# The Equilibrium Constant of the System Ethanol, Aldehyde, DPN$^+$, DPNH and H$^+$

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The equilibrium constant for the ethylalcohol + DPN$^+$ $\rightleftarrows$ acetaldehyde + DPNH + H$^+$ reaction has been determined at different pH's, temperatures and ionic strengths, using yeast ADH as catalyst. At 20°C and ionic strength 0.1, K is found to be 0.801 $\pm$ 0.014 $\times$ 10^{-11} M in the pH range 7—10. The free-energy change for the reduction of DPN has been calculated by combining the experimental values with reliable free-energy data for alcohol and acetaldehyde. At 25°C and zero ionic strength, $\Delta F° = 5.47$ kcal mole^{-1}. Calculation of the oxidation-reduction potential for the DPN—DPNH system gives $E'_o = -0.326$ V at pH 7, 25°C and zero ionic strength, which is in good agreement with recent data in the literature.

Some values have been reported previously for the equilibrium constant of the enzyme catalysed reaction

$$CH_3CH_2OH + DPN^+ \xrightarrow[ADH]{} CH_3CHO + DPNH + H^+$$
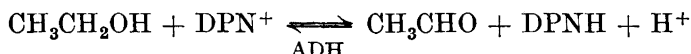
By using a crude enzyme preparation from Lebedew juice, von Euler et al.[1] found the inverse equilibrium constant,

$$k = \frac{[DPN]\ [CH_3CH_2OH]}{[DPNH]\ [CH_3CHO]}$$

to have the value 0.73 $\times$ 10^4 at pH 7.6. As could be expected from the reaction formula, this constant was found to be strongly dependent on pH. Negelein and Wulff[2] using a crystalline preparation of yeast ADH, found the same constant to have the value 1.35 $\times$ 10^3 at pH 7.9 and 20°C. For the overall equilibrium constant

$$K = [DPNH]\ [CH_3CHO]\ [H^+]/[DPN^+]\ [CH_3CH_2OH]$$

Racker[3] found $K = 1.15 \times 10^{-11}$ at 25°C, the value being independent of pH throughout the range investigated. Theorell and Bonnichsen[4] determined K at low concentrations of liver ADH and found, on an average, $K = 0.86 \times 10^{-11}$ at 20°C over the pH range 7 to 10.

Because of the discrepancies between the values, which could be due to the influence of temperature and ionic strength, the value of K for the yeast ADH catalysed reaction has been determined at 20°C for the pH range 7—10 with $\mu = 0.1$; at different ionic strengths at pH 7 and 20°C and at different temperatures in the pH range 7 to 9 with $\mu = 0.1$. The effect of sodium chloride on K has also been determined.

MATERIALS AND METHODS

Yeast ADH was prepared according to the method of Racker [3] and recrystallized several times. Before use the enzyme was dialysed against the buffer. At pH 10, some loss of activity could not be avoided.

A commercial sample of DPN from the Sigma Chemical Company which was found to be 84 % pure was used.

All buffers ($\mu = 0.1$) were stabilized with Versene (0.001 M), phosphate being used at pH 7 and 8 and glycine-NaOH at pH 9 and 10.

The experiments were carried out in 1 cm Beckman cuvettes (3 ml), the temperature being controlled by water circulated from a thermostat through coils on both sides of the cell compartment of a Beckman DU spectrophotometer.

To a cuvette containing 2.8 ml of buffer, 0.1 ml of a dilute alcohol solution and 0.1 ml of a DPN solution, 5—10 μl of the enzyme solution were added with rapid stirring. The concentration of DPNH at equilibrium was determined by measuring the light absorption at 340 mμ. The molecular extinction coefficient [5] for reduced DPN was taken as 6.22×10^6 cm²/mole.

The temperature dependence of K was determined by measuring the light absorption at 340 mμ and hence K first at 15°C, and then for each 5°C rise in temperature. It was thus possible to determine K in one and the same reaction mixture at all the temperatures. In order to prevent evaporation of the acetaldehyde, the cuvettes had to be covered with lids.

Corrections were made for the pH dependence on temperature of the different buffers [6]. These were checked in separate experiments.

The method unaccountably failed at pH 10, but gave good results from pH 7 to 9.

EXPERIMENTAL RESULTS

Varying pH. Temperature and ionic strength constant. Some of the experimental results for the pH range 7 to 10 (20°C, $\mu = 0.1$) are collected in Tables

Table 1. Equilibria at 20°C, pH 7.0 and $\mu = 0.1$.

[ADH] × 10⁴	[EtOH] × 10⁴	[DPN]$_{tot}$ × 10⁴	% Red	$K \times 10^{11}$
0.0043	5 470	0.29	72.8	0.846
0.0043	1 087	0.53	33.8	0.778
0.0045	547	0.49	24.3	0.623
0.0086	1 087	0.53	33.1	0.760
0.0215	1 087	0.53	33.1	0.747
0.0223	1 090	0.49	35.1	0.760
0.0356	5 470	0.50	65.9	0.940
0.0416	1 050	0.51	34.6	0.812
0.0430	5 470	0.33	71.8	0.912
0.0430	1 087	0.53	34.4	0.798
0.0864	1 090	0.33	42.3	0.793
0.1296	109	0.52	13.1	0.770
			Mean:	0.795

Table 2. Equilibria at 20°C, pH 8.0 and $\mu = 0.1$.

$[ADH] \times 10^4$	$[EtOH] \times 10^4$	$[DPN]_{tot} \times 10^4$	% Red	$K \times 10^{11}$
0.0043	1 087	0.53	69.7	0.790
0.0045	1 090	0.50	63.8	0.782
0.0086	543	0.53	57.5	0.768
0.0215	543	0.53	59.5	0.856
0.0223	109	0.50	29.2	0.840
0.0356	547	0.49	52.7	0.835
0.0534	109	0.49	29.4	0.878
0.0712	54.7	0.49	22.3	0.908
0.1120	547	0.49	55.8	0.848
0.1680	547	0.49	56.7	0.896
			Mean:	0.840

Table 3. Equilibria at 20°C, pH 9.0 and $\mu = 0.1$.

$[ADH] \times 10^4$	$[EtOH] \times 10^4$	$[DPN]_{tot} \times 10^4$	% Red	$K \times 10^{11}$
0.0020	51.8	0.49	60.0	0.822
0.0020	55.0	0.53	62.1	0.820
0.0045	109.0	0.53	82.0	0.871
0.0045	10.7	0.53	43.3	0.775
0.0080	109.0	0.53	73.0	0.806
0.0200	109.0	0.53	73.4	0.826
0.0200	10.7	0.53	38.2	0.777
0.0400	54.0	0.53	60.3	0.757
0.0600	54.0	0.53	61.6	0.808
			Mean:	0.807

Table 4. Equilibria at 20°C, pH 10.0 and $\mu = 0.1$

$[ADH] \times 10^4$	$[EtOH] \times 10^4$	$[DPN]_{tot} \times 10^4$	% Red	$K \times 10^{11}$
0.0037	2.74	0.51	39.1	0.710
0.0183	5.47	0.51	50.9	0.722
0.0356	10.90	0.50	61.7	0.829
0.0356	5.47	0.50	51.3	0.813
0.0367	10.90	0.53	66.3	0.881
0.0533	2.74	0.50	38.3	0.733
0.0712	5.47	0.50	49.7	0.830
0.0712	2.74	0.50	38.9	0.764
0.0712	2.74	0.50	38.3	0.830
			Mean:	0.795

1—4. As stated previously, K is seen to be independent of pH. An average value of $K_{20°, \mu=0.1} = 0.801 \pm 0.014 \times 10^{-11}$ M has been calculated from a larger number of experiments.

Varying ionic strength. pH and temperature constant. The results of these experiments, carried out in phosphate buffer (pH 7, 20°C) are summarized

Table 5. Equilibria at 20°C. Phosphate buffers of different ionic strengths at pH 7.0 and added sodium chloride.

[ADH] $\times 10^{-4}$	[EtOH] $\times 10^{-4}$	[DPN]$_{tot}$ $\times 10^{-4}$	% Red	[NaCl]	μ	$K \times 10^{11}$
0.0062	1 062	0.398	35.2	—	0.096	0.801
0.0062	5 393	0.398	63.8	—	0.096	0.813
0.0062	5 393	0.398	59.8	—	0.050	0.736
0.0062	5 393	0.398	69.8	—	0.189	0.927
0.0062	5 393	0.398	74.2	—	0.376	0.997
0.0062	5 393	0.398	57.8	0.167	0.259	0.925
0.0062	5 393	0.398	56.6	0.333	0.423	1.047
0.0062	5 393	0.398	52.3	0.667	0.749	1.184

in Table 5. Fig. 1, where for a temperature of 20°C log K has been plotted against $\sqrt{\mu}$, shows that an increase of ionic strength causes an increase of K. Addition of sodium chloride also increases K in the same manner. Perhaps the increase of K is due to the effect of ionic strength on the activity coefficients of the reactants. Similar results have been reported by Burton and Wilson [7] for the malic dehydrogenase system.

Statistical analysis gave a regression line with a slope of 0.315 and an intercept of log $K + 12 = 0.8085$ at $\sqrt{\mu} = 0$, which corresponds to a value of $K_{20°, \mu=0} = 0.646 \times 10^{-11}$ M.

Varying temperature and pH. Ionic strength constant. The experimental results for the temperature dependence of K at different pH's when $\mu = 0.1$ are shown in Fig. 2, where $K_{\mu=0.1}$ has been plotted against $1/T$. The equation for the regression line is log $K = (1554/T) - 5.80$.
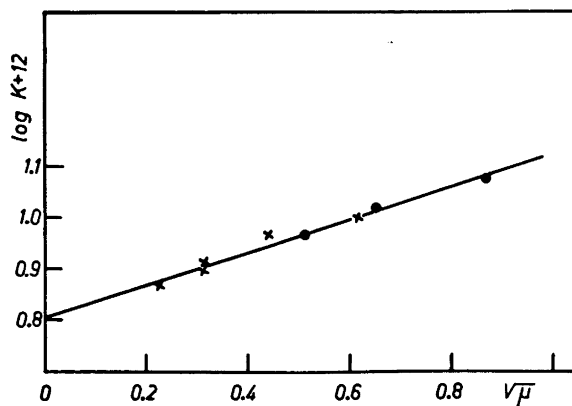

Fig. 1. Equilibrium constant, K, at varied ionic strength. Temp. 20°C; log $K + 12$ plotted *versus* $\sqrt{\mu}$; $\times$ phosphate buffers pH 7; ● added NaCl.

Table 6. $K \times 10^{11}$ at different temperatures, $(\mu = 0.1)$, and the related thermodynamic constants

pH	Slope of the line	15°C	20°C	25°C	30°C	35°C	$\Delta H°$ kcal/mole	$\Delta S°$ cal/mole · degr.
7.0	−1.55	0.646	0.800	0.977	1.202	1.445	7.09	−26.6
8.0	−1.52	0.642	0.795	0.966	1.175	1.413	6.96	−27.1
9.0	−1.60	0.635	0.794	0.972	1.200	1.454	7.32	−25.8
Mean:	−1.55	0.641 ±0.005	0.796 ±0.004	0.972 ±0.006	1.192 ±0.020	1.437 ±0.020	7.13 ±0.20	−26.5 ± 0.7
$\Delta F°$ kcal/mole		14.76	14.89	15.02	15.16	15.29		

Table 6 gives the mean value of $K_{\mu=0.1}$ for different temperatures and pH's and the corresponding thermodynamic constants, obtained by applying the general formulas

$$\frac{d(\log K)}{d(1/T)} = - \frac{\Delta H°}{R \ln 10}\,; \; \Delta F° + RT\ln K = 0;\; \Delta H° = \Delta F° + T\,\Delta S°$$

from which

$\Delta H° = 7.13 \pm 0.20$ kcal mole^{-1} at $\mu = 0.1$
$\Delta S° = -26.5$ cal mole^{-1} degree^{-1} at $\mu = 0.1$
$\Delta F° = 14.89$ kcal mole^{-1} at 20°C (15.02 kcal mole^{-1} at 25°C) and $\mu = 0.1$

Extrapolation to zero ionic strength gives $\Delta F° = 15.01$ kcal mole^{-1} at 20°C (15.15 kcal mole^{-1} at 25°C).

Burton [8] has calculated that for the reaction

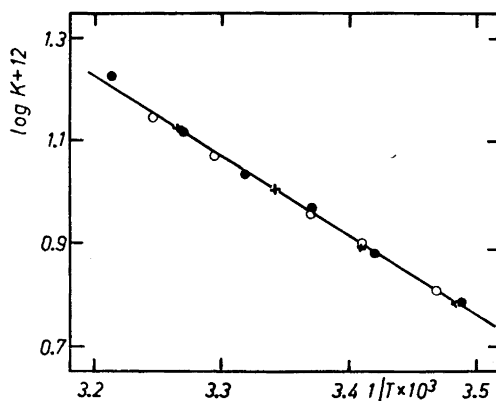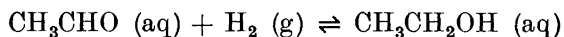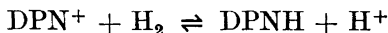$$CH_3CHO\ (aq) + H_2\ (g) \rightleftharpoons CH_3CH_2OH\ (aq)$$

Fig. 2. Equilibrium constant, K, at different temperatures and pH's; $\mu = 0.1$. log K + 12 plotted *versus* $1/T \times 10^3$; ● pH = 7; ○ pH = 8; × pH = 9.

$\Delta F^\circ = -9.68 \pm 0.4$ kcal mole^{-1} at 25°C. Combining this value of ΔF° with the above value of $\Delta F^\circ = 15.15$ kcal mole^{-1} gives for the reaction

$$DPN^+ + H_2 \rightleftharpoons DPNH + H^+$$

$\Delta F^\circ = 5.47$ kcal mole^{-1} (± 0.4 kcal mole^{-1} according to Burton [8]) at 25°C and $\mu = 0$. This in turn gives $E_0' = -0.326$ V (± 0.008 V) as the value of the oxidation-reduction potential for the DPN — DPNH system at pH 7, 25°C and $\mu = 0$, calculated by using the formula

$$E_0' = - \frac{\Delta F^\circ}{2F} - \frac{RT \ln 10 \text{ pH}}{2F}$$

DISCUSSION

Recalculating the value of the equilibrium constant reported by Negelein and Wulff[2] and correcting for the erronous molecular weight of DPN (N. and W. used 700 instead of 663) and molecular extinction coefficient of DPNH ($\varepsilon = 7.7 \times 10^6$ instead of 6.22×10^6 cm^2/mole), gives for a 50 % reduction of DPN, $K = 0.896 \times 10^{-11}$ M at pH 7.9 and 20°C. This value agrees with ours if they worked at an ionic strength of $\mu = 0.20$.

When recalculating the value reported by von Euler et al.[1], K is found to be $= 0.342 \times 10^{-11}$ M at pH 7.6. No temperature data are reported, but this value is certainly too low and would be still lower if recalculated [5] with $\varepsilon = 6.22 \times 10^6$ cm^2/mole instead of the value 4.05×10^6 cm^2/mole used by von Euler et al. The low value could be due to the crude enzyme preparation used.

Under the conditions of ionic strength (0.04) and temperature (25°C) used by Racker [3], K as calculated from our values should be $= 0.92 \times 10^{-11}$ M, the assumption being made that the influence of ionic strength on K is constant in the temperature interval 20—25°C. The value of $K = 1.15 \times 10^{-11}$ M reported by Racker [3] therefore seems to be too high, particularly as he used the higher value for the molecular extinction coefficient of reduced DPN ($\varepsilon = 6.28 \times 10^6$ cm^2/mole) reported by Ohlmeyer [9].

The value of $K = 0.86 \times 10^{-11}$ M at 20°C and $\mu = 0.1$ reported by Theorell and Bonnichsen [4] should be corrected for the protein concentration. If 50 % reduction of the 40 μM DPN used at a protein concentration of 0.004×10^{-4} M ($= 0.8$ μN) is assumed, $K = 0.83 \times 10^{-11}$ M which is close to the value found in this investigation.

The value reported here for the oxidation-reduction potential of the DPN—DPNH system is in good agreement with the value $E_0' = -0.320$ V at pH 7 and 25°C recently reported by Burton [8] from investigations of the isopropanol-DPN-acetone system. Rodkey [10] has found, by potentiometric titration, the value $E_0' = -0.318 \pm 0.001$ V at pH 7 and 30°C. The formerly accepted value for the DPN-DPNH potential (—0.282 V at pH 7 and 30°C) reported by Borsook [11] thus again seems to be too high.

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