

Amperometric method for simultaneous monitoring of oxygen and hydrogen peroxide concentrations

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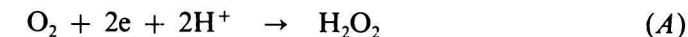
A new method of continuous analysis based on polarographic principle and an apparatus built for this purpose are described. The method is suitable for systems with partly overlapping polarographic waves and it was used for monitoring oxygen consumption and hydrogen peroxide formation in kinetic studies.

Описывается новый метод континуального анализа, основанный на полярографическом принципе, а также прибор, созданный для его проведения. Данный метод применим для систем с частично перекрывающимися полярографическими волнами и был использован для регулировки потребления кислорода и образования перекиси водорода в кинетических исследованиях.

In mechanistic investigations of oxidation by molecular oxygen, the kinetics of hydrogen peroxide formation and consumption is of importance, being very often indicative of reaction pathway [1]. Polarographic analysis of oxygen and hydrogen peroxide was found suitable for some systems [2], but its classical form is inconvenient for monitoring systems in which chemical reactions occur with medium velocity. A method of quasicontinuous analysis of both components based on polarographic principle was therefore developed and an apparatus built for this purpose [3—5]. From the electrochemical point of view, the method should be considered as a continuous amperometric analysis with dropping mercury electrode (DME) and it can be used for analysis of multicomponent systems the polarographic waves of which are partly overlapping. Its principle will be explained on the O_2 — H_2O_2 system.

Principles

Polarographic reduction of dissolved oxygen on a dropping mercury electrode proceeds in two steps (Fig. 1, full line) corresponding to electrode reactions



The limiting currents at potentials E_1 and E_2 are i_1 and $2i_1$, respectively, and they are proportional to oxygen concentration. When hydrogen peroxide is added to the solution, the limiting current at E_2 increases by the value of i'_2 , proportional to hydrogen peroxide concentration (Fig. 1, dotted line), while the current i_1 remains unchanged. Consequently, concentration of oxygen is proportional to i_1 , concentration of hydrogen peroxide in solution to $i_2 - 2i_1 = i'_2$, where the factor 2 incorporates the stoichiometry of the electrode reactions.

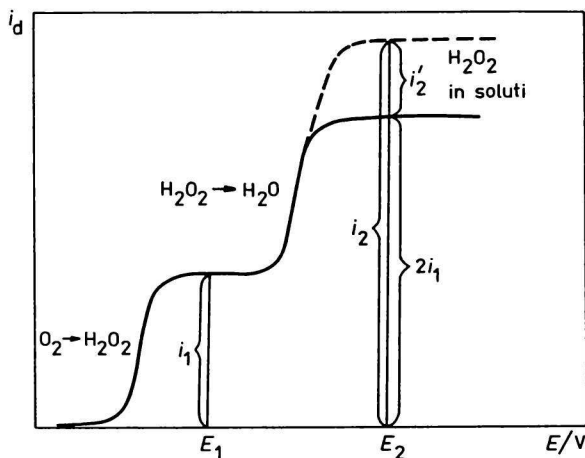


Fig. 1. Polarographic reduction of oxygen and hydrogen peroxide.

A quasicontinuous analyzer of O_2 — H_2O_2 solutions should contain a polarograph, a control unit and a computing device. On each odd drop of the electrode, potential E_1 is imposed, current i_1 measured and stored in an analogue memory. On even drops, the potential is set to E_2 , current i_2 is measured and the value $i_2 - 2i_1$ stored in the other memory. At the output of the memories, signals proportional to concentrations of oxygen and hydrogen peroxide are available as functions of time.

The analyzer can perform the classical polarography as well. In this case, the potential is changed stepwisely by means of a digital-to-analogue converter. A differential polarogram is available from the computing network if current i_1 is not multiplied by any factor when subtracted from the value i_2 .

Experimental

All chemicals were of anal. grade purity. Reconstituted cytochrome P-450 LM-2 from rabbit liver and benzphetamine (*N*-benzylmethylphenylethyl amine) were granted by the Central Institute of Molecular Biology, Academy of Sciences of the GDR, Berlin.

The analyzer

In Fig. 2, the idealized block diagram of an analyzer based on this principle is shown. First, it contains a two-electrode or three-electrode polarograph 1 with the polarographic vessel 2 and a current-to-voltage converter 3. The polarization voltage is set to the value E_1 by a helical potentiometer R; in all even periods the potential is increased by means of an electronic switch S_E to the value E_2 . The voltage increment is set by another potentiometer R'

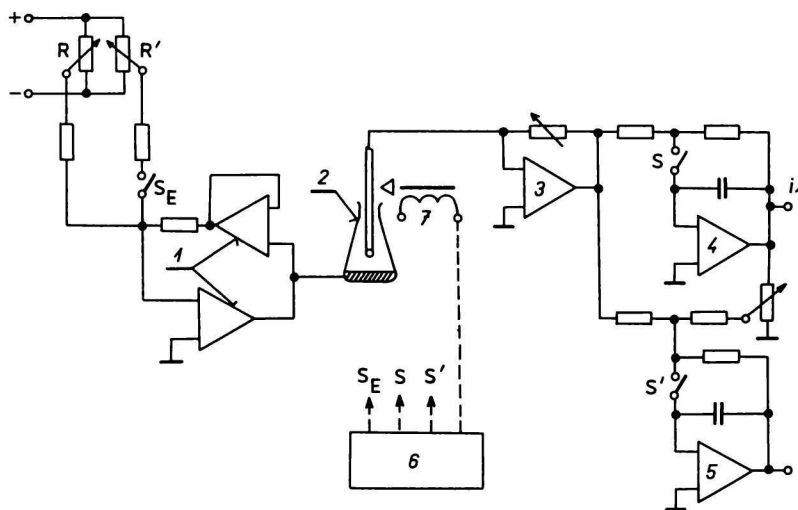


Fig. 2. Principle of the analyzer.

1. Polarograph; 2. polarographic cell; 3. current-to-voltage converter; 4, 5. analogue memories; 6. control unit; 7. hammer; R, R' — setting potential E_1 and $\Delta E = E_2 - E_1$; S_E , S, S' — electronic switches.

The current response is obtained on the output of the converter 3 and it is lead to inputs of two controlled integrators used as sample-and-hold amplifiers 4 and 5. They are controlled by electronic switches S and S' so that current samples are taken in a proper instant of life of the dropping electrode. The amplifier 4 is activated in odd periods, so that it contains the value proportional to i_1 , while the other amplifier 5 is activated in even periods of DME. The subtraction is done by a simple analogue network of the amplifier 5. The digital-to-analogue converter, necessary for classical polarography, is not involved in Fig. 2.

The analyzer is controlled by a timing unit 6, which alters the potential, activates amplifiers 4 and 5 and the hammer 7 of the DME. The timing unit controls also the digital-to-analogue converter and modifies the network of amplifier 5 if classical analysis is performed.

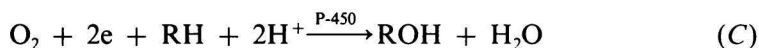
Procedure

The reactions were studied directly in a polarographic vessel without free space above the solution, so that changes in oxygen concentration due to diffusion from gaseous phase were eliminated. The temperature was kept at 25.0 °C. First, classical polarogram of oxygen was recorded in order to obtain setting of both potentials E_1 and E_2 . Then, the reaction was started by injecting the substance to be oxidized into the solution, and both outputs from amplifiers 4 and 5, *i.e.* profiles of $[O_2]$ and $[H_2O_2]$ vs. time, were recorded. Calibration was carried out by KCl solutions for which solubilities of oxygen are tabulated [6].

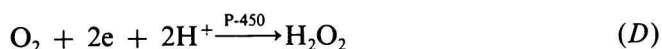
Results

The analyzer was used for kinetic investigations of oxidations by molecular oxygen. The oxidation (hydroxylation) of benzphetamine catalyzed by the enzyme cytochrome P-450 was chosen as an example.

In biological systems removal of numerous toxic substances RH rests in their hydroxylation catalyzed by the enzymic system cytochrome P-450. The hydroxylation proceeds according to the equation [7]



A simultaneously occurring reaction, called uncoupling mechanism



diminishes the yield of the hydroxylated product ROH (reaction C) and produces hydrogen peroxide which is toxic for organisms. Hence, the partition of oxygen between both reactions is of importance.

Hydroxylation of benzphetamine, a tertiary amine widely used as model substance, was studied by means of the amperometric analyzer. Reconstituted enzyme system P-450 LM-2 was used as a catalyst. Both oxygen consumption and hydrogen peroxide formation (Fig. 3, curves 1 and 2) were found to be reactions of the first order with rate constants $k_{O_2} = 3.44 \times 10^{-4} \text{ s}^{-1}$ and $k_{H_2O_2} = 2.51 \times 10^{-4} \text{ s}^{-1}$, respectively. Hydrogen peroxide concentration remains constant for about 15 min after total consumption of oxygen. From this we conclude that no hydrogen peroxide reactions manifest themselves during the followed reaction period. The fraction of oxygen inserted into the substrate was calculated from the mass balance of oxygen in the course of the reaction (Fig. 3, curve 3).

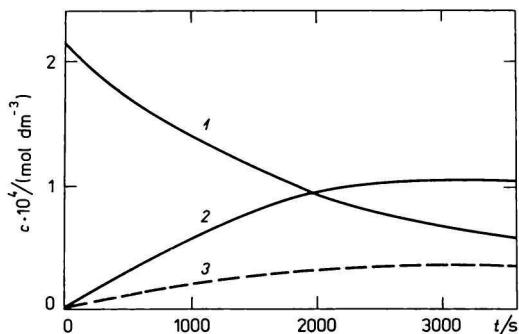


Fig. 3. Variation in O_2 and H_2O_2 concentrations with time in the system O_2 —benzphetamine—reconstituted P-450 LM-2.

$c(\text{P-450 LM-2}) = 1.95 \times 10^{-6} \text{ mol dm}^{-3}$; $c(\text{benzphetamine})_0 = 2.6 \times 10^{-3} \text{ mol dm}^{-3}$; $c(\text{reductase})_0 = 3.8 \times 10^{-7} \text{ mol dm}^{-3}$; $c(\text{NADPH})_0 = 1.1 \times 10^{-3} \text{ mol dm}^{-3}$; $g(\text{dilauryl lecithin}) = 0.63 \text{ mg dm}^{-3}$ Phosphate buffer pH = 6.86. Potential: oxygen $E_1 = 0.9 \text{ V}$, hydrogen peroxide $E_2 = 1.6 \text{ V (vs. Hg)}$. 1. O_2 ; 2. H_2O_2 ; 3. calculated fraction of O_2 inserted into product ROH.

Kinetic data of oxygen consumption and hydrogen peroxide formation were obtained for the first time in this system and along with a more detailed study [8] contribute to elucidation of cytochrome P-450 action.

Conclusion

The proposed principle of amperometric analysis was found useful for investigation of reaction kinetics of multicomponent system, namely O_2 — H_2O_2 . It has been used for several years in our laboratories and enabled, as *e.g.* in the case of cytochrome P-450, access to unique information. The prototype used was built on LSI level circuitry. A microprocessor control, however, would convert it in a versatile analytical tool. Moreover, its principle would be easily incorporated as one of operating modes of any microprocessor controlled polarographic analyzer.

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