

Liquid Chromatography

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1 Purpose

The purpose of this experiment is to use liquid chromatography to separate the component substances that are contained in grape-flavored Kool-Aid.

2 Procedure

There are two parts to this lab. In part one, the two dyes in the drink are separated using 10 mL of 70% isopropanol as the eluant. One mL of Kool-Aid is injected into the chromatography column and then a constant supply of the eluant is applied so that the dyes are flushed through the column at different rates. Measurements are recorded of the amount of effluent collected as the first and last drops of each dye emerge. After each of the three trials, the column is washed with distilled water.

In part two, the components of the Kool-Aid are separated using a gradient of isopropanol dilution. Starting with eluting with distilled water, each component is separated by polarity as the concentration of the eluent is increased. Qualitative observations are then recorded of the eluted fractions.

3 Data

The following data were collected in the first part.

	Red Dye			Blue Dye		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Start of Band (mL)	1.5	2.1	1.3	2.7	3.3	2.0
End of Band (mL)	2.7	3.3	2.0	5.9	6.1	4.0

In the second part, the following data were collected.

Beaker	Eluant	Observations of the Eluted Fractions
1	H ₂ O	Clear
2	5% isopropanol	Light red
3	28% isopropanol	Light blue
4	70% isopropanol	Clear

4 Calculations

Now, the selectivity and resolution (α and R) of the separation in the first part must be calculated.

First, the bandwidths (W) are to be calculated. For each run of each dye

$$W = V_{end} - V_{start} \quad (1)$$

For the red dye

$$W_1 = 2.7 - 1.5 = 1.2$$

$$W_2 = 3.3 - 2.1 = 1.2$$

$$W_3 = 2.0 - 1.3 = 0.7$$

For the blue dye

$$W_1 = 5.9 - 2.7 = 3.2$$

$$W_2 = 6.1 - 3.3 = 2.8$$

$$W_3 = 4.0 - 2.0 = 2.0$$

Then, the average bandwidth for the red dye is $W_{avg} = \frac{1.2+1.2+0.7}{3} = 1.0$ and for the blue dye $W_{avg} = \frac{3.2+2.8+2.0}{3} = 2.7$

The average retention volume ($V_{R_{ave}}$) is

$$V_{R_{ave}} = V_{start_{ave}} + \frac{W_{ave}}{2} \quad (2)$$

The average starting values are, for red and blue dyes, respectively

$$V_{start_{ave}} = \frac{1.5 + 2.1 + 1.3}{3} = 1.6$$

$$V_{start_{ave}} = \frac{2.7 + 3.3 + 2.0}{3} = 2.7$$

For the red dye, $V_{R_{ave}} = 1.6 + \frac{1.0}{2} = 2.1$, and for the blue dye, $V_{R_{ave}} = 2.7 + \frac{2.7}{2} = 4.1$

Using the estimation of $V_M = 0.49$ mL being one-half of the volume of the Sep-Pak cartridges, the capacity factor (k') is

$$k' = \frac{V_{R_{ave}} - 0.49}{0.49} \quad (3)$$

For the red and blue dyes,

$$k'_{red} = \frac{2.1 - 0.49}{0.49} = 3.3$$

$$k'_{blue} = \frac{4.1 - 0.49}{0.49} = 7.4$$

The separation factor (α) is

$$\alpha = \frac{k'_{blue}}{k'_{red}} \quad (4)$$

which is

$$\alpha = \frac{7.4}{3.3} = 2.2$$

The resolution of the separation (R) is given by

$$R = \frac{2(V_{R_{ave}(blue)} - V_{R_{ave}(red)})}{W_{blue} + W_{red}} \quad (5)$$

So,

$$R = \frac{2(4.1 - 2.1)}{2.7 + 1.0} = 1.1$$

Therefore, in this experiment, $\alpha = 2.2$ and $R = 1.1$

5 Discussion

1. Polarity of molecules is the difference in charge across a given molecule. For a molecule to be polar, it must have a permanent dipole with one end having a positive charge and the other end having a negative charge. The cause of polarity is in the alignment of the atoms which can make an uneven electron distribution. When electron charge is uneven, there is a difference which makes the permanent dipole.

2. When solubility is discussed, the rule "like dissolves like" is frequently used. This means that compounds that are similar in polarity are more likely to dissolve in one another. Polar solvents will dissolve polar solutes and non-polar solvents will dissolve non-polar solutes. In a polar compound, the molecules are attracted due to their poles, while in a non-polar compound the molecules are attracted due to the dispersion forces. These forces do not attract each other, so the compounds that employ each are not attracted to each other.

3. Isopropyl alcohol differs in polarity from water in that it is completely surrounded by hydrogen, making an even distribution in electrons so there is no difference in charge, and likewise very little polarity compared to water, whose hydrogens are all on one side. Since the electrons are pulled from the hydrogen, this side becomes very negative which makes water polar. Also, since water basically has two -OH groups on one side and isopropyl alcohol only has one (the hydrocarbons do not add to a charge since the hydrogen's charges cancel each other out around their respective tetrahedrons), water is much more polar.

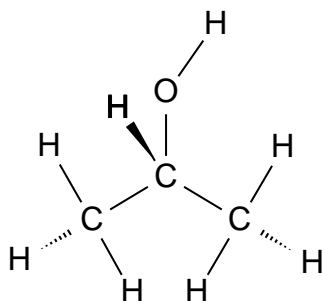


Figure 1: **Isopropyl alcohol structural formula**

4. The value of the resolution of separation of the two dyes was calculated to be 1.1. The dyes did overlap as they emerged from the column, but not by too much. So, the resolution probably was not as high as it should have been.

5. The four eluting solvents decreased in polarity from one run to the next (water and then isopropyl alcohol starting from 5% to 70%). The water removed the most polar compounds

from the column, and as the eluting solvent decreased in polarity, the less polar compounds emerged since "like dissolves like."