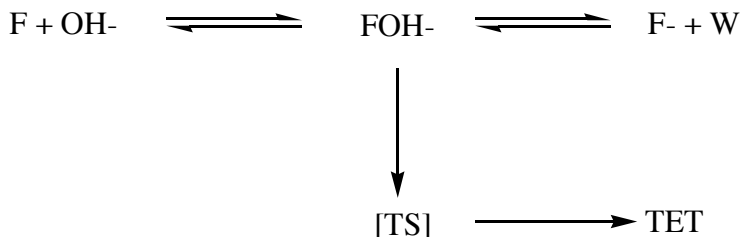


In the base reaction, we are dealing with an equilibrium between 3 “species” according to:



Where F=formamide, OH⁻=hydroxide, FOH⁻=formamide/OH⁻ adduct, F⁻=formamide anion, W=water, [TS]=formamide/OH⁻ transition state and TET=tetrahedral intermediate. From FOH⁻ the reaction evolves to the [TS] and then to the tetrahedral intermediate.

What is measured in the kinetic experiments is

$$-d[\text{FOH}^-]/dt = k [\text{FOH}^-]$$

You measure the concentration change of total formamide [F]_t, but the concentration change is determined by the disappearance of FOH⁻, the other species decrease their concentration following their respective equilibrium.

You can express [FOH⁻], at any time, as:

$$[\text{F}]_t = [\text{FOH}^-] + [\text{F}] + [\text{F}^-] = [\text{FOH}^-] + K_1[\text{FOH}^-]/[\text{OH}^-] + K_2[\text{FOH}^-]/[\text{W}]$$

From which:

$$[\text{FOH}^-] = \{[\text{F}]_t / (1 + K_1/[\text{OH}^-] + K_2/[\text{W}])\}$$

In the experiments you determine k from the slope of d[FOH⁻]/dt. You will determine a k_{obs} that will depend on K_i (where i=1,2) according to:

$$d[\text{FOH}^-]/dt = k [\text{F}]_t / (1 + K_1/[\text{OH}^-] + K_2/[\text{W}]) = k_{\text{obs}} [\text{F}]_t$$

k_{obs} depends on K₁ and K₂ according to the previous equation. However, if the species are in equilibrium, K₁ and K₂ do not change with the temperature. Moreover, if they do, you cannot get a linear Arrhenius plot as you observe in the experiments results reported in the literature.

Why do we chose [FOH⁻] in our analysis? Because in kinetic experiments you always determine the slow reaction, which is the one involving the transformation of this species

to the TS. In other words, the referee is right in saying that the species are in equilibrium, but not in saying that in our treatment of this equilibrium has not been taken into account in our data analysis. If we write the exponential kinetic equations including the previous equilibria, the K_i ($i=1,2$) dependent terms will appear in the preexponential factor, and will not influence the value of ΔG .

In the acid catalyzed reaction the same reasoning holds. Equations similar to those given above for the base catalyzed reactions can be written. Moreover, we believe that the referee is incorrect in saying that we are calculating the free energy difference between the protonated amine and the TS. While for the base catalyzed reaction, the reference state is an adduct.