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A new potentiometric sensor for the determination of α-amylase activity

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ABSTRACT

A platinum redox sensor for the direct potentiometric determination of α-amylase concentration has been described. The sensor measured the amount of triiodide released from a starch–triiodide complex, which was correlated with the α-amylase activity after biocatalytic starch degradation. The composition and stability of the potassium triiodide solution was optimized. The starch–triiodide complex was characterized potentiometrically at variable starch and triiodide concentrations. The response mechanism of the platinum redox sensor towards α-amylase was proposed and the appropriate theoretical model was elaborated. The results obtained using the redox sensor exhibited satisfactory accuracy and precision and good agreement with a standard spectrophotometric method and high-sensitive fully automated descret analyser method. The sensor was tested on pure α-amylase (EC 3.2.1.1, Fluka, Switzerland), industrial granulated α-amylase *Duramyl 120 T* and an industrial cogranulate of protease and α-amylase *Everlase/Duramyl 8.0 T/60 T*. The detection limit was found to be 1.944 mU for α-amylase in the range of 0–0.54 U (0–15 μg), 0.030 mKNU for *Duramyl 120 T* in the range of 0–9.6 mKNU (0–80 μg) and 0.032 mKNU for *Everlase/Duramyl 8.0 T/60 T* in the range of 0–9.24 mKNU (0–140 μg).

1. Introduction

α-Amylase (endo-1,4-a-D-glucan glucanohydrolase, EC 3.2.1.1), an enzyme that cleaves internal α-1,4-glycosidic linkages in starch to produce glucose, maltose, or dextrins, exists extensively in plants, animals and microorganisms. It is widely used in the industrial conversion of starch into sugars, such as in the food industry, and also has a significant role in the detergent industry [1]. In clinical chemistry, the activity of α-amylase in serum and urine indicates the presence of parotitis and pancreatitis [2] and reveals the occlusion of the pancreas. In saliva, it increases the production of dental caries [3].

Because of its importance in diagnostics and food production, it is important that assays to measure α-amylase activity be objective and highly selective. Some published methods have been applied to determine the catalytic activity of α-amylase, including spectrophotometry [4], fluorometry [5], amperometry [6], electrophoresis [7], isoelectric focusing [8], chromatography [9] and immunological methods [10].

The difficulties in determining amylolytic activity are reflected by the variety of analytical procedures that have been developed. There are four common types of assays (listed in order of citation frequency): (1) assays measuring the increase in reducing power by measuring reducing sugars by the dinitrosalicylic acid (DNS) assay [11,12] or the Nelson–Somogyi [13–15] method; (2) assays measuring the stainability of residual starch by forming complexes with iodine, a method developed by Fuwa [14,16–18]; (3) a colorimetric method [4] in which starch is covalently labeled with various dyes [19] and hydrolyzed by α-amylase to give soluble fragments that are measured colorimetrically after the removal of the unhydrolyzed substrate and (4) assays based on the decrease in viscosity of starch solutions during enzymatic reaction [20].

These methods, most of which are spectrophotometric, have the disadvantage that they are sensitive to turbidity and require the coloration of the test solution. In contrast, electrochemical measurements do not have these problems. Electrochemical measurements include electrochemical sensors for α-amylase determination, which rely on the detection of starch [21], starch hydrolysis products, such as glucose and maltose [22], and maltose hydrolysis product glucose [23], which are detected using enzyme-modified electrodes [24] using complex flow injection-type systems [25,26] and magnetoelastic principles [27].

The aim of this investigation was to develop a theoretical approach for measuring the α-amylase activity using direct potentiometry sensing principles. The approach (model) was tested based on the measurement of the triiodide released from a

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starch–triiodide complex after biocatalytic starch degradation by α-amylase.

2. Experimental

2.1. Reagents and solutions

Soluble starch was purchased from Kemika (Croatia). α-Amylase (EC 3.2.1.1) from *Aspergillus oryzae* (its activity was 36 U/mg) was purchased from Fluka (Switzerland) and was used as the standard amylase reagent without purification. Two commercial granulated industrial bleach resistant α-amylases (bacterial amylase, GM *Bacillus* sp.) Duramyl were obtained from Novozymes A/S (Denmark). *Duramyl 120 T* was reported to have an amylolytic activity of 120 KNU/g, and cogranulate made of α-amylase Duramyl and proteolytic enzyme Everlase named *Everlase/Duramyl 8.0 T/60 T* was reported to have an amylolytic activity of 66 KNU/g. 1 KNU (Kilo Novo Unit) is the amount of enzyme that degrades 4870 mg starch dry matter per hour under standard conditions, pH 5.6, 37 °C, and Ca^{2+} concentration 0.3 mM. Duramyl is an α-amylase used in detergent industry.

Iodine (I_2), purchased from Kemika (Croatia), and potassium iodide (KI), purchased from Sigma–Aldrich (Germany), were used to prepare the potassium triiodide solution.

Glacial acetic acid (CH_3COOH) was purchased from Panreac (Spain), sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$) was purchased from J.T. Baker (Holland), calcium chloride ($CaCl_2$) was purchased from Kemika (Croatia) and sodium chloride (NaCl) was purchased from Sigma–Aldrich (Germany).

2.1.1. Starch solution preparation

The initial 5 g/L starch concentration was prepared by adding 5 g of dried soluble starch to a 0.1 M acetate buffer solution (pH 6.0) in a closed 100 mL volumetric flask. After heating and stirring the solution for 5 min and cooling to room temperature, the solution was finally made up with deionized water in a 100 mL volumetric flask and used for further investigations. Starch solutions were prepared freshly every day.

2.1.2. Preparation of α-amylase solution

A standard α-amylase solution was prepared by dissolving 5 mg of α-amylase (Fluka, Switzerland) in 500 mL (0.36 U/mL) of a 0.1 M acetate buffer (pH 6.0) solution containing 6 mM $CaCl_2$ and 20 mM NaCl. The addition of $CaCl_2$ and NaCl to the amylase solution stabilized enzyme activity. The decrease in amylase activity by self-decomposition was less than 3% in 3 days when stored in a refrigerator. The Duramyl standard solutions were prepared in the same way as the standard α-amylase solution, in accordance with their amylase activity.

These solutions were stored in a refrigerator as stock solutions.

2.1.3. Conditioning solution (CS) preparation

The conditioning solution was prepared in the same way as the α-amylase solution but without amylase addition.

2.1.4. Acetic acid–triiodide solution

Potassium triiodide solution was prepared by dissolving solid iodine ($c = 100\ \mu M$) in potassium iodide solution ($c = 0.05$ M). Glacial acetic acid was added to this solution and diluted with deionized water until the final concentration reached 1 M. This acetic acid–triiodide solution (ATIS) was used for the inhibition of amylase activity and the reaction of triiodide with the nonhydrolyzed starch.

2.2. Apparatus

A Metrohm 780 pH meter, a 728 Stirrer, a Metrohm 765 Dosimat (all from Metrohm, Switzerland), homemade software and a platinum redox electrode IJ64 (Ionode, Australia) were all used for the response measurements. A silver/silver (I) chloride reference electrode (Metrohm, Switzerland) was used as a reference.

A thermostat (PolyScience, USA) was used for the amylase incubation. Direct potentiometric measurements were performed using an eDAQ 186 Quad Amp pH/mV, eDAQ e-corder 821 8-channel data acquisition system, eDAQ EChem 1.5 software (all from eDAQ, Australia), an IJ64 platinum redox electrode and a silver/silver (I) chloride reference electrode.

2.3. Procedures

2.3.1. Optimization of the potassium triiodide solution

Potassium triiodide solutions containing fixed iodine concentration ($c = 5$ mM) and variable potassium iodide concentrations ($c = 0.02$, 0.05, 0.1 and 0.5 M) were prepared in ultrapure deionized water. Potassium iodide solutions with the above concentrations were prepared separately.

The responses of the platinum redox sensor were measured by accurate incremental addition of the triiodide solutions to 20 mL of potassium iodide solution of an equal iodide concentration. In this way the triiodide response was always measured at constant iodide concentrations, resulting in a pure triiodide response of the redox sensor. The solution was continuously stirred during triiodide addition and measurement.

2.3.2. Selection and optimization of the starch–triiodide complex

The responses of the platinum redox sensor were measured by accurate incremental addition of the starch solutions at variable concentrations (2, 3 and 5 g/L) to 8 mL of potassium triiodide solution of different triiodide concentrations and constant iodide concentration (0.05 M). During measurements, the solution was continuously stirred.

2.3.3. Measurement of α-amylase concentration/activity

Starch hydrolysis by α-amylase was carried out in the test tubes submerged in the thermostat bath. The buffered amylase solutions of variable concentrations were added to the starch solution and incubated at varying temperature, pH and time. The volume of the reaction mixtures was kept constant and adjusted by using of CS solution when necessary. The reaction was terminated using 5 mL ATIS solution. After cooling the mixture to room temperature, the redox potential was measured using the platinum sensor described.

2.3.4. Optimization strategy using solver

Solver is a spreadsheet optimization modeling system incorporated into Microsoft Excel for Windows. It can be used to solve a wide spectrum of linear, nonlinear or integer problems. When applied to the same data set, it can yield the same results as expensive commercial software packages.

Solver was activated by choosing *Add ins*. . . in the *Tools* menu. It was used to compare an array of data predicted by the model with an initial set of parameter values over a range of dependent variable values with a set of experimental data. Then, the sum of squared residuals (SSR) between the two arrays was calculated by varying the parameter values according to an iterative search algorithm to minimize the error (SSR) between the two data sets.

The following steps in the *solver* optimization procedure were used:

(a) A worksheet was generated containing the data, fit with an independent variable E (redox potential in mV) and dependent variable mA (mass of α-amylase in μg).
(b) A column was added containing E_{calc}, which was calculated by means of Eq. (13), to describe the response of the platinum amylase sensor and include the appropriate number of parameters to be varied (changing cells). These parameters include the sensor slope (S) the constant potential term (Const), and the proportionality factor (k). Instead of a very low k value (on the order 1×10^{-4}), the log k value should be used. Providing different sets of initial conditions ensured that *solver* found a global minimum.
(c) A column was added to calculate the squares of the residuals, $E - E_{calc}$, for each data point.
(d) The sum of the squares of the residuals (target cell) was calculated.
(e) *Solver* was used to minimize the sum of the squares of the residuals (target cell) by changing the selected parameters of Eq. (13) (changing cell). No constraints were applied to the variables.

The macro SolvStat [28] provided the regression statistics for *solver* by calculation of the standard deviations of the parameters, correlation coefficients and standard errors of the y estimate SE(y).

3. Results and discussion

3.1. Optimization of iodide/triiodide composition

Potassium triiodide was prepared by dissolving iodine in potassium iodide solution according to the following equation:

$$I_2 + I^- \leftrightharpoons I_3^- \quad (1)$$

The corresponding equilibrium constant can be described as follows:

$$K = \frac{a_{I_3^-}}{a_{I_2} a_{I^-}} \cong \frac{C_{I_3^-}}{C_{I_2} C_{I^-}}. \quad (2)$$

The low value of the formation constant ($K_f = 710$) predicts a large excess of iodide in order to keep the triiodide concentration constant.

Eq. (2) can be rearranged to Eq. (3):

$$K = \frac{C_{I_3^-}}{(C_{(I_2)_0} - C_{I_3^-})(C_{(I^-)_0} - C_{I_3^-})}, \quad (3)$$

where the subscript 0 denotes the initial concentrations of the iodine and iodide species after final dilutions and before equilibrium has been attained.

The equilibrium triiodide concentration $C_{I_3^-}$ can be calculated by solving the quadratic Eq. (3):

$$(C_{I_3^-})_{1,2} = \frac{\left[K(C_{(I^-)_0} + C_{(I_2)_0}) + 1\right] \pm \sqrt{\left[K(C_{(I^-)_0} + C_{(I_2)_0}) + 1\right]^2 - 4K^2 C_{(I^-)_0} C_{(I_2)_0}}}{2K}. \quad (4)$$

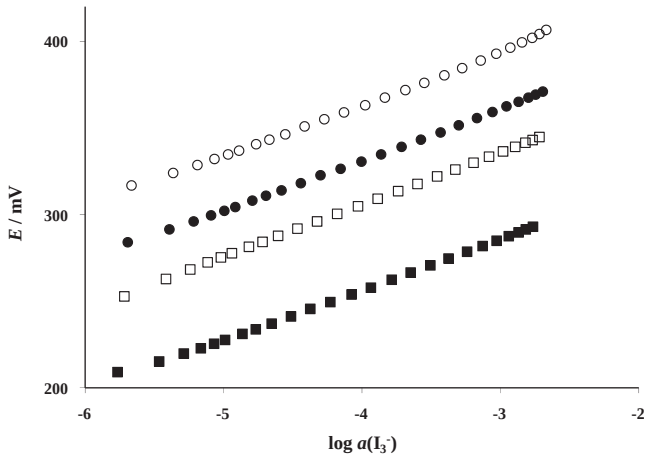

Fig. 1. The response characteristics of the platinum redox sensor to the triiodide ion at four constant iodide concentration levels [$c = 0.02$ M (○); $c = 0.05$ M (●); $c = 0.1$ M (□); $c = 0.5$ M (■)].

The real solution of Eq. (4) is:

$$(C_{I_3^-}) = \frac{\left[K(C_{(I^-)_0} + C_{(I_2)_0}) + 1\right] - \sqrt{\left[K(C_{(I^-)_0} + C_{(I_2)_0}) + 1\right]^2 - 4K^2 C_{(I^-)_0} C_{(I_2)_0}}}{2K}. \quad (5)$$

The following equations were used to measure the response of the redox sensor towards the triiodide ion.

$$I_3^- + 2e^- \leftrightharpoons 3I^-, \quad (6)$$

the corresponding redox potential can be described using the Nernst equation:

$$E = E^0 + \frac{RT}{2F} \ln \frac{C_{I_3^-}}{C_{I^-}^3} = E^0 + S \log \frac{C_{I_3^-}}{C_{I^-}^3}, \quad (7)$$

where $S = 0.0296$ V.

The response characteristics of the platinum redox sensor to the triiodide ion at different constant iodide concentrations ($c = 0.02$–0.5 M) are given in Fig. 1 and the corresponding statistics is shown in Table 1.

It can be seen that the sensor exhibited a Nernstian response and a high correlation $E - \log(a_{I_3^-})$ at all iodide concentration levels investigated. The triiodide concentrations for amylase determination were always within the linear response range of the sensor.

3.2. Preparation of the starch–triiodide complex and selection of substrate concentration

Starch forms a violet–blue complex with the triiodide ion, and the color depends on the nature of the starch [1].

The formation of starch–triiodide complexes can be described by the following equation:

$$[KI_3]_0 + [KI]_0 + \text{Starch} \rightleftharpoons [KI_3 \times \text{Starch}] + [KI_3]_f + [KI]_0, \quad (8)$$

Table 1
Response statistics of the platinum redox sensor to the triiodide ion at different constant iodide concentration levels ± confidence limits ($p = 0.95$).

Parameters	Potassium iodide concentration (M)			
	0.02	0.05	0.1	0.5
Slope (mV/decade of activity)	29.7 ± 0.4	29.5 ± 0.3	30 ± 0.1	29.1 ± 0.2
Correlation coeff. (R^2)	0.9993	0.9995	0.9999	0.9997
Constant potential term (const)	482 ± 2	449 ± 1	426 ± 0.3	373 ± 1
Linear conc. range (mol/L)	7.5×10^{-6} to 1.7×10^{-3}	5×10^{-6} to 2.5×10^{-3}	5×10^{-6} to 2.5×10^{-3}	5×10^{-6} to 2.5×10^{-3}

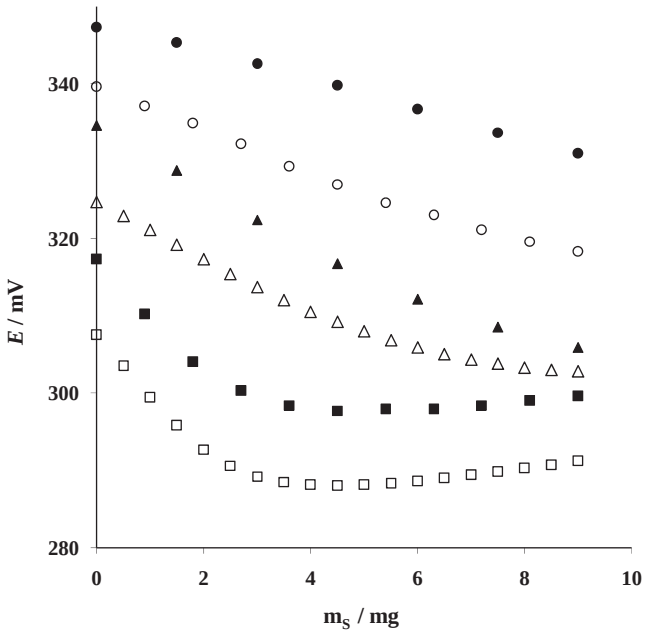

Fig. 2. Response characteristics of the platinum redox sensor to starch at different triiodide and starch solution concentrations and fixed iodide ion concentration, $c(I^-) = 0.05$ M [$c(I_3^-) = 25$ μM, $c_S = 2$ g/L (□); $c(I_3^-) = 25$ μM, $c_S = 3$ g/L (■); $c(I_3^-) = 100$ μM, $c_S = 2$ g/L (△); $c(I_3^-) = 100$ μM, $c_S = 5$ g/L (▲), $c(I_3^-) = 125$ μM, $c_S = 3$ g/L (○); $c(I_3^-) = 250$ μM, $c_S = 5$ g/L (●)].

where

$[KI_3]_0$ and $[KI]_0$ = initial triiodide and iodide concentration, respectively and
$[KI_3]_f$ = free (unbound) triodide concentration.

Iodide concentrations should be present in great excess (more than 500× higher than the initial triiodide concentration) and can be considered as constant.

After addition of a defined amount of starch solution to the solution of iodide–triiodide containing a fixed concentration of triiodide ion at a constant excess of iodide ion, the following equilibria will be established:

$$(C_{I_3^-})_f = (C_{I_3^-})_0 - (C_{I_3^-})_b, \quad (9)$$

where

$(C_{I_3^-})_f$ = free triiodide concentration,
$(C_{I_3^-})_0$ = initial triiodide concentration, and
$(C_{I_3^-})_b$ = concentration of triiodide bound to starch.

In order to determine the sensitivity of the proposed method, the response of the platinum redox sensor was investigated at different triiodide and starch solution concentrations (used as a substrate), simulating the changes caused by the biocatalytic starch-degradation process (Fig. 2).

The results of these investigations are given in Table 2.

The relative sensitivity has been defined as the ratio of the change of *emf* value in the linear response region and the change of the corresponding starch concentration. Due to the high ionic strength of the solutions investigated, the response of the platinum redox sensor in all the investigations was extremely stable and highly reproducible. It is evident from Table 2 that the sensitivity increased with a decrease in the concentration of starch solution used, but the linear response range became narrower. This result means that lower amylase concentrations/activities

Table 2
Sensitivity characteristics of the platinum redox sensor toward starch at different triiodide and starch solution concentrations and fixed iodide concentration (0.05 M).

$(C_{I_3^-})_0$ (μm)	C_S (mg/L)	ΔE (linear range) (mV)	m_S (linear range) (mg)	Relative sensitivity (mV/mg)
25	2	13.1	2	6.55
100	2	12.7	4	3.18
25	3	13.4	1.8	7.44
125	3	15.2	5.4	2.81
100	5	22.1	6	3.68
250	5	16.6	9	1.84

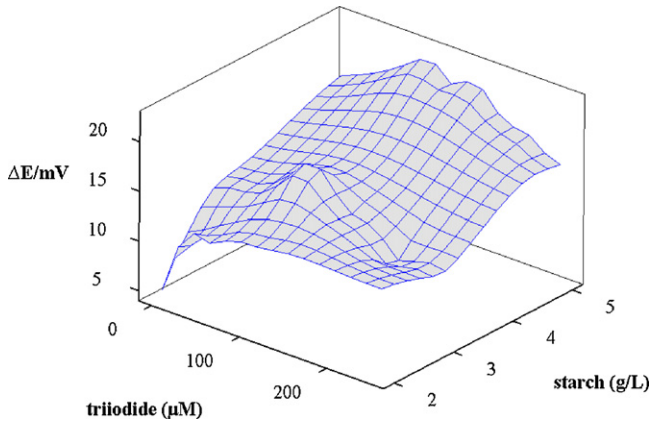

Fig. 3. The relative changes of the redox potential of the platinum sensor as a function of the triiodide and starch concentrations.

can be more accurately and precisely determined by the use of lower starch/substrate concentrations, which cause higher relative changes of *emf*s.

A surface 3D plot, which defines the relative changes of the redox potential of the platinum sensor as a function of triiodide and starch concentration, is shown in Fig. 3. Maximum changes were observed when using a triiodide solution with a concentration of 100 μM and a starch solution (substrate) with a concentration of 5 g/L.

3.3. Direct potentiometric determination of α-amylase concentration

3.3.1. Response mechanisms of the platinum redox sensor toward α-amylase

α-Amylase catalyzes the hydrolysis of starch. The nonhydrolyzed starch, which is inversely proportional to the α-amylase concentration, forms a complex with triiodide (Eq. (8)), reducing the initial triiodide concentration.

The change of the triiodide ion concentration (which exists only in excess of iodide ion) causes the change of the triiodide/iodide redox couple ratio, which results changes to the electrode potential of the redox sensor according to Eq. (7). These changes are directly correlated with the α-amylase concentration.

After rearrangement of Eq. (7), the following expression was obtained:

$$E = \text{const} + S \log C_{I_3^-}, \quad (10)$$

where

$$\text{Const} = E^0 - S \log C_{I^-}^3 = E^0 - S \log C_{I^-}. \quad (11)$$

Iodide concentration is present in large excess (>500× higher than the initial triiodide concentration) and can be considered constant.

Initially, before amylase addition and at a fixed starch concentration, the following expression was obtained:

$$E = \text{const} + S \log (C_{I_3^-})_0 \quad (12)$$

where

E = electrode potential (mV),
S = slope of the sensor (mV/decade of activity), and
$(C_{I_3^-})_0$ = initial triiodide concentration (mol/L).

After addition of α-amylase at a concentration CA, the following expression was obtained:

$$E = \text{const} + S \log (C_{I_3^-})_f, \quad (13)$$

where

$$(C_{I_3^-})_f = (C_{I_3^-})_0 - (C_{I_3^-})_I. \quad (14)$$

After inserting Eq. (14) in (13), the following expression was obtained:

$$E = \text{const} + S \log \left[(C_{I_3^-})_0 + (C_{I_3^-})_I\right], \quad (15)$$

where$(C_{I_3^-})_I$ = increase in triiodide concentration (mol/L).

An increase in triiodide concentration proportional to the α-amylase concentration (quantity) added:

$$(C_{I_3^-})_I = k \times m_A, \quad (16)$$

where

k = proportionality factor
m_A = α-amylase concentration (μg)

By inserting of Eq. (16) in (14), the following equation is obtained:

$$(C_{I_3^-})_f = (C_{I_3^-})_0 = k \times m_A. \quad (17)$$

After insertion of Eq. (16) in (15), the following expression is obtained:

$$E = \text{const} + S \log \left[(C_{I_3^-})_I = k \times m_A\right]. \quad (18)$$

From Eq. (17), the α-amylase concentration can be calculated as follows:

$$m_A = \frac{(C_{I_3^-})_f - (C_{I_3^-})_0}{k}. \quad (19)$$

3.3.2. Optimization of the analytical procedure (for direct potentiometric determination of α-amylase concentration)

α-Amylase activity is affected by temperature, pH, and the presence of heavy metal ions.

The kinetics of starch hydrolysis by amylase were investigated in order to define optimum conditions for an assay procedure.

3.3.2.1. Influence of pH. The effect of pH on the rate of starch hydrolysis was investigated at a range of pHs between 4 and 8. It was found that the maximum hydrolysis rate occurred at pH 5.5, which was selected as the optimum pH for the investigations described.

3.3.2.2. Influence of temperature and selection of reaction time. In order to reduce the analysis costs, the selection of the proper temperature and hydrolysis time was very important.

The aim of this investigation was to determine the minimum hydrolysis temperature and the shortest incubation time which would still deliver analytically usable data. The investigations of starch hydrolysis at fixed starch (5 mg/mL) and α-amylase concentrations (10 μg) demonstrated that α-amylase almost completed the starch hydrolysis in 10 min at 40 °C, and these parameter values were used in further investigations.

Increasing hydrolysis time and temperature insignificantly influenced the magnitude of the sensor-response change.

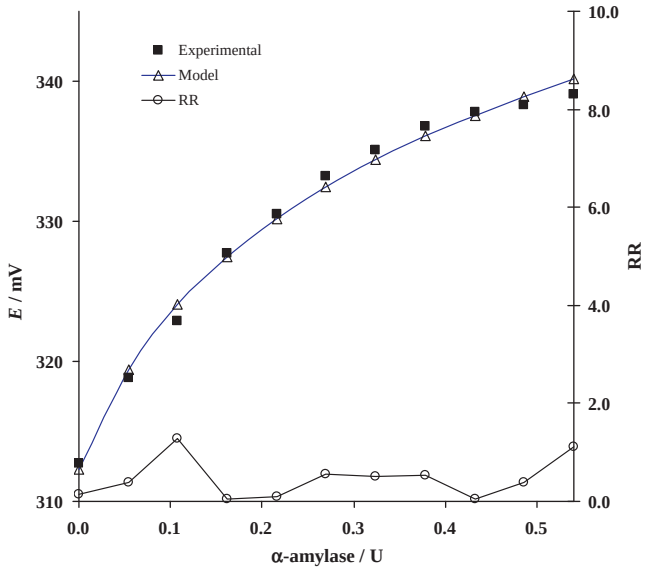

Fig. 4. Comparison of experimental (■) and modeled (△) response characteristics of a platinum amylase sensor toward α-amylase (triiodide concentration 100 μM, iodide concentration 0.05 M, starch concentration 5 mg/mL). The values of the squared residuals RR (○) are presented on the secondary Y-axis.

3.3.3. Determination of α-amylase concentration/activity in pure and industrial samples

3.3.3.1. Pure α-amylase. The responses of the platinum amylase sensor to different concentrations of α-amylase (EC 3.2.1.1, Fluka, Switzerland), are presented in Fig. 4.

The experimental data (Fig. 4) were compared with an appropriate theoretical model (Eq. (13)) in which the sensor and analyte properties (sensor slope S, constant potential term Const, proportionality factor k) were optimized with *solver* using the least-squares criterion to fit a theoretical curve to the experimental data set. *Solver* (Excel) may be used to determine the values of the variables that minimize the sum of the squares of the differences between the theoretical and experimental curve.

The values of those parameters and the corresponding regression statistics obtained by macro SolvStat are given in Table 3.

The theoretical model satisfactorily fit the experimental values for the α-amylase concentrations investigated. The value of the slope obtained by modeling is in accordance with the theoretical Nernstian value from Eq. (7).

The results of α-amylase determination using between 0 and 0.54 U (0–15 μg) α-amylase and employing the standard addition method are given in Table 4. The detection limit was found to be 1.944 mU α-amylase.

Table 3
Sensor and analyte parameters obtained by modeling potentiometric data after the optimization procedure using *solver* and the corresponding regression statistics obtained by SolvStat.

Slope[a]/(mV/decade)	Const.[a]/mV	Log (k)[a]	Correlation coefficient (R^2)	SE (y)
28 ± 1	425 ± 5	3.28 ± 0.05	0.9984	0.405

[a] Values ± standard deviation

Table 4
Results and the statistics of the direct potentiometric determination of α-amylase activity.

Taken (μg)	α-Amylase activity		Recovery[a] (%)	Stand. dev.	Confid. interval
	Expected[b] (U)	Found[a] (U)			
4.50	0.162	0.163	100.6	0.006	±0.004
6.00	0.216	0.224	103.8	0.004	±0.003
9.00	0.324	0.339	104.8	0.010	±0.009
12.00	0.432	0.445	103.0	0.010	±0.007
13.50	0.486	0.488	100.5	0.009	±0.007

[a] Average values of 5 measurements.
[b] Based on the enzyme activity of 36 U/mg (Fluka, Switzerland).

3.3.3.2. Industrial α-amylase. The sensor was also tested on two samples of granulated industrial α-amylase Duramyl (Novozymes A/S, Denmark): *Duramyl 120 T* and enzyme cogranulate of α-amylase and protease *Everlase/Duramyl 8.0 T/60 T*. Duramyl standard obtained from the reference laboratory of the producer was used as a standard.

The experimental and modeled responses of the platinum amylase sensor to different concentrations of these two enzymes samples are shown in Figs. 5 and 6. The results obtained with platinum amylase sensor compared with the results obtained on the fully automated discrete analyser (Thermo Clinical Labsystems, Konelab Corporation Espoo, Finland, Model Konelab Arena 30) are listed in Table 5.

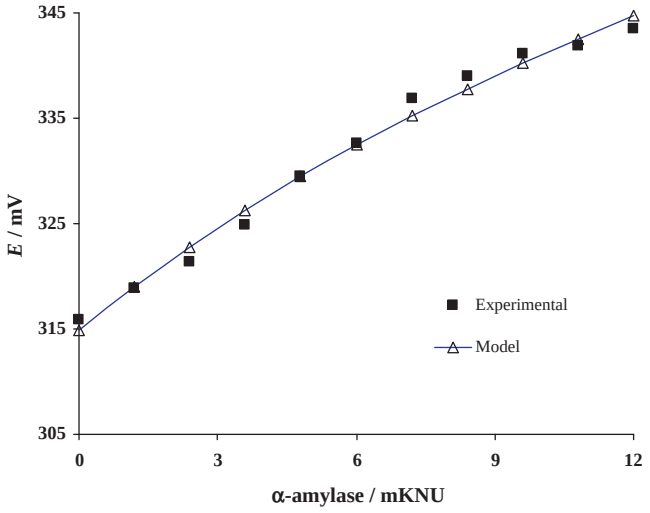

Fig. 5. Comparison of the experimental (■) and modeled (△) response characteristics of a platinum amylase sensor toward Duramyl (triiodide concentration 100 μM, iodide concentration 0.05 M, starch concentration 5 mg/mL).

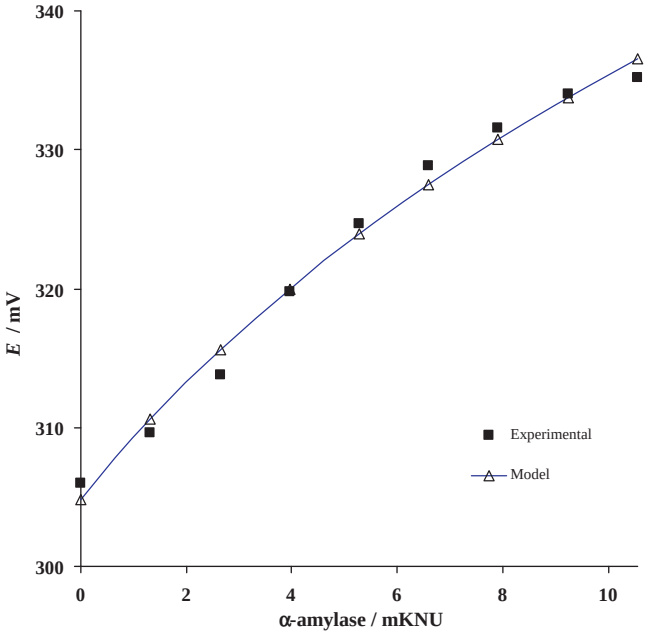

Fig. 6. Comparison of the experimental (■) and modeled (△) response characteristics of a platinum amylase sensor toward industrial amylase and a protease mixture (triiodide concentration 100 μM, iodide concentration 0.05 M, starch concentration 5 mg/mL).

The analyser method is based on the cleavage of 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)-α,D-maltoheptaoside by α-amylase and subsequent hydrolysis of degradation products to *p*-nitrophenol with the aid of α-glucosidase, resulting with liberation of the chromophore. The measurements are performed at wavelength of 420 nm.

The detection limits for platinum amylase sensor were found to be 0.030 mKNU for *Duramyl 120 T* in the range of 0–9.6 mKNU

Table 5
Results for α-amylase activity of granulated commercial enzymes for powder detergents (α-amylase: *Duramyl 120 T* and protease and amylase cogranulate: *Everlase/Duramyl 8.0 T/60 T*) obtained with direct potentiometric determination and *Konelab Arena* fully automated discret analyser.

α-Amylase activity							
Duramyl 120 T				*Everlase/Duramyl 8.0 T/60 T*			
Taken (μg)	Expected[a] (mKNU)	Discret analyser method (mKNU)	Direct potentiometry[b] (mKNU)	Taken (μg)	Expected[c] (mKNU)	Discret analyser method (mKNU)	Direct potentiometry[a] (mKNU)
20	2.40	2.35	2.27	40	2.64	2.56	2.48
30	3.60	3.64	3.60	60	3.96	3.90	3.85
40	4.80	4.80	4.80	80	5.28	5.38	5.40
50	6.00	6.00	5.99	100	6.60	6.61	6.78
60	7.20	7.20	7.45	120	7.92	7.92	7.98

[a] Calculated from the data obtained from *Novozymes* laboratory (Bagswærd, Denmark); α-amylase activity is 120 KNU/g.
[b] Average values of 5 measurements.
[c] Calculated from the data obtained from *Novozymes* laboratory (Bagswærd, Denmark); α-amylase activity is 66 KNU/g.

Table 6
Comparison of the results obtained with Fuwa spectrophotometric method and redox sensor method.

Sample	α-Amylase activity[a]	
	Fuwa method	Redox sensor
α-Amylase (Fluka) (U)	0.157 ± 0.007	0.150 ± 0.006
	0.296 ± 0.004	0.292 ± 0.003
Duramyl 120 T (KNU)	2.48 ± 0.12	2.32 ± 0.11
	4.91 ± 0.08	4.80 ± 0.07
Everlase/Duramyl 8.0 T/60 T (KNU)	4.20 ± 0.16	3.99 ± 0.14
	4.99 ± 0.12	4.88 ± 0.10

[a] Average of 5 determinations $\pm\sigma_{N-1}$.

(0–80 μg) and 0.032 mKNU for industrial cogranulate of protease and α-amylase *Everlase/Duramyl 8.0 T/60 T* in the range of 0–9.24 mKNU (0–140 μg).

The responses of the new sensor were stable and reproducible due to the high ionic strength of the redox buffer and the sample solutions investigated, which enabled the determination of α-amylase with satisfactory accuracy and precision.

The results obtained using the proposed sensor were compared with those obtained using the spectrophotometric Fuwa method [16] and are listed in Table 6. There are no significant differences between mean values at the 95% confidence level after evaluation using Student's *t*-test. The lower precision of the results obtained with both methods for industrial α-amylase products *Duramyl 120 T* and *Everlase/Duramyl 8.0 T/60 T* can be assigned to the impurities and product complexity (*Everlase/Duramyl 8.0 T/60 T*).

4. Conclusions

An theoretical approach for measuring α-amylase activity accurately and rapidly based on potentiometric principles was proposed and used to design a sensing methodology involving a platinum redox electrode. The sensor functioning was based on the measurement of the triiodide released from a starch–triiodide complex after biocatalytic starch degradation by α-amylase. All these parameters were optimised, resulting in a significant reduction of measuring time and costs of analysis.

Experimental measurements on both purified and industrial α-amylase samples showed satisfactory accuracy and precision and good correlation with both the standard method and the fully automated discrete analyser method.

The proposed sensor uses a simple and inexpensive apparatus and can be miniaturized and incorporated as a detector in microfluidic systems.

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