Measuring Lipophilicity with NMR
# Contents

- Objectives 1
- Introduction 1
-Determining log $P_{ow}$ for some common solvents 2
- Example 1H spectra 3
-References 9

Manuscript prepared by Dr. Hemi Cumming.
School of Chemical and Physical Sciences, Victoria University of Wellington, New Zealand.
Objectives

The aim of this experiment is to determine the 1-octanol/water partition coefficient ($P_{ow}$) of some common solvents (which will be the analyte in this experiment). Students will quantify the amount of the chosen analyte in water, before and after the addition of 1-octanol layer by NMR spectroscopy using the benchtop Spinsolve NMR spectrometer (Figure 1). This information will allow the calculation of the $P_{ow}$ and log $P_{ow}$ of the analytes.

Introduction

The lipophilic (“fat-loving”), or conversely the hydrophilic (“water-loving”) behaviour of a molecule is an important physicochemical property. From a pharmaceutical perspective, this property for a drug-like molecule strongly influences its distribution within the body. The typical method for determining the lipophilicity of a substance is to measure its distribution between two immiscible solvents, usually 1-octanol and water.

This allows for the calculation of the 1-octanol/water partition coefficient ($P_{ow}$) and its logarithm (log $P_{ow}$). As stated by Lipinski’s “Rule of 5” for orally active drugs, a substance will have better drug-likeness if its log $P_{ow}$ is no greater than 5.1

In this experiment, the log $P_{ow}$ for some common solvents will be experimentally determined by $^1$H NMR and compared to literature values.

---

**Figure 1.** Representative diagram of analyte distribution between the water and 1-octanol layers upon addition of 1-octanol.
Determining log $P_{ow}$ for some common solvents

**Safety**
Acetone, acetonitrile, 2-propanol, THF, ethanol, tert-butanol and 1-octanol are highly flammable, handle with caution.

**Procedure**
Place approximately 200 μL of acetone and exactly 500 μL of water into a clean, dry NMR tube, cap the tube and invert to mix.

Record $^1$H NMR spectrum of the solution, obtain integration values for the acetone and water peaks. Normalise the water peak to a value 1.00.

Add exactly 500 μL of 1-octanol to the NMR tube, cap the tube and invert 15-20 times to mix well. Allow the layers to separate (approximately 5 minutes).

Record another $^1$H NMR spectrum of the solution, and again obtain integration values for the acetone and water peaks. Normalise the water peak to a value 1.00.

Use integration values to calculate the $P_{ow}$ and the log $P_{ow}$ of acetone and write on a large whiteboard.

Using at least 4 other values (obtained by other students), calculate the mean and standard deviation.

Repeat the above procedure, replacing acetone with one of the following: ethanol, 2-propanol, acetonitrile, THF or tert-butanol.

**Calculations**
The octanol/water partition coefficient ($P_{ow}$) is equal to the concentration of analyte in the organic layer ($C_0$) divided by the concentration of the analyte in the water layer ($C_w$).

The amount of water should be the same before and after the extraction, because water is insoluble in 1-octanol. Therefore the two integral values we have obtained for the analyte are directly proportional to the concentration before and after the extraction.

Thus, the following equation can be used:

\[
P_{ow} = \frac{C_0}{C_w} = \frac{I_w - I_{wo}}{I_{wo}},
\]

where $I_w$ and $I_{wo}$ are the peak values of the analyte in water and in water with 1-octanol added.
Example $^1$H NMR spectra

Acetone

Figure 2. Spectrum of acetone in water (top) and with a layer of 1-octanol (bottom).

$$P_{ow} = \frac{C_o}{C_w} = \frac{0.197 - 0.124}{0.124} = 0.589$$

$$\log P_{ow} = -0.23 \text{ (literature value = -0.24)}$$
Figure 3. Spectrum of ethanol in water (top) and with a layer of 1-octanol (bottom).

\[ P_{ow} = \frac{C_o}{C_w} = \frac{0.183 - 0.121}{0.121} = 0.512 \]

\[ \log P_{ow} = -0.29 \text{ (literature value = -0.30)} \]
Figure 4. Spectrum of THF in water (top) and with a layer of 1-octanol (bottom).

\[ P_{ow} = \frac{C_0}{C_W} = \frac{0.152 - 0.041}{0.041} = 2.707 \]

\[ \log P_{ow} = 0.43 \text{ (literature value = 0.46)}^2 \]
Figure 5. Spectrum of tert-butanol in water (top) and with a layer of 1-octanol (bottom).

\[ P_{ow} = \frac{C_o}{C_w} = \frac{0.197 - 0.060}{0.060} = 2.28 \]

\[ \log P_{ow} = 0.36 \text{ (literature value = 0.35)}^2 \]
2-Propanol

Figure 6. Spectrum of 2-propanol in water (top) and with a layer of 1-octanol (bottom).

\[ P_{ow} = \frac{C_o}{C_w} = \frac{0.249 - 0.109}{0.109} = 1.28 \]

\[ \log P_{ow} = 0.11 \text{ (literature value = 0.05)} \]
Figure 7. Spectrum of acetonitrile in water (top) and with a layer of 1-octanol (bottom).

\[
P_{\text{ow}} = \frac{C_0}{C_W} = \frac{0.098 - 0.075}{0.075} = 0.307
\]

\[
\log P_{\text{ow}} = -0.51 \quad \text{(literature value = -0.34)}^2
\]

Acetonitrile
References
