SI Organic Chemistry - Full Discipline Demo

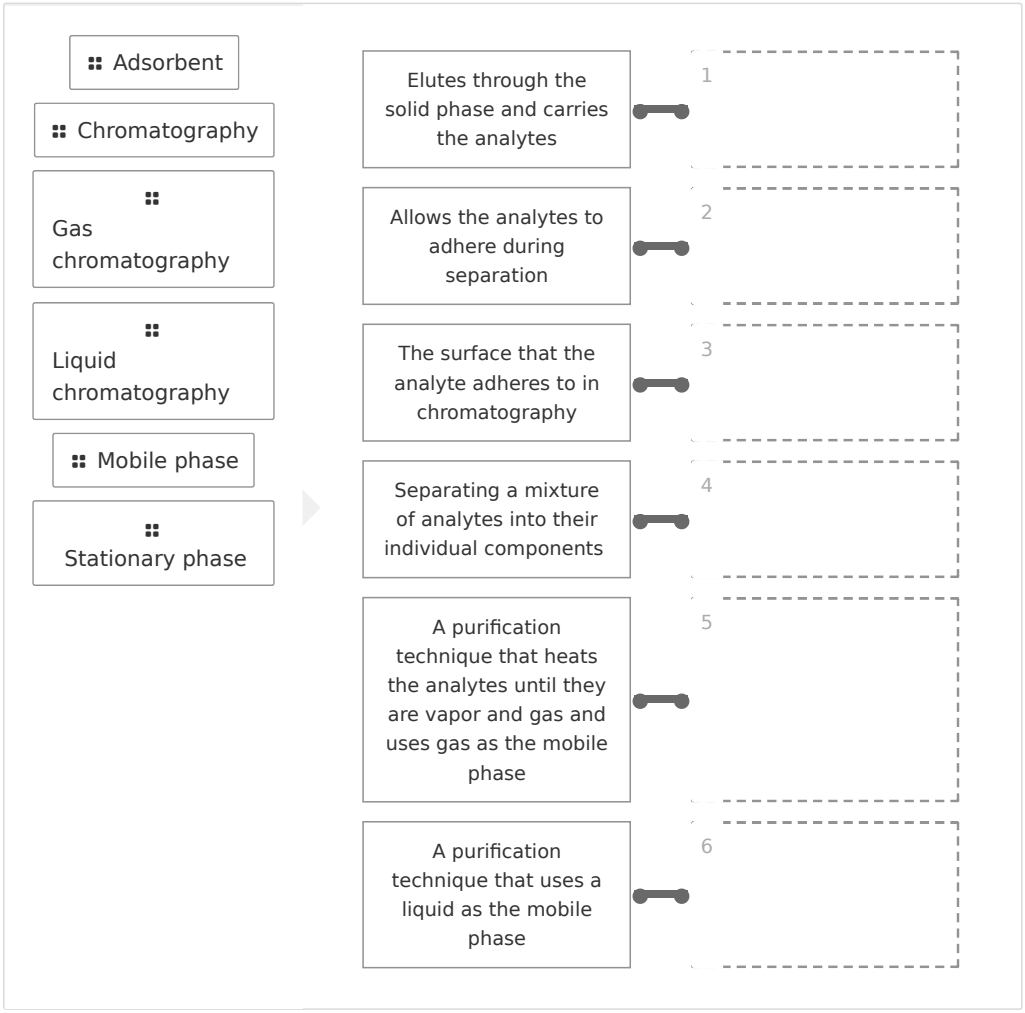
Column Chromatography to Separate Dyes and Analysis by a Colorimeter

Final Report - Answer Guide

Institution	Science Interactive University
Session	SI Organic Chemistry - Full Discipline Demo
Course	SI Organic Chemistry - Full Discipline Demo
Instructor	Sales SI Demo

Test Your Knowledge

Match each term with the best description.

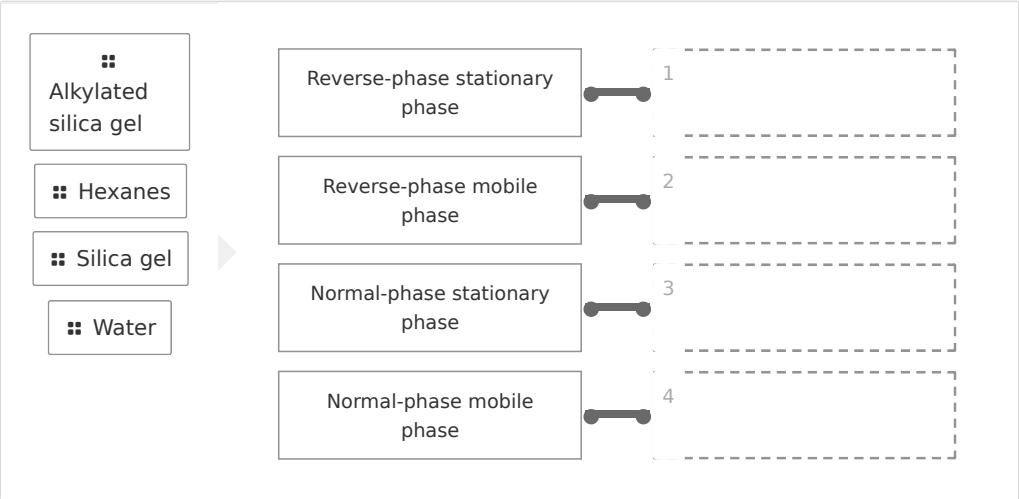


Correct answers:

1 Mobile phase 2 Stationary phase 3 Adsorbent

4 Chromatography 5 Gas chromatography 6 Liquid chromatography

Match each compound with the corresponding phase.



Correct answers:

1 Alkylated silica gel 2 Water 3 Silica gel 4 Hexanes

Exploration

Chromatography is used to separate a _____ into _____.

- pure compound; its individual atoms
- mixture of compounds; their individual compounds ✔
- mixture of compounds; difference solution concentrations
- None of the above

_____ is used as a stationary phase for chromatography.

- Gas
- Liquid
- Paper ✔
- All of the above

Normal phase column chromatography uses a polar stationary phase and a nonpolar mobile phase.

- [x] True ✔
- [ ] False

A(n) _____ elution involves maintaining a constant ratio of the solvent for the entire run.

- [x] isocratic ✔
- [ ] gradient
- [ ] variable
- [ ] None of the above

Red-40 Dye and Blue-1 Dye are the compounds responsible for giving grape soda its purple color.

- [x] True ✔
- [ ] False

A(n) _____ elution involves maintaining a constant ratio of the solvent for the entire run.

- [x] isocratic ✔
- [ ] gradient
- [ ] variable
- [ ] None of the above

**A colorimeter is a type of spectrometer which is used to measure the _____ transmitted
through a sample at a specific _____.**

- light; wavelength ✔
- magnetism; polarity
- vibration; time
- None of the above

Exercise 1

Based on your results, were you successful in separating the dyes? How could you tell if they were not separated? Reference Data Table 1 and Photo 1 in your answer.

The dyes were successfully separated because the fractions has a distinct color change as can be seen in Photo 1. The red dye eluted in fractions 2-3 and the blue dye eluted in fractions 4-5 in Data Table 1. If the two dyes were not separated then the fractions would have appeared purple.

What type of column chromatography was used to separate the dyes? Why were water and isopropanol chosen for the mobile phase?

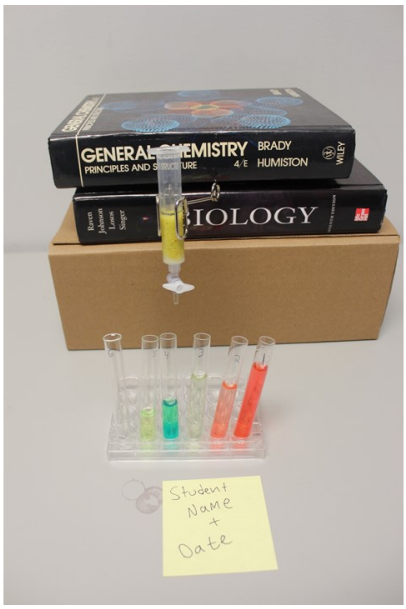
The sand acts as a reverse phase stationary phase. This means it is nonpolar. The water and isopropanol were chosen for the mobile phase because they are polar.

Data Table 1: Volume of samples
(SAMPLE ANSWER BELOW)

	Volume (mL)
Grape soda	5.0
Fraction 1	7.7
Fraction 2	6.0
Fraction 3	6.6
Fraction 4	4.2

Fraction 5	3.2
Fraction 6	0.0

Photo 1: Fraction colors
(SAMPLE ANSWER BELOW)



Exercise 2

Based on your results in Data Tables 4 and 7, which dye existed in the highest quantity (number of moles)?

Student answers will vary, but most grape sodas have around double as much red dye as blue dye.

Ex: The amount of red dye in the grape soda sample was $1.0x10^{-6}$ moles. The total amount of red dye recovered from the column was $9.0x10^{-7}$ moles. Comparatively, the amount of blue dye in the grape soda sample was $4.4x10^{-7}$ moles, and the total amount of blue dye recovered from the column was $4.0x10^{-7}$ moles. Therefore, there were more moles of red dye present as measured both in the original sample and in the recovered samples.

What would happen to the absorbance value if you used the wrong color LED?

The LED's were chosen because the maximum absorbance is at that wavelength. If a different wavelength was chosen then the absorbance value would be decreased.

What percentage of each dye did you recover based on your results in Table 8? During what procedures did you lose dye?

Variable: dripped during a transfer; some stuck inside of pipet; overfilled a test tube; stuck on column.

Data Table 2: Resistance of Samples with Green LED
(SAMPLE ANSWER BELOW)

Test Tube	Resistance for dH_2O Blank (Ω)	Resistance for sample (Ω)	Overall Resistance
dH_2O	0.7	0.7	1

GS	0.7	7.9	11.3
1	0.7	1.9	2.7
2	0.7	1.0	1.4
3	0.7	0.7	1
4	0.7	0.9	1.3
5	0.7	0.7	1
6	No sample answer	No sample answer	No sample answer

Data Table 3: Current and Percent Transmittance for Samples with Green LED
(SAMPLE ANSWER BELOW)

Test Tube	Current for blank (A)	Current for sample (A)	Percent Transmittance (%T)
dH_2O	9	9	100
GS	9	0.79	8.8
1	9	3.3	36
2	9	6.3	70
3	9	9	100
4	9	7	77
5	9	9	100
6	9	N/A	N/A

Data Table 4: Absorbance and Concentration with Green LED of Red Dye
(SAMPLE ANSWER BELOW)

Test Tube	Absorbance	Concentration (M)	Number of moles
dH_2O	0	0	0
GS	1.05	0.00020	0.0000010
1	0.43	0.000082	0.00000063
2	0.15	0.000029	0.00000018
3	0	0	0
4	0.19	0.000021	0.000000087
5	0	0	0
6	N/A	N/A	N/A
Total (test tubes 1-6)			0.00000090

Data Table 5: Resistance for samples with Red LED
(SAMPLE ANSWER BELOW)

Test Tube	Resistance for dH_2O Blank (Ω)	Resistance for sample (Ω)	Overall Resistance
dH_2O	0.4	0.4	1
GS	0.4	4.7	11.75
1	0.4	0.4	1

2	0.4	0.4	1
3	0.4	0.5	1.25
4	0.4	4.0	10
5	0.4	0.4	1
6	0.4	N/A	N/A

Data Table 6: Current and % Transmittance with Red LED
(SAMPLE ANSWER BELOW)

Test Tube	Current for blank (A)	Current for sample (A)	Percent Transmittance (%T)
dH_2O	9	9	100
GS	9	0.77	8.5
1	9	9	100
2	9	9	100
3	9	7.2	80
4	9	0.9	10
5	9	9	100
6	9	N/A	N/A

Data Table 7: Absorbance and Concentration with Red LED of Blue dye
(SAMPLE ANSWER BELOW)

Test Tube	Absorbance	Concentration (M)	Number of moles
dH_2O	0	0	0
GS	1.07	0.000088	0.00000044
1	0	0	0
2	0	0	0
3	0.096	0.0000079	0.000000053
4	1	0.000082	0.00000035
5	0	0	0
6	N/A	N/A	N/A
Total (test tubes 1-6)			0.00000040

Data Table 8: Percent Isolation of Dyes
(SAMPLE ANSWER BELOW)

Dye	Percent Isolated (%)
Red-40	90
Blue-1	91

Competency Review

Chromatography is a purification technique used to separate individual compounds from a mixture.

- True ✔
- False

In normal phase chromatography the mobile phase is _____ and the stationary phase is ____.

- polar; polar
- polar; nonpolar
- nonpolar; polar ✔
- None of the above

In column chromatography, the compound that requires the longest time to elute adheres less tightly to the stationary phase.

- True
- False ✔

A UV-Vis spectrometer measures the amount of light that the sample absorbs.

- True ✔
- False

_____ dye elutes first when separating grape soda colors using water as a mobile phase in column chromatography.

- Red-40 ✔
- Blue-1
- Yellow-5
- None of the above

The Beer's Law plot shows the relationship between ___ and ____.

- mass; absorbance
- concentration; absorbance ✔
- volume; % transmittance
- None of the above

Blue 1 dye has a higher concentration than Red 40 dye in grape soda.

- True
- False ✔

Extension Questions

Indigo is another blue dye but is not used in food like Blue-1 dye. Research the structure of indigo and explain what functional groups Blue-1 dye and indigo have in common. What industrial use does indigo have? (SAMPLE ANSWER BELOW)

Indigo has a highly congugated pi system like blue-1 dye. They both have several aromatic rings as part of their chemical structure. Indigo is used in the fabric and clothing industry. It is most commonly used in blue jeans to give them their distinct color.

Orange soda is typically formulated with Red-40 and Yellow-6 as food coloring. Look up the structures of these dye molecules. Will this same experiment be effective to separate the two dyes in orange soda? Why or why not? (SAMPLE ANSWER BELOW)

Red-40 and Yellow-6 are very similar molecules. While Blue-1 has substantially more ring systems, which make it significantly more hydrophobic than Red-40, Yellow-6 has basically the same overall structure as Red-40 with only two small functional group differences. Therefore, no, these two dyes would be very difficult to separate using this method, as their overall polarities are very similar.