A Spectrophotometric Analysis of Salicylate in Face Washes

In this Laboratory Exercise, we will determine the amount of active ingredient in a commercial face wash; Sodium Salicylate. We will accomplish this by conducting a spectrophotometric analysis of the preparation at the wavelength of maximal absorbance for this compound when complexed with Fe^{3+}. The resulting absorbance measurement will be correlated against calibration standards, using Beer’s Law, to determine the concentration of Salicylate in the preparation.

Spectrophotometric analyses rely on the ability of the analyte (molecule, atom, or ion) to absorb or emit electromagnetic radiation. In Absorbance Spectroscopy an incoming photon interacts with the analyte, and if the energy of the photon matches the energy difference between two of the analyte's quantum states, then the photon is likely to be absorbed.

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Na^+  

(Sodium Salicylate)
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\begin{center}
\includegraphics[width=0.3\textwidth]{image.png}
\end{center}
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Thus, Absorbance can occur if:

\[
\Delta E = E_{\text{photon}} = h \nu = \frac{hc}{\lambda}
\]  

(Eq. 1)

where \(c\), \(\lambda\), and \(\nu\) are the speed, wavelength and frequency of the photon, respectively. \(h\) is Planck’s constant. Note, the larger the energy gap between the quantum states, the shorter must be the wavelength of the absorbed photon. So, different regions of the electromagnetic spectrum, different \(\lambda\)'s, correspond to different types of quantum transitions. Low energy photons cause molecular rotational transitions, whereas high energy photons cause electronic transitions. This correspondence between the photon wavelength and the type of transition is diagramed below:

### Electromagnetic Spectrum

\[\lambda\]

\[10^{-4}\text{m}\] \[750\text{nm}\] \[350\text{nm}\] \[10^{-8}\text{m}\]

- Radio
- Microwave
- Infrared
- Visible
- Ultraviolet
- X-Ray
- Gamma Ray

- Molecular Rotational Transitions
- Molecular Vibrational Transitions
- Electronic Transitions

We will focus here on electronic transitions due to absorbance of photons in the Visible and Ultraviolet regions of the spectrum. Additionally we will restrict ourselves to analytes dissolved in solution. In these cases, a solution of the analyte is subject to UV-Vis radiation at a given wavelength, with Power \(P_o\), and the Transmitted beam's intensity, Power \(P\), is measured.

The solution's Transmittance (T) is then defined as:

\[
T = \frac{P}{P_o}
\]  

(Eq. 2)
and its Absorbance (A) is:

\[ A = - \log T \quad \text{(Eq. 3)} \]

In practical terms, the Transmittance is usually not measured in this simple fashion because beam intensity can be diminished by a number of factors associated with the fact that the analyte solution must be placed in a sample cell. A percentage of the radiation will suffer from reflection at the Air-Cell and Cell-Solution interfaces, attenuating the intensity of the beam. Additionally, there are usually some scattering losses from within the solution itself and the container may absorb some radiation as well. (The latter is particularly true for glass containers in the Ultraviolet region of the spectrum; hence Quartz cuvettes are typically used in this spectral region.) So, in practice, the Transmittance is typically measured by comparing the power output of the beam transmitted by the analyte solution with the power of the beam transmitted by an identical cell containing only solvent. (If two different cells are used for these measurements, then it is important the two cells be optically matched. Optically matched cells are very expensive.)

Analytically, the Absorbance is useful because for dilute solutions, the Absorbance is proportional to the Concentration (c) of the analyte via the Beer-Lambert Law:

\[ A = \varepsilon b c \quad \text{(Eq. 4)} \]

where the Path Length \( b \) [cm], and the ability of the analyte to absorb light at the given wavelength, the Molar Absorptivity \( \varepsilon \) [M\(^{-1}\)cm\(^{-1}\)] also influence the absorbance.

Beer’s Law has a number of limitations. At higher concentrations, solvent-solute and solute-solute interactions begin to affect the analyte environment and thus its absorptivity. Chemical reactions causing the analyte to dissociate, associate, or react with the solvent will cause an apparent deviation in Beer’s Law as well. Finally, because the Molar Absorptivity \( \varepsilon \) depends on the wavelength of the radiation, and because the incident radiation is not completely monochromatic (it is instead a range of wavelengths passing through the slits), deviations from Beer’s Law will also become apparent because of slight changes in \( \varepsilon \) over the spectral range of the radiation passing through the slits.

Lastly, a major complication enters the picture at this point. Because, for the case under consideration, the analyte is in a solution, it is surrounded by constantly jostling solvent molecules. Thus, each analyte molecule/ion/atom finds itself in a slightly different environment than its brothers. Thus, the energy gap between the quantum states responsible for the absorbance will be slightly different for each analyte molecule/ion/atom. This means we will
have a series of very, very closely spaces absorbance bands. Practically, this means the Absorbance Spectrum will be broad, as diagramed below:

Thus, both $A$ and $\varepsilon$ depend on the wavelength:

$$A_\lambda = \varepsilon_\lambda \cdot b \cdot c \quad \text{(Eq. 4)}$$

To analyze for a given analyte, $\varepsilon_\lambda$ is first determined using a series of standard solutions of known $c$. (Typically $\lambda_{\text{max}}$ is chosen as the wavelength for measuring the absorbance values.) Then the absorbance of the analyte solution is determined. The unknown concentration is then determined via:

$$c = \frac{A_\lambda}{\varepsilon_\lambda} \cdot b \quad \text{(Eq. 5)}$$

Alternatively a calibration curve is constructed and used directly to determine the analyte’s concentration. A series of standard solutions containing the analyte at known concentrations are measured. Then, the least squares method can be used to determine the “best fit” line to a plot of absorbance vs. concentration. This calibration curve is the used to determine the analyte concentration in an unknown solution whose absorbance has been measured.
Our analyte, Salicylate, is an organic compound that was historically derived from the Soaponification of Salicin found in the bark of the willow tree.

Salicylate, and Salicylate derivatives like Aspirin, acts as an analgesic and anti-inflammatory. Topically it is used against acne, psoriasis, corns and warts. “It works as both a keratolytic and a comedolitic agent by causing the cells of the epidermis to shed more readily, opening clogged pores and neutralizing bacteria within, preventing pores from clogging up again by constricting pore diameter, and allowing room for new cell growth.” (Wikipedia)

Salicylate itself only absorbs in the Ultraviolet region of the spectrum, creating a bit of an experimental challenge. However, when complexed with Ferric Ion ($Fe^{3+}$):

\[ x \text{ Sal}(aq) + n \text{ Fe}^{3+}(aq) \rightarrow \text{Fe}_n\text{Sal}_x(aq) \]

it absorbs intensely in the Visible region at 535 nm, having produced a purple colored solution. This chelation between the Ferric Ion and the Salycilate is a common reaction of phenolic compounds (R-OH) and metal cations and is dependent upon the acidity of the reaction system. Under the acidic conditions we will be working with the Salicylate will be fully protonated and the Ferric Ion will occur as the hexahydrate $Fe(H_2O)_6^{3+}$.

Our first task will be to determine the stoichiometry of the Iron-Salicylate complex. We will do this using the Method of Continuous Variation of Job. Here, this means we will
take the absorbance measurement of a series of solutions whose mole fraction ratio for the reactants varies, but whose total number of moles remains constant. The maximal absorbance will occur at the ratio that is closest to the stoichiometric ratio for the reactants.

Finally, we will measure Absorbance values using a double beam Shimadzu UV-2550 scanning UV-VIS spectrometer. This spectrometer uses a double beam arrangement to correct for stray radiation problems associated with the sample cells, etc.

This instrument’s spectral range is from 190 – 900nm; operating with a resolution of 0.1nm. It uses 50W Halogen (VIS) and Deuterium (UV) lamps as lights sources. Wavelength dispersal is via a double-blazed holographic grating monochrometer. Radiation is detected using an R-928 Photomultiplier. The instrument itself is interfaced to a computer data acquisition system and is under control of the acquisition software.
Pre-Lab Calculation

1. Bi$^{3+}$ complexes with Thiourea to form a colored Complex which Absorbs light at $\lambda_{\text{max}} = 470$ nm with an Molar Absorptivity of $\varepsilon = 9320$ M$^{-1}$cm$^{-1}$. What is the Concentration of the Complex in a solution that has a measured Absorbance $A = 0.265$ in 1.00 cm cell. What is the measured Transmittance $T$?

2. For the data below, use Excel to determine a linear regression line. Use this trendline to determine the concentration of analyte in the unknown (unk).

<table>
<thead>
<tr>
<th>Analyte [M]</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>0.164</td>
</tr>
<tr>
<td>0.10</td>
<td>0.147</td>
</tr>
<tr>
<td>0.060</td>
<td>0.078</td>
</tr>
<tr>
<td>0.030</td>
<td>0.050</td>
</tr>
<tr>
<td>0.010</td>
<td>0.018</td>
</tr>
<tr>
<td>unk</td>
<td>0.098</td>
</tr>
</tbody>
</table>
Procedure

Adapted from “Salicylate Detection by Complexation with Iron (III) and Optical Absorbance Spectroscopy” by Jeremy T. Mitchell-Koch, Kendra R. Reid and Mark E. Meyeroff *Journal of Chemical Education* 85 (2008) 1658.

**Salicylate Determination**

1. Prepare five standard solutions of Sodium Salicylate in deionized Water. Use a 100 mL volumetric flask to prepare an initial stock solution of 0.1M. By dilution, prepare standards of 10 mM (at least 100 mL needed), 20 mM, 40 mM 60 mM and 80 mM.

2. Obtain a sample of a commercial face wash. Record the concentration of active ingredient as listed on the product label.

3. Into separate test tubes, pipet exactly 0.1 mL of each standard and the face cream solution. Repeat this procedure two more times for the face wash solution. Add 10.00 mL of acidic 10 mM Ferric Nitrate solution to each test tube. Mix the solutions well.

4. Measure the absorbance of each solution at 535 nm.

5. Prepare an appropriate calibration curve using *Excel* or some other graphics software. Add a trendline. Determine the slope and intercept and the standard deviations of the slope, intercept and regression line.

6. Determine the concentration of the commercial face wash solution and its standard deviation. Report the final concentration in units consistent with the product label.

**Job’s Plot Determination**

1. Obtain ~ 20 mL 10 mM acidic Ferric Nitrate, 50 mL of dilute Nitric Acid and the 10 mM Sodium Salicylate solution from above.

2. Prepare solutions of the following mixes:

<table>
<thead>
<tr>
<th>Fe$^{3+}$/Salicylate Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 : 1</td>
</tr>
<tr>
<td>4 : 1</td>
</tr>
<tr>
<td>3 : 1</td>
</tr>
<tr>
<td>2 : 1</td>
</tr>
<tr>
<td>1.5 : 1</td>
</tr>
<tr>
<td>1 : 1.5</td>
</tr>
<tr>
<td>1 : 2</td>
</tr>
<tr>
<td>1 : 3</td>
</tr>
</tbody>
</table>
It is suggested each mix be a total of 1 mL. This can be accomplished using the following volumes:

<table>
<thead>
<tr>
<th>Vol. [mL] 10 mM Sal.</th>
<th>Vol. [mL] 10 mM Fe^{3+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>0.25</td>
<td>0.75</td>
</tr>
<tr>
<td>0.33</td>
<td>0.67</td>
</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>0.67</td>
<td>0.33</td>
</tr>
<tr>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>0.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

3. Add 4.00 mL of dilute Nitric Acid to each mixture.

4. Measure the absorbance of each solution at 535 nm.

5. Prepare a Job’s plot for this data; plot the absorbance vs. mole fraction Fe^{3+}.

6. Determine the stoichiometry of the Fe_{n}Sal_{x} complex.