Temperature and Composition Coefficients of the Density, Refractive Index, and Viscosity of the Methyl Alcohol–Dioxane System

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Since the dielectric constants of methyl alcohol–dioxane mixtures have been determined over some range of temperature and composition,[1] the authors determined to measure the density, viscosity and refractive index at several temperatures of methyl alcohol, dioxane and their mixtures, and to derive empirical equations representing these properties as functions of composition and temperature.

Materials.—The 1,4-dioxane used in this work was manufactured by the Eastman Kodak Company. Three grades were used, P 2144-practical, 2144X-histological, and 2144-m. p. 10.5–11°. All were carefully purified by the methods described by Hess and Frahm.[2] The practical and histological grades were refluxed for a day with 100 ml. of normal hydrochloric acid per liter of dioxane. At the same time a slow current of air was drawn through the condenser to sweep out any acetaldehyde originally present as an impurity and any formed by hydrolysis of ethylene acetal.[3] The dioxane was now treated with solid potassium hydroxide and the aqueous layer which settled out was separated in a separatory funnel. After two or three treatments in this manner, the dioxane was refluxed over metallic sodium until the bright luster of the sodium indicated that there was no more chemical reaction. It was then carefully distilled through a fractionating column 115 cm. long and fitted with an efficient still head of the total condensation partial take off type. The initial boiling points were always low, usually below 90°, whereas the boiling point of dioxane is 101.31°.[3] Usually 15 to 20% of the dioxane would distill over before the boiling point reached 101.3°. The next portion, about 60% boiling within a range of 0.02°, was collected. This fraction was further purified by fractional recrystallization in an ice-bath. In order to protect the dioxane from moisture in the air, glass stoppered flasks equipped with calcium chloride drying tubes were used. Recrystallization was repeated until no further increase in the density was observed. This would often take as many as six or eight recrystallizations. In the case of the best grade of dioxane, the hydrochloric acid treatment was omitted.[2] The same distillation procedure was followed. The initial boiling point was considerably higher than in the case of the other two and as much as 80% would distill over within 0.02° boiling point range. Usually four or five recrystallizations of this product were sufficient to bring it to the correct density. After this final treatment, the dioxane was stored over metallic sodium, from which it was distilled as needed. It may be pointed out that the densities of the three grades of dioxane, after distillation but before recrystallization, were not the same nor could they be made to agree by repeated rectification. All three grades, however, gave the same density after recrystallization.

The methyl alcohol used in this investigation contained not more than 0.003% aldehydes and acetone. It was further purified by the method of Lund and Bjerrum.[4] One hundred grams of magnesium turnings and 1 g. of iodine per liter of c. p. methyl alcohol was refluxed until the iodine disappeared. Four liters of c. p. methyl alcohol was then added and refluxed for about half an hour. The alcohol was then distilled through the fractionation column previously described, and the middle 80% fraction, which distilled over within 0.02°, was collected.

This procedure was repeated and the final product stored over magnesium methylate, prepared as above and distilled off immediately before use. No difficulty was ever experienced in obtaining the same density with different batches.

The solutions were made up by weight just before use and kept in 250-cc. Pyrex bottles. The solvents and solutions were never poured from bottle to bottle but always pumped by means of pressure from dry air. Every precaution was taken in all the experimental stages to prevent absorption of moisture from the air.

Procedure.—The temperature was held to $\pm0.005°$. The thermometers and weights were checked against standards calibrated by the Bureau of Standards. The calibration of the weights included the correction for the buoyancy of air, using for the density the average value of 0.0012 g. per ml.

Following the technique of Hartley and Barrett,[5] three pycnometers were prepared and adjusted so that the external volume of all three agreed within 0.5 ml. One of these was used as a counterpoise. At least two determinations were made using different pycnometers. If the average deviation of the mean was greater than 0.00001, more determinations were made in order to keep it within this figure. On the pure liquids many more measurements were made than were required to give this precision.

Temperature control for the measurement of refractive index was accomplished by pumping water from the thermostats through the prisms of the Abbe refractometer. In each determination several independent readings on different samples of liquid were observed and the average taken as the correct value.

The dimensions of the Ostwald viscometer were made to conform with the recommendations of Bingham,[6] Jones and Fornwalt,[7] and Cannon

* Now employed by The Ethyl Gasoline Corporation, Baton Rouge, La.

(1) Benoit and Ney, Compt. rend., 208, 1888–90 (1939).
(2) Hess and Frahm, Ber., 71B, 2627–36 (1938).
(3) Oxford Biochem. J., 28, 1325–29 (1934).

(4) Lund and Bjerrum, Ber., 64B, 210–213 (1931).
(5) Hartley and Barrett, J. Chem. Soc., 1072, 99 (1911).
(6) Bingham, "Fluidity and Plasticity," McGraw-Hill Book Co., Inc., New York, N. Y., 1922.
(7) Jones and Fornwalt, THIS JOURNAL, 60, 1684–92 (1938).

and Fenske,[8] and were as follows:

Volume of efflux	$V = 8.09$ ml.
Loading volume	$v = 11.34$ ml.
Fluid head	$H = 24.69$ cm.
Length of capillary	$l = 29.7$ cm.
Radius of capillary	$R = 0.02805 \pm 0.00003$ cm
Radius of upper and lower bulb	$r = 0.7$ cm.

That flow in the viscometer was not turbulent may be shown by the criteria of Reynolds.[9] That is

$$V/\pi R^2 t \text{ must be less than } 1000\eta/Rd \quad (1)$$

where t is in seconds and the other symbols are given above. For the extreme case of methyl alcohol at 50°, we have from Eq. 1

$8.09/3.142 \times 0.02805^2 \times 181.4$ is less than $1000 \times$
$0.003913/0.02805 \times 0.76266$ or 18.1 is less than 183

The condition of Bingham[6] that the kinetic energy term be small in comparison with the main term of viscosity in the equation

$$\eta = \pi g H d R^4 t/8 V l - m d V/8\pi l t \quad (2)$$

where g is the gravitational constant, d the density, and m the kinetic energy coefficient is also satisfied, as shown by dividing the kinetic energy term by the main term. For the extreme case of methyl alcohol at 50°, the kinetic energy correction is about 1.6%. A small error in the kinetic energy coefficient will, therefore, have a negligible influence on the main term.

Surface tension effects, as suggested by Jones and Fornwalt,[7] and Cannon and Fenske,[8] have been eliminated as far as possible by making the diameter of the upper and lower bulbs the same.

Viscosities are often calculated by the formula

$$\eta = \eta' d H t/d' H' t' + C_1 d/t [(H t^2/H' t'^2) - 1] \quad (3)$$

where η', d', H' and t' are the values for the reference liquid and C_1 is the constant in the kinetic energy term and equal to $mV/8\pi l$. In our experimental procedure H and H' are not constant since the viscometer was always filled at 30° and the fluid head is different at other temperatures. Equation (3) can be modified in the following manner to take care of the change in fluid head at temperatures other than 30°. Since the last term is the kinetic energy correction term and since it is small and H and H' do not differ greatly, their ratio is taken as unity in the term. Also let H_{30}, d_{30} and v_{30} be the fluid head, density, and load-

(8) Cannon and Fenske, Ind. Eng. Chem., Anal. Ed., 10, 297-301 (1938).
(9) Reynolds, Phil. Trans., 174, 935-83 (1883).

ing volume, respectively, at 30° and H, d, v the same quantities at temperature t, then

and

$$v = v_{30} d_{30}/d \quad (4)$$

$$\Delta v = v - v_{30} = v_{30} d_{30}/d - v_{30} \quad (5)$$
$$\Delta H = \Delta v/\pi r^2 = v_{30}/\pi r^2 [d_{30}/d - 1] \quad (6)$$
$$= k(d_{30}/d - 1) \quad (7)$$

Remembering that in an Ostwald viscometer the fluid head decreases as the loading volume increases, we have

$$H = H_{30} - \Delta H \quad (8)$$

Therefore from Eqs. 3, 7 and 8 we obtain

$$\eta = \eta' \frac{dt}{d't'} \left[\frac{H_{30} - k(d_{30}/d - 1)}{H'_{30} - k(d'_{30}/d' - 1)} \right] + \frac{C_1 d}{t} \left[\frac{t^2}{t'^2} - 1 \right] \quad (9)$$

Each recorded value of viscosity represents the mean of at least two measurements made upon separate samples. If the difference in the observed efflux time was more than 0.2 second other observations were made until the average deviation was of this order of precision. Two-tenths of a second represents a maximum of two parts in eighteen hundred. This minimum precision occurs for methyl alcohol at 50° since this is the lowest viscosity measured.

Discussion of Data.—In Table I are given the measured values of the viscosities of methyl alcohol–dioxane mixtures at various temperatures and compositions together with the viscosities of the pure substances at various temperatures. The small number in parentheses to the right of each value is the quantity to be added algebraically to the value calculated from the composition coefficient equation in order to make this calculated value agree with the one observed. These composition coefficient equations were derived by the method of least squares, and will be given later.

Densities and refractive indexes as a function of composition are represented by the general parabolic equation

$$f(m) = a + bm + cm^2 + dm^3 + em^4 \quad (10)$$

where m is the weight per cent. of methyl alcohol. Tables II and III contain the constants to be inserted in the above equation when representing densities and refractive indexes, respectively. These constants are to be incorporated in the equation along with the sign as indicated in the table. The standard error of estimate between the calculated and observed quantities, although small, as shown in the last column in Table II is still greater than the measured precision. The

TABLE I

MEASURED VISCOSITIES OF METHYL ALCOHOL–DIOXANE MIXTURES AT VARIOUS TEMPERATURES AND COMPOSITIONS: MEASURED VISCOSITIES OF THE PURE SUBSTANCES AT VARIOUS TEMPERATURES (*CALCULATED)

Per cent. by wt. of MeOH	10.04°	20.00°	30.00°	40.00°	50.00°
0.000	0.015941 *(0)	0.013133 (0)	0.011040 (0)	0.009421 (0)	0.008190 (0)
9.392	.011809 (−253)		.008759 (−116)	.007572 (−84)	
10.635		.009992 (−140)		.007401 (−79)	.006508 (−80)
19.231	.010169 (−125)	.008686 (−49)			.005742 (−56)
20.010			.007425 (−24)	.006436 (−43)	
30.410	.008882 (−6)	.007639 (+36)		.005763 (−4)	.005093 (−28)
30.586			.006573 (−8)		
40.782			.006056 (+26)	.005294 (+14)	
40.925	.008111 (+46)	.006972 (+47)			.004701 (+3)
49.975	.007612 (+42)	.006579 (+53)			.004449 (+10)
50.695			.005694 (+33)	.004990 (+26)	
60.206	.007242 (+47)	.006268 (+53)			.004249 (+21)
60.624			.005455 (+48)	.004782 (+32)	
61.785			.005434 (+50)		
69.931	.007005 (+42)	.006069 (+48)			.004107 (+18)
70.353			.005288 (+46)	.004627 (+28)	
80.058			.005172 (+36)		
80.666	.006847 (+30)	.005935 (+35)		.004520 (+25)	.004003 (+15)
90.119			.005105 (+21)		
90.569	.006787 (+21)	.005877 (+29)		.004460 (+13)	.003949 (+15)
100.000	.006783 (0)	.005858 (0)	.005080 (0)	.004438 (0)	.003913 (0)

TABLE II

CONSTANTS FOR EQUATIONS DERIVED BY THE METHOD OF LEAST SQUARES FOR THE COMPOSITION COEFFICIENTS OF THE DENSITIES OF METHYL ALCOHOL–DIOXANE MIXTURES

$$d_4^t = a + bm + cm^2 + dm^3 + em^4$$

Temp., °C.	a	$b \times 10^3$	$c \times 10^6$	$d \times 10^9$	$e \times 10^{12}$	Standard error of estimate
10.04	+1.04451	−2.9460	+5.814	−7.07	−4.6	0.00005
20.00	+1.03362	−2.9524	+7.122	−28.48	+102.1	.00007
30.00	+1.02227	−2.9262	+6.629	−18.52	+44.4	.00003
40.00	+1.01071	−2.9147	+7.631	−39.84	+166.7	.00006
50.00	+0.99949	−2.9016	+7.643	−37.45	+144.2	.00008

TABLE III

CONSTANTS FOR THE EQUATIONS DERIVED BY THE METHOD OF LEAST SQUARES FOR THE COMPOSITION COEFFICIENTS OF THE REFRACTIVE INDEXES OF METHYL ALCOHOL–DIOXANE MIXTURES

$$n_D^t = a + bm + cm^2 + dm^3 + em^4$$

Temp., °C.	a	$b \times 10^3$	$c \times 10^6$	$d \times 10^9$	$e \times 10^{11}$	Standard error of estimate
20.00	+1.4223	−1.188	+4.40	−34.1	+15.5	0.0001
30.00	+1.4176	−1.129	+2.75	−13.3	+5.8	.0002
40.00	+1.4134	−1.167	+3.68	−19.7	+6.6	.0002
50.00	+1.4088	−1.140	+2.17	+8.3	−8.1	.0002

standard error of estimate was calculated by the formula $\sqrt{\Sigma D^2/(n-k)}$ where n is the number of observations and k is the number of constants in the equation. The deviation is always in the fifth place of measured density and values calculated from the equation are good for most purposes. However, if more accurate densities are desired the calculated values can be corrected by using the deviation graphs, Fig. 1.

The standard error of estimate given in the last column of Table III for the difference between calculated and observed values of refractive index is about the same as the experimental accuracy. The deviations are represented by the curves of Fig. 2.

In the case of either density or refractive index, to make calculated and experimental results agree, the calculated value must have added to or subtracted from it the ordinate of the point on the deviation curve corresponding to that function and concentration. The ordinate of the point is added to the calculated value if the point falls above the zero line and subtracted from the calculated result if the point falls below the zero line.

It is extremely difficult to represent viscosity as a function of concentration. For our purposes Bingham's formula[6] was adopted as being the most satisfactory. This equation can be written

$$1/\eta = V_1\%/\eta_1 + V_2\%/\eta_2 + K(V_1\% - W_1\%) \quad (11)$$

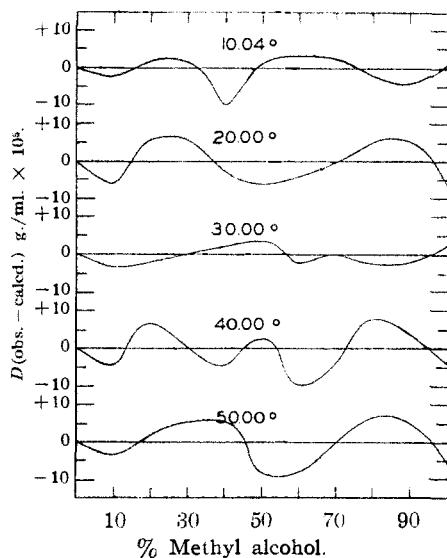where η_1 and η_2 are the viscosities, and $V_1\%$ and

Fig. 1.—Deviation curves of the composition coefficient equations for density.

Fig. 2.—Deviation curves of the composition coefficient equations for refractive index.

$V_2\%$ the volume per cent. of the pure components, $W_1\%$ the weight per cent. of methyl alcohol, and K is a constant at a given temperature. Table IV contains K at various temperatures to be used in the above equation and the standard error of estimate between calculated and observed values. When it is remembered that percentage deviations of one to three per cent. are considered good by many investigators (see Bingham,[6] page 169) it is evident that our calculated and observed values are in surprisingly good agreement. Furthermore, viscosities calculated from this equation are superior in accuracy to those obtained by graphical means. Calculated values, sensibly in agreement with observed values, can be obtained from Eq. 11 and deviation plots prepared from the numbers given in parentheses in Table I.

ties as a function of temperature. These equations are of the form

$$f(t) = a + bt + ct^2 + \cdots \qquad (12)$$

The constants for these equations are tabulated in Tables V, VI and VII, and are to be incorporated along with the indicated signs in the above general equation.

In the last column of Table V is given the standard error of estimate between the densities calculated by the temperature coefficient equations and those obtained from the composition coefficient equations and deviation graphs. If greater accuracy is required, the values of density must be calculated at even percentage composition using Table II and the curves in Fig. 1. Using the temperature coefficient equations in Table V values for density for the given temperatures must be calculated at even percentage compositions. These values given by the temperature coefficient equations compared with the values obtained using

TABLE IV

CONSTANTS FOR THE EQUATIONS FOR THE COMPOSITION COEFFICIENTS OF THE VISCOSITIES OF METHYL ALCOHOL–DIOXANE MIXTURES

$$\frac{1}{\eta} = \frac{V_1\%}{\eta_1} + \frac{V_2\%}{\eta_2} + K(V_1\% - W_1\%)$$

Temp., °C.....	10.04°	20.00°	30.00°	40.00°	50.00°
K..............	327.1	354.8	377.5	403.9	409.3
Standard error of estimate..	0.000094	0.000059	0.000047	0.000041	0.000034

Using these methods, we have calculated the densities, refractive indexes and viscosities at even percentage compositions, and from these calculated values have derived by the method of least squares empirical equations for these proper-
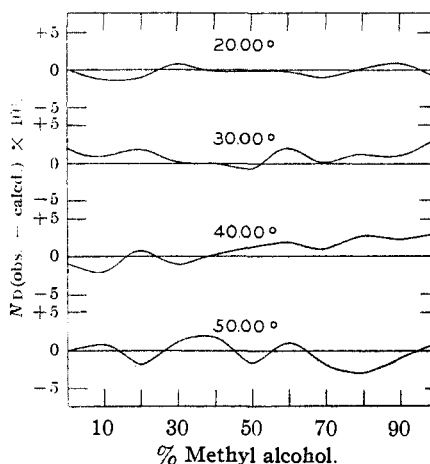
TABLE V

CONSTANTS FOR THE EQUATIONS DERIVED BY THE METHOD OF LEAST SQUARES FOR THE TEMPERATURE COEFFICIENTS OF THE DENSITIES OF METHYL ALCOHOL–DIOXANE MIXTURES AND FOR THE PURE SUBSTANCES

$$d_4^t = a + bt + ct^2 + dt^3$$

Per cent. by wt. of MeOH	a	$b \times 10^4$	$c \times 10^6$	$d \times 10^8$	Standard error of estimate
0.000	+1.05459	−9.477	−6.244	+6.314	0.00005
10.000	+1.02592	−9.917	−4.148	+4.144	.00009
20.000	+0.99813	−9.906	−3.349	+3.232	.00005
30.000	+ .97111	−9.515	−4.012	+3.991	.00006
40.000	+ .94491	−9.040	−4.746	+4.670	.00003
50.000	+ .92054	−9.445	−2.422	+1.917	.00004
60.000	+ .89649	−9.004	−3.746	+3.597	.00005
70.000	+ .87346	−8.819	−3.900	+3.854	.00013
80.000	+ .85127	−8.723	−3.512	+3.358	.00008
90.000	+ .82993	−8.656	−3.109	+2.861	.00008
100.000	+ .80974	−9.130	−5.586	.....	.00001

TABLE VI

CONSTANTS FOR THE EQUATIONS DERIVED BY THE METHOD OF LEAST SQUARES FOR THE TEMPERATURE COEFFICIENTS OF THE REFRACTIVE INDEXES OF METHYL ALCOHOL–DIOXANE MIXTURES AND FOR THE PURE SUBSTANCES

$$n_D^t = a + bt + ct^2$$

Per cent. by wt. of MeOH	a	$b \times 10^4$	$c \times 10^7$	Standard error of estimate
0.000	+1.4313	−4.50	....	0.0001
10.000	+1.4190	−4.03	− 5.0	.0004
20.000	+1.4057	−2.28	−30.0	.0001
30.000	+1.3980	−3.86	− 7.5	.0003
40.000	+1.3879	−3.77	− 7.5	.0002
50.000	+1.3769	−2.76	−22.5	.0001
60.000	+1.3675	−2.58	−23.0	.0004
70.000	+1.3596	−3.22	−12.5	.0002
80.000	+1.3514	−3.32	−10.0	.0002
90.000	+1.3444	−4.00	....	.0000
100.000	+1.3368	−4.00	....	.0001

TABLE VII

CONSTANTS FOR THE EQUATIONS DERIVED BY THE METHOD OF LEAST SQUARES FOR THE TEMPERATURE COEFFICIENTS OF THE VISCOSITIES OF METHYL ALCOHOL–DIOXANE MIXTURES AND FOR THE PURE SUBSTANCES

$$1/\eta = a + bt + ct^2$$

Per cent. by wt. of MeOH	a	b	c	Standard error of estimate
0.000	+ 49.18	+1.2727	+0.003727	0.000014
10.000	+ 69.59	+1.3686	+ .005643	.000023
20.000	+ 84.14	+1.4794	+ .007192	.000022
30.000	+ 94.82	+1.6582	+ .007202	.000024
40.000	+103.58	+1.8232	+ .006729	.000017
50.000	+111.86	+1.8671	+ .007918	.000015
60.000	+117.88	+1.9134	+ .008722	.000014
70.000	+122.31	+1.9378	+ .009841	.000013
80.000	+125.11	+1.9596	+ .010646	.000011
90.000	+125.68	+2.0318	+ .010448	.000016
100.000	+125.88	+2.0252	+ .011457	.000007

Table II and Fig. 1 give the deviations to be plotted for correcting the density calculated by the equations from Table V at any given temperature.

The standard error of estimate of the values calculated by means of the temperature coefficient equations of refractive index are given in Table VI.

As indicated by the standard error of estimate in the last column of Table VII, we have succeeded in formulating an equation for the temperature coefficients of viscosities which, although not as good as could be desired, is superior to the composition coefficient equation in the accuracy with which it will reproduce our data. More accurate viscosities can be obtained in a manner similar to that discussed in the case of density and using the values of viscosity and the deviation numbers given in Table I.

In the temperature range from 10 to 25°, our values of the densities of methyl alcohol are somewhat lower than those given in the "International Critical Tables," but in excellent agreement between 30 and 50°. At 25°, where the greatest number of comparisons can be made, our value is almost identical with that of Lund and Bjerrum,[4] and Jones and Fornwalt,[7] who obtained 0.78651 and 0.786525, respectively. The "International Critical Tables" value of 0.78660 at this temperature is the highest of any of the recorded values.

The densities of dioxane for any temperature show a wider divergence than those of methyl alcohol. The probable explanation for the variation of the observed densities is due to the difficulty of obtaining pure dioxane. Even among authors who use the same method of purification there is a relatively wide range of observed density. For example, Meisenheimer and Dorner,[10] Hovorka, Schaefer and Dreisbach,[11] Hess and Frahm,[2] and the authors used similar methods of purification including recrystallization yet obtained 1.0296, 1.03318, 1.03375, and 1.03339, respectively, for the density of dioxane at 20°. The other values recorded at 20° are consistently lower than those of Hess and Frahm and of the authors. At other temperatures, similar variations are observed in the data of various investigators.

The refractive indexes for both methyl alcohol and dioxane obtained in this investigation agree well with the observations of other investigators.

The viscosities of both methyl alcohol and dioxane vary widely, even at the same temperatures. At 25° the value 0.005445 for methyl alcohol recorded in this paper agrees very well with the values recorded by Jones and Fornwalt,[7] Ewart and Raikes,[12] Goldschmidt and Aarflot,[13] and Dunstan, Thole and Benson,[14] who give 0.005445, 0.00545, 0.00544 and 0.00546, respectively. In the temperature range from 20 to 50°, the viscosities of dioxane observed by the authors closely parallel those of Geddes.[15] The wide variation in viscosity for the same substances at

(10) Meisenheimer and Dorner, Ann., 482, 130 (1930).
(11) Hovorka, Schaefer and Dreisbach, THIS JOURNAL, 58, 2264–67 (1936).
(12) Ewart and Raikes, J. Chem. Soc., 129, 1907–12 (1926).
(13) Goldschmidt and Aarflot, Z. physik. Chem., 122, 374 (1926).
(14) Dunstan, Thole and Benson, J. Chem. Soc., 105, 782–95 (1914).
(15) Geddes, THIS JOURNAL, 55, 4833 (1933).

the same temperature strongly emphasizes the difficulties involved in viscometry.

Summary

The densities, refractive indexes and viscosities of mixtures of methyl alcohol and dioxane were measured over a complete range of composition and at several temperatures. The densities, refractive indexes and viscosities of the pure substances were measured at several temperatures.

The composition coefficient equations for the densities, refractive indexes, and viscosities are given and the constants of these equations tabu-

lated along with the numbers for plotting deviation graphs so that computed values agreeing with observed values may be obtained.

Temperature coefficient equations for densities, refractive indexes, and viscosities of the pure substances and their mixtures are derived and the constants of these equations are tabulated.

Comparison of our experimentally observed values of densities, refractive indexes and viscosities of pure methyl alcohol and pure dioxane is made with the values of these properties of the pure substances recorded in the literature.

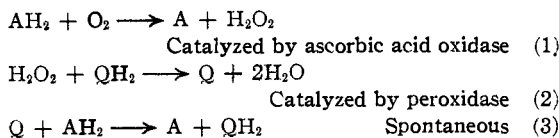# On the Mechanism of the Ascorbic Acid–Ascorbic Acid Oxidase Reaction. The Hydrogen Peroxide Question

BY HARRY G. STEINMAN AND CHARLES R. DAWSON

In 1937, St. Huszák,[1] while investigating the function of peroxidase in plants, came to the conclusion that since ascorbic acid and ascorbic acid oxidase are generally found associated with peroxidase they must play an integral part in the peroxidase system. In support of this hypothesis he cited experimental evidence which he interpreted as indicating that hydrogen peroxide was produced during the enzymatic oxidation of ascorbic acid by ascorbic acid oxidase. This hydrogen peroxide was assumed to be utilized by the peroxidase to oxidize an accompanying flavone derivative, the quinoid form of which then further oxidized a molecule of ascorbic acid. The proposed mechanism can be summarized as follows:

$$AH_2 + O_2 \longrightarrow A + H_2O_2$$
Catalyzed by ascorbic acid oxidase   (1)
$$H_2O_2 + QH_2 \longrightarrow Q + 2H_2O$$
Catalyzed by peroxidase   (2)
$$Q + AH_2 \longrightarrow A + QH_2$$ Spontaneous   (3)

where AH_2 represents ascorbic acid, A dehydroascorbic acid, QH_2 the reduced form of the flavone and Q the oxidized form. This theory of ascorbic acid oxidation appears to have been widely accepted by other workers in the field.[2-5]

Since many oxidizing enzyme systems are known to produce hydrogen peroxide, e. g., xanthine oxidase, d-amino acid oxidase and uricase,[6] and since the formation of hydrogen peroxide has been demonstrated in the oxidation of ascorbic acid catalyzed by cupric ion,[7,8] there was, until recently, little reason to question St. Huszák's theory. As soon as it was demonstrated, however, that ascorbic acid oxidase is a copper-protein,[9,10] it seemed desirable to reinvestigate the alleged formation of hydrogen peroxide during the enzymatic oxidation of ascorbic acid. Other metallo-protein oxidases have been shown to produce no hydrogen peroxide, e. g., cytochrome oxidase,[6,11] and tyrosinase.[12] In fact, formation of hydrogen peroxide has been suggested as a criterion for the identification of respiratory systems lacking terminal metallo-protein oxidases.[13]

The study reported in this paper deals fundamentally with the question of hydrogen peroxide formation during the aerobic oxidation of ascorbic

(1) St. Huszák, Z. physiol. Chem., 247, 239 (1937).

(2) A. Szent-Györgyi, "On Oxidation, Fermentation, Vitamins, Health and Disease," The Williams and Wilkins Co., Baltimore, Md., 1939.

(3) E. A. H. Roberts, Biochem. J., 33, 836 (1939).

(4) M. F. Jayle, Bull. soc. chim. biol., 21, 14 (1939).

(5) T. Ebihara, J. Biochem. (Japan), 29, 199 (1939).

(6) D. Keilin and E. F. Hartree, Proc. Roy. Soc. (London), B119, 114 (1936).

(7) C. M. Lyman, M. O. Schultze and C. G. King, J. Biol. Chem., 118, 757 (1937).

(8) A. O. Dekker and R. G. Dickinson, THIS JOURNAL, 62, 2165 (1940).

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