LXII. NOTES ON REGULATOR MIXTURES, RECENT INDICATORS, ETC. II.

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In preparing the second edition of this chart I have taken the opportunity, in these notes, of attaching to it recent additions to the data which it is intended to present.

The first chart [1910] owed its origin almost entirely to a paper by Sörensen [1909, 2]: the accompanying matter was explanatory, and gave in addition a technique for applying the indicator method of Friedenthal and Salm [1907] to coloured fluids, without the use of neutral dyes.

On this occasion I have been able to avail myself of a later monograph by Sörensen [1912] and also one by Michaelis [1914]. In these are collected an invaluable store of information on the subject of indicators, solutions of standard reaction, the electrometric measurement of hydrogen ion concentration, and the properties of amphoteric electrolytes which form the foundations of so much biochemical work. In addition I have included the results of some determinations in these laboratories which are here published for the first time; and data from the general literature which are not to be found in either of the sources mentioned.

INDICATORS.

Confusion arises in descriptions of indicators and their relative practical utility because they are regarded by different authors from one or other of two essentially different points of view.

In earlier books, *e.g.* Cohn [1899] and Glaser [1901] and even in many contemporary papers indicators are described as "good" for certain titrations when their colour change occurs at a point where the reacting

substances are in chemically equivalent proportions. It is natural, therefore, that phenolphthalein should be described as "no good with ammonia," but admirable for titrating acetic acid with caustic soda.

There is, however, an alternative standard whereby an indicator may be judged, *i.e.* the fidelity with which it, by its tint, represents the [H] of a solution into which it is introduced.

From this standpoint these notes have been written and an indicator will be the more highly valued the less its accuracy is impaired by the presence of protein, neutral salts or other condition of practical expediency. It may be remarked in passing that phenolphthalein works perfectly well as a quantitative indicator of reaction over its sensitive range even in solutions containing ammonia. The following experiment may be cited:

20 cc. of Sörensen borate solution (NaH_2BO_3) containing phenolphthalein gave against the 0·10 N calomel electrode, with saturated KCl as connecting fluid, at 13°, a hydrogen E.M.F. 0·8630 volt ($P_H^+ = 9{\cdot}26$).

20 cc. 1 per cent. ammonium chloride solution containing the same amount of phenolphthalein + 0·95 cc. N NaOH gave a liquid having the same tint. Its hydrogen E.M.F. was 0·8620 volt at 14° ($P_H^+ = 9{\cdot}21$).

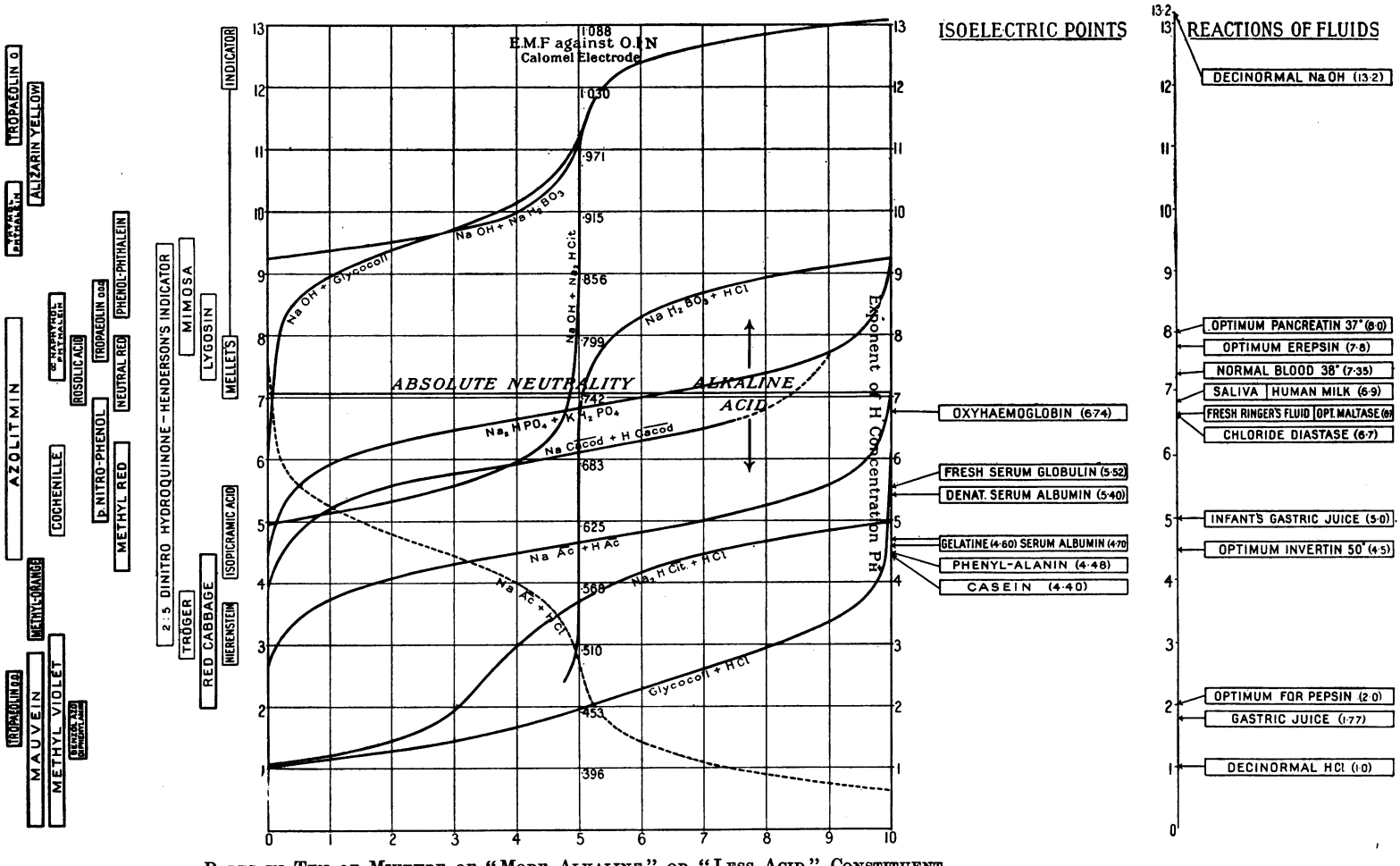
As an extreme example of those dyes which may give misleading and useless results when used as *indicators of reaction* may be mentioned *alizarin-sulphonic acid* which is even more accurate than methyl orange, when used to titrate distillates from the Kjeldahl process, especially when artificial light is used.

Adding equal quantities of it to 10 cc. each of the following Sörensen standard mixtures,

P_H^+	
7·66	(9 alk. phosphate + 1 acid phosphate) (5·26 borate + 4·74 HCl)
8·30	(9·74 alk. phosphate + 0·26 acid phosphate) (0·30 NaOH + 9·70 glycocoll) (6 borate + 4 HCl)
9·24	(1·68 NaOH + 8·32 glycocoll) (10 borate)
9·63	(2·74 NaOH + 7·26 glycocoll) (2·74 NaOH + 7·26 borate)
10·00	(4 NaOH + 6 borate),

it was seen that the dye behaved in an abnormal fashion, giving colours increasing in depth from yellow to brown, with increasing alkalinity, in the solutions containing boric acid. In the other solutions it gave purple colours

CHART OF HYDROGEN ION CONCENTRATION DATA



except in the case of the dotted curve ($NaAc + HCl$).

The Sörensen solutions are: 0·10N HCl; 0·10N NaOH; 7·505 g. glycocoll + 5·85 g. NaCl per litre; 11·876 g. $Na_2HPO_4 . 2H_2O$ per litre; 9·078 g. KH_2PO_4 per litre; 21·008 g. citric acid in 1 litre of 0·20N NaOH; 12·404 g. boric acid in 1 litre 0·10N NaOH. The other solutions are: 0·20N sodium acetate, acetic acid, sodium cacodylate, cacodylic acid. For details of plotting of dotted acetate-HCl curve, see notes.

increasing in intensity in a normal and regular fashion with the P_H^+ of the solution.

Such dyes as this, therefore, though they may be used for particular purposes under conditions thoroughly understood, merit no place in any list of approved indicators for $H^{\cdot}$ concentration measurement.

Sörensen made preliminary tests of over 100 indicators but discarded so many (pyrogallol-phthalein, azo-acid-blue, chrysamine, alizarinsulphonic acid, etc.) that only 36 find places in his tabulated results. Of these he was able to recommend 20 for general use. It is significant that neither cochenille, Congo-red, alizarin nor any litmus preparation is included in the list.

In the chart, I have inserted as many as possible of these 20 approved indicators—marked specially—and, in addition, litmus (azolitmin) because it is so much used, and several others which are of interest at present in view of their recent description or for other reasons.

The list of indicators here given, when added to those in the monograph by Sörensen [1912], brings complete to the end of 1914 the whole of the indicators of which notes have been published in the available literature. In cases where a sample of the material could be obtained a test has been made of its range of sensitiveness if this had not been already done. My thanks are due to those authors who have kindly sent me specimens of their preparations. Rectangles representing the sensitive range of some of these indicators are to be found in the chart grouped immediately on the left-hand side of the curves. Where a heavy line has been used in drawing the rectangle the indicator whose name is enclosed has been approved for the colorimetric method.

2 : 5-*Dinitroquinol* [Lawrence G. Henderson and Alexander Forbes, 1910]. This indicator, obtainable from Schuchardt, is extremely useful, as it gives at a glance the approximate reaction of any solution from $P_H^+ = 3$ to $P_H^+ = 9{\cdot}5$. Over this extremely long range it shows a steady change of colour. In dilute acid it is green. If the solution be made progressively more alkaline it commences to assume a yellowish tinge when $P_H^+ = 3$, at absolute neutrality it is a bright reddish brown; when $P_H^+ = 9{\cdot}5$ a purple tint is reached which undergoes no further change with the addition of caustic alkali. The change from brown to purple is very sharp between $P_H^+ = 8{\cdot}3$ and $P_H^+ = 9{\cdot}3$: so is that from green to brown in the region $P_H^+ = 3{\cdot}4$ to $4{\cdot}7$. The convenience of having at hand an indicator working over the ranges of methyl orange, litmus and phenolphthalein will be readily appreciated.

Di-o-hydroxystyryl ketone (*Lygosin*) $CO(CH:CH.C_6H_4.OH)_2$ [Ferenz Aron, Kolosvar, 1913]. The sodium salt of this indicator is marketed by the Zimmer Factory under the name "natrium lygosinatum." For the sample supplied to me I am indebted to Prof. Aron. I found the range of its change of colour from brownish yellow to green to be exactly that of α-naphthol-phthalein ($P_H^+ = 7{\cdot}3$ to $8{\cdot}7$)—a comparative experiment with both indicators using the same regulator mixtures confirms this. If more concentrated solutions of indicator are used the colour in alkaline solution is the brown-reddish tinge described by the author. Its behaviour was examined in the series of solutions detailed on page 629. It behaved quite normally except that it was bleached, losing colour entirely after a few hours, in every series *except* those containing borate. In solutions containing borate it appeared to retain its colour indefinitely.

1-*Oxy-naphtho-chinomethane* [M. Nierenstein, Bristol. Private communication]. A sample of this indicator was kindly submitted by Dr Nierenstein for examination and its sensitive range was determined. It is colourless in solutions $P_H^+ = 2{\cdot}7$, showing a gradual increase of purple colour to $P_H^+ = 3{\cdot}7$. It is similar therefore to some of the azo indicators described by Sörensen but is of a sharper colour change than any indicator previously described which changes in this region.

6-*Sulpho-α-naphthol*-1-*azo*-m-*hydroxybenzoic acid* [M. R. Mellet, Lausanne, 1910, 1911, 1913]. The sodium salt is a violet black powder very soluble in water. In excess of alkali it is cherry red passing on the addition of acid through blue violet to bright red. Its colouring power is intense, so that quite brilliantly coloured solutions can be used without loss of accuracy in the result. The orange blue change is observed over the range $P_H^+ = 7$ to $P_H^+ = 8$: the second change, violet to red, is seen in solutions comparatively very alkaline—$P_H^+ = 12$ to $P_H^+ = 13$. I am indebted to Prof. Dr Mellet, who informs me that his indicator is still under examination, for a sample with which I have been able to confirm his earlier results.

2:6-*Dinitro*-4-*aminophenol* (*Isopicramic Acid*) [Raphael Meldola and A. J. Hale, 1912]. An alcoholic solution of this indicator is used. From the results of titrations using solutions accurately prepared gravimetrically the authors concluded that "although useless for weak acids it is possibly better than methyl orange for alkalis, of equal sensitiveness to litmus, and better than methyl orange for carbonates."

Using a sample supplied to me by Prof. Meldola I found that in solutions

more acid than $P_H^+ = 4{\cdot}1$ its colour was pink and in solutions more alkaline than $P_H^+ = 5{\cdot}6$ its colour was uniformly yellow. At intermediate reactions the changes of tint are reminiscent of methyl orange.

A sulphonic acid $C_{14}H_{15}N_4SO_3H$ [Julius Tröger and W. Hille, Brunswick, 1903]. This indicator, soluble in water, is not perfectly stable, so that a fresh solution must be prepared from time to time. Like methyl orange it is destroyed rapidly by strong alkali. It is described by the authors as being more sensitive than helianthin (methyl orange) though in its scope of application and colourations it resembles it. The sample supplied to me by the author showed the following colour changes: $P_H^+ = 2{\cdot}78$ orange pink; 2·93 ditto, but much more intense; 3·12 pale yellowish pink; 3·42 very pale brown; 3·76 yellow; 3·92 primrose yellow; 4·00 and upwards greenish primrose yellow. The range of this indicator is entered on the chart as $P_H^+ = 2{\cdot}8$ to $P_H^+ = 3{\cdot}9$. With the same regulator mixtures the methyl orange range—using two samples, one "commercial" and the other purified by repeated recrystallisation—$P_H^+ = 3{\cdot}1$ to 4·4 was confirmed. The conclusions to be drawn from my measurements are that it differs little from methyl orange in sensitiveness, and that its sensitive range is more acid than that over which methyl orange shows its colour changes.

Alizarinmonosulphonic acid [Geo. E. Knowles, 1907; compare Glaser, 1901]. This indicator, to be found amongst those described by Glaser, was discarded by Sörensen [1909, 1, 2] as being useless for the Friedenthal and Salm colorimetric method. Knowles states that it is a good substitute for methyl orange and that it can be used by artificial light. In very dilute solution I found that a sharp colour change took place between $P_H^+ = 3{\cdot}7$ (yellow) and $P_H^+ = 4{\cdot}2$ (pink). A direct comparison showed that it was even more sensitive than methyl orange over this range. Its curious behaviour with borates was noted by Knowles, and has been referred to on page 629.

3-*Amino*-2-*methylquinoline* [O. Stark, Kiel, 1907] is described as an excellent indicator which can replace methyl orange.

Compound obtained when 1 *molecule diazotised* p. *nitroaniline* + 1 *molecule of* 2-*aminonaphthol*-5: 7-*disulphonic acid is boiled with benzaldehyde, hydrochloric acid and water* [J. R. Woods, 1905]. According to the author this indicator—colourless in acid; orange in alkali—is sensitive to CO_2. This suggests a range somewhere on the alkaline side of absolute neutrality.

Dimethyl brown [Emmanuel Pozzi-Escott, 1909]. Yellow in alkaline; brown in neutral and acid solutions.

An indicator of formula $C_{11}H_8O_4$ [H. J. H. Fenton, 1906]. This indicator, and derived substances which may also be used as indicators, would appear, from the author's statements, to be of greater importance as specific reagents for amines than instruments for H˙ concentration measurement.

Indicators from vegetable sources. The discovery of a valuable indicator in red cabbage and the continued use of litmus to-day suggest attention to the accounts of indicators from natural sources. Of those noted below I was able to examine the alcoholic extracts of radish skins and mimosa flowers.

Extract of red cabbage [L. E. Walbum, Copenhagen, 1913, 1, 2]. This indicator is valuable as it works accurately in the presence of proteins, neutral salts, toluene, etc. Its sensitive range is from $P_H^+ = 2$ to $P_H^+ = 4{\cdot}5$.

Radish skins [J. F. Sackur, 1910]. The radish skins were extracted with their own weight of 96 per cent. alcohol. The colour changes observed in this case in passing from normal acid to normal soda were so bewildering in number as to surpass Henderson's indicator in this respect. In addition, the colours changed rapidly although retaining their brilliance and at certain reactions showed a dichroism unequalled by any other solution I have seen not excluding eosin. The use of this extract as an indicator is attended with difficulty on account of the brilliance of these phenomena.

Mimosa flowers [Lucien Robin, 1904]. Yellow dye from mimosa flowers. Its colour change, sensitive range $P_H^+ = 7{\cdot}7$ to $9{\cdot}6$, and behaviour with borates are so strikingly reminiscent of turmeric as to suggest the identity of the colouring matter in the two cases. The two dyes tested with the solutions given on page 629 show that the colour changes of neither depend solely on the H˙ concentration even when borate is not present. In the borate tubes they show brown to black colours of increasing intensity with increasing alkalinity though after some hours this colour fades but at unequal rates, leaving the greatest intensity at $P_H^+ = 9{\cdot}24$.

Iris flowers [A. Ossendowsky, 1903]. Extract of iris flowers is red in the presence of acids; green in the presence of alkalis.

Black pansy flower [E. Pozzi-Escott, 1913]. Alcoholic infusion of crushed black pansy flower: red in the presence of mineral acids passing through bluish violet with successive alkali addition till it becomes green.

Juice of the blueberry [G. N. Watson, 1913]. Alkaline—olive green: acid—rose; "sensitive to CO_2. May be used instead of cochenille or litmus."

Inorganic indicators. There may also be mentioned two "inorganic" indicators descriptions of which have been found.

Cyanogen iodide [J. H. Kastle, 1903]. Cyanogen iodide, potassium iodide and starch are recommended as very sensitive to acids.

Bismuth oxyiodide [C. Reichard, 1912]. This indicator is reported as being not sensitive to CO_2, colourless in alkaline solution and in acid yellow.

SERIES OF MIXTURES OF STANDARD REACTION.

The hydrogen E.M.F.'s of these series have been in some cases very carefully determined. Except when the curves run steep vertically the mixtures they represent are "regulator mixtures," *i.e.* they may be subjected to addition of certain quantities of acids or bases with only minimal changes in [H].

The seven series originally worked out by Sörensen are plotted on the chart as before. The tables connecting the composition of individual mixtures of these series, their E.M.F.'s against the 0·1 N calomel electrode, and the P_H^+ values calculated therefrom are to be found in the sources mentioned [Sörensen, 1909, 1, 2]. Similar tables referring to the curves added to the original seven in this second edition of the chart are to be found below.

The abscissae of every curve on the chart refer to the volumes in ten of the mixture of the "more alkaline" or "less acid" constituent excepting only the curve which is drawn as a dotted line. This curve refers to mixtures made up in a different fashion as explained on page 637.

Cacodylic acid-sodium cacodylate mixtures. Michaelis and Davidsohn [1912] have employed mixtures of cacodylic acid and caustic soda to obtain solutions of high "reaction inertia" which are not so acid as those obtained by mixing acids of greater dissociation constants with their salts. The solutions are not very stable. The values given in the table below are only approximate but are sufficiently accurate to indicate the range over which these mixtures can be used. They were determined by the electrometric method in these laboratories at room temperature: saturated KCl was used as connecting fluid and no correction for contact potential was made.

Composition of mixture							E.M.F.	*t.*	P_H^+
10 cc.	0·2 N	cacodylic acid	+	0 cc.	0·2 N	sodium cacodylate	0·5575	14	3·86
9	,,	,,		1	,,	,,	0·6375	18	5·20
7	,,	,,		3	,,	,,	0·6675	16	5·76
5	,,	,,		5	,,	,,	0·6880	16	6·11
3	,,	,,		7	,,	,,	0·7090	16	6·48
1	,,	,,		9	,,	,,	?	—	?
0	,,	,,		10	,,	,,	—	—	—

The solutions were made by taking successive portions of 20 cc. of a 6·9 per cent. aqueous solution of cacodylic acid (0·5 N), adding 0, 1, 3 cc. of 0·5 N NaOH and then diluting to 50 cc. in each case [compare Michaelis, 1914, p. 186].

Mixtures of acetic acid and sodium acetate,—total acetate 0·20 *normal.* Solutions of this type give hydrogen potentials which change only slightly on dilution. They may be calculated approximately from their composition by a simple formula. Results of such calculations are given by Sörensen [1912] and Michaelis [1910, 1914]. The accurate experimental results for the particular series in which the total acetate is 0·20 normal are given herewith. In the original communication [Walpole, 1914, 1, 2] are given data relating to the extent to which the hydrogen potentials of these solutions change on dilution. The correction for diffusion potential for these solutions, whether diluted or not, is negative and in no case exceeds 1·5 millivolt. It is generally much less.

Acetic acid-sodium acetate mixtures,—total acetate 0·20 *normal.*

Composition of mixture						E.M.F. (fully corrected) against 0·10 N electrode	P_H^+
0·125 cc.	0·20 N	acetic acid +	9·875 cc.	0·20 N	sodium acetate	0·7138	6·518
0·25	,,	,,	9·75	,,	,,	0·6961	6·211
0·375	,,	,,	9·625	,,	,,	0·6853	6·024
0·5	,,	,,	9·5	,,	,,	0·6778	5·894
1	,,	,,	9	,,	,,	0·6593	5·574
1·5	,,	,,	8·5	,,	,,	0·6478	5·374
2	,,	,,	8	,,	,,	0·6393	5·227
2·5	,,	,,	7·5	,,	,,	0·6316	5·093
3	,,	,,	7	,,	,,	0·6256	4·990
4	,,	,,	6	,,	,,	0·6148	4·802
5*	,,	,,	5	,,	,,	0·6046	4·626
6	,,	,,	4	,,	,,	0·5947	4·454
7	,,	,,	3	,,	,,	0·5841	4·272
8	,,	,,	2	,,	,,	0·5712	4·047
9	,,	,,	1	,,	,,	0·5525	3·723
9·25	,,	,,	0·75	,,	,,	0·5450	3·592
9·5	,,	,,	0·5	,,	,,	0·5348	3·416
9·6	,,	,,	0·4	,,	,,	0·5290	3·315
9·7	,,	,,	0·3	,,	,,	0·5225	3·202
9·75	,,	,,	0·25	,,	,,	0·5193	3·147
9·8	,,	,,	0·2	,,	,,	0·5155	3·081
9·85	,,	,,	0·15	,,	,,	0·5105	2·994
9·9	,,	,,	0·1	,,	,,	0·5057	2·912
9·95	,,	,,	0·05	,,	,,	0·4995	2·804
10	,,	,,	—	,,	,,	0·4931	2·696

The mixture marked * is "standard acetate."

Hydrochloric acid-sodium acetate series,—total acetate 0·20 *normal—total sodium* 0·20 *normal.* This series, like the one preceding, has been thoroughly worked out. It has been extended, however, so that it may be conveniently considered in two parts. The first, obtained by adding 0, 1, 2....5 cc. of N hydrochloric acid to 5 cc. N sodium acetate and making up to 25 cc. with water in each case, is the same series as the acetic-acetate series just described except that NaCl is added to each solution in amount equivalent to the acetic acid present. The result of this is to have the total sodium as well as the total acetate 0·20 normal, and each solution is for that reason just a little more acid than the corresponding solution of the series preceding. The second part is obtained by adding 5, 6, 7....10 cc. N hydrochloric acid to 5 cc. N sodium acetate and making up to 25 cc. with water in each case, and these solutions contain increasing quantities from 0 to 0·2 N of

Hydrochloric acid-sodium acetate series,—total acetate 0·20 *normal—total sodium* 0·20 *normal.*

Composition of mixture	E.M.F. (fully corrected) against 0·10 N electrode	P_H^+
0·10 cc. N HCl + 5 cc. N sodium acetate + water to 25 cc.	0·7015	6·31
0·25 " " " " "	0·6762	5·87
1 " " " " "	0·6375	5·20
1·5 " " " " "	0·6235	4·95
2 " " " " "	0·6126	4·76
2·5 " " " " "	0·6019	4·58
3 " " " " "	0·5908	4·39
3·5 " " " " "	0·5793	4·19
4 " " " " "	0·5654	3·95
4·25 " " " " "	0·5564	3·79
4·5 " " " " "	0·5461	3·61
4·625 " " " " "	0·5389	3·49
4·725 " " " " "	0·5297	3·33
4·75 " " " " "	0·5274	3·29
4·85 " " " " "	0·5159	3·09
4·875 " " " " "	0·5129	3·04
4·975 " " " " "	0·4949	2·72
5 " " " " "	0·4902	2·64
5·097 " " " " "	0·4715	2·32
5·222 " " " " "	0·4550	2·03
5·25 " " " " "	0·4525	1·99
5·35 " " " " "	0·4442	1·85
5·475 " " " " "	0·4372	1·72
5·5 " " " " "	0·4362	1·71
6 " " " " "	0·4194	1·42
6·5 " " " " "	0·4090	1·24
7 " " " " "	0·4007	1·09
8 " " " " "	0·3904	0·91
9 " " " " "	0·3812	0·75
10 " " " " "	0·3750	0·65

hydrochloric acid in addition to acetic acid and sodium chloride with respect to each of which they are all 0·2 N.

It is necessary in preparing solutions near the middle of this series to standardise the sodium acetate and the hydrochloric acid solutions against each other carefully—and an electrometric method has been devised for this purpose—and in their use to recognise that although the range of possible change of hydrogen potential under ordinary conditions is minimal the "reaction inertia" of such solutions is small. The figures below are taken from the paper referred to.

Care must be taken not to consider more or less concentrated solutions of weak acids as regulator mixtures. The addition of very little strong base leads to the freeing of so many of the anions of the weak acid that its dissociation is reversed with the consequent fall in [H], *e.g.* 1 cc. of ·01 N NaOH added to 20 cc. 0·2 N acetic acid will change the P_H^+ value by 0·11.

As the addition of strong base proceeds, however, the reaction inertia rapidly increases until an excellent regulator mixture is obtained. The shape of each of the curves is, in fact, an index of the reaction inertia of the individual members of the series of mixtures the curve represents: the tangent to the curve at any point might well be taken as the measure of the reaction inertia of the mixture represented by that point if a factor were included representing the normality of its constituents so that mixtures of different series should be comparable one with another.

ISOELECTRIC POINTS.

These data are taken from the papers of Michaelis and his collaborators. The actual figure taken in each case is that which had been obtained by the transport method.

Oxyhaemoglobin, $P_H^+ = 6{\cdot}74$ [Michaelis and Takahashi, 1910; Michaelis and Davidsohn, 1912].

Fresh serum globulin, $P_H^+ = 5{\cdot}52$ [Michaelis and Rona, 1910, 1, 2].

Denaturated serum albumin, $P_H^+ = 5{\cdot}40$ and serum albumin, $P_H^+ = 4{\cdot}7$ [Michaelis and Davidsohn, 1911, 2].

Gelatin, $P_H^+ = 4{\cdot}60$ [Michaelis and Grineff, 1912].

Caseinogen, $P_H^+ = 4{\cdot}4$ [Michaelis and Rona, 1910, 1, 2; Michaelis and Pechstein, 1912].

Phenylalanine, $P_H^+ = 4{\cdot}48$ [Michaelis, 1912].

OPTIMUM REACTIONS FOR ENZYMES.

These data, and much accompanying and explanatory matter, are for the most part collected in Michaelis' monograph [1914]. See also Sörensen [1912]. They do not all appear on the chart for lack of space.

The following references are to original papers.

Tryptic enzymes [Kurt Meyer, 1911; Palitsch and Walbum, 1912; Michaelis and Davidsohn, 1911, 1].

Invertin [Sörensen, 1909, 1, 2; Michaelis and Davidsohn, 1911, 3].

Peptic and Ereptic enzymes [Rona and Arnheim, 1913; Sörensen, 1909, 1, 2; Michaelis and Davidsohn, 1911, 4].

Lipase [Davidsohn, 1912, 1, 2; Rona and Bien, 1914, 1, 2].

Catalase [Sörensen, 1909, 1, 2; Michaelis and Pechstein, 1913; Waentig, Percy, and Steche, 1911].

Maltase [Michaelis and Rona, 1913, 1, 2].

Diastase [Michaelis and Pechstein, 1914; Norris, 1913, 1, 2].

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Saliva [Michaelis and Pechstein, 1914].

Gastric Juice [Fränkel, 1905; Michaelis and Davidsohn, 1910].

Infants' Gastric Juice [Allaria, 1908; Davidsohn, 1912, 2].

Intestinal Juice [Auerbach and Pick, 1912].

Blood [Hasselbalch and Lundsgaard, 1912].

Human Milk [Davidsohn, 1913].

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