was precipitated twice with 30 % (v/v) ethanol and then recrystallized 5 times with 7—10 % ethanol. The relative values of the activity/E_{280} ratio obtained for the 5 crystalline products were, successively, 0.80, 0.89, 0.97, 1.00, 0.98 and 0.99.

A solution of the final product, after thorough dialysis against 0.01 M phosphate, pH 7.3, was found by dry weight to contain 15.5 mg protein/ml, and was diluted 10 fold for activity and E_{280} measurements. From the latter a value of 0.450 was obtained for the extinction of 1 mg/ml in 1 cm optical depth, in good agreement with Bonnichsen's [2,3] value of 0.455. From the activity measurements (Fig. 1) the relationship between the volume in ml (v) of an enzyme solution used in the assay, the value of $t_{0.2}$ in seconds, and the concentration of the enzyme solution is mg/ml $= 1.05/(t_{0.2} \times v)$. If E_{280} is measured on the same solution in 1 cm optical depth, the % purity of the sample relative to the above preparation will be $0.47/(E_{280} \times t_{0.2} \times v)$. These data have been reproduced to within a few per cent with many other crystalline preparations.

In the activity test of Theorell and Bonnichsen [4], our enzyme preparations gave an increase of E_{340} of 0.058 in 3 min per μg enzyme per ml, which is significantly higher than values previously reported (0.036[4], 0.045[5]). The corresponding value of the maximum initial rate of the reaction of ethanol and DPN at pH 10.0 was $k_2 = 8.0$ sec^{-1}, compared with 5.5 sec^{-1} obtained by Theorell and Bonnichsen [4]. In spite of the high activity, evidence of inhomogeneity in the present preparations was obtained in prolonged electrophoresis, indicating the presence of about 10 % of a second component. Further work on the preparation and properties of the enzyme is in progress.

The author is grateful to Professor Hugo Theorell and Dr. Roger Bonnichsen for guidance and helpful criticism.

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Received January 22, 1957.

The Sedimentation Constant of Crystalline Horse Liver Alcohol Dehydrogenase

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The sedimentation constant of crystalline alcohol dehydrogenase (ADH) prepared from horse liver [1,2] was found by Pedersen to be $S^0_{20} = 4.9$ S as the mean of two runs in the oil driven ultracentrifuge at Uppsala, with *ca.* 10 mg protein/ml at pH 7.8 [3]. This gives 4.5 S after correction as suggested by Pedersen [4]. In connection with the interpretation of marked effects of pH and chloride ion concentration on the kinetics of the enzyme reaction, DPNH + acetaldehyde + H$^+$ $\rightleftharpoons$ DPN + ethanol, reported by Theorell and his collaborators [3,5], it is of interest to test the possibility that the enzyme itself undergoes some change, such as dissociation or polymerisation, with change of ionic milieu. Such changes might be revealed by variations of the sedimentation constant.

Table 1. Sedimentation constants of liver alcohol dehydrogenase.

ADH mg/ml	NaCl M	Buffer (ionic strength = 0.1)	pH	S^0_{20} Svedberg units
7.8	0.075	phosphate	7.15	4.91
7.8	0.15	phosphate	7.15	4.90
7.8	0.30	phosphate	7.15	4.85
13.3	0.075	phosphate	7.15	4.69
13.3	0.30	phosphate	7.15	4.71
7.8	0.15	phosphate	5.85	4.93
7.8	0.15	glycine	9.9	4.82

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Alcohol dehydrogenase was prepared by the method of Bonnichsen and Brink [6] and recrystallized 5 times to constant activity/protein ratio, as described earlier [7]. The sedimentation constant was measured in the Spinco ultracentrifuge at 59 780 r.p.m. and 20°C with various protein, chloride and hydrogen ion concentrations. The results are shown in Table. 1. There is no evidence of any significant effect of chloride or hydrogen ion concentration on the sedimentation constant. Extrapolation to infinite dilution of the protein gives $S^0_{20} = 5.1\ S$.

These data give a value of 4.8 S at a protein concentration of 10 mg/ml, slightly greater than Pedersen's value. The difference is probably to be ascribed to the greater purity of our material indicated by higher specific activity [7]. More complete data on the molecular weight will be reported shortly.

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Received January 22, 1957.

The Equilibrium Between Yellow and Blue Nickel(II) Triethylenetetramine Ions in Strong Salt Solutions

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Lifschitz *et al.*[1,2] prepared solid nickel(II)bis(stilbenediamine) complexes in two isomers, one yellow, diamagnetic, and one blue, paramagnetic. The equilibrium between two such forms in solution has not yet been studied by means of the absorption spectra. Jonassen and Douglas [3] investigated nickel(II) complexes of triethylenetetramine (= trien) and prepared solid salts of $Ni_2trien_3^{+3}$, but not of Ni trien++. The latter violet-blue ion turns brownish yellow in strong salt solutions. Fig. 1 gives the absorption spectra at 19°C in different concentrations of sodium perchlorate. The absorption band at 443 mμ, which increases in the yellow solutions, corresponds to the planar, diamagnetic complex. Basolo *et al.*[4] prepared the yellow Nitemeen$_2$++ (temeen = C, C, C′, C′ tetramethyl-ethylenediamine) which has a Gaussian shaped band at 433 mμ with $\varepsilon = 67$. It is interesting that Ni temeen$_2$++ does not exhibit other spin-allowed, Laporte-forbidden ligand field bands below the ultra-violet absorption limit ($\varepsilon = 8$ at 260 mμ), since the ligand field theory [5] would predict three transitions at not very different wavenumbers (the analogous difficulty * occurs in Cu en$_2$++). The two

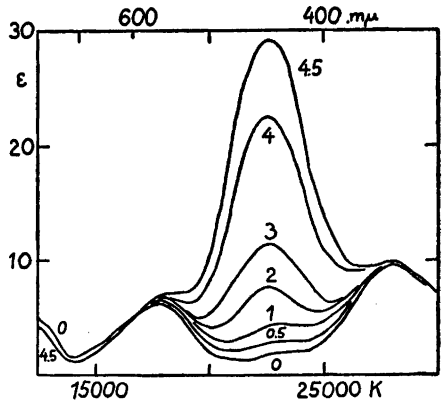

Fig. 1. The absorption spectra of 0.1 M Ni trien$(NO_3)_2$ in water and aqueous solutions of sodium perchlorate. The numbers on the curves indicate the molarity of $NaClO_4$. The spectra were measured at 19°C on a Cary spectrophotometer.

* However, the band at 422 mμ of Ni(II) in ethanolic sodio-succinimide has a shoulder at 470 mμ, and the band at 440 mμ of Ni(II) in 1 M NaOH with caseine has a shoulder at 510 mμ. These orange solutions do not seem to contain paramagnetic nickel, according to the spectra. Sodium caseinate is very effective for formation of tetragonal complexes; its biuret reaction with Cu(II) has the band maximum at 547 mμ.