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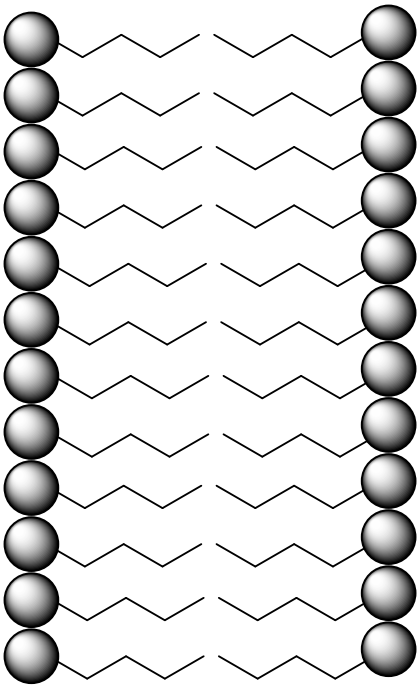
# Measuring Lipophilicity with NMR



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## Contents




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## 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.

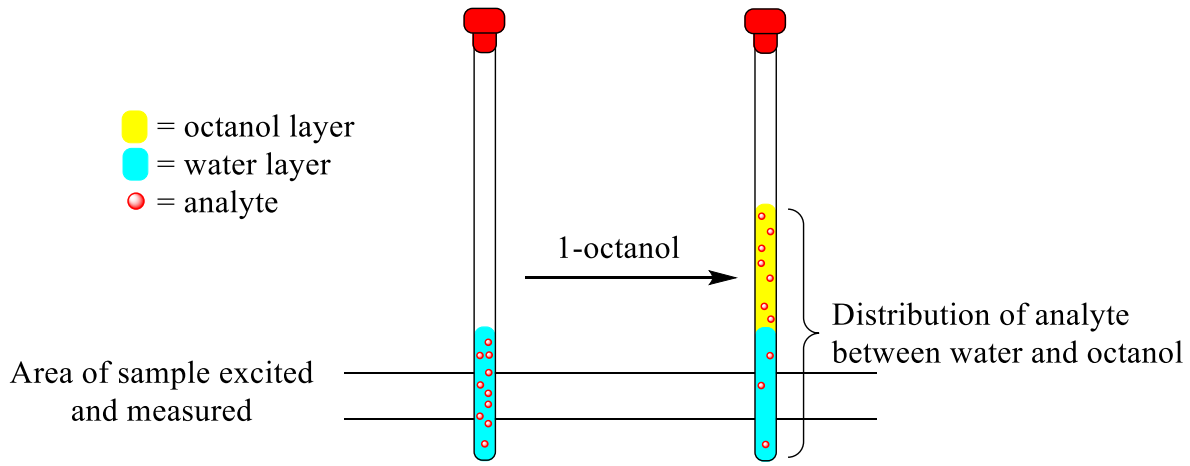


Figure 1. Representative diagram of analyte distribution between the water and 1-octanol layers upon addition of 1-octanol.

# 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 ($C_o$) 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:

$$P_{ow} = \frac{C_o}{C_w} = \frac{I_w - I_{wo}}{I_w} ,$$

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**

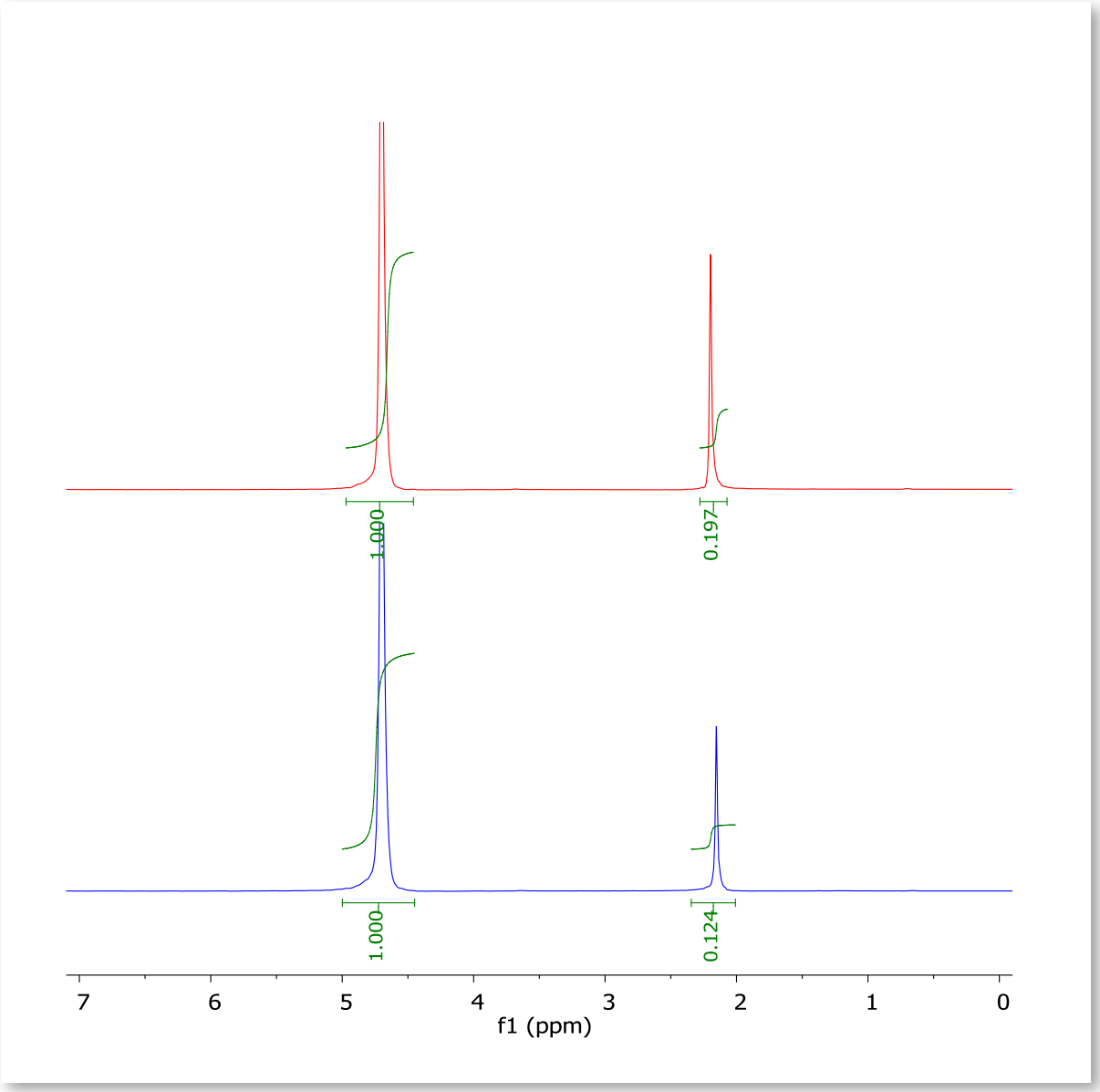


Figure 2. Spectrum of acetone in water (top) and with a layer of 1-octanol (bottom).

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

log $P_{ow}$ = -0.23 (literature value = -0.24)[2]

**Ethanol**

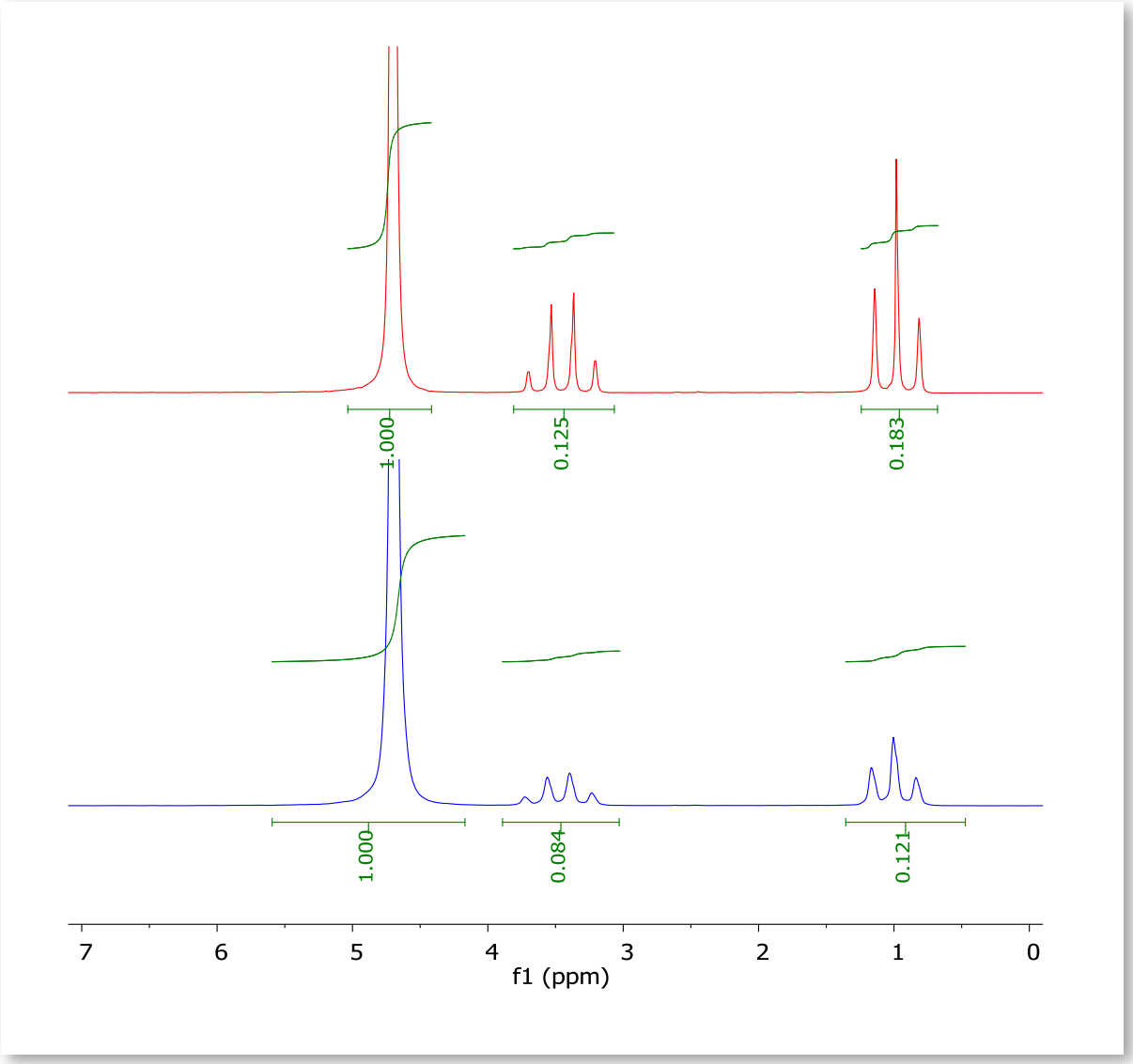


Figure 3. Spectrum of ethanol in water (top) and with a layer of 1-octanol (bottom).

$$P_{ow} = C_O/C_W = \frac{0.183 - 0.121}{0.121} = 0.512$$

log $P_{ow}$ = -0.29 (literature value = -0.30)[2]

**THF**

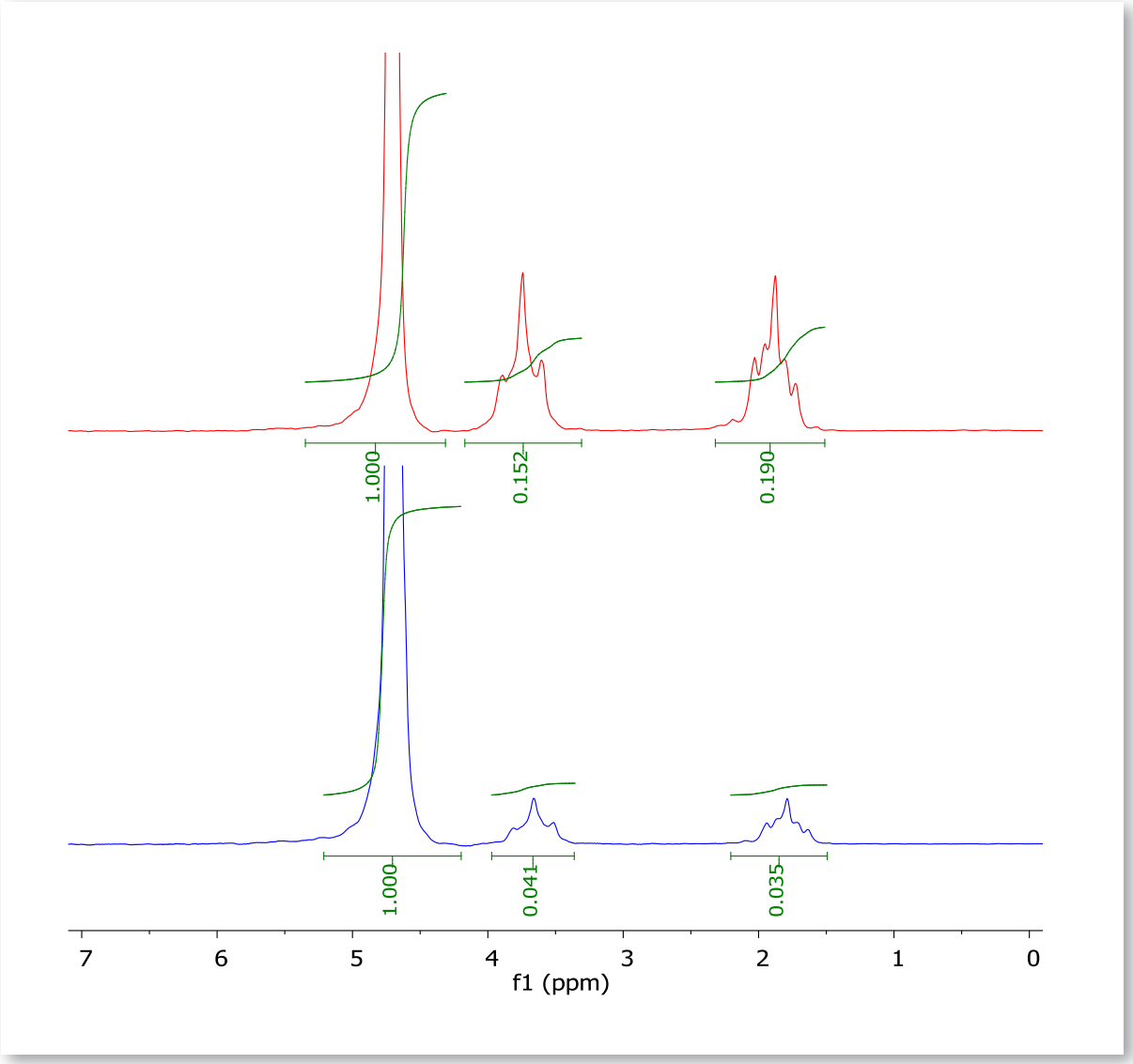


Figure 4. Spectrum of THF in water (top) and with a layer of 1-octanol (bottom).

$$P_{ow} = C_O/C_W = \frac{0.152 - 0.041}{0.041} = 2.707$$

log $P_{ow}$ = 0.43 (literature value = 0.46)[2]

**Tert-butanol**

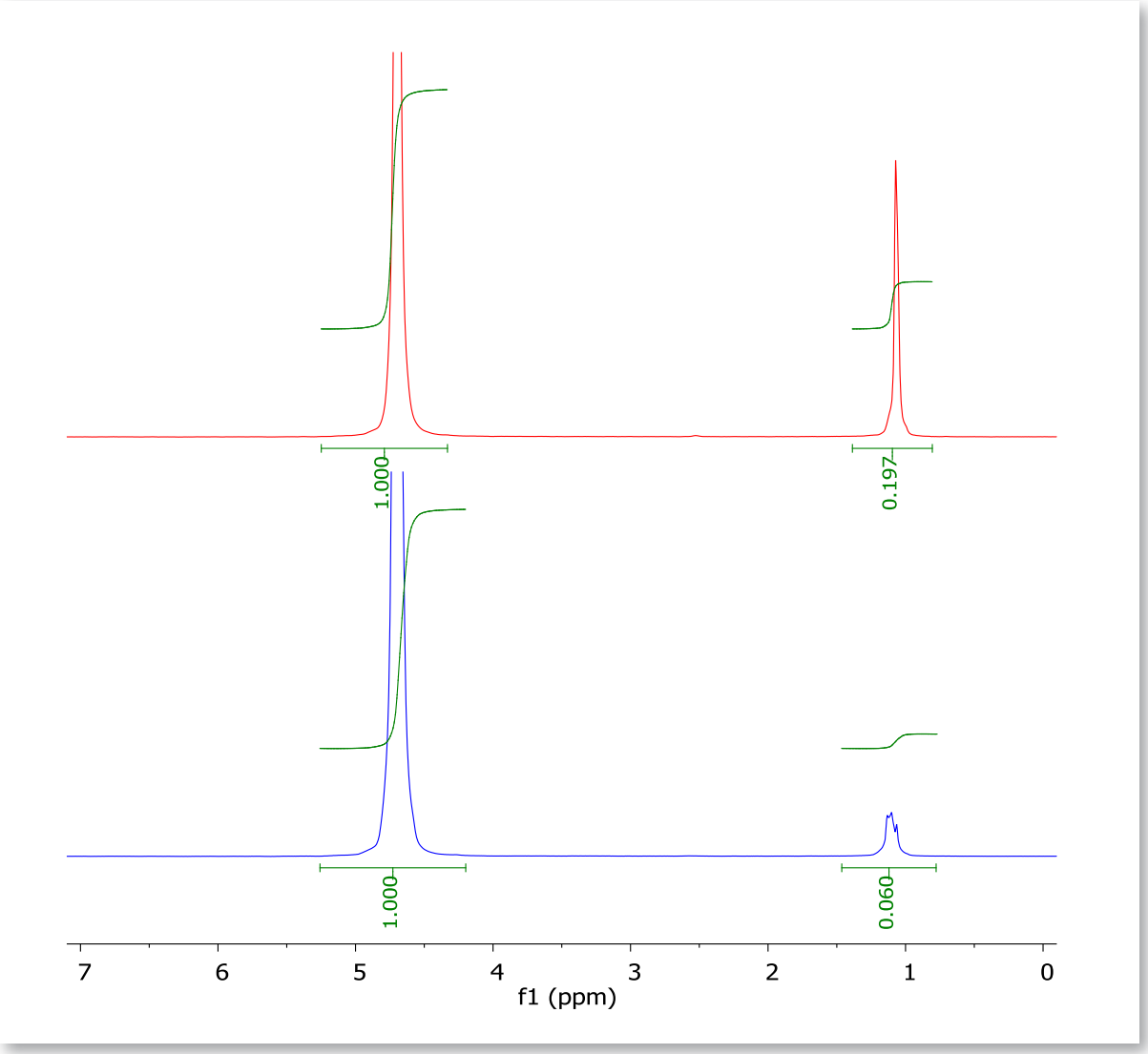


Figure 5. Spectrum of *tert*-butanol in water (top) and with a layer of 1-octanol (bottom).

$$P_{ow} = C_o/C_w = \frac{0.197 - 0.060}{0.060} = 2.28$$

log $P_{ow}$ = 0.36 (literature value = 0.35)[2]

## 2-Propanol

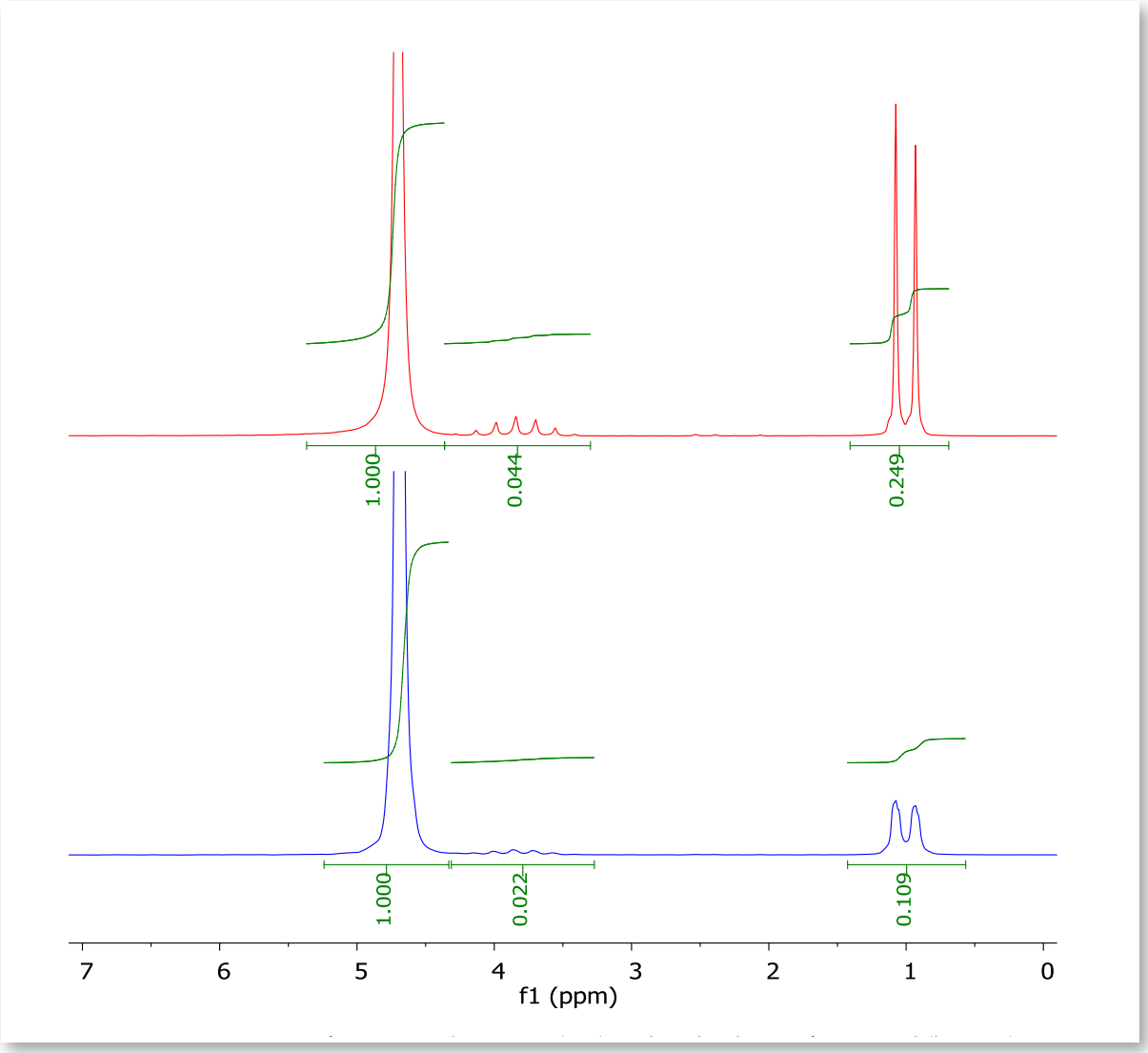


Figure 6. Spectrum of 2-propanol in water (top) and with a layer of 1-octanol (bottom).

$$P_{ow} = C_O/C_W = \frac{0.249 - 0.109}{0.109} = 1.28$$

log $P_{ow}$ = 0.11 (literature value = 0.05)[2]

**Acetonitrile**

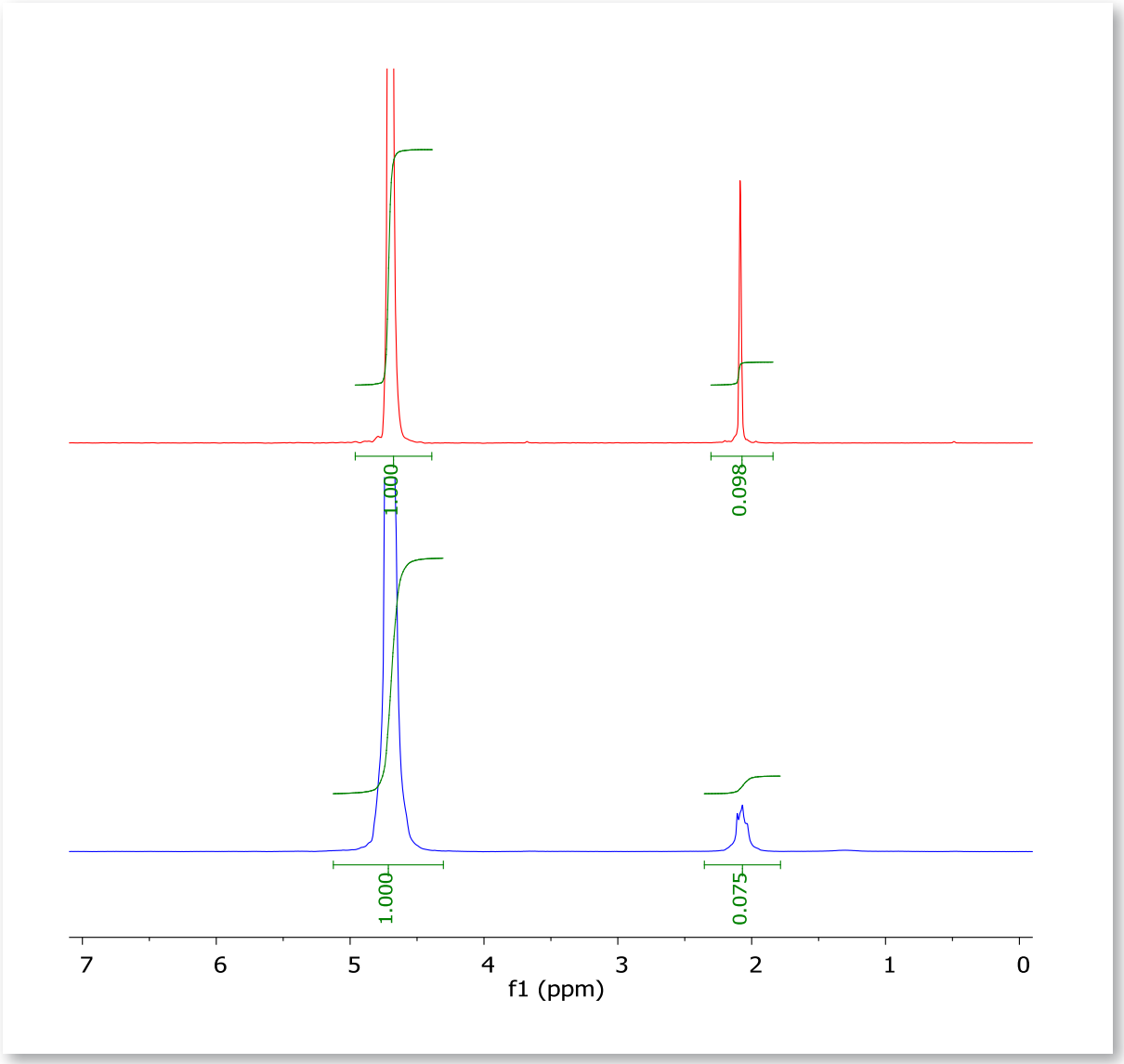


Figure 7. Spectrum of acetonitrile in water (top) and with a layer of 1-octanol (bottom).

$$P_{ow} = C_o/C_w = \frac{0.098 - 0.075}{0.075} = 0.307$$

L og $P_{ow}$ = -0.51 (literature value = -0.34)[2]

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## References


1) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, 23 (1-3), 3-25.

2) Sangster, J., Octanol-water coefficients of simple organic compounds. *J. Phys. Chem. Ref. Data.* **1989**, 18, 1111-1227.



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