# 4.3: Dry Lab

## Drylab Procedure:

This section contains FIDs measured using a 600 MHz Bruker Avance spectrometer. The FIDs can be downloaded and processed to quantify the concentration of malic acid in a $D_2O$ solution and in apple juice. Inversion-recovery spectra measured for KHP are provided so that you can calculate the $T_1$ relaxation time using the resonance intensity. The FIDs are provided in JCAMP format which can be processed using most modern vendor software programs. If you do not have access to an NMR spectrometer, a free NMR processing software package wxNUTS can be downloaded to use with Mac OSX and Windows:

http://www.acornnmr.com/nuts.htm

### A. Preparation of KHP solution and determination of the $T_1$ relaxation times of the KHP protons

A small amount of potassium hydrogen phthalate (KHP) was placed a beaker put into an oven at 110 ºC for 4 hrs. The beaker was then removed, covered in aluminum foil and placed in a dessicator to cool. The KHP was weighed using an analytical balance, transferred to a 5 mL volumetric flask and diluted with to the mark with $D_2O$ to prepare a stock solution.

Mass of weighing paper = 0.2219 g

Mass of weighing paper + KHP = 0.3533 g

To measure the $T_1$ relaxation times of the KHP protons, a 600 µL aliquot of this stock solution was transferred to an NMR tube. A series of inversion recovery spectra were acquired as a function of the variable delay between the $180^o$ and $90^o$ pulses. The spectrometer frequency was 599.923 MHz. This experiment used an acquisition time of 2 sec, and an additional relaxation delay of 35 sec. 8 FIDs were coadded for each of the following spectra. Download and analyze these spectra to determine the $T_1$ relaxation times of the KHP protons.

To download a file click the file name and once the text window opens go to File and Save As to save the file as a text file. To process the downloaded file using NUTS, open the wxNUTS program and under the File menu click Import and then select the file to process.

| Variable delay | JCAMP File |
|---|---|
| 0.005 (s) | T1-measurement-KHP-051708_0s.dx |
| 2 | T1-measurement-KHP-051708_2s.dx |
| 2.5 | T1-measurement-KHP-051708_2p5s.dx |
| 3 | T1-measurement-KHP-051708_3s.dx |
| 3.5 | T1-measurement-KHP-051708_3p5s.dx |
| 4 | T1-measurement-KHP-051708_4s.dx |
| 6 | T1-measurement-KHP-051708_6s.dx |
| 10 | T1-measurement-KHP-051708_10s.dx |
| 15 | T1-measurement-KHP-051708_15s.dx |
| 20 | T1-measurement-KHP-051708_20s.dx |

### B. Determination of the malic acid concentration in a $D_2O$ stock solution

To test our ability to quantitatively measure the malic acid concentration in an unknown apple juice sample using KHP as an internal standard, a solution containing a known malic acid concentration was prepared by transferring a known mass of malic acid to a 5 mL volumetric flask and diluting to the mark with $D_2O$.

Mass of paper = 0.1897 g

Mass of paper + Malic acid = 0.3324 g

The solution for Q-NMR was prepared by combining 1.00 mL of the KHP stock solution and 1.00 mL of the malic acid stock solution. The solution was mixed well and a 600 µL aliquot transferred to an NMR tube for analysis. The frequency of the spectrometer was 599.923 MHz. The spectrum was measured using a 2 s acquisition time and an additional 35 s relaxation delay. 64 FIDs were coadded.

The FID below was acquired for the quantitative analysis of the malic acid standard solution using KHP as an internal standard. Download and analyze this spectrum to determine the concentration of the malic acid in this stock solution.

Q-NMR-Malic-JHP-061108_Run2.dx

### C. Determination of the malic acid concentration in a fruit juice solution

The KHP stock solution was diluted by mixing 1.00 mL of the stock solution prepared in part A with 1.00 mL of $D_2O$. After mixing, a pipettor was used to add 100 µL of the diluted KHP solution to 900 µL of apple juice obtained from the grocery store (note that better accuracy and precision would have been achieved if a 1 mL glass pipette were used instead of a pipettor). The pH of the solution was adjusted to approximately 1.35 using HCl. A 600 µL aliquot of this solution was transferred to an NMR tube and the spectrum recorded at a frequency of 599.923 MHz. The spectrum was measured using a 2 s acquisition time and an additional 35 s relaxation delay. The solvent resonance was suppressed by saturation during the relaxation delay. 480 FIDs were coadded.

The FID below was acquired for the quantitative analysis of the malic acid in apple juice using KHP as an internal standard. Download and analyze this spectrum to determine the concentration of the malic acid in the apple juice sample.

Q-NMR-Apple-JHP-061108A_Run1.dx

## Dry Lab Report:

1. Using the mass and volume used to make the stock solution in part A, calculate the KHP concentration.
2. Using the inversion-recovery data, determine the $T_1$ relaxation times of the two KHP resonances.
3. Using the mass and volume used to make the stock solution in part B, calculate the malic acid concentration.
4. What is the concentration of malic acid determined from the Q-NMR experiment using KHP as an internal standard?
5. What is the concentration of the malic acid in the apple juice?

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