Objectives

1) To isolate caffeine from dry tea leaves by extraction and purify the crude extract by recrystallization.

2) To determine the mass percentage of caffeine in the tea leaves.

Introduction

Caffeine is probably the most commonly used addictive drug. It belongs to a large class of organic compounds called alkaloids, which vary widely in structure and reactivity. All alkaloids have a nitrogen atom that allows most of them to accept a proton (H+) and act as a base, or alkaline substance. The name “alkaloid” comes from this characteristic. Alkaloids are usually found in plants. They usually taste bitter and often are physiologically active in humans. The names of some of these compounds are familiar to you even if the structures are not: nicotine, morphine, strychnine, and cocaine. The roles these compounds play in the plants in which they are found is not well understood. In some cases they may act as pesticides; nicotine is found in tobacco and has been sprayed onto other plants to function as an insecticide. The structure of caffeine is shown below. It can be considered to be constructed from purine ring system, which is important biologically, being found in nucleic acids and elsewhere.

Caffeine is found in a number of foods and beverages. The table below lists some of them. Caffeine acts as a stimulant. It stimulates the heart, respiration, the central nervous system, and is a diuretic. Its use can cause nervousness, insomnia and headaches. It is physically addictive. A person who drinks as few as 4 cups of coffee a day and who attempts to
stop “cold turkey” may experience headache, insomnia, and possibly nausea as the result of withdrawal.

<table>
<thead>
<tr>
<th>Food</th>
<th>Caffeine Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee</td>
<td>80-125 mg per cup</td>
</tr>
<tr>
<td>Coffee, decaf</td>
<td>2-4 mg per cup</td>
</tr>
<tr>
<td>Tea</td>
<td>30-75 mg per cup</td>
</tr>
<tr>
<td>Cocoa</td>
<td>5-40 mg per cup</td>
</tr>
<tr>
<td>Milk chocolate</td>
<td>6 mg per ounce</td>
</tr>
<tr>
<td>Baking chocolate</td>
<td>35 mg per ounce</td>
</tr>
<tr>
<td>Coca-Cola</td>
<td>46 mg per 12 ounces</td>
</tr>
</tbody>
</table>

Tea has been consumed as a beverage for almost 2,000 years, starting in China. It is a beverage produced by steeping in freshly boiled water the young leaves and leaf buds of the tea plant, *Camellia sinensis*. Today, two principal varieties are used, the small-leaved China plant (*C. sinensis sinensis*) and the large-leaved Assam plant (*C. sinensis assamica*). Hybrids of these two varieties are also grown. The leaves may be fermented or left unfermented. Fermented teas are referred to as black tea, unfermented teas as green tea, and partially fermented teas as oolong. As trade routes opened to Asia in the 17th century, tea was imported to Europe.

In this experiment, you are going to make a small but strong cup of tea and extract the caffeine from it. Also, you are going to purify the caffeine by recrystallization and calculate the mass percentage of caffeine in the tea sample using the equation below.

\[
\text{Mass \%} = \left( \frac{\text{wt. of purified caffeine}}{\text{wt. of tea sample}} \right) \times 100
\]

Tea leaves consist mostly of cellulose, a water-insoluble polymer of glucose, which is a simple sugar (a monosaccharide). Cellulose performs a function in plants similar to that of fibrous proteins in animals: it is structure building material. Along with the cellulose are found a number of other things including caffeine, tannins (phenolic compounds, compounds that have an \(-\text{OH}\) directly bonded to an aromatic ring) and a small amount of chlorophyll.

The idea in this experiment is to extract the water soluble materials in the tea leaves into hot water. The solubility of caffeine in water is 22 mg/mL at 25 C, 180 mg/mL at 80 C, and 670 mg/mL at 100 C. The hot solution is allowed to cool and the caffeine is then extracted from the water with dichloromethane (methylene chloride), which is an organic solvent that is
insoluble in water. Since caffeine is more soluble in dichloromethane (140 mg/mL) than it is in water (22 mg/mL), it readily dissolves in the dichloromethane. However, the tannins are also slightly soluble in the dichloromethane and we want to separate the caffeine from the tannins by having the caffeine dissolve in the dichloromethane and the tannins remain in the water. We can do this by taking advantage of the fact that phenols are acidic enough to be converted to their salts (deprotonation of the -OH group) by reaction with sodium carbonate. So, we will add sodium carbonate to the water and the tannins will be converted to phenolic anions, which are not soluble in the dichloromethane but are soluble in highly polar water.

There is one practical disadvantage in converting the tannins to their salts – they become anionic surfactants. Detergents and soap are surfactants and these cause materials that do not dissolve in water (like oil, grease and dichloromethane) to form an emulsion with water. As we want to be able to separate the aqueous phase from the dichloromethane phase, it is better not to let the emulsion to occur during the extraction. Consequently, as you extract the caffeine from water into dichloromethane, do not shake the separatory funnel too vigorously.

The flow chart below summarizes the details of this experiment.

**Experimental Procedure**

1) Place 5 g of dry tea leaves into a clean 150 mL beaker. Record the exact weight of tea leaves. Add 30 mL of distilled water and 2.0 g of sodium carbonate.
2) Gently boil the contents for 10 minutes on a hot plate. Place a watch glass on top of the beaker to prevent excessive evaporation.
3) Filter the hot tea through a glass funnel plugged with a small piece of cotton and keep the hot tea in a 50 mL Erlenmeyer flask.
4) Transfer the tea leaves back into the beaker. Add 20 mL of distilled water and again bring the contents to a boil.

5) Filter and combine the hot tea into the 50 mL Erlenmeyer flask. Discard the tea leaves.

6) When the tea is cooled to room temperature, transfer the tea into a 125 mL separatory funnel that is supported by a ring on a stand.

7) Add 10 mL of dichloromethane to the separatory funnel. Stopper the funnel, shake and release any pressure that may have built up inside the funnel. Close the stopcock and swirl the content in the funnel without vigorous shaking. Place the separatory funnel on the ring stand.

8) Allow the contents in the separatory funnel to settle and remove the stopcock. There should be two distinct mostly clear layers. If there is an emulsion (cloudy) layer between two clear layers, it is sometimes possible to break the emulsion by swirling the contents of the funnel or stirring the contents using a glass rod. If the emulsion persists seek your instructors help.

9) Carefully drain the lower (dichloromethane) layer into a clean 50 mL Erlenmeyer flask. Try not to include any of the aqueous (upper) layer. If there is a lot of emulsion, keep it in the Erlenmeyer flask.

10) Repeat steps 7 through 9 using another 10 mL portion of dichloromethane.

11) Add half a spoon of anhydrous magnesium sulfate to the combined dichloromethane extracts in the Erlenmeyer flask. Swirl the contents of the flask. The anhydrous sodium sulfate will absorb the small amount of water that is dissolved in the dichloromethane and small amounts of water from the aqueous layer that may have gotten into the flask by accident. [If you collected a substantial amount of emulsion in the Erlenmeyer flask, the sodium sulfate should help to “break” the emulsion. You may need to separate the aqueous material from the dichloromethane solution at this point. Seek your instructor's help.]

12) Decant the liquid from the flask into a clean and dry beaker. Place the beaker on a steam bath to evaporate dichloromethane. Record the weight of caffeine before recrystallization.

13) Recrystallize the crude caffeine with hot ethanol and vacuum filter the crystals.

14) Transfer the crystals on a watch glass. Dry the crystals on a steam bath.

15) Place the crystals into a pre-weighed plastic bag. Record the amount of your isolated caffeine. Calculate the recovery percentage and mass percentage of caffeine in your original tea leaves.
Laboratory Safety Precaution

1) Wear safety goggles and lab coat at all times while working in the laboratory.
2) Dichloromethane is irritating and toxic. Sodium carbonate is corrosive.
3) Wash your hands thoroughly with soap or detergent before leaving the laboratory.