A CATALASE MEMBRANE ELECTRODE FOR THE DETERMINATION OF HYDROGEN PEROXIDE

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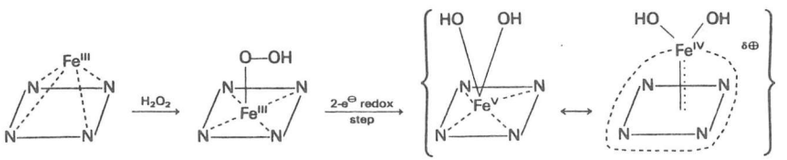
There is a great need for a direct method to determine hydrogen peroxide due to its wide and different applications in all areas. Enzyme sensors have been developed for that purpose. The use of an enzyme electrode enables us to combine the specificity of enzyme reactions with substrates with electrochemical detection of the product. In this case hydrogen peroxide is the preferred substrate of the enzyme catalase which causes oxidation to oxygen following the overall reaction

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

and the mechanism proposed [1] suggests that the two peroxide molecules react with the enzyme in sequence. There is the formation of compound I with the first H_2O_2



compound I

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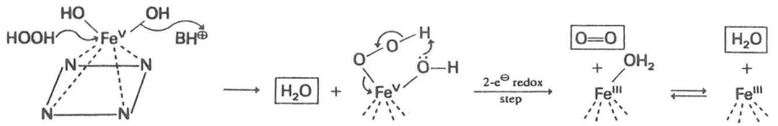
and following this compound I reacts with the second H_2O_2 giving the products.



Previously double membranes were used: an enzyme containing membrane placed over the membrane of an oxygen electrode. In this study catalase was covalently immobilised directly onto the oxygen permeable modified Teflon membrane used in the oxygen electrode. The catalase used was from bovine liver (H_2O_2:H_2O_2 oxidoreductase; EC 1.11.1.6), supplied by Sigma.

The graft copolymer, Teflon-g-co-acrylic acid, was prepared as follows. The Teflon membrane was irradiated in 30cm^3 of an acrylic acid solution (10% in distilled water) for 72 hours at 18.3 rad s^{-1}. It was then removed from solution, washed thoroughly with water and dried to constant weight in vacuum at 40ºC.

In order to immobilise the enzyme, the graft copolymer was treated with an enzyme solution containing 40mg of enzyme and 20mg of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodi-imide metho-p-toluene-sulphonate (CMC) as described earlier [2]. The activity of the enzyme was determined using hydrogen peroxide.

A membrane with an initial specific enzyme activity of around 7000U/g was used in the studies described below.

The Teflon membrane containing immobilised catalase was placed over the gold electrode of the standard Clark-type oxygen electrode (Metrohm EA-541) in the usual way, and connected to a Metrohm 627

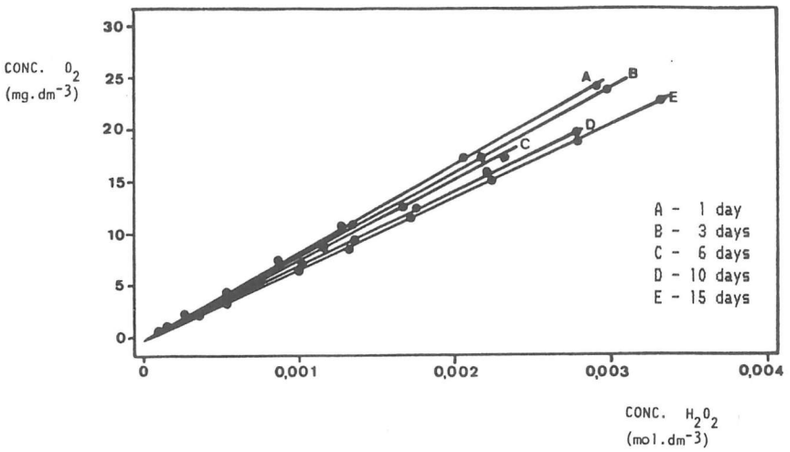


Fig.1 Calibration curves for hydrogen peroxide oxidation at the catalase enzyme sensor.

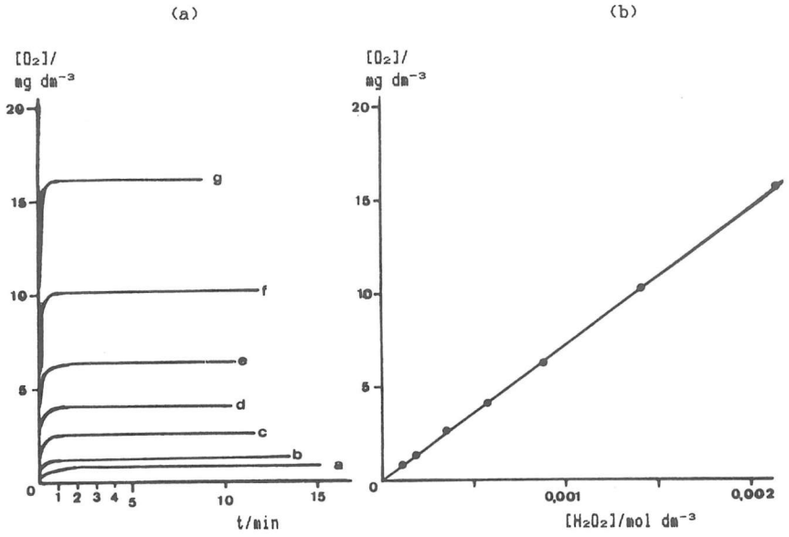


Fig.2 (a) Response time and stability of the sensor after successive additions of H_2O_2. (b) Corresponding calibration curve.

O_2-meter. All measurements were done in a 0.1M phosphate buffer solution of pH=6.87.

In Fig.1 are shown calibration curves of the enzyme sensor, which show good linearity for concentrations from 10^{-5}M to 3.10^{-3}M, and good lifetime of one month. The difference in initial response and response after 15 days is small. The sensor is very precise and can be applied to non coloured solutions, thus having an advantage over the colorimetric method [3].

Response times are shown in Fig.2 for different concentrations together with the corresponding calibration plot. The final potential is reached within less than a minute, and does not alter with time.

This catalase sensor is quite robust and portable enabling its use for measuring hydrogen peroxide *in situ*. The quantity of sample used is very small. Comparing with the methods normally used for hydrogen peroxide determination the electrochemical method seems a very reliable one.

In conclusion, this enzyme sensor responds linearly to hydrogen peroxide over a wide concentration range, has a good detection limit, a very short response time and a reasonable lifetime for the immobilised enzyme.

References

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STRIPPING VOLTAMMETRY AT HYDRODYNAMIC MERCURY THIN FILM ELECTRODES

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In anodic stripping voltammetry from mercury electrodes, mercury thin film electrodes (MTFE) offer advantages over hanging mercury drop electrodes (HMDE), in that the current peak in redissolution is narrower and the peak current higher, offering improved sensitivity, detection limits and reproducibility. Glassy carbon substrates are generally used, and pre-deposition or co-deposition of mercury with the metal ion successfully employed. An important advantage of the MTFE is that it is directly usable in forced convection systems with electrode movement or solution flow, thus being applicable to on-line electroanalysis. Additionally, at positive potentials the bare glassy carbon electrode can be used for other electrode reactions, since the mercury is readily removed electrochemically or mechanically.

Conventionally, although the pre-concentration (or deposition) step is carried out with forced convection, this is stopped during stripping, ostensibly to give better reproducibility. The increasing use of electrochemical detectors in flow systems, particularly on-line, makes it desirable to continue solution flow. We have therefore re-examined the question with respect to linear scan stripping, theoretically and experimentally, at the rotating disc electrode (uniformly accessible and with electrode movement) and at the wall-jet

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