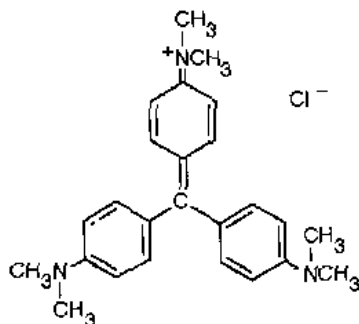


Partition Coefficient of a Dye/Water/Alcohol Mixture

Objective: To determine the partition coefficient of crystal violet in a series of water/alcohol mixtures, and to interpret the trend in the values.

Introduction: The difference in solubility of a solute between two phases is utilized in many branches of chemistry, biology and their associated technologies. The various chromatographic techniques are based on the partitioning of solutes between two phases, usually designated as a stationary and a mobile phase. The purification of pharmaceuticals and other fine chemicals is often affected by extraction methods. In extraction, a mixture containing the desired solute is contacted with a second immiscible phase (liquid or solid) for which the solute has a greater affinity. This results in the removal (extraction) of the solute from the mixture. In the field of microbiology, staining techniques are employed to classify microbes. Gram-negative microbes are covered by a lipophilic coating; they can be identified by the absorption of nonionic organic dyes. Gram-positive microbes are covered by a hydrophilic layer; they can be stained with ionic dyes. Crystal violet is an example of a Gram-positive dye.

Crystal violet is a cationic dye that is sold as a chloride salt. One of its resonance structures is given below. The dye readily dissolves in water, giving rise to an intense



violet solution ($\lambda_{\text{max}} = 588 \text{ nm}$). When the dye is introduced to water/hexane mixtures the hexane layer is completely clear, while the water layer is violet. This simple experiment demonstrates the preferential partitioning of crystal violet into aqueous solutions relative to hydrophobic solvents.

The straight chain primary alcohols exhibit diminishing solubility in water as the length of the hydrocarbon tail of the molecule is increased. Methanol, ethanol and 1-propanol are all completely miscible with water. 1-butanol exhibits limited solubility in water (~2 mole % at 25 °C). 1-octanol behaves more like an oil than an alcohol. In mixtures of water/1-octanol, crystal violet is expected to preferentially partition into the water phase. In this experiment you will determine the partition coefficient of crystal violet in the two-phase mixtures of water with the homologs 1-hexanol and 1-octanol.

The partition coefficient for a solute "i" between two phases, α and β , is defined as

$$K_i = \frac{x_i^a}{x_i^b} \quad (1)$$

Here, x_i^α refers to the mole fraction of species "i" in phase α . The partition coefficient is independent of concentration at low concentrations of solute. To demonstrate this, we begin with the description of the chemical potential, μ_i , of the solute in the solution.

$$\mu_i = \mu_i^\circ + RT \ln(\gamma_i x_i) \quad (2)$$

Here μ_i° refers to the chemical potential of a specific reference state. The combination $\gamma_i x_i$ is called the activity of the solute in the solution. The activity can be thought of as an effective concentration. At a sufficiently low concentration of the solute, the activity is equal to the mole fraction. Solutions for which the activity coefficient, $\gamma_i = 1$, are referred to as ideal solutions. If solution ideality occurs as a consequence of dilution of the solute, we say that the solution is an ideally dilute solution. Expression (2), for the ideally dilute solution becomes

$$\mu_i = \mu_i^\circ + RT \ln(x_i) \quad (3)$$

The chemical potential of the ideally dilute solution approaches μ_i° as x_i approaches 1. Therefore the reference state for the solute chemical potential is that of pure solute at infinite solution! These, fictitious reference states are very common in chemical thermodynamics. But, what does it mean? If the solute molecule were truly in its pure state it would be entirely surrounded by other solute molecules. The bonding and structure that give rise to μ_i is a consequence of the surrounding molecules. In the ideally dilute solution reference state the structure and bonding are those of solvent, rather than solute molecules. This is the state to which the molar Gibbs free energy, μ_i° , refers.

If a solution containing a solute is contacted with a second immiscible solvent, equilibrium will be established between the solute in the two phases. The criterion for this equilibrium is that the chemical potential of the solute in the two contacting phases must be the same (i.e. $\mu_i^\alpha = \mu_i^\beta$). Substituting into expression (3)

$$\mu_i^{\circ,\alpha} + RT \ln(x_i^\alpha) = \mu_i^{\circ,\beta} + RT \ln(x_i^\beta) \quad (4)$$

Solving for the partition coefficient, K_i , gives:

$$K_i = \frac{x_i^a}{x_i^b} = \exp \left[\frac{\mu_i^{\circ,b} - \mu_i^{\circ,a}}{RT} \right] \quad (5)$$

Since the reference state chemical potentials are independent of concentration, the partition coefficient is only dependent on temperature.

The partition coefficient is generally recorded as $\text{Log}K$, and is assumed to represent the ratio of the mole fraction of solute in the less polar phase to that in the more

polar phase (in our case alcohol to water). Since crystal violet is a Gram-positive stain, we shall put forth the following hypotheses.

LogK for crystal violet will be negative for the 1-hexanol/water and 1-octanol/water systems.

LogK for the 1-octanol/water system < LogK for the 1-hexanol/water system.

Experimental: In this experiment you will use UV-Vis spectroscopy to determine the partition coefficient of crystal violet in water/alcohol mixtures. To accomplish this, you will need to determine the relationship between absorbance and molar concentration for the dye in the two phases of these mixtures. The ability of a dye to absorb light depends on the solvent environment that it is surrounded by. Therefore, a Beers law plot must be constructed for each phase of the two-phase water/alcohol mixtures.

Reagents:

Crystal Violet
1-hexanol
1-octanol
Deionized water

Apparatus:

Shimadzu UV-Vis spectrophotometer
2 25-mL Volumetric flasks
1 100-mL Volumetric flask
3 60-mL Separatory Funnels or burets (very clean)
Disposable UV-Vis cuvetts
5-mL graduated pipets
50- μ L LC syringe

Development of Beers Law Plots:

Solutions:

- A) 100 mL of a 1.0×10^{-3} M solution of crystal violet in water
- B) 25 mL of a 1.0×10^{-3} M solution of crystal violet in 1-hexanol
- C) 25 mL of a 5.0×10^{-3} M solution of crystal violet in 1-octanol

- 1) Add 2 mL of deionized water to a disposable UV-Vis cell; then add 10 μ L of solution A to the same cell using an LC syringe. (Be sure to thoroughly clean the syringe between uses.)
- 2) Record the spectrum for crystal violet to locate λ_{\max} . It should be located near 588 nm. If the absorbance is greater than 0.2, you will have to reduce the amount of dye you use.
- 3) Add 2 mL of water to a new, clean UV-Vis cell and place it in the spectrometer. Auto-zero the instrument at the λ_{\max} for the dye.
- 4) Add 10 μ L of solution A to the cell and record the absorbance at the λ_{\max} for the dye. (Use less if the absorption is too great.)

- 5) Repeat step 4) for a total of 5 additions.
- 6) Repeat steps 1 through 5 for 1-hexanol (with solution B) and 1-octanol (with solution C).

Measurement of the Partition Coefficient

- 1) Thoroughly clean a 60-mL separatory funnel. Rinse the funnel multiple times with distilled water to remove any traces of soap.
- 2) Prepare a mixture of 10 mL of solution A and 10 mL of solution B in the separatory funnel.
- 3) Remove 5 mL of the top phase and place it in a labeled vial. Remove 5 mL from the bottom phase and place it in a labeled vial.
- 4) In a second clean 60-mL separatory funnel, add 15 mL distilled water and 15 mL 1-hexanol. Then add 5 mL of solution A and 5 mL of solution B. Mix well and repeat step 3).
- 5) In a third clean 60-mL separatory funnel, repeat step 4) using 2 mL of solution A and B, respectively.
- 6) Auto-zero the spectrometer with water at the λ_{max} you recorded in water.
- 7) Record the absorbance for your three lower phases. You may have to dilute the samples to get into the absorbance range of your Beers law plots.
- 8) Auto-zero the spectrometer with 1-hexanol at the λ_{max} you recorded for 1-hexanol.
- 9) Record the absorbance for your three upper phases. You may have to dilute the samples to get into the absorbance range of your Beers law plots.
- 10) Repeat steps 1 through 9 for 1-octanol.

Analysis and Questions:

- 1) Generate the Beers law plots for crystal violet in water and in the two alcohols. Place all the curves on one graph to enable you to compare them. Use the peak near 588 nm to prepare these plots.
- 2) Fit regression lines through the curves of part 1, forcing the intercept through zero. Give 95% confidence limits for the molar extinction coefficient.
- 3) Determine the average molar concentrations of crystal violet in the three samples of the 1-hexanol/water system using the appropriate Beers law regression lines.
- 4) Determine the mole fraction of crystal violet in the upper and lower layers of the 1-hexanol/water system. Assume that water and 1-hexanol are immiscible.
- 5) Calculate $\log K$ for the three trials of the experiment. Is there a trend, or is the difference within the variability of the experiment?
- 6) Repeat parts 3 to 5 for the 1-octanol/water system.
- 7) Compare the average $\log K$ value for 1-octanol/water to that of 1-hexanol/water. Are the values significantly different? What does this say about the hypothesis of the experiment?

- 8) Are your results consistent with the use of crystal violet as a Gram-positive stain?
- 9) Compare the three molar extinction coefficients of crystal violet in water, 1-hexanol and 1-octanol. Are the values significantly different? Give a plausible explanation for the trends in the data.