STUDIES ON SOIL REACTION. II.

THE COLORIMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION IN SOILS AND AQUEOUS SOIL EXTRACTS. (PRELIMINARY COMMUNICATION.)

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The methods used for the determination of $-\log[H^{\cdot}]$ fall naturally into two groups:

(*a*) Electrometric, and
(*b*) Colorimetric methods.

Electrometric methods were first introduced into analytical practice in 1897 by Böttger(1) who determined the neutral point in titrating acids with alkalis by using a gas chain; subsequent improvements were made by Hildebrand(16), Cumming and Gilchrist(10), Hasselbalch(15), W. M. Clark(6), Michaelis(19), Walpole(28,29) and others. The method has been applied to the measurement of $[H^{\cdot}]$ of biological fluids with considerable success*. It was first applied to the measurement of $[H^{\cdot}]$ of soil suspensions by G. Fischer(12) in Germany in 1914 and subsequently in America by Sharp and Hoagland(23), and by Gillespie(13,14) and his co-workers.

The colorimetric method was introduced by Sörensen(24,25) in 1909, and was improved and applied to biological fluids by Sörensen(26), by Palitzsch(21), and in this country by Walpole(28). Basing their work on the same principles but using a different set of indicators and of buffer mixtures Clark and Lubs(7,8) have further improved the method. Gillespie and his co-workers(13,14) applied both methods to soils with excellent agreement in spite of the fact that they employed aqueous soil extracts in the colorimetric method, but mixtures of soil and water in the electrometric method, soil extracts alone being unsuitable in the latter case as they are relatively poor in buffer action. Further the NO'_3-ions were reduced to NH_3 by the hydrogen of the electrode, thus

* See detailed account of whole method in Michaelis, L., *Die Wasserstoffionen-Konzentration*, J. Springer, Berlin, 1914.

rendering a constant potential impossible of attainment, and in some cases even changing the reaction of the whole fluid to indicators. This is the only systematic comparison so far made between the two methods as applied to soils and it deserves repetition on account of its fundamental importance*.

The Colorimetric Method.

The colorimetric method of determining hydrogen-ion concentration depends on the fact that for every indicator there is a particular zone of $[H^{\cdot}]$ or of $-\log[H^{\cdot}]$ within which its colour changes but gradually. A large number of indicators are known each having its own particular zone of change which differs from that of most other indicators. Thus methyl red changes its colour gradually from yellow through brown to red within the zone of $-\log[H^{\cdot}]$ of 6·0 to 4·4; phenol phthalein changes colour between $-\log[H^{\cdot}]$ 10 to 8·5, litmus between 8 and 5, methyl orange 4·0 to 3·0. Moreover many of these ranges overlap so that the tints produced on a particular indicator by a particular solution, A, allow of direct comparison of its $-\log[H^{\cdot}]$ with reference to that of a standard solution B. At certain points where the ranges of two or more indicators overlap the results can be checked by using more than one indicator for the determination of the same $-\log[H^{\cdot}]$. The method is not absolute; it does not really measure the $-\log[H^{\cdot}]$ of a solution but only shows that this function is identical with that of a particular standard. Ultimately the absolute $-\log[H^{\cdot}]$ of the standards must be determined by the more fundamental electrometric method. If the standard solutions are strongly "buffered" by the presence of reaction regulators they will maintain their $-\log[H^{\cdot}]$ unchanged for considerable periods since small quantities of impurities from the air, glass, or slight mould growth, etc., have but little effect. Further, the standard buffer solutions are generally easily prepared and once made up and their $-\log[H^{\cdot}]$ measured electrometrically they can easily be renewed without making fresh electrometric determinations. Where a

* Rice and Osugi(22) showed that the inversion of sucrose by soils is invariably greater than that of soil extracts, results which apparently militate against the validity of the electrometric method. These authors however did not prove that nothing else was present in soils capable of inverting sucrose except hydrogen-ions so that the validity of their method rests upon a somewhat precarious foundation. On the other hand the electrometric method would appear to measure only the $[H^{\cdot}]$ of the liquid aqueous phase, the work of Bovie (3) indicating that any hydrogen-ions adsorbed on the surface of the solid phase play no part in the electrical potential generated. This point however requires further work as it would appear to present difficulties.

considerable degree of accuracy is required however the standard buffer solutions should be examined electrometrically at frequent intervals, but for routine work or where fairly accurate comparisons are required without a high degree of accuracy in the absolute values the solutions need not necessarily be checked electrometrically but should be renewed at frequent intervals and the new ones checked against the old.

The essentials of the method are: first a set of indicators that can be used to cover the required ranges of $-\log[H^{\cdot}]$ and, secondly, a set of standard buffer solutions for use within these ranges.

The standard buffer solutions are generally mixtures of some acid and its alkali salt. Clark and Lubs (7, 8) standardised the following series:

Acid potassium phthalate and hydrochloric acid.
Acid potassium phthalate and sodium hydroxide.
Acid potassium phosphate and sodium hydroxide.
Boric acid, potassium chloride and sodium hydroxide.

This series possibly has certain advantages over the older ones of Sörensen (24, 25, 26), Palitzsch (21) and Walpole (28). The simplicity and ease of preparation is a real advantage in ordinary laboratory routine. Only four substances are involved to cover a range of $-\log[H^{\cdot}]$ from 2·2 to 10·0. The technique of the preparation is simple especially in comparison with that of the acetate mixtures of Walpole (29)*. Both acid potassium phthalate and acid potassium phosphate have no water of crystallisation and can therefore be oven-dried at 110° C. And only one alkaline substance is involved and therefore only one that need be protected against the CO_2 of the air.

As will be seen from Fig. 1 the phthalate and phosphate curves and the phosphate and borate curves overlap, hence at $-\log[H^{\cdot}] = 5{\cdot}8$, 6·0 and 6·2, and 7·8 and 8·0 we get two series of solutions of the same $-\log[H^{\cdot}]$ which serve as very useful checks on the accuracy with which the standard solutions have been made up.

Acid potassium phthalate is also excellent for standardising the sodium hydroxide†, and hence indirectly the hydrochloric acid which can further be checked by precipitation as AgCl. The potassium phosphate, potassium chloride and boric acid can all be accurately weighed.

* In this case in particular the elaborate method of purification and the difficulty in really preventing mould growth are serious troubles; in the latter respect the glycocoll mixtures are no better.

† Acid potassium phthalate is now on the market for this purpose, but in spite of its high price should *always* be recrystallised once before use at a temperature above 20° C. Below this temperature the so-called tri-phthalate separates out.

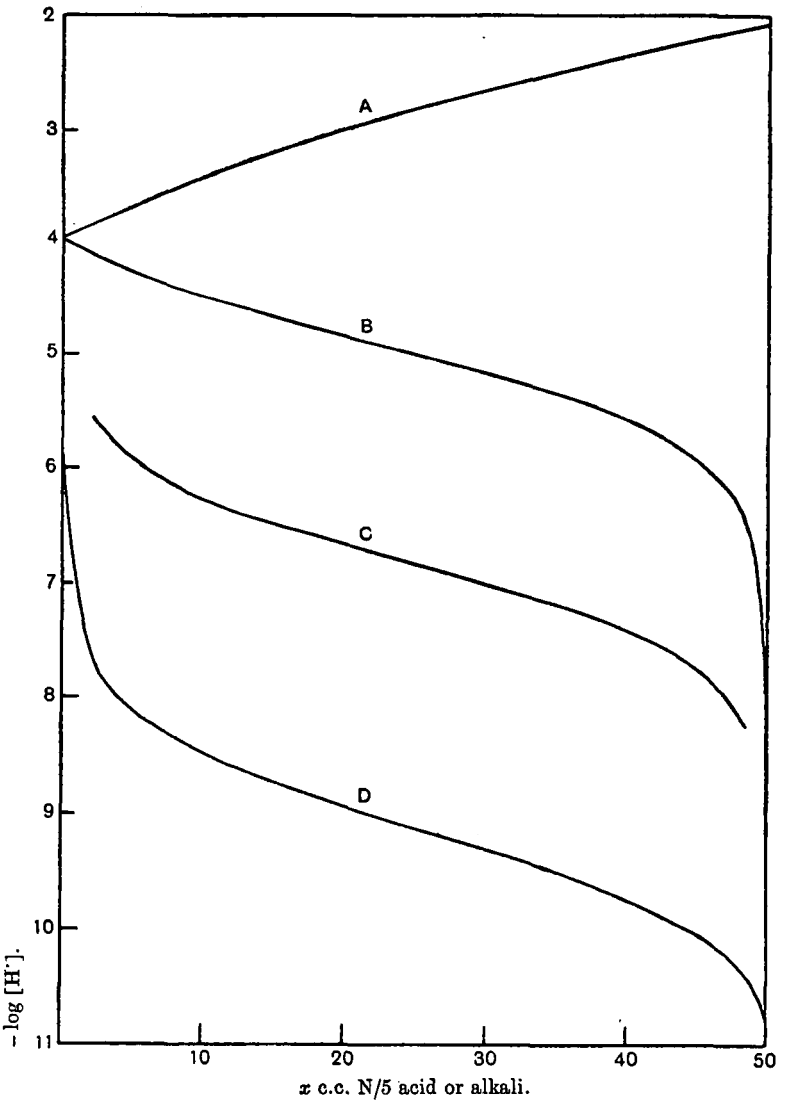

Fig. 1. Showing variation in $-\log [H^{\cdot}]$ of standard buffer solutions with amounts of HCl or NaOH added.

A. 50 c.c. M/5 H-K-Phthalate + x c.c. M/5 HCl, the whole diluted to 200 c.c.
B. 50 c.c. M/5 H-K-Phthalate + x c.c. M/5 NaOH, the whole diluted to 200 c.c.
C. 50 c.c. M/5 KH_2PO_4 + x c.c. M/5 NaOH, the whole diluted to 200 c.c.
D. 50 c.c. M/5 (H_3BO_3 + KCl) + x c.c. M/5 NaOH, the whole diluted to 200 c.c.

A slight disadvantage this series possesses in comparison with the older ones: in standard buffer mixtures the $-\log[H^{\cdot}]$ depends entirely on the ratio of the acid to base. Sörensen used Na_2HPO_4 as the base in his mixtures and Palitzsch used $Na_2B_4O_7$ in his borate mixtures. In Clark and Lubs' series the base is the much stronger NaOH, so that very accurate standardisation is necessary to avoid serious errors at the alkaline end of the series.

PREPARATION OF STANDARD BUFFER SOLUTIONS.

The mode of preparation of the standard solutions was essentially that given by Clark and Lubs(7,8). The following stock solutions were used:

M/5 HCl, M/5 KH_2PO_4, M/5 $HKC_8H_4O_4$, M/5 H_3BO_3 with M/5 KCl, and M/5 NaOH.

The water used throughout the investigation was "conductivity" water made according to the directions of Bourdillon(2)*. All weights, burettes, pipettes and measuring flasks were calibrated and in all cases the purest salts obtainable were purchased. The HCl was purified by diluting pure concentrated HCl to 20 per cent. and distilling. An M/5 stock solution was made up and standardised by the AgCl method and checked with the standard soda which had been standardised by Dodge's method described below.

The potassium chloride was recrystallised three times from "conductivity" water and dried in a hot air oven at 120° C. for two days. An M/5 solution contains 14·912 gms. KCl per litre.

Acid potassium phthalate ($HKC_8H_4O_4$) was recrystallised three times from "conductivity" water, the crystals being drained with suction on a Buchner funnel after each crystallisation. The salt was dried by heating in an air oven at 110–115° C. until after cooling in a desiccator over $CaCl_2$ the weight remained constant. An M/5 solution contains 40·828 gms. $KHC_8H_4O_4$ per litre.

Acid potassium phosphate (KH_2PO_4) was recrystallised three times

* The "conductivity" water made by the Bourdillon still is of exceptionally good quality. According to Bourdillon seven or eight litres can be obtained by a single distillation from ordinary tap water of a conductivity of less than 0·2 gemmho ($=0{\cdot}2\times10^{-6}$ reciprocal ohms). The present writer was not in a position to measure its conductivity but always measured the $-\log[H^{\cdot}]$ colorimetrically of every fresh supply, and generally daily. This was rarely found to be less than 6·8 if the measurement was carried out as rapidly as possible. The water however was so sensitive to the action of CO_2 of the air that the $-\log[H^{\cdot}]$ fell rapidly on exposure, reaching in the course of a few seconds the value of about 6 or less.

from conductivity water, the crystals being drained with suction on a Buchner funnel after each crystallisation and dried by heating in an air oven at 110–115° C. to constant weight. An M/5 solution contains 27·232 gms. KH_2PO_4 per litre. (As a rough test of purity the solution should be distinctly red to methyl red and distinctly blue to brom phenol blue.)

Boric acid was recrystallised three times from "conductivity" water. It was air-dried in thin layers between filter paper (as it loses "water of constitution" above 50° C.) and finally dried to constant weight in thin layers in a desiccator over $CaCl_2$ for several weeks. An M/5 solution contains 12·4048 gms. boric acid and also 14·912 gms. KCl. The borate curve overlaps the phosphate curve and the object of having the solution M/5 to KCl as well as to boric acid is to make the salt content comparable with the salt content of the phosphate solutions as it is well known that neutral salts themselves have a distinct, though small, effect on the colours of indicators.

The sodium hydroxide is the most difficult of the series to obtain pure. It was made as follows: 100 gms. of the purest NaOH were dissolved in 100 c.c. "conductivity" water in a conical flask. The mouth of the flask was loosely corked with a cork covered with tin foil, and allowed to stand over night. Most of the carbonate settles and the solution was filtered as follows: a "hardened" filter paper on a Buchner funnel was treated with a warm 50 per cent. solution of NaOH for a few minutes. The NaOH solution was decanted off and the paper washed first with absolute alcohol, then with diluted alcohol, and finally with conductivity water. At the end of the process gentle suction was applied until most of the water had evaporated but not to the extent that the paper began to curl. The concentrated alkali was then poured upon the middle of the paper, spread with a glass rod so that the paper under gentle suction adhered well to the funnel and the solution was then drawn through by increasing the suction. The clear filtrate was diluted (after rough calculation) to a strength of about N, 10 c.c. were withdrawn and titrated with standard acid and from this preliminary standardisation the dilution required to bring the concentration down to M/5 was calculated. This dilution was made and, with as little exposure as possible, the whole was transferred to a large bottle* fitted with a calibrated burette and soda lime guard tubes.

* This bottle should either be an *old* one, preferably one that has been used for years for storing soda; or if new should be thickly coated on the inside with paraffin wax, about 1 lb. to a five litre bottle.

The soda was then accurately standardised by Dodge's(11) method, using the purified acid potassium phthalate. Several portions of $KHC_8H_4O_4$ of about 1·6 gms. were weighed out and each portion was dissolved in about 20 c.c. conductivity water to which was added four drops of phenol phthalein solution. A stream of CO_2-free air was drawn through the solutions (or the CO_2 can be boiled off) which were then titrated with the soda till a faint but distinct and permanent pink colour developed.

Five or six litres of each of these M/5 stock solutions were prepared and the phosphate and phthalate solutions were protected against mould growth by the addition of a little calomel.

From these stock solutions the standard buffer solutions were made up according to the table below. 200 c.c. of each solution were made and kept in a stoppered bottle. In the case of the borate mixtures the bottles were paraffined on the inside.

Table I. *Composition of mixtures giving* $-\log[H^{\cdot}]$ *values at intervals of* 0·2.

A. *Phthalate-HCl mixtures*[1].

$-\log[H^{\cdot}]$	2·20	2·40	2·60	2·80	3·00	3·20	3·40	3·60	3·80
x c.c. M/5 HCl	46·70	39·60	32·95	26·47	20·32	14·70	9·90	5·97	2·63

B. *Phthalate-NaOH mixtures*[2].

$-\log[H^{\cdot}]$	4·00	4·20	4·40	4·60	4·80	5·00	5·20	5·40	5·60	5·80	6·00	6·20
x c.c. M/5 NaOH	0·40	3·70	7·50	12·15	17·70	23·85	29·95	35·45	39·85	43·00	45·45	47·00

C. *K-H_2-Phosphate-NaOH mixtures*[3].

$-\log[H^{\cdot}]$	5·80	6·00	6·20	6·40	6·60	6·80	7·00	7·20	7·40	7·60	7·80	8·00
x c.c. M/5 NaOH	3·72	5·70	8·60	12·60	17·80	23·65	29·63	35·00	39·50	42·80	45·20	46·80

D. *Boric Acid, KCl-NaOH mixtures*[4].

$-\log[H^{\cdot}]$	7·80	8·00	8·20	8·40	8·60	8·80	9·00	9·20	9·40	9·60	9·80
x c.c. M/5 NaOH	2·61	3·97	5·90	8·50	12·00	16·30	21·30	26·70	32·00	36·85	40·80

[1] 50 c.c. M/5 H-K-Phthalate + x c.c. M/5 HCl, the whole diluted to 200 c.c.
[2] 50 c.c. M/5 H-K-Phthalate + x c.c. M/5 NaOH, the whole diluted to 200 c.c.
[3] 50 c.c. M/5 KH_2PO_4 + x c.c. M/5 NaOH, the whole diluted to 200 c.c.
[4] 50 c.c. M/5 (H_3BO_3 + KCl) + x c.c. M/5 NaOH, the whole diluted to 200 c.c.

The consistency of the series was always tested by checking the phthalate-NaOH mixtures of $-\log[H^{\cdot}]$ 5·8, 6·0 and 6·2 against the phosphate-NaOH mixtures of the same $-\log[H^{\cdot}]$ using brom cresol purple as indicator, and the phosphate-NaOH mixtures of $-\log[H^{\cdot}]$ 7·8 and 8·0 against the boric acid-NaOH mixtures of the same $-\log[H^{\cdot}]$ using phenol red as indicator.

The standard buffer solutions will not keep indefinitely but need not be made up weekly, as recommended by Clark and Lubs(6), if a trace of calomel is added. Such solutions keep easily for considerable periods although it is inadvisable in the absence of electrometric control to keep them in use for longer than a month or six weeks. In every case newly made up standards should be compared colorimetrically with those about to be discarded in order to see whether any appreciable alteration in $-\log[H^{\cdot}]$ has occurred during the period of use. The writer was never able to detect any such change.

Indicators used.

Of the many indicators studied by Clark and Lubs the following series* was recommended by them to cover the whole range of $-\log[H^{\cdot}]$ from 1·2 to 9·8:

Table II. *List of indicators.*

Indicator	Common name	Concentration %	Colour change	Range of $-\log[H^{\cdot}]$
Thymol sulphone phthalein (acid range)	Thymol blue	0·04	Red-yellow	1·2–2·8
Tetra-bromo-phenol sulphone phthalein	Brom phenol blue	0·04	Yellow-blue	3·0–4·6
O-carboxy benzene-azo-dimethyl aniline	Methyl red	0·02	Red-yellow	4·4–6·0
O-carboxy benzene-azo-di propyl aniline	Propyl red	0·02	Red-yellow	4·8–6·4
Di-bromo-o-cresol sulphone phthalein	Brom cresol purple	0·04	Yellow-purple	5·2–6·8
Di-bromo-thymol-sulphone phthalein	Brom thymol blue	0·04	Yellow-blue	6·0–7·6
Phenol sulphone phthalein ...	Phenol red	0·02	Yellow-red	6·8–8·4
O-cresol sulphone phthalein...	Cresol red	0·02	Yellow-red	7·2–8·8
Thymol sulphone phthalein (alkaline range)	Thymol blue	0·04	Yellow-blue	8·0–9·6
O-cresol phthalein	Cresol phthalein	0·02	Colourless-red	8·2–9·8

Of this series propyl red, cresol red and cresol phthalein are not absolutely necessary as the remaining six form a complete overlapping series in themselves. In the present investigation therefore the former were not used.

* For their preparation and purification see Lubs and Clark(18), and for a critical study of their usefulness as indicators see Clark and Lubs (8). These indicators are now on the market.

The methyl red solution was made by dissolving 0·1 gm.* in 300 c.c. re-distilled alcohol and diluting to 500 c.c. with conductivity water.

The other indicators were all used in aqueous solution as the mono-sodium salts: brom cresol purple should dissolve easily in 1·5 equivalents of soda, the others in 1·1 equivalents. Stock solutions were prepared of 0·6 per cent. strength in the case of phenol red and of 1·2 per cent. strength in the case of the others. Such stock solutions are now on the market, but it is always best to make up the solutions oneself. In fact for accurate work it is very necessary to eliminate every trace of the acetic acid used in the preparation or purification, otherwise the indicator requires more than 1·1 equivalents† of base for its solution, and further the sodium acetate produced (different in amount according to the degree of purity of the material) may exert appreciable and variable "salt action‡" on the colour changes produced by the various standard and other solutions to be tested. By making up the stock solutions oneself one has a useful check on the purity of the indicators supplied.

Before using, 10 c.c. of each of these solutions (except of course the methyl red which is ready for use) should be diluted to 300 c.c. with water to make the solutions used in the tests.

Of the six indicators used in this investigation two, *viz.* brom phenol blue and brom phenol purple, show considerable dichromatism under ordinary conditions. With suitable precautions this does not present any difficulties when the indicators are used in daylight, but real difficulty occurs in using them for colorimetric comparisons in ordinary

* The methyl red of commerce sometimes contains acetate from which it must be freed as it is one of those indicators the colour change of which is affected by "neutral salt action." The methyl red was recrystallised from toluene.

† 1·5 equivalents in the case of brom cresol purple.

‡ In determining $-\log[H^{\cdot}]$ electrometrically and colorimetrically differences have been frequently observed between the two methods which have been traced to the influence of proteins or of neutral salts on the colour changes of the indicators. Some indicators are to some extent precipitated or adsorbed by protein or colloid bodies, *e.g.* congo red by the casein of milk. Such an effect will result not only in a decrease of intensity of colour but in the case of dichromatic indicators and in all cases of turbid or slightly coloured media an alteration in quality of the colour as well. This is the so-called *protein effect.* Many salts exert a similar effect on indicators which has been discussed at some length by Michaelis and Rona (20), by Sörensen (24, 25), and especially by Sörensen and Palitzsch (27). The mechanism of this *salt action* is not understood and in general such salt errors cannot be eliminated although in certain specific cases they can be estimated and empirical corrections applied as in the work of Sörensen and Palitzsch (27) on sea water. According to Clark and Lubs (8) the sulphone phthalein indicators have very small salt errors. See also Brightman, Meacham and Acree (4).

electric light. Daylight is rich, while ordinary electric light is poor, in blue rays, so that brom phenol blue and brom cresol purple will appear blue in daylight and reddish in the electric light, thereby introducing difficulties especially in the case of turbid or slightly coloured solutions. Most of the determinations recorded in this paper were carried out in daylight. All the indicators of the sulphone phthalein series exhibit dichromatism to a certain extent and under certain conditions. Also thymol blue and brom thymol blue, changing as they do from yellow to blue, show much greater contrasts when viewed in light rich in blue rays, so that when any of these four indicators had to be used in artificial light the light of the mercury vapour lamp was invariably employed.

On the other hand methyl red shows up better in the ordinary electric light (rich in red) than in the light of the mercury lamp (poor in red) and phenol red has its incipient dichromatism brought out and made more pronounced in the light of the mercury lamp which was not therefore used with these two indicators.

Method of using Colour Standards.

The actual colour standards were made up as follows: a series of test tubes 4 inches by $\frac{5}{8}$ inch were selected so that the internal diameters were exactly the same throughout the series. 10 c.c. of standard buffer solution were transferred to each test tube, four drops of the indicator solution added to each*, the test tubes were carefully shaken with a rotary motion, closed by rubber caps and stored in a large test tube rack. These solutions have to be renewed at least once a week as the indicators at this small concentration tend to fade in sunlight especially at the paler end of each series of standards, the yellow and brownish ends of the methyl red series being particularly bad in this respect. In this case also each freshly made series was compared colorimetrically with the previous one.

Biological fluids and soil extracts present two main difficulties: the interfering effects of the turbidity and of the natural colour of the fluid. These were overcome by a slight modification of Walpole's(28) method. A small white test tube rack was made to hold six test tubes in two rows of three each. A wooden partition separated each pair from the others in such a way that no light could pass through except actually through the test tubes and the coloured solutions contained

* In the case of methyl red six drops were used as the size of the drop in this case was much smaller than with the other indicator solutions owing to the reduced surface tension due to the high alcohol content.

in them. The test tubes A, B and C contained 10 c.c. each of the solution whose $-\log[H^{\cdot}]$ was to be measured and which was generally turbid and often slightly yellowish or brownish in colour; while E contained 10 c.c. of distilled water. D and F were two of the colour standards with $-\log[H^{\cdot}]$ differing by 0·2. Four drops of indicator solution (or six drops in the case of the methyl red) were added to the solution in B

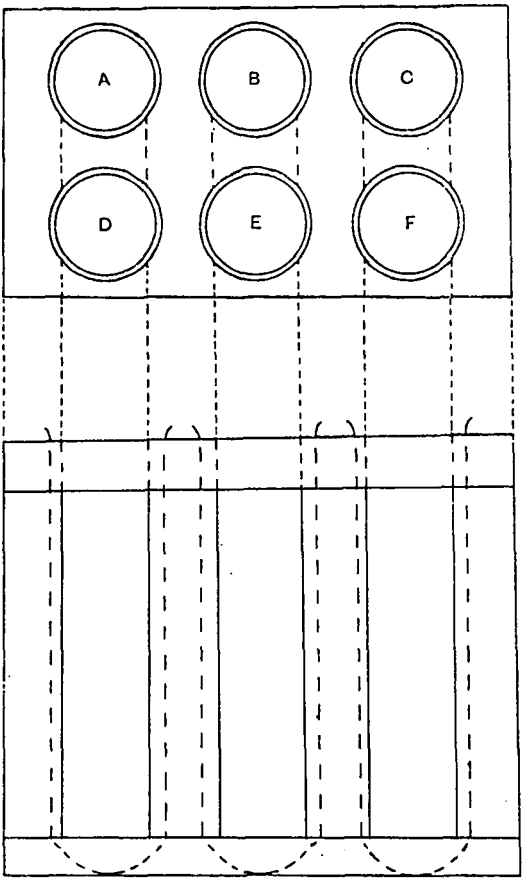

Fig. 2.

and the colour compared with those of D and F. By this arrangement the colour comparison could be fairly accurately carried out without any difficulty and with very little disturbing effect due to turbidity or natural colour. It will be noticed that in each of the three pairs of tubes the light must pass through the same total amount of liquid, of indicator, of turbidity and of natural colour, any disturbing effects due to

the last two factors being compensated for. The tubes *A*, *B*, *C* and *E* were closed with rubber caps similar to those on *D* and *F* to prevent any light from entering from above; if this occurred such light would be scattered by the particles in suspension and would be likely to affect the tint of an indicator showing dichromatism. A white paper or cardboard screen pinned to the front of the rack and just low enough to cut off the menisci from view is also desirable and has a sensible effect on the accuracy with which the colours can be matched or compared. With turbid liquids and especially indicators showing dichromatism such an arrangement possibly has an advantage over the Walpole tintometer and other types in which the light enters at the bottom and passes up the length of two superimposed tubes, in that errors due to dichromatism are very much reduced when the solutions are viewed in thin layers. There is an advantage also in using three pairs of tubes rather than two because the comparison tubes *D* and *F* can be so chosen that the colour of the solution to be tested (in *B*) is intermediate between them. With a little practice it is easy to judge whether the colour in *B* is exactly midway between that of *D* and *F* or whether it is more nearly that of *F* or *D*, so that, by a kind of visual interpolation, one can estimate the $-\log[H^{\cdot}]$ of the solution in *B* to 0·05 with a fair degree of accuracy.

As the approximate $-\log[H^{\cdot}]$ of the solution to be tested is generally not known preliminary tests were always made in test tubes of portions of the solutions with different indicators so as to find out the correct indicator to use. It was then merely a matter of finding a pair of standards such that one has a darker tint and one a lighter than the solution to be tested when all three are viewed side by side in the colorimeter. In all cases where two indicators overlap measurements were made with both indicators separately. There was generally good agreement except in those cases (of which there were few) where the turbidity or natural colour was so great that only approximate measurements of $-\log[H^{\cdot}]$ could be made. In such cases however the measurements were also made by the dilution method; that is the solution to be tested was diluted with conductivity water five or ten times and the $-\log[H^{\cdot}]$ measured in the usual way against standard solutions which had been themselves diluted to the same extent*.

* It can be shown theoretically as well as demonstrated that at the concentrations commonly employed in colorimetric work diluting ten times has a negligible effect on the $-\log[H^{\cdot}]$ of the solution. The present writer could detect no measurable effect on the colour of an indicator by diluting the standard solution ten times before adding the indicator.

The soil samples were taken with the usual precautions from the Rothamsted Experimental Grass Plots on the same day in August 1919; they were air dried, passed through the 3 mm. sieve (round holes) and stored in bottles.

Two courses were then open; $-\log[H^{\cdot}]$ measurements could be made on the filtered extracts or on the centrifuged extracts. The former were almost perfectly clear liquids, while the centrifuged extracts were always more or less turbid. Little is definitely known as to the effect the filter may have on the $[H^{\cdot}]$ of a solution passed through it, although some effect of adsorptive or other factors might be expected. Further it is uncertain whether turbidity has any effect on the $-\log[H^{\cdot}]$ of an extract other than that compensated for in the type of colorimeter used. It has been assumed generally that no such effects occur and the assumption has been supported in the case of culture media by the careful electrometric and colorimetric comparisons of Clark and Lubs and in the case of soils by the work of Gillespie and Hurst*.

In the case of extracts filtered through paper or Pasteur-Chamberland thimbles it was found that as filtration proceeded the acidity of the filtrate increased so that the first portions of the filtrate always showed a larger $-\log[H^{\cdot}]$ than the later portions collected; while all gave different values from the centrifuged extracts. In consequence of this determinations were always made on the centrifuged extracts and in the preliminary work the procedure was as follows: 50 gms. soil (3 mm. sample) were mixed with 100 c.c. conductivity water in a stoppered bottle and shaken in an end-over-end shaker for an hour. They were allowed to stand a few minutes and the supernatant liquid decanted off and centrifuged for ten minutes at a speed of 3000 revs. per minute. Determinations were then made on the centrifugate as described above.

The results of preliminary experiments seemed to indicate little difficulty and perfectly definite values for $-\log[H^{\cdot}]$ were obtained, thus in a general way apparently confirming the results of other workers.

DETERMINATION OF LIME REQUIREMENTS COLORIMETRICALLY.

It was decided to extend the work so as to measure lime requirements as well as reaction alone. This could be done by adding varying amounts of calcium oxide to a series of mixtures of soil with twice its weight of water, shaking for an hour and measuring the $-\log[H^{\cdot}]$ after centrifuging. By plotting the values and interpolating at $-\log[H^{\cdot}] = 7{\cdot}07$ the amount of lime required to bring the reaction

* See however later, p. 64.

Table III, A. *Samples of considerable Lime Requirements.*

Plot No. → c.c. N/5 $Ba(OH)_2$ added	9 (limed)	9 (unlimed)	10 (limed)	11–1 (limed)	11–2 (limed)	4–2 (limed)	18
0	5·70 –	5·10	5·80 +	5·60 +	5·60 +	5·20 –	5·40 +
5	—	—	—	—	—	5·80 +	—
10	6·30	5·45	6·75	6·10	6·30 +	6·80 –	6·70 +
15	6·50 –	—	7·40 –	6·70 –	6·80	7·60 +	7·40
20	6·70 –	6·00 –	8·40	7·00 –	7·20	8·45	8·00 +
25	—	—	—	7·30	7·60 +	—	—
30	7·40	7·40	—	—	8·40	—	—
% CaO required to make $-\log [H^{\cdot}] = 7·07$	0·295	0·317	0·129	0·244	0·207	0·129	0·141

Table III, B. *Samples* of low Lime Requirements.*

Plot No. → c.c. N/5 $Ba(OH)_2$ added	1	2	3	4–1	7	8	13	16	19	20
0	6·00 –	6·70	6·70 –	6·60 –	6·60	6·60 –	5·50 +	6·70 –	5·80	6·10
0·5	—	7·00 –	7·10 –	—	7·00 +	—	—	7·10	—	—
1·0	—	7·40 +	—	7·20	7·50 –	7·20 +	—	—	—	—
1·5	—	7·80 –	—	7·60 –	—	7·60 –	—	—	7·10 –	7·10 –
2·0	—	—	—	7·90 –	—	7·90 +	—	—	—	—
3·0	7·30 –	—	—	—	—	—	—	—	—	—
4·0	7·70	—	—	—	—	—	—	—	—	—
5·0	8·20	—	—	—	—	—	7·10 –	—	—	—
% CaO required to make $-\log [H^{\cdot}] = 7·07$	0·029	0·0067	0·0064	0·0084	0·0062	0·0084	0·056	0·0060	0·0224	0·0224

* All these samples were from the limed plots.

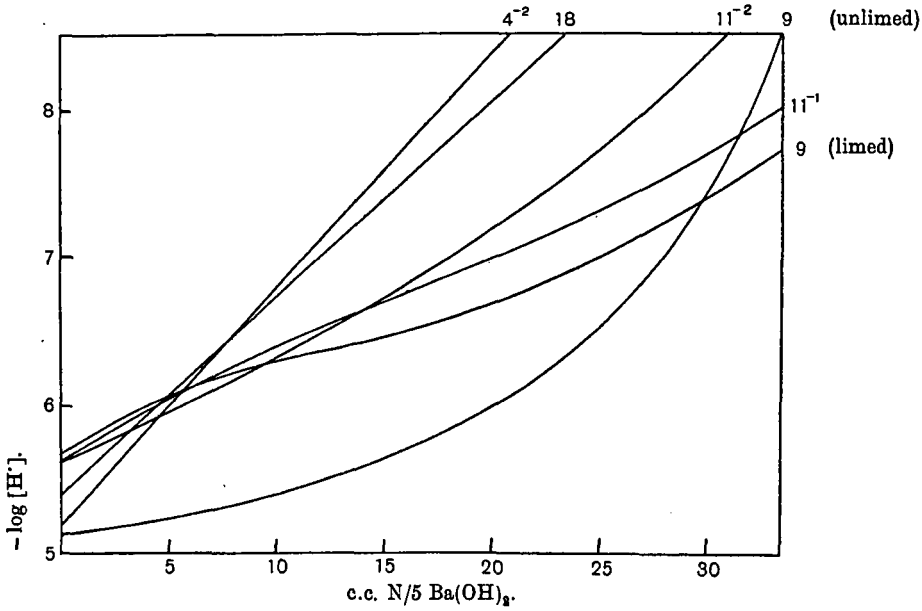

Fig. 3.

of the soil to this value could be calculated. Lime itself could not very well be used in practice. Saturated lime water was too dilute except where the lime requirement was extremely small. To weigh out numerous small quantities of pure CaO without contamination by moisture or atmospheric CO_2 is too tedious an operation, while if $CaCO_3$ were employed there is always the possibility that the decomposition would be slow or incomplete. Consequently until the validity of the colorimetric method could be established it was decided to use $Ba(OH)_2$ but to calculate the results in terms of lime.

It is evident of course that the $-\log[H^{\cdot}]$ values obtained colorimetrically do not represent the true values for the actual soil solution. They are the values obtained by shaking one part of soil with two parts of water for a definite time (one hour), always assuming that equilibrium has been attained during that time and that the method of determination is valid. The ratio soil/water = 1/2 was employed purely as a matter of convenience. But even if values for the $-\log[H^{\cdot}]$ so found are not strictly accurate they should be comparable from sample to sample and moreover the degree of dilution should not affect the value for the lime requirement obtained since once the mixture is neutral no reasonable amount of dilution should affect the reaction.

The values for $-\log[H^{\cdot}]$ and for percentage lime requirements (obtained by interpolation from the graphs) of 17 of the Grass Plot samples are given in Table III, A and B; and some of the results in Table III, A are shown graphically in Fig. 3.

EFFECT OF FINENESS OF DIVISION ON MEASUREMENT OF $-\log[H^{\cdot}]$.

The possible influence of fineness of division on the lime requirement of soil seems to have been almost completely overlooked and only three papers on the subject appear to have been published. Brown and Johnson (5) found that with certain sandy Iowa soils the lime requirement as found by the Veitch method diminished on grinding and to such an extent that in some cases soils having a high lime requirement before grinding actually became alkaline afterwards. Cook (9), on the other hand, with New Jersey soils using the same method found that in every case the lime requirement increased with grinding, and recommended in consequence that soils should not be ground if used for determination of lime requirement by the Veitch method. Sharp and Hoagland (23), using six soils from four American states, showed that the $-\log[H^{\cdot}]$ measured electrometrically was unaltered by grinding except in one instance when the value increased from 6·40 to 7·15.

This anomalous result, like those of Brown and Johnson, can apparently only be explained by the supposition that the interior of the soil particles concerned was of a different chemical composition from the exterior partially weathered layers*.

If fineness of division does affect the $-\log[H^{\cdot}]$ of a soil and consequently the lime requirement as determined colorimetrically then the values given in Tables III, A and B, will probably be inaccurate. Moreover the supposition that fineness of division is a factor to be reckoned with is supported by the considerable curvature shown by some of the curves in Fig. 3, *e.g.* the curves for Plots 9 (limed and unlimed), and 11–2. As indicated in Fig. 1†, when considerable buffer action is present, neutralisation curves show very little curvature. In the case of soil-water mixtures with large buffer action and over a small range of

Table IV.

	Plot 10 (limed)			Plot 9 (unlimed)			
	3 mm. samples		100 mesh sample	3 mm. samples		100 mesh samples	
c.c. N/5 $Ba(OH)_2$	I	II	I	I	II	I	II
0	5·80 +	5·30 –	5·40 –	5·10	5·10	5·40 –	5·30 +
10	6·80 – ; 6·70 +	5·50	5·50	5·40 +	5·50 –	5·40 +	5·40
15	7·40 –	—	—	—	—	5·50 +	5·60 –
20	8·40 +	6·30 – ; 6·60 –	5·80 + ; 6·00	6·50 – ; 6·40 +	6·00 –	5·80 +	5·80 +
25	—	7·00	6·30 +	—	—	—	—
30	—	7·80 +	6·90	7·40 + ; 7·40 –	7·40	6·50; 6·60	6·50
35	—	—	7·50	—	—	—	—
40	—	—	8·00	—	—	—	—

$-\log[H^{\cdot}]$ very slight, if any, curvature should be shown. This was shown to be the case in the recent work of Knight. Moreover although it was generally easy to get a series of experimental points lying on or near a continuous curve when the whole series was done at the same time, if the series was repeated under slightly different conditions as to duration, manner and violence of shaking, quite different values and curves were obtained. If the samples were ground so that the greater part passed through the 100 mesh to the inch sieve little difficulty was

* In all these cases the soil was actually ground up, *e.g.* in a porcelain mortar, so that a considerable amount of attrition occurred. In this investigation (see also Part I, pp. 41, 42) a *wooden* pestle was used so as to avoid actual attrition as the effect desired was a mere separation of the larger soil crumbs into the ultimate soil particles without any breaking up of these latter. It is unlikely that any fresh unweathered surface was exposed under these conditions and the case is not strictly comparable with those of the American workers.

† See also Figs. 1 to 3 in No. I of this paper.

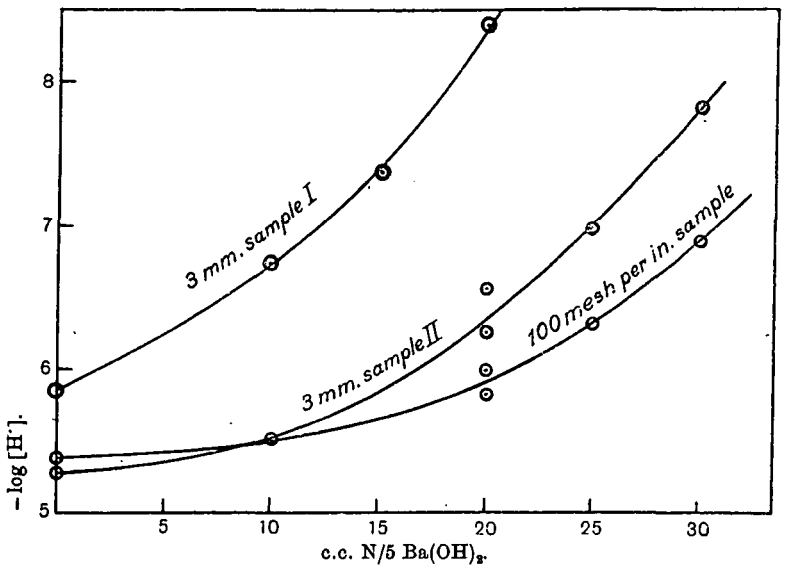

Fig. 4. Neutralisation curves for Plot 10 (limed end).

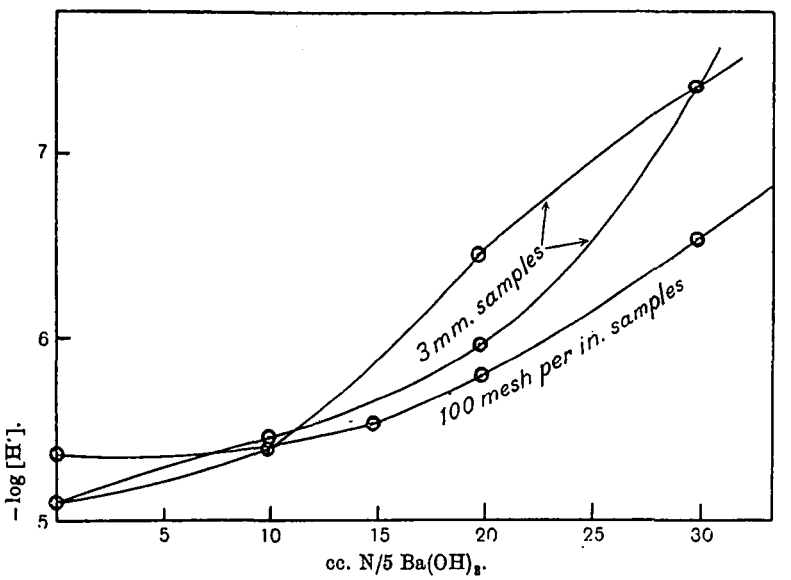

Fig. 5. Neutralisation curves for Plot 9 (unlimed end).

experienced in obtaining reproducible results. Table IV and Figs. 4 and 5 illustrate this point and bring out quite clearly the fact that fineness of division is apparently a serious factor to be reckoned with in acidity determinations.

Presumably the irregularities of the results from the 3 mm. samples are due to the alteration in effective absorbing surface resulting from the breaking up of the larger soil crumbs during the shaking. If this is so then the duration of the shaking should also have an effect on the apparent reaction and lime requirements of a soil, and no neutralisation curves should be accepted as correct unless they can be shown to be real equilibrium curves. Gillespie and his co-workers (14) used unground soil samples, the soil-water mixtures were shaken by hand fifty times and the extract centrifuged and its $-\log[H^{\cdot}]$ measured colorimetrically. In later papers the colorimetric results were compared with those obtained electrometrically, but apart from the satisfactory agreement found between the two lots of results there is nothing to show that equilibrium had really been attained. On the other hand, Gillespie did not attempt to measure lime requirements nor plot neutralisation curves, but only the actual $-\log[H^{\cdot}]$ of the soils investigated, and the disturbing effect of fineness of division and of time of shaking might be expected to be less on the latter than on the former. In this connection Sharp and Hoagland (23) in their electrometric measurements of soil reaction state with regard to the time taken for constant voltmeter readings to be obtained: "This occurs in the case of acid soils within a few minutes, but for soils approximately neutral a slightly longer time will be required. In the case of titrations prolonged shaking is required after each addition of the titrating solution, in order to obtain constant readings." They interpret this as being due to the "slow rate of solubility possessed by the acid constituents of the soil," but it can be equally well, if not more satisfactorily, explained as due to an increase in effective surface resulting from a breaking up of the crumbs during the shaking, especially as it is not at once evident why the rate of solubility of the acid constituents of the soil should be slower in the presence of an alkaline titrating liquid than in the presence of water alone. The results for Plot 4–2 (unlimed) given in Table V, columns *A*, *B* and *C*, and the corresponding curves in Fig. 6 were thought at first to throw some light on this matter: the 100 mesh sample gave consistently smaller values for $-\log[H^{\cdot}]$ than the 3 mm. sample shaken for the same time (one hour). On the other hand, the 3 mm. sample shaken for 30 hours gave very much smaller values for $-\log[H^{\cdot}]$ than either of the other samples.

Another explanation of this difference is however possible: it was noticed that the more finely ground a soil was or the longer the shaking the greater was the turbidity even after centrifuging, and it was thought that the variations observed might be due to some action of the suspended clay particles. To test this point a series of determinations was carried out on the same Plot 4–2 (unlimed), finely ground sample,

Table V. *Plot* 4–2 (*unlimed*).

c.c. N/5 $Ba(OH)_2$	100 mesh sample shaken 1 hour (Curve *B*)*	3 mm. sample shaken 1 hour (Curve *A*)*	3 mm. sample shaken 30 hours (Curve *C*)*	100 mesh sample shaken 24 hours and flocculated with CaAc (Curve *D*)*
0	5·50	5·50 +	—	6·10
10	5·80	5·90 +	5·50	—
20	6·20	6·50 –	5·80 +	6·60
25	—	—	—	6·70 +
30	6·60	7·10	6·20 –	—
40	7·00	—	6·50	—

* See Fig. 6.

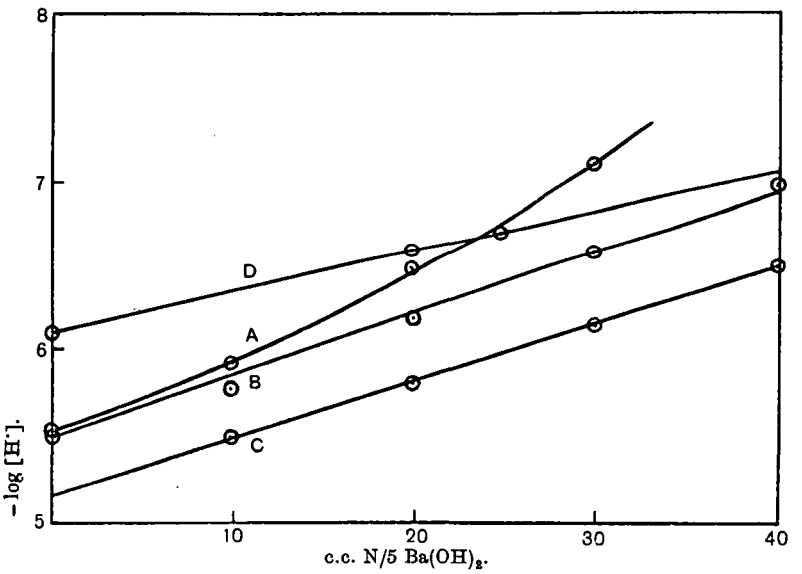

Fig. 6. Neutralisation curves for Plot 4–2 (unlimed end).

shaken for 24 hours. The soil-water mixtures were centrifuged for ten minutes, the extracts decanted from the sediment into a second lot of tubes, made N/100 with carefully neutralised calcium acetate solution and re-centrifuged for ten minutes. The calcium acetate causes a tolerably complete flocculation of the suspended particles thus eliminating the turbidity. The $-\log[H^{\cdot}]$ of these extracts was measured and the results are given in the last column of Table V and curve *D* of Fig. 6.

Clarifying with calcium acetate considerably decreased the acidity and also the apparent lime requirement from which it might be inferred that there is a turbidity effect due to the small amount of clay in suspension. No such turbidity effect was noticed in measuring the $-\log[H^{\cdot}]$ of bacterial culture media where the turbidity was due to agar. But agar is relatively non-reactive in comparison with the colloidal alumino-silicates that presumably make up the greater part of the turbidity of aqueous soil extracts, and it is possible that such compounds may exert some action on the indicator used such as selective absorption of acid or base or some disturbance of the tautomeric equilibrium determining the colour change. At the same time such effects are generally accompanied by difficulty in colour matching, and no such difficulty was observed.

The most probable explanation is to be sought in the nature of the substances present in the extract. If the acidity is due to sulphuric acid (produced, for example, by the hydrolysis of $Al_2(SO_4)_3$) or acid phosphates capable of forming insoluble salts of lime, then the addition of Ca-acetate will result in the precipitation of an insoluble Ca-salt while a corresponding amount of acetic acid will be liberated. The $[H^{\cdot}]$ of the acetic acid produced would be diminished by the 'buffer action' of the Ca-acetate present. This would account satisfactorily for the observed differences in $-\log[H^{\cdot}]$ and is supported by the history of the soil used which had been manured for many years with sulphate of ammonia and superphosphate. Some other flocculant than Ca-acetate, preferably one of an insoluble or non-electrolytic character, is therefore desirable in eliminating turbidity. Possibly dialysed colloidal ferric hydroxide might answer the purpose as suggested by Gillespie.

Evidently a thorough investigation of the hydrogen-ion concentration of soils and aqueous soil extracts is very desirable. The present writer however has had to abandon the work at an early stage but it is hoped that the results so far obtained, although preliminary in character, may be found of interest.

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