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THE NON-EQUILIBRIUM OXIDATION-REDUCTION POTENTIAL

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The question as to the nature and importance of the redox-potential determined in non-equilibrium and irreversible redox-systems is nowadays one of the most actual problems of the theory of the oxidation-reduction (redox) potential. That is easy to understand if we call to mind that a large majority of biochemical and biological redox-systems are non-equilibrious. However, the theory of the redox-potentials, founded on propositions of the classical electrochemistry, can be directly applied only to reversible, equilibrious redox-systems. We have thus to state a gap between the theory of the redox-potentials and the experimental material obtained in the course of biological and physico-chemical investigations.

This gap impedes our further progress and more than once attempts have been made in literature to «theoretically conceive» the redox-potentials determined in non-equilibrium biochemical redox-systems, for example, in solutions of substances containing the sulfhydryl group (cystine-cysteine, glutathione), in sugar-solutions, etc. (R. Wurmser, L. Michaelis and others [1, 2, 3, 4]]. But these attempts have resulted in merely indefinite and ungrounded advancements.

If we want to through light on the physical nature of the redox-potential measured in non-equilibrium and irreversible redox-systems, we must call to mind the process of potential formation on an indifferent electrode immersed in an aqueous redox-system.

In aqueous media there corresponds to any potential of indifferent electrode a definite superficial concentration of hydrogen or oxygen on the electrode. However may proceed the process of saturation of the surface of the electrode, this process proceeds up to the time (or, more exactly, up to such concentrations of hydrogen or oxygen on the electrode) where it is counterbalanced by an opposite process leading the hydrogen (oxygen) off from the electrode. If the electrode reacts with a reversible redox-substance, this opposite process is also a reverse one with respect to the former. So, say, the action exerted on the electrode by the reduced form (Red) of a reversible oxidizing-reducing agent (for instance, hydrochinon), leads to an increase of hydrogen concentration on the electrode, while the action of the corresponding oxidized form—Ox—(for instance chinon) results in a decrease of that concentration. In the course of their «reactions» with the electrode the Ox- and Red-forms undergo a mutual transformation which phenomenon is to be regarded as a fundamental characteristic of the true (labile) chemical equilibrium. The process is quite different in the case of irreversible systems: the Ox-form of cysteine—cystine for example is not reduced under ordinary conditions and, although the Red-form of cystein displaces the electrode-potential in the negative direction and performs consequently the same function as the Red-form of any reversible redox-substance, the processes limiting this potential—displacement can obviously not result

in the regeneration of the primary Red-form, being therefore not inverse, but only opposite with respect to their action on the electrode. Thus on the latter a typical stationary condition is created.

Let us now examine one of the most simple examples of a redox-system, characterized by a complete absence of reactionable Ox-forms. The electrode potential can be displaced in this case in the positive direction: first, under the influence of molecular oxygen (if the latter is present in the electrode surrounding medium, or dissolved in the metal which it is made of), and secondly, owing to a decrease in the amount of hydrogen on the electrode which may be due to direct diffusion of hydrogen into the surrounding medium, or recombination into H_2-molecules, followed by their subsequent diffusion.

The electrode potential attains a stable value when the summed up velocity of the above processes becomes equal to the velocity of the potential displacement in the negative direction by the non-equilibrious Red-form, e. g. by cysteine.

In accordance with the above let us assume that

$$E = \frac{RT}{F} \ln \frac{[H+]}{aC_H} + E_0, \qquad (1)$$

where E—the electrode potential; a—the coefficient of proportionality; C_H—the hydrogen concentration on the electrode, and E —the normal potential*.

The dependence of E on the different factors can be expressed as

$$\frac{dC_H}{dt} = k_1[\text{Red}] - k_2 \cdot C_H[O_2], \qquad (2)$$

k_1—the velocity constant of the reaction $\text{Red} \rightarrow (\text{Ox}) + H$, where (Ox) represents the inactive form; k_2—the velocity constant of the oxidation reaction between hydrogen and oxygen on the surface of the electrode. After a stationary condition, i. e. a limiting potential, has been established, the following relations are valid: $\frac{dC_H}{dt} = 0$, i. e. $k_1[\text{Red}] = k_2 \cdot C_H[O_2]$; $C_H = \frac{k_1[\text{Red}]}{k_2[O_2]}$, and consequently

$$E = \frac{RT}{F} \ln \frac{k_2(O_2)}{k_1[\text{Red}]} + \frac{RT}{F} \ln [H^+] + E_0. \qquad (3)$$

It may appear at first sight that there exists no difference between the above equation and the usual thermodynamic equation of the same process, provided that the part of the Ox-form is played by oxygen. In reality there does exist an essential difference.

It is known that precisely in the case of a reversible reaction the relations between the velocity constants of the direct and inverse process can be expressed by the equation $\frac{k'}{k''} = K$, where K is the constant of the reaction equilibrium $K = \frac{[\text{Ox}]\,[H]}{[\text{Red}]}$. This relation ensues from the mass law.

Hence the equation for E can be transcribed as follows:

$$E = \frac{RT}{F} \ln \frac{k''[\text{Ox}]}{k'[\text{Red}]} + \frac{RT}{F} \ln [H^+] + E_0 = \frac{RT}{F} \ln \frac{[\text{Ox}]}{K[\text{Red}]} +$$
$$+ \frac{RT}{F} \ln [H^+] + E_0 = \frac{RT}{F} \ln \frac{[H+]}{[H]} + E_0. \qquad (4)$$

* The stoichiometric ratios of the reaction between the hydrogen adsorbed on the electrode and the oxygen do not matter to the object of our reasoning. The second term of the right part of the equation is, therefore, given the simplest possible expression.

Thus it is obvious that the final potential of an equilibrium redox-system is not influenced by kinetic factors.

As to the equation given for the non-equilibrium system, there exists, as a rule, no connection between the constants k_1 and k_2, and therefore they cannot be elimitated from the equation deduced for E.

The situation we have before us when determining E in a non-equilibrium redox-system after the indicator method may be analysed by expressions of the same type as used by O. Warburg and Christian[5] for the deduction of the relation existing between the degree of oxidation of the «yellow oxidizing ferment» and the oxygen content of the liquid.

Let be: C—the total concentration of the redox-indicator, equal to $C_{Ox} + C_{Red}$, [Red]—the concentration of the reduced form of the irreversibly oxidixed substance, and $[O_2]$—the concentration of oxygen. We get for the stationary state:

$$\frac{C_{Ox}}{C} = \frac{k_2 [O_2]}{k_1 [Red] + k_2 [O_2]}, \qquad (5)$$

where $\frac{C_{Ox}}{C}$ is the degree of colouring of the indicator (Färbungsgrad) after which, according to the indicator method, the redox-potential is calculated. From the above equation it may be seen that in non-equilibrium systems this quantity depends directly on the velocity constants of the reactions taking place between the indicator and the redox-components of the system.

Thus, in whatever way we measure the quantity of the redox-potential in a non-equilibrium system, the value obtained will necessarily reflect not only the concentrations and relationships of the redox-components, but also the kinetic characteristics of the system which are identical with the velocity constants of the corresponding redox-reactions. From the dependence of the velocity constants on the concentrations of the «accelerators»—catalyzers and ferments follows that an addition to the system of a ferment, accelerating the splitting of hydrogen off the reducing agent, will displace the potential in the negative direction exactly in the same way as an increase of the Red-concentration*.

The question suggests itself: may this dependence be observed in the experiment? Fortunately, one is able to give a positive answer. Although the lack of definite theoretical interpretation of the nature of non-equilibrium redox-potentials prevented a systematic experimental study of non-equilibrium redox-systems, we can point out a number of observations made with other purpose in view which show an obvious dependence of the redox-potential measured in stationary (and consequently non-equilibrium) systems on the velocity of corresponding redox processes. For example, it has been established that:

1) The addition to cysteine solutions of substances known as catalyzing their oxidation (dehydrogenation) results in a decrease of the limiting redox-potential ([1], [3]).

2) The addition to acetaldehyde solutions of dehydrase preparations has the same effect ([1], [3]).

3) The same dependence is observed in a number of experiments on intercellular redox-potentials ([4], [6], [7]). The experimenters added to aqueous cell suspensions very small quantities of substances endowed with a desactivating power with respect to one or the other of the different redox-enzymic cell systems. It has been found that the reagents inhibiting the oxidizing enzymes (KCN, CO, H_2S) greatly depress the intracellular redox potential in aerobic conditions but do not exert any influence in anaerobic conditions.

* It should be noted that strictly speaking the composition of a stationary redox-system can remain unchangeable only under the condition that the «sources» of oxidation and reduction are not subject to a decrease. In many cases oxygen is supplied by the atmosphere which may of course be considered as a permanent source. The source of the reducing agent is limited and can be considered as permanent only within a relatively short period of time. However, in biological systems, as, for instance, in bacteria cultures, the vital activity of the microorganisms and tissues may produce new and new quantities of substances endowed with reducing ability.

The reagents inhibiting the dehydrogenases (ethyluretane, sodiumpyrophosphate) displace the aerobic limiting potential in the positive direction. Analogous results were obtained by Korr([8]) who determined the redox-potential in a culture of phosphorescent bacteria, and by other experimenters.

The equation for E, which has been discussed above under the aspect of formation of a definite potential on the electrode, is valid also in the absence of any electrode. In this case C_H will express the concentration, or (in the case of sufficiently strong positive potential values) the «probability of appearance» of hydrogen in the solution itself. The stationary (limiting) redox-potential represents the measure of the a c t u a l work which can be performed by a system in the course of a redox-reaction, provided that the values of the velocity constants remain the same as during the potential determination. It is evident that this work cannot be equal to the change in the free energy of the system but it expresses nevertheless the a c t u a l intensity of the oxidizing and reducing action which can be developed by the system at the given redox-potential concentrations and k i n e t i c c h a r a c t e r i s t i c s.

The above conclusions comprise not only redox-systems. They are also valid for reversible redox-systems containing s l o w l y r e a c t i n g components, as, for example, for the redox-couples known in the biological physico-chemistry (succinic acid—fumaric acid; tartaric acid, lactic acid, and others) if those systems are activated by the corresponding enzymes. With the aid of equations analogous to the aforecited, it can be easily shown that the redox-potential of such systems must be stationary and will therefore markedly depend upon the kinetic factors and concentrations of O_2, provided $k'_2\,[Ox] \leqslant k_2\,[O_2]$, where [Ox] is the concentration of the oxidized component, e. g. fumaric acid, acetaldehyde, and others, and k'_2, the velocity constant of the reaction $Ox + nH \rightarrow Red$.

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