CL. GLYOXALINES: THE DETERMINATION OF THEIR pK VALUES AND THE USE OF THEIR SALTS AS BUFFERS

BY A. H. M. KIRBY AND A. NEUBERGER¹

*From the Department of Pathological Chemistry, University College
Hospital Medical School, London*

(Received 23 May 1938)

THE work described in this paper was undertaken for two reasons: first, there is a lack of buffers for use between pH 6 and 9 in experiments where phosphates are excluded. Further, it is generally desirable, particularly in physico-chemical investigations, to use buffers of a symmetrical valency type; these should preferably be uni-univalent salts.

The pH of buffer solutions is usually determined by the electrometric technique involving cells with liquid junctions, although it is now recognized that measurements with such cells do not generally yield accurate values for either the concentration or the mean activity of H^+ ions. The magnitude which we call pH is really a function which depends on the concentrations, valencies and mobilities of all the ions present in the solution and is generally not an accurate measure of the acidity. Only in cases where the total ionic concentration is low and the different ions are of low valency type, can the Debye-Hückel approximations be applied. If in such cases precautions are taken to make the liquid junction potential small, the pH values obtained represent a fairly good approximation of the mean activity of H^+ ions which is the true measure of the acidity of the solution.

In physico-chemical work with compounds of high valency such as proteins the advantage of using uni-univalent buffers is even more obvious. The Debye-Hückel formulae, which already fail with concentrated solutions of uni-univalent salts, break down for dilute solutions of salts of higher and unsymmetrical valency type [La Mer *et al.* 1927]. It is certainly not to be expected that the Debye-Hückel formulae and the principle of ionic strength should apply to protein solutions [*v.* Hartley, 1935]. Ions of higher valency interact with oppositely charged protein ions much more strongly than can be accounted for on the basis of Debye-Hückel formulae, a phenomenon which is predictable from Bjerrum's theory of ionic association: such a type of Coulomb interaction affords an explanation of the experiments of Tiselius [1930; 1937], who found that the electric mobilities of protein ions at constant pH were altered by substituting multivalent for univalent buffers. Shifts in the isoelectric points of proteins produced by salts of different valency type [Smith, 1935; 1936] and the differences between "isoionic" and "isoelectric" points as in the experiments of Adair & Adair [1934] can also be explained by the fact that in protein systems the principle of ionic strength breaks down and has to be replaced by theories of the type suggested by Bjerrum [1926] and Hartley [1935]. In all these cases the difficulties of even qualitative interpretation are much enhanced by the use of buffers of unsymmetrical valency type. The use of uni-univalent buffers will

¹ Beit Memorial Research Fellow.

not eliminate all difficulties, since specific properties of ions may come into play in colloidal systems, but it is reasonable to expect that the interpretation of measurements of this character will be greatly facilitated.

For substances to be suitable as the basis of a system of buffers of the type desired certain criteria must be fulfilled. The required range can scarcely be covered with less than three compounds which should have pK approximately 6, 7 and 8; the compounds should be stable and easily accessible and, since the buffer solutions are likely to be employed in biological experiments, they should if possible be biologically indifferent.

A search of the literature indicated that these requirements were probably most nearly satisfied by bases of the glyoxaline series; glyoxaline itself has pK in the neighbourhood of 7 and it seemed likely, particularly in view of the few data concerning the dissociation constants of substituted glyoxalines which are already recorded in the literature, that the introduction of suitable electron-attractive or electron-repellent groups into the molecule would yield derivatives having the required pK of about 6 and 8. As will be seen below this expectation has been fulfilled.

So far as stability is concerned the glyoxalines leave little to be desired; buffer solutions prepared from them have remained unchanged over 3 weeks without any elaborate precautions and there is no reason to suppose that their actual period of stability is not much longer. A large variety of substituted glyoxalines have recently become readily accessible through the simple synthetic method of Weidenhagen & Herrmann [1935]. Finally, so far as is at present known, the glyoxalines used in the present work have no such specific effects on enzyme systems as have been found for instance to be troublesome in the case of the veronal buffers of Michaelis [Maeda, 1937].

EXPERIMENTAL

Preparation of compounds

Glyoxaline was prepared from nitrotartaric acid according to the method of Fargher & Pyman [1919], being purified by distillation and finally by recrystallization from benzene. 4 (or 5)-Methylglyoxaline was prepared from glucose by the method of Windaus & Knoop [1905] and purified by distillation followed by conversion into the picrate; the latter was decomposed with HCl and benzene and the aqueous solution evaporated; the residual hydrochloride was crystallized from absolute alcohol. The hydrochloride, which is hygroscopic, has m.p. 118° . (Found: N (Kjeldahl) 23.7; Cl, 30.6%. $C_4H_7N_2Cl$ requires N, 23.6; Cl, 30.0%.)

4(or 5)-Hydroxymethylglyoxaline was prepared according to Weidenhagen & Herrmann [1935] from dihydroxyacetone (oxantin); it was purified through the picrate and finally converted into the hydrochloride, which had m.p. 110° (values given in the literature range from 93 to 109°).

2,4(or 5)-Dimethylglyoxaline was obtained by the method of Weidenhagen & Herrmann [1935] except that acetol (*Org. Syntheses*, **10**, 1) was used instead of acetol acetate. This base was either purified by distillation or converted via the picrate into the hydrochloride, which, after recrystallization from alcohol, had m.p. 207° (uncorr.). (Found: N, 21.2; Cl, 27.0%. $C_5H_9N_2Cl$ requires N, 21.2; Cl, 26.8%.)

All the other glyoxaline derivatives were prepared exactly according to Weidenhagen & Herrmann [1935], and had m.p. in agreement with the values recorded in the literature.

Table I

Substance	$E_{\text{corr.}}$	pH	α	μ	pK_0
Glyoxaline	0.7533	7.074	50.0	0.1050	6.95
	0.7525	7.062	50.0	0.0750	6.96
	0.7513	7.041	50.0	0.0450	6.95
	0.7506	7.028	50.0	0.0250	6.96
	0.7496	7.011	50.0	0.0150	6.96
	0.7490	7.001	50.0	0.0090	6.96
	0.7221	6.546	75.0	0.0475	6.93
	0.7790	7.509	25.0	0.0425	6.95
2-Methylglyoxaline	0.8066	7.977	50.0	0.1050	7.86
	0.8057	7.960	50.0	0.0750	7.85
	0.8049	7.947	50.0	0.0450	7.86
	0.8037	7.927	50.0	0.0250	7.86
	0.8032	7.918	50.0	0.0150	7.86
	0.8024	7.905	50.0	0.0090	7.86
	0.7746	7.435	75.0	0.0475	7.82
	0.8322	8.410	25.0	0.0425	7.85
4(or 5)-Methylglyoxaline	0.7871	7.645	50.0	0.1050	7.52
	0.7857	7.623	50.0	0.0750	7.52
	0.7841	7.595	50.0	0.0450	7.51
	0.7832	7.580	50.0	0.0250	7.51
	0.7830	7.577	50.0	0.0150	7.52
	0.7830	7.577	50.0	0.0090	7.53
	0.7556	7.112	75.0	0.0475	7.50
	0.8133	8.089	25.0	0.0425	7.53
2:4(or 5)-Dimethylglyoxaline	0.8366	8.482	50.0	0.1050	8.36
	0.8355	8.465	50.0	0.0750	8.36
	0.8345	8.448	50.0	0.0450	8.36
	0.8334	8.429	50.0	0.0250	8.36
	0.8327	8.418	50.0	0.0150	8.36
	0.8322	8.410	50.0	0.0090	8.37
	0.8057	7.960	75.0	0.0475	8.35
	0.8618	8.910	25.0	0.0425	8.35
4(or 5)-Hydroxymethylglyoxaline	0.7200	6.510	50.0	0.1050	6.39
	0.7184	6.483	50.0	0.0750	6.38
	0.7174	6.468	50.0	0.0450	6.38
	0.7166	6.454	50.0	0.0250	6.39
	0.7160	6.443	50.0	0.0150	6.39
	0.7160	6.443	50.0	0.0090	6.40
	0.6887	5.981	75.0	0.0475	6.37
	0.7454	6.940	25.0	0.0425	6.38
2-Phenylglyoxaline	0.7177	6.473	51.0	0.0550	6.40
	0.7164	6.449	51.0	0.0350	6.39
	(0.7166)	—	51.0	0.0250	—
	0.7157	6.439	51.0	0.0190	6.40
	0.7161	6.445	51.0	0.0150	6.41
	0.6881	5.971	76.5	0.0375	6.40
	0.7460	6.950	25.5	0.0325	6.41
	0.6973	6.127	50.0	0.1050	6.01
4(or 5)-Phenylglyoxaline	0.6949	6.087	50.0	0.0750	5.98
	0.6942	6.074	50.0	0.0450	5.99
	0.6940	6.071	50.0	0.0250	6.00
	0.6933	6.059	50.0	0.0150	6.00
	0.6653	5.586	75.0	0.0475	5.97
	0.7254	6.603	25.0	0.0425	6.04

Table II

Substance	Mean pK_0 value
Glyoxaline	6.95
2-Methylglyoxaline	7.86
4(or 5)-Methylglyoxaline	7.52
2:4(or 5)-Dimethylglyoxaline	8.36
4(or 5)-Hydroxymethylglyoxaline	6.38
2-Phenylglyoxaline	6.39
4(or 5)-Phenylglyoxaline	6.00

Measurements of the dissociation constants

The dissociation constants were measured in cells of the type:

Pt, H ₂	0.01 <i>M</i> base	3.5 <i>M</i>	0.1 <i>M</i>	Hg
	<i>X M</i> HCl	KCl	KCl	
	<i>Y M</i> KCl		HgCl	

where $X < 0.01$ and Y varied between 0.1 and 0.004 *M*. Except in the most dilute solutions the concentrations of K⁺ and Cl⁻ were much larger than the sum of all the other ions. The liquid junctions were made inside a tube of internal diameter 3 mm. and were very sharp initially. The potentiometer was a Tinsley instrument Type 4025; the galvanometer was of the D'Arsonval type with an internal resistance of 400 ohms. All the measurements were made at $25^\circ \pm 0.05$.

The main difficulty in measurements with cells containing liquid junctions is that errors due to liquid junction potentials introduce uncertainty about the correct value of E_0 . The liquid junction potentials in systems of the type described above are certainly not greater than 0.5 mV. Both half-cells contain K⁺ and Cl⁻ ions and are nearly symmetrical and the two liquid junctions should cancel each other to a very large extent. Variations in the KCl concentration in the one half-cell will cause definite changes in the liquid junction potential, but these may be neglected as shown by the fact that extrapolation of the apparent pK values to ionic strength leads to fairly constant pK_0 values. Liquid junction potentials have been in fact neglected throughout this work.

The values for E_0 given in the literature vary considerably. Guggenheim & Schindler [1934] chose a value of 333.7 mV., whilst in an earlier paper by one of us [Neuberger, 1937] a value of 334.4 mV. was used. A few experiments on the acetic acid-sodium acetate system showed that the best average pK value of acetic acid was obtained when E_0 was taken as 335.1 mV., if the liquid junction potential is neglected. Taking into account the slight remaining uncertainty which cannot be completely eliminated it is assumed that the pK values will be correct within 0.01.

The results obtained with the different compounds are recorded in Table I. E is corrected for barometric pressure, α is the degree of neutralization of the base with acid and μ is the ionic strength. pK_0 is calculated from the apparent pK by the formula

$$pK_0 = pK - \frac{0.5 \sqrt{\mu}}{1 + \sqrt{\mu}}.$$

Table II contains the mean values for pK_0 obtained from the data of Table I.

DISCUSSION

The introduction of the electron-repelling methyl group increases the basicity very considerably. The effect of substitution in position 2 is much stronger than the effect of substitution in position 4 (or 5), whilst substitution of two methyl groups in positions 2 and 4 (or 5) increases the pK values even more strongly. The difference in pK values between glyoxaline and the dimethyl derivative is nearly equal to the sum of the differences between glyoxaline and the 2 and the 4 (or 5) monomethyl derivatives respectively, indicating that the effect of substitution is additive.

The electron-attracting phenyl group produces the opposite effect, but here substitution in position 4 (or 5) causes a smaller change in pK than substitution in position 2, an observation which is difficult to interpret. The diphenyl derivative is too insoluble in water to allow any accurate measurements to be made by the technique used in this work.

As might be expected from its electronegative character the hydroxymethyl group causes a decrease of pK . Generally it may be noted that the effect of substitution on the pK of glyoxalines is very much larger than the effect of substitution in aliphatic systems or even in the benzene ring. Effects of similar order of magnitude have already been observed, however, in other heterocyclic ring systems, such as pyridine and pyrazole. It seems that electronic displacements in systems containing conjugated double bonds are transmitted to the dissociating centres much more readily than in more saturated compounds.

A further point which makes a comprehensive interpretation of the effect of substitution on pK in glyoxalines more difficult is the existence of tautomerism. The free bases may exist in the forms

In glyoxaline itself and in the bases substituted in position 2 only, the two forms are identical. But for 4 (or 5) monosubstituted bases and for 2:4 (or 5) disubstituted compounds the two forms are different and will have different dissociation constants. The pK values obtained give a measure of the basicity of the equilibrium mixtures only and it is not possible to derive any dissociation constants for the two bases separately. For the corresponding cations it may be assumed that no tautomerism but only resonance is involved, as is the case with guanidine and amidine salts. The charge then will not reside at one particular nitrogen atom but will be distributed over the molecule as a whole.

Substitution would then influence the pK not only by the intrinsic effect of substitution as such, but also by shifting the equilibria between the tautomeric forms of the bases and by the effects caused on the resonance of the cations.

BUFFERS

The substances which were finally selected as buffers were the hydrochlorides of 4(or 5)-methylglyoxaline, 4(or 5)-hydroxymethylglyoxaline and 2:4(or 5)-dimethylglyoxaline; these compounds are very soluble in water, are fairly easily accessible and together cover the desired pH range satisfactorily. Three series of solutions were made up as shown in Table III; the E.M.F. values were measured at once for each solution and measured again after 3 weeks' storage in the cold room. The potentials found and the pH values calculated from these measurements are recorded in Table III¹ and it can be seen that the buffers have remained unaltered over this period. The buffers have a constant ionic strength; if any other buffer concentration is desired the pH values can be calculated from the formula

$$pH = pK - \log \frac{[B \cdot HCl]}{[\text{Free base}]} + \frac{0.5 \sqrt{\mu}}{1 + \sqrt{\mu}}.$$

Table III shows that the range between pH 5.4 and 9.3 is satisfactorily covered by the new buffers and it is now possible to use uni-univalent buffers in aqueous systems throughout, since the range from pH 3.6 to 5.6 is covered by acetic acid-sodium acetate, whilst glycine-sodium hydroxide mixtures are available for the range 8.6–11.5.

¹ It must be remembered that the figures in Table III are valid for 25° only; the temperature coefficient may be of considerable magnitude.

Table III. *Data for the preparation of buffer solutions*

In each case 25 ml. *N*/10 solution of the base were treated with the number of ml. *N*/10 NaOH shown in col. 2 and the whole made up to 50 ml.

Substance	ml. of <i>N</i> /10 NaOH	<i>E</i>		<i>pH</i>		Mean <i>pH</i>
		observed initially	observed after 3 weeks	observed initially	observed after 3 weeks	
4(or 5)-Hydroxymethylglyoxaline hydrochloride	2.5	0.6578	0.6581	5.46	5.47	5.47
	5.0	0.6795	0.6808	5.83	5.85	5.84
	7.5	0.6944	0.6955	6.09	6.10	6.10
	10.0	0.7061	0.7070	6.28	6.30	6.29
	12.5	0.7175	0.7188	6.48	6.50	6.49
	15.0	0.7282	0.7291	6.64	6.67	6.66
	17.5	0.7418	0.7419	6.88	6.88	6.88
	20.0	0.7567	0.7568	7.14	7.14	7.14
4 (or 5)-Methylglyoxaline hydrochloride	5.0	0.7499	0.7508	7.02	7.04	7.03
	7.5	0.7631	0.7640	7.24	7.26	7.25
	10.0	0.7751	0.7760	7.45	7.46	7.46
	12.5	0.7861	0.7868	7.64	7.65	7.65
	15.0	0.7960	0.7978	7.80	7.83	7.82
	17.5	0.8077	0.8093	7.98	8.03	8.01
	20.0	0.8221	0.8220	8.24	8.24	8.24
	22.5	0.8365	0.8367	8.49	8.50	8.49
2:4(or 5)-Dimethylglyoxaline hydrochloride	7.5	0.8112	0.8109	8.06	8.05	8.06
	10.0	[0.8178]	0.8225	—	8.25	8.25
	12.5	0.8330	0.8334	8.43	8.44	8.44
	15.0	0.8428	0.8440	8.59	8.62	8.61
	17.5	0.8541	0.8552	8.79	8.80	8.80
	20.0	0.8670	0.8679	9.00	9.03	9.02
	22.5	0.8850	0.8857	9.31	9.32	9.32
	25.0	0.8985	0.8987	9.56	9.57	9.56

SUMMARY

1. It is pointed out that buffers of a symmetrical valency type, especially uni-univalent buffers, give the best-defined *pH* values; the particular advantage of the use of such buffers in the physico-chemical study of proteins is discussed.

2. The *pK* values of glyoxaline and some methyl-, phenyl- and hydroxymethyl-substitution products have been measured and are discussed in relation to the structures of the compounds.

3. 4(or 5)-Methyl-, 2:4(or 5)-dimethyl- and 4(or 5)-hydroxymethyl-glyoxaline with hydrochloric acid are proposed as suitable buffers for the range between *pH* 5.4 and 9.3. The whole *pH* range from *pH* 3.6 to 11.5 can thus now be covered with uni-univalent buffer systems.

The authors wish to thank Prof. C. R. Harington for his advice and help in the preparation of the compounds used. Grateful acknowledgement is made to the Rockefeller Foundation for a grant from which the cost of the potentiometer used in this work was defrayed.

REFERENCES

- Adair & Adair (1934). *Biochem. J.* **28**, 1230.
 Bjerrum (1926). *K. danske vidensk. Selsk. Skr.* **7**, 9.
 Fargher & Pyman (1919). *J. chem. Soc.* **115**, 227.
 Guggenheim & Schindler (1934). *J. phys. Chem.* **38**, 533.
 Hartley (1935). *Trans. Faraday Soc.* **31**, 31.
 La Mer, King & Mason (1927). *J. Amer. chem. Soc.* **49**, 363, 410.
 Maeda (1937). *Bull. Chem. Soc., Japan*, **12**, 319.
 Neuberger (1937). *Proc. roy. Soc. A*, **158**, 68.
 Smith (1935). *J. biol. Chem.* **108**, 189.
 — (1936). *J. biol. Chem.* **113**, 473.
 Tiselius (1930). Dissertation, Upsala.
 — (1937). *Biochem. J.* **31**, 313.
 Weidenhagen & Herrmann (1935). *Ber. deutsch. chem. Ges.* **68**, 1960.
 Windaus & Knoop (1905). *Ber. deutsch. chem. Ges.* **38**, 1166.