>
<td>92 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>ΔG (kJ)</td>
<td>-12.0</td>
<td>-13.5</td>
<td>-14.0</td>
<td>-13.5</td>
<td>-12.0</td>
<td></td>
</tr>
<tr>
<td>ΔH (kJ)</td>
<td>37.8</td>
<td>12.5</td>
<td>-13.8</td>
<td>-41.1</td>
<td>-69.2</td>
<td></td>
</tr>
<tr>
<td>ΔS (kJ/K)</td>
<td>0.20</td>
<td>0.10</td>
<td>-0.001</td>
<td>-0.10</td>
<td>-0.20</td>
<td></td>
</tr>
<tr>
<td>TΔS (kJ)</td>
<td>49.8</td>
<td>26.0</td>
<td>0.2</td>
<td>-27.6</td>
<td>-57.2</td>
<td></td>
</tr>
</tbody>
</table>
Table 2-6: Cinnamon Bark Oil Experimental Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Temperature</th>
<th>-20°C</th>
<th>-10°C</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon Bark Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Cinnamaldehyde)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a Volume of Upper Phase (mL)</td>
<td>4.0</td>
<td>3.5</td>
<td>3.0</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>b Volume of Lower Phase (mL)</td>
<td>8.0</td>
<td>8.5</td>
<td>9.0</td>
<td>9.5</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>a/b Phase Ratio</td>
<td>0.50</td>
<td>0.41</td>
<td>0.33</td>
<td>0.26</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Partition Coefficient (Krc)</td>
<td>31 ± 5.0</td>
<td>44 ± 2.0</td>
<td>28 ± 2.2</td>
<td>17 ± 0.6</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>% Recovery</td>
<td>94 ± 1.1</td>
<td>95 ± 0.2</td>
<td>90 ± 0.7</td>
<td>82 ± 0.5</td>
<td>60 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>ΔG (kJ)</td>
<td>-5.5</td>
<td>-6.0</td>
<td>-5.6</td>
<td>-4.4</td>
<td>-2.4</td>
</tr>
<tr>
<td></td>
<td>ΔH (kJ)</td>
<td>16.1</td>
<td>-5.1</td>
<td>-27.1</td>
<td>-49.9</td>
<td>-73.5</td>
</tr>
<tr>
<td></td>
<td>ΔS (kJ/K)</td>
<td>0.09</td>
<td>0.003</td>
<td>-0.08</td>
<td>-0.16</td>
<td>-0.24</td>
</tr>
<tr>
<td></td>
<td>TAS (kJ)</td>
<td>21.6</td>
<td>0.9</td>
<td>-21.4</td>
<td>-45.5</td>
<td>-71.1</td>
</tr>
</tbody>
</table>
lowered to -10°C. The percent recovery was the same within experimental error (94%) at -20°C, which indicated that the analyte was not trapped in the solid matrix.

For all six essential oils, the partition coefficients in the temperature range of -20 to 20°C were always >1. This was a result of the phenomenon of phase separation so long as the conditions are favorable for phase separation; the hydrophobic analyte will migrate into the organic phase. Thus, the main compounds present in each essential oil have a greater affinity for the acetonitrile-rich organic phase than the glycerol-rich aqueous phase. The trend observed for all six of the essential oils was lowering the temperature favors the partitioning of compounds to the organic phase simply because the phase ratio increases at lower temperatures. Based on the results obtained, the optimal temperature range for phase separation was below -10°C and includes -20°C even though partial freezing of the sample occurred. In the -10 to -20°C temperature range, the % recovery for all six essential oils was >95%.

2.3.2. Thermodynamics

Since $K_{PC}$ is temperature dependent, the thermodynamic properties for this process were determined. Using $K_{PC}$ values in the -20 to 20°C temperature range, van’t Hoff plots were obtained by plotting $\ln K_{PC}$ vs. $1/T$. The van’t Hoff plots for all six essential oils shown in Figure 2-4 indicated that a nonlinear trend occurs. This is consistent with the observation that the sample partially freezes at -20°C which is consistent with a change in mechanism of the phase separation process whereby there is a liquid-liquid phase separation in the -10 to 20°C
Figure 2-4: van’t Hoff plot of Essential Oils
temperature range, and, at -20°C, there is a liquid-liquid-solid phase separation as shown in Figure 2-5. The enthalpy change at any temperature is provided by the slope of the van’t Hoff plot, assuming linearity. Since the van’t Hoff plot was not linear, treating the data points where freezing is not observed (-10°C to 20°C) is one approach to obtaining thermodynamic parameters. However, another approach is to use a Gibbs-Helmholtz plot as depicted in Figure 2-6 and subject the resulting curve to a polynomial regression analysis procedure as described by Seelig et al. By solving for the polynomial regression parameters $\alpha_1, \alpha_2,$ and $\alpha_3$ in Equation 2-4, enthalpy (Equation 2-5) and entropy (Equation 2-6) can be obtained.

$$\Delta G^\circ = -RT \ln K_{eq} = \alpha_1 + \alpha_2 T + \alpha_3 T^2$$  \hspace{1cm} \text{(Equation 2-4)}

$$\Delta H^\circ = \frac{\delta \left( \Delta G^\circ \right)}{\delta T} = \frac{\delta (\Delta G^\circ)}{\delta (1/T)} = \alpha_1 - \alpha_3 T^2$$  \hspace{1cm} \text{(Equation 2-5)}

$$\Delta S^\circ = \frac{\delta \Delta G^\circ}{\delta T} = -\alpha_2 - 2\alpha_3 T$$  \hspace{1cm} \text{(Equation 2-6)}

The thermodynamic data results for eugenol extracted from clove buds are shown in Table 2-1. $\Delta G^\circ$ (free energy) is negative at all temperatures which is consistent with partitioning of the essential oil into the upper organic phase. However, $\Delta G^\circ$ is clearly temperature dependent. As the temperature is decreased, $\Delta G^\circ$ becomes more negative and goes from -6.0 to -9.4 kJ/mol; at -20°C the trend in $\Delta G^\circ$ changes and it becomes less negative (-8.0 kJ/mol) for the same reason discussed previously for the trend in $K_{PC}$. Thus, at -20°C, the sample partially freezes.
Figure 2-5: Phase Separation at Different Temperatures
Figure 2-6: Gibbs-Helmholtz Plot of Essential Oils
but the analyte is not trapped in the newly formed solid matrix causing a slight increase in the concentration of the analyte in the lower aqueous liquid phase. Because the analyte is not trapped in the solid matrix, it appears that freezing may be condensing water as a solid. The decrease in the amount of water in the lower aqueous solution results in an increase in $C_{EO}$, i.e., an increase in the concentration of the eugenol relative to the pre-freezing condition. Such an increase affects only $C_{EO}$ at $-20^\circ$C and results in a decrease in $K_{PC}$. Consequently, $\Delta G^\circ$ at $-20^\circ$C is less negative than at $-10^\circ$C.

From equation 2-5, the thermodynamic parameters of enthalpy ($H^\circ$) and entropy ($S^\circ$) are obtained. Enthalpy is temperature dependent and it shifts from negative (-91 kJ/mol at $20^\circ$C) to positive (40 kJ/mol at $-20^\circ$C). This indicated that above 0°C where $\Delta H^\circ$ is -23.0 kJ/mol, the partitioning of the analyte into the upper organic phase is driven by enthalpy and below 0°C, the partitioning is driven by entropy. Thus, below 0°C, $\Delta S^\circ$ switches from negative to positive (from -50 J/K at 0°C to 190 J/K at $-20^\circ$C). The $T\Delta S^\circ$ term shows the same trend increasing as it becomes the dominant term at $-10^\circ$C (18.3 kJ) and $-20^\circ$C (48 kJ) where the partition coefficient and the percent recovery is the highest.

The trend that enthalpy becomes more positive and entropy becomes more negative as temperature decreases is consistent for all six essential oils while free energy is always negative in the 20 to $-20^\circ$C temperature range. This indicates that when the conditions are sufficient for phase separation, the hydrophobic analyte consistently partitions into the upper organic phase. Below 0°C, entropy is positive and $T\Delta S^\circ$ is greater than $\Delta H^\circ$, thus, when the conditions are
sufficient for the highest partition coefficient and highest percent recovery, the partition process is driven by entropy.

How can the phenomenon that at the best conditions for phase separation process are driven by entropy be rationalized? Initial evidence came from the phase ratio as at the best conditions for separation, this ratio is the largest (0.53), meaning that greatest possible amount of analyte exists in the upper phase. This is a condition of increasing entropy relative to a condition where there is a smaller amount of material in the upper phase such as at 20°C where the phase ratio is 0.24. Additional evidence is suggested by the increase in the number of phases where at -20°C there are three phases (two liquid and one solid) which is an overall increase in entropy relative to the initial conditions in which there is one phase which occurs before addition of the polyol and at higher temperatures. It is suggested that it is these two factors that are driving the increase in entropy and thus driving the partitioning of the analyte into the upper phase.

Negative ΔG° values and positive partition coefficients values for all essential oils demonstrate that PIE is a spontaneous process in the temperature range of -20 to 20°C. Furthermore, it indicates that partitioning is a combination of both endothermic and exothermic contributions with the net ΔH° being negative. As temperature decreases, enthalpy values shift from negative to positive. At 20°C, the kinetic energy of the system is high and there is less discrimination between ACN and glycerol in the hydrogen bonding to water. As temperature decreases, the kinetic energy of the system is lower which causes glycerol to more effectively hydrogen bond to water displacing the ACN molecules. At these lower temperatures, the majority of the
glycerol and water molecules have formed hydrogen bonds already and the bond breaking energies between ACN and water dominates, hence an endothermic process and positive $\Delta H^\circ$ values.

For entropy, as temperature decreases, $\Delta S^\circ$ values shift from negative to positive (ordered to less ordered). At 20°C, the bonding between ACN and water is a very ordered complex. (negative $\Delta S^\circ$ values). As glycerol is added to the mixture (greater bonding affinity with water) and the temperature is lowered, the water-ACN bonds break and ACN molecules are forced out and become less ordered (positive $\Delta S$ values).

Figures 2-7 through 2-12 show the influence of temperature on $\Delta H^\circ$ and $\Delta S^\circ$. The slopes of these graphs as a function of temperature are negative. From these slopes, we can determine where $\Delta H^\circ$ and $\Delta S^\circ$ are equal to 0, which is the point where the reaction mechanism changes.

Figure 2-7 shows that in the extraction of eugenol from cloves buds, the enthalpy mechanism changed at -7.4°C and the entropy mechanism changed at -4.2°C. Figure 2-8 shows that in the extraction of $d$-carvone from caraway seeds, the enthalpy mechanism changed at -9.0°C and the entropy mechanism changed at -6.8°C. Figure 2-9 shows that in the extraction of menthol from caraway seeds, the enthalpy mechanism changed at -9.8°C and the entropy mechanism changed at -5.5°C. Figure 2-10 shows that in the extraction of $l$-carvone from spearmint leaves, the enthalpy mechanism changed at -9.8°C and the entropy mechanism changed at -5.5°C.
Figure 2-7: Enthalpy and Entropy: Clove Bud Oil
Figure 2-8: Enthalpy and Entropy: Caraway Seed Oil

![Graph showing enthalpy and entropy of caraway seed oil.

The graph plots ΔH° (kJ/mol) against T (K) on the x-axis and ΔS° (J/mol) against T (K) on the y-axis.

Key points:
- ΔH° (-9.0°C) at 264.2 K
- ΔH° (-6.8°C) at 266.4 K

Legend:
- Red circle: Enthalpy
- Blue square: Entropy]
Figure 2-9: Enthalpy and Entropy: Peppermint Leaf Oil
Figure 2-10: Enthalpy and Entropy: Spearmint Leaf Oil
Figure 2-11 shows that in the extraction of anethole from anise seeds, the enthalpy mechanism changed at -5.6°C and the entropy mechanism changed at 0.05°C. Figure 2-12 shows that in the extraction of cinnamic aldehyde from cinnamon bark, the enthalpy mechanism changed at -12.5°C and the entropy mechanism changed at -9.6°C.

The general trend shown is that between 0°C and -10°C, the reaction mechanism reached a change-over point, which is also shown in the ΔG° values as they are very close for 0°C and -10°C. Once the temperature is decreased further, there is a change in the mechanism of the system, which is apparent from the van’t Hoff plot shown in Figure 2-4 as there is a change in linearity of the plot between -10°C and -20°C.

It should be noted that the entropic contribution is very relevant and is the dominating factor for the PIE process. (Net ΔS° value is negative and TΔS° value is greater than ΔH° at the optimal extraction temperature.) The results obtained show that the effect of temperature on the PIE process is highly significant and that it is necessary to precisely control the temperature at which the extraction is performed in order to obtain maximum reproducibility. The presence of these maximum values as a function of temperature implies that the partitioning effect is driven by opposite effects that result from the temperature dependence of entropic and enthalpic contributions.
Figure 2-11: Enthalpy and Entropy: Anise Seed Oil
Figure 2-12: Enthalpy and Entropy: Cinnamon Bark Oil
2.3.3. Essential Oil Composition

The distribution of the chemical components extracted by PIE from each essential oil was compared with traditional extraction methods. The organic phases of each essential oil at -10°C (optimal extraction temperature) were analyzed and the main components of each essential oil were identified using GC/MS. The percentages of the major constituents present in each essential oil were compared to that of an essential oil extracted using steam-distillation and solvent extraction with a polar and non-polar solvent.

The results for the comparison of techniques for the extraction of eugenol from clove buds are shown in Table 2-7. The percentage of eugenol present was 78% via PIE, 70% via steam-distillation, 70% via DCM solvent extraction and 71% via ethanol extraction. Table 2-7 also shows us that PIE reduced the amount of eugenol acetate extracted in comparison to steam distillation by 7% and the amount of caryophyllene extracted using solvent extraction by 8-10%.

The results for the comparison of techniques for the extraction of d-carvone from caraway seeds are shown in Table 2-8. d-carvone was extracted at 80% via PIE, 47% via steam-distillation, 47% via DCM extraction and 49% via ethanol extraction. The major impurity present in caraway seed oil (limonene) was limited in the extraction to only 17% in PIE while the other techniques ranged from 22-50%. Limonene is readily soluble in polar solvents (e.g. DCM, ethanol) and is sparingly soluble in ACN. Since the lower aqueous layer is made up of
Table 2-7: GC/MS compositional profile of Clove Bud Oil: Comparison of different extraction techniques

<table>
<thead>
<tr>
<th>Clove Bud Oil Main Constituents</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Retention Time (min.)</th>
<th>% PIE</th>
<th>% Steam Distillation</th>
<th>% Solvent Extraction with DCM</th>
<th>% Solvent Extraction with EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td>164.20</td>
<td>C_{10}H_{12}O_{2}</td>
<td>17.107</td>
<td>78</td>
<td>70</td>
<td>70</td>
<td>71</td>
</tr>
<tr>
<td>Eugenol Acetate</td>
<td>206.24</td>
<td>C_{12}H_{14}O_{3}</td>
<td>19.411</td>
<td>15</td>
<td>22</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>204.35</td>
<td>C_{15}H_{24}</td>
<td>18.242</td>
<td>4.1</td>
<td>4.5</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>204.35</td>
<td>C_{15}H_{24}</td>
<td>18.731</td>
<td>0.6</td>
<td>0.4</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Caryophyllene Oxide</td>
<td>220.35</td>
<td>C_{15}H_{26}O</td>
<td>20.520</td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table 2-8: GC/MS compositional profile of Caraway Seed Oil: Comparison of different extraction techniques

<table>
<thead>
<tr>
<th>Caraway Seed Oil Main Constituents</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Retention Time (min.)</th>
<th>% PIE</th>
<th>% Steam Distillation&lt;sup&gt;60&lt;/sup&gt;</th>
<th>% Solvent Extraction with DCM</th>
<th>% Solvent Extraction with EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Carvone</td>
<td>150.22</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;14&lt;/sub&gt;O</td>
<td>15.234</td>
<td>80</td>
<td>47</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>Limonene</td>
<td>136.24</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
<td>10.472</td>
<td>17</td>
<td>50</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>(E)-Dihydrocarveol</td>
<td>154.25</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>14.895</td>
<td>0.6</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>(E)-Dihydrocarvone</td>
<td>152.23</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O</td>
<td>14.302</td>
<td>0.3</td>
<td>0.6</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Carveol</td>
<td>152.23</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O</td>
<td>14.677</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>136.23</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
<td>9.370</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>280.46</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>25.804</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>282.46</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>25.846</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>
glycerol and water, limonene exhibits a greater solubility in the aqueous phase than the organic phase, which contributes to its limited presence in the compositional profile.

The results for the comparison of techniques for the extraction of menthol from peppermint leaves are shown in Table 2-9. The percentage of menthol present was 57% via PIE, 33% via steam-distillation, 5.8% via DCM extraction and 6.6% via ethanol extraction. PIE was able to extract more menthol than all of the other techniques while also limiting the amount of impurities extracted such as menthone, eugenol, caryophyllene, and phytol.

The results for the comparison of techniques for the extraction of l-carvone from spearmint leaves are shown in Table 2-10. l-carvone was extracted at 82% via PIE, 67% via steam-distillation, 63% via DCM extraction and 64% via ethanol extraction. Limonene was limited in the extraction to only 0.4% in PIE while steam distillation showed limonene being present at 9.3%. Major non-polar impurities such as β-bourbenene, Caryophyllene, and α-farnesene were extracted at greater amounts via solvent extraction (1.6-3.3%) while PIE reduced the amount of unwanted impurities (0.4-0.7%).

The results for the comparison of techniques for the extraction of anethole from anise seeds are shown in Table 2-11. Anethole was extracted at 87% via PIE, 85% via steam-distillation, 83% via DCM extraction and 56% via ethanol extraction. PIE, steam-distillation and extraction with DCM all showed similar results with PIE extracting anethole at a slightly greater amount. Extraction with ethanol extracted the least amount of anethole and while also extracting large
Table 2-9: GC/MS compositional profile of Peppermint Leaf Oil: Comparison of different extraction techniques

<table>
<thead>
<tr>
<th>Peppermint Leaf Oil Main Constituents</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Retention Time (min.)</th>
<th>% PIE</th>
<th>% Steam Distillation&lt;sup&gt;44&lt;/sup&gt;</th>
<th>% Solvent Extraction with DCM</th>
<th>% Solvent Extraction with EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-Menthol</td>
<td>156.27</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O</td>
<td>13.769</td>
<td>57</td>
<td>33</td>
<td>5.8</td>
<td>6.6</td>
</tr>
<tr>
<td>(E)-Menthone</td>
<td>154.25</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>13.409</td>
<td>11</td>
<td>21</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>(Z)-Menthone</td>
<td>154.25</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>13.652</td>
<td>4.4</td>
<td>-</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>(E)-Menthyl Acetate</td>
<td>198.31</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>16.032</td>
<td>3.8</td>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Z)-Menthol</td>
<td>156.27</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O</td>
<td>13.617</td>
<td>3.7</td>
<td>-</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Caryophyllene Oxide</td>
<td>220.35</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;O</td>
<td>20.677</td>
<td>1.6</td>
<td>-</td>
<td>2.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Veridiflorol</td>
<td>222.37</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O</td>
<td>20.780</td>
<td>1.5</td>
<td>0.3</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Phytol</td>
<td>296.53</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O</td>
<td>25.633</td>
<td>-</td>
<td>-</td>
<td>4.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Eugenol</td>
<td>164.20</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>17.011</td>
<td>-</td>
<td>-</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>204.35</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
<td>18.229</td>
<td>-</td>
<td>-</td>
<td>2.9</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Table 2-10: GC/MS compositional profile of Spearmint Leaf Oil: Comparison of different extraction techniques

<table>
<thead>
<tr>
<th>Spearmint Leaf Oil Main Constituents</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Retention Time (min.)</th>
<th>% PIE</th>
<th>% Steam Distillation$^{a2}$</th>
<th>% Solvent Extraction with DCM</th>
<th>% Solvent Extraction with EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$l$-Carvone</td>
<td>150.22</td>
<td>C$<em>{10}$H$</em>{14}$O</td>
<td>15.240</td>
<td>82</td>
<td>67</td>
<td>63</td>
<td>64</td>
</tr>
<tr>
<td>(E)-Sabinene Hydrate</td>
<td>154.25</td>
<td>C$<em>{10}$H$</em>{16}$O</td>
<td>11.387</td>
<td>3.2</td>
<td>1.3</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Veridiflorol</td>
<td>222.37</td>
<td>C$<em>{13}$H$</em>{26}$O</td>
<td>20.783</td>
<td>2.6</td>
<td>0.2</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>(E)-Jasmone</td>
<td>164.25</td>
<td>C$<em>{11}$H$</em>{16}$O</td>
<td>17.847</td>
<td>1.8</td>
<td>0.3</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Caryophyllene Oxide</td>
<td>220.35</td>
<td>C$<em>{15}$H$</em>{24}$O</td>
<td>20.691</td>
<td>0.8</td>
<td>-</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>204.35</td>
<td>C$<em>{13}$H$</em>{24}$</td>
<td>18.398</td>
<td>0.7</td>
<td>0.9</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>$\alpha$-Farnesene</td>
<td>204.35</td>
<td>C$<em>{13}$H$</em>{24}$</td>
<td>18.548</td>
<td>0.4</td>
<td>-</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>$\beta$-Bourbonene</td>
<td>204.36</td>
<td>C$<em>{13}$H$</em>{24}$</td>
<td>17.824</td>
<td>0.5</td>
<td>1.4</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Limonene</td>
<td>136.24</td>
<td>C$<em>{10}$H$</em>{16}$</td>
<td>10.482</td>
<td>0.4</td>
<td>9.3</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>(Z)-Sabinene Hydrate</td>
<td>154.25</td>
<td>C$<em>{10}$H$</em>{16}$O</td>
<td>12.130</td>
<td>0.4</td>
<td>1.3</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>(Z)-Dihydrocarvone</td>
<td>152.23</td>
<td>C$<em>{10}$H$</em>{16}$O</td>
<td>14.685</td>
<td>0.3</td>
<td>1.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Carvyl Acetate</td>
<td>194.26</td>
<td>C$<em>{12}$H$</em>{18}$O$_{2}$</td>
<td>17.177</td>
<td>0.3</td>
<td>2.0</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 2-11: GC/MS compositional profile of Anise Seed Oil: Comparison of different extraction techniques

<table>
<thead>
<tr>
<th>Anise Seed Oil Main Constituents</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Retention Time (min.)</th>
<th>% PIE</th>
<th>% Steam Distillation</th>
<th>% Solvent Extraction with DCM</th>
<th>% Solvent Extraction with EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-Anethole</td>
<td>148.20</td>
<td>C₁₀H₁₂O</td>
<td>15.963</td>
<td>87</td>
<td>85</td>
<td>83</td>
<td>56</td>
</tr>
<tr>
<td>p-Methoxy Benzaldehyde</td>
<td>136.15</td>
<td>C₈H₁₀O₂</td>
<td>15.417</td>
<td>2.4</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Anethole-2-Methyl Butyrate</td>
<td>284.14</td>
<td>C₁₀H₁₅O₃</td>
<td>23.266</td>
<td>3.4</td>
<td>-</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>4-Methoxy-2-(3-methyloxiranyl)</td>
<td>264.32</td>
<td>C₁₅H₂₀O₄</td>
<td>23.903</td>
<td>1.9</td>
<td>-</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>phenyl-2-Methyl Butyrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Allyl Anisole</td>
<td>148.20</td>
<td>C₁₀H₁₂O</td>
<td>14.257</td>
<td>1.8</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>(Z)-Anethole</td>
<td>148.20</td>
<td>C₁₀H₁₂O</td>
<td>13.013</td>
<td>0.2</td>
<td>2.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>282.46</td>
<td>C₁₈H₃₂O₂</td>
<td>25.789</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.8</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>282.46</td>
<td>C₁₈H₃₄O₂</td>
<td>25.831</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.0</td>
</tr>
<tr>
<td>Mannitol</td>
<td>182.17</td>
<td>C₆H₁₄O₈</td>
<td>22.825</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.6</td>
</tr>
</tbody>
</table>
amounts of polar compounds such as linoleic acid (2.8%), oleic acid (7.0%) and mannitol (8.6%) that were not extracted by any other technique.

The results for the comparison of techniques for the extraction of cinnamic aldehyde from cinnamon bark are shown in Table 2-12. Cinnamic aldehyde was extracted at 79% via PIE, 70% via steam-distillation, 80% via DCM extraction and 73% via ethanol extraction. Major impurities that were present were coumarin and eugenol. Coumarin was extracted at greater amount in PIE at 16% compared to 0.5% in steam distillation, 8.5% in DCM extraction and 6.8% in ethanol extraction. Eugenol was extracted at a lesser amount in PIE at 0.9% compared to 8.0% in steam distillation, 5.2% in DCM extraction and 5.9% in ethanol extraction.

For all of the essential oils examined, the main analytes present were extracted at greater amounts than traditional techniques. In addition to more of the main component being extracted, PIE reduces the amount of unwanted high molecular weight matrix interferences and impurities that are present and more prominent in steam distillation and solvent extraction. Figures 2-13 through 2-18 show the GC/MS chromatograms of the six essential oils tested.

Figure 2-13 shows the comparison of eugenol extracted from clove buds using PIE and solvent extraction with DCM and ethanol. From the GC/MS chromatogram, high molecular weight matrix interferences are present starting at retention time 25.0 minutes and are more abundant in solvent extraction compared to PIE. Impurities present in solvent extraction include high boiling saturated and unsaturated organic carboxylic acids that are present in the botanical matter.
Table 2-12: GC/MS compositional profile of Cinnamon Bark Oil: Comparison of different extraction techniques

<table>
<thead>
<tr>
<th>Cinnamon Bark Oil Main Constituents</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Retention Time (min.)</th>
<th>% PIE</th>
<th>% Steam Distillation$^{64}$</th>
<th>% Solvent Extraction with DCM</th>
<th>% Solvent Extraction with EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-Cinnamaldehyde</td>
<td>132.16</td>
<td>C$_9$H$_8$O</td>
<td>15.747</td>
<td>79</td>
<td>70</td>
<td>80</td>
<td>73</td>
</tr>
<tr>
<td>Coumarin</td>
<td>146.14</td>
<td>C$_9$H$_6$O$_2$</td>
<td>18.623</td>
<td>16</td>
<td>0.5</td>
<td>8.5</td>
<td>6.8</td>
</tr>
<tr>
<td>Eugenol</td>
<td>164.20</td>
<td>C$<em>{10}$H$</em>{12}$O$_2$</td>
<td>17.147</td>
<td>0.9</td>
<td>8.0</td>
<td>5.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Cinnamyl Acetate</td>
<td>176.22</td>
<td>C$<em>{11}$H$</em>{14}$O$_2$</td>
<td>18.419</td>
<td>0.6</td>
<td>5.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>154.25</td>
<td>C$<em>{10}$H$</em>{18}$O</td>
<td>10.595</td>
<td>0.4</td>
<td>0.5</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>154.25</td>
<td>C$<em>{10}$H$</em>{18}$O</td>
<td>14.151</td>
<td>0.3</td>
<td>1.4</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>4-Terpineol</td>
<td>154.25</td>
<td>C$<em>{10}$H$</em>{18}$O</td>
<td>13.914</td>
<td>0.2</td>
<td>0.8</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>204.35</td>
<td>C$<em>{15}$H$</em>{24}$</td>
<td>18.376</td>
<td>0.2</td>
<td>2.3</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Cinnamic Acid</td>
<td>148.16</td>
<td>C$_9$H$_8$O$_2$</td>
<td>17.967</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>2.1</td>
</tr>
</tbody>
</table>
Figure 2-13: GC/MS chromatogram of Clove Bud Oil: Comparison of different extraction techniques
Figure 2-14 shows the comparison of $d$-carvone extracted from caraway seeds using PIE and solvent extraction with DCM and ethanol. From the GC/MS chromatogram, the major polar impurities linoleic and oleic acids at retention time 25-26 minutes are present in the solvent extracted sample while not being present in the PIE extracted sample. These impurities are very soluble in the polar solvents DCM and ethanol which is why they are present at higher abundances.

Figure 2-15 shows the comparison of menthol extracted from peppermint leaves using PIE and solvent extraction with DCM and ethanol. From the GC/MS chromatogram, high molecular weight matrix interferences are present starting at retention time 25.0 minutes and are more abundant in solvent extraction compared to PIE. Most notably in these samples is the impurity phytol that is present at a higher abundance at retention time 45 minutes in both solvent extracted samples while it is absent in the PIE sample.

Figure 2-16 shows the comparison of $l$-carvone extracted from spearmint leaves using PIE and solvent extraction with DCM and ethanol. From the GC/MS chromatogram, high molecular weight matrix interferences are present starting at retention time 25.0 minutes and are more abundant in solvent extraction compared to PIE. Major impurities present in the solvent extracted samples include non-polar sesquiterpene and unsaturated long chain hydrocarbon compounds.

Figure 2-17 shows the comparison of anethole extracted from anise seeds using PIE and solvent extraction with DCM and ethanol. From the GC/MS chromatogram, many
Figure 2-14: GC/MS chromatogram of Caraway Seed Oil: Comparison of different extraction techniques
Figure 2-15: GC/MS chromatogram of Peppermint Leaf Oil: Comparison of different extraction techniques
Figure 2-16: GC/MS chromatogram of Spearmint Leaf Oil: Comparison of different extraction techniques
Figure 2-17: GC/MS chromatogram of Anise Seed Oil: Comparison of different extraction techniques
impurities starting at retention time 23 minutes are present in both solvent extracted samples while not being present in the PIE extracted sample. Major impurities once again include high boiling organic carboxylic acids such as linoleic and oleic acid as well as the high boiling C6 sugar alcohol mannitol.

Figure 2-18 shows the comparison of cinnamic aldehyde extracted from cinnamon bark using PIE and solvent extraction with DCM and ethanol. The GC/MS chromatogram for all three extractions showed that there are not many matrix interferences present as seen in previous samples. The major impurity coumarin was extracted at higher amounts in PIE versus solvent extraction using both DCM and ethanol, which is most likely due to coumarin having a higher solubility in ACN than DCM and ethanol. Eugenol, another impurity present, had a higher abundance in DCM and ethanol compared to that of PIE, which is also most likely due to the solubility of eugenol in each individual solvent.

In general, all six essential oils extracted showed less matrix interferences present using PIE than solvent extraction. Since the bottom aqueous layer has glycerol and water present (both polar solvents), the majority of the polar impurities present that are extracted from the plant matter, (e.g. oleic acid, linoleic acid, mannitol, phytol) are “trapped” and don’t migrate into the organic phase. This phenomenon makes PIE highly effective for removing matrix interferences which leads this extraction technique to have potential to be used in many other applications.
Figure 2-18: GC/MS chromatogram of Cinnamon Bark Oil: Comparison of different extraction techniques
2.4 Conclusions

In this work, a partitioning based extraction system for essentials oils based on acetonitrile/aqueous solvent systems and glycerol was demonstrated. Polyol induced extraction (PIE) is a useful chemical process for the extraction of essential oils where heat, cost, and time is a concern. In all situations, the main compounds present in each essential oil partition to the acetonitrile-rich organic phase. Additionally, a decrease in temperature leads to an increase on $K_{PC}$. The highest partitioning and percent recovery occurred at -10°C, thus, -10°C is considered as the optimal temperature for a high yielding extraction. The transition of the main compounds present in each essential oil to the organic phase was spontaneous and an overall exothermic process with entropic contribution dominating the phase separation.
CHAPTER 3 - EXTRACTION OF ESSENTIAL OILS via PIE AND QuEChERS: A COMPARISON AND VALIDATION STUDY

3.1 Introduction

This chapter discusses the QuEChERS extraction technique and the methodology behind it. QuEChERS is one of the most widely used extraction techniques for a variety of different analytes and applications. This study compares and contrasts PIE to QuEChERS and assesses the validity of both techniques for the extraction of essential oils.

3.1.1 QuEChERS Background

QuEChERS stands for Quick, Easy, Cheap, Effective, Rugged and Safe and it was developed by Michelangelo Anastassiades while performing his post-doctoral research work under Steven J. Lehotay. The method was originally designed for the extraction of veterinary drugs from animal tissues but the ability of the method to successfully and efficiently extract polar and basic compounds led it to be adopted for pesticide extraction in plant materials. This newly developed method was presented by Anastassiades and Lehotay at the European Pesticide Workshop in 2002, with the first QuEChERS publication coming in 2003. Since this method was developed, it has evolved into two other methods, the AOAC 2007.01 method and the European Standard EN 15662 method, which makes use of buffering salts to increase the recovery of pH dependent analytes.
Although QuEChERS was originally developed for pesticide extraction from botanical matrices, it is very well suited for extraction of many different chemical compounds in a wide variety of matrices. Research has been performed using QuEChERS in the extraction of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), veterinary and pharmaceutical drugs in matrices that include food and animals, sewage, soil, dietary supplements, biological matrices, breast milk and baby formula.69-74

3.1.2 QuEChERS Theory and Methodology

The QuEChERS method is a combination of a two-step extraction that includes a liquid-liquid extraction (LLE) and a dispersive solid phase extraction (d-SPE) clean up step. In the first step, an organic solvent is used to extract an aqueous mixture of analytes and then salts are used in order to induce a phase separation. In the second step, a d-SPE sorbent is added to bind unwanted matrix interferences which provide a cleaner sample for analysis.

As mentioned earlier, there are three commonly used methods that have given rise to all of the current variations in QuEChERS methods. These methods are the original method, the AOAC 2007.01 method and the European Standard EN 15662 method. Figure 3-1 shows a flowchart of the steps involved for all three methods. The methods all follow the same initial steps, with a LLE between an organic solvent and water followed by introduction of salts to generate the liquid-liquid partitioning. The sample is shaken and centrifuged and then an aliquot of the organic extract is removed and subjected to a d-SPE clean-up step using magnesium sulfate.
### Step 1: Liquid-Liquid Extraction Methodology

<table>
<thead>
<tr>
<th>Original QuEChERS Method</th>
<th>AOAC QuEChERS Method</th>
<th>Buffered QuEChERS Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anastassiades and Lehotay</strong></td>
<td><strong>AOAC 2007.01</strong></td>
<td><strong>EN 15662</strong></td>
</tr>
<tr>
<td>Add 10mL ACN to 10g homogenized/hydrated sample</td>
<td>Add 15 mL 1% Acetic Acid in ACN to 15mL homogenized/hydrated sample</td>
<td>Add 10mL of ACN to 10g homogenized/hydrated sample</td>
</tr>
<tr>
<td>Shake</td>
<td>Shake</td>
<td>Shake</td>
</tr>
<tr>
<td>Add 4g MgSO₄ and 1 g NaCl</td>
<td>Add 6g MgSO₄ and 1.5g NaOAc</td>
<td>Add 4g MgSO₄ 1 g NaCl, 1g Na₃Citr, 0.5g Na₂HCitr</td>
</tr>
<tr>
<td>Shake vigorously for 1 minute</td>
<td>Shake vigorously for 1 minute</td>
<td>Shake vigorously for 1 minute</td>
</tr>
<tr>
<td>Centrifuge for 5 minutes (5000 rpm)</td>
<td>Centrifuge for 1 minute (&gt;1500 rcf)</td>
<td>Centrifuge for 5 minutes (5000U/min)</td>
</tr>
</tbody>
</table>

### Step 2: Dispersive Solid Phase Extraction Clean-up

<table>
<thead>
<tr>
<th>Transfer 1mL of supernatant to clean-up tube (150mg MgSO₄ and 50mg PSA)</th>
<th>Transfer 1mL supernatant to a dispersive clean-up tube with MgSO₄ and PSA</th>
<th>Transfer 1 mL of supernatant to a d-SPE clean up tube with 25 mg PSA and 150 mg MgSO₄ (plus 2.5 or 7.5 mg GCB to remove pigments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shake for 1 minutes</td>
<td>Shake for 30 seconds</td>
<td>Shake for 30 seconds</td>
</tr>
<tr>
<td>Centrifuge for 1 minute at 6000 rpm</td>
<td>Centrifuge for 1 minute (&gt;150Orct)</td>
<td>Centrifuge for 5 minutes (3000U/min)</td>
</tr>
</tbody>
</table>

Transfer 0.5mL to vial for GC or LC analysis

For GC/MS: Preserve with toluene
For LC/MS/MS: Preserve with 6.7mM formic acid

Preserve with 5% formic acid in ACN
Analyze by GC/MS or LC/MS/MS

---

**Figure 3-1: Comparison of QuEChERS Methods.** Reprinted with permission from Schmidt, Michelle L., "QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) Extraction – Gas Chromatography for the Analysis of Drugs" (2015), Seton Hall University Dissertations and Theses (ETDs). Paper 2060.

http://scholarship.shu.edu/dissertations/2060
(MgSO₄) and a sorbent such as primary-secondary amine (PSA), graphitized carbon black (GCB) or end capped C18 silica, in order to bind the unwanted matrix components. After this, the sample can then be analyzed using GC or LC.

In the LLE step, acetonitrile, ethyl acetate, or acetone are the three most commonly used organic solvents in QuEChERS. Acetonitrile is used most frequently as it has the broadest extraction range while also minimizing the amount of unwanted interferences. The salts used to aid in the phase separation while also driving the analyte of interest into the organic phase vary depending on which method is being followed. Sodium chloride (NaCl) and magnesium sulfate (MgSO₄) are used in the original and European methods, with the European method also using citrate buffering salts that include sodium citrate dibasic sesquihydrate (Na₂HCitr-1.5H₂O) and sodium citrate tribasic dihydrate (Na₃Citr-2H₂O).66,68 The addition of salt aids in the partitioning of polar compounds by increasing the ionic strength that can lead to salting out. Also, salt increases the polarity of the solvent being used, which in turn increases the solubility of the analyte in that solvent.

Sodium chloride decreases the amount of polar interferences extracted while magnesium sulfate works to improve the polar analyte recover and also aids in the solvent partitioning during the LLE step. Most methods use a 4:1 ratio of sodium chloride to magnesium sulfate, but different salts can be used depending on the nature of the analytes being extracted. The AOAC method uses magnesium sulfate and sodium acetate (NaAc) rather than sodium
chloride and also uses acetonitrile with 1% acetic acid as a buffer for base sensitive pesticide compounds.

The d-SPE step is similar in all three methods where a clean-up sorbent is added to remove matrix interferences and magnesium sulfate is added to remove any residual water that might be present in the organic phase. The choice of sorbent can be optimized and is greatly dependent on the analytes being extracted as each different sorbent removes a specific interference. Some examples of sorbents are shown in Figures 3-2 to 3-4. They include primary secondary amine (PSA), which removes polar interferences such as sugars, fatty and organic acids, and pigments based upon weak ion exchange, graphitized carbon black (GCB) which works to bind planar analytes and remove pigments and end-capped C18 to remove non-polar interferences. A combination of sorbents can also be used to remove certain compounds while leaving other intact for analysis.

The objective of the work presented in this chapter is to demonstrate that PIE can be an analogous extraction technique to QuEChERS. Since QuEChERS is considered to be the “gold standard” in sample preparation for the extraction of analytes present in botanical matter, alternate techniques such as PIE have potential to be investigated as a sample preparation technique. Both techniques are very similar as they make use of an induced phase separation to extract analytes of interest and partition them into the organic phase for analysis. This similarity presented an excellent opportunity to compare and contrast both techniques.
Primary Secondary Amine (PSA) for cleanup of fatty acids and polar interferences

Palmitic Acid

Stearic Acid

Figure 3-2: Primary Secondary Amine (PSA)
Figure 3-3: Graphitized Carbon Black (GCB) for removal of pigments
End capped C18 Silica for cleanup of sterols and non-polar interferences

Figure 3-4: Endcapped C18 Silica
3.2 Materials and Methods

3.2.1. Chemicals, Reagents and Samples

The reagents used in this experiment were all reagent grade and purchased from Sigma Aldrich (St. Louis, MO): acetonitrile (HPLC-grade with a purity of 99.9%), glycerol (purity of > 99.5%), anhydrous sodium chloride (purity of ≥ 99.0%), and anhydrous magnesium sulfate (purity of ≥ 99.5%). Q-sep® QuEChERS tubes containing 1200 mg MgSO₄ and 400 mg PSA were purchased from Restek Corporation (Bellefonte, PA) and 15 ml high-density polyethylene (HDPE) conical centrifuge tubes with screw caps were purchased from VWR International (Radnor, PA). Distilled and deionized water was used in all experiments.

The essential oils investigated in this work were isolated from dried clove buds [main component: eugenol (4-allyl-2-methoxy phenol)], cinnamon bark [main component: cinnamaldehyde (3-phenyl-2-propenal)], caraway seeds [main component: d-carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexenone)], spearmint leaves [main component: l-carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexenone)], peppermint leaves [main component: menthol (2-isopropyl-5-methyl cyclohexanol)], and anise seeds [main component: anethole (1-methoxy-(4-propenyl) benzene)], all of which were purchased from internet suppliers.
3.2.2 Sample Preparation

The procedure outlined below was used for extraction of the main compounds present in six essential oils and was performed in three replicates for both the PIE and QuEChERS procedures. Dried plant matter was homogenized and approximately 2 grams was added to a 15 mL glass vial containing 10 mL of deionized water. The sample was vortexed for 1 minute and sonicated for 10 minutes. After sonication and centrifugation, a 5 mL aliquot of the aqueous mixture was transferred to a 15 mL HDPE centrifuge tube and 5 mL of acetonitrile was added. The samples were vortexed once again for 1 minute to homogenize the sample. At this point in the procedure, the difference in the methodology between PIE and QuEChERS is observed.

For PIE, glycerol (20 %, w/v) is added and the sample is vortexed for 1 minute. The sample was then equilibrated for 30 minutes at -10°C. After the two phases separated, the entire organic layer was removed via pipette and transferred to a GC autosampler vial for analysis.

For QuEChERS, a 1:1 mixture of NaCl / MgSO₄ (25%, w/v) is added and the sample is vortexed for 1 minute. After vortexing, the sample is centrifuged for 3 minutes at 4,000 rpm. Once centrifugation is complete, the entire organic top layer is removed and transferred to a 15 mL HDPE centrifuge tube. A 2:3 mixture of PSA/anhydrous MgSO₄ (10%, w/v) is added to the tube, vortexed for 1 minute and centrifuged for 3 minutes at 4,000 rpm. The entire top organic layer is then removed via pipette and transferred to a GC autosampler vial for analysis.
3.2.3 Instrumental Parameters

The instrumentation utilized for this study was an Agilent 7890B gas chromatograph (GC) (Santa Clara, CA) equipped with a CTC Analytics CombiPAL Autosampler (Zwingen, Switzerland) and an Agilent 5977A Inert Mass Selective Detector (MSD) (Santa Clara, CA) according to the below mentioned GC/MS conditions. The data were interpreted using Agilent’s MassHunter software. The identities of the compounds were determined by the similarity of their mass spectra with those from the Wiley Flavors and Fragrances of Natural and Synthetic Compounds 3 (3rd edition) and the NIST 11-MS mass spectral databases.

The GC/MS analysis was performed using an Agilent DB-5ms capillary column (60 m, 0.25 mm id, 0.25 μm) with the following parameters: He (g) constant flow, 1.0 mL/min; inlet temperature, 300°C; injection volume 3μL (split 50:1); initial oven temperature, 50°C, held for 7 min, then a 10°C/min ramp to 250°C and held for 25 min. The quadrupole mass analyzer (electron impact ionization type, 70 eV) was operated in both full scan mode and selected ion monitoring (SIM) mode simultaneously. Full scan mode was operated at a rate of 3.89 scans/sec and a range of 35-400 amu. For SIM mode, the ions monitored for each of the main components in the six different essential oils are shown in Table 3-1. These ions were selected based on the known mass spectral fragmentation patterns for each analyte. The source temperature was set at 230°C and the transfer line temperature set at 280°C.
Table 3-1: SIM Parameters for Essential Oils

<table>
<thead>
<tr>
<th>Essential Oil Main Component</th>
<th>Parent Ion (m/z)</th>
<th>Daughter Ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td>164</td>
<td>165,149</td>
</tr>
<tr>
<td>Anethole</td>
<td>148</td>
<td>147,149</td>
</tr>
<tr>
<td>d-Carvone</td>
<td>150</td>
<td>151,82</td>
</tr>
<tr>
<td>l-Carvone</td>
<td>150</td>
<td>151,82</td>
</tr>
<tr>
<td>Cinnamic Aldehyde</td>
<td>132</td>
<td>131,103</td>
</tr>
<tr>
<td>Menthol</td>
<td>156</td>
<td>81,71</td>
</tr>
</tbody>
</table>
3.2.4 Experimental Parameters

3.2.4.1 Extraction Efficiency

In order to evaluate the extraction efficiency of the two methods, the partition coefficient and percent recovery were determined. Essential oil standards containing eugenol, anethole, d-carvone, l-carvone, cinnamic aldehyde and menthol were prepared in HPLC grade acetone with concentrations ranging from 0.005 ppm to 5000 ppm and analyzed via GC/MS in order to generate calibration curves for each of the six essential oils. Calibration curves for all six of the main analytes present in the six essential oil are shown in Figures 3-11 to 3-16. The calibrations curves used varied based on the essential oil being assessed. For clove bud and cinnamon bark oil, a calibration curve ranging from 5 to 5000 ppm was used. For caraway seed and spearmint leaf oil, a calibration curve ranging from 0.5 to 500 ppm was used. For aniseed and peppermint leaf oil, a calibration curve ranging from 0.5 ppm to 50 ppm was used. The $R^2$ value and equation of the line were obtained using Microsoft Excel for all the resulting calibration curves. Once the calibration curves were constructed, the equation of the line could be used to determine the concentration of the analytes in the organic and aqueous phases, which were then used to determine the partition coefficient using the same equation as shown in Chapter 2.

$$K_{PC} = \frac{C_{EO\,(UP)}}{C_{EO\,(LP)}}$$

(Equation 3-1)

The partition coefficients and percent recovery were determined for each sample in triplicate and an average was taken.
Figure 3-5: Eugenol Calibration Curve

Calibration Curve: Eugenol

\[ y = 80938x + 3 \times 10^6 \]

\[ R^2 = 0.9989 \]
Figure 3-6: Anethole Calibration Curve

\[ y = 83766x + 3 \times 10^6 \]

\[ R^2 = 0.999 \]
Figure 3-7: $d$-Carvone Calibration Curve

The calibration curve for $d$-Carvone is shown with the equation $y = 38462x + 1 \times 10^6$ and $R^2 = 0.9989$. The concentration is given in parts per million (ppm).
Figure 3-8: $\alpha$-Carvone Calibration Curve
Figure 3-9: Cinnamic Aldehyde Calibration Curve

Calibration Curve: Cinnamic Aldehyde

\[ y = 120283x + 3 \times 10^6 \]

\[ R^2 = 0.9992 \]
Figure 3-10: Menthol Calibration Curve

Calibration Curve: Menthol

\[ y = 46452x + 1 \times 10^6 \]

\[ R^2 = 0.9989 \]
3.2.4.2 Method Validation

Method validation was assessed in terms of precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). All parameters were evaluated using the same calibration curves as shown in Figures 3-11 to 3-16. Precision was determined as the percent relative standard deviation (%RSD), which was calculated from the partition coefficients for all six essentials oils extracted that were run in triplicate. (Equation 3-2)

\[
\% \text{RSD} = \left( \frac{s}{\bar{x}} \right) \times 100
\]

Accuracy was assessed as the percent recovery of the main components in each essential oil and this was calculated from the partition coefficient and phase ratio according to the same equation that was used in Chapter 2. (Equation 3-3)

\[
R_T = \frac{100}{1 + \frac{1}{K_{PC} \times R_v}}
\]

Limit of detection (LOD) and limit of quantitation (LOQ) were assessed using the data analysis software where the signal to noise ratio (S/N) for each analyte peak was 3 and 10. The concentrations of the analyte ([A]) associated with each peak as well as the peak width (P_w) in time (mins) were also used to establish the detection limits. The equations used for LOD (Equation 3-4) and LOQ (Equation 3-5) are shown below.

\[
\text{LOD} = \frac{3 \times ([A] / P_w)}{S/N}
\]

\[
\text{LOQ} = \frac{10 \times ([A] / P_w)}{S/N}
\]
3.3 Results and Discussion

3.3.1 Extraction Efficiency

The data obtained for the main analytes extracted from the six essential oils using PIE and QuEChERS are shown in Tables 3-2 and 3-3. The data shown is the amount of the analyte in the organic phase and the amount of analyte in the aqueous phase which is used to determine the partition coefficient. Figures 3-17 through 3-22 show the GC/MS/SIM chromatograms comparisons of the analytes extracted from each essential oil using PIE and QuEChERS.

For the extraction of eugenol from clove buds, eugenol was present at 4590 ppm in the organic phase using PIE compared to 3430 ppm in the organic phase using QuEChERS. In the aqueous phase, eugenol was present at 13 ppm using PIE while only present at 1 ppm in the aqueous phase using QuEChERS. These values led to partition coefficients for eugenol of 350 for PIE and 3430 for QuEChERS. In Figure 3-17, in the organic phase, eugenol has a peak abundance of $2.1 \times 10^6$ using PIE while only having a peak abundance of $1.6 \times 10^6$ for QuEChERS. In the aqueous phase, eugenol has a peak abundance of $2.0 \times 10^4$ using PIE and $6.6 \times 10^2$ for QuEChERS.

For the extraction of anethole from anise seeds, anethole was present at 20 ppm in the organic phase using PIE compared to 16 ppm in the organic phase using QuEChERS. In the aqueous phase, anethole was present at 0.3 ppm using PIE while only present at 0.08 ppm in the aqueous phase using QuEChERS. These values led to partition coefficients for anethole of 67 for PIE
### Table 3-2: PIE Extraction Efficiency Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Polyol Induced Extraction (PIE)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount in Organic Phase (ppm)</td>
<td>Amount in Aqueous Phase (ppm)</td>
<td>Partition Coefficient ($K_{PC}$)</td>
<td></td>
</tr>
<tr>
<td>Clove Bud Oil</td>
<td>4590 ± 108</td>
<td>13 ± 0.30</td>
<td>350 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td>20 ± 0.9</td>
<td>0.3 ± 0.1</td>
<td>67 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td>930 ± 99</td>
<td>12 ± 1.3</td>
<td>78 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td>420 ± 11</td>
<td>3 ± 0.04</td>
<td>140 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td>280 ± 19</td>
<td>0.9 ± 0.05</td>
<td>310 ± 11</td>
<td></td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td>13 ± 0.31</td>
<td>0.5 ± 0.03</td>
<td>26 ± 1.7</td>
<td></td>
</tr>
</tbody>
</table>
## Table 3-3: QuEChERS Extraction Efficiency Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>QuEChERS</th>
<th></th>
<th>Partition Coefficient ($K_{PC}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount in Organic Phase (ppm)</td>
<td>Amount in Aqueous Phase (ppm)</td>
<td></td>
</tr>
<tr>
<td>Clove Bud Oil</td>
<td>3430 ± 74.3</td>
<td>1.0 ± 0.30</td>
<td>3430 ± 694</td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td>16 ± 1.7</td>
<td>0.08 ± 0.01</td>
<td>200 ± 50</td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td>430 ± 15</td>
<td>0.8 ± 0.1</td>
<td>540 ± 81</td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td>260 ± 9.4</td>
<td>0.2 ± 0.1</td>
<td>1300 ± 470</td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td>200 ± 5</td>
<td>0.06 ± 0.01</td>
<td>3330 ± 695</td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td>9 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>23 ± 8.6</td>
</tr>
</tbody>
</table>
Figure 3-11: GC/MS/SIM chromatogram of Clove Bud Oil: Eugenol
and 200 for QuEChERS. In Figure 3-18, in the organic phase, anethole has a peak abundance of $1.9 \times 10^4$ using PIE while only having a peak abundance of $1.7 \times 10^4$ for QuEChERS. In the aqueous phase, anethole has a peak abundance of $2.0 \times 10^4$ using PIE and $4.0 \times 10^2$ for QuEChERS.

For the extraction of cinnamic aldehyde from cinnamon bark, cinnamic aldehyde was present at 930 ppm in the organic phase using PIE compared to 430 ppm in the organic phase using QuEChERS. In the aqueous phase, cinnamic aldehyde was present at 12 ppm using PIE while only present at 0.8 ppm in the aqueous phase using QuEChERS. These values led to partition coefficients for cinnamic aldehyde of 78 for PIE and 540 for QuEChERS. In Figure 3-19, in the organic phase, cinnamic aldehyde has a peak abundance of $7.0 \times 10^6$ using PIE while only having a peak abundance of $3.4 \times 10^6$ for QuEChERS. In the aqueous phase, cinnamic aldehyde has a peak abundance of $1.5 \times 10^5$ using PIE and $1.2 \times 10^4$ for QuEChERS.

For the extraction of $d$-carvone from caraway seeds, $d$-carvone was present at 420 ppm in the organic phase using PIE compared to 260 ppm in the organic phase using QuEChERS. In the aqueous phase, $d$-carvone was present at 3 ppm using PIE while only present at 0.2 ppm in the aqueous phase using QuEChERS. These values led to partition coefficients for $d$-carvone of 140 for PIE and 1300 for QuEChERS. In Figure 3-20, in the organic phase, $d$-carvone has a peak abundance of $2.4 \times 10^4$ using PIE while only having a peak abundance of $1.5 \times 10^4$ for QuEChERS. In the aqueous phase, $d$-carvone has a peak abundance of $1.4 \times 10^4$ using PIE and $1.3 \times 10^3$ using QuEChERS.
Figure 3-12: GC/MS/SIM chromatogram of Aniseed Oil: Anethole
Figure 3-13: GC/MS/SIM chromatogram of Cinnamon Bark Oil: Cinnamic Aldehyde
Figure 3-14: GC/MS/SIM chromatogram of Caraway Seed Oil: $d$-Carvone
For the extraction of \(l\)-carvone from spearmint leaves, \(l\)-carvone was present at 280 ppm in the organic phase using PIE compared to 200 ppm in the organic phase using QuEChERS. In the aqueous phase, \(l\)-carvone was present at 0.9 ppm using PIE while only present at 0.06 ppm in the aqueous phase using QuEChERS. These values led to partition coefficients for \(l\)-carvone of 310 for PIE and 3330 for QuEChERS. In Figure 3-21, in the organic phase, \(l\)-carvone has a peak abundance of \(2.4 \times 10^4\) using PIE while only having a peak abundance of \(1.5 \times 10^4\) for QuEChERS. In the aqueous phase, \(l\)-carvone has a peak abundance of \(1.4 \times 10^4\) using PIE and \(1.3 \times 10^3\) using QuEChERS.

For the extraction of menthol from peppermint leaves, menthol was present at 13 ppm in the organic phase using PIE compared to 9 ppm in the organic phase using QuEChERS. In the aqueous phase, menthol was present at 0.5 ppm using PIE while only present at 0.4 ppm in the aqueous phase using QuEChERS. These values led to partition coefficients for menthol of 26 for PIE and 23 for QuEChERS. In Figure 3-22, in the organic phase, menthol has a peak abundance of \(1.5 \times 10^4\) using PIE while only having a peak abundance of \(1.2 \times 10^4\) for QuEChERS. In the aqueous phase, menthol has a peak abundance of \(8.1 \times 10^3\) using PIE and \(9.5 \times 10^2\) using QuEChERS.
Figure 3-15: GC/MS/SIM chromatogram of Spearmint Leaf Oil: l-Carvone
Figure 3-16: GC/MS/SIM chromatogram of Peppermint Leaf Oil: Menthol
3.3.2 Method Validation

The method validation comparison data for the extraction of the six essentials oils via PIE and QuEChERS is shown in Tables 3-4 and 3-5. To validate the accuracy and precision of the methods, the percent recovery and percent relative standard deviations (RSD) were assessed and the % RSD is shown in Figure 3-23. The limit of detection (LOD) and limit of quantitation (LOQ) were also assessed for all six essential oil and are shown in Tables 3-4 and 3-5.

In the extraction of eugenol from clove buds, the % RSD was found to be 0.9% using PIE compared to 21% using QuEChERS. The percent recovery for eugenol was found to be 99.6% using PIE and 99.9% using QuEChERS. The LOD for eugenol extracted using PIE was calculated to be 4.1 ppb compared to 5.0 ppb using QuEChERS. The LOQ for eugenol extracted using PIE was calculated to be 13.5 ppb compared 16.8 ppb using QuEChERS.

In the extraction of anethole from anise seeds, the % RSD was found to be 4.4% using PIE compared to 22% using QuEChERS. The percent recovery for anethole was found to be 98.1% using PIE and 99.3% using QuEChERS. The LOD for anethole extracted using PIE was calculated to be 12 ppb compared to 4.9 ppb using QuEChERS. The LOQ for anethole extracted using PIE was calculated to be 13.5 ppb compared 16.3 ppb using QuEChERS.
Table 3-4: PIE Method Validation Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Polyol Induced Extraction (PIE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision (%)</td>
</tr>
<tr>
<td>Clove Bud Oil</td>
<td>0.9</td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td>4.4</td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td>7.0</td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td>2.3</td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td>3.4</td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Table 3-5: QuEChERS Method Validation Data

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>QuEChERS</th>
<th>Precision (% RSD)</th>
<th>Accuracy (% Recovery)</th>
<th>LOD (ppb)</th>
<th>LOQ (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove Bud Oil</td>
<td></td>
<td>21</td>
<td>99.9 ± 0.01</td>
<td>5.0 ± 0.7</td>
<td>16.8 ± 2.4</td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td></td>
<td>22</td>
<td>99.3 ± 0.16</td>
<td>4.9 ± 0.7</td>
<td>16.3 ± 2.3</td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td></td>
<td>31</td>
<td>92.5 ± 2.6</td>
<td>24 ± 8.9</td>
<td>80 ± 30</td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td></td>
<td>14</td>
<td>99.8 ± 0.04</td>
<td>207 ± 48</td>
<td>690 ± 161</td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td></td>
<td>28</td>
<td>99.9 ± 0.04</td>
<td>18 ± 1.9</td>
<td>58 ± 6.5</td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td></td>
<td>19</td>
<td>99.9 ± 0.01</td>
<td>17 ± 3.4</td>
<td>58 ± 11.4</td>
</tr>
</tbody>
</table>
Figure 3-17: % RSD for Essential Oils
In the extraction of menthol from peppermint leaves, the % RSD was found to be 7.0% using PIE compared to 31% using QuEChERS. The percent recovery for menthol was found to be 92.4% using PIE and 92.5% using QuEChERS. The LOD for menthol extracted using PIE was calculated to be 68 ppb compared to 24 ppb using QuEChERS. The LOQ for menthol extracted using PIE was calculated to be 225 ppb compared 80 ppb using QuEChERS.

In the extraction of cinnamic aldehyde from cinnamon bark, the % RSD was found to be 2.3% using PIE compared to 14% using QuEChERS. The percent recovery for cinnamic aldehyde was found to be 98.3% using PIE and 99.8% using QuEChERS. The LOD for cinnamic aldehyde extracted using PIE was calculated to be 88 ppb compared to 207 ppb using QuEChERS. The LOQ for cinnamic aldehyde extracted using PIE was calculated to be 293 ppb compared 690 ppb using QuEChERS.

In the extraction of d-carvone from caraway seeds, the % RSD was found to be 3.4% using PIE compared to 28% using QuEChERS. The percent recovery for d-carvone was found to be 98.7% using PIE and 99.9% using QuEChERS. The LOD for d-carvone extracted using PIE was calculated to be 0.9 ppb compared to 18 ppb using QuEChERS. The LOQ for d-carvone extracted using PIE was calculated to be 3.0 ppb compared 58 ppb using QuEChERS.

In the extraction of l-carvone from spearmint leaves, the % RSD was found to be 3.6% using PIE compared to 19% using QuEChERS. The percent recovery for l-carvone was found to be
99.4% using PIE and 99.9% using QuEChERS. The LOD for l-carvone extracted using PIE was calculated to be 0.6 ppb compared to 17 ppb using QuEChERS. The LOQ for l-carvone extracted using PIE was calculated to be 2.0 ppb compared 58 ppb using QuEChERS.

For all six essential oils extracted and analyzed, the % RSD is much less in PIE compared to QuEChERS as shown in Figure 3-23. Overall, the % RSD ranges from 0.9 to 7.0 in PIE while ranging from 14 to 31 in QuEChERS. The percent recovery for all six essential oils show very similar results using both PIE and QuEChERS, with QuEChERS showing slightly higher results for some essential oils. The extraction recoveries were > 98% for all essential oils, with the exception of peppermint leaf oil which has a percent recovery of 92%. LOD and LOQ were also very similar for both extraction techniques, with PIE showing lower limits of detections for some essential oils as discussed previously.

### 3.3.3 Essential Oil Composition

The compositional profiles for all six of the essential oils extracted using the two methodologies were compared and are shown in Figures 3-24 and 3-25. Figure 3-24 shows a graphically representation of the peak abundance of the main component present in each essential oil. Figure 3-25 shows the comparison FID chromatograms of the main analyte present in each essential oil extracted using PIE and QuEChERS. For all six essential oils, both techniques show similar results in the essential oil compositional profiles, but PIE was able to extract a greater abundance of the main component present in the essential oil.
Figure 3-18: Abundance of Main Component in Essential Oils
Figure 3-19: FID Chromatograms of Main Components in Essential Oils
In addition to extracting more of the main component present in each essential oil, PIE was comparable to QuEChERS in its ability to reduce the amount of matrix interferences present in each of the six essential oils. Figures 3-25 to 3-31 shows the overlaid comparison TIC chromatograms of all six essential extracted using PIE and QuEChERS. For eugenol, cinnamic aldehyde, \(d\)-carvone, and \(l\)-carvone, as shown in Figures 3-25, 3-26, 3-27 and 3-28, there are no matrix impurities present in either chromatogram as both techniques were able to remove them effectively. For anethole and menthol as shown in Figures 3-20 and 3-31, matrix effects are still present but the chromatograms for both PIE and QuEChERS are identical as both techniques were not able to remove matrix interferences for these essential oils.

### 3.4 Conclusions

In this study, PIE was compared to the widely known extraction technique QuEChERS. From the results obtained, it is shown that PIE is a comparable technique to QuEChERS for the extraction of essential oils. PIE is able to extract the main component of each essential oil analyzed at a greater abundance in the organic phase than QuEChERS. Conversely, more of the main component is present in the aqueous phase which leads to much higher partition coefficients in QuEChERS compared to PIE. In an analytical extraction technique, the organic phase is usually only considered to be of interest, so PIE proves to be a promising technique.

The accuracy and precision of both methods was also comparable with PIE showing better results. In terms of accuracy, the percent recovery for all six essential oil in both methods was
Figure 3-20: Matrix Effects for Clove Bud Oil: Eugenol
Figure 3.21: Matrix Effects for Cinnamon Bark Oil: Cinnamic Aldehyde
Figure 3-22: Matrix Effects for Caraway Seed Oil: \textit{d}-Carvone
Figure 3-23: Matrix Effects for Spearmint Leaf Oil: $l$-Carvone
Figure 3-24: Matrix Effects for Aniseed Oil: Anethole
Figure 3-25: Matrix Effects for Peppermint Leaf Oil: Menthol
similar with all recoveries being > 95% with QuEChERS being minutely greater in some cases. For precision, PIE proved to be a better technique. In terms of %RSD, PIE had much lower % RSD for all six essential oils tested. This is due to the fact the PIE is a single-tube extraction technique with less steps than QuEChERS which limits the amount of human error and analyte loss while transferring sample to different tubes.

Finally, PIE is also able to reduce the amount of matrix interferences on a comparable level to that of QuEChERS. The glycerol used in PIE acts as a polar sorbent which allows for the reduction of polar interferences present similar to the use of PSA in QuEChERS. Glycerol also is used as a MSA separating agent, which binds and complexes water in order to generate a phase separation, which salts are used for in QuEChERS. Overall, it is shown that PIE can be used as an alternative extraction technique to QuEChERS for the extraction of essentials oils. PIE is a simpler, more cost effective, less error prone method with the potential for reusable solvents while also not having the need for an additional clean-up step.
CHAPTER 4 - EXTRACTION OF ESSENTIAL OILS via PIE WITH
GENERALLY RECOGNIZED AS SAFE (GRAS) SOLVENTS

4.1 Introduction

This chapter discusses the Flavor and Extract Manufactures Association (FEMA) and background information of their beginning and how they operate in order to determine if a substance can be deemed to be generally recognized as safe (GRAS). The potential for the extraction of essentials oils via PIE with FEMA/GRAS solvents was assessed and validated.

4.1.1 FEMA/GRAS Background

FEMA is comprised of flavor manufactures, flavor users, flavor ingredient suppliers and other parties with interests in the flavor industry. It is the national association of the US flavor industry. FEMA was founded in 1909 in response to the FDA Pure Food and Drug Act of 1906 which prohibited interstate commerce of adulterated and misbranded food and drugs. FEMA’s sole goal is to work with regulatory agencies in order to provide safety evaluations of flavor ingredients through its established critical objectives. By doing this, FEMA assists small companies and businesses who do not have the capability and expertise to make these types of determinations on their own. FEMA is comprised of individuals such as industry scientists and chemists, flavor company executives, regulatory agencies, academic institutions and scientific professional organizations who all collaborate to address a wide range of specific interests of the flavor industry. FEMA has also founded the International Organization of the Flavor
Industry (IOFI), which is the international association of national and regional flavor associations and companies who represent the interest of the international flavor industry.

Abiding and operating based on their mission statement, “FEMA furthers the business interests of its members through a sound scientific program designed to promote the safe use of flavors. Through effective representation of its members, FEMA fosters a global environment in which the flavor industry can create, innovate, and compete.” FEMA’s critical objectives are broken down into four categories: science, advocacy, communication, and intellectual property protection.

The goals of these objectives are to achieve and maintain a scientifically valid approach to the safety evaluations of flavor ingredients through continued support of the FEMA expert panel who are making these safety evaluations. By doing this, FEMA is serving as an advocate for FEMA members in the flavor industry by communicating key flavor industry issues that are currently being addressed while also constantly educating members of new regulatory developments that arise.

The generally recognized as safe (GRAS) program was established by FEMA in 1959 in response to 1958 Food Additives Amendments to the Federal Food, Drug, and Cosmetic Act, the Federal law governing the regulation of flavors and other food ingredients. This program began by polling the flavor industry ingredients to assess the amounts of substances being used
to manufacture flavors. From this, the FEMA Expert Panel was established in 1960 for the evaluation of the safety of flavor ingredients. This panel consists of several members with expert qualifications in the fields of toxicology, organic chemistry, biochemistry, metabolism, and pathology.75

In the United States, the regulations of a substance as a food ingredient is determined either through the GRAS perception stated by the FDA in Section 201(s) of the Federal Food, Drug, and Cosmetic Act or through a GRAS determination by a qualified private party. In order for a substance to be considered GRAS, it must meet four crucial criteria. These criteria state that a substance must be recognized as safe by experts, the experts making this determination must be qualified to do so, this determination must be based on a scientifically accepted method or criteria and the final use of the substance must be taken into account. If all these criteria are met, all of the parties review the basis for the GRAS determination and it concluded to be valid by all, the material is assigned a FEMA number. There are currently over 4800 substances with FEMA numbers published in 27 GRAS flavoring substance lists that have been approved for use as flavor ingredients. These lists provide recommended usage levels for substances in different flavoring categories and are constantly being updated as new flavoring substances are evaluated.
4.1.2 Polyol Induced Extraction (PIE) with FEMA/GRAS Solvents

In Chapter 2, PIE using acetonitrile as a solvent was demonstrated to be effective in the extraction of essential oils. Although acetonitrile is considered to be safer and “greener” than halogenated solvents, it is still not considered to be GRAS. For PIE with GRAS solvents, acetone and isopropyl alcohol (IPA) were of interest since they are fully miscible with water, polar solvents with high dielectric constants while also being considered GRAS. Table 4-1 shows a solvent comparison of acetone, Isopropyl Alcohol, acetonitrile, and dichloromethane in terms of Permissible Exposure Limit (PEL), Recommended Exposure Limit (REL), Threshold Limit Value (TLV) and lethal dose (LD$_{50}$). From the table, it shows that acetone and IPA have significantly higher PEL, REL, TLV, and LD$_{50}$ compared to that of acetonitrile and dichloromethane. Acetone is designated by FEMA number 3326 and was published in the FEMA GRAS List 6. Isopropyl Alcohol is designated by FEMA number 2929 and was published in FEMA GRAS list 3.

From the PIE patent$^{22}$ that was filed, it was shown that 1:1 (v/v) mixtures of water and acetone do not separate with the addition of glycerol at any amount. However, it stated that a 6:4 (v/v) mixture of acetone and water was able to be separated with addition of 20% sorbitol (Figure at -18°C). There was no mention or testing of 1:1 (v/v) mixtures of water and isopropyl alcohol. Based on these results for acetone, mixtures of acetone/water and isopropyl alcohol/water (6:4, v/v) were chosen as the starting point. Initial attempts at separation of these mixtures failed as phase separation were not observed with 20% sorbitol at room temperature, -10°C and -20°C. Successful phase separation was observed with the addition of 20% sorbitol to 7:3 (v/v)
Table 4-1: Toxicity comparison of common extraction solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Permissible Exposure Limit (PEL)</th>
<th>Recommended Exposure Limit (REL)</th>
<th>Threshold Limit Value (TLV)</th>
<th>LD$_{50}$ (rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>1000 ppm</td>
<td>250 ppm</td>
<td>500 ppm</td>
<td>5800 ppm</td>
</tr>
<tr>
<td>IPA</td>
<td>400 ppm</td>
<td>400 ppm</td>
<td>200 ppm</td>
<td>5045 ppm</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>40 ppm</td>
<td>20 ppm</td>
<td>20 ppm</td>
<td>2730 ppm</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>12.5 ppm</td>
<td>Lowest feasible concentration</td>
<td>50 ppm</td>
<td>1600 ppm</td>
</tr>
</tbody>
</table>
mixtures of acetone/water and isopropyl alcohol/water at room temperature. This result was used as the phase separation conditions for the experiments performed.

4.2 Materials and Methods

4.2.1 Chemicals, Reagents and Samples

The reagents used in this experiment were all reagent grade and purchased from Sigma Aldrich (St. Louis, MO): acetone (HPLC-grade with a purity of 99.9%), isopropyl alcohol (HPLC-grade with a purity of 99.8%) and D-sorbitol (purity of ≥ 98.0%). 15 mL high-density polyethylene (HDPE) conical centrifuge tubes with screw caps were purchased from VWR International (Radnor, PA). Distilled and deionized water was used in all experiments.

The essential oils investigated in this work were isolated from dried clove buds [main component: eugenol (4-allyl-2-methoxy phenol)], cinnamon bark [main component: cinnamaldehyde (3-phenyl-2-propenal)], caraway seeds [main component: d-carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexenone)], spearmint leaves [main component: l-carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexenone)], peppermint leaves [main component: menthol (2-isopropyl-5-methyl cyclohexanol)], and anise seeds [main component: anethole (1-methoxy-(4-propenyl) benzene)], all of which were purchased from internet suppliers.
4.2.2 Sample Preparation

The procedure outlined below was used for extraction of the main compounds present in six essential oils and was performed in three replicates. Dried plant matter was homogenized and approximately 0.5 grams was added to a 15 mL HDPE conical centrifuge tube with screw caps. 10 mL of a 7:3 (v/v) mixture of isopropyl alcohol and deionized water was added to the tube and vortexed for 15 mins. After vortexing, the solid plant matter was filtered off and D-sorbitol (20%, w/v) was added to the tube. The samples were vortexed once again for 10 minutes and equilibrated for 30 minutes at -10°C. After the two phases separated, the entire organic layer was removed via pipette and transferred to a GC autosampler vial for analysis. The same procedure was repeated using the acetone/water (7:3, v/v) solvent system.

4.2.3 Instrumental Parameters

The instrumentation utilized for this study was an Agilent 7890B gas chromatograph (GC) (Santa Clara, CA) equipped with a CTC Analytics CombiPAL Autosampler (Zwingen, Switzerland) and an Agilent 5977A Inert Mass Selective Detector (MSD) (Santa Clara, CA) according to the below mentioned GC/MS conditions. The data were interpreted using Agilent’s MassHunter software. The identities of the compounds were determined by the similarity of their mass spectra with those from the Wiley Flavors and Fragrances of Natural and Synthetic Compounds 3 (3rd edition) and the NIST 11-MS mass spectral databases.

The GC/MS analysis was performed on an Agilent DB-5ms capillary column (60 m, 0.25 mm id, 0.25 µm) with the following parameters: He (g) constant flow, 1.0 mL/min; inlet
temperature, 300°C; injection volume 3μL (split 50:1); initial oven temperature, 50°C, held for 7 min, then a 10°C/min ramp to 250°C and held for 25 min. The quadrupole mass analyzer (electron impact ionization type, 70 eV) was operated in both full scan mode and selected ion monitoring (SIM) mode simultaneously. Full scan mode was operated at a rate of 3.89 scans/sec and a range of 35-400 amu. For SIM mode the ions monitored for each of the main components in the six different essential oils are the same as shown in Chapter 3, Table 3-1. The source temperature was set at 230°C and the transfer line temperature set at 280°C. Essential oil compositional profiles were determined using all the same parameters as mentioned previously with the exception of using a polar Agilent DB-FFAP capillary column (60 m, 0.25 mm id, 0.25 μm).

4.2.4 Experimental Parameters

4.2.4.1 Extraction Efficiency

In order to evaluate the extraction efficiency of the two methods, the partition coefficient and percent recovery were determined. Essential oil standards containing eugenol, anethole, d-carvone, l-carvone, cinnamic aldehyde and menthol were prepared in HPLC grade acetone with concentrations ranging from 0.005 ppm to 5000 ppm and analyzed via GC/MS in order to generate calibration curves for each of the six essential oils, which were shown previously in Chapter 3. The calibrations curves used varied based on the essential oil being assessed. For clove bud oil, a calibration curve ranging from 50 to 50000 ppm was used. For caraway seed, cinnamon bark, and aniseed oil, a calibration curve ranging from 50 to 5000 ppm was used. For spearmint leaf oil, a calibration curve ranging from 5 ppm to 500 ppm was used. For
peppermint leaf oil, a calibration curve ranging from 0.5 ppm to 50 ppm was used. The $R^2$ values and equation of the line were obtained using Microsoft Excel for all the resulting calibration curves. Once the calibration curves were constructed, the equation of the line could be used to determine the concentration of the analytes in the organic and aqueous phases, which were then used to determine the partition coefficient using the same equation as shown in Chapter 2. The partition coefficients and percent recovery were determined for each sample in triplicate and an average was taken.

4.2.4.2 Method Validation

The method validation was assessed in terms of precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). All parameters were evaluated using the same calibration curves as shown previously in Figures 3-2 to 3-7 of Chapter 3. Precision was determined as the percent relative standard deviation (% RSD), which was calculated from the partition coefficients for all six essentials oils extracted that were run in triplicate. Accuracy was assessed as the percent recovery of the main components in each essential oil and this was calculated from the partition coefficient and phase ratio. Limit of detection (LOD) and limit of quantitation (LOQ) were assessed using the data analysis software where the signal to noise ratio (S/N) for each analyte peak was 3 and 10, respectively. The concentrations of the analyte ([A]) associated with each peak as well as the peak width ($P_w$) in time (minutes) were also used to establish the detection limits. All calculations were performed using the same equations as shown previously in Chapter 3.
4.3 Results and Discussion

4.3.1 Extraction Efficiency

The data obtained for the main analytes extracted from the six essential oils using PIE with acetone and isopropyl alcohol are shown in Tables 4-2 and 4-3. The data shown is the amount of the analyte in the organic phase and the amount of analyte in the aqueous phase which is used to determine the partition coefficient. Figures 4-1 through 4-6 show the GC/MS/SIM chromatograms comparisons of the analytes extracted from each essential oil using PIE and QuEChERS.

For the extraction of eugenol from clove buds, eugenol was present at 26200 ppm in the organic phase using PIE with acetone compared to 20400 ppm in the organic phase using PIE with IPA. In the aqueous phase, eugenol was present at 95 ppm using PIE with acetone while present at 190 ppm in the aqueous phase using PIE with IPA. These values led to a partition coefficient for eugenol of 280 for PIE with acetone and 110 for PIE with IPA. In Figure 4-1, in the organic phase, eugenol has a peak abundance of $2.3 \times 10^6$ using PIE with acetone while only having a peak abundance of $1.8 \times 10^6$ for PIE with IPA. In the aqueous phase, eugenol has a peak abundance of $1.9 \times 10^5$ using PIE with acetone and $2.8 \times 10^5$ using PIE with IPA.
Table 4-2: PIE Extraction Efficiency: Acetone

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Polyol Induced Extraction (PIE): Acetone</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount in Organic Phase (ppm)</td>
<td>Amount in Aqueous Phase (ppm)</td>
<td>Partition Coefficient (K&lt;sub&gt;PC&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td>Clove Bud Oil</td>
<td>26200 ± 1020</td>
<td>95 ± 2.3</td>
<td>280 ± 15</td>
<td></td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td>3100 ± 460</td>
<td>88 ± 11</td>
<td>35 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td>1200 ± 68</td>
<td>5.1 ± 3.2</td>
<td>240 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td>1970 ± 169</td>
<td>15 ± 1.2</td>
<td>131 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td>590 ± 13</td>
<td>4.7 ± 0.12</td>
<td>130 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td>33 ± 0.16</td>
<td>0.2 ± 0.01</td>
<td>170 ± 5.2</td>
<td></td>
</tr>
</tbody>
</table>
### Table 4-3: PIE Extraction Efficiency: Isopropyl Alcohol

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Amount in Organic Phase (ppm)</th>
<th>Amount in Aqueous Phase (ppm)</th>
<th>Partition Coefficient (K&lt;sub&gt;PC&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove Bud Oil</td>
<td>20400 ± 884</td>
<td>190 ± 17</td>
<td>110 ± 12</td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td>2200 ± 43</td>
<td>110 ± 5.5</td>
<td>20 ± 0.7</td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td>770 ± 10</td>
<td>6.7 ± 0.21</td>
<td>120 ± 2.4</td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td>1400 ± 29</td>
<td>20 ± 2</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td>480 ± 2.6</td>
<td>9.3 ± 0.46</td>
<td>52 ± 2.7</td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td>28 ± 1.1</td>
<td>0.2 ± 0.02</td>
<td>140 ± 9.1</td>
</tr>
</tbody>
</table>
Figure 4-1: GC/MS/SIM Chromatogram of Clove Bud Oil: Eugenol
For the extraction of cinnamic aldehyde from cinnamon bark, cinnamic aldehyde was present at 3100 ppm in the organic phase using PIE with acetone compared to 2200 ppm in the organic phase using PIE with IPA. In the aqueous phase, cinnamic aldehyde was present at 88 ppm using PIE with acetone while present at 110 ppm in the aqueous phase using PIE with IPA. These values led to a partition coefficient for cinnamic aldehyde of 35 for PIE with acetone and 20 for PIE with IPA. In Figure 4-2, in the organic phase, cinnamic aldehyde has a peak abundance of $2.5 \times 10^7$ using PIE with acetone while only having a peak abundance of $1.9 \times 10^7$ for PIE with IPA. In the aqueous phase, cinnamic aldehyde has a peak abundance of $1.0 \times 10^6$ using PIE with acetone and $1.4 \times 10^6$ using PIE with IPA.

For the extraction of anethole from anise seeds, anethole was present at 1200 ppm in the organic phase using PIE with acetone compared to 770 ppm in the organic phase using PIE with IPA. In the aqueous phase, anethole was present at 5.1 ppm using PIE with acetone while present at 6.7 ppm in the aqueous phase using PIE with IPA. These values led to a partition coefficient for anethole of 240 for PIE with acetone and 120 for PIE with IPA. In Figure 4-3, in the organic phase, anethole has a peak abundance of $1.0 \times 10^7$ using PIE with acetone while only having a peak abundance of $6.9 \times 10^6$ for PIE with IPA. In the aqueous phase, anethole has a peak abundance of $3.6 \times 10^4$ using PIE with acetone and $6.4 \times 10^4$ using PIE with IPA.

For the extraction of $d$-carvone from caraway seeds, $d$-carvone was present at 1970 ppm in the organic phase using PIE with acetone compared to 1400 ppm in the organic phase using PIE with IPA. In the aqueous phase, $d$-carvone was present at 15 ppm using PIE with acetone while
Figure 4-2: GC/MS/SIM Chromatogram of Cinnamon Bark Oil: Cinnamic Aldehyde
Figure 4-3: GC/MS/SIM Chromatogram of Aniseed Oil: Anethole
present at 20 ppm in the aqueous phase using PIE with IPA. These values led to a partition coefficient for \(d\)-carvone of 131 for PIE with acetone and 70 for PIE with IPA. In Figure 4-4, in the organic phase, \(d\)-carvone has a peak abundance of \(6.2 \times 10^6\) using PIE with acetone while only having a peak abundance of \(4.6 \times 10^6\) for PIE with IPA. In the aqueous phase, \(d\)-carvone has a peak abundance of \(8.0 \times 10^4\) using PIE with acetone and \(9.9 \times 10^4\) using PIE with IPA.

For the extraction of \(l\)-carvone from spearmint leaves, \(l\)-carvone was present at 590 ppm in the organic phase using PIE with acetone compared to 480 ppm in the organic phase using PIE with IPA. In the aqueous phase, \(l\)-carvone was present at 4.7 ppm using PIE with acetone while present at 9.3 ppm in the aqueous phase using PIE with IPA. These values led to a partition coefficient for \(l\)-carvone of 130 for PIE with acetone and 52 for PIE with IPA. In Figure 4-5, in the organic phase, \(l\)-carvone has a peak abundance of \(2.8 \times 10^6\) using PIE with acetone while only having a peak abundance of \(2.3 \times 10^6\) for PIE with IPA. In the aqueous phase, \(l\)-carvone has a peak abundance of \(3.4 \times 10^4\) using PIE with acetone and \(5.5 \times 10^4\) using PIE with IPA.

For the extraction of menthol from peppermint leaves, menthol was present at 33 ppm in the organic phase using PIE with acetone compared to 28 ppm in the organic phase using PIE with IPA. In the aqueous phase, menthol was present at 0.2 ppm using PIE with acetone and present at 0.2 ppm in the aqueous phase using PIE with IPA. These values led to a partition coefficient for menthol of 170 for PIE with acetone and 140 for PIE with IPA. In Figure 4-6, in the organic phase, menthol has a peak abundance of \(1.2 \times 10^5\) using PIE with acetone while only having a
**Figure 4-4:** GC/MS/SIM Chromatogram of Caraway Seed Oil: $d$-Carvone

<table>
<thead>
<tr>
<th>$d$-Carvone Organic Phase</th>
<th>$d$-Carvone Aqueous Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Graph showing chromatogram]</td>
<td>[Graph showing chromatogram]</td>
</tr>
</tbody>
</table>

- **Acetone**
- **IPA**
Figure 4-5: GC/MS/SIM Chromatogram of Spearmint Leaf Oil: \textit{l}-Carvone
Figure 4-6: GC/MS/SIM Chromatogram of Peppermint Leaf Oil: Menthol
peak abundance of $9.9 \times 10^4$ for PIE with IPA. In the aqueous phase, menthol has a peak abundance of $4.7 \times 10^4$ using PIE with acetone and $3.5 \times 10^4$ using PIE with IPA.

### 4.3.2 Method Validation

The method validation comparison data for the extraction of the six essentials oils via PIE with acetone and isopropyl alcohol is shown in Tables 4-4 and 4-5. To validate the accuracy and precision of the methods, the percent recovery and percent relative standard deviations (RSD) were assessed and the % RSD is shown in Figure 4-7. The limit of detection (LOD) and limit of quantitation (LOQ) were also assessed for all six essential oil and are shown in Tables 4-4 and 4-5.

In the extraction of eugenol from clove buds, the % RSD was found to be 5.5\% using PIE with acetone compared to 11\% using PIE with IPA. The percent recovery for eugenol was found to be 99.5\% using PIE with acetone and 98.7\% using PIE with IPA. The LOD for eugenol extracted using PIE with acetone was calculated to be 16 ppb compared to 23 ppb using PIE with IPA. The LOQ for eugenol extracted using PIE with acetone was calculated to be 53 ppb compared to 76 ppb using PIE with IPA.

In the extraction of anethole from anise seeds, the % RSD was found to be 1.2\% using PIE with acetone compared to 2.1\% using PIE with IPA. The percent recovery for anethole was found to be 99.4\% using PIE with acetone and 98.8\% using PIE with IPA. The LOD for
anethole extracted using PIE with acetone was calculated to be 13 ppb compared to 4.9 ppb using PIE with IPA. The LOQ for anethole extracted using PIE with acetone was calculated to be 42 ppb compared to 17 ppb using PIE with IPA.

In the extraction of menthol from peppermint leaves, the % RSD was found to be 2.6% using PIE with acetone compared to 7.0% using PIE with IPA. The percent recovery for menthol was found to be 99.3% using PIE with acetone and 98.9% using PIE with IPA. The LOD for menthol extracted using PIE with acetone was calculated to be 120 ppb compared to 93 ppb using PIE with IPA. The LOQ for menthol extracted using PIE with acetone was calculated to be 410 ppb compared to 310 ppb using PIE with IPA.

In the extraction of cinnamic aldehyde from cinnamon bark, the % RSD was found to be 2.3% using PIE with acetone compared to 3.5% using PIE with IPA. The percent recovery for cinnamic aldehyde was found to be 96.1% using PIE with acetone and 93.2% using PIE with IPA. The LOD for cinnamic aldehyde extracted using PIE with acetone was calculated to be 32 ppb compared to 23 ppb using PIE with IPA. The LOQ for cinnamic aldehyde extracted using PIE with acetone was calculated to be 110 ppb compared to 76 ppb using PIE with IPA.

In the extraction of $d$-carvone from caraway seeds, the % RSD was found to be 3.9% using PIE with acetone compared to 6.7% using PIE with IPA. The percent recovery for $d$-carvone
Table 4-4: PIE Method Validation Data: Acetone

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Polyol Induced Extraction (PIE): Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision (% RSD)</td>
</tr>
<tr>
<td>Clove Bud Oil</td>
<td>5.5</td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td>1.2</td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td>2.6</td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td>2.3</td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td>3.9</td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td>1.3</td>
</tr>
</tbody>
</table>
Table 4-5: PIE Method Validation Data: Isopropyl Alcohol

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Polyol Induced Extraction (PIE): Isopropyl Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision (% RSD)</td>
</tr>
<tr>
<td>Clove Bud Oil</td>
<td>11</td>
</tr>
<tr>
<td>Aniseed Oil</td>
<td>2.1</td>
</tr>
<tr>
<td>Peppermint Leaf Oil</td>
<td>7.0</td>
</tr>
<tr>
<td>Cinnamon Bark Oil</td>
<td>3.5</td>
</tr>
<tr>
<td>Caraway Seed Oil</td>
<td>6.7</td>
</tr>
<tr>
<td>Spearmint Leaf Oil</td>
<td>5.2</td>
</tr>
</tbody>
</table>
Figure 4-7: % RSD of Essential Oils
was found to be 98.9% using PIE with acetone and 98.1% using PIE with IPA. The LOD for 
d-carvone extracted using PIE with acetone was calculated to be 22 ppb compared to 36 ppb 
using PIE with IPA. The LOQ for d-carvone extracted using PIE with acetone was calculated 
to be 72 ppb compared to 119 ppb using PIE with IPA.

In the extraction of l-carvone from spearmint leaves, the % RSD was found to be 1.3% using 
PIE with acetone compared to 5.2% using PIE with IPA. The percent recovery for l-carvone 
was found to be 98.9% using PIE with acetone and 97.4% using PIE with IPA. The LOD for 
l-carvone extracted using PIE with acetone was calculated to be 30 ppb compared to 83 ppb 
using PIE with IPA. The LOQ for l-carvone extracted using PIE with acetone was calculated 
to be 99 ppb compared to 223 ppb using PIE with IPA.

For all six essential oils extracted and analyzed, the % RSD is much less in PIE using acetone 
as a solvent than PIE using IPA as a solvent, which is shown in Figure 4-7. Overall, the % 
RSD ranges from 1.2 to 5.5 in PIE using acetone while ranging from 2.1 to 11 in PIE using 
IPA. The percent recovery for all six essential oils are much higher when using acetone as a 
solvent rather than IPA in the PIE process. The extraction recoveries were > 98% for all 
essential oils using PIE with acetone, with the exception of cinnamon bark oil which has a 
percent recovery of 96%. For PIE, the extraction recoveries were > 98% for all essential oils 
with the exception of cinnamon bark oil and spearmint leaf oil which had percent recoveries 
of 93% and 97%. LOD and LOQ also showed the same trend with PIE using acetone showing 
lower limits of detections and quantitation for some essential oils as discussed previously.
4.3.3 Essential Oil Composition

The compositional profiles for all six of the essential oils extracted using the two methodologies were compared and are shown in Figures 4-8 and 4-9. Figure 4-8 shows a graphically representation of the peak abundance of the main component present in each essential oil. Figure 4-9 shows the comparison FID chromatograms of the main analyte present in each essential oil extracted using PIE with acetone and IPA as solvents. For all six essential oils, both techniques show similar results in the essential oil compositional profiles, but PIE using acetone was able to extract a greater abundance of the main component present in the essential oil.

While PIE with acetone was able to extract more of the main component present in each essential oil, both PIE with acetone and IPA were not able to reduce the amount of matrix interferences present in each of the six essential oils. Figures 4-10 to 4-13 shows the overlaid comparison TIC chromatograms of select essential oils extracted using PIE with acetone and IPA as solvents. For eugenol, cinnamic aldehyde, anethole and d-carvone, as shown in Figures 4-10, 4-11, 4-12 and 4-13, there are many matrix impurities present in both chromatogram as both PIE with acetone and IPA were not able to remove them effectively. In all chromatograms, looking at the retention time of 21 minutes to 46 minutes, there are several high molecular weight matrix impurities that are still in the organic phase as they were not sufficiently removed by the extraction process.
Figure 4-8: FID Peak Area of Essential Oils
Figure 4-9: FID Chromatograms of Main Components in Essential Oils
Figure 4-10: Matrix Effects in Clove Bud Oil: Eugenol

TIC Chromatogram: Eugenol Organic Phase

Acetone
IPA
Figure 4-11: Matrix Effects in Cinnamon Bark Oil: Cinnamic Aldehyde
Figure 4-12: Matrix Effects in Aniseed Oil: Anethole
Figure 4-13: Matrix Effects in Caraway Seed Oil: $d$-Carvone
4.4 Conclusions

In this study, PIE of essential oils using FEMA/GRAS solvents was explored. Initially it was shown the mixtures of acetone/water and Isopropyl Alcohol/water (1:1, v/v) cannot be separated using glycerol as a mass separating agent. In order to induce a phase separation between acetone/water and isopropyl alcohol/water mixtures, sorbitol needed to be added (20%, w/v) to 7:3 (v/v) mixtures of acetone/water and isopropyl alcohol/water. Acetone and isopropyl alcohol are fully miscible with water and form very strong hydrogen bonds with water which is evident from the heat given off (exothermic reaction) when mixing them together. Glycerol (C3 sugar alcohol) is not strong enough to break the bonds and bind with water. By employing sorbitol (C7 sugar alcohol), it is able to bind to water therefore forcing out the acetone and isopropyl alcohol, which causes a phase separation. However, sorbitol is a solid at standard temperature and pressure so it is not very efficient to work with. Also, significant matrix effects are present as we can see in Figures 4-11 to 4-13. Polar compounds such as palmitic, linoleic, and oleic acid were observed at the end of the TIC chromatogram, so an additional clean up step to remove these inferences would be needed. Combinations of polyols could also be explored to help remedy this situation and induce a more efficient phase separation. Mixtures of sorbitol and glycerol or higher order sugar alcohols (C7, C12, C18, and C24 sugar alcohols) would be good candidates to start with, but these would most likely require heating, which is a process that we are trying to avoid.
Acetone was shown to be able to extract the main component of each essential oil analyzed at a greater abundance in the organic phase than isopropyl alcohol while also leaving behind less residual analyte in the aqueous phase, which led to higher partition coefficients compared to isopropyl alcohol. Acetone has a higher dielectric constant than isopropyl alcohol (20.7 vs. 17.9)\textsuperscript{76}, so based on this, it would act as a better solvent, which is evident from the results obtained.

The accuracy and precision of PIE using both acetone and Isopropyl Alcohol was also comparable with acetone showing better results. In terms of accuracy, acetone showed higher percent recovery for all six essential oil at > 96% compared to only > 93% using isopropyl alcohol. For precision, both solvents were within the experimental error range of ± 20%, with acetone showing better results. In terms of % RSD, acetone had a lower % RSD for all six essential oils tested at < 5.5% compared to < 11% for isopropyl alcohol.

In general, PIE with FEMA/GRAS solvents can be used in the extraction of essentials oils. However, further studies are needed to optimize the extraction conditions (i.e. polyol mass separating agent combinations) to achieve a phase separation of 1:1 (v/v) mixtures of acetone/water and isopropyl alcohol/water in order to achieve the highest abundance of analyte extracted while also being able to reduce the matrix interferences present without the need for an additional clean-up step.
CHAPTER 5 - pH OPTIMIZATION OF PIE

5.1 Introduction

This chapter discusses the pH optimization of Polyol Induced Extraction (PIE). Since many of the constituents present in essential oils are ionisable at a certain pH, it was thought that by adjusting the pH of the extraction technique, a cleaner and purer essential oil with a lesser amount of impurities can be obtained.

5.1.1 Acid –Base Extractions: Background

Acid base extractions are a variation of liquid-liquid extractions where the only difference is that the aqueous layer is not neutral but rather acidic or basic. The fundamental theory behind these types of extractions is the fact that the protonated or deprotonated ions of the compounds that are formed will be ionic and therefore water soluble. For most organic functional groups, acid-base extractions have no effects, but for carboxylic acids, phenols and amine functional groups, an acid base reaction will reverse its solubility characteristics.

Most carboxylic acids are insoluble in water. However, when a carboxylic acid is extracted with an aqueous base the carboxylic acid is deprotonated to produce a carboxylate salt which is more readily soluble in aqueous solvent rather than an organic solvent, as shown in equation
5-1. The two layers can then be separated with the aqueous layer containing the analytes of interest.

\[
\text{RCOOH} + \text{NaOH (aq)} \rightarrow \text{RCO}^- \text{Na}^+ (aq) + \text{H}_2\text{O} \quad \text{(Equation 5-1)}
\]

The carboxylate ion can then be restored to its original carboxylic acid form by employing another acid-base reaction with addition of an acid, as shown in Equation 5-2.

\[
\text{RCO}^- \text{Na}^+ (aq) + \text{HCl (aq)} \rightarrow \text{RCOOH} + \text{NaCl(aq)} \quad \text{(Equation 5-2)}
\]

Equation 5-3 shows a similar reaction that occurs with phenols, except strong bases are needed to deprotonate them, since they are less acidic than carboxylic acids. This property is very advantageous as this would enable the separation of phenols from carboxylic acids as long as the differences in their pK_a or pK_b values are large enough. Compounds containing an amine functional group can be converted into their hydrochloride salt (which is soluble in water) with the addition of aqueous HCl as shown in Equation 5-4.

\[
\text{ArOH} + \text{NaOH (aq)} \rightarrow \text{ArO}^- \text{Na}^+ (aq) + \text{H}_2\text{O} \quad \text{(Equation 5-3)}
\]

\[
\text{RNH}_2 + \text{HCl (aq)} \rightarrow \text{RNH}_3 + \text{Cl}^- (aq) \quad \text{(Equation 5-4)}
\]

As a drawback, this technique is only applicable for acids and bases that have large differences in solubility of their charged and uncharged forms. Limitations of this extraction technique
include zwitterions that contain acidic and basic functional groups in the same molecule, very
lipophilic amines and acids that don’t easily dissolve in the aqueous phase in their charged
state, lower amines that are soluble with water at most pH’s and hydrophilic and inorganic
acids that are readily water soluble.77

In summary, standard extraction of carboxylic acids, amines, phenols, and other organic
compounds will all be soluble in the organic phase. When an acid base extraction is performed,
one can selectively remove the phenol with an aqueous strong base, the carboxylic acid with
an aqueous weak base and the amine with an aqueous acid.

5.1.2 Acid–Base Extractions: Essential Oils

The majority of essential oils contain compounds that are classified as phenolic terpenoids or
phenylpropanoids, which contain a phenolic functional group. Figure 5-1 depicts a base
extraction of phenol. By applying an acid-base extraction with strong base, the phenolic
functional group will be deprotonated and the sodium phenoxide ion will be formed, which is
water soluble. In clove bud oil, the main compound present is eugenol, which contains an
ionisable proton. Eugenol has a pKa of 10.3, so in theory a solution with a pH greater than the
pKa of eugenol will deprotonate eugenol to form its ionic counterpart, which will make it
readily soluble in the aqueous phase. A basic schematic of an acid-base extraction coupled to
PIE to extract the main component eugenol from clove bud oil is shown in Figure 5-2. By
Figure 5-1: Base Extraction of Phenol
optimizing the pH for the PIE extraction technique, the main component present in the essential oil will be extracted at a greater abundance while limiting the amount of impurities in the final extract.

5.2 Materials and Methods

5.2.1 Chemicals, Reagents and Samples

The reagents used in this experiment were all reagent grade and purchased from Sigma Aldrich (St. Louis, MO): acetonitrile (HPLC-grade with a purity of 99.9%), sodium hydroxide pellets (reagent grade with a purity of ≥ 98.0%) and hydrochloric acid (ACS reagent grade with a purity of 37%). 15 ml high-density polyethylene (HDPE) conical centrifuge tubes with screw caps were purchased from VWR International (Radnor, PA).

Distilled and deionized water was used in all experiments. The essential oils investigated in this work were Clove Bud Oil (Syzygium aromaticum L.) [main component: eugenol (4-allyl-2-methoxy phenol)], Thyme Oil (Thymus vulgaris) [main component: thymol (2-isopropyl-5-methylphenol)] and Oregano Oil (Origanum compactum) [main component: carvacrol (5-isoproypl-2-methylphenol)] which were all steam-distilled essential oils purchased from Healing Solutions, LLC (Scottsdale, AZ) via the internet.
Figure 5-2: Base Extraction of Eugenol in Clove Bud Oil
5.2.2 Sample Preparation

The procedure outlined below was used for the pH optimized PIE extraction of the main compounds present in clove bud, thyme, and oregano essential oils. Approximately 0.5 g of dried clove buds were added to a 15 mL HDPE conical centrifuge tube with a screw cap. 10 mL of an acetonitrile and deionized water mixture (1:1, v/v) was added to the tube and vortexed for 15 minutes. After vortexing, the solution was filtered, basified using 5M sodium hydroxide and vortexed again for 1 min. The mass separating agent was added (25% glycerol (w/v)), vortexed for 1 minute and then equilibrated for 10 minutes at -10°C. After the two phases separated, the entire organic layer was removed via pipette and transferred to a GC autosampler vial for analysis. (1st extraction) The aqueous layer was then acidified using 5M HCl and vortexed for 1 minute. After vortexing, acetonitrile (3 mL) was added to the sample, vortexed again for 1 minute and then equilibrated at -10°C for 10 minutes. After the two phases separated, the entire organic layer was removed via pipette and transferred to a GC autosampler vial for analysis. (2nd extraction) Figure 5-3 shows the initial essential oil before the acid-base extraction and the organic phases (1st and 2nd) after extraction. Thyme and oregano essential oils were also subjected to the same extraction procedure as outlined above.

5.2.3 Instrumental Parameters

The instrumentation utilized for this study was an Agilent 7890B gas chromatograph (GC) (Santa Clara, CA) equipped with a CTC Analytics CombiPAL Autosampler (Zwingen, Switzerland) and an Agilent 5977A Inert Mass Selective Detector (MSD) (Santa Clara, CA)
Figure 5-3: Acid Base Extraction of Clove Bud Oil: Initial Extraction (A), 1st Extraction (B), and 2nd Extraction (C)
according to the below mentioned GC/MS conditions. The data were interpreted using Agilent’s MassHunter software. The identities of the compounds were determined by the similarity of their mass spectra with those from the Wiley Flavors and Fragrances of Natural and Synthetic Compounds 3 (3rd edition) and the NIST 11-MS mass spectral databases.

The GC/MS analysis was performed on an Agilent DB-5ms capillary column (60 m, 0.25 mm id, 0.25 µm) with the following parameters: He (g) constant flow, 1.0 mL/min; inlet temperature, 300°C; injection volume 3µL (split 50:1); initial oven temperature, 100°C, held for 7 min, then a 5°C/min ramp to 250°C and held for 25 min. The quadrupole mass analyzer (electron impact ionization type, 70 eV) was operated in full scan mode. Full scan mode was operated at a rate of 3.89 scans/sec and a range of 35-400 amu. The source temperature was set at 230°C and the transfer line temperature set at 280°C.

5.2.4 Experiments Performed

In order to assess the applicability of pH optimization of PIE for essential oil extraction, two different types of experiments were conducted. First, clove bud oil was extracted from dried clove buds using an acid-base extraction coupled with PIE. The comparison of the main component eugenol and the major impurities present in the organic phases (1st and 2nd extraction) was then compared to the initial clove bud extract.

For the second experiment, steam distilled thyme and oregano essential oils were subjected to the pH optimized PIE extraction. The goal was to see if a premium and pure therapeutic grade
essential oils (as stated by the manufacturer) can be refined and purified further through the use of PIE with pH optimization. After receipt, the essential oils were initially analyzed by GC/MS and the main components of each essential oil as well as the major impurities present were compared to the 1st and 2nd organic extraction phases.

5.3 Results and Discussion

5.3.1 Essential Oil Composition Profile: Clove Bud Oil

The results obtained from the extraction of clove bud oil from dried clove buds are shown in Table 5-1 and Figure 5-4. The initial analysis was performed on dried clove buds suspended and vortexed in an acetonitrile/water (1:1, v/v) solvent system. The GC/MS profile of the organic phase showed eugenol at 95% with the major sesquiterpene impurities of caryophyllene, α-humulene, and caryophyllene oxide being present at 2.7%, 0.6%, and 0.4%, respectively.

The 1st extraction consisted of an alteration of the extraction solvent system to a basic pH of 11-12 using 5M sodium hydroxide. After addition of base, the organic layer was once again analyzed using GC/MS. Figure 5-6 shows the GC/MS profile of the organic phase showed eugenol at 39% with the major sesquiterpene impurities of caryophyllene, α-humulene, and caryophyllene oxide being present at 46%, 6.8%, and 2.5%, respectively. The solvent system was then returned to its neutral state by the addition of 5M hydrochloric acid. The TIC chromatograms in figures 5-5 and 5-7 shows the peak abundance and peak area of the main
Table 5-1: PIE with pH optimization: Clove Bud Oil

<table>
<thead>
<tr>
<th>Extraction (organic phase)</th>
<th>Eugenol (%)</th>
<th>Caryophyllene (%)</th>
<th>α-Humulene (%)</th>
<th>Caryophyllene Oxide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>95</td>
<td>2.7</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>1st extraction</td>
<td>39</td>
<td>46</td>
<td>6.8</td>
<td>2.5</td>
</tr>
<tr>
<td>2nd extraction</td>
<td>98</td>
<td>0.4</td>
<td>0.05</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Figure 5-4: Percent Composition of Clove Bud Oil
Figure 5-5: pH Optimization of Clove Bud Oil: Eugenol - Overlaid TIC Chromatograms
Figure 5-6: GC/MS Compositional Profile of Clove Bud Oil
Figure 5.7: GC/MS Compositional Profile of Clove Bud Oil: Peak Area of Main Components
components present in organic phase after the 2\textsuperscript{nd} extraction. Eugenol is present at 98\% with the major sesquiterpene impurities of caryophyllene, \(\alpha\)-humulene, and caryophyllene oxide being present at only 0.4\%, 0.05\%, and 0.04\%, respectively. By altering the pH of the system, eugenol was able to be extracted at a greater abundance than the initial amount while also limiting the presence of unwanted impurities in the final organic phase.

5.3.2 *Purification of Steam-Distilled Essential Oils*

The application of PIE with pH optimization was assessed in order to evaluate its potential as a purification technique for steam-distilled essential oils. The two essential oils that were examined were oregano and thyme essential oils.

5.3.2.1 *Oregano Oil (Origanum compactum)*

The results obtained for the purification of steam-distilled oregano oil are shown in Table 5-2 and Figure 5-8. For oregano oil, initial GC/MS analysis of the steam distilled oil showed the main component carvacrol being present at 84\%. The major impurities present were \(p\)-cymene at 6.1\%, linalool at 1.8\%, caryophyllene at 1.7 \%, and \(\gamma\)-terpinene at 1.6\%. The essential oil was then subjected to PIE at basic pH in order to remove the ionisable proton from carvacrol to form its corresponding sodium salt, which is soluble in the aqueous phase.
<table>
<thead>
<tr>
<th>Extraction (organic phase)</th>
<th>Carvacrol (%)</th>
<th>p-Cymene (%)</th>
<th>Linalool (%)</th>
<th>Caryophyllene (%)</th>
<th>γ-Terpinene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>84</td>
<td>6.1</td>
<td>1.8</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>1st extraction</td>
<td>35</td>
<td>24</td>
<td>4.1</td>
<td>10</td>
<td>9.0</td>
</tr>
<tr>
<td>2nd extraction</td>
<td>95</td>
<td>1.6</td>
<td>0.3</td>
<td>0.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 5-2: PIE with pH optimization: Oregano Oil
Figure 5-8: Percent Composition of Oregano Oil
In the first extraction, the amount of carvacrol significantly decreased while the abundance of the non-polar impurities increased as shown in Figures 5-9 and 5-10. Carvacrol was present at 35% with the major impurities of p-cymene, linalool, caryophyllene, and γ-terpinene being present at 24%, 4.1%, 10%, and 9.0%. Neutralizing the aqueous phase with hydrochloric acid returned the extraction system to its original pH to which additional organic solvent was added for the second extraction. Analysis of the organic phase after the second extraction showed carvacrol at 95% with the major sesquiterpene impurities of p-cymene, linalool, caryophyllene, and γ-terpinene being reduced to 1.6%, 0.3%, 0.7%, and 0.6%, as shown in Figure 5-11. Through the use of pH optimization, the essential oil obtained via steam distillation was further purified by increasing the abundance of the main component by more than 10% while decreasing the abundance of major impurities that were present in the original analysis by 8%.

5.3.2.2 Thyme Oil (Thymus vulgaris)

The results obtained for the purification of steam-distilled thyme oil are shown in Table 5-3 and Figure 5-12. For thyme oil, initial GC/MS analysis of the steam distilled oil showed the main component thymol being present at 62%. The major impurities present were p-cymene at 16%, γ-terpinene at 16%, β-pinene at 1.3% and α-pinene at 0.2%. The essential oil was then subjected to PIE at basic pH in order to remove the ionisable proton from thymol to form its corresponding sodium salt, which is soluble in the aqueous phase. In the first extraction, the amount of thymol present significantly decreased while the impurity compounds present significantly increased as shown in Figures 5-13 and 5-14. Thymol was present at 16% with
Figure 5-9: pH Optimization of Oregano Oil: Carvacrol - Overlaid TIC Chromatograms
Figure 5-10: GC/MS Compositional Profile of Oregano Oil
Figure 5-11: GC/MS Compositional Profile of Oregano Oil: Peak Area of Main Components
Table 5-3: PIE with pH optimization: Thyme Oil

<table>
<thead>
<tr>
<th>Extraction (organic phase)</th>
<th>Thymol (%)</th>
<th>γ-Terpinene (%)</th>
<th>p-Cymene (%)</th>
<th>β-Pinene (%)</th>
<th>α-Pinene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>62</td>
<td>16</td>
<td>16</td>
<td>1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; extraction</td>
<td>16</td>
<td>46</td>
<td>29</td>
<td>3.5</td>
<td>0.6</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; extraction</td>
<td>91</td>
<td>4.2</td>
<td>2.4</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Figure 5-12: Percent Composition of Thyme Oil
Figure 5-13: pH Optimization of Thyme Oil: Thymol - Overlaid TIC Chromatograms
Figure 5-14: GC/MS Compositional Profile of Thyme Oil
Figure 5-15: GC/MS Compositional Profile of Thyme Oil: Peak Area of Main Components
the major impurities of \( p \)-cymene, \( \gamma \)-terpinene, \( \beta \)-pinene, and \( \alpha \)-pinene being present at 29\%, 46\%, 3.5\%, and 6.0\%. Neutralizing the aqueous phase with hydrochloric acid returned the extraction system to its original pH to which additional organic solvent was added for the second extraction. Figure 5-15 shows the comparison of TIC chromatogram of all three extractions. The organic phase after the second extraction showed thymol at 91\% with the major sesquiterpene impurities of \( p \)-cymene, \( \gamma \)-terpinene, \( \beta \)-pinene, and \( \alpha \)-pinene being reduced to 2.4\%, 4.2\%, 0.3\%, and 0.1\%. Through the use of pH optimization, the essential oil obtained via steam distillation was further purified by increasing the abundance of the main component by more than about 30\% while decreasing the abundance of major impurities that were present in the original analysis by 27\%.

### 5.4 Conclusions

From the experiments performed, it was demonstrated that the pH optimization of PIE was successful in extracting the main components present in clove bud oil at greater amounts. The technique also proved to be successful as a purification technique for steam-distilled essential oils as PIE was able to reduce the amount of impurities present in the essential oil while increasing the abundance of the main components through the use of pH optimization.

For all three essentials oils examined (clove bud, oregano, and thyme) the overall purity of each essential oil was increased in the final extraction compared to the initial analysis. For
clove bud oil, the initial GC/MS analysis showed eugenol at 95% which was increased to 98%. For oregano oil, the initial GC/MS analysis showed carvacrol at 84% which was increased to 95%. For thyme oil, the initial GC/MS analysis showed thymol at 62% which was increased to 91%. The increase in abundance of the main components present in each essential oil was due to the fact that by altering the pH from neutral to basic, the sodium salts of each compound were more soluble in the aqueous phase. Since the main component was solubilized in the aqueous phase, all sesquiterpene (hydrocarbons without ionisable protons) impurities that were present were extracted into the organic phase in the first extraction, which in turn led to a higher purity of the main component in the final extraction.

As a drawback, pH optimization is only applicable for compounds that contain ionisable protons, which limits this optimization technique to essential oils that contain phenolic terpenoids or phenylpropanoids type compounds. However, if the main component itself is not ionisable, the impurities present in the essential oils might contain ionisable protons, so the technique would work in reverse as the impurities would remain in the aqueous phase and the purified main component would be present in the first extracted organic phase.
CHAPTER 6 - FUTURE WORK FOR PIE

6.1 PIE Method Automation

PIE has proven to be an effective technique for the extraction and purification of essential oils. The next step for this technique would be to fully automate this process for extraction of essential oils on an industrial scale. Automation of the technique is highly plausible as the solvents (acetonitrile, acetone, isopropyl alcohol, water) and mass separating agents (glycerol, sorbitol) that are used in the process all have the potential to be recycled and reused. An overall process flow diagram of automation of PIE on an industrial scale is shown in Figure 6-1.

The process begins with the botanical matter containing the essential oil of interest being placed in a large mixing vessel. Next, water and the organic solvent of choice are added into the vessel in their intended ratios. After mixing and shaking for an appropriate amount of time, the polyol of choice is added to the same vessel. The mixture is then shaken and stirred further until all components present in the vessel are homogenized. The vessel is then cooled to -10°C via a cooling jacket around the outside of the vessel and is equilibrated for a set amount of time. After equilibration, the vessel is drained and the aqueous and organic layers are separated through the use of a sensor device that detects the difference in refractive index and/or specific gravity of the two layers. The layers are then pumped away to different separation chambers where they undergo purification through fractional distillation techniques.
Figure 6-1: PIE of Essential Oils on an Industrial Scale
In the organic layer, the residual aqueous medium present will be the heaviest fraction, so it will remain at the bottom of the distillation apparatus to be pumped away to the same vessel where the rest of the aqueous layer resides. The essential oil of interest and the organic solvent can then be separated via fractional distillation. The organic solvent will be the most volatile, so it will be distilled off first and then collected in a vessel to be recycled and reused. The essential oil can then be collected in a separate vessel to be used as it or subjected to further purification techniques for isolation of the individual components.

The aqueous layer is treated in the same fashion as the organic layer. The water and polyol present in the aqueous layer are separated via fractional distillation. Polyols have a higher boiling point than water so then can be separated through distillation or by recrystallization if the polyol is a solid. Water and the polyols are then collected in their own separate vessels to be reused. Residual waste from the botanical matter is also separated from the aqueous layer and pumped away for disposal.

The procedure can be programmed to recycle and reuse the solvents for multiple extractions of the same botanical matter which allows for optimal extraction efficiency of the analytes present. Once the automated batch run is complete, the main vessel is cleaned and prepared for the next run. Automation of PIE would reduce operating costs in the long run while also providing highly purified essential oils without subjecting them to heat, which allows for heat labile compounds present in the oil to remain intact.
6.2 Biochemical Applications

PIE also has the potential to be used in bioanalysis applications in two major fashions. The first aspect is using PIE for sample preparation of analytes for analysis and the second is using PIE with pH optimization for sample purification.

Currently the most widely used technique in bioanalysis for sample preparation is QuEChERS as it is highly efficient in removing matrix interferences. As we saw previously in Chapter 3, PIE was a comparable sample preparation technique to QuEChERS but surpassed it as PIE does not need an additional clean up step to remove matrix interferences while also being more cost effective and less error prone. PIE can be employed in the use of sample preparation for analysis of pharmaceutical and illegal drugs of abuse and their corresponding metabolites, which can then be subjected to liquid chromatography-mass spectrometry (LC/MS) techniques for quantitation and identification.

As we saw in Chapter 5, PIE with pH optimization is an effective technique to purify essentials oils. This technique can also be applied to protein extraction purification. When synthesizing proteins, more often than not, purification and isolation of the individual protein that is desired is hard to achieve. Proteins are made up of numerous different amino acids which all have different pKa and pI values. By altering the pH of extraction system and performing multiple extraction steps, the protein of interest can be isolated. A highly purified protein can be
achieved while leaving behind unwanted impurity remnants that were generated as a result of the synthesis.

6.3 Organic Solvent Dehydration

PIE can also prove to be useful for water removal from organic solvents. Organic solvents used for synthesis often have to be anhydrous as water can have detrimental effects to the chemical reaction. Current techniques to remove water for organic solvents include the use of drying agents which consist of anhydrous inorganic salts such as calcium chloride ($\text{CaCl}_2$), magnesium sulfate ($\text{MgSO}_4$), calcium sulfate ($\text{CaSO}_4$), potassium carbonate ($\text{K}_2\text{CO}_3$) and sodium sulfate ($\text{NaSO}_4$). These salts are able to become hydrated and attract residual water that is present in the organic phase.

By employing PIE, the polyol present can be an alternative method to remove water from organic solvents. Polyols have multiple hydroxyl groups (three for glycerol and six for sorbitol) that have to ability to be act as both hydrogen bond donors and acceptors which give them the ability to form strong bonds with water. Based on these chemical characteristics, polyol have the potential to serve as organic solvent dehydrating agents.
6.4 Extraction of Rare Metals

Platinum group metals (PGM’s) are exceptionally rare naturally occurring metals whose versatile chemical properties lead them to be used in a variety of different applications. The PGM’s are comprised of six metals which include Iridium (Ir), Osmium (Os), Palladium (Pd), Platinum (Pt), Rhodium (Rh) and Ruthenium (Ru). Of the six, platinum, palladium, and rhodium are of the most interest and there has a push in recent years to develop new techniques in order to recover and recycle these metals. 78 Zhang and colleagues have already successfully investigated three liquid phase systems (TLPS) and one-step separation of Pt(IV), Pd(II), and Rh(III) through the use of salting out, sugaring out, and ionic liquids in acetonitrile-water solvent systems. 79-81 Based on their experimental results, it would be of interest to investigate the use of PIE for the extraction and purification of the platinum group metals.

6.5 Conclusions

Polyol Induced Extraction (PIE) is a novel patented extraction technique that has untapped possibilities waiting to be investigated. This method has the potential to be applied to a wide variety of very unique applications that should all be examined further. The technique proved to be successful in the extraction of essential oils using acetonitrile with glycerol and also acetone/IPA with sorbitol. It was also shown the pH optimization of PIE can lead to highly purified essential oils through reducing the amounts of naturally occurring impurities. On a further note, PIE was also shown to be a comparable extraction technique to QuEChERS. Further research using PIE should focus on industrial scale up of the process as if this technique
proves useful with other applications on a micro scale, the potential to use this on an industrial scale is more cost effective as all reagents can be recycled and reused.
CHAPTER 7 - OVERALL CONCLUSIONS

As a result of this work, Polyol Induced Extraction (PIE) has demonstrated to be an excellent technique for the extraction of essential oils. Chapter 1 discussed how essential oils are a significant and important commodity in today’s society. Based on the widespread use of essentials oils in consumer products or as therapeutic agents, a cost effective, highly efficient, environmentally friendly extraction technique is of great necessity.

In Chapter 2, it was demonstrated that polyols are great candidates for use as mass separating agents (MSA) as they proved to be able to induce a phase separation in acetonitrile/water solvent mixtures. PIE of essential oils was evaluated in terms of extraction efficiency, thermodynamics parameters and the essential oil compositional profiles. For extraction efficiency, the trend observed for all six essential oils is that partition coefficients were $> 1$ in the temperature range of $-20^\circ$ to $20^\circ$C. Lowering the temperature favors the partitioning of the main analytes to the organic phase with the optimal extraction temperature being $-10^\circ$C. For the thermodynamic properties, the trend observed for all six essential oils is that $\Delta G$ values are all $< 1$ in the temperature range of $-20^\circ$ to $20^\circ$C, meaning the phase separation is a spontaneous process. PIE is a combination of a two and three phase mechanism with the change in mechanism being driven by lowering the temperature. The overall PIE process is an exothermic process with entropic contribution dominating the phase separation. PIE is a comparable extraction technique to traditional methods for extraction of essential oils. It surpasses
traditional techniques as it extracts more of the main analyte present in the essential oil while also limiting the amount of matrix impurities that are present.

In Chapter 3, PIE and QuEChERS were compared and contrasted. Both techniques proved to be analogous extraction techniques, however PIE showed some unique advantages. The major advantage was that PIE is a one tube extraction technique that does not need a separate additional clean-up step. PIE uses one unique reagent compared to QuEChERS, which uses three unique reagents, and all reagents used in PIE have the potential to be recycled for further use. These advantages translate to PIE into being an overall simpler method with less steps, resulting in a more cost effective and less error prone extraction technique.

In Chapter 4, PIE using FEMA/GRAS solvents was evaluated. From the experiments performed, the following conclusions were drawn: 1:1 mixtures of acetone/water or IPA/water cannot be separated using glycerol as a MSA. To achieve a phase separation, the ratio of solvent to water needs to be adjusted to 7:3 (v/v) and the nature and amount of polyol used needs to changed (D-sorbitol, 25% (w/v)). Acetone proved to be a more efficient solvent than IPA for the extraction of essential oils via PIE. However, a major drawback of using GRAS solvents is that matrix interferences are not completely removed from the sample.

In Chapter 5, the pH optimization of PIE of essential oils was assessed. By altering the pH of the extraction system, the main analyte present in the essential oils was able to be purified. For clove bud oil, the purity of eugenol was increased from 95% to 98%. For oregano oil, the purity
of carvacrol was increased from 84% to 95%. For thyme oil, the purity of thymol was increased 62% to 91. As a drawback, pH optimization is only applicable for essential oils that contain ionisable compounds, such as phenolic terpenoids and phenylpropanoids.

Chapter 6 discussed the future potential uses for PIE. Automation of the technique is highly plausible as the solvents (acetonitrile, acetone, isopropanol and water) and mass separating agents (glycerol and sorbitol) that are used in the process all have the potential to be recycled and reused. Other potential future directions include sample preparation for identification and quantitation of different analytes, sample purification of proteins and pharmaceutical drugs that contain ionisable functional groups, removal of water from organic solvents, and extraction and purification of metal complexes.
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