Worksheet for Fumarase Mediated Conversion of Disodium Fumarate to Malate
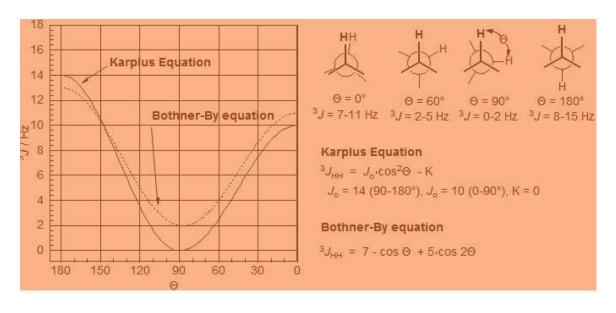
1.   Assign the NMR spectrum of the fumarate/malate/fumarase/D_2O buffer solution.

2. Considering the part of the NMR that is due to malate, what is the hydrogen--hydrogen coupling constant in Hertz.

3. Consider the addition of D_2O occurring in a syn fashion (the D and OD coming in on the same face of the double bond) and draw the most stable conformer for the addition product in a Newman projection. What factors are you considering in your evaluation of conformational stability? Are there forces or factors that are different than those you learned about in lecture?

4. Consider the addition of D_2O occurring in an anti fashion (D and OD coming in on opposite sides of the double bond) and draw the most stable conformer for the addition product in a Newman projection. What factors are you considering in your evaluation of conformational stability? Are there forces or factors that are different than those you learned about in lecture?

5. Considering the following equations developed by Karplus and Bothner‑‑‑By to describe the relationship between the torsional angle between two vicinal hydrogens and coupling constant. Calculate the coupling constants for syn addition modeled above and anti addition modeled above. Which model most closely matches your experimental coupling constant.

$\Theta = 0°$ $\Theta = 60°$ $\Theta = 90°$ $\Theta = 180°$

$^3J = 7\text{-}11\text{ Hz}$ $^3J = 2\text{-}5\text{ Hz}$ $^3J = 0\text{-}2\text{ Hz}$ $^3J = 8\text{-}15\text{ Hz}$

Karplus Equation

$$^3J_{HH} = J_o \cdot \cos^2\Theta - K$$

$J_o = 14\ (90\text{-}180°),\ J_o = 10\ (0\text{-}90°),\ K = 0$

Bothner‑By equation

$$^3J_{HH} = 7 - \cos\Theta + 5 \cdot \cos 2\Theta$$

6. To what to you attribute the differences between your experimental value and the predicted value using the Karplus equations.

7.   Using the Henderson---Hasselbalch equation explain the fact that fumarate and malate are considered to be in a ---2 charge state at physiological pH.

pH=pK$_a$+ log[base]/[acid]