

Oxygen Supply to Immobilized Biocatalysts. A Model Study*

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Enzymes and whole cells can carry out a wide variety of chemical reactions. Technical processes have been designed for large scale production of different substances with catalysts of biological origin (enzymes or whole cells). The production media used are aqueous solutions. When reactions are to be performed, which demand oxygen, problems arise due to the poor solubility of oxygen in water (the concentration of oxygen in water saturated with air is about 0.25 mM). The problems are especially serious when the catalyst is immobilized and used in packed bed reactors.

One obvious way of increasing the oxygen supply is to modify the medium so that it contains more oxygen. This report presents the use of the enzyme thermistor to monitor such modifications of the medium in a quick and reliable way. The oxidation of glucose by glucose oxidase (E.C. 1.1.3.4.) and catalase (E.C. 1.11.1.6.) was used as an indicator reaction. The enzyme preparation was quite stable and was used for several months without replacing the enzymes.

Substances added to increase the oxygen supply must, of course, be biocompatible. Two different potential oxygen carriers were tested. One is of natural origin, hemoglobin, and one is synthetic, the perfluorochemical FC-75.

Experimental. Materials. Hemoglobin was pre-

pared from pig erythrocytes. FC-75 was obtained from 3M and Pluronic F-68 from Montoil AB. All other chemicals were of analytical grade.

Preparation of FC-75-emulsions. The buffer solution (water phase), the perfluorochemical (organic phase) and Pluronic F-68 (emulgator) were mixed in appropriate proportions. 10 ml of the mixture (in a 25 ml beaker) was cooled with crushed ice. The mixture was sonicated for about 30 sec (a probe was used on a A 350G from Ultrasonics Ltd., England).

Enzyme immobilization. Glucose oxidase (1,000 U) and catalase (300,000 U) were immobilized on 1 ml activated glass beads as described earlier.¹

Experimental set-up. The enzyme thermistor has been used for analyses of many different substances.² With this technique, a suitable enzyme is immobilized on a solid support and the preparation is packed in a small column. When a sample containing a substrate of the enzyme is pumped through the column, heat is evolved. The increase in temperature is measured with a sensitive thermistor.

Several models of the enzyme thermistor have been used.² In the present investigation a standard enzyme thermistor was modified for oxygen analyses (Fig. 1). It was equipped with tubing of stainless steel to prevent leakage of oxygen. The buffer solution (saturated with nitrogen) was kept in a pressure vessel connected to a nitrogen bomb *via* a reduction valve. A pressure of 1.5 atm in the pressure vessel gave a suitable flow rate (1 ml/min) through the system. To assure that the flow rate remained constant, a peristaltic pump was connected to the effluent of the system. With these precautions the flow rate remained at 1.0 ml/min throughout the experiments. A sample loop of stainless steel (vol. 0.5 ml) was used.

The immobilized enzymes used were glucose oxidase and catalase. Glucose was added to the samples. The oxygen of the samples oxidized some of the glucose added in the reaction catalyzed by glucose oxidase. In this reaction hydrogen peroxide

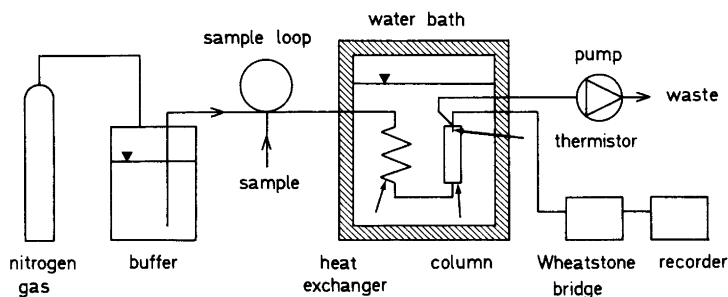
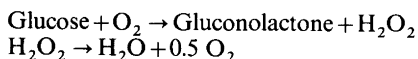
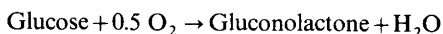


Fig. 1. Enzyme thermistor used for oxygen analyses.

is formed. Catalase catalyzes the decomposition of hydrogen peroxide to oxygen and water. The reactions are:



The oxygen formed in the second reaction is used to oxidize more glucose. The net reaction is:



The thermistor was connected to a Wheatstone bridge unit, which in turn was connected to a recording devise. The response of the thermistor varied with the amount of glucose oxidized. When glucose was present in excess of oxygen, the thermistor response was proportional to the oxygen content of the sample. The enzyme thermistor was chosen for the oxygen analyses because it measures the oxygen *content* of a sample and not the oxygen *pressure* like, e.g., a polarographic oxygen electrode.

Results and discussion. To calibrate the enzyme thermistor, buffer solutions with varying concentrations of dissolved oxygen were analyzed. The same solutions were also analyzed with a polarographic oxygen electrode. The response of

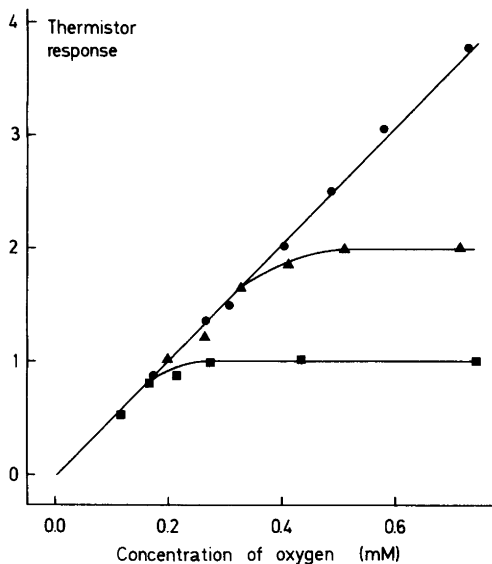


Fig. 2. Correlation between oxygen measurements with a polarographic oxygen electrode (Beckman) and with the enzyme thermistor. The samples were buffer solutions (0.1 M K-phosphate, pH 7.0) with varying concentrations of glucose and oxygen: 10 mM glucose (●), 1.0 mM glucose (▲) and 0.5 mM glucose (■).

the enzyme thermistor was plotted against the concentration of oxygen measured with the oxygen electrode (Fig. 2). When glucose was present in excess of oxygen (10 mM glucose) a straight line was obtained. When lower concentrations of glucose were used the curves levelled off and reached constant values. These plateau-values depended on the glucose concentration.

The effect of oxygen carriers in the medium was then tested. When hemoglobin supplied oxygen to the enzymes in the thermistor, the thermistor response varied with the concentration of hemoglobin in the solution (Fig. 3). Some levelling off was observed at higher concentrations of hemoglobin. This was probably due to incompleteness of the enzyme catalyzed reactions, caused by the short residence time in the column with immobilized enzymes.

The solubility of oxygen from air in the solution without hemoglobin was approximately 0.25 mM. Assuming that the addition of hemoglobin does not

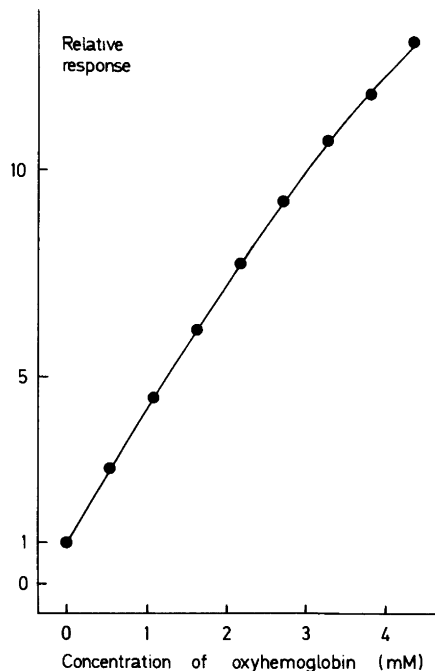


Fig. 3. Effect of hemoglobin concentration on the response of the glucose oxidase-catalase thermistor. Samples containing 0.1 M glucose and varying amounts of oxy-hemoglobin (expressed as concentration of heme-groups) were analyzed. The samples were saturated with air (750 mm Hg, 22 °C). The flow rate through the thermistor unit was 1.0 ml/min. The peak height of the sample without hemoglobin was set to 1.0.

alter the solubility of oxygen very much, the maximal concentration of oxygen in the 2.16 mM oxyhemoglobin solution (2.16 mM with respect to heme-groups) was 2.41 mM. The thermistor response for this solution was 7.8 times that of the solution without added hemoglobin. Assuming that the oxygen in this control was completely used up, the utilized concentration of oxygen in the 2.16 mM hemoglobin solution can be calculated to be 1.95 mM, corresponding to an efficiency of 81%. This efficiency can probably be raised by decreasing the flow rate and thereby increasing the residence time of the sample in the column. However, this would increase the time required for the analyses. The time routinely required per analysis was about 8 min with the conditions reported.

Hemoglobin proved to be a good oxygen carrier in the model system, however, in a potential technical process the oxygen carrier must be rather stable so that it can be used for a long time. Hemoglobin is a rather complex and sensitive substance, so perhaps it is not the ideal oxygen carrier for technical purposes. Other possible substances are perfluorochemicals.

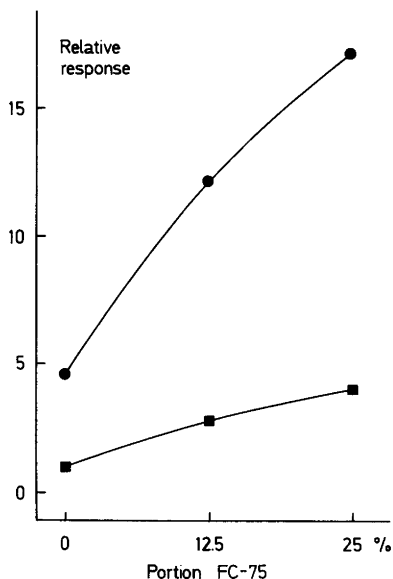


Fig. 4. Response of the glucose oxidase-catalase thermistor as a function of the percentage (v/v) of perfluorochemical in the emulsion analyzed. The perfluorochemical phase of the emulsion consisted of FC-75, while the water phase contained 40 mM glucose in 0.1 M K-phosphate buffer (pH 7.0). The emulgator used was Pluronic F-68 (0.21 g/ml FC-75). The samples were saturated with air (■) or oxygen (●). The atmospheric pressure was 760 mm Hg and the temperature of the samples was 22 °C. The response of the sample without perfluorochemical saturated with air was set to 1.0.

Perfluorochemicals are organic compounds in which all hydrogen atoms have been replaced by fluorine atoms. These compounds are very nonpolar, heat-stable and very chemically inert. Many gases, for example oxygen and carbon dioxide, have a high solubility in perfluorochemicals. These properties make perfluorochemicals suitable as blood substitutes. In this application, pure perfluorochemicals cannot be used, but emulsions of these have successfully been used in "blood-transfusions" to several different animals³ and recently also to humans.^{4,5}

The ability of perfluorochemical emulsions to transport oxygen was tested with the enzyme thermistor. The oxygen-transporting capacity of an FC-75 emulsion increased with the amount of perfluorochemical added (Fig. 4). Since the solubility of oxygen in perfluorochemicals is proportional to the partial pressure of oxygen in the gas phase, the oxygen content of an emulsion can be increased by a factor of approximately 5 if it is saturated with pure oxygen instead of air. The response of the thermistor was 4–4.5 times greater for the emulsion saturated with oxygen compared to that saturated with air. The highest response (25% emulsion of FC-75 saturated with oxygen) was 17 times that of the buffer solution saturated with air.

The solubility of oxygen from air in FC-75 is reportedly 4.6 mM.⁶ A calculation similar to that shown for hemoglobin reveals that the efficiency of oxygen transfer from the 25% emulsion saturated with air to the enzymes in the thermistor unit was 82%.

Conclusions. The enzyme thermistor proved to be a quick and reliable method for determining the oxygen content of a solution, even when an oxygen carrier was present.

Both hemoglobin and FC-75 could carry oxygen to the enzymes in the thermistor unit. The oxygen content of the media was increased by a factor of approximately 15.

Perfluorochemicals like FC-75 do not seem to be deleterious to enzymes. The immobilized enzyme preparation in the thermistor unit was stable for several months at room temperature.

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