CHEM 322: Azo Dyes: Combinatorial Synthesis of Dyes

Introduction:

Compounds containing one or more azo groups (-N=N- linked to two carbon atoms) have a variety of uses. Aliphatic azo compounds, like azobisisobutyronitrile (AIBN), can be as radical initiators in polymerization of alkenes to make plastics. Aromatic azo compounds are used as acid-base indicators, biological stains, and commercial colorants for clothing, plastics, cosmetics, and food beverages. Many azo-dyes, such as methyl red, methyl orange, and congo red, can be used as acid-base indicators due to their ability to function as weak acids or bases. Color changes are caused by changes in extent of delocalization of electrons: more delocalization shifts the absorption max to longer wavelengths and makes the light absorbed redder, while less delocalization shifts the absorption max to shorter wavelengths. Color changes can also be due to geometrical isomerism of the azo group. UV radiation can cause a trans azo group to become cis. This can lead to photochromism, a light-induced reversible color change. Some azo dyes with this property (and which can revert slowly to the trans isomer in the dark) are used in sunglasses and car sunroofs. Many azo dyes, like Sudan red and scarlet red, can be used as biological dyes because they are fat-soluble and can be absorbed into fat cell tissues on microscope slides. Azo dyes form 60-70% of all synthetic dyes used as commercial colorants. Azo dyes have several advantages over other commercial dyes including their wide color range, good color fastness and ability to absorb light. They can also be synthesized cheaply because the starting materials are readily available, inexpensive compounds; most of the chemistry is completed at or below room temperature; and the environmental impact is low due to the use of water as a solvent in all of the reactions. Cost advantages tend to compensate for the lower resistance to bleaching and lower brilliance of azo dyes compared to anthraquinones, the second most used dye class.

In theory, a collection of different azo dyes should be able to make a complete rainbow of colors. In practice, azo dye compounds come in yellows, oranges, reds, browns, and blues. The color differences are caused by different substituents on the aromatic rings which lead to differences in the extent of conjugation of the π system in the azo dye. In general, the less extensive the conjugated π system of a molecule, the shorter the wavelength of visible light it will absorb.

colorless (shortest π system) → yellow → orange → red → green → blue (longest π system)

The eye perceives color because dyes absorb some wavelengths and reflect others. For example, if a compound absorbs in the green visible region, the combination of the remaining wavelengths which are reflected makes the compound appear red or purple.

The synthesis of an azo dye requires two organic compounds - a diazonium salt and a coupling component. The general synthesis of azo dyes is shown below.

\[
\text{Diazonium Salt} + \text{Coupling Component} \rightarrow \text{Azo Dye}
\]

{HONO was used in a prior step to generate the diazonium salt}
When sodium nitrite is mixed with hydrochloric acid, it produces nitrous acid. The nitrous acid can be protonated under acidic conditions to cause the loss of water and produce the nitrosonium ion:

\[
\begin{align*}
\text{N}\text{O}_2^- + \text{H}^+ & \rightarrow \text{HNO}_2^- \\
\text{HNO}_2^- & \rightarrow \text{H}^+ + \text{NO}_2^- \\
\text{NO}_2^- & \rightarrow \text{H}^+ + \text{NO}_2\text{H} \\
\text{NO}_2\text{H} & \rightarrow \text{H}_2\text{O} + \text{NO}_2^-
\end{align*}
\]

An aromatic amine can attack the electrophilic nitrosonium ion to form a nitrosoamine:

\[
\begin{align*}
\text{H}_2\text{N} & - \text{aryl group} \\
\text{N} & \text{O} \\
\text{H} & + \text{HNO}_2^- \\
\text{H}_2\text{N} & - \text{aryl group} + \text{HNO}_2^- \\
\end{align*}
\]

The nitrosoamine can undergo a proton transfer (tautomerism):

\[
\begin{align*}
\text{H}_2\text{N} & - \text{aryl group} \\
\text{N} & \text{O} \\
\text{H} & + \text{HNO}_2^- \\
\text{H}_2\text{N} & - \text{aryl group} + \text{HNO}_2^- \\
\end{align*}
\]

Under acidic conditions, the OH group can be protonated to cause loss of water. The diazonium salt is resonance stabilized:

\[
\begin{align*}
\text{aryl group} & \rightarrow \text{aryl group} \\
\text{N} & \text{N} \\
\text{H}_2\text{O} & \rightarrow \text{H}_2\text{O} \\
\end{align*}
\]

Many diazonium salts are unstable and can be explosive when dry. Therefore, they are always prepared as needed under acidic conditions with good stirring, kept at or near 0°C to minimize the reaction with water to produce a phenol, and used immediately in the coupling reaction.

To complete the synthesis of the azo dye, the diazonium salt reacts as an electrophile with an electron-rich coupling component, like a phenol or an aniline, through an electrophilic aromatic substitution mechanism. The -OH or -NH₂ group direct the aryl diazonium ion to the para site unless that position is occupied, in which case the ion attaches ortho.
In this experiment, you will synthesize an azo dye as part of a parallel combinatorial synthesis scheme using a coupling reaction of an aromatic amine or phenol with an aryl diazonium ion, and will dye a piece of fabric using the direct dyeing process.

Combinatorial chemistry is heavily used in drug discovery research. Combinatorial methods allow for the simultaneous synthesis of many potentially valuable compounds. These are screened for desirable biological activity. In a “parallel synthesis”, a variety of reactants (several nucleophiles which are assigned to the rows) is allowed to react with a variety of substrates (several electrophiles which are assigned to the columns). In this way, an array of chemicals can be synthesized.

In this combinatorial synthesis, five different aminobenzenes and six different “activated” aromatic compounds will be used (See the Table of Possible Azo Dye Combinations). Each student choosing to do this experiment will be assigned one of the azo compounds to synthesize. NOTE: There are 2 different procedures according to which type of aniline you are using to make the diazonium salt. One procedure is for 4-nitroaniline. The other procedure is for the sulfonic acid substituted anilines.

The azo dye will be used to dye a piece of fabric using the direct dyeing process. Direct dyeing is achieved by treating a fabric with a diazo component and a coupling component to make an insoluble azo dye. The final color is controlled by the choice of the diazo and coupling components. In direct dyeing, the basic or acidic groups on the dye interact with the fabric to form a salt. That makes fabrics whose fibers have ionized surfaces, such as wool or silk, absorb the dye better than other materials like cotton.

Safety:
Avoid skin contact with all the chemicals, work under the hood, and wear gloves throughout the experiment.
- The nitroanilines and naphthols are highly toxic. Prevent skin contact.
- Sodium hydroxide and hydrochloric acid are corrosive.
- Sodium nitrite is a toxic oxidizer.
- Diazonium salts are explosive in the solid form. Keep them in solution and cold.
- Azo dyes are irritants.
### Experimental Procedure:

**Table of Possible Azo Dye Combinations:**
This table shows the possible combinations for this experiment. Some may be unavailable this year. Contact Katie ([ksours@linfield.edu](mailto:ksours@linfield.edu)) to determine what combination you should prepare for.

<table>
<thead>
<tr>
<th></th>
<th>Aniline (NH₂)</th>
<th>Orthanilic acid (Aniline-2-sulfonic acid)</th>
<th>Metanilic acid (Aniline-3-sulfonic acid)</th>
<th>Sulfanilic acid (Aniline-4-sulfonic acid)</th>
<th>4-nitroaniline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>1-naphthol</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>2-naphthol</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>N,N-dimethyl aniline</td>
<td>26</td>
<td>27</td>
<td>28</td>
<td>29</td>
<td>30</td>
</tr>
</tbody>
</table>

Para Red

Methyl Orange
**Diazonium Salt Preparation:**

**Procedure A: Aniline**

In a large test tube, mix 2.1 mmol of aniline and 7 mL of water. In another test tube, mix 1 mL of concentrated HCl and 6 mL of ice. Keep this HCl solution at 0 °C – 5 °C using an ice-water bath. In a third test tube, make a solution of 0.20 g of sodium nitrite in 0.5 mL of water. Add the sodium nitrite solution to the first test tube containing the aniline solution. Use a Pasteur pipette to slowly add this suspension to the second test tube containing the HCl solution, with constant stirring using a glass rod. Make sure to maintain the temperature below 10 °C of the HCl solution throughout the addition. Once all of the suspension has been added, keep stirring the mixture occasionally for about 2 minutes. To make sure that enough sodium nitrite has been added, test the solution with starch-iodide paper. If the test paper does not turn blue-violet (after 45 sec), make more aliquots of the sodium nitrite solution, and slowly add small portions to the test tube with aniline and water. Continue to add more sodium nitrite solution and test the solution until the paper turns blue-violet. If there are any precipitates, then filter those and discard them. Keep the diazonium salt solution cold and covered to minimize evaporation of the solution (see safety notes).

**Procedure B: Aniline Sulfonic Acids:**

In a large test tube, mix 2.1 mmol of aniline sulfonic acid and 7 mL of water. Add 0.13 g of sodium carbonate to neutralize the acid. Use a stirring rod and a warming bath (you can prepare this on a stirrer/hotplate) to dissolve as much of the solid as possible. Once the majority of the solid is dissolved, remove the test tube from water bath, and let the test tube slowly cool to approx. room temperature. In another test tube, make a solution of 0.20 g of sodium nitrite in 0.5 mL of water. Add the sodium nitrite solution to the first test tube containing the aniline sulfonic acid solution. In a third test tube, add 0.50 mL of concentrated HCl and 3 g of ice. Use a Pasteur pipette to slowly add the aniline sulfonic acid suspension to the HCl solution with constant stirring using a glass rod. Maintain the temperature of the HCl / aniline sulfonic acid suspension mixture below 10 °C throughout the addition. Once all of the suspension has been added, keep stirring the mixture occasionally for about 2 minutes. To make sure that enough sodium nitrite has been added, test the solution with starch-iodide paper. If the test paper does not turn blue-violet (after 45 sec), make more aliquots of the sodium nitrite solution, and slowly add small portions to the test tube with aniline and water. Continue to add more sodium nitrite solution and test the solution until the paper turns blue-violet. If there are any precipitates, then add more water to dissolve them. Keep the diazonium salt solution cold and covered to minimize evaporation of the solution (see safety notes).

**Procedure C: 4-nitroaniline:**

Mix 2.0 mL of concentrated HCl and 2.0 mL of water in a test tube. Weigh 0.08 g (0.58 mmol) of 4-nitroaniline. Use a stirring rod and a warming bath to dissolve as much of the 4-nitroaniline as possible in the HCl solution. Chill the 4-nitroaniline / HCl solution to 0 °C – 5 °C. In another test tube, weigh 0.11 g (1.59 mmol) of sodium nitrite and add 0.5 mL of water. Using a Pasteur pipette, slowly add some of the sodium nitrite solution to the 4-nitroaniline / HCl solution with constant stirring using a glass rod. To know if enough sodium nitrite solution has been added,
test the overall solution (4-nitroaniline / HCl / some sodium nitrite) with starch-iodide paper. If the test paper does not turn blue-violet (after 30 sec), make more aliquots of the sodium nitrite solution, and slowly add small portions to the test tube with 4-nitroaniline and HCl. Continue to add more sodium nitrite solution and test the solution until the paper turns blue-violet. Once the overall solution has passed the starch-iodide paper test, stir the mixture occasionally for about 10 min. Keep the diazonium salt solution cold and covered to minimize odor and evaporation of the solution (see safety notes).

**Coupling Agent Preparation:**

If the coupling agent is a phenol:

Dissolve 2.1 mmol of the phenol in 10 mL of 3 M NaOH (resorcinol and the two naphthols may need more 3 M NaOH, and may need to be slightly warmed to dissolve) in a small beaker, and place the beaker in an ice-water bath to cool to 5 °C.

If the coupling agent is N,N-dimethyl aniline:

Dissolve 2.1 mmol of N,N-dimethyl aniline in 10 mL of 3 M HCl in a small beaker, and place the beaker in an ice-water bath to cool to 5 °C.

**To dye a piece of cloth:**

Soak the piece of cloth in the coupling agent solution. Remove piece of cloth from the solution and pat to dryness. Use a Pasteur pipette to paint a design on the fabric with the diazonium salt solution. Dry the fabric overnight in a student hood. If there are any clumps of the azo dye, rinse the fabric well using tap water.

**Clean-up and Disposal**

Put all solutions containing aromatic amine or azo dye in a container labeled “Azo Dye Recycle.”

**Laboratory Report:**

- Use the introduction to help you make an educated guess about the mechanism and structure for your azo dye.
- Discuss conjugation of pi systems, delocalization of electrons, and the different effects of substituent positions on these systems.
- Evaluate the effectiveness of the synthesized dye in the direct dyeing of the cloth: How well did the colors “stick” to the cloth? Describe the color of the solutions of coupling agent and diazonium salt. How do these colors compare to the color of the azo dye itself?
- Insert a photo of the dyed fabric (labeled as a figure) in your lab report.
References:


