

Log P and MR

Reading Assignment: Please read about log P and MR in the Computer Aided Molecular Design Tutorial (<http://www.colby.edu/chemistry/CompChem/CAMD.pdf>)

Introduction

The Computer Aided Molecular Design Tutorial discusses the definition of the octanol-water partition coefficient and the molar refractivity, MR. The Tutorial also discusses the importance of log P and MR in QSAR studies. In this lab we will determine the log P value and MR for 1-methyl-2-pyrrolidinone.

The traditional method for determining log P is called the shake-flask method. The compound of choice will be equilibrated in the two-phase octanol-water system in a small vial. HPLC will be used to determine the concentration of the compound in each phase. The integral under the peak in the HPLC chromatogram is proportional to the concentration, $c_i = k I_i$, where I_i is the integral under the peak. The proportionality constant k is called the response factor. The ratio of the integrals for the octanol and water phase will give the partition coefficient:

$$P = \frac{c_i(\text{octanol})}{c_i(\text{water})} = \frac{kI_i(\text{octanol})}{kI_i(\text{water})} = \frac{I_i(\text{octanol})}{I_i(\text{water})} \quad (1)$$

For Eq. 1 to work, the proportionality constant must be the same for each determination in each solvent. For very careful work, a separate calibration curve for the sample in octanol and for the sample in water would be constructed to verify that the response factors are equal. In this exercise, we will measure the peak width at half height from each phase to verify similar chromatographic behavior.

Molar Refractivity, MR

MR is defined by the Lorentz-Lorentz equation:

$$MR = \frac{(n^2 - 1)}{(n^2 + 2)} \left(\frac{M}{d} \right) \quad (2)$$

Where n is the index of refraction, M is the molecular weight, and d is the density. For QSAR studies, the value of MR is usually divided by 10 so that the range of typical MR values is similar to the range of logP and other typical descriptors¹. We will use the literature value for the density in this exercise. The index of refraction will be determined in this lab using an Abbe' refractometer.

Let us consider some of the underlying ideas leading to Eq 2. The derivation of Eq. 2 starts with the relative permittivity, ϵ_r :

$$\epsilon_r = \frac{\epsilon}{\epsilon_0} \quad (3)$$

which is the ratio of the dielectric constant for the compound to the dielectric constant of vacuum. The relative permittivity or dielectric constant should be familiar to you from the

Coulomb Potential, where the $4\pi\epsilon_r\epsilon_0$ or $4\pi\epsilon$ determines the ability of the substance to "communicate" electrostatic forces. The relative permittivity can be determined through the index of refraction of the substance:

$$\epsilon_r = n^2 \quad (4)$$

where the index of refraction is the ratio of the speed of light in the sample to the speed of light in vacuum. The effect of the electric field of light on a sample is to polarize the sample. The molar polarization due to the electric field of light is:

$$P_m = \frac{(\epsilon_r - 1)}{(\epsilon_r + 2)} \left(\frac{M}{d} \right) \quad (5)$$

Substituting in the index of refraction squared for the relative permittivity gives Eq 2. Therefore, the MR and molar polarization are equivalent.² For low frequencies (constant electric fields or frequencies into the MHz range) both the permanent dipole moment and the polarizability contribute to the molar polarization. For high frequencies only the polarizability of the molecule, α , contributes to the molar polarization since the molecules can't reorient quickly enough to respond to the rapidly oscillating electric field. At high frequencies then Eq. 5 can be rewritten in terms of the molecular polarizability:

$$\alpha = \frac{(\epsilon_r - 1)}{(\epsilon_r + 2)} \left(\frac{M}{d} \right) \left(\frac{3\epsilon_0}{N_A} \right) \quad (5)$$

where N_A is Avagadro's number. Eq. 5 shows that MR and the molecular polarizability as molecular descriptors in QSAR studies should be strongly correlated, since they only differ by a constant. The molecular polarizability as a QSAR descriptor, A_{pol} , is often approximated as the sum of the Van der Waals A coefficients for all the atoms in the molecule.

For polar or highly polarizable molecules, MR, P_m , ϵ_r , and α (A_{pol}) should all be large.

Procedure

Prepare a 1×10^{-3} M solution of 1-methyl-2-pyrrolidinone in water. Make up 200 mL of this stock solution. Now using this stock solution and an autopipettor, prepare three standard solutions of the compound in water in autosampler vials that are 0.333×10^{-3} , 0.5×10^{-3} , and 1.0×10^{-3} M. The total volume in each vial should be 1.5 mL. These aqueous only samples will be used to construct a calibration curve. The purpose of the calibration curve is to verify that the HPLC method provides a linear calibration. The calibration curve will also help determine an estimate of the experimental uncertainty in the final concentrations.

The phase ratio in a partitioning experiment is the ratio of the volumes of the two phases, V_{oct}/V_{H_2O} . The logP value should be independent of the phase ratio. We will use two different phase ratios to check this expectation. Prepare two samples with different phase ratios using octanol and the 1-methyl-2-pyrrolidinone stock solution. The phases will be sampled at 2 mm from the bottom of the vial and 10 mm from the bottom of the vial. So in preparing your samples make sure that the 2 mm and 10 mm heights in both samples will be in different phases. The total volume in each vial should be 1.5 mL. Place the two-phase samples on their sides in a shaker bath for 30 minutes. If the samples are cloudy after they have equilibrated, centrifuge in the sample vials for about 30 sec.

After equilibration, place all the vials in the HPLC autosampler tray. Instructions for running the HPLC will be provided in the laboratory. Set the detector at 200 nm. Use a flow rate of 1.00 mL min⁻¹ and a mobile phase composition of 50:50 acetonitrile and water. A run time of 5 min is sufficient.

Determine the index of refraction for 1-methyl-2-pyrrolidinone. Instructions on using the refractometer will be provided in the laboratory.

Calculations

Calculate the logP value for the two-phase samples. Two or three replicates are too small to calculate a standard deviation. For small numbers of trials it is best to simply give the range. Of course, if you only have two replicates, the range is just the two results. Report the average and range for logP. Are the results for the two different phase ratios sufficiently similar that we can assume the results are the same, within experimental error? To answer this question we need to have a way of estimating the experimental error in logP.

We are assuming that the response factors for the aqueous and octanol phases are the same in this experiment. Therefore, the calibration results that you acquired are not used in the calculation of logP. However, the calibration data is useful for estimating the uncertainty in the results. By plotting the peak integral versus concentration, verify that the HPLC method provides a linear calibration plot for aqueous solutions. We can use the standard deviation of the y-values as an estimate of the uncertainty of the HPLC integral from each run. Assuming the relative uncertainty in the aqueous and the octanol integrals are the same, estimate the uncertainty in your logP. Does the experimental uncertainty based on the two different phase ratios match the expected uncertainty based on the calibration curve uncertainties? Can you assume that the results for the two different phase ratios are the same, within experimental error?

Calculate MR using the literature value of the density and your experimental index of refraction. Don't forget to divide by ten to get the range used for QSAR descriptors. Compare your index of refraction with the literature value (see the CRC). For comparison, calculate MR using the literature value of the index of refraction.

Use MOE to calculate the logP and MR value. How well does MOE do in predicting these values? Leo and Hansch have developed a more detailed prediction algorithm for both logP and MR.² Convert the structure of 1-methyl-2-pyrrolidinone into Smiles notation (see the Molecular Mechanics Tutorial). Use the Daylight, Inc. Website to obtain the Leo and Hansch ClogP and CMR values:

<http://www.daylight.com/release/index.html>

The "C" in ClogP and CMR just indicates the Leo and Hansch algorithm has been used in the calculation.

The logPstar value listed in the ClogP printout gives the literature value. Don't worry about looking up the literature value for MR, just use the MR calculated from the literature index of refraction as the literature value. Make a table of the literature, your experimental, the MOE predicted, and the Leo and Hansch predicted values. Comment on the differences. Are they significant? How well do the predictions work? What influence do these variations play in

QSAR studies? What do these values tell you about this compound in terms of its solution properties?

References Cited:

1. Corwin Hansch, Albert Leo, Exploring QSAR: Fundamentals and applications in chemistry and biology, American Chemical Society, Washington, DC, 1995.

2. P. W. Atkins, J de Paula, "Physical Chemistry," W. H. Freeman, New York, NY, 2002, Sections 21.2-4.

Appendix 1: MOE Database Construction

Create a database containing this compound and use the QuaSar-Descriptors application to calculate the logP and MR. Use the following instructions to create the database:

1. With your molecule in the MOE main window, pull down the File menu, slide right on New and choose Database. In the file librarian dialog box enter the database file name as "D:/moefiles/XXXpyrrolidinone.mdb", where XXX are your initials, or on an OSX system switch to the Documents/moefiles folder and enter the file name.

2. In the Database Viewer, pull down the Field menu and choose Create Field.

3. In the dialog bar at the top of spreadsheet window pull down the Create Field type menu and choose molecule. In the adjacent Name dialog box enter "mol". Press enter. A column label "mol" should appear.

4. Pull down the Entry menu and choose Add Entry... In the New Entry window click OK. The current molecule in the MOE window should be transferred into the database.

5. You can now add your descriptors by pulling down the Compute menu and choosing Descriptors.