Sugar Identification using Polarimetry

Safety Concerns:
No special safety precautions need to be implemented. Standard organic laboratory safety measures need to be in place.

Purpose
The purpose of this lab is to become familiar with use of a polarimeter and to use optical rotation as a method of determining the identity of unknown sugars.

Introduction:
Approximately 25% of all drugs are marketed as either racemates (mixtures of two enantiomers) or mixtures of diasteromers\(^1\). The orientation around a chiral center can have a dramatic impact on the pharmacological response of that drug in the human body. A worst case scenario is one which the non desired enantiomer causes serious toxicity. The drug Thalidomide, was prescribed to millions of women to suppress morning sickness associated with pregnancy during the late 50’s and early 60’s. Horrible birth defects, including missing limbs, resulted. The cause of these birth defects was assigned to the S-(\(-\))-enationmer, which did not undergo clinical trials\(^2\). This assessment is currently being questioned. None the less, this brought about severe tightening in the laws surrounding the introduction of new drugs. Chiral synthesis and purification is a crucial aspect of all successful drug manufacture.

Chirality
Chiral molecules have an asymmetrical center which respond to light as a lens and rotate the light. The ability to rotate light is termed optical activity. Enantiomeric compounds rotate light by exactly the same amount but in the opposite direction. The degree to which a substance rotates light may be used to determine a) the identity of the substance, b) the enantiomeric purity of the substance or c) the concentration of a known substance in a solution. In order to observe rotation, the light which is passed through the solution must be plane polarized. Ordinary light has have waves which are oriented in all directions. Plane polarized light is made up of waves which are oriented parallel to a defined plane.

![Ordinary Light and Plane Polarized Light](image-url)
When a beam of plane polarized light passes through a solution of optically active material the light will rotate.

![Diagram of light rotation through optically active material]

Each pure chiral material has a set specific rotation \([\alpha]\) which is a fixed physical characteristic for that material. The specific rotation describes how far a material will rotate light. A \([\alpha]\) of +87.6 means that pure material will rotate light in the clockwise manner by 87.6 degrees. The enantiomer will rotate the plane of polarized light by exactly the same amount but in the opposite direction. An \([\alpha]\) of -87.6 would result in a rotation in the counterclockwise direction by 87.6 degrees.

![Diagram of light rotation through optically active material]

Racemic mixtures (equal parts of two enantiomers) will have no net rotation because the equal but opposite rotations cancel each other.

**Practicalities**
If the two enantiomers are not present in equal amounts, the amount of this enantiomeric excess (ee) may be calculated using the observed rotation \((\alpha_{\text{obs}})\) compared to the specific rotation \([\alpha]\).

\[
\text{e.e.} = \frac{|R-S|}{(R+L)} \times 100\% = \frac{|\alpha_{\text{obs}}|}{|\alpha|} \times 100\%
\]

The e.e. divided by 100 and multiplied by the absolute value of the specific rotation of the pure compound will result in the absolute value of the observed rotation by the mixture. For example, if a pure R material has a specific rotation of -16.70, a mixture of 40% R and 60% S will result in a 20% enantiomeric excess (The 40%R cancels 40% of the S, and leaves 20% excess S). The 20% e.e. is then divided by 100 to result in 0.20, and multiplied by the absolute value of \([\alpha]\) of |-16.70| or 16.70 to show this mixture will rotate light by |3.35|. Knowing that the R material rotates in the negative direction, and knowing that this mixture has an excess of the S enantiomer, means that this material will rotate light in the positive or clockwise direction by 3.35 degrees.
e.e. = |R-S| / (R+S) * 100% = |60-40|/(60 +40) *100% = 20%  
20% of |-16.70| = |3.35|, and an excess of S means +3.35.

Of more practical use is the assessment of the enantiomeric excess given an observed rotation and a known specific rotation. If a mixture of this R and S has an observed rotation (\(\alpha_{\text{obs}}\)) of +3.97, then the percent composition is calculated as follows. The overall rotation is positive so there is an excess of the S enantiomer. We know from the above equation that ee = \(\alpha_{\text{obs}} / [\alpha] \times 100\%\), therefore ee is equal to 3.97/16.7 * 100 or 23.8%. This means that the rest of the material (100.0 – 23.8 = 76.2%) is equally divided between the R and S (100 % - %e.e. = % of balanced material). Therefore there is 76.2% R material, and 38.1% +23.8 % or 61.9% S material. To check, 61.9% + 38.1% is 100 %, and the difference between the two is 23.8%.

The specific rotation ([\(\alpha\)])of a compound is a fixed physical property of that compound (as is its boiling point or melting point or density). The observed rotation (\(\alpha_{\text{obs}}\)) depends on the concentration of the sample in solution (c) and the length of the cell (\(\ell\)) as well as the specific optical rotation of the compound [\(\alpha\)]. Doubling the concentration of a material in a solution will double the observed rotation. Cutting the cell length in half will half the observed rotation. The specific rotation [\(\alpha\)] remains the same regardless of the concentration or cell length.

\[
[\alpha] = \frac{\alpha_{\text{obs}}}{c \times \ell}
\]

[\(\alpha\)] = specific rotation of the compound  
\(\alpha_{\text{obs}}\) = observed rotation of light in degrees  
c = concentration in grams per milliliter (g/ml)  
\(\ell\) = cell length in decimeters (dm)

**Experimental**

In this experiment a sugar solution of known concentration (c), but unknown identity will be prepared (note the units of concentration used). The observed rotation (\(\alpha_{\text{obs}}\)) will be obtained by using a polarimeter. This data will be used to calculate the specific rotation [\(\alpha\)] and the identity of the sugar will be hypothesized.
**Chemicals:**

<table>
<thead>
<tr>
<th>Name (other names)</th>
<th>Structure (Fisher, Haworth)</th>
<th>Specific Rotation $[\alpha]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D- Fructose (D-Levulose)</td>
<td><img src="image" alt="Structure" /></td>
<td>-86</td>
</tr>
<tr>
<td>D-Glucose</td>
<td><img src="image" alt="Structure" /></td>
<td>+98</td>
</tr>
<tr>
<td>D- Galactose</td>
<td><img src="image" alt="Structure" /></td>
<td>+ 82</td>
</tr>
<tr>
<td>D-Allose</td>
<td><img src="image" alt="Structure" /></td>
<td>+15</td>
</tr>
<tr>
<td>Sucrose</td>
<td>glucose-fructose</td>
<td>+64.5</td>
</tr>
<tr>
<td>Maltose</td>
<td>glucose-glucose</td>
<td>+118</td>
</tr>
</tbody>
</table>

**Sample Preparation, Set Up and Procedure:**

1) Weigh out approximately 0.5 g of unknown. Record **all the digits** of this mass in your notebook.
2) In 50ml beaker, dissolve your material in approximately 10ml of deionized water. Swirl the contents until all of the solid has dissolved.
3) Carefully transfer this solution to a 25ml volumetric flask.
4) Rinse beaker with approximately 2 ml of deionized water. Transfer this solution to volumetric flask. Repeat.
5) Carefully drop wise add deionized water to volumetric flask until bottom of meniscus is exactly on line. This is your solution.
6) In notebook, calculate the concentration in **g/ml**.
7) Obtain the $\alpha_{obs}$ by analyzing your solution in the polarimeter using the instructions mounted adjacent to machine and reviewed by your instructor.
8) After an acceptable measurement is obtained, empty volumetric flask, rinse volumetric with deionized water, and return volumetric flask to supply bench.
9) Using the above equation, calculate the specific rotation $[\alpha]$.
10) Select the identity of your material from the list provided.
11) Fill out the relevant spaces on the data sheet.

References:
2. http://www.chromatography-online.org/topics/thalidomide.html February 27, 2008
p.