

1/11/23

Kinetics of enzymes catalyzed reaction

Ch-7 Bailey Ollis


mathematical expression for reaction rate



'reaction rate' $\rightarrow v$ (quasi steady state approx)

$$v = -\frac{ds}{dt} = \frac{dp}{dt}$$

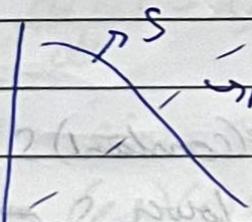


molar/unit volume/time

enzyme kinetics \rightarrow "initial rate data"

"S+E" \rightarrow Isothermal vessel \downarrow

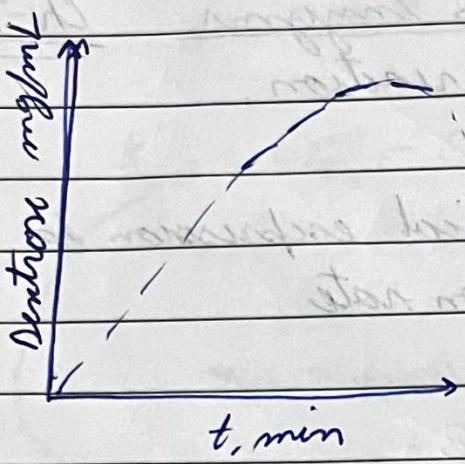
& buffer solⁿ- pH



$$\frac{dp}{dt}_{t=0} = -\frac{ds}{dt}_{t=0} = v_{t=0}$$

$$c=c_0, S=S_0, P=0$$

↓
enzyme



Batch reactor data

"hydrolysis"

glucoamylase

 $E_0 = 11,600 \text{ unit}$

arbitrary unit
to because difficult to bind
exact conc. in body

 $E_0 \rightarrow$ checked in "unit of activity"

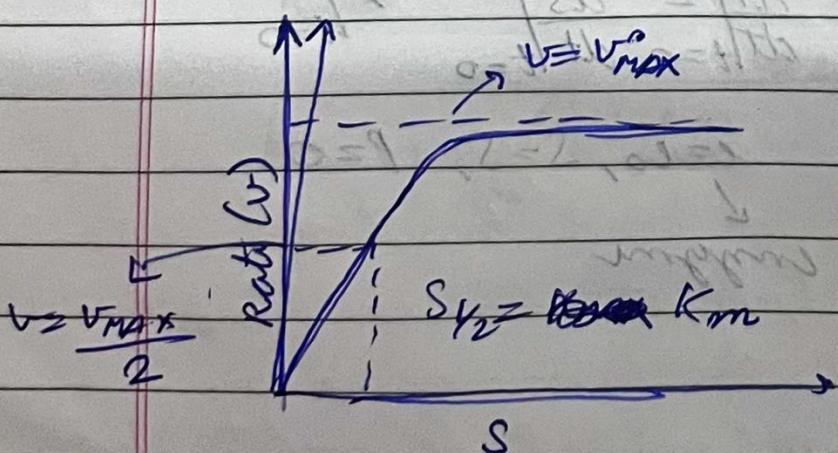
amount of enzyme which gives
 a certain amount of activity under

"stir stir a set of conditions"

Michaelis - Menten Kinetics

$$v = V_{\max} S$$

$$K_m + S$$

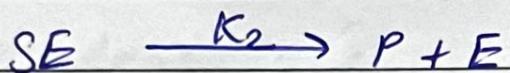
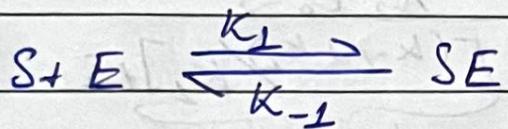


$$v = (constant) S^n$$

- at lower S , ~~not~~ $n=1$

- $S > n = 0$

* Derivation of Michaelis-Menten?



As per mass-action law of kinetics, if this reaction is in equilibrium,

$$\frac{SE}{(ES)} = \frac{k_1}{k_{-1}} = K_m \text{ (diss cont.)}$$

$$v = \frac{dp}{dt} = k_2 (se)$$

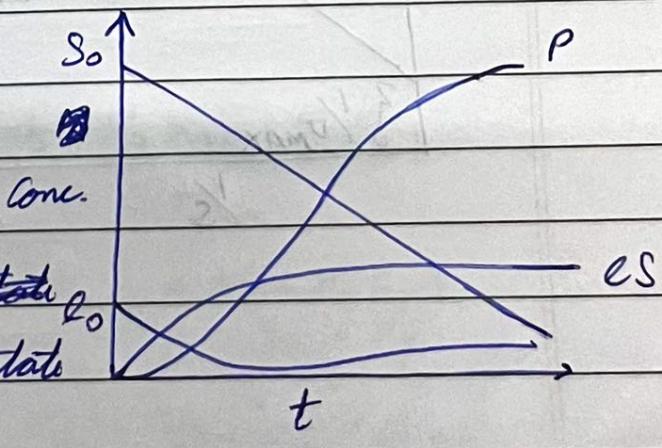
$$e_0 = e + (es)$$

$$v = -\frac{ds}{dt} = k_1 se - k_2 (es)$$

$$\frac{d(es)}{dt} = k_1 se - (k_1 + k_2)(es)$$

$$s(0) = S_0$$

$$(es)(0) = 0$$



May be
one of
the question
in end sem
- Sin

$$\frac{d(es)}{dt} = 0$$

quasi steady state approx

essential assumption for this calculation

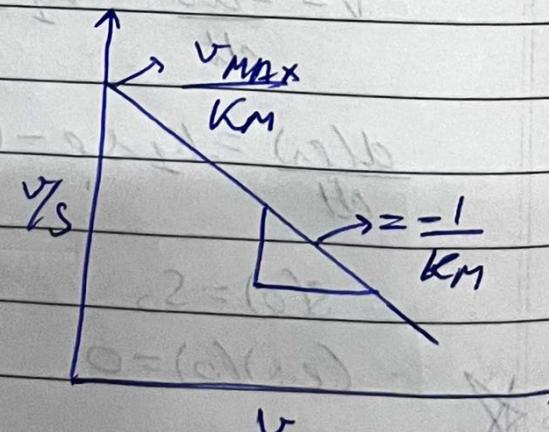
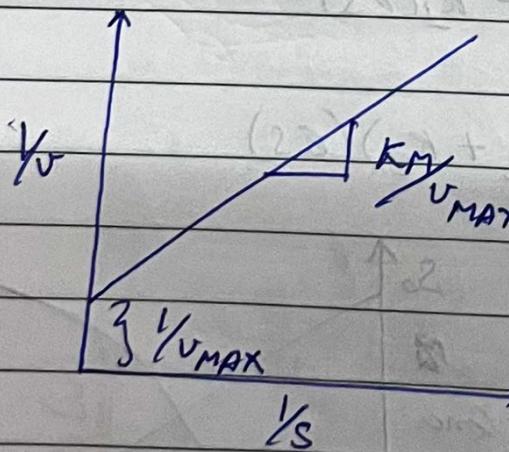
$$v = \frac{ds}{dt} = \frac{k_1 e_0 s}{\underbrace{[(k_2 + k_1) / k_1] + s}_{K_m}} + s$$

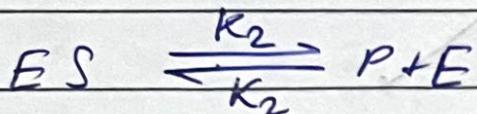
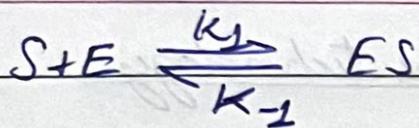
$$v = \frac{ds}{dt} = \frac{v_{max} s}{K_m + s}$$

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{K_m}{v_{max}} \cdot \frac{1}{s} \quad \left. \begin{array}{l} \text{→ Liner Weizzen-Burk} \\ \text{plot} \end{array} \right.$$

$$\frac{s}{v} = \frac{K_m}{v_{max}} + \frac{1}{v_{max}} \cdot \frac{s}{s}$$

$$v = v_{max} - K_m \frac{v}{s}$$





Might ask derivation ↗

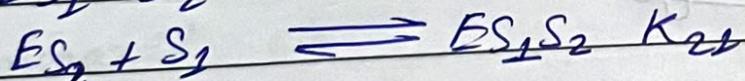
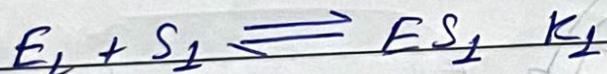
$$v = -\frac{dS}{dt} = \frac{dP}{dt} = \frac{\left(\frac{v_s}{K_s}\right)S - \left(\frac{v_p}{K_p}\right)P}{1 + \frac{S}{K_s} + \frac{P}{K_p}}$$

$$v_s \rightarrow v_{max}$$

$$k_s \rightarrow k_m$$

$$v_p = \frac{k_1 k_2}{K_2}$$

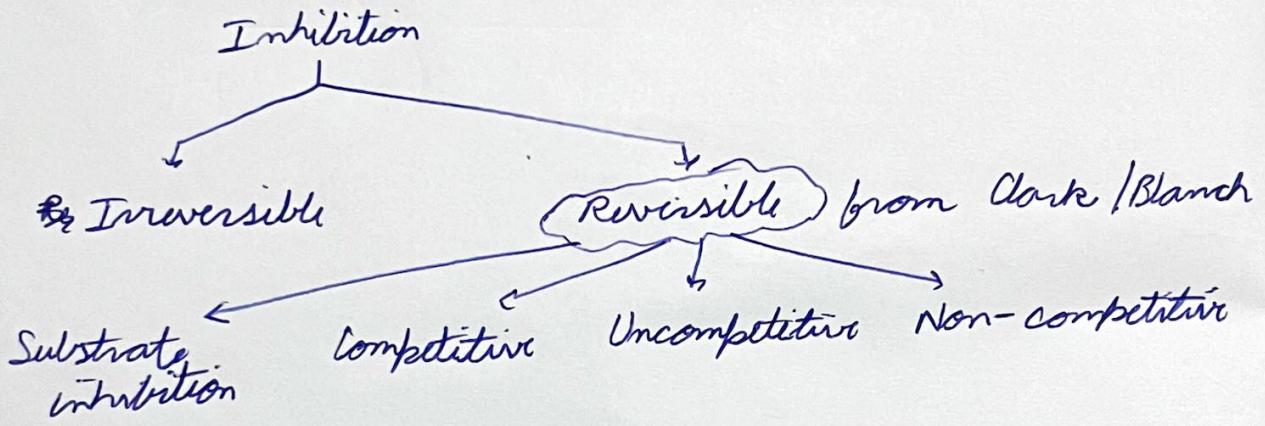
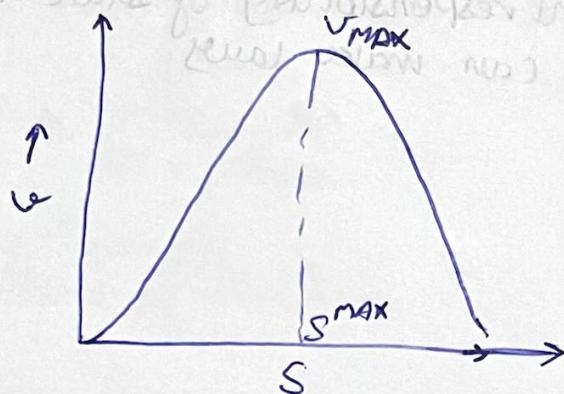
$$K_p = \frac{k_1 k_s}{K_{-2}}$$



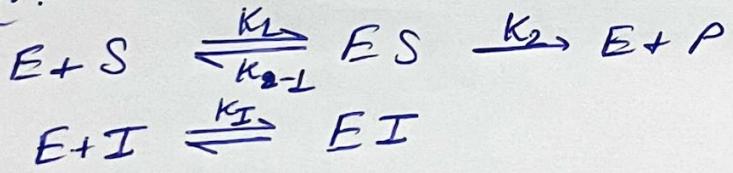
$$v = \frac{K_p}{1 + \frac{K_{21}}{S_1} + \frac{K_{12}}{S_2} + \frac{1}{S_1 S_2} (K_2 K_{21} + K_1 K_{12})}$$

$$= \frac{v_{max} S_1}{K_1 + S_1} \quad v_{max} = \frac{K_p K_{12} S_2}{S_2 + K_{12}}$$

$$K_1 = \frac{K_{21} S_2 + K_1 K_{12}}{S_2 + K_{12}}$$

Enzyme inhibition

* Competitive :-



$$K_I = \frac{[E][I]}{[EI]} \quad \textcircled{1}, \quad v = \frac{dP}{dt} = K_2 [ES] \quad \textcircled{2}$$

$$K_m = \frac{[E][S]}{[ES]} = \frac{K_2 + K_1}{K_1} \quad \textcircled{3}$$

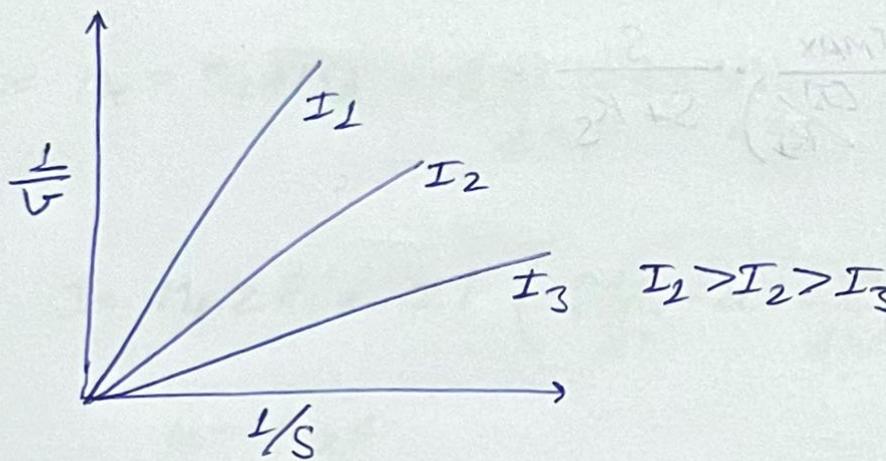
$$[E_o] = [E] + [ES] + [EI]$$

$$[E_0] = K_m \frac{[ES]}{S} \left[1 + \frac{[I]}{K_I} \right] + [ES]$$

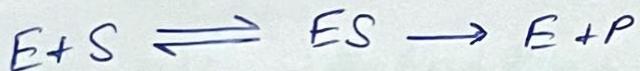
Substituting this in ②,

$$v = \frac{K_2 [E_0] [S]}{S + K_m \left[1 + \frac{[I]}{K_I} \right]} \xrightarrow{\text{Let } v_{max}}$$

$$\frac{1}{v} = \frac{K_m \left(1 + \frac{[I]}{K_I} \right)}{v_{max}} \cdot \frac{1}{[S]} + \frac{1}{v_{max}} = \frac{K_m}{v_{max}} \cdot \frac{1}{[S]} + \frac{1}{v_{max}}$$



* Uncompetitive:



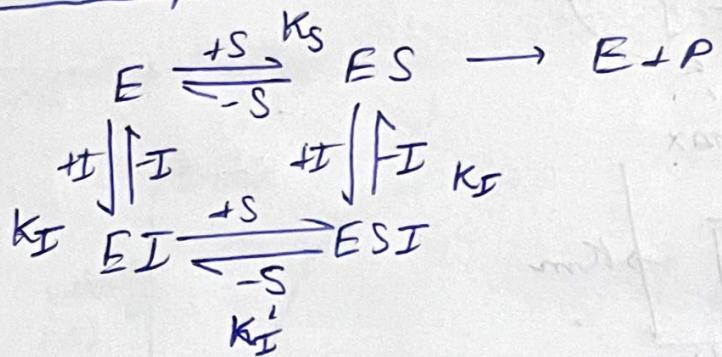
$$K_I = \frac{[ES][I]}{[ESI]}, \quad v = K_2 [ES]$$

$$[E_0] = [E] + [ES] + [ESI]$$

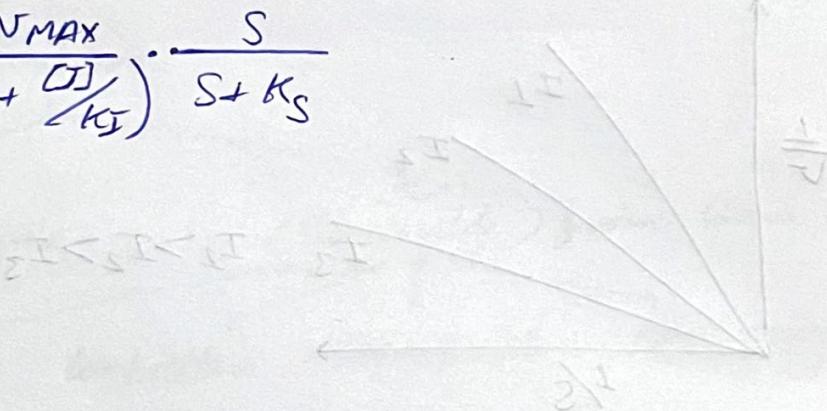
$$v = \frac{K_2 [E] \frac{v_{max} \cdot [S]}{(1 + [I]/K_I)}}{S + \frac{K_m}{(1 + [I]/K_I)}} \xrightarrow{\text{Let } v_{max} = \frac{v_{max}}{1 + [I]/K_I}}$$

$$K_m^{\text{app}} = \frac{K_m}{1 + [I]/K_I}$$

* Non-competitive :-



$$V = \frac{V_{MAX}}{\left(1 + \frac{[I]}{K_I}\right)} \cdot \frac{S}{S + K_S}$$



$$[ES]_0 = v, \quad \frac{[E][S]}{[ES]} = k_I$$

$$[ES]_0 + [ES] + [E] = [E]$$

$$\frac{[E][S]}{[E][S] + [E] + [S]} = \frac{v}{v + k_I}$$

6/11/23

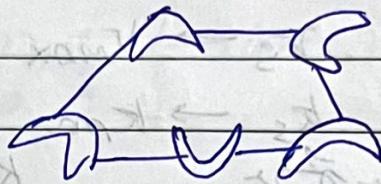
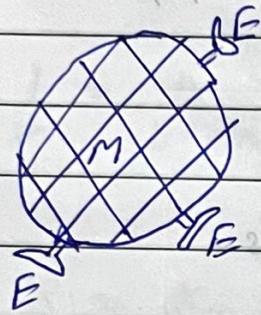
BT303

Immobilized enzyme technology

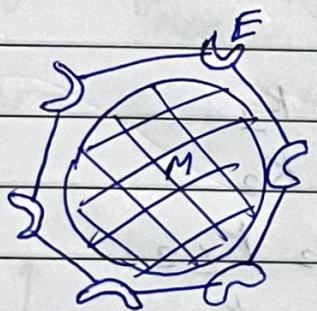
Chemical
covalent bond

physical
weaker interaction/adsorption

Chemical method:-

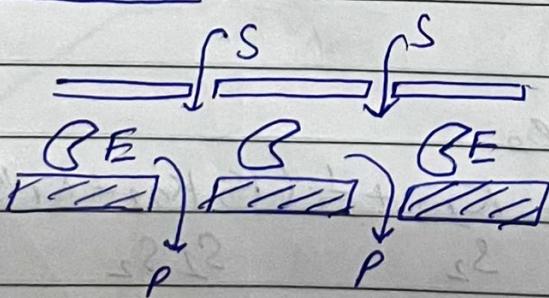


cross-linked enzyme matrix



Enzyme cross linking by multifunctional reagent

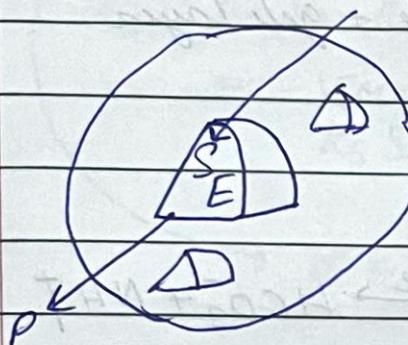
Physical method



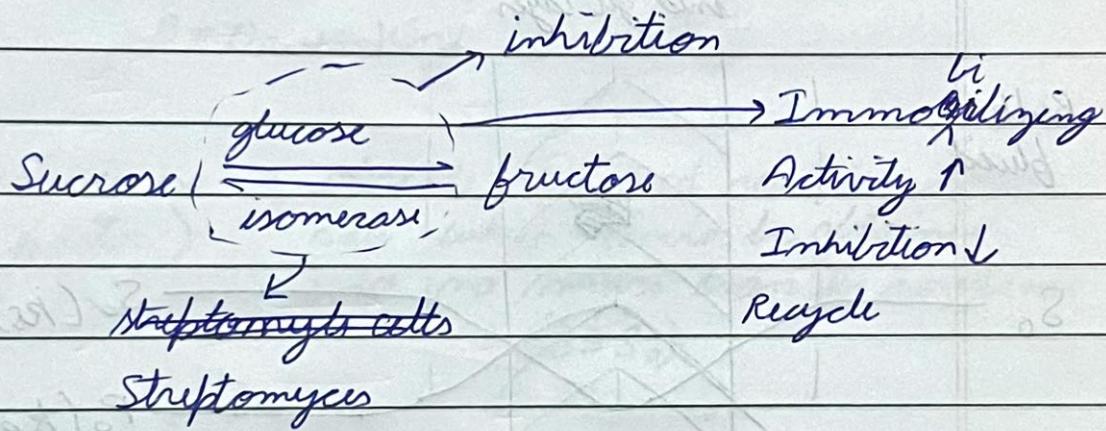
entrapped in a porous hollow fiber



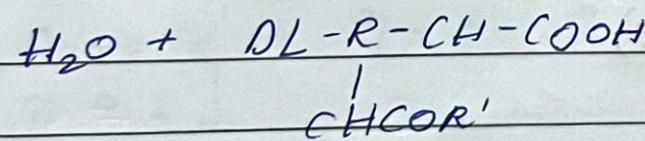
enforcement within insoluble gel matrix



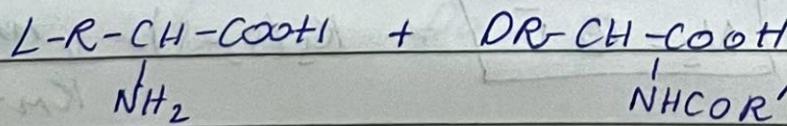
Microcapsules



L-aminoacid 4/0



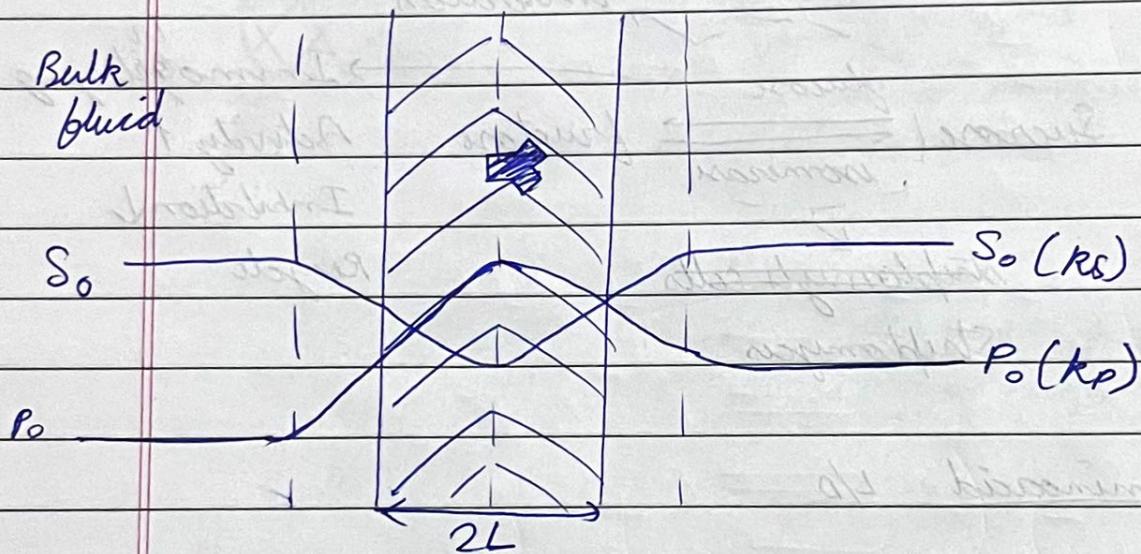
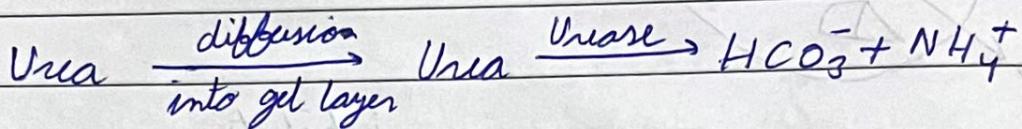
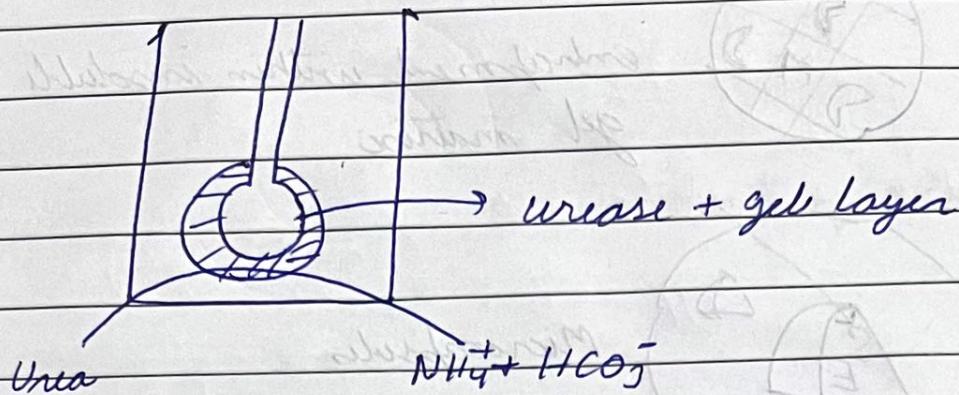
aminoacyl ester DL-acylamino acid



L-amino acid

D-acylamino acid

Analytical:- Urea electrode



$$N_s = k_s (S_0 - S)$$

$$v = \frac{v^{\max} s}{K_m + s}$$

$$S \rightarrow [N_s = v \bar{t}] \Rightarrow k_s (S_0 - S) = \frac{v^{\max} s}{K_m + s} \bar{t}$$

$$n = \frac{s}{S_0} \quad D_a = \frac{v^{\max}}{K_m + s} \bar{t}$$

$$K_2 = \frac{K_m}{S_0}$$

$$1 - n = n$$

$$Da \quad K + n$$

$$0 < n \leq 1$$

α (Dam Kohler number)

$$= \frac{V_{max}}{K_S S_0} = \frac{\text{Max. reaction rate}}{\text{Max. mass transfer rate}}$$

$$\alpha = \frac{\beta}{2} \left(\pm \sqrt{1 + \frac{4KC}{\beta^2}} - 1 \right)$$

$$\beta = Da + \frac{K-1}{2}$$

η (effectiveness factor) = ~~observed~~ observed reaction rate / rate which would be obtained with no mass transfer resistance ($S = S_0$)

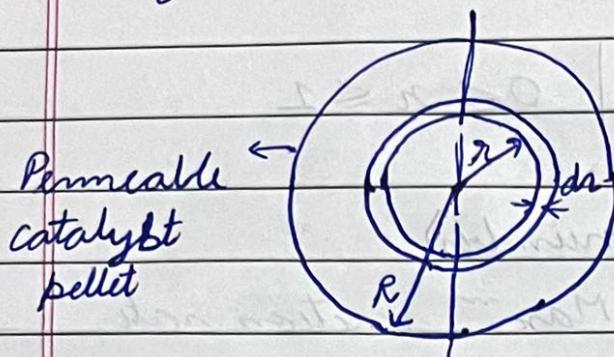
$$\eta = \frac{n}{(K+n)}$$

$$Da \rightarrow 0 \Rightarrow \eta = 1, \bar{v} = \frac{V_{max} \times S}{K_m + S_0}$$

$$Da \rightarrow \infty \Rightarrow \eta = \frac{1+K}{Da}, \bar{v} = K_S S_0$$

8/11/23

BT303

Analysis of intraparticle diffusion & reaction

n = local rate of substrate localization

D_{eff} = substrate effective coefficient

(i) Porosity: $\epsilon_p \rightarrow$ experimentally bound

(ii) Tortuosity: $\gamma \rightarrow 1.4 - 7$

(iii) Restricted diffusion situation: $\frac{k_p}{k_d} \approx \left(1 - \frac{r_{\text{sub}}}{r_{\text{pore}}} \right)^4$

$$V = V_{\text{max}} \cdot S \quad . \quad V_{\text{max}} = \epsilon_{\text{pore}} \cdot S_p \cdot f_{E, \text{max}}$$

$$\frac{k_m + S}{K_m + S}$$

enzyme loading
($\mu\text{mol enzyme}$)
 $\left(\frac{\mu\text{mol enzyme}}{\text{g support}} \right)$

Particle density
(g of support)
($\text{Vol}^m \text{ of support}$)

activity
(μmol)

* Steady state mass balance

$$\left. \left(-D_{\text{eff}} \frac{ds}{dr} 4\pi r^2 \right) \right|_R - \left. \left(-D_{\text{eff}} 4\pi r^2 \frac{ds}{dr} \right) \right|_{R+dr} = V 4\pi r^2 dr$$

$$\rightarrow \text{Des} \left(\frac{r^2 ds}{dr} \Big|_{r+dr} - \frac{r^2 ds}{dr} \Big|_r \right) = r^2 v$$

$$dr \rightarrow 0$$

$$\rightarrow \text{Des} \frac{d}{dr} \left(\frac{r^2 ds}{dr} \right) = r^2 v$$

$$\rightarrow \text{Des} \left(\frac{d^2 s}{dr^2} + \frac{2}{r} \frac{ds}{dr} \right) = v(s)$$

$$\frac{ds}{dr} \Big|_{r=0} = 0$$

$$s \Big|_{r=R} = S_0 \quad (\text{bulk surface conc.})$$

Boundary concentration

~~Observed~~ observed overall rate of 'S' utilization

v_o = 'S' diffusive flux

$$v_o = \left(\frac{A_p}{V_p} \right) \left(\text{Des} \frac{ds}{dr} \Big|_{r=R} \right) \quad (1)$$

$\eta = \frac{v_o}{v_o(s)} = \frac{\text{observed rate}}{\text{rate which would obtained}}$

with no. conc. gradient

$$\bar{s} = \frac{s}{S_0} \quad \bar{r} = \frac{r}{R}$$

$$\Rightarrow \frac{d^2\bar{S}}{dr^2} + 2 \sum \frac{d\bar{S}}{dr} = \frac{\nu R^2}{S_0 \text{Des.}} = \frac{q \phi^2}{1 + BS} \bar{S}$$

Dimensionless

⑤

$$\phi = \frac{R}{3} \quad \frac{v_{\max}/\text{Km}}{\text{Des.}}, \quad B = \frac{S_0}{\text{Km.}}$$

Thiele modulus Saturation parameters

$$\bar{S} \Big|_{\bar{r}=1} = 1 \quad \frac{d\bar{S}}{dr} \Big|_{\bar{r}=0} = 0$$

$$\phi^2 = \frac{q}{3} \nu T R^3 \left(\frac{v_{\max}}{\text{Km}} \right) S_0 \xrightarrow{\text{reaction rate}} \text{Might be wrong}$$

1st order

$$4 \nu T R^2 \text{ Des. } S_0 \xrightarrow{-\text{Des.}} \text{diffusion rate}$$

$$\gamma = \left(\frac{d\bar{S}}{dr} \right) \Big|_{\bar{r}=1} - ⑥$$

$$= 3\phi^2 \left[2 / (1 + B) \right] - ⑦$$

$$V_o = \frac{A_p}{V_p} \left(\text{Des.} \frac{d\bar{S}}{dr} \Big|_{r=R} \right)$$

$$= \frac{A_p}{V_p} \left(\text{Des.} \frac{S_0}{R} \cdot \frac{8 d\bar{S}}{dr} \Big|_{\bar{r}=1} \right)$$

$$= \frac{3}{R^2} \text{ Des. } S_0 \frac{d\bar{S}}{dr} \Big|_{\bar{r}=1}$$

$$\rightarrow \left. \frac{d\bar{S}}{dr} \right|_{r=1} = \frac{V_0 R^2}{3 D_{es} S_0} = 0$$

Substitute this in ⑥.

$$\rightarrow \phi = \left[\frac{V_0 R^2 (1+B)}{9 \eta D_{es} S_0} \right]^{1/2} - ⑧$$

$$\bar{\phi} = V_0 \left(\frac{V_p}{A_p} \right)^2 - ⑨$$

$$\eta = g(\bar{\phi}, \beta) - ⑩$$

η for large ϕ ,

criterium

$$\phi < 0.3$$

η -value

$$\propto \phi^{-1}$$

$$\phi > 3$$

$$\rightarrow D_{es} \left(\frac{d^2 S}{dr^2} + \frac{2}{r} \frac{dS}{dr} \right) = v$$

$$\rightarrow D_{es} \frac{d^2 S}{dr^2} = v$$

$$\rightarrow \frac{1}{2} \frac{d}{ds} \left(\frac{ds}{dr} \right)^2 = v$$

$$\left. \frac{ds}{dr} \right|_{r=R} = \left[\frac{2}{D_{es}} \int_{S_0}^S v(s) ds \right]$$

hence \downarrow conc.

$$\phi = \frac{3D_{es}}{R} \left[\frac{\frac{2}{\rho_{es}} \int_0^S v_s ds}{v(S_0)} \right]$$

9/11/23 BT303

Syllabus \Rightarrow After midsem - Instrumentation, mass transfer, enzyme kinetics, reactor design
 40 marks \rightarrow 10 marks theory

Interparticulate mass transfer

$$\gamma|_{large \phi} = \frac{3D_{es}}{R} \left[\frac{\frac{2}{\rho_{es}} \int_0^R v(s) ds}{v(S_0)} \right]^{1/2}$$

$$\gamma|_{\phi \gg 1} = \frac{1}{\phi} \cdot \frac{1+\beta}{\beta} \sqrt{2} \left[\beta - \ln(1+\beta) \right]^{1/2}$$

$$v = \frac{V^{\max} \cdot S}{K_m + S} \cdot \left(2.5 \cdot \frac{S}{K_m} + 2^3 \cdot \frac{h}{K_m} \right)$$

① $K_m \gg S$

1st order

$$v = K \cdot S \quad (K = \frac{V^{\max}}{K_m})$$

$$\gamma = \frac{1}{\phi} \left(\frac{1}{\tanh h \frac{1}{2} \phi} - \frac{1}{\phi} \right) \cdot \phi = \frac{V_p}{A_p} \sqrt{\frac{K}{D_{es}}}$$

② $S > K_m$

zero order

$$V = \begin{cases} K_0 = \text{constant} & S > 0 \\ 0, S = 0 & \end{cases}$$

$$K_0 = V^{\max}$$

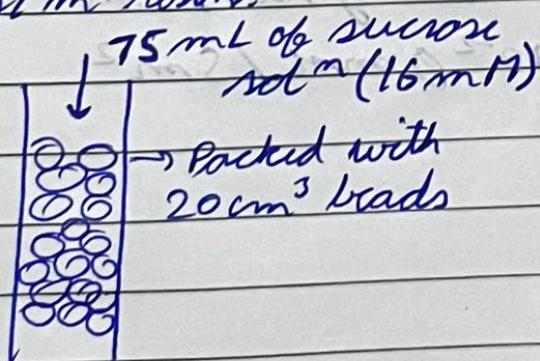
$$S = S_0 - \frac{K_0}{60 \text{ s}} (R^2 - r^2)$$

$$\gamma = \frac{4}{3} \frac{J(R^3 - R_c^3)}{4\sqrt{3} D R^3 K_0} = 1 - \left(\frac{R_c}{R} \right)^3$$

$R_c \rightarrow$ critical radius, $R_c \leq r \leq R$

$\rightarrow r$ at which $R_c \cancel{S=0}$

Q) Invertase is immobilized on ion exchange resin ($d = 1 \text{ mm}$). The amount of 'E' loaded = 0.05 kg/m³ resin



In a different reactor,
free enzyme is mixed
with same volume of
~~sucrose soln~~

$$K_m = 8.8 \text{ mM}$$

Turnover number = $2.4 \times 10^{-7} \text{ g}^{-1} \text{ mol e of sucrose}$
 $D_{\text{eff}} = 2 \times 10^{-6} \text{ (gE)}^{1/2} \text{ s}^{-1}$

- (a) Rate of reaction using free enzyme
 (b) "immobile" enzyme.

Ans

$$(a) v = \frac{v_{\text{MAX}} s}{k_m + s}, k_m = 8.8 \text{ mM} = 8.8 \times 10^{-3} \text{ mol/L}$$

$$= 8.8 \text{ mol/m}^3$$

$$D_{\text{eff}} = 2 \times 10^{-6} \text{ cm}^2/\text{s} = 2 \times 10^{-10} \text{ m}^2/\text{s}$$

$$R = 5 \times 10^{-9} \text{ m}$$

$$8 = 16 \text{ mM} = 16 \text{ mol/m}^3$$

$$\text{Mass of enzyme} = 0.05 \text{ kg/m}^3 \times \frac{20}{1000}$$

$$= 10^{-6} \text{ kg}$$

$$v_{\text{MAX}} = k_{\text{cat}}$$

$$\text{Enzyme conc.} = \frac{10^{-6}}{75} = 1.33 \times 10^{-2} \text{ kg/m}^3$$

$$v_{\text{MAX}} = k_{\text{cat}} \cdot \text{L-alanine} \cdot \text{nutrient}$$

$$= 2.4 \times 10^{-2} \times 1.33 \times 10^{-2}$$

$$= 3.19 \times 10^{-2} \text{ g/mol/s m}^2$$

$$v = 2.06 \times 10^{-2} \text{ g/mol/s m}^2$$

$$M_{\text{m}} 8.8 = 1$$

$$2.06 \times 10^{-2} = 0.02$$

$$(4) \eta = \frac{V_{obs}}{V_0}$$

$$\begin{aligned} V^{MAX} &= 2.4 \times 10^{-3} \text{ g mol/g.s} \times (0.05 \text{ kg/m}^2) \times 10^2 \\ &= 0.12 \text{ g mol/sm}^2 \end{aligned}$$