MEETING OF THE LONDON SECTION, HELD AT THE HORSE SHOE HOTEL, TOTTENHAM COURT ROAD, ON MONDAY, OCTOBER 8TH, 1934.

Mr. L. E. SIMPKIN in the chair.

The following paper was read and discussed :

r_H AND ITS APPLICATIONS IN BREWING.

By JEAN DE CLERCK.

ANYONE familiar with scientific investigations previous to formulation of the conception of p_H will remember how difficult it was to understand the results when questions of acidity and alkalinity arose. It was evident that acidity played an important part in many chemical and biological phenomena but when attempts were made to study its effects more closely one was faced with contradictions. The conceptions of electrolytic dissociation, of p_H and buffer action cleared up all these confusions, and enabled great progress to be made in chemistry, and particularly in biochemistry, from which brewing has greatly profited.

What happened 10 or 20 years ago in regard to acidity and alkalinity is now taking place with oxidation and reduction. These terms have acquired an entirely different meaning. Oxidising and reducing agents are now arranged on a simple scale and graded according to their activity, just as acids and alkalis were ranged according to their dissociation constants and just as the degree of acidity of a liquid was expressed by the symbol p_H so now is its level of oxidation designated by that of r_H.

Such rather imperfect measurements of r_H as I have carried out with beer have shown that its determination has real value. In this new scientific conception we appear to have the means to take another step towards the comprehension of phenomena which occur in brewing.

OXIDATION—REDUCTION POTENTIAL.

The old definition of oxidation was rather vague. Fixation of oxygen, loss of hydrogen and increase of valency or positive charge, dissimilar though they might appear, were all regarded as oxidations since they could all be caused by oxygen. Even when oxygen took no part they were still referred to as oxidations. Thus when cuprous chloride is exposed to the air it is oxidised to cupric chloride, but cuprous chloride could also be oxidised by ferric chloride without the intervention of oxygen according to the equation.

$$CuCl + FeCl_3 = CuCl_2 + FeCl_2.$$

The fundamental characteristic common to all these forms of oxidation was unknown. Modern knowledge of the constitution of atoms was necessary to elucidate this. It is held that atoms consist of a central nucleus charged with positive electricity around which gravitate corpuscles carrying a negative charge and called electrons.

Normally, the negative charges of the electrons are in equilibrium with the central positive charge but it may happen that an atom loses one or more electrons, or rather that it passes them to another atom. In such a case the atom which has lost an electron appears to be positively charged and that which has received the electron becomes negatively charged. These are what we call positive and negative ions. The ferrous ion, to which two positive charges are attributed (Fe^{++}) is actually an atom of iron which has lost two electrons. In the ferric state it has lost three. When ferrous iron passes to the ferric state it is said to be oxidised and we see that it has lost an electron. The fundamental characteristic of all oxidations is loss of electrons as can be proved by the following experiment.

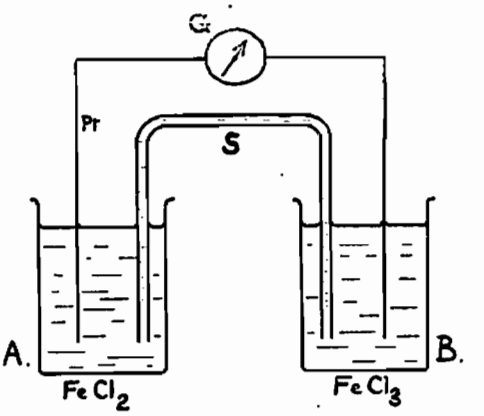


FIG. 1.

Place a solution of ferrous chloride in the beaker A and another of ferric chloride in B. Join them by a syphon S containing, for example, a solution of potassium chloride. So far no change takes place in the solutions, but if they are connected by means of platinum wire it may be shown that ferric chloride immediately commences to form in A and ferrous chloride in B. If a sensitive galvanometer is placed in the circuit a deviation of the needle will be noted.

The explanation is that the ferrous ions in the solution A are richer in electrons than the ferric ions in solution B. There is a tendency to pass this excess of electrons to the Fe +++ ions. In other words Fe ++ has a greater electronic tension. The electrons, therefore, escape from the solution by way of the platinum wire, causing the galvanometer needle to deviate, and attach themselves to the iron in solution B. The negative Cl ions, which cause the equilibrium with the positive charge of the iron are driven back through the syphon to the solution A where they replace the electrons which have disappeared. This goes on until a condition of equilibrium is reached. This equilibrium is determined by a constant K as in all chemical reactions.

If a current from an external source, such as an accumulator, is then passed through the beakers in the direction opposite to that in the platinum wire, as indicated in Fig. 2, it will be noted that the changes are reversed. $FeCl_2$ disappears from B and $FeCl_3$ from A and the galvanometer deviates in the opposite direction. This proves that oxidation is related to the transfer of electrons.

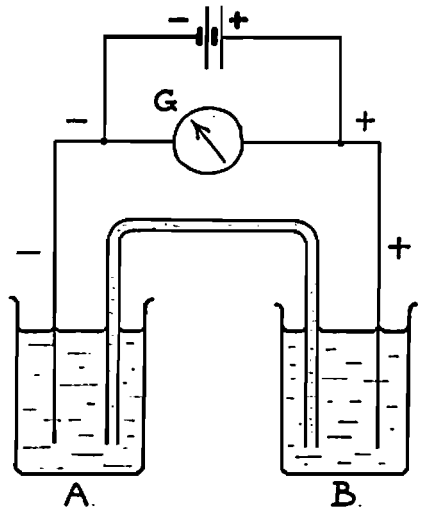


FIG. 2.

Experiment proves that every oxidation, however it is produced, is accompanied by similar electrical phenomena. The general definition of oxidation is therefore *loss of electrons*. The reverse reaction, reduction, is a *gain of electrons*. As the two must necessarily occur at the same time, *oxidation-reduction* is usually referred to, and then is defined by *the passage of electrons from the oxidised to the reduced substance*.

A difference of potential obviously exists between the two ends of the platinum wire and this may be measured by the method of opposition employed in the electrometric measurement of p_H. This difference of potential gives a measure of the intensity of the oxidation-reduction phenomenon. It is not necessary to connect the solution of $FeCl_2$ and $FeCl_3$ in order to measure it. The same result is obtained by connecting one after the other to the same reference electrode, a calomel electrode, for example. The difference between these two potentials will evidently be the same as that measured directly between the two solutions. If a platinum electrode is then immersed in mixtures of $FeCl_2$ and $FeCl_3$, potentials intermediate to those of the pure solutions will be set up. This potential will be higher according as the proportion of the oxidised form $FeCl_3$ is greater.

Substances which can pass reversibly from the oxidised to the reduced state, such as $Fe^{++}=Fe^{+++}$, are known as oxidation-reduction systems.

The potential of an equimolecular mixture of the oxidised and reduced forms is known as the "Normal Potential" of the oxidation-reduction system. This potential varies from one system to another. The following values are taken from L. Michaelis' book, "Oxidation-Reduction Potential," Berlin, 1933 :—

CO^{+++}/CO^{++}	1·76 volts		
Fe^{+++}/Fe^{++} (slightly acid)	0·742 volts	at	18° C.
Ti^{++++}/Ti^{+++} (in 4N. H_2SO_4)	0·056 „	„	18° C.
Sn^{++++}/Sn^{++} (with HCl)	−0·426 „	„	25° C.
Sn^{++++}/Sn^{++} (in 0.6N. NaOH)	−0·854 „	„	16° C.
Indigo Disulphonate at p_H 5 ..	−0·010 „	„	18° C.
„ „ at p_H 9 ..	−0·199 „	„	18° C.
Methylene blue at p_H 5 ..	−0·101 „	„	18° C.
„ „ at p_H 5 ..	−0·101 „	„	18° C.

The potential of a normal hydrogen electrode is always taken as the basis of comparison in these measurements of oxidation-reduction potential, that is to say, a hydrogen electrode in which the hydrogen is under a pressure of an atmosphere, and which is plunged in a solution of normal concentration of H ions. The potential of this electrode is thus taken arbitrarily as the zero point of the scale of comparison.

In the above table each substance is an oxidiser towards those which have a lower potential, and a reducing agent to those with a higher potential, from which it appears the oxidation and reduction are relative terms.

For any given oxidation-reduction system there is a constant relation between the potential and the proportions of the oxidised and reduced forms. This is given by the formula

$$E = \frac{0{\cdot}0002\,T}{n} \log \frac{(Ox)}{(Re)} + \text{Constant} \quad (1)$$

in which T is the absolute temperature (273° at 0° C.).

(Ox)=Concentration of the oxidised form.
(Re)= ,, ,, reduced ,,
n=number of electrons exchanged in the reaction.

and the constant is the normal potential of the system.*

If the variations in the potential of a system passing from the oxidised to the reduced state are plotted diagrammatically,

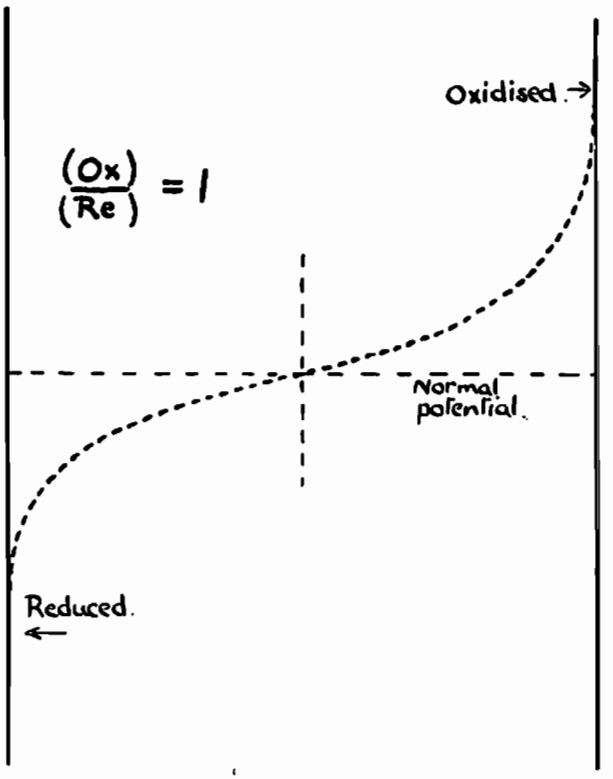


FIG. 3.

a curve will be obtained which varies with $\log \frac{(Ox)}{(Re)}$. It thus has the same form as a curve representing the neutralisation of an acid by a base in terms of p_H which varies according to $\log \frac{(Salt)}{(Acid)}$. The slope of the

* Proof of this formula can be found in Michaelis' book or in a paper published by the present author. (*Bulletin de l'Assoc. Anc. Louvain*, 1933, 34, No. 2.)

curve expresses the buffering effect of the oxidation-reduction system which evidently increases with the concentration, but at equal concentration it is greatest at half oxidation.

In mixtures of oxidation-reduction systems the potential is the resultant of the normal potentials and concentrations of the systems present. If a small quantity of methylene blue, of which the normal potential is moderate is mixed with a concentrated solution of the system Sn^{++++}/Sn^{++}, with a very low potential, the methylene blue will be reduced. In Fe^{+++}/Fe^{++} it will, on the contrary, be oxidised. Now methylene blue and quite a number of other dyes are colourless in the reduced state, or rather their reduced form is colourless, and it is possible to judge the potential existing in a liquid to which they are added from the colour they give. Indicators indeed exist for different zones of potential by means of which the potential can be determined in a very simple manner.

The Expression r_H. Up to this point the oxidation-reduction potential has been expressed in volts, but it is more frequently expressed by the symbol r_H. It is consequently necessary to explain why this manner of expression is preferred and exactly what it signifies.

It will be noted from the table already given that the potential of oxidation-reduction systems varies with the p_H. This is because oxidation-reduction is the passage of electrons from the oxidised to the reduced substance. These must, therefore, have different electric charges, and one of them at least must be ionised. Oxidation-reduction is thus related to ionisation, and everything which influences the latter will affect the former.

Take, for example, the oxidation-reduction system, hydroquinone/quinone. Formerly the oxidation of hydroquinone was formulated as $C_6H_6O_2 = C_6H_4O_2 + 2\,H$

In reality hydroquinone is a weak dibasic acid which dissociates into $C_6H_4O_2^{--} + 2\,H^+$.

The oxidation of hydroquinone actually consists in the loss of negative charges or electrons of its ion.

$$C_6H_4O_2^{--} \rightleftharpoons C_6H_4O_2 + 2 \text{ electrons.}$$

The reduced form of the system is thus the ion $C_6H_4O_2^{--}$ and the concentration of this ion alone counts for the value of the potential. If the acidity of the solution is increased, the ionisation of hydroquinone will be reversed and the value of the relation $\frac{(Ox)}{(Re)}$, on which the potential depends, will diminish.

In acid media, hydroquinone only dissociates to the extent of one H^+ ion, and the oxidation reaction then becomes

$$C_6H_5O_2^{--} \rightleftharpoons C_6H_5O_2 + 1 \text{ electron.}$$

The potential depending also on the number of electrons exchanged (n in the formula 1) varies again on account of this.

Sometimes ionisation of the oxidised and reduced forms vary in parallel under the influence of p_H. Such is the case to a certain extent in the system Fe^{+++}/Fe^{++}. In such cases the potential evidently remains the same.

Therefore the ionisation of an oxidation-reduction system must be given before the system is completely known. As p_H is by far the most important factor in ionisation its effects on the potential of a number of systems have been studied. Curves for the variations in the normal potentials of a few systems are given in Fig. 4.

The zero potential in the above diagram represents that of the normal hydrogen electrode which is the generally adopted point of comparison. It will be noticed that the effects of p_H on the systems Fe^{++}/Fe^{+++} and hydroquinone-quinone, for example, is quite different, and that it may vary in one and the same system from one zone of p_H to another.

This diagram is indispensable if it is desired to deduce the proportions of oxidised and reduced forms in a system from the potential, and also to measure potentials by means of indicators. Thus, for example, if methylene blue is half decolorised in a liquid, if the p_H of this liquid is 6, the diagram indicates a potential of about $+0{\cdot}060$ volt, but if the p_H is 4, the potential would be about $+0{\cdot}190$.

It is, however, somewhat inconvenient always to state the p_H with the oxidation-reduction system, and then to have recourse to this diagram. Attempts have been made to find a simpler method of expression, the reasoning being :—The diagram shows the variations in potential of the hydrogen electrode with p_H while the hydrogen electrode is in fact a reversible oxidation-reduction system which may be expressed as :—

$$H \text{ gas} = H^+ + 1 \text{ electron.}$$

In this system the hydrogen gas is the reduced form (Re) and the ions H^+, which are produced from it by the loss of an electron, constitute the oxidised form (Ox). Formula (1) would thus become :—

$$E = 0{\cdot}0002\ T.\log\frac{(H^+)}{\text{pressure } H_2} + \text{constant}.$$

When the p_H increases by 1 unit, (H^+) becomes 10 times less, and since the pressure of hydrogen is always 1 atmosphere, $\log\frac{(H^+)}{\text{Pressure } H_2}$ will decrease by one unit. The potential of the hydrogen electrode thus diminishes by 0·0002 T for each increase of one unit in p_H.

If the curve for the system hydroquinone-quinone is followed in the diagram, it will be observed that it is parallel to that of the

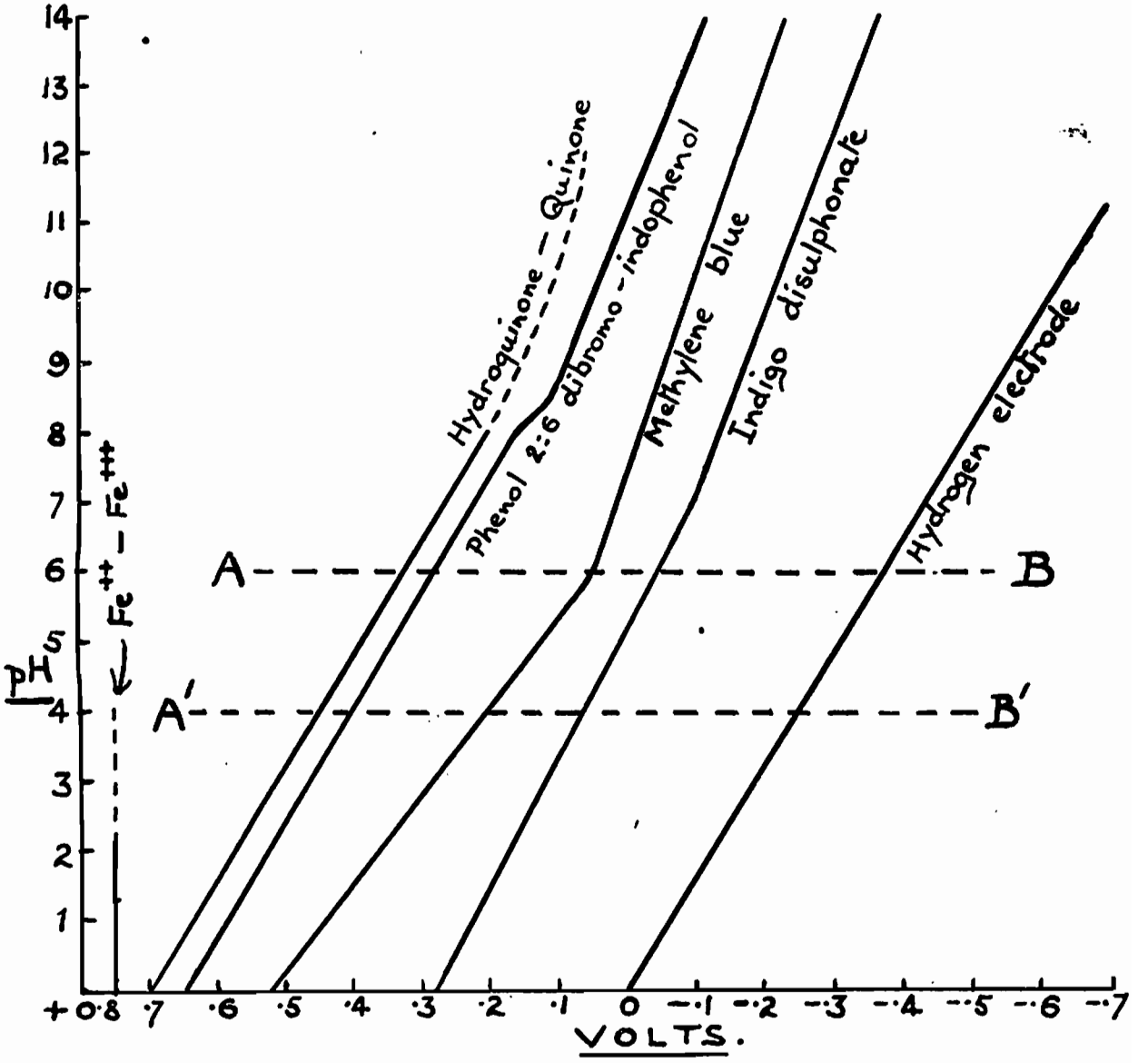


Fig. 4.

hydrogen electrode up to p_H 8. Below this p_H hydroquinone only dissociates into one H^+ ion, and the dissociation is very slight, so much so that the product $[C_6H_5O_2^-] \times [H^+]$ will be practically constant. When the H^+ ion concentration is decreased ten times, the concentration of the reduced form $C_6H_5O_2^-$ is increased ten times, and by application of the formula (1) the same variation of potential will be found as for the hydrogen electrode. The difference of potential between the hydrogen electrode and a mixture of hydroquinone and quinone is therefore constant below p_H 8. (*Note:* The measurement of p_H by means of the quinhydrone electrode is based on the foregoing :— The quinhydrone when dissolved in water is divided into equimolecular proportions of hydroquinone and quinone. The liquid to be examined is saturated with quinhydrone, and the p_H is deduced from measurement of the oxidation-reduction potential.)

It happens that a great number of oxidation-reduction systems, notably those met with in physiology, and many dyes are of the same type as hydroquinone-quinone. The variations in their potential are also parallel, or almost so, with those of the hydrogen electrode in an acid medium. This makes it possible to compare them one with another independently of p_H. The potential of the hydrogen electrode system can therefore be taken as the point of comparison for measuring oxidation-reduction power in place of an arbitrarily selected potential.

If a line A—B parallel to the axis of the abscissae is drawn on the diagram (Fig. 4), the section of this line between the curve of the hydrogen electrode and that of another system represents the oxidation-reduction power of that system. If a line A^1—B^1 is drawn at another level of the axis of p_H, the corresponding sections will have the same value as at the level A—B, for parallel systems, and this method of measurement is consequently independent of p_H.

This reasoning has been pursued further, and all oxidation-reduction systems have been considered as similar to the hydrogen electrode.

If a very strong reducing agent, metallic sodium for example, is placed in water, a violent evolution of hydrogen occurs

$$Na + H_2O = NaOH + H$$

In ionic notation this would be written

$$Na + H^+ = Na^+ + H.$$

The sodium passes electrons to the H^+ ions of the water, and these are reduced to gaseous hydrogen.

This reaction should take place with any reducing agent, however weak it may be. With strong reducing agents such as titanous salts a manometrically measurable hydrogen pressure is obtained and their reducing power could be measured by this pressure. With weak reducing agents this hydrogen pressure should still exist. Thus the ions of hydroquinone should give rise to the following reaction in presence of H^+ ions.

$$C_6H_4O_2^{--} + 2H^+ = C_6H_4O_2 + H_2 \text{ (gas)}$$

The pressure of hydrogen resulting from this equilibrium is so very small that there can be no question of measuring it manometrically, but certain physicists contend that it can be measured by the potential of a platinum electrode plunged in the oxidation-reduction medium. This electrode, they say is not a simple receiver of electrons, as it has here been so far considered, but it becomes charged with the hydrogen gas present in the liquid and there functions as a simple hydrogen electrode, but at a very low pressure. It has been shown that the potential of the hydrogen electrode varies with $\log \frac{H^+}{\text{pressure } H_2}$

When the hydrogen pressure decreases the potential must therefore increase.

The difference of potential between two hydrogen electrodes at the same p_H but at different hydrogen pressures, is given by the formula

$$E_{p_1} - E_{p_2} = 0{\cdot}0001 \text{ T. } \log \frac{p_1}{p_2}$$

In the present case, p_1 the pressure of hydrogen in the hydrogen electrode is one atmosphere and p_2 is the hydrogen pressure of the platinum electrode to be calculated. This gives

$$E_1 \text{ atm} - E_{p_2} = 0{\cdot}0001 \text{ T. } \log \frac{1}{p_2}$$

$$= 0{\cdot}0001 \text{ T} \times (-\log p_2)$$

$$\text{and} - \log p_2 = \frac{E_1 \text{ atm.} - E_{p_2}}{0{\cdot}0001 \text{ T}}$$

If a difference of potential of 0·300 volt at 18 deg. C. is found between the platinum electrode and the hydrogen electrode at the same p_H, it follows that

$$-\log p_2 = \frac{0{\cdot}300}{0{\cdot}0291} = 10{\cdot}3$$

for which $p_2 = 10^{-10{\cdot}3}$ atmospheres

W. M. Clark proposed to simplify this expression by denoting the pressure by its negative logarithm, and represented the figure by the symbol r_H. This simplification is analogous to that introduced by Sörensen for hydrogen ion concentration, representing the negative logarithm by the symbol p_H

This gives

$$r_H = -\log\ p_2$$

and in the example taken $r_H = 10 \cdot 3$.

The r_H is thus the negative logarithm of the pressure of reducing hydrogen existing in the liquid. It expresses the degree of oxidation just as p_H expresses the degree of acidity.

It is deduced from the difference of potential between the platinum electrode plunged in the liquid, and the hydrogen electrode at the same p_H. When the potential of the liquid under test varies in parallel with that of the hydrogen electrode according to the p_H, the r_H is independent of the p_H. In physiology r_H is utilised as an independent measure of p_H. This is justified, according to Michaelis, because the potentials of physiological systems vary almost in parallel with the hydrogen electrode within the restricted limits of p_H found in physiological media. It is nevertheless necessary to guard against generalisation which may lead to serious errors of interpretation.

The indicators* used for the determination of r_H vary in potential almost in parallel with the hydrogen electrode. When added to a liquid they therefore indicate its r_H directly. Thus methylene blue changes from blue to colourless between r_H $15 \cdot 5 - 13 \cdot 5$, cresol blue between r_H $17 - 15$, etc.

Applications in Brewing.

It has been known for a long time that aëration is of considerable importance in brewing. Lengthy discussions are to be found in the literature on the influence of oxidation on the cooler, at the refrigerator, during fermentation and at racking or bottling. The views expressed are most contradictory. This is due to the fact that they all have an empirical basis and because no method of measuring oxidation was available. All that was possible was measurement of the quantity of dissolved oxygen. It is now known that it is the r_H which is of importance in oxidation. Oxygen in contact with a liquid tends to oxidise it, to increase its r_H, but the increase is very different in different cases, just as the increase of p_H produced by addition of the same quantity of a base may vary greatly. It is obvious that it was impossible to avoid contradictions until the conception of r_H permitted a methodical and precise study of the phenomena of aëration.

It is not only aëration which influences the r_H. It has been known for a long time that yeast had a reducing power, but it was not possible to measure it. It will be shown later that light affects the r_H of beer and that the change in r_H is accompanied by marked alterations in its flavour. There are, no doubt, many more phenomena for which an explanation has not yet been found, but which will be cleared up by consideration of r_H.

Changes in r_H of beer in absence of air.—I employed the following indicators in my first attempts to measure the r_H of beer :—

Janus Green, which changes from blue to red between $r_H 15$ and 13, and becomes colourless about $r_H 5$. The first change with this indicator is irreversible.

Methylene Blue which becomes colourless between $r_H 15 \cdot 5$ and $13 \cdot 5$.

Nile Blue, which becomes colourless between $r_H 10$ and 8 approximately.

Small quantities of these indicators, dissolved in distilled water, were placed in white glass sterilised bottles. These were afterwards filled with beer taken from the outlet of the filter and closed with a crown cork. The concentration of the indicators were one in 300,000 for Janus Green and Methylene Blue, and one in 600,000 in the case of Nile Blue.

The beers were all green at first on account of the mixture of their own yellow colour with the blue of the indicator. The indicators were thus oxidised and the r_H was evidently higher than $15 \cdot 5$. After a few days in the laboratory the colours commenced to change in some of the bottles. Those containing Janus Green became olive-green, then reddish brown, and finally red, after 5 or 6 days. Those containing methylene blue became gradually paler and lost the blue colour after about the same lapse of time, but those with Nile Blue retained their colour even after many weeks.

*Note.—A list of indicators with the r_H values at which they change colour and curves of variation with p_H will be found in a pamphlet issued by The British Drug Houses, Ltd., and entitled "The Colorimetric Determination of Oxidation-Reduction Balance."

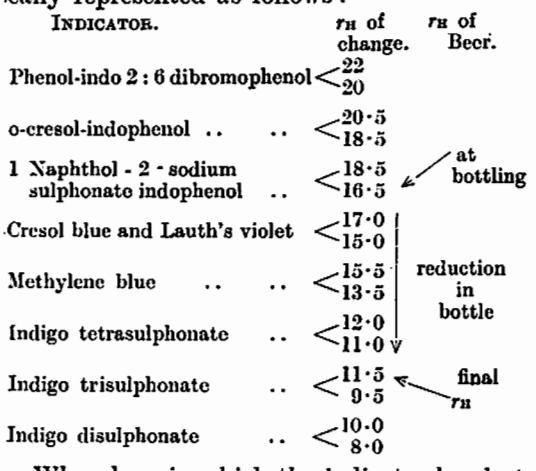
It thus appeared that the r_H of beer decreased gradually when it was kept out of contact with air, but the reduction did not go so far as the colour change of Nile Blue. It stopped above a value of 10.

Later I tried other indicators. Cresol blue and Lauth's violet, which change from blue to colourless between r_H17 and 15, give the same results as Methylene blue, except that they lose their colour more rapidly because they change at a higher r_H. But they also were always coloured at first. 1-naphthol—2-sodium sulphonate-indophenol, which changes from red to colourless between r_H18·5 and 16·5, gives a red colour with beer, but it is difficult to see as the colour is too close to that of beer.

o-cresol-indophenol and phenol-indo-2 : 6 dibromophenol, which change from red to colourless between r_H20—18 and 22—20 respectively, are decolourised even in beer which has been strongly aërated. Thus the r_H of beer is always above 17, but apparently never higher than 18–19.

For the lower r_H ranges I used, in addition to Nile blue, the tetrasulphonate, trisulphonate and disulphonate of indigo which change from blue to colourless between r_H13–11, 11·5–9·5 and 10–8 respectively. The tetrasulphonate and trisulphonate sometimes became colourless but only after a considerable lapse of time. The disulphonate was never entirely decolourised. The r_H consequently often goes as low as 10–12, but very rarely to 9.

These experiments may be diagrammatically represented as follows :—



When beer in which the indicator has lost its colour is poured from a bottle into a glass, the colour comes back after a short time, in about ¼ or ½ hour. The surface in contact with air becomes coloured first. The same thing happens when the beer is poured from one bottle to another which is closed immediately. Simple contact with air is sufficient to bring the beer back to an r_H higher than 17.

In some of these experiments I added 2 to 5 times more indicator, when the decolourisation was much slower. Thus a bottle with methylene blue at the rate of 1 in 60,000 became paler at the end of 3 weeks but did not decolourise. This shows that the reducing power of beer is slight, since it does not decolourise this very small quantity of indicator. Also when the beer is a little too much aërated at bottling the reduction does not take place.

I have said that the bottles in which reduction took place were filled at the outlet of the filter. When the sample is taken from a cask, after the beer has been in contact with air at racking, the reduction becomes much slower or even fails to take place at all. The same thing happens when a large air space is left over the beer in bottles.

It may be asked at what r_H does the beer leave the storage tank. Is it at a higher r_H than 17, or does it become oxidised to this r_H by contact with air when the sample is taken.

It would appear that reduction should take place in the tank just as it does in bottle, but that is not the case with bottom fermentation beers, since if samples taken as previously indicated are placed in the cellars at 0° C., the reduction does not take place. There is no reduction, or certainly very little at 0° C. It is known, however, that yeast has a strong reducing power. Fermenting beers rapidly decolourise the indicators even in the cold cellars. I have fermented beers in conical flasks closed with rubber stoppers but allowing the gas to bubble through distilled water. The indicators were rapidly decolourised in these beers, but after fermentation was finished they coloured again in a few days. This was not due to admission of air since an excess pressure was always shown in the flasks. The re-oxidation, however, appears to be due to the small quantity of air which remains in the flasks above the beer, or which diffuses into the flask, since it occurs more rapidly when the flasks contain less beer, and the

colouration first appears at the surface. Samples taken from a tank immediately after it was filled were reduced rapidly at 0° C., but became coloured again after a few days or weeks, probably through air contained in the neck of the bottle.

It is thus very probable that beer in tanks is normally in a reduced state but, in order to follow the r_H, it would be necessary to take samples completely protected from air.

The reduction is frequently very different in duplicate samples, because aëration is not always the same at sampling. I have always taken samples at least in triplicate in order to have as fair a sample as possible.

These experiments show that there are great variations in the r_H of beer at bottling. The r_H tends to diminish but the fall varies greatly according to the condition of aëration and temperature.

Influence of r_H on Yeast Turbidities.— It has long been known that aëration is unfavourable to stability and that it favours yeast turbidities in particular.

It has been shown that aëration causes the r_H to vary greatly. Biological studies have shown that there is a favourable r_H zone for every organism, beyond which it does not develop. In this way precision has been given to the old view of aërobic and anaërobic organisms. It will, therefore, be interesting to follow the development of micro-organisms in beers of which the r_H has been determined by means of indicators.

It may be mentioned that the indicators used do not themselves hinder the growth of micro-organisms in the experiments since they developed to the same extent in control samples without indicators.

It is necessary to distinguish the nature of the organism before considering the effect of r_H on its growth. Only yeasts and rods have developed in the beers which I have examined, the former almost always culture yeasts, I can consequently only speak of these.

Yeasts only develop rapidly when the r_H remains high, that is in aërated beers. There is no yeast development or only very slight growth, when reduction takes place. Below r_H13, that is to say when methylene blue is decolourised, growth is certainly very slow, and even below r_H15, when Cresol blue is decolourised.

A light deposit of yeast often forms in beer after which reduction takes place and the development then ceases, and the deposit remains very small. When such a sample is transferred aseptically into another bottle the contact with air raises the r_H, this is shown by reappearance of the colour, and after a few days there is abundant yeast growth.

Even in unfiltered beers taken directly from the tank, the development of yeast is slight when reduction takes place.

Since I made these observations I have always added methylene blue to samples taken for the daily control of stability. The results have fully confirmed the earlier experiments. When samples became quickly turbid from yeast it was always in beer which remained blue.

Another observation made in connection with this method of control was that the reduction in stronger beers was generally more rapid than in light beers. Their reducing power was stronger, and their stability was also greater. The greater stability of strong beers is usually explained by their higher alcohol content, but they are always found on analysis to contain more degraded nitrogen which might be expected to reduce their stability. I consider that the reducing power provides a much better explanation of the difference in stability, at least in respect of yeast development.

It may be added that yeasts also develop in beers attenuated to the limit when the r_H remains high.

In some countries brewers are allowed to add a certain quantity of SO_2 to beer. This has the reputation of having an effective action in reducing yeast growth. This might be expected as it is a reducing agent. I have made some experiments on its effect by determining the r_H, and find when it is added in very small quantity, insufficient to affect the flavour of the beer, that it adds to the reducing power of the latter. On the other hand, the r_H remains high when the beer contains too much air and the yeast develops just as if there was no SO_2. Its so-called antiseptic action on yeast thus appears to be due solely to its reducing power.

So far only the part played by aëration in the development of yeast has been discussed, as if the degree of infection had no importance. In order to determine its importance relative to that of aëration, I have counted the cells in five bottlings,

and at the same time examined the r_H and stability in samples taken at various stages from racking and bottling. These experiments were made with the lightest beer which is the most sensitive to aëration. The beers were first racked into casks and bottled from these. A number of samples were taken from the filter, from cask and after bottling from the same casks. Two of these samples at each stage served for cell counts on wort gelatine. The number of cells in duplicate bottles agreed well, but there were big differences between one racking and another. At the filter outlet the number of cells varied from 3 to 420; in the cask from 170 to 1820 per cc. The beers from the filter outlet reduced rapidly and were stable, even those which contained 420 cells per cc., while those from the casks and bottling machine did not reduce, and even those which contained many less than 420 cells per cc. gave a deposit and all in practically the same time.

It is not really surprising that such great differences in the degree of infection produced so little difference in the stability. Yeast multiplies in geometric progression and, with equal rates of growth, it would consequently be necessary to diminish the infection 10 times in order to double the stability and 100 times in order to prolong the life three times.

The important point in regard to stability is that reduction of the beer should take place before the yeast has had time to develop. Air at bottling or racking is the oil to the flame, and it is absolutely necessary to replace it by something else. The CO_2 collected from the fermenting vessels might have been specially designed for the purpose.

All the beers of the experiments described were very sensitive to air, but beers which are not do exist. I have had light beer in which yeast did not develop or only to a small extent after they had been aërated. I had no knowledge of r_H at the time, and cannot say whether they had a particularly strong reducing power or whether some other factor caused the resistance to yeast growth. It will be necessary to wait until similar cases are again met with before the explanation can be found.

Rod bacteria are not influenced by r_H to the same extent as yeasts, and they develop readily below r_H 13, when methylene blue is decolourised. I have not been able to find out whether they grow better at higher r_H, since the beers which I examined were then rapidly invaded by yeast.

r_H and the "sun flavour" in the beer. During my experiments on reduction I found by chance that a beer exposed to the sun was reduced very rapidly. While several days were necessary for reduction in darkness, this occurred in less than half an hour in sunlight unless the beer was strongly aërated, in which case it was not reduced.

Although the reducing power is very much weakened, if not non-existent, at 0° C., beer at this temperature is reduced in a short time in sunlight.

It is well known that beer exposed to sunlight develops a very disagreeable odour and flavour, known as "sun flavour," which makes it undrinkable. This flavour and smell were very distinct in beers reduced in sunlight, but did not develop in those reduced in darkness. On the other hand, light had no effect on the flavour when reduction was prevented by aëration.

I have made many experiments on reduction in sunlight with beers containing methylene blue. The beers were always tasted by several people unaware of the conditions, and their reports clearly showed that the flavour came with reduction. The following is a striking experiment. Beer coloured with methylene blue was poured partly into Petri dishes and partly into ordinary glasses and exposed to sunlight for half an hour. The beer in the glasses was reduced except at the surface, where the contact with air caused a green layer of 1 or 2 cm. to persist. In Petri dishes all the beer remained green on account of exposure in such a thin layer. The beer in the glasses had a pronounced "sun flavour." That in the Petri dishes had not, although it offered much greater surface to penetration of the rays of light. Sun flavour is therefore connected with reduction.

Beer which had been some time in cask or bottle and which had already been reduced to some extent, acquired the objectionable flavour much more readily than freshly-bottled beer.

A glass of beer on the terrace of a café can easily get the sun taste if the customer is slow in drinking it. Spacious terraces and cafés with large windows are consequently not the ideal places for serving beer. In this, again, our ancestors were right with their cool dark taverns, and their tankards or mugs.

Sun flavour is connected with reduction, but it does not disappear when the beer oxidises again. Glasses of beer which have been reduced in the sunlight and afterwards allowed to re-oxidise by exposure to air retain their bad flavour. This reduction effect is, therefore, irreversible.

Sun flavour is produced not only by direct sun rays, but also by diffused and artificial light, but very much more slowly. A series of bottles of the same beer to which Cresol blue or methylene blue had been added was divided. Some were placed in sunlight, others in diffused light, other 8 or 10 inches from a 100 watt electric light, and a few in darkness. The beers in direct sunlight were reduced in less than half an hour; those exposed to the electric light in 2 to 4 days; those in diffused light in 6 to 8 days, while those in darkness were not reduced until between 14 and 20 days. In this experiment the development of sun flavour corresponded with the reduction.

We have seen that reduction prevents the development of yeast. In carrying out stability tests it is consequently necessary to avoid exposure to light in the thermostat previously to putting the beers in it.

Conclusions.

Determination of r_H has already made it possible to add precision to the effect of aëration at racking or bottling, and to discover that sun flavour in beer is also connected with reduction.

It is not only at racking and bottling that aëration has deleterious effects. There are probably many other phenomena during brewing which, like sun flavour, are connected with reduction or oxidation without our knowledge. r_H gives us the means to study these. We know, for example, that hop resins are readily oxidisable. It is probable that the r_H of the wort and beer has some effect on them.

The rudimentary technique which has been followed in the experiments described here will probably be insufficiently precise to continue the studies much further. It will be necessary, in particular, to take samples and carry out experiments in the absence of air. Without this a precise and methodical study of the subject is impossible. Usually a current of pure nitrogen is bubbled through liquids during determinations of r_H, but in the case of beer the frothing would be too troublesome to make this possible.

It will also be necessary to try and titrate the oxidation-reduction systems present in beer. Great difficulties will probably be encountered in this. The titration of buffer systems is very simple in acidimetry, because these systems are, with a few rare exceptions, reversible. They can be brought at will to the acid or alkaline state and back again, and they react instantaneously. Reversible oxidation-reduction systems are, on the other hand, rare. They are always accompanied in physiological liquids by irreversibly oxidisable or reducible substances, or by slowly reversible systems or, in other cases, reversible only in presence of catalysts. All this makes this study very difficult, and much more complicated than that of p_H and acid-base systems.

Discussion.

Mr. J. L. Baker said they were greatly indebted to M. de Clerck for submitting his paper to the Institute—a paper which he (the speaker) considered one of the most valuable and suggestive that had been read in recent years. M. de Clerck's findings opened up an extensive vista of research, for it would be necessary to study in what manner the different organisms concerned with beer would affect the r_H. Certain bacteria and yeasts associated with abnormalities in beer developed equally well under aërobic or anaërobic conditions, and it would be of interest to know how the r_H would be affected in such cases.

Mr. H. F. E. Hulton said that a point in the paper which especially interested him was the reference to sulphites and their influence on the stability of beer. It was curious that the addition of minute amounts of sulphurous acid had a beneficial effect on beer stability, though the quantities added could hardly have any real antiseptic value in the ordinary sense, especially when considered in relation to the fact that increasing the dose above a relatively small amount proved no advantage (see Baker and Day, this *Journ.*, 1911, 465). An explanation now seemed to be forthcoming in the favourable action which sulphurous acid might have upon the r_H of the beer in removing traces of free oxygen.

Mr. A. J. C. Cosbie said he would like to associate himself with Mr. Baker's view that

M. de Clerck's paper was one of the most important contributions the Institute had had for many years. A new weapon had been placed in the hands of the brewer's chemist, and it might perhaps help in attacking the question of "stability." He was particularly interested in Mr. Hulton's remarks about bisulphites, and he agreed that beyond a certain limit little benefit was to be derived from the use of those compounds.

Mr. W. J. WATKINS said he had been in touch with Mr. Hind in connection with M. de Clerck's work, and he had carried out some rough experiments, without very successful results, but it had taught him that if there was anything in the idea of the amount of air that was absorbed by the beer which was to be chilled and filtered and bottled, there was far too much in that which he was handling at the present time. He had used methylene blue as an indicator, but he had failed to get it to come back to anything like its proper colour except at the filters. He had been able to produce some slight differences in colour by aërating the beers excessively. He always took great care to avoid the presence of air in the conditioning and cold store tanks. During bottling the beer was moved about by air pressure. The diffusion of air and CO_2 was almost instantaneous, and the idea that because there was a cushion of CO_2 on the top of the tank of beer, it would keep the air on the top of the CO_2 was erroneous. Then again there was the question of what was to be used as a counter pressure on the bright tank and on the bottling machine ; most bottling machines were counterpoised partially by the air which was displaced by beer in the bottle. It was well known that if gas containing air was used for carbonating, apart from the nuisance of fobbing when bottling, it would cause an early haze or greyness in the beer. It would be of interest to know if there was any relation between oxidation-reduction and the appearance of protein haze ?

Mr. H. L. HIND said that M. de Clerck thought there was a relation between protein haze and oxidation reduction potential, and it was quite possible that oxygen was a factor concerned.

Mr. W. J. WATKINS asked what was the lowest value for r_H which might be expected in a beer.

Mr. H. L. HIND replied that the lowest figure was about 11·5 to 9·5.

Mr. G. C. GREEN asked whether M. de Clerck had extended his studies to the r_H in the fermenting vessel, how he has followed it, and what the course of the r_H was during the actual fermentation. Was it possible to determine the r_H of a stout or a coloured beer ?

Mr. H. L. HIND said that M. de Clerck had followed the changes in oxidation-reduction potential during fermentation in a small flask, and the colour disappeared in a few hours. For stouts an electrometrical method had to be used.

Mr. G. C. GREEN said that assuming it was possible to adjust the r_H in the fermenting vessel, could it be done without at the same time altering the p_H ?

MR. H. L. HIND said he did not think that it would have any effect on the p_H.

Mr. B. DIXON said that one of the points which struck him was the fact that W. Mansfield Clark insisted that the use of the term r_H originally introduced by himself was erroneous. His reason for so doing was that it had been used unjustifiably to describe the condition of biological systems of unknown constitution and incompletely studied properties. He stated that all such determinations should be given in terms of ^E_H. As the ^E_H was in some way dependent upon the p_H, a determination of the p_H was required. It was generally understood that colorimetric determinations of the oxidation-reduction potential applied to biological systems introduce uncontrollable errors and were never likely to reach the same standard of accuracy as colorimetric p_H determinations. The reactions concerned in the oxidation-reduction processes in beers and worts were incompletely studied. Hence it was obvious that all useful investigations must be carried out by means of electrometric methods. There were, however, difficulties in the way of electrometric determinations such as the effect of pressure on the agar in the bridge between the two half-cells.

Mr. H. L. HIND said M. de Clerck was aware of that difficulty, and it was for that reason that he prefers the colorimetric method.

Mr. DIXON said that a quicker result could be obtained by means of the electrometric method. Time was rather important.

Mr. H. L. HIND said that the decolouration was slow because the r_H of the beer was changing slowly. The colour of the methylene blue would return as soon as that r_H was reached. Consequently, if the electrometric method was used, it would take an equally long time.

Mr. DIXON said that owing to the difficulty of observing the colour change of most of the available indicators in even a moderately dark beer, and further, owing to the effect of p_H on these indicators, an excess of indicator had to be added. That prejudiced the determination of the r_H itself and might also have a toxic effect on micro-organisms present. He had tried experiments on taking samples of beer from tanks without contamination by air. It was most difficult and he thought it would be more satisfactory if a standard method were devised. He was aware of the frothing that occurred when certain gases were passed through beer. He had been fairly successful with the following method :—A screw-stoppered bottle with a specially devised sealing apparatus in which an electrode, an agar bridge, and two tubes were fitted, was filled with distilled water and chilled. The water was forced out by means of a slight pressure of CO_2 which was as nearly pure as possible. The beer was then let in against this slight counter pressure. The beer was then introduced into the bottle without contamination by air. The agar was securely held in place by means of the special apparatus that he employed. It was advisable, when carrying out the experiment, to bear in mind the effect of CO_2 gas on the agar in the bridge and on the p_H value.

Mr. H. HERON said that apparently the reducing power in beers would appear from the evidence brought forward to be traced to the hop resins. If that were the case it might be expected that English beers, on account of their higher hop rate, would have a rather greater reducing value than the beers with which the author had been carrying out his experiments, and it would be necessary to use those indicators which gave higher values. It was evident than English beers were not so sensitive to sunlight as were beers of the lager type ; he had certainly not noticed any marked alteration in flavour after exposure to sunlight for comparatively short intervals of time.

Mr. C. W. MCHUGO said that the paper opened up a new vista of scientific work in connection with brewing, and one which, no doubt, in time would lead to valuable practical results. In connection with the examination of bottled beers he gathered that the determination of the r_H, or oxidation-reduction ratio, was a more exact and scientific method of measuring the amount of oxygen not actually absorbed by the beer. The stability of bottled beer, in his opinion, depended very largely upon three factors. The first of these was the number of living organisms present in the beer at the time of bottling. The second, the amount of oxygen left unabsorbed in the bottle, and the third, the degree of the attenuation of the beer itself. He considered that the last of these factors was by far the most important, and it was his experience that where beers were specially brewed and suitably attenuated for bottling, and also, of course, all reasonable care taken in the bottling itself, such beers usually had a high degree of stability.

Mr. H. L. HIND, in reply to Mr. Heron, said that unfortunately there was so much air in English beers that they were not more reducing than lagers. Bottled beers—with which trials were made—retained the colour of methylene blue, they were too fully oxidised, but that was the first indication that such a method of control was possible. Consequently only one or two trials had been made.

Mr. J. STENHOUSE proposed a cordial vote of thanks to M. de Clerck for his paper, and for coming so far to be present when it was read. He would also like to thank Mr. L. Hind for the excellent translation of the original paper and for the reading of it.