Thermodynamic Quantities for the Dissociation Equilibria of Biologically Important Compounds

4. THE SECOND ACID DISSOCIATION OF GLUCOSE 1-PHOSPHORIC ACID

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The acid dissociations of glucose 1-phosphoric acid are important because of its key position at the beginning of the glycolysis cycle. The phosphorylase equilibria have been studied by Trevelyan, Mann & Harrison (1952) at 30°, and any extension of this work to other temperatures will require further data on the acid dissociations. Further, glucose 1-phosphoric acid falls in the intermediate range of free energies of hydrolysis, having nearly twice as large a free energy of hydrolysis as glycerol 2-phosphoric acid. It was therefore of interest to see whether the increased energy of hydrolysis was reflected in an altered dissociation constant or in the alteration of any of the thermodynamic quantities of ionization. Glucose 1-phosphoric acid may be used as a buffer and as such has certain advantages over phosphoric acid. More extensive data on the system are therefore desirable, in particular for standardization purposes.

Only the second dissociation constant has been measured. The prolonged equilibration periods of the cells and the low first pK, coupled with the acid lability of glucose 1-phosphoric acid, render the first dissociation constant inaccessible to the present technique.

EXPERIMENTAL

Glucose 1-phosphoric acid. The preparation was based on McCready & Hassid's (1944) modification of Hanes's (1940) method, but with considerable modifications in detail which were found essential in order to obtain good yields of material of high purity. Three solutions were prepared: (a) 100 g. soluble starch suspended in 500 ml. water poured slowly into 3.5 l. boiling water and boiled for 2 min. Much less viscous and more even suspensions were obtained with soluble starch than with potato starch. (b) A buffer solution pH 6.8, by dissolving 1 mole $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (136 g.) and 1 mole $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (358 g.) in 3.0 l. water. (c) Potato extract, 1100 ml., made by scraping 6 lb. of potatoes, mincing finely and expressing the juice into 100 ml. 0.01 M-KCN (neutralized), allowing it to settle and decanting. The cyanide is essential to prevent the formation of quinones, which discolour the final product. No significant effect of the age of the potatoes was observed. The three solutions were mixed, 50 ml. of toluene added, and the final mixture was incubated at 25° for 24 hr. At the end of the incubation, the mixture was boiled to precipitate the proteins and,

without filtering, a solution of Mg acetate added (85 g. MgO in 500 ml. water and 250 ml. acetic acid), followed by ammonia (sp.gr. 0.88) to pH 8.4. The mixture was placed in the cold room for 48 hr. and the combined precipitates of protein and inorganic phosphate were filtered off. The precipitation of inorganic phosphate is not complete until 48 hr. and if the period of standing in the cold is reduced, it contaminates the final product.

The glucose 1-phosphate was separated from acetate, excess starch, etc., by means of ion exchange. The solution was passed through a 'Zeocarb 215' cation-exchange resin column of bed volume 1080 ml. at the rate of 5 bed vol./hr. and at once through an 'Amberlite IR-4B' anion-exchange column saturated with acetate (see below) at the rate of 8 bed vol./hr. The bed volume of this column was 690 ml. It is essential that there should be no delay once the cations are removed, since the solution is then sufficiently acid to cause slow hydrolysis of the glucose 1-phosphoric acid and this leads to contamination of the final product with inorganic phosphate. With the reagent concentrations used here, the 'Zeocarb 215' becomes saturated after approx. 3 bed vol. have passed through. It is preferable to allow most of the effluent to flow directly into the anion exchanger, testing only the last portions to find when the cation exchanger needs regeneration. Similarly, it is preferable to arrange the bed volumes so that the anion exchanger does not require regeneration during the course of a run. The ester is then removed in a single batch, yields are improved and contamination lessened. When all the ester was attached to the anion exchanger, the column was washed with 3-4 l. water to remove soluble impurities, starch and dextrans. The ester was then eluted by passing M-KOH at 3 bed vol./hr. through the column. McCready & Hassid (1944) recommend ammonia for the elution, but no advantage is apparent in its use. KOH gave sharper limits for the elution and yielded the K salt directly, thus eliminating the necessity for boiling off the ammonia with KOH. Resinous decomposition products were thereby avoided. Acetate is first removed from the column and then the greater part of the ester follows in the next 500 ml. of effluent. This solution was treated with 50 g. K acetate and 10% (w/v) KOH to pH 12.0. The solution was heated to 70-80° and 1.3 vol. of boiling methanol were added. Dipotassium glucose 1-phosphate, $2\text{H}_2\text{O}$ crystallized at once. The solution was allowed to stand overnight in the refrigerator, and filtered, the crystals washed with methanol and ether and dried *in vacuo* over CaCl_2 . The yield was generally 40 g. The reduction of the volume of the methanol from the 1.5 vol. recommended by McCready & Hassid slightly reduces the yield, but has the advantage of giving crystals

Table 1. Glucose 1-phosphoric acid; second dissociation. Molalities (m) and ionic strength (I) of solutions, e.m.f.'s of cells (E) in absolute volts (corrected to 1 atm. pressure of hydrogen and rounded temperatures) and extrapolation functions (y)

m_1 = molality of HCl, m_2 = molality of dipotassium glucose 1-phosphate, $I = 3m_2 - m_1$ (one value for I suffices for all temperatures, since m_{H^+} is negligible)

$$y = 2.3026 \frac{F}{RT} + \log \frac{m_2^2}{m_2 - m_1} + \frac{2AI}{1 + I}.$$

Two sets of values appear for 25° in set 1, on one day the values from 5 to 25° were determined, on the next day the values from 25 to 50° were determined on the same dilutions. In sets 2 and 3 the e.m.f.'s were determined with no break in the measurements.

		Set 1									
	$10^2 m_1$										
	$10^2 m_2$										
	$10^2 I$										
5°	E	0.28107	0.45021	0.54891	0.68049	0.81899	1.14029	1.36864			
	y	0.53352	0.85460	1.04195	1.29173	1.55462	2.16450	2.59860			
	E	1.31950	2.11359	2.57694	3.19470	3.84487	5.35321	6.42716			
	y	0.72593	0.71338	0.70792	0.70244	0.69737	0.68841	0.68343			
10°	E	0.51283	0.51351	0.51186	0.51899	0.51968	0.52462	0.52755			
	y	0.73177	0.71907	0.71359	0.70782	0.70270	0.69357	0.68850			
	E	6.50756	6.50971	6.50954	6.51336	6.51489	6.51986	6.52288			
15°	E	0.73760	0.72478	0.71911	0.71320	0.70802	0.69872	0.69354			
	y	6.50447	6.50863	6.50692	6.51019	6.51234	6.51737	6.52017			
20°	E	0.74351	0.73038	0.72470	0.71860	0.71339	0.70391	0.69871			
	y	6.50598	6.50885	6.50877	6.51064	6.51394	6.51885	6.52298			
25°	E	0.74938	0.73601	0.73027	0.72401	0.71874	0.70909	0.70375			
	y	6.50968	6.51248	6.51312	6.51418	6.51807	6.52305	6.52844			
	E	0.74933	—	0.73017	0.72409	0.71866	0.70908	0.70367			
	y	6.50884	—	6.51143	6.51553	6.51671	6.52289	6.52508			
30°	E	0.75526	0.74166	0.73565	0.72947	0.72401	0.71418	0.70868			
	y	6.51585	6.51879	6.51665	6.52091	6.52324	6.52814	6.53049			
35°	E	0.76117	0.74721	0.74123	0.73487	0.72936	0.71933	0.71374			
	y	6.52599	6.52695	6.52704	6.53015	6.53325	6.53778	6.54029			
40°	E	0.76713	0.75291	0.74677	0.74028	0.73472	0.72455	0.71894			
	y	6.53880	6.53947	6.53866	6.54149	6.54535	6.55050	6.55429			
45°	E	0.77306	0.75856	0.75235	0.74564	0.74002	0.72976	0.72399			
	y	6.55348	6.55357	6.55333	6.55445	6.55890	6.56546	6.56829			
50°	E	0.77899	0.76418	0.75804	0.75106	0.74543	0.73498	0.72910			
	y	6.56855	6.56763	6.57014	6.56884	6.57465	6.58103	6.58371			

Set 2

$10^2 m_1$	0.48654	0.57212	0.73609	0.78686	0.90469	1.02023	1.35337	1.48090	1.90750	2.11031	2.23824
$10^3 m_2$	0.72556	0.85318	1.09770	1.17340	1.34912	1.52143	2.01822	2.20839	2.84457	3.14700	3.33779
$10^3 I$	1.69014	1.98742	2.55701	2.73334	3.14268	3.54406	4.70128	5.14427	6.62621	7.33070	7.77513
E	0.69736	0.69315	0.68646	0.68463	0.68075	0.67752	0.66991	0.66761	0.66055	0.65776	0.65616
5°	0.60729	0.59973	0.59158	0.59172	0.51063	0.51203	0.51628	0.52030	0.52184	0.52338	0.52483
E	0.70283	0.69835	0.69158	0.68976	0.68594	0.68255	0.67478	0.67237	0.66522	0.66238	0.66077
y	0.50461	0.50365	0.50678	0.50745	0.50856	0.50819	0.51220	0.51505	0.51739	0.51900	0.52083
E	0.70828	0.70366	0.69675	0.69488	0.69106	0.68746	0.67963	0.67709	0.66987	0.66696	0.66532
y	0.50389	0.50195	0.50485	0.50506	0.50759	0.50467	0.51021	0.51157	0.51507	0.51640	0.51824
20°	0.71366	0.70896	0.70191	0.70001	0.69617	0.69244	0.68448	0.68186	0.67456	0.67161	0.66993
E	0.50515	0.50327	0.50596	0.50625	0.50966	0.50565	0.51145	0.51226	0.51671	0.51830	0.51998
y	0.71907	0.71426	0.70708	0.70513	0.70125	0.69745	0.68933	0.68662	0.67931	0.67625	0.67454
25°	0.50977	0.50748	0.51011	0.51406	0.51025	0.51001	0.51557	0.51568	0.52225	0.52289	0.52461
y	0.72442	0.71956	0.71221	0.71025	0.70633	0.70246	0.69414	0.69140	0.68396	0.68087	0.67912
30°	0.51520	0.51400	0.51595	0.51639	0.52082	0.51674	0.52143	0.52186	0.52849	0.52958	0.53114
E	0.72987	0.72493	0.71743	0.71534	0.71141	0.70747	0.69902	0.69619	0.68866	0.68551	0.68374
E	0.52677	0.52514	0.52676	0.52565	0.53105	0.52696	0.53198	0.53173	0.53910	0.54011	0.54187
y	0.73530	0.73028	0.72259	0.72034	0.71653	0.71253	0.70382	0.70093	0.69334	0.69009	0.68831
40°	0.53934	0.53781	0.53850	0.53539	0.54385	0.53989	0.54313	0.54273	0.55130	0.55162	0.55374
y	0.74072	0.73561	0.72777	0.72540	0.72161	0.71749	0.70859	0.70566	0.69794	0.69472	0.69288
45°	0.55412	0.55253	0.55292	0.54854	0.55836	0.55361	0.55625	0.55603	0.56468	0.56639	0.56806
E	0.74616	0.74096	0.73290	0.73065	0.72676	0.72254	0.71315	0.71055	0.70269	0.69940	0.69755
y	0.56962	0.56797	0.56701	0.56513	0.57442	0.56920	0.56661	0.57233	0.58091	0.58240	0.58443

Set 3

$10^2 m_1$	0.45790	0.56729	0.65144	0.72797	0.76903	0.88568	1.24728	1.38777	1.61826	1.80308	2.11431	2.19992
$10^2 m_2$	0.64252	0.79602	0.91410	1.02148	1.07911	1.24278	1.75018	1.94732	2.27074	2.53008	2.96679	3.08693
$10^3 I$	1.46967	1.82077	2.09085	2.33648	2.46828	2.84286	4.00325	4.45420	5.19397	5.78716	6.78607	7.06086
5°	E	0.69452	0.68898	0.68529	0.68225	0.68078	0.67691	0.66476	0.66063	0.65781	0.65330	0.65221
10°	E	0.50843	0.51168	0.51219	0.51150	0.51184	0.51141	0.51602	0.51883	0.52288	0.52285	0.52356
15°	E	0.69987	0.69420	0.69037	0.68729	0.68581	0.68190	0.67254	0.66957	0.66245	0.65791	0.65671
20°	E	0.50448	0.50726	0.50652	0.50614	0.50680	0.50695	0.51129	0.51225	0.51474	0.51968	0.51880
25°	E	0.70513	0.69935	0.69547	0.69233	0.69085	0.68687	0.67734	0.67432	0.66999	0.66244	0.66119
30°	E	0.50130	0.50401	0.50364	0.50321	0.50435	0.50456	0.50901	0.51007	0.51240	0.51763	0.51618
35°	E	0.71045	0.70453	0.70061	0.69743	0.69590	0.69185	0.68217	0.67906	0.67466	0.66695	0.66570
40°	E	0.50240	0.50452	0.50468	0.50457	0.50532	0.50559	0.51050	0.51097	0.51349	0.51832	0.51736
45°	E	0.71581	0.70975	0.70579	0.70252	0.70099	0.69686	0.68703	0.68385	0.67938	0.67150	0.67025
50°	E	0.50704	0.50860	0.50928	0.50861	0.50984	0.51000	0.51536	0.51562	0.51832	0.52305	0.52212
	E	0.72113	0.71493	0.71097	0.70762	0.70606	0.70186	0.69186	0.68863	0.68408	0.67604	0.67483
	E	0.51332	0.51434	0.51620	0.51517	0.51635	0.51659	0.52208	0.52246	0.52519	0.52929	0.52977
	E	0.72646	0.72013	0.71613	0.71277	0.71113	0.70692	0.69672	0.69344	0.68863	0.68067	0.67939
	E	0.52323	0.52391	0.52626	0.52603	0.52635	0.52764	0.53279	0.53330	0.53639	0.54090	0.54059
	E	0.73181	0.72536	0.72126	0.71784	0.71622	0.71195	0.69923	0.69823	0.69352	0.68525	0.68391
	E	0.53534	0.53585	0.53772	0.53747	0.53858	0.54009	0.54491	0.54572	0.54853	0.55303	0.55272
	E	0.73719	0.73061	0.72641	0.72299	0.72130	0.71701	0.70643	0.70304	0.69828	0.69501	0.68985
	E	0.55029	0.55050	0.55189	0.55258	0.55302	0.55539	0.56023	0.56085	0.56417	0.56815	0.56853
	E	0.74260	0.73584	0.73160	0.72812	0.72641	0.72207	0.71128	0.70786	0.70298	0.69969	0.69302
	E	0.56611	0.56524	0.56713	0.56779	0.56837	0.57109	0.57551	0.57658	0.57932	0.58388	0.58339

which are free from the brown material eluted from the resin by the alkali. Any ester remaining in solution can be recovered by including the mother liquors in the next batch of filtrate which is put through the columns. The presence of the methanol appears not to have any deleterious effect upon the performance of the resins. Ester intended for potentiometric work was recrystallized 3 times by dissolving 40 g. in 500 ml. water and adding methanol as before.

The cation-exchange columns were 5 cm. in diameter and those for the anion exchanger 7 cm. in diameter. The large cross-section of the anion exchanger has two advantages: first, the acid solution has only a short distance to travel before encountering fresh resin, so that the time during which hydrolysis of the ester could occur is minimized; secondly, the swelling of the Amberlite IR-4B which follows its absorption of anions was less likely to burst the wide column. Troubles with swelling of the resin were finally overcome by passing 5% (w/v) acetic acid upwards through the column after it had been prepared for use followed by the removal of the excess acid with distilled water. The resin was then fully expanded and the displacement of acetate by glucose 1-phosphate was effected with but little volume change. This technique has the added advantage of freeing the column from CO_2 which may otherwise be evolved during the absorption of the ester, with disruption of the column and reduced efficiency of absorption.

The 'Zeocarb 215' was prepared for use by passing 4% (w/v) HCl through the column at 6 bed vol./hr. until the pH of the effluent fell below pH 1. This took approximately 2 l. of acid; the resin was then washed free of acid with dist. water at 10 bed vol./hr. until the pH rose to that of the water. The 'Amberlite IR-4B' was generated by passing 5% (w/v) KOH at 4 bed vol./hr., until the effluent was at pH 11-12. The resin was then washed free of alkali with dist. water at 10 bed vol./hr. until the pH dropped to near 7. If the resin is to be used in the acetate form it is unnecessary to wash so thoroughly before introducing the acetic acid.

After the passage of the solution through the cation exchanger, it contains only free acids and its pH is near 1. The exhaustion of the resin is indicated by a sudden sharp rise in the pH of the effluent to near 4. The solution in the column was then displaced with 1 bed vol. of water and the column regenerated. The elution of the ester from the columns was followed by boiling a few drops of the effluent with acid molybdate at intervals.

The purity of the potassium glucose 1-phosphate could not be assayed by incineration to $\text{K}_4\text{P}_2\text{O}_7$ as was possible with sodium glycerol 2-phosphate. The melting point of the potassium salt was low and the decomposing melt entrained carbon, which it was subsequently impossible to incinerate. Instead, the salt was assayed by weight titrations with standard HCl to an end point at pH 3.4 (glass electrode). Glucose (by Benedict's method after hydrolysis) and the specific rotation were also determined. The rotation found was higher than any recorded in the literature. Typical analytical figures were: weight titration, 99.98-100.06% pure; glucose, expected 48.4%, found 48.1%; $[\alpha]_D^{25}$, literature value; 78°; found: 79.7°. Chromatography in a *tert.*-amyl alcohol-water-*p*-toluene sulphonic acid solvent (Hanes & Isherwood, 1949) showed only a single spot. Controls indicated that less than 1% of inorganic phosphate or other esters could be detected.

There is thus good evidence that the material was highly purified $\text{C}_6\text{H}_{11}\text{O}_6 \cdot \text{PO}_3\text{K}_2 \cdot 2\text{H}_2\text{O}$. It is usually assumed that

the ester prepared by phosphorylase has the α -configuration and that assumption is followed here.

Potentiometry. This was carried out using the same apparatus and technique as for glycerol 2-phosphoric acid (Ashby, Crook & Datta, 1954a). Three sets of experiments were done on different batches of glucose 1-phosphoric acid, the results being combined as before by averaging the coefficients of the orthogonal polynomials used to fit the thermodynamic pK values to the temperature curve. During the early part of this investigation it became necessary, owing to leakage of paraffin from the thermostat bath into the platinum resistance thermometer, to obtain a new thermometer from the Cambridge Instrument Company. It was subsequently discovered that the calibration of this thermometer was incorrect. A more suitable platinum resistance thermometer was therefore constructed according to the design of Barber (1950), and this was calibrated by the National Physical Laboratory. At the same time the two standard resistances and the two standard cells were also checked by the National Physical Laboratory. The resistance of the thermometer at the triple point of water (273.15° K) was rechecked frequently during the course of this work using a triple-point cell (Barber, Handley & Herington, 1954).

RESULTS

The measurements and calculations of the three sets of experiments are summarized in the tables. Table 1 gives the molalities of the solutions, the e.m.f. measurements and the calculated extrapolation functions for each of the three sets of dilutions. Table 2 contains the values of the thermodynamic pK values obtained from the least squares extrapolations of the results in Table 1, together with the calculated values of the pK values from the equation

$$\text{pK} = A/T - D + CT,$$

the constants of this equation, and the minimum pK and the temperature at which it occurs. The slopes of the regression lines ' β ' are also included as they will be of value to those wishing to calculate the pH of glucose 1-phosphate-chloride buffers. Table 3 contains the thermodynamic quantities of the ionization calculated from the constants of the above equation, together with the standard errors of the results, calculated according to Please (1954).

DISCUSSION

The present results lend weight to the previous conclusion that the methods of Harned and his collaborators are applicable to phosphate esters of biological importance. The errors found in this work are of the same order of magnitude as for glycerol 2-phosphoric acid (Ashby, Crook & Datta, 1954b), and the wider experience now obtained suggests that the chief source of variance lies in the difficulty of preparing the esters in a sufficiently high degree of purity, especially as regards water content of the

Table 2. *Glucose 1-phosphoric acid; second dissociation. Observed and calculated values of pK_2 at rounded temperatures, the values of ' β ' the slope of the extrapolation, the constants A, C and D of the equation $pK_2 = A/T - D + CT$, where T is the absolute temperature, the minimum value of pK_2 and the temperature at which it occurs*

The constants of the equation $pK_2 = A/T - D + CT$ were obtained by the method of least squares and the calculated values of pK_2 were obtained by using this equation.

The values of pK_2 observed are given to four decimal places, as these figures were used to calculate the constants of the equation relating pK_2 to temperature.

Temp. (°)	Set 1		Set 2		Set 3		pK (calc.)
	pK (obs.)	β	pK (obs.)	β	pK (obs.)	β	
5	6.5070	0.32	6.5036	0.28	6.5055	0.26	6.506
10	6.5029	0.31	6.4995	0.27	6.5004	0.28	6.500
15	6.5006	0.31	6.4982	0.25	6.4973	0.29	6.499
20	6.5010	0.34	6.4992	0.26	6.4983	0.30	6.500
25	6.5045	0.33	6.5032	0.27	6.5025	0.30	6.504
30	6.5113	0.30	6.5092	0.28	6.5086	0.32	6.510
35	6.5207	0.31	6.5198	0.27	6.5184	0.34	6.519
37	—	—	—	—	—	—	6.524
40	6.5322	0.33	6.5320	0.27	6.5301	0.35	6.531
45	6.5464	0.33	6.5462	0.26	6.5445	0.37	6.545
50	6.5613	0.34	6.5611	0.27	6.5599	0.37	6.561

$\sigma = \pm 3 \times 10^{-4}$

$A = 1432.1569$; $D = 3.4213$; $C = 0.017177$; $pK_{min.} = 6.498$; $T_{min.} = 15.6^\circ$.

Table 3. *Glucose 1-phosphoric acid; second dissociation. Thermodynamic quantities*

Temp. (°)	ΔG°		ΔH°		$-\Delta S^\circ$		$-\Delta C_p^\circ$	
	Cal.	Joule	Cal.	Joule	Cal. deg. ⁻¹	Joule deg. ⁻¹	Cal. deg. ⁻¹	Joule deg. ⁻¹
5	8277	34 626	474	1980	28.1	117.4	43.7	183
10	8419	35 221	254	1060	28.85	120.7	44.5	186
15	8566	35 832	29	120	29.6	124.0	45.3	189
20	8716	36 460	-199	-830	30.4	127.3	46.1	193
25	8870	37 105	-431	-1800	31.2	130.5	46.8	196
30	9028	37 765	-668	-2790	32.0	133.8	47.6	199
35	9190	38 443	-908	-3800	32.8	137.1	48.4	203
37	9255	38 718	-1005	-4200	33.1	138.4	48.7	204
40	9355	39 136	-1152	-4820	33.6	140.4	49.2	206
45	9525	39 847	-1400	-5860	34.35	143.7	50.0	209
50	9699	40 573	-1652	-6910	35.1	147.0	50.8	212

Errors

$\sigma_5, \sigma_{25}, \sigma_{50}$, = standard errors at 5, 25 and 50°, respectively

σ_5	0.7	3.1	21.7	91	0.08	0.32	0.90	3.8
σ_{25}	0.45	1.9	6.3	26	0.02	0.09	0.97	4.1
σ_{50}	0.7	3.1	24.0	100	0.08	0.32	1.05	4.4

Table 4. *Thermodynamic pK_2 values of orthophosphoric acid and ester phosphates at 30°*

Acid	pK_2	Author
Orthophosphoric	7.190	Bates & Acree (1943)
Glycerol 2-phosphoric	6.657	Ashby, Crook & Datta (1954b)
Glucose 1-phosphoric	6.510	This paper
Glucose 1-phosphoric	6.51 ± 0.01	Trevelyan, Mann & Harrison (1952)
Glucose 1-phosphoric	6.47*	Cori, Colowick & Cori (1937)

* Calculated from the apparent pK_2 using the Guntelberg approximation for the activity coefficients (Ashby, Crook & Datta, 1954a).

crystals. Thus a difference in the water of crystallization of 2 parts in 1000 between batches would produce errors in pK of the same order of magnitude as those actually observed. In view of this, the close agreement between batches must be regarded as very satisfactory. With glucose 1-phosphoric acid the most important source of error to be suspected is that due to hydrolysis. Tests showed that under the conditions here used no hydrolysis could be detected during the course of a set of measurements.

No values of the thermodynamic quantities are available in the literature for comparison, but, as with glycerol 2-phosphoric acid, these fall within the range of Harned & Owen's (1950) data. Comparison with glycerol 2-phosphoric acid shows the relation to be complex, but the most striking difference is the larger value of $-\Delta C_p^\circ$ for glucose 1-phosphoric acid. The minimum pK and the temperature at which they occur are similar for the two esters.

The only value of the thermodynamic pK_2 for glucose 1-phosphoric acid in the literature is that obtained by Trevelyan *et al.* (1952) at 30° , by the extrapolation of glass electrode measurements to infinite dilution. An apparent pK_2 has also been determined by Cori, Colowick & Cori (1937). This value has been recalculated to an approximate thermodynamic value by use of the Guntelberg approximation for the activity coefficients (Ashby *et al.* 1954*a*). The results at 30° , together with the thermodynamic pK_2 values for glycerol 2-phosphoric acid and orthophosphoric acid, are shown in Table 4. The agreement between the present results and those of Trevelyan *et al.* is very good indeed, and that with the results of Cori *et al.* is reasonably close considering the inaccuracy of correcting, without extrapolation for the effects of ionic strength on the activity coefficients.

In view of the greater standard free energy of hydrolysis, it is interesting that the second acid dissociation constant of glucose 1-phosphoric acid is stronger than that of glycerol 2-phosphoric acid by approximately 0.15 pK unit.

SUMMARY

1. The second thermodynamic dissociation constant of glucose 1-phosphoric acid has been measured at 5° intervals from 5 to 50° using hydrogen and silver-silver chloride electrodes in cells without liquid junction.

2. From these measurements have been calculated the standard free energy, heat content, entropy and heat capacity for the ionization over the whole temperature range, together with their standard deviations.

3. The data presented allow the use of glucose 1-phosphoric acid as a standard buffer.

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