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Immobilized enzymes – A simple calorimetric method for the determination of the catalytic activity[1]

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Abstract

The results communicated in this paper show that rapid and reliable information about the activity of immobilized enzymes follows from calorimetric measurements. The study was done using spherical and plain carriers as well as different enzymes (urease, glucose-oxidase, invertase). The enzyme thermistor developed by Danielsson et al. was used as a measuring system. This measuring system was applied to investigate the activity of enzyme carrier complexes produced by the sol–gel technique. The influence of processing parameters could be pointed out at complexes of different forms (xerogel, gel on ceramic carrier, thin gel layers on foil, etc.). With the described calorimetric method, a fast and reliable technique for comparative determination of the activity of immobilized enzymes is available. A special advantage of this method is its variability in carriers and the generally applicable thermal measuring principle. Therefore, it seems useful for the development of new immobilization techniques. © 1998 Elsevier Science B.V.

Keywords: Activity; Calorimetry; Immobilized enzyme; Plain carriers; Sol–gel

1. Introduction

A simple and fast method is required for determination of catalytic activities of immobilized enzymes. The application of photometric techniques developed for soluble enzymes resulted in different restrictions on their use for investigation with immobilized enzymes. In particular, the development of new procedures for enzyme immobilization requires rapid, comparable statements about the enzyme activity. In the present study, a flow calorimeter [1] was used as a calorimetric measuring system for the determination of enzyme activity. The spherical and plain carrier systems were both investigated. Of particular interest are the several enzyme carrier complexes produced by the sol–gel technique. The properties of these enzyme carrier complexes were varied by using different additives or substituted precursors (type: $RSi(OR')_3$).

2. Experimental

2.1. Materials and equipment

A split-flow enzyme thermistor [1] was used as the measuring device.

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The following enzyme carrier complexes were investigated:

– urease at Eupergit C®
– invertase at polysulfone membranes
– glucose-oxidase in different sol–gel materials

- xerogel
- thin layers at SIRAN®
- thin layers at cellulose acetate foils

2.2. Enzymes used

Urease (E.C. 3.5.1.5.) from jack bean, Serva, 260 U/mg, glucose-oxidase (E.C. 1.1.3.4.), from *Aspergillus niger*, Biozyme, 180 U/mg, invertase (β-fructofuranosidase, E.C. 3.2.1.26.), from yeast, Merck.

2.3. Carrier materials

Eupergit C®; Oxirane acrylic beads, Röhm Pharma, Weiterstadt, Germany, SIRAN®, macroporous glass beads, bead diameter 1–2 mm, pore diameter 60–300 μm, Schott Mainz, Germany.

All reagents used (buffer substances, enzyme substrates, etc.) were of analytical grade (p.A.).

2.4. Method of measuring

Granular enzyme carrier complexes (Eupergit C®, crushed xerogels, layers at SIRAN®) were fitted into the calorimetric column as fixed beds between porous filter material.

To investigate plain enzyme carrier complexes, a device was developed that permits the reproducible arrangement of such systems in the calorimetric column. Small discs were punched out from the carrier, the diameter of which corresponds exactly to the inner diameter of the calorimetric column. A crescent-shaped piece was cut off at the edge of these discs. The discs were staggered by 180° at a steel spike. Fig. 1 illustrates the arrangement.

Measurements were carried out at 25° or 30°C with a flow velocity of 1.2 ml/min. Substrate solutions were introduced with an injection valve using a 0.5 ml sample loop.

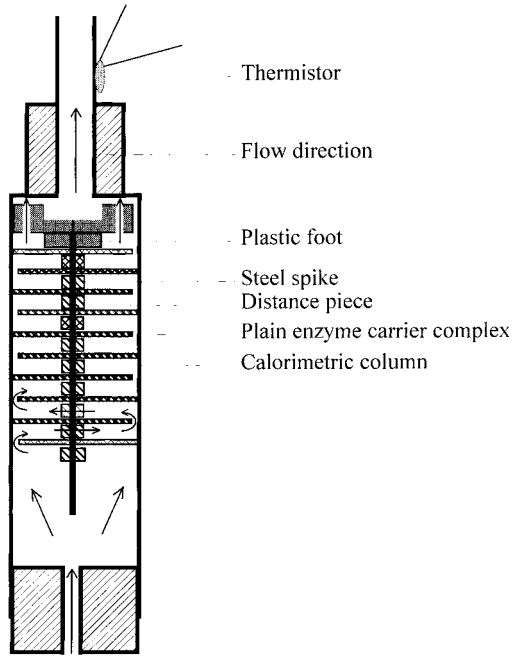

Fig. 1. Device for fitting in plain enzyme carrier complexes.

This method produces rapid results for qualitative assessment of the catalytic activity of enzyme carrier complexes. Several measurements are possible over a longer time range (from several hours to days) using the same sample of enzyme carrier complex.

The temperature difference occurring between the exit of enzyme column and reference column, respectively, is proportional to the heat exchange in the flowing medium. The released heat is proportional to turnover of substrate in the column. The turnover of a catalytic reaction is determined by the activity of the catalyst. The heights of resulting signals (ΔT_{max}, peak heights) can, therefore, be used as a measurement parameter for catalytic or enzyme activities [2].

3. Results

Three different enzyme-catalysed reactions for which the reaction enthalpies are reliably known from the literature were selected for the calorimetric investigations.

Urease-catalysed hydrolysis of urea:

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COO^- + NH_4^+ \rightarrow \text{products of hydrolysis}$$

$\Delta_R H = -58$ kJ mol^{-1} (phosphate buffer)[3]

Glucose-oxidase-catalysed oxidation of glucose:

$$\beta\text{-glucose} + O_2 \rightarrow H_2O_2 + \text{D-glucono-}\delta\text{-lactone}$$

$$\text{D-glucono-}\delta\text{-lactone} + H_2O \rightarrow \text{D-gluconic acid}$$

$\Delta_R H = -127$ kJ mol^{-1} (phosphate buffer)[4]

Invertase-catalysed hydrolysis of saccharose:

$$\text{saccharose} + H_2O \rightarrow \text{D-glucose} + \text{D-fructose}$$

$\Delta_R H = -15$ kJ mol^{-1} (acetate buffer)[5]

3.1. Investigation of spherical enzyme carrier complexes

The tests for the applicability of the calorimetric arrangement for the determination of the enzyme activity were done for immobilized enzymes on Eupergit C®, a frequently used carrier material [6]. The urease-catalysed hydrolysis of urea was selected as enzymatic reaction. Results of calorimetric investigations of this reaction are given by Hüttl et al. [3]. The activity of the catalyst bed was varied by using different amounts of enzyme carrier complex. To obtain identical flow conditions for all samples, the different beds were prepared with equal heights by filling with inactive carrier material. Fig. 2 shows the correlation between the peak heights and the used amounts of the enzyme carrier complex, up to a plateau value, which depends on the applied measuring conditions. The proportional area has to be ascertained for each enzyme carrier complex. Therefore, relevant measuring parameters are substrate concentration, flow velocity, sample volume and packing density.

This knowledge was used for comparative investigations of sol–gel immobilized enzymes. The sol–gel technique is very suitable for the immobilization of enzymes in different fields due to its mild reaction conditions and to its flexibility with respect to the shaping of products [7].

The sol–gel enzyme complexes used in this work were made from silicon alkoxides. An influence on

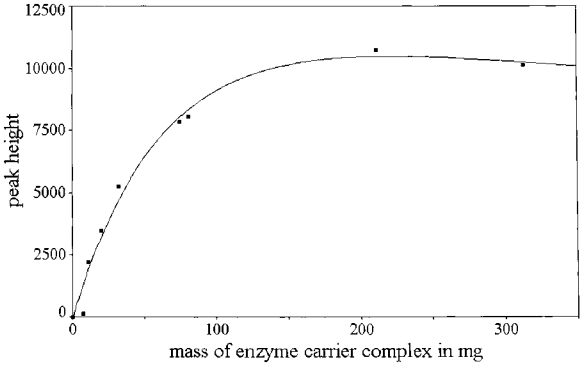

Fig. 2. Peak height (in mm) vs. mass of enzyme carrier complex.

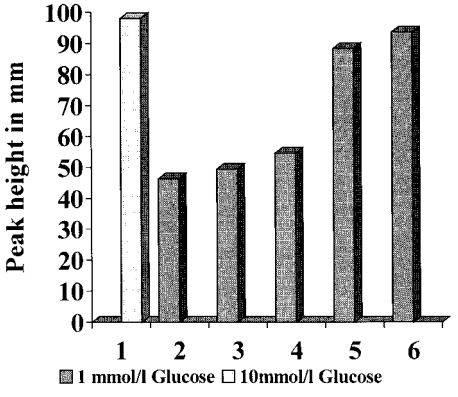

Fig. 3. Dependence of enzyme activity in xerogels from additives; (1) – additive-free xerogel; (2) – urotropin; (3) – veratraldehyde; (4) – glyoxal; (5) – glutaraldehyde; and (6) – aminocaproic acid.

enzyme activity should be achieved by the variation of the precursors and use of different additives in the sol preparation [8]. Fig. 3 shows the results of the measurements with crushed xerogels using different additives. Xerogels with additives show a significantly higher activity in comparison to the additive-free xerogel (comparable peak heights at 1/10 of substrate concentration). Glutaraldehyde and aminocaproic acid proved to be specially advantageous additives; therefore, they were used for further immobilization procedures.

Sol–gel layers applied by dip-coating on SIRAN®, a commercially available macroporous carrier material, served as an additional testing enzyme carrier complex. The determination of the activity carried out at these enzyme carrier complexes shows the influence

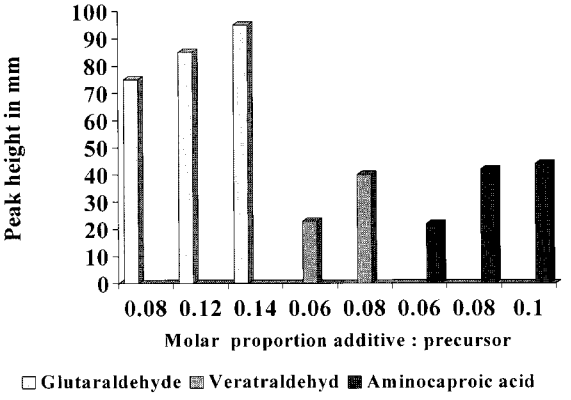

Fig. 4. Dependence of enzyme activity from additive concentration (const. proportion GOD : SiO_2, 10 mmol/l glucose)

of the additive concentration on the resulting enzyme activity. Increase of the additive concentration leads to an improvement of the activity of the enzyme carrier complexes. The results obtained with aminocaproic acid clearly show that an optimal proportion between precursor and additive exists. A further increase of additive amount does not seem to be useful (Fig. 4).

3.2. Investigation of plain enzyme carrier complexes

The plain enzyme carrier complexes were fitted into the calorimetric column using the device described here (Fig. 1). The cellulose–acetate foils show a sufficient mechanical stability. When fitting in the polysulfone membranes, it was avoided to press together the discs in the flowing medium by using supporting foil.

The ultrafiltration membranes made by Artelt and Staude [9] contained the enzyme invertase. In addition to the calorimetric measurements, the activity of these enzyme carrier complexes was investigated using different methods [10].

The results of the calorimetric measurements are presented in Fig. 5.

Membranes made from sulfonated polysulfone (S3 and SPSU) proved to be specially active. The differences in activity of immobilized invertase, determined calorimetrically at the polysulfone membranes, correspond to the differences already known [11]. Therefore, the applicability of the presented method for a comparative determination of the activity in plain enzyme carrier complexes could be demonstrated.

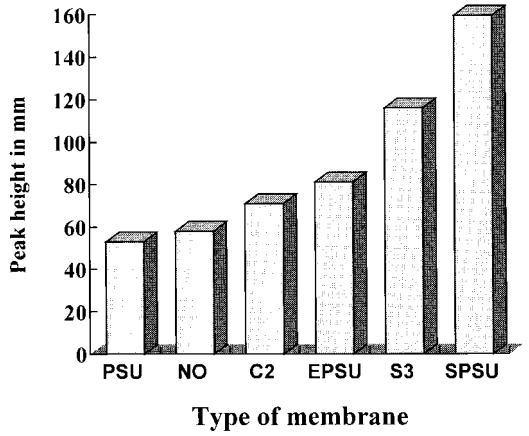

Fig. 5. Investigation of the enzyme activity for polysulfone membranes, given by peak height vs. type of membrane, (concentration of saccharose 100 mmol/l). PSU – un-modified polysulfone; NO – NO_2-modified polysulfone; C2 – chloromethyl-polysulfone; EPSU – lithiated polysulfone, converted with glycidyle-4- oxoetherhexyleether; and S3 and SPSU – different membranes made from sulfonated polysulfone.

Thin layers containing glucose-oxidase were investigated by this method. The layers were prepared by the sol–gel technique on cellulose-acetate foils.

It is noteworthy that, in spite of using additives, a sufficient activity could not be achieved with the precursor tetraethoxysilane.

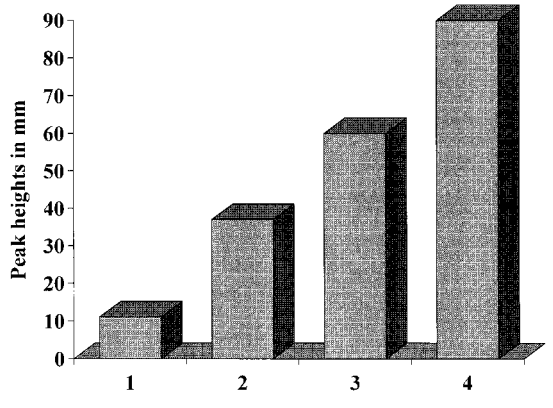

Fig. 6. Enzyme activity of thin layers on cellulose acetate: (1) – sol made from tetraethoxysilan, additive: glutaraldehyde; (2) sol made from aminopropyltriethoxysilane (APTES), no additive; (3) – sol made from tetraethoxysilane and APTES, no additive; and (4) – sol made from methyletriethoxysilane and APTES, no additive.

The use of modified silicon alkoxides (aminopropyltriethoxysilane) enables the preparation of sufficiently active layers. A further increase could be achieved by using mixtures of different precursors. The results are presented in Fig. 6.

4. Conclusions

The results of the presented investigations clearly show that flow calorimetry is a reliable method for the determination of the activity of immobilized enzymes on very different carriers and, consequently, for the optimization of the immobilization techniques. The enzymatic reactions can be followed directly. The use of consequent reactions, additional enzymes and dyes does not apply. Sources of errors can be avoided.

The enzyme activity can be judged by these method in a rapid and efficient way. The influence of the preparation parameters can be detected. A great advantage is to measure the activity of individual samples of the enzyme carrier complex over a longer time range (from several hours up to days) deriving information on stability of the immobilized enzymes.

Based on the ascertained calorimetric parameters, comparable investigations can be done using much less complicated calorimetric devices. By use of integrated circuits as calorimeter (IC-calorimeter) [12], investigations with easy-to-handle, low-cost devices seem to be possible.

Acknowledgements

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