A PHYSICO-MATHEMATICAL MODEL FOR BISUBSTRATE ENZYME MEMBRANE ELECTRODES

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ABSTRACT : *A physico - mathematical model has been developed that is suitable for biosensors incorporating membranes and bisubstrate enzymes. The set of equations regarding the transport of reactants across the membrane, the bisubstrate enzymatic reaction, back diffusion of reactants and reaction products, are written down and simultaneously solved and transient response of the electrode is determined as a function of time. The model is assessed experimentaly employing a simple glucose enzyme pH electrode.*

In the range practical for the enzyme pH electrode (0.01-0.1 M) there is satisfactory agreement between the theoretical predictions and experimental results. The model described is general and as well allows the determination of enzyme activity as a function of pH, thereby reflecting the range of substrate concentration that is measurable.

KEY WORDS : *Biosensor, Modeling, Biosubstrate enzyme electrode, Glucose sensor, pH electrode, Activity - pH relation.*

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INTRODUCTION :

Presently there are a number of fine and sensitive probes for determining chemical and biochemical substances among which biosensors represent the most recent development [1]. Biosensors are increasingly being used in various laboratory and industrial applications.

A biosensor can be defined as an analytical probe incorporating a biological component that is connected to, or integrated with, a tranceducer. The specificity and sensivity of the biological system which is complemented by the tranceducer, renders possible the attainment of an amplified electronic signal. Many possible biological elements can be combined with various tranceducers to construct the biosensor [2].

An important class of biosensors is the enzyme electrodes. In one typical design the enzyme electrode is made by placing a membrane containing an immobilized enzyme over a conventional electrode that is sensitive either to one of the products, or one of the consumed substances in an enzymatic reaction.

Fig. 1 shows the schematic diagram of the type of enzyme electrode simulated in this work. The electrode is dipped in the solution to be monitored where the substrate sought after, penetrates through the semipermeable membrane located at the tip of the electrode, whereupon it encounters the confined enzyme solution layer and a predesigned reaction takes place causing a proportionate signal at electrode:

$$\text{substrate} + \text{Co-substrate} \xrightarrow{\text{Enzyme}} \text{products} + R'$$

Consumption or generation of electroactive species leads to electrical signal at the electrode

As can be appreciated the sequence of the events leading to signal generation are each uniquely time dependent up until the steady-state situation.

MODELING :

In efforts such as biosensor research and development the use of theoretical models, in a predictive manner, helps to identify which, of themany controlling variables are most likely to be influential in obtaining the desired results in real systems [3]. Towards this end modeling of a system can be achieved only when one has a clear and detailed understanding of simultaneous phenomena taking place during the electrode's functioning.

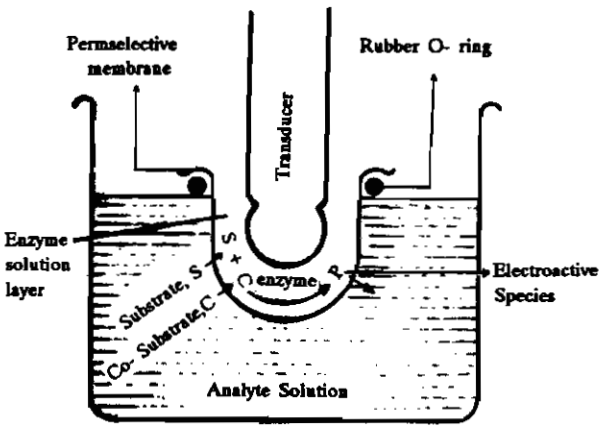

Fig. 1 : Schematic diagram of a bisubstrate- enzyme membrane electrode

In this work an attemptis made to develope a suitably general physico- mathematical model describing the response of bisubstrate enzymes as a function of the time and substrate concentration. The major improvement planned to be achieved is to consider the role of the second substrate (co- substrate), which inevitebaly results in a more complex set of equations [4], which heretofore has not been analyzed.

A schematic representation of the kinetic events in a bisubstrate enzyme- pH electrode is shown in Fig. 2.

The key events that need to be considered in formulating the mathematical model, are :

a) Transport of substrate and co- substrate from the analyte solution into the confined enzyme solution layer.
b) The bisubstrate- enzyme reaction that generates produets p_1 (an acid product) and

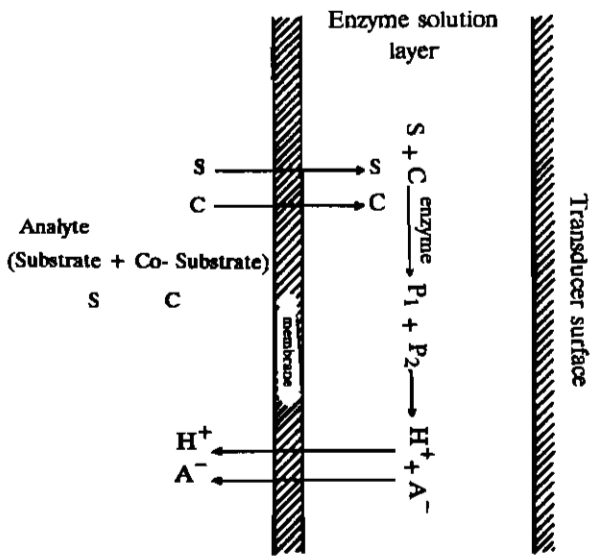

Fig. 2 : Schematic diagram of kinetic events in a typical enzyme membrane electrode (P_1 & P_2 = Intermediates)

p_2 in the confined solution layer.

c) Dissociation of p_1 and formation of electroactive species H_3O^+ in the confined solution layer.

d) Back diffusion of H_3O^+ and its conjugate anion A^- from the confine enzyme solution layer across the membrane and into the bulk solution.

Equations governinig the transient- state of the system can be derived using the principle of mass conservation. The relevant differential equations are :

$$d[S]/dt = (a\,k_s/v)\{[S]_{inf} - [S]\} - V \quad (1)$$

$$d[C]/dt = (a\,k_c/v)\{[C]_{inf} - [C]\} - V \quad (2)$$

$$d[H_3O^+]/dt = -(a\,k_H/v)\{[H_3O^+] - [H_3O^+]_{inf}\} + V \quad (3)$$

$$d[A^-]/dt = -(a\,k_A/v)[A^-] - [A^-]_{inf}\} + V \quad (4)$$

where the terms in square brackets represent concentration of species concerned (A^-= anion, H_3O^+ = proton); a= membrane surface area, v= volume of enzymatic solution layer, V= rate of bisubstrate enzymatic reaction.

To determine the electrode's transient response as a function of time, Eqs. (1-4) must be simultaneously solved. It is convenient to cast Eqs. (1-4) into dimensionless forms. At first, according to mechanism of bisubstrate reaction the rate law must be determined. If the reaction mechanism is double- displacement (Ping-Pong)type [5-8], the mechanism may be summarized as follows:

$$S + E \xrightarrow{k_1} EP_1 \xrightarrow{k_2} P_1 + E^*$$

$$E^* + C \xrightarrow{k_3} E^*P_2 \xrightarrow{k_4} P_2 + E$$

The rate law that verifies the above mechanism is as follows:

$$d[EP_1]/dt = k_1[E][S] - k_2[EP_1] \quad (5)$$

$$d[E^*P_2]/dt = k_3[E^*][C] - k_4[E^*P_2] \quad (6)$$

$$d[E]/dt = -k_1[E][S] + k_4[E^*P_2] \quad (7)$$

$$d[E^*]/dt = -k_3[E^*][C] + k_2[EP_1] \quad (8)$$

and:

$$[E]_0 = [E] + [E^*] + [EP_1] + [E^*P_2] \quad (9)$$

Assuming the steady state, substitution of Eq. (9) into Eqs. (5-8) leads to :

$$[EP_1] = \frac{(1/k_2)[E]_0}{1/k_2 + 1/k_4 + 1/k_1[S] + 1/k_3[C]} \quad (10)$$

$$[E^*P_2] = \frac{(1/k_4)[E]_0}{1/k_2 + 1/k_4 + 1/k_1[S] + 1/k_3[C]} \quad (11)$$

$$[E] = \frac{(1/k_1[S])[E]_0}{1/k_2 + 1/k_4 + 1/k_1[S] + 1/k_3[C]} \quad (12)$$

$$[E^*] = \frac{(1/k_3/[C])[E]_0}{1/k_2 + 1/k_4 + 1/k_1[S] + 1/k_3[C]} \quad (13)$$

Since the rate determining step in the sequence is the dissociation of the active complex and formation of products, thus:

$$V = k_4[E^*P_2] \quad (14)$$

Substituating Eq. (7) into Eq. (10) leads to the following rate law :

$$V = \frac{[E]_0}{1/k^* + 1/k_2\,[S] + 1/k_4\,[C]} \qquad (15)$$

$$1/k^* = 1/k_2 + 1/k_4 \qquad (16)$$

Substituation of Eq. (15) into Eqs. (1-5)shows that in determining the electrode's transient response as a function of time, Eqs. (1-3) must be simultaneously solved. It is convenient to cast Eqs. (1-3) into the following dimensionless forms:

$$dS^*/dt^* = S^*_{inf} - S^* - \frac{E^*_s S^* C^*}{S^* + C^* + S^* C^*} \qquad (17)$$

$$dC^*/dt^* = C^*_{inf} - C^* - \frac{E^*_c S^* C^*}{S^* + C^* + S^* C^*} \qquad (18)$$

$$dH^*/dt^* = H^*_{inf} - H^* + \frac{\text{sqrt}\,(E^*_s E^*_c)\, S^* C^*}{S^* + C^* + S^* C^*} \qquad (19)$$

where :

$S^* = [S]\,k_1 / k^*$, $C^* = [C]\,k_3/k^*$, $H^* = [H_3O^*]$ $\text{sqrt}(k_1k_3)/k^*$, $t^* = (aK/v)t$, $E = k_1[E]_0/(aK/v)$, $E=k_3[E]_0/(aK/v)$.

Parameters E_c and E_s represent the characteristic ratios of the rate of enzymatic reaction (of substrate and co- substrate) to those of diffusion.

EXPERIMENTAL ASSESMENT :

The model was evaluated using data obtained from a simple and readily fabricable glucose enzyme pH- electrode.

The principal reactions taking place in this electrode are :

$$\beta - D - \text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} D- \text{gluconic acid} + H_2O_2$$

$$D - \text{gluconic acid} \longrightarrow \text{gluconate} + H_3O^+$$

$$H_2O_2 \longrightarrow H_2O + (1/2)\,O_2$$

Gluconic acid ($pK_a \approx 3.5$) undergoes complete dissociation at pH > 4. Thus, the overall reaction may be represenred by :

$$\beta - D- \text{glucose} + (1/2)\,O_2 \xrightarrow{\text{glucose oxidase}} H_3O^+ + \text{gluconate} \qquad (20)$$

Materials and Methods

1-Materials: All chemicals used were reagent grade obtained from commerical sources (mostly from Merck, Germany). The enzyme, glucose oxidase (E.C.1.1.3.4.) was purchased from Sigma (St. Louis, U.S.A).

2- Methods : The enzyme activity was assayed at ambient temperature, pH, etc according to standard procedures [9]. An ordinary glass electrode was used for pH measurments. In order to have an optimized membrane structure, it was decided to tailor homogeneous cellulose acetate membranes following the procedure described by *Sarbolouki* and *Miller* [10]. A casting solution consisting of 10/9/81 (wt. percent) cellulose acetate/ formamide/acetone, respectively, yields the desired membrane with proper pore radius [11].

Fabrication of the enzyme electrode was made according to *Nilson* et al. [12]:

a 5 × 5 cm piece of wet cellulose acetate membrane is layed flat and 0.01 g of enzyme together with a drop of 0.001 M phosphate buffer in 0.1 M sodium sulfate is placed at its center. The membrane is carefully lifted and placed over the sensor bulb at the tip of the pH glass electrode and kept in place tight with a rubber O- ring. To condition the electrode assembly, it is dipped in 0.1 M phosphate buffer solution for at least 1 hour. The electrode response was read as a function of time at five glucose concentrations in the range of 0.001- 0.1 M. The experimental pH data reported are the average of at least three(oftenmore) independent measurements.

MATHEMATICAL TREATMENT :

According to the overall reaction (20) and

Eqs. (1-4), the relevant differential equations are:

$$d[G]/dt = (a / v)\, k_G \{[G]_{inf} - [G]\} - V \quad (21)$$

$$d[O_2]/dt = (a / v)\, k_O \{[O_2]_{inf} - [O_2]\} - V/2 \quad (22)$$

$$d[H_3O^+]/dt = -(a / v)\, k_H \{[H_3O^+]_{inf} - [H_3O^+]\} + V \quad (23)$$

$$d[G^-]/dt = -(a / v)\, k_G - \{[G^-]_{inf} - [G^-]\} + V \quad (24)$$

A bisubstrate double- displacement (ping - pong) mechanism best describes the enzymatic oxidation of glucose by oxygen. The mechanism may be summarized as follows :

$$\beta - D - \text{glucose} + E_{ox} \xrightarrow{k_1} E_{red}P_1 \xrightarrow{k_2} D - \beta - \text{gluconate} + E_{red}$$

$$D - \beta - \text{gluconate} + H_2O \longrightarrow D - \text{gluconic acid}$$

$$E_{red} + O_2 \xrightarrow{k_3} E_{ox}P_2 \xrightarrow{k_4} E_{ox} + H_2O_2$$

The rate law Eq. (15), when appropriately applied gives:

$$V = \frac{[E]_0}{(1/k^*) + (1/k_1) / [G] + (1/k_3) / [O_2]} \quad (25)$$

To determine the electrode transient response as a function of time, Eqs. (21-23)must be simultaneously solved. These equations may be cast into their dimensionless forms in accordance with Eqs. (17-19):

$$dG^*/dt^* = G^*_{inf} - G^* - \frac{E_g\, G^*\, O^*}{G^* + O^* + G^*\, O^*} \quad (26)$$

$$dO^*/dt^* = O^*_{inf} - O^* - \frac{0.5\, E_O\, G^*\, O^*}{G^* + O^* + G^*\, O^*} \quad (27)$$

$$dH^*/dt^* = H^*_{inf} - H^* + \frac{sqrt\,(E_O E_g)\, G^*\, O^*}{G^* + O^* + G^*\, O^*} \quad (28)$$

where:

$G^* = [G]\,(k_1/k^*)$, $O^* = [O_2](k_3/k^*)$, $H^* = [H_3O^+](sqrt(k_1k_3)/k^*)$, $t^* = (aK/v)t$, $E_g = k_1[E]_O/(aK/v)$, $E_o = k_3[E]_O/(aK/v)$

Where parameters E_o and E_g represent the ratios of the characteristic rate of enzymatic reactions to those of diffusion. Because the kinetic parameters needed were not available, an attempt was made to obtain them by solving the equations. In order to decrease the number of unknown parameters, it is assumed that K represents an average of mass transfer coefficients for various species. Notice should be made that glucose and oxygen can freely diffuse through the membrane, whereas the proton is somewhat prevented from free diffusion by its conjugate (gluconate) anion.

The complexity of these differential equations prevents their straight analytical solution and thus, use was made of numerical methods, employing the Runge- Kutta method [13].

RESULTS AND DISCUSSION :

Fig. 3 shows solutions to equations (26-28). As can be seen the reaction rate of oxygen is higher than that of glucose. Thus, when glucose diffuses into the enzyme solution layer it immediately reacts and quickly reaches a steady-state whereafter the glucose concentration stays constant. Simultaneously the concentration of proton increases while the concentration of

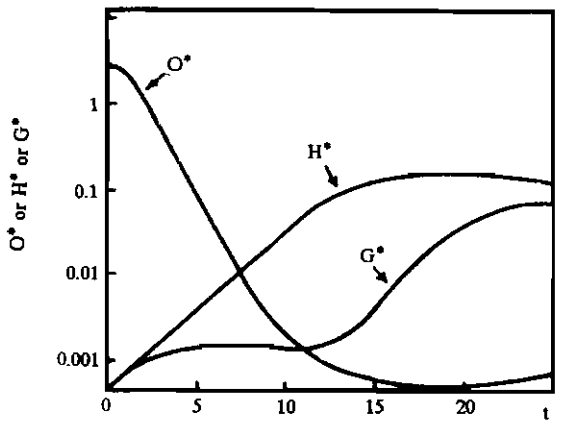

Fig. 3 : Expected variation of species

oxygen decreases until it reaches a very low level and hence the enzymatic reaction stops and proton concentration levels off, whereas the oncentration of glucose increases until it approaches the concentration of bulk solution. Solution of the differential equations and determination of the relevant parameters for fitting the theoretical with experimental data, were carried out as follows .

The rate law expression, Eq. (25) can be rewritten as :

$$V = \frac{V_{max}}{1 + K^{G}_{M}/[G] + K^{O}_{M}/[O_2]} \qquad (29)$$

The predicted electrode response i.e. the changes in pH as a function of time at each glucose concentration, can be obtained by simultaneous solution of diferential Eqs. (21-23)using numerical fourth- order of Runge-Kutta method. The difference between theoretical and experimental results is then minimized, for example, by using Nelder- Mead direct search method [14].

$$\text{Error} = \Sigma\ ([H_3O^+]_{th.} - [H_3O^+]_{exp})^2 \qquad (30)$$

Since the differential equations are strongly nonlinear and stiff, the nearest approximation to the answer is to be gussed. Once the minimization of error is achieved, system parameters can be determined. This way K , V_{max} , K^{G}_{M} , K^{O}_{M} were obtained at each glucose concentration. By plotting V_{max} vs. the average pH at each glucose concentraion, Fig. 4, the followong expression was considered :

$$V_{max} = \frac{a}{1 + [H_3O^+]/b + c/[H_3O^+]} \qquad (31)$$

and upon solving it, the following results were obtained :

a = 38.52 (mol/lit)

b = 0.678×10^{-7} (mol/lit)

c = 17.316×10^{-7} (mol/lit)

K = 1.98×10^{-5} (1/s)

K^{G}_{M} = 0.15701 (mol/lit)

K^{O}_{M} = 0.00598 (mol/lit)

E_o/E_g = 26

The activity- pH relationship found here coincides very well with those reported by *Bright* and *Weibel* [15].

Figs. 5 and 6 show the comparison of the experimental with theoretical pH as a function of time. Considering the simplifying assumptions

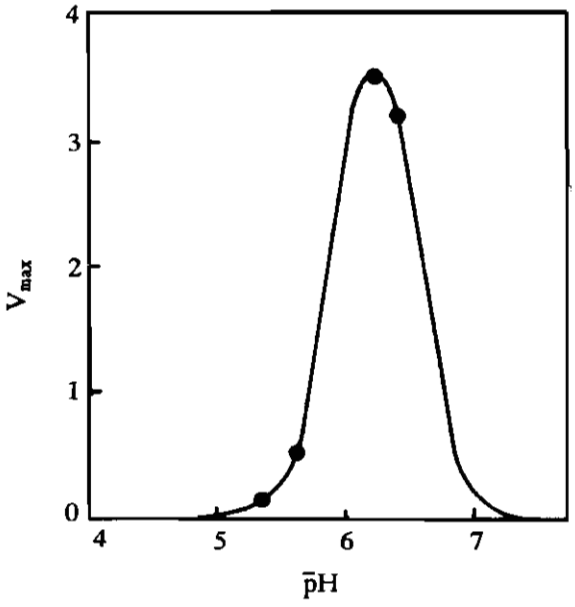

Fig. 4 : V_{max} as a function of $\bar{p}H$

• experimental

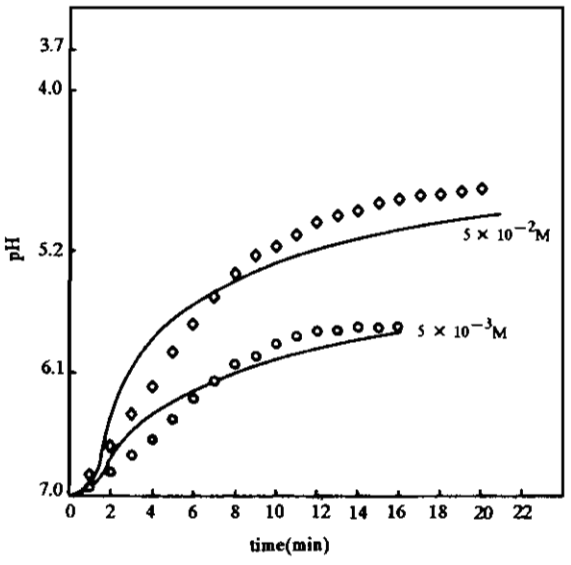

Fig. 5 : Comparison of transient response of glucose-enzyme pH electrode

—— theoretical

○ , ◇ experimental

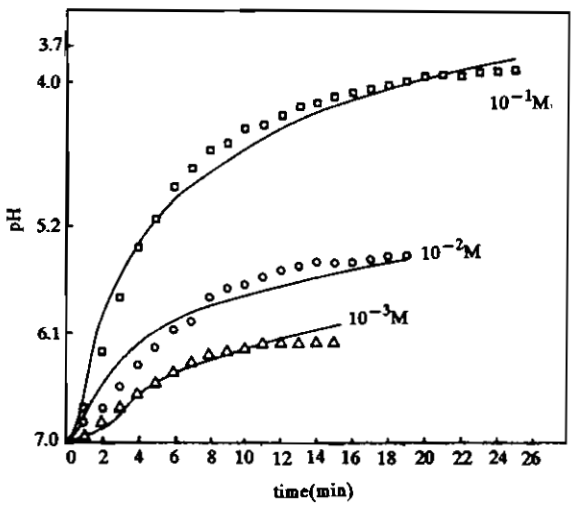

Fig. 6 : Comparison of transient response of glucose-enzyme pH electrode

—— *theoretical*

□, ○, ∇ *experimental*

involved, indeed a rather satisfactory agreement is observed.

CONCLUSIONS :

The model proposed, based on a bisubstrate enzyme reaction mechanism and appropriate assumptions, leads to a satisfactory prediction of the electrode response in the range where the pH electrode is itself capable of responding (0.001- 0.1 M glucose). The model proposed not only describes the transient response of the glucose pH electrode, but its steady- state response as well. In this model, it is assumed that the confined enzyme solution layer is a homogenous phase and as such its properties can be considered lumped, thus the key variables become only functions of time thereby making the equations simple to solve. Despite of this simplifying assumption the results are quite satisfactory. The approximate solution predicts that the larger the ratio E_g/E_o the faster the electrode response. The slow and rate determining step during the response of the electrode is the diffusion of species across the membrane. Thus by varying the parameters that accelerate the rate of diffusion (e. g. decreasing the membrane thickness) a faster response can be obtained. The model described is general and may suitably be used for enzyme electrodes of similar construct, with the unique feature that it allows as well the determination of enzyme activity as a function of pH. In the case of the enzyme electrode studied here, the narrow range of glucose oxidase activity vs the pH clearly reflects the limited range of glucose concentration that can be measured.

Notation

a
membrane surface area cm^2

$[E]_0$
total enzyme concentration (includes all forms of the enzyme and enzyme- substrate complexes), mol/lit

k_1 , k_3
the rate constant for formation of enzyme-product lit/mol/sec

k_2 , k_4
the rate constants for decomposition of enzyme-product complex into products 1/s

k_G , k_O , k_H , k_{G-}, k_s , k_c
mass transfer coefficient of glucose, oxygen, proton, gluconate, substrate, co- substrrate, anion across membrane cm/s

k
average of mass transfer coefficient of species cm/s

$K = (a/v) \times k$ 1/s

K^G_M , K^O_M
Michaelis constants for glucose and oxygen mol/lit

K^S_M , K^O_M
Michaelis constants for substrate and co-substrate mol/lit

$[G]$, $[O_2]$, $[H_3O^+]$, $[G^-]$, $[S]$, $[C]$, $[A^-]$
concentration of glucose, oxygen, proton, gluconate, substrate, co- substrate, anion in the bulk solution mol/lit

G^*, O^*, H^*
dimensionless concentration of glucose, oxygen,

proton,

S^* , C^*

dimensionless concentration of substrate, co-substrate,

$\bar{p}H$

average ph at each bulk concentration of glucose,

R'

other products,

t^*

dimensionless time,

v

volume of enzyme solution layer, cm^3

V

rate of enzymatic reaction, maximum rate, mol/lit/s

Charactristic symbols

E_g , E_o The characteristic ratio of rate of enzymatic reaction to the diffusion for glucose and oxygen

E_s , E_c the characteristic ratio of rate of enzymatic reaction to the diffusion for substrate and co- substrate

sqrt squared root

Subscripts

exp experimental

th theoretical

inf concentration of species in the bulk solution

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