ENZIM DAN BIOTEKNOLOGI

Sumardi

PERANAN

1. Cocok untuk industri karena mempunyai efisiensi yang tinggi, spesifik dan selektif

KELEMAHAN

1. Protein : Rapuh, tidak tahan kondisi ekstrim

KEAMANAN

- TIDAK MENIMBULKAN MASALAH
- KONTAMINAN MUNGKIN DITIMBULKAN OLEH MIKROBA YANG IKUT DALAM ENZIM ITU SENDIRI

MENGUKUR AKTIVITAS ENZIM

- PRODUK YANG TERBENTUK
- SISA SUBSTRAT

- E + S === ES --→ P + E

- E tidak aktif + S === > ????

PRODUKSI DAN HARGA JUAL ENZIM

Jenis	Produksi (ton/th)	Nilai jual (jt dolar/th)
Protease bakteri	530	66
Glukoamilase	350	36
Alfa amilase	320	12
Glukoisomerase	70	56
Renin	26	64

ENZIM KOMERSIAL DAN NAMA DAGANGNYA

ENZIM	SUMBER	NEGARA
amilase	*A. oryzae*	Denmark, Belanda, USA
	B. Subtilis	Denmark, Inggris, Jepang
Selulase	*Aspergillus sp*	Jerman, Jepang
	Trichoderma reesei	Jerman, Jepang
Glukoamilase	*A. niger*	Denmark, Inggris, Jepang
	Rhizopus	Jepang
Lipase	*Aspergillus sp*	Jepang
	Candida cylindraccae	Jepang
	B. licheniformis	Denmark, Belanda.
Pektinase	*Aspergillus sp*	Denmark, Belanda, USA, Swis
Protease	*A. niger*	Denmark, Inggris, Jepang, inggris

DIMANA INDONESIA ?

PEMBENTUKAN PROTEIN/ ENZIM

DNA

transkripsi

mRNA

translasi

Protein/ Enzim

CONTOH MIKROBA PENGHASIL ENZIM MANANASE

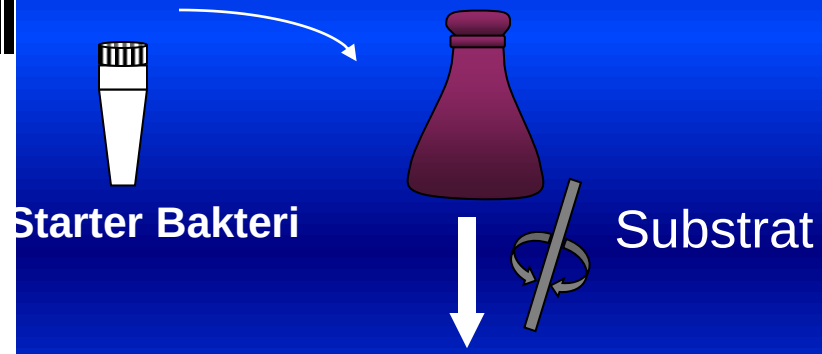
α-Galaktosidase

α-D-Gal
↓(1→6)
β-D-Man(1→ 4) β-D-Man(1→ 4) β-D-Man(1→ 4) β-D-Man(1→ 4) β-D-Man(1→ 4)
α-D-Gal
↓ (1→6)

β-Manosidase

β-Mananase

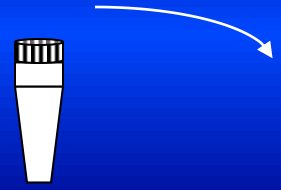
Produksi Enzim Ekstraseluler



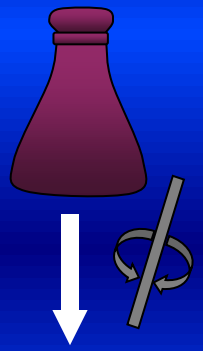
Supernatan

Uji Enzim

Produksi Enzim Intraselluler



Starter mikrba



Substrat

Sel mikroba

Pecah sel

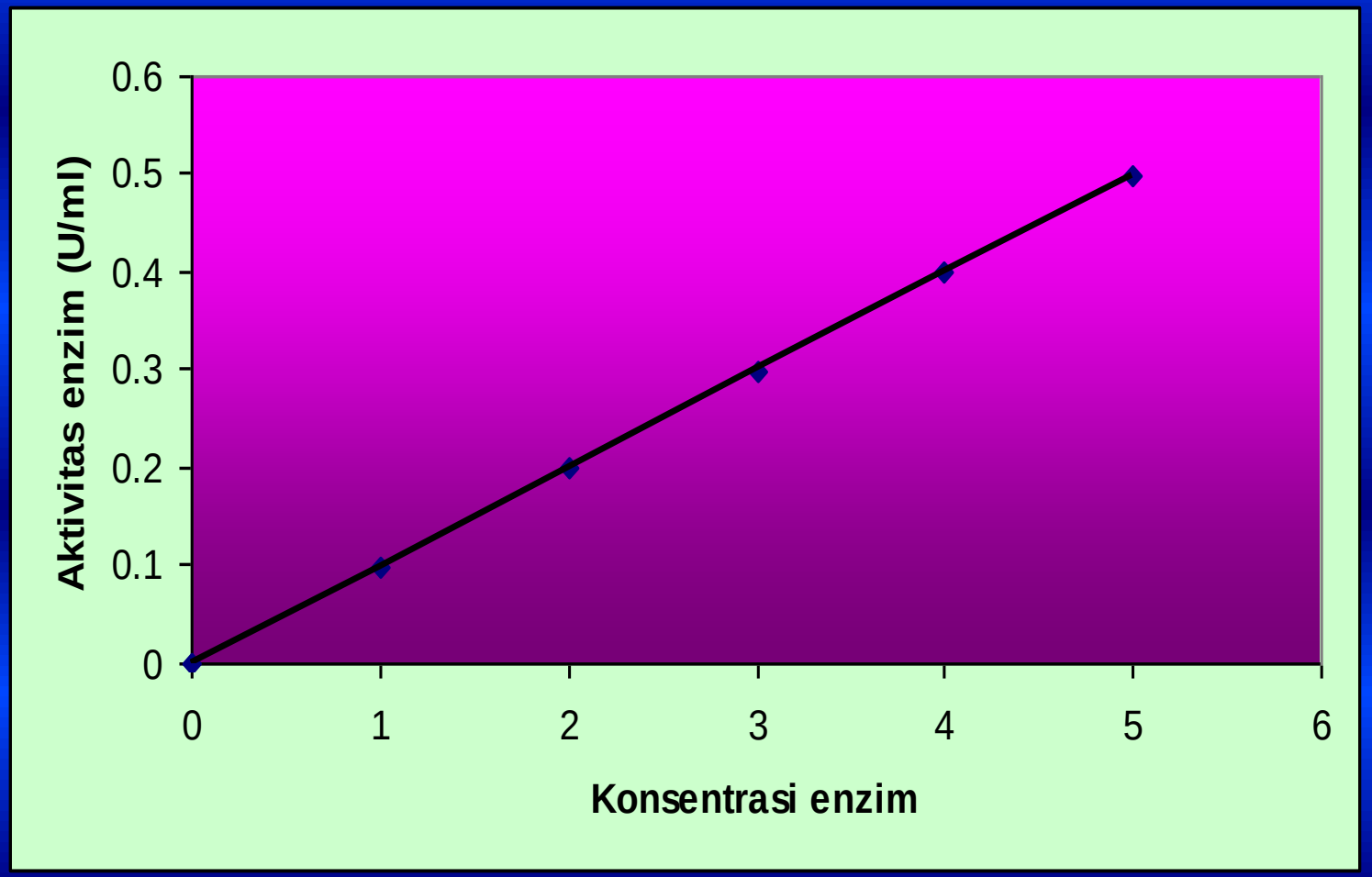
supernatan

Uji enzim

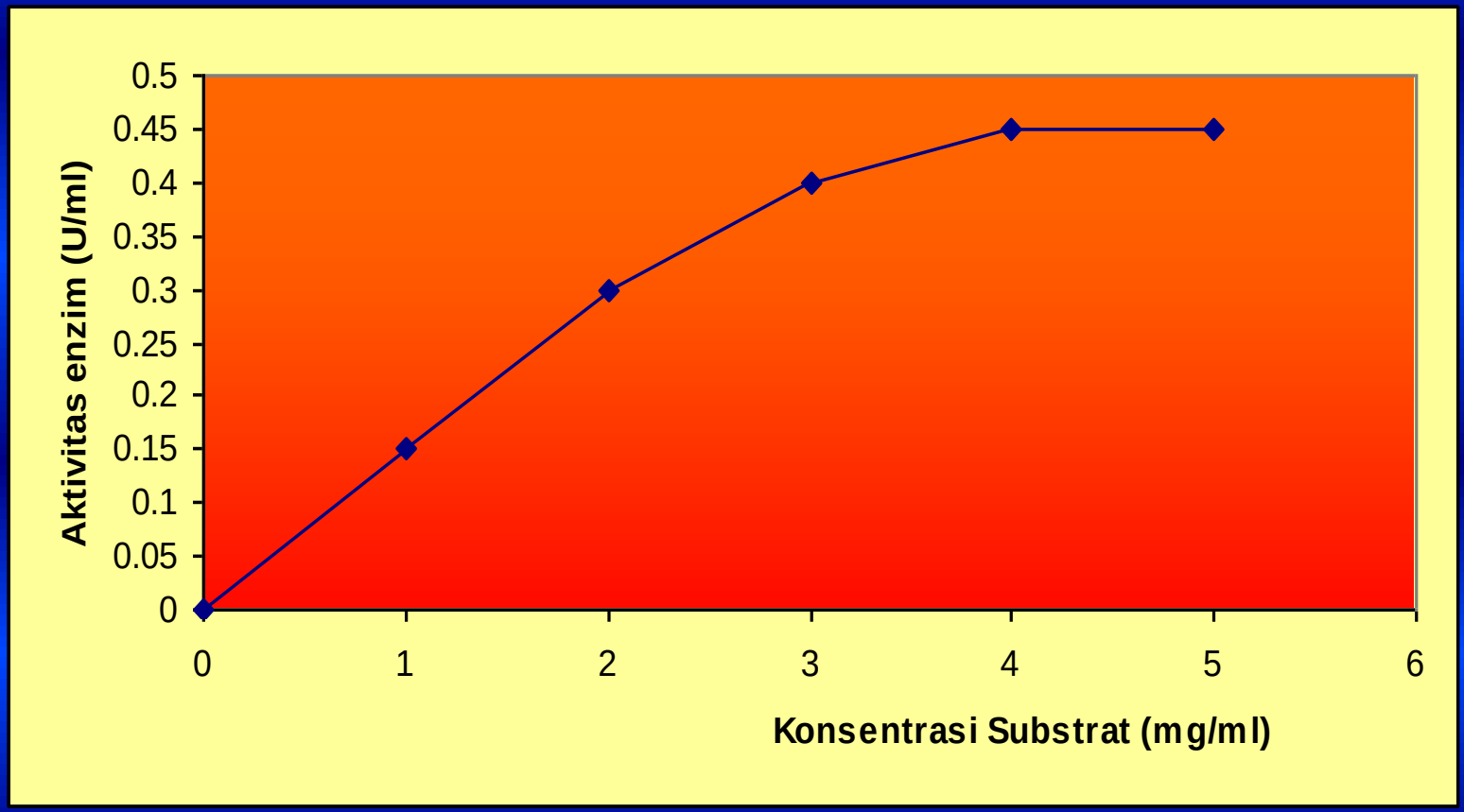
YANG MEMPENGARUHI AKTIVITAS ENZIM

- KONSENTRASI ENZIM
- KONSENTRASI SUBSTRAT
- SUHU
- pH

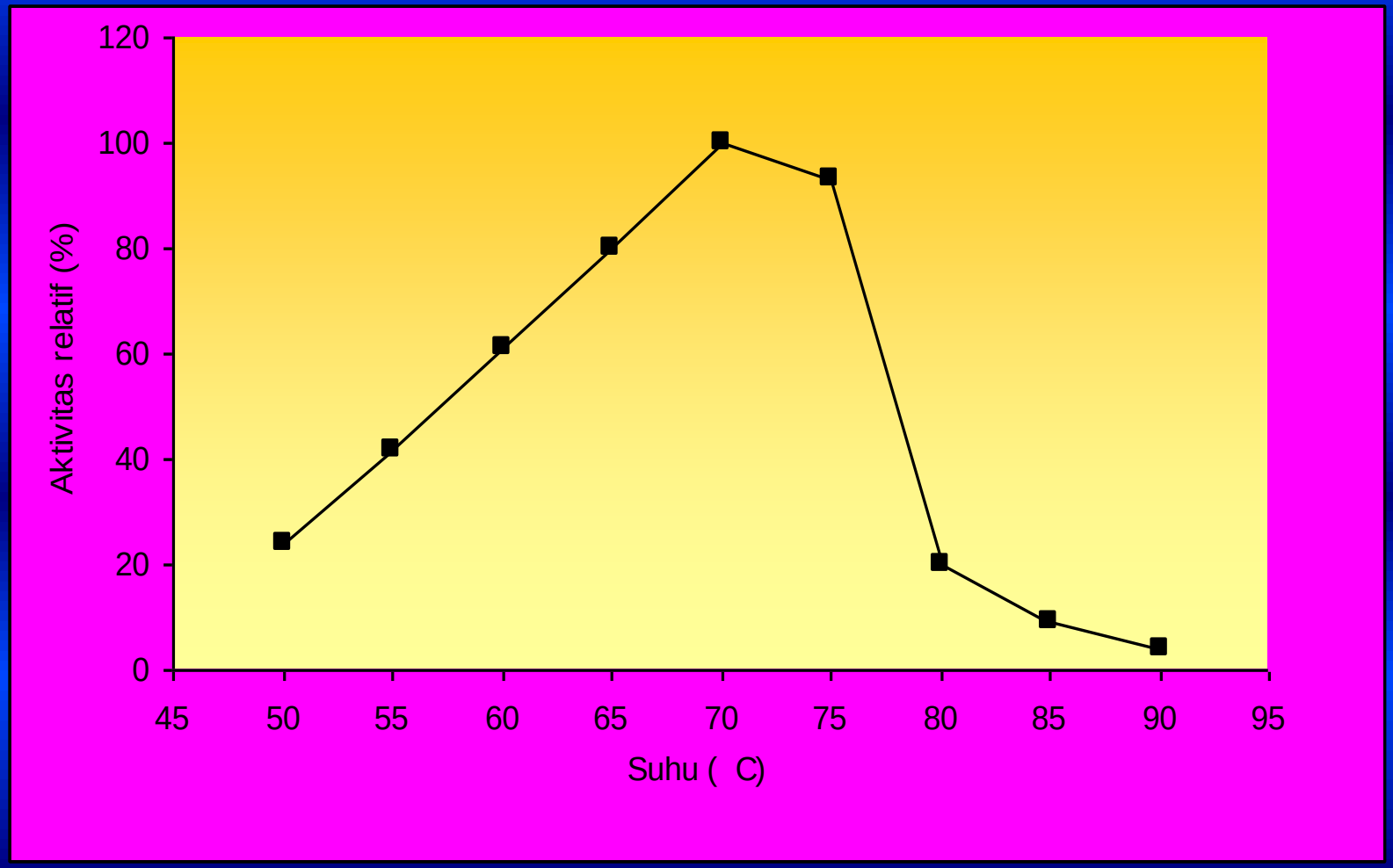
PENGARUH KONSENTRASI ENZIM TERHADAP AKTIVITAS ENZIM



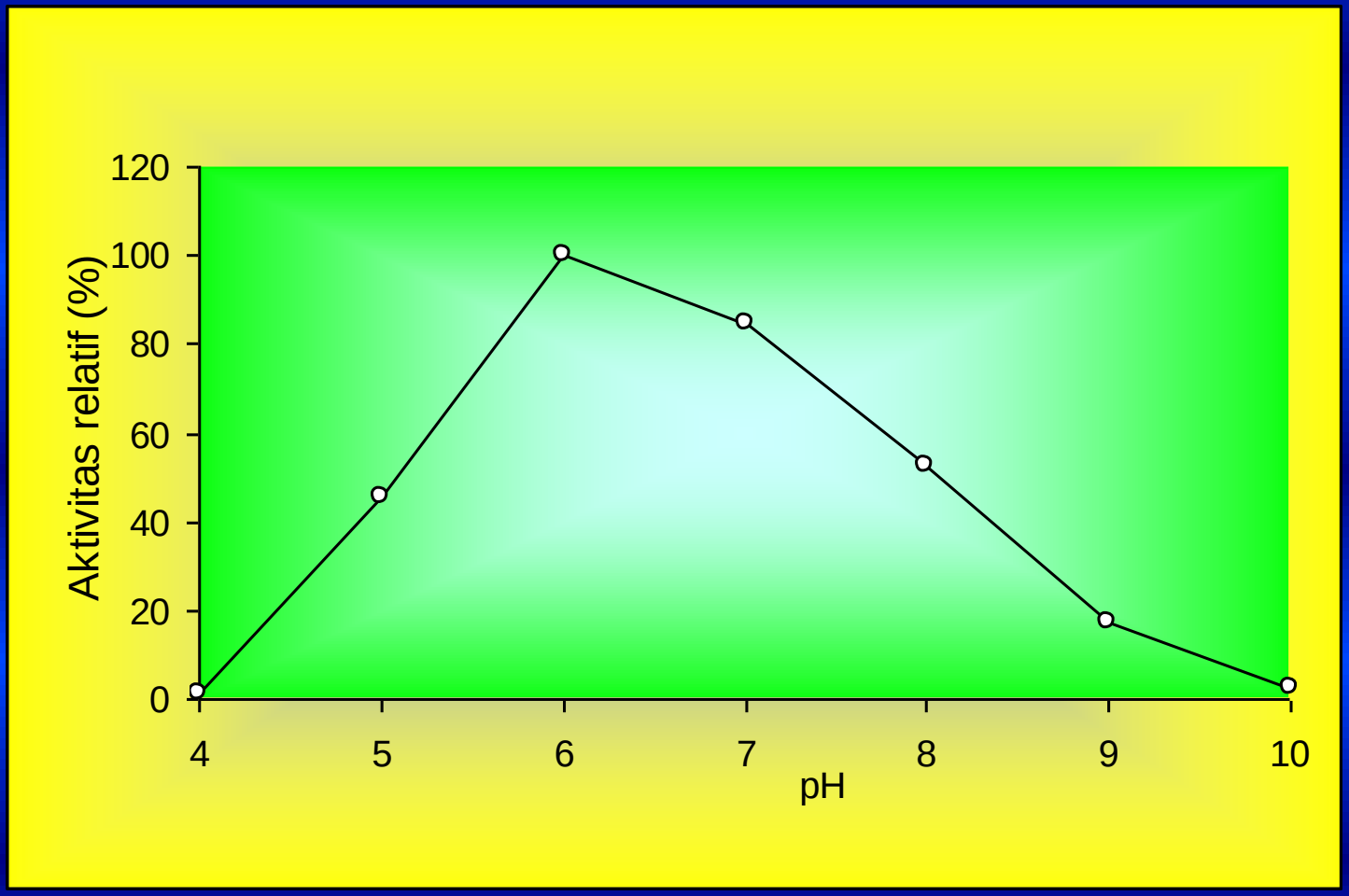
PENGARUH KONSENTRASI SUBSTRAT TERHADAP AKTIVITAS ENZIM



PENGARUH SUHU TERHADAP AKTIVITAS MANANASE L-07



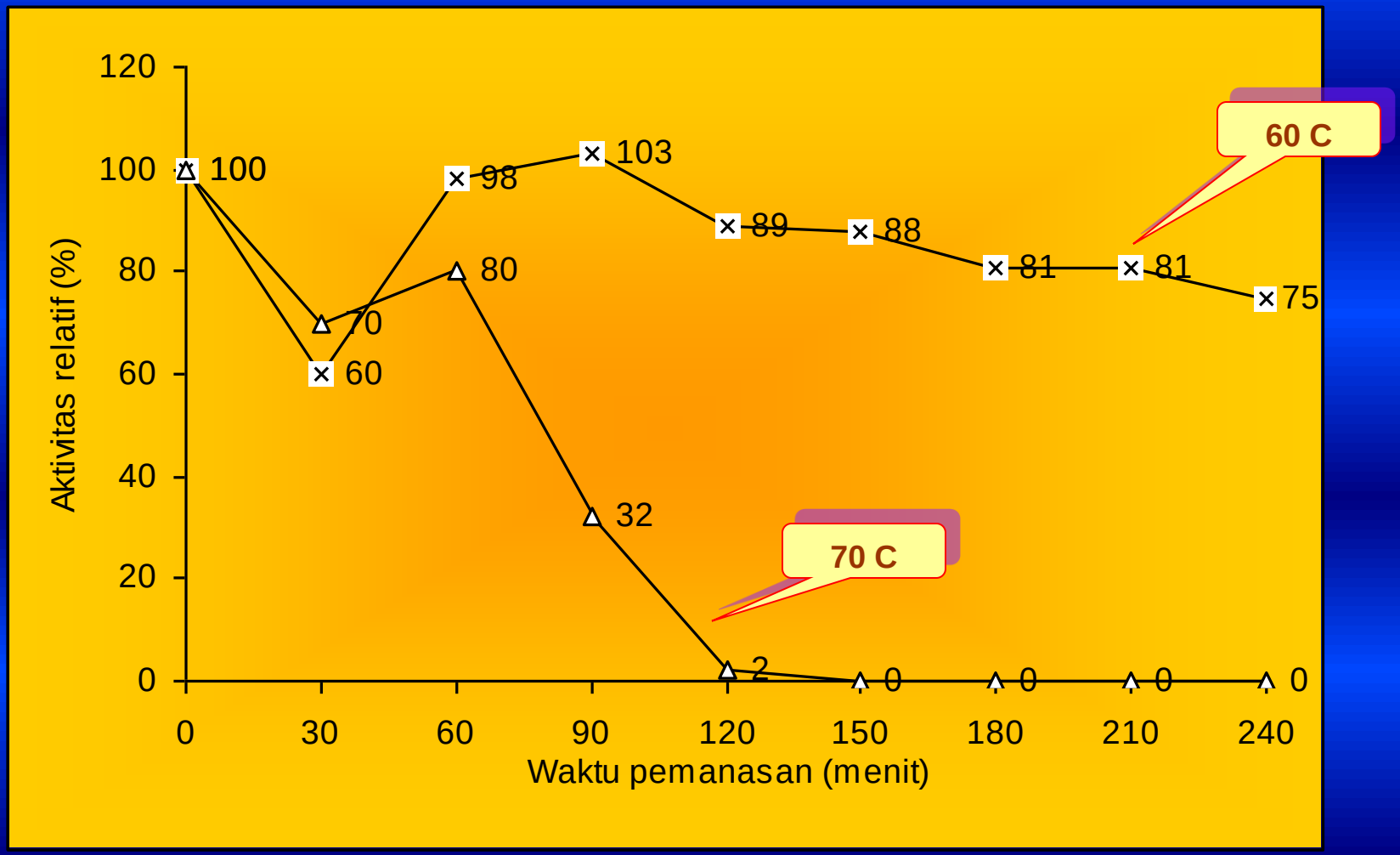
PENGARUH pH TERHADAP AKTIVITAS RELATIF MANANASE L-07



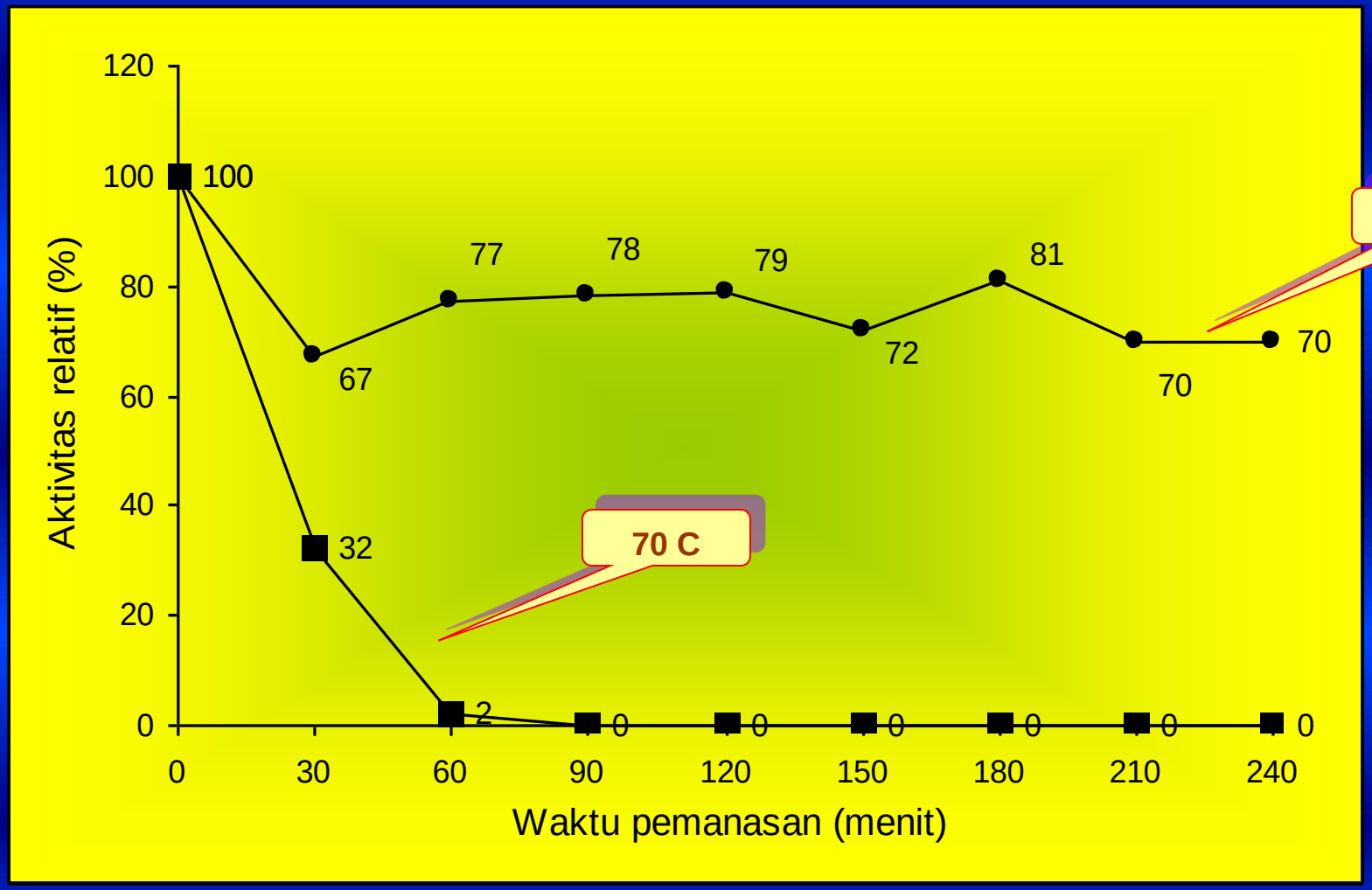
SIFAT YANG DIINGINKAN DARI ENZIM

- STABIL PADA SUHU DAN pH TERTENTU
- STABIL PADA PENGARUH ION LOGAM

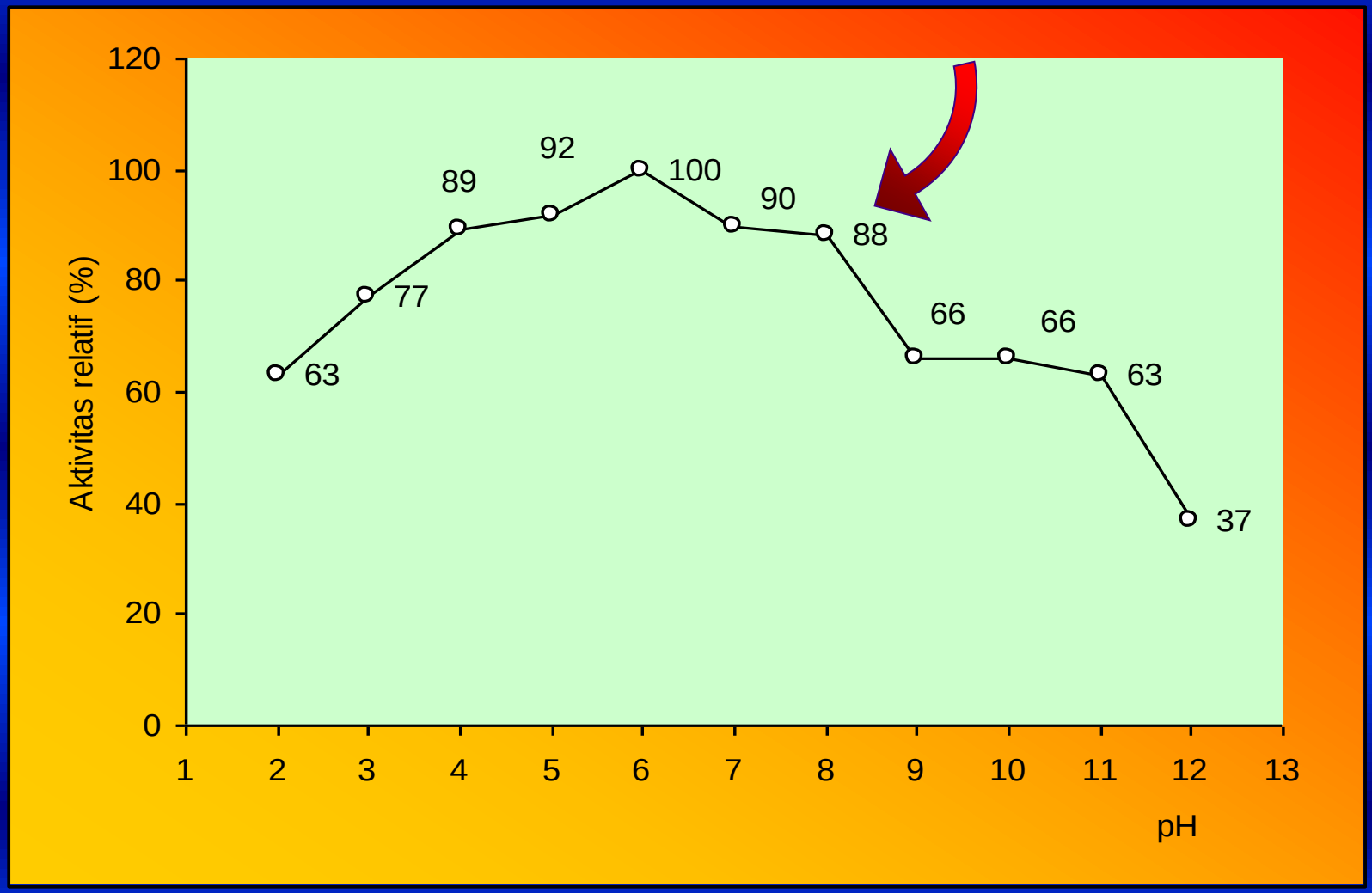
TERMOSTABILITAS MANANASE L-07 HASIL PURIFIKASI DENGAN DEAE SEPHADEX A-50



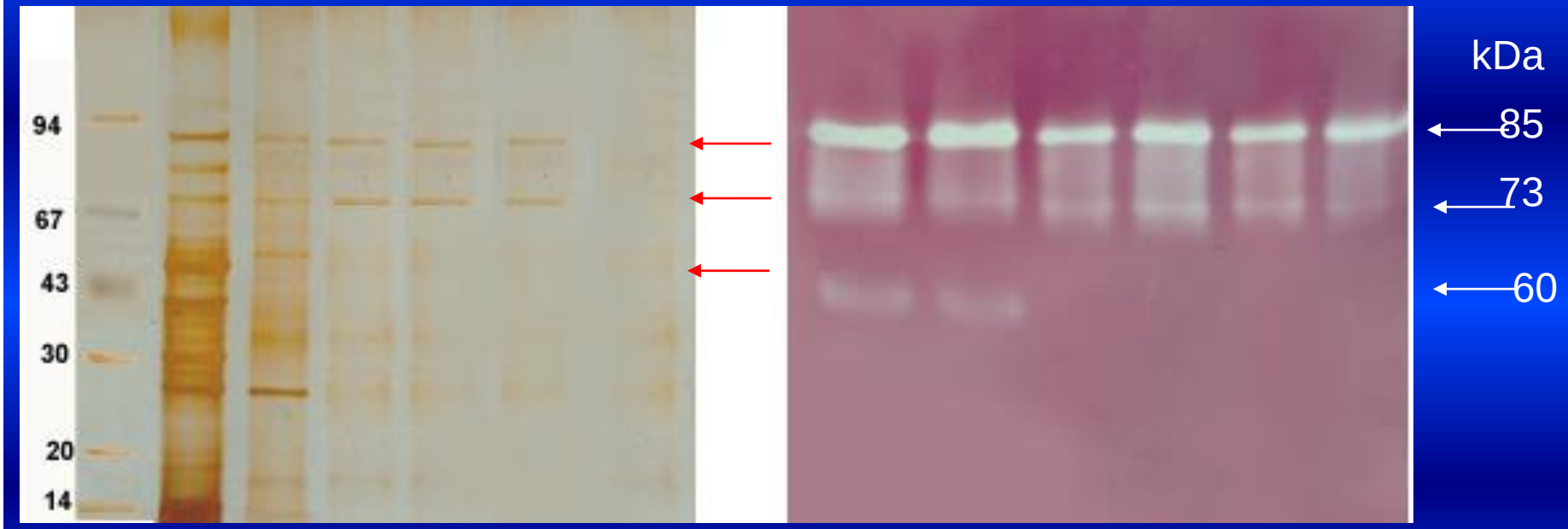
TERMOSTABILITAS MANANASE L-07 HASIL PURIFIKASI DENGAN SEPHADEX G-100



STABILITAS pH MANANASE KASAR L-07



SDS PAGE DAN ZYMOGRAM



M = marker

M-as = Pemekatan dg Am. Sulfate

M-2p = Pemekatan fraksi II - A-50

M-1 = Fraksi 1 - A-50

M-2 = Fraksi 2- A-50

M-3 = Fraksi 3- A-50

M-g = G-100

DALAM PENELITIAN ENZIM

TUJUAN

1. Seleksi, isolasi, identifikasi, dan karakterisasi mikroba penghasil enzim
2. Purifikasi dan karakterisasi enzim
3. Pelajari produksi enzim

MANFAAT

1. Diharapkan diperoleh novel (baru/unik) enzim
2. Enzim untuk biokonversi substrat
3. Mengetahui aplikasi enzim

19 sept 2019

ENZIM DAN FUNGSINYA

Protease/ amilase	Dalam deterjen utk menghilangkan kotoran yang bersifat protein/karbohidrat
Renin	Menggumpalkan susu (keju)
Papain /protease	Melunakkan daging
Glukosa oksidase	Utk mengukur kandungan glukosa dlm darah (diabetes)
Enzim hidrolisis (protease, lipase, selulase, amilase)	Obat untuk membantu proses pencernaan
Streptokinase	Melarutkan gumpalan darah, utk pengobatan radang dan luka
Laktase	Membuat susu berlaktosa rendah

KINETIKA ENZIM

Adalah suatu suatu cabang enzimologi yang membahas faktor-faktor yang mempengaruhi kecepatan reaksi enzimatis

Faktor yang mempengaruhi : pH, suhu, substrat, produk, senyawa inhibitor, dan aktivator

KINETIKA ENZIM

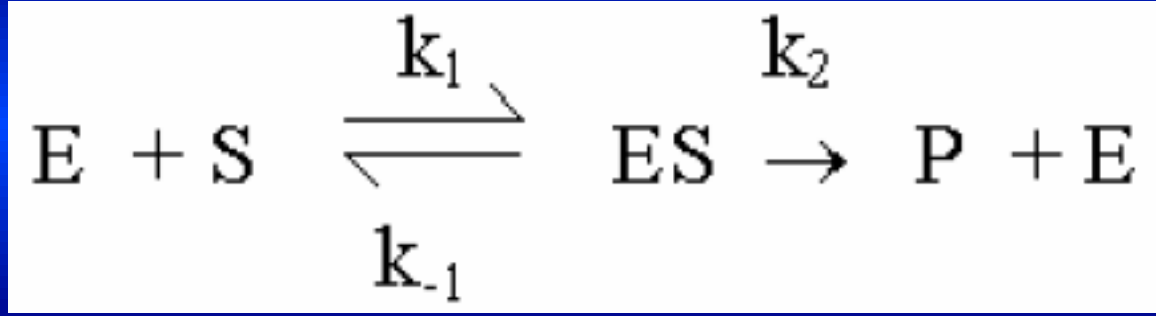
Kinetika Enzim

Kinetika adalah ilmu yang mempelajari tentang laju reaksi

- Sel-sel mempunyai enzim dengan kemampuan yang luar biasa yang dikontrol dengan potensi termodinamika
- Enzim berperan dalam berfungsinya metabolisme sel
- Apa yang ingin diketahui :
  - **Kecepatan Maximum**
  - **Afinitas Substrat**
  - **Afinitas Inhibitor**

Persamaan Michaelis-Menten

$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$



Kesimpulan Kinetika Michaels - Menten

- when [S]= K_M, the equation reduces to

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{V_{max}[S]}{[S] + [S]} = \frac{V_{max}}{2}$$

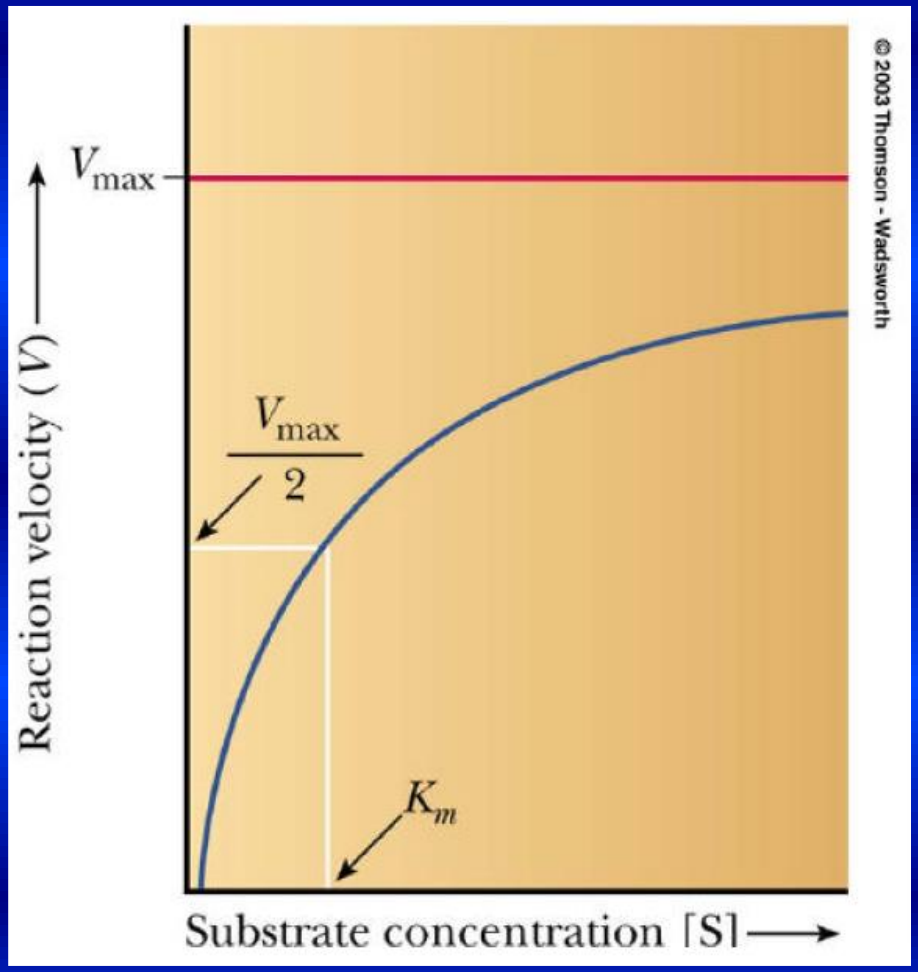
- when [S] >> K_M, the equation reduces to

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{V_{max}[S]}{[S]} = V_{max}$$

- when [S] << K_M, the equation reduces to

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{V_{max}[S]}{K_M} = \frac{V_{max}}{K_M}[S]$$

Kesimpulan Kinetika Michaels - Menten



3 Desember 2020

- E + S =====ES ------- E + P

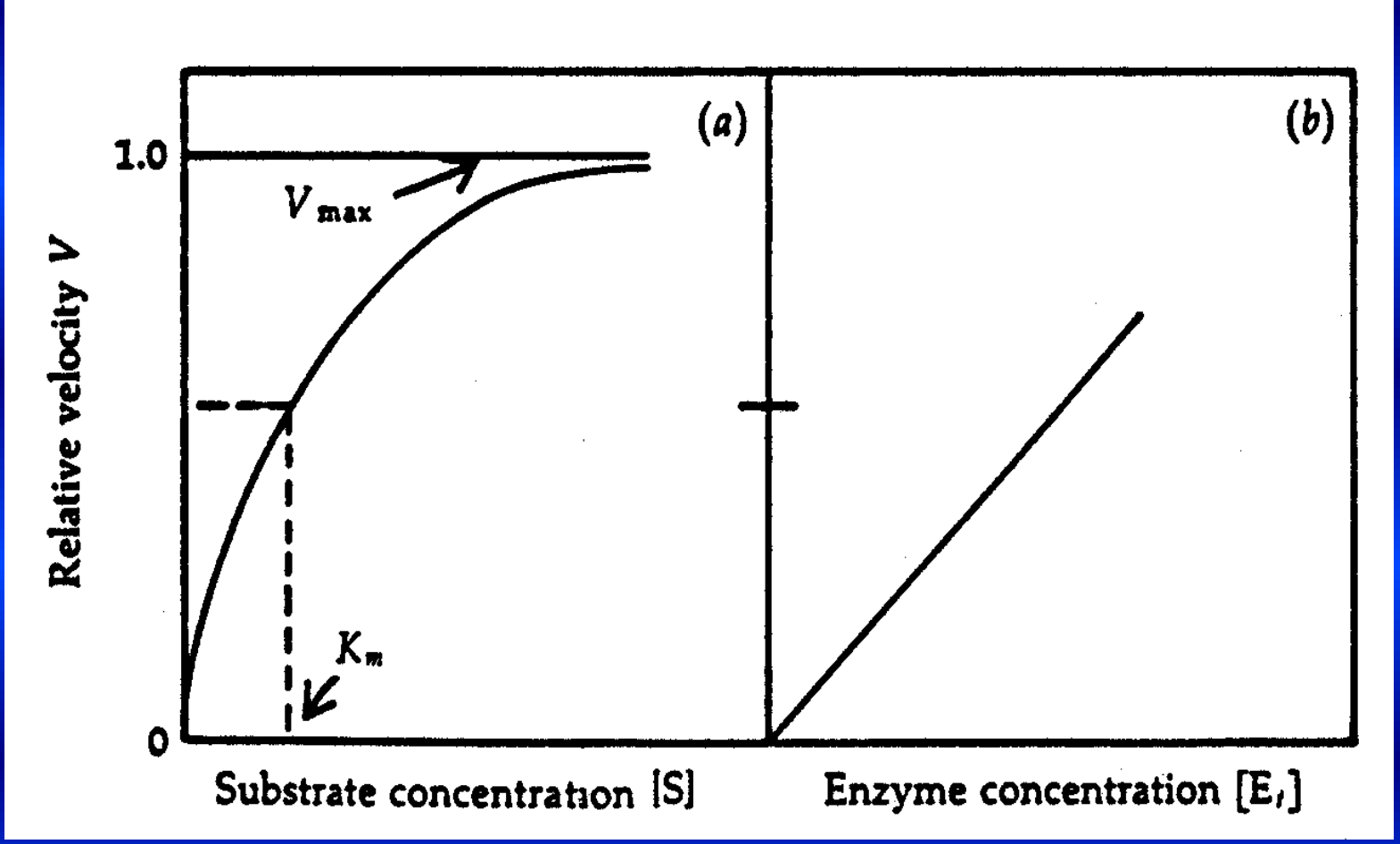


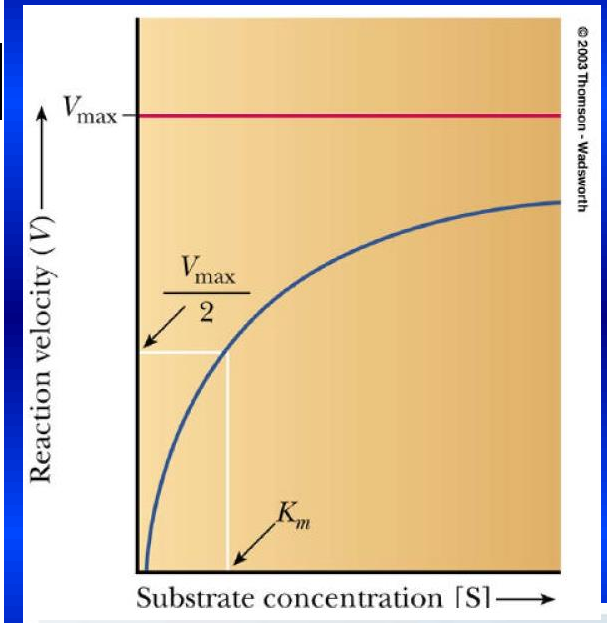
Figure 8.3. The relative velocity (a) as a function of substrate concentration and (b) as a function of enzyme concentration. V_{max} is 1.0, and the substrate concentration required to achieve ½V_{max} is equal to K_m. In contrast to the hyperbolic curve relating V to [S], V as a function of [E_t] is linear.

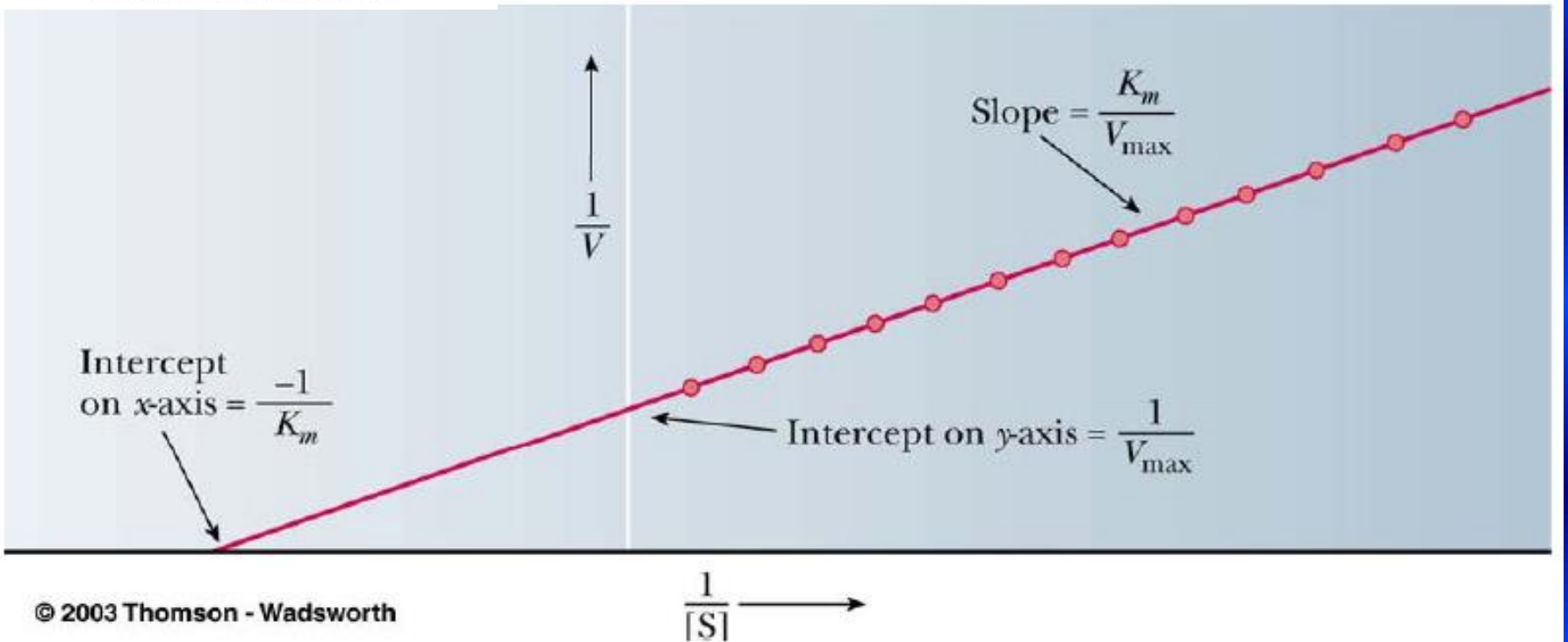
Persamaan Garis Lineweaver – Burk

- Ada kesulitan menentukan Vmax dlm percobaan
- Persamaan tersebut adl hyperbola yang ditransformasi dalam persamaan garis lurus
- Persamaan garis adl **y = mx + b**

- Sebuah grafik 1/V terhadap 1/[S] akan memberikan garis lurus dg kemiringan Km/Vmax dan titik potong y adalah 1/Vmax
- Grafik tersebut dikenal sebagai persamaan garis Lineweaver-Burk

Persamaan garis Lineweaver – Burk





Arti K_m

- Km sifatnya konstan
- Km kecil berarti ikatannya kuat; Km besar berarti ikatannya lemah
- Useful to compare Km for different substrates for one enzyme

  Hexokinase : D-fructose – 1.5 mM

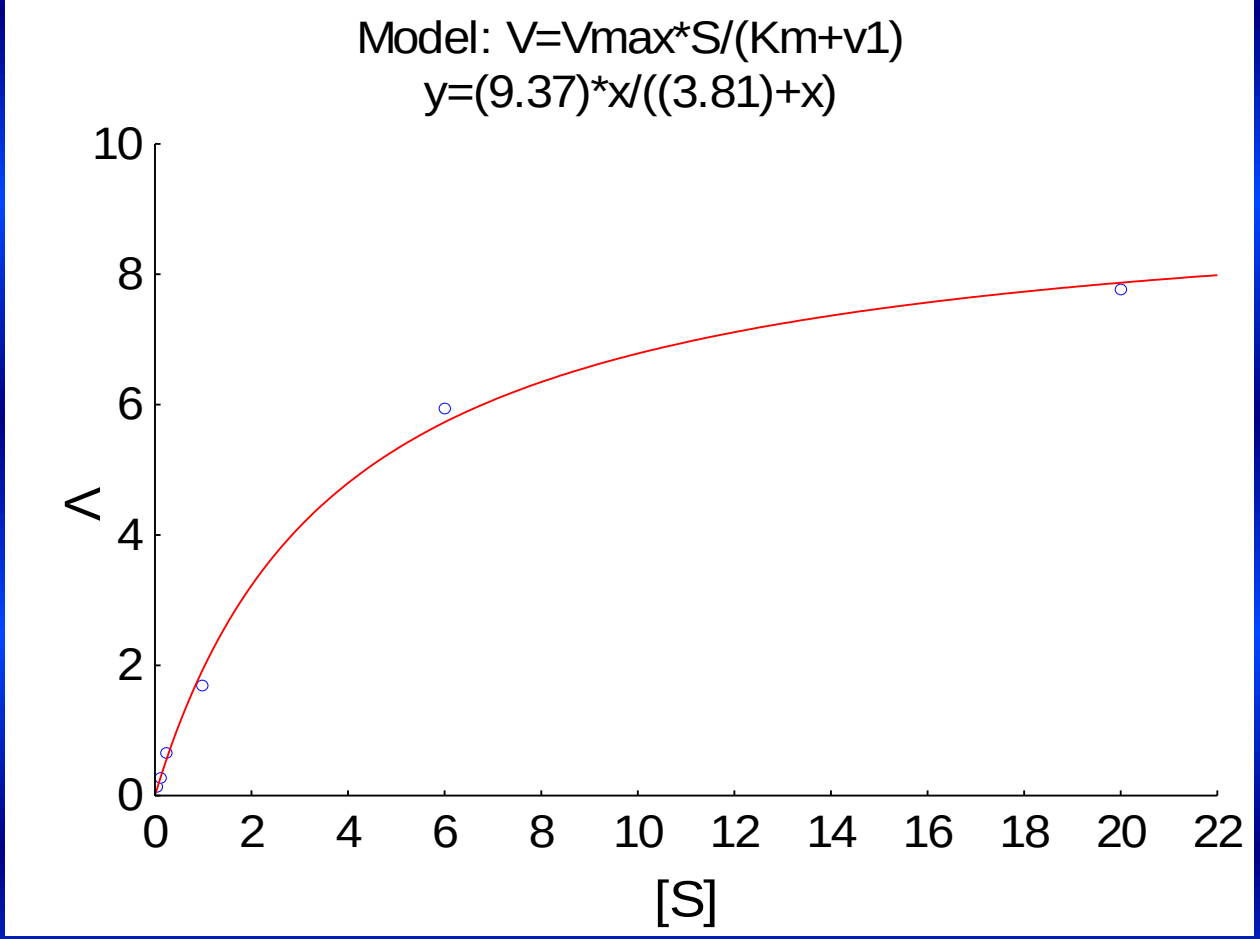
  D-glucose – 0.15 mM
- Useful to compare Km for a common substrate used by several enzymes

  Hexokinase: D-glucose – 0.15 mM

  Glucokinase: D-glucose – 20 mM

Perbandingan Kinetika Enzim terhadap Mekanisme perubahan kimiawi

- Mekanisme kinetika enzim adalah penambahan substrat dan pelepasan produk dlm reaksi yang dikatalisis oleh reaksi enzim
- Mekanisme perubahan kimiawi adalah jalur perubahan kimiawi dari S $\rightarrow$ P, termasuk beberapa struktur antara



(From Statistica)

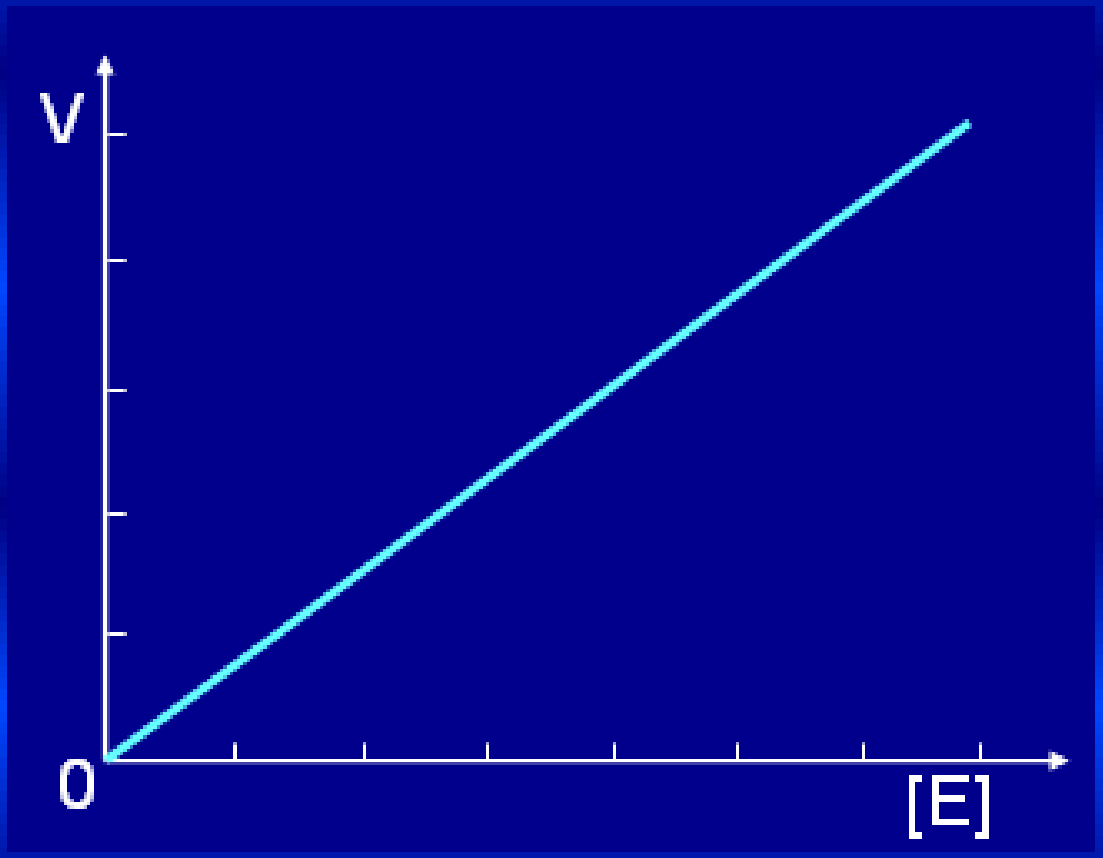
	Estimate	Standard	t-value	p-level	Lo. Conf	Up. Conf
Vmax	9.371080	0.341119	27.47159	0.000010	8.423982	10.31818
Km	3.812651	0.453108	8.41444	0.001092	2.554622	5.07068

Actual parameters: Vmax=10, K_m=4

Influence of enzyme concentration

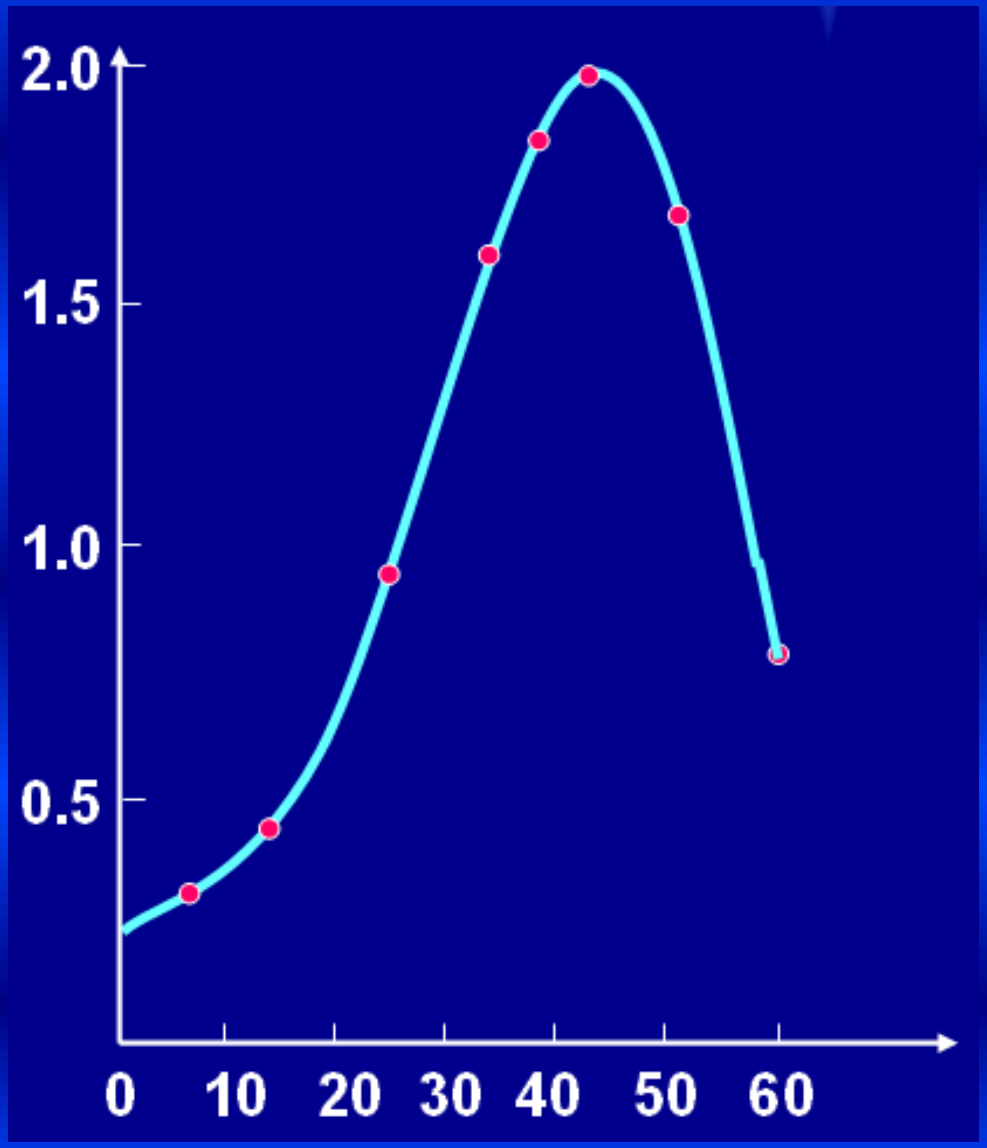
$$V = \frac{K_3[E][S]}{K_m + [S]}$$

$v = k_3 [E]$, as $[S] >> [E]$

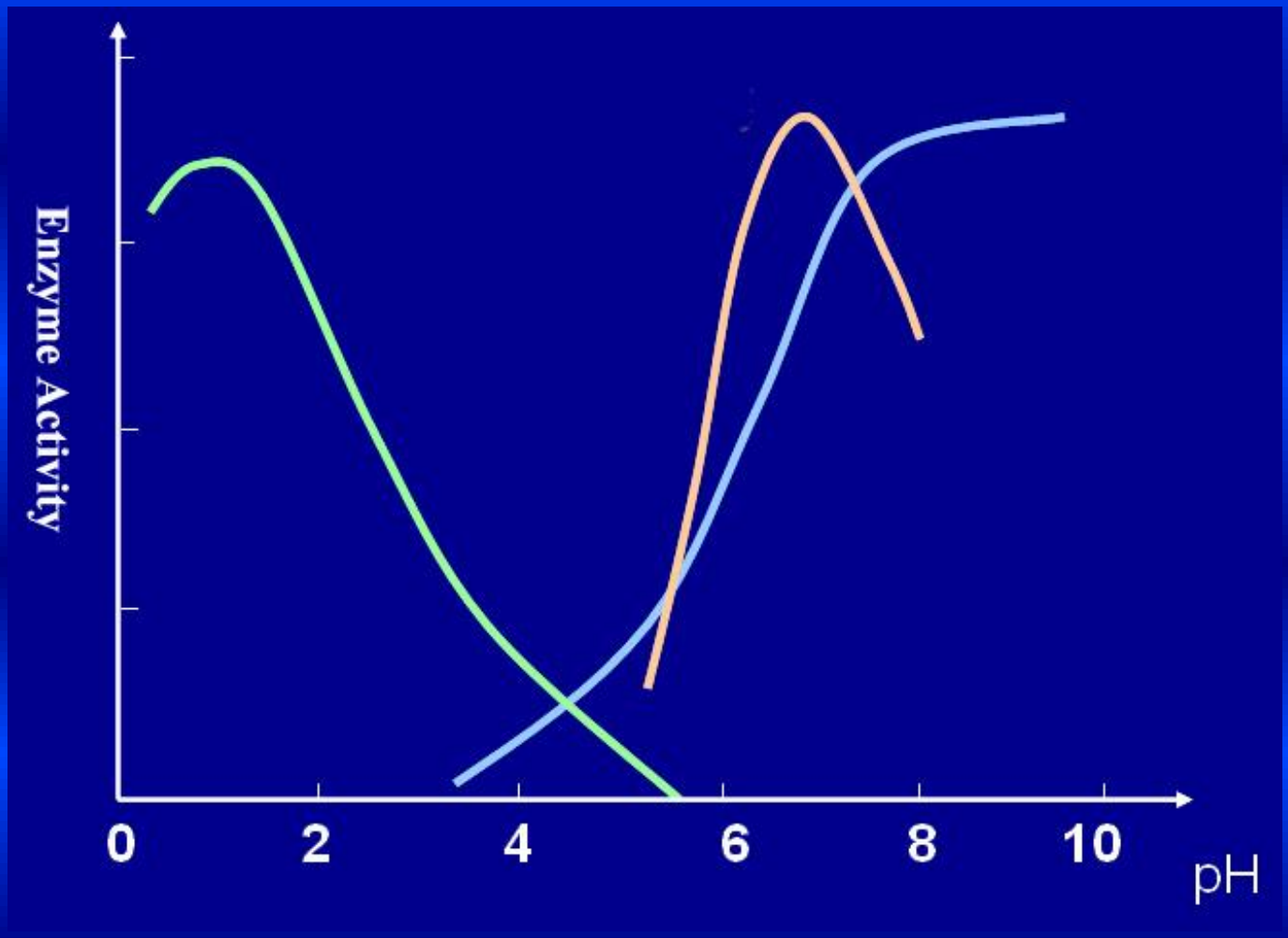


Influence of temperature

Optimum temperature，most of them are in the range from 35 to 45℃ .



Influence of pH



Optimum pH

Penghambatan Enzim

Penghambatan enzim sangat penting diketahui untuk berbagai alasan :

1) Penghambatan digunakan untuk memperoleh informasi ttg bentuk situs aktivitas dan residu as amino pada situs aktif tersebut.
2) Penghambatan digunakan untuk memperoleh informasi ttg mekanisme reaksi kimia.
3) Penghambatan digunakan untuk memperoleh informasi ttg regulasi dan pengontrolan jalur metabolisme
4) Penghambatan digunakan untuk memperoleh informasi ttg design obat

Enzyme Inhibition

- **Reversible inhibitor: a substance that binds to an enzyme to inhibit it, but can be released**
  - **usually involves formation of non-covalent bonds**

- **Irreversible inhibitor: a substance that causes inhibition that cannot be reversed**
  - **usually involves formation or breaking of covalent bonds to or on the enzyme**

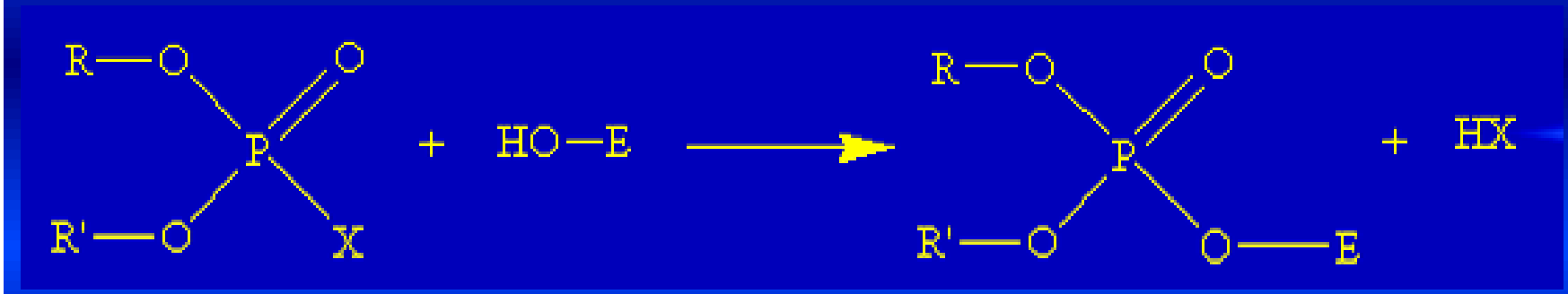
Inhibitors

- Irreversible inhibition
- Reversible inhibition
  - competitive inhibition
  - non-competitive inhibition
  - uncompetitive inhibition

Irreversible inhibition

- Irreversible inhibition:

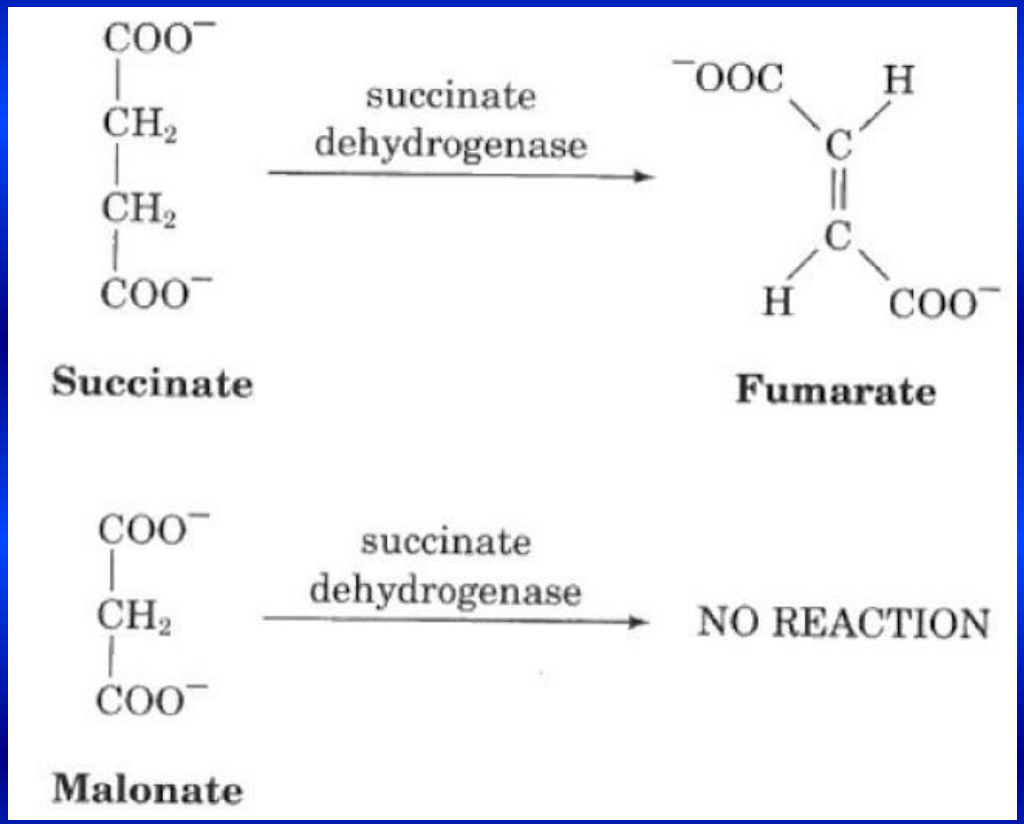
  The inhibitor combine with essential group of enzyme active center by covalent bond, resulting in enzymatic activity loss.



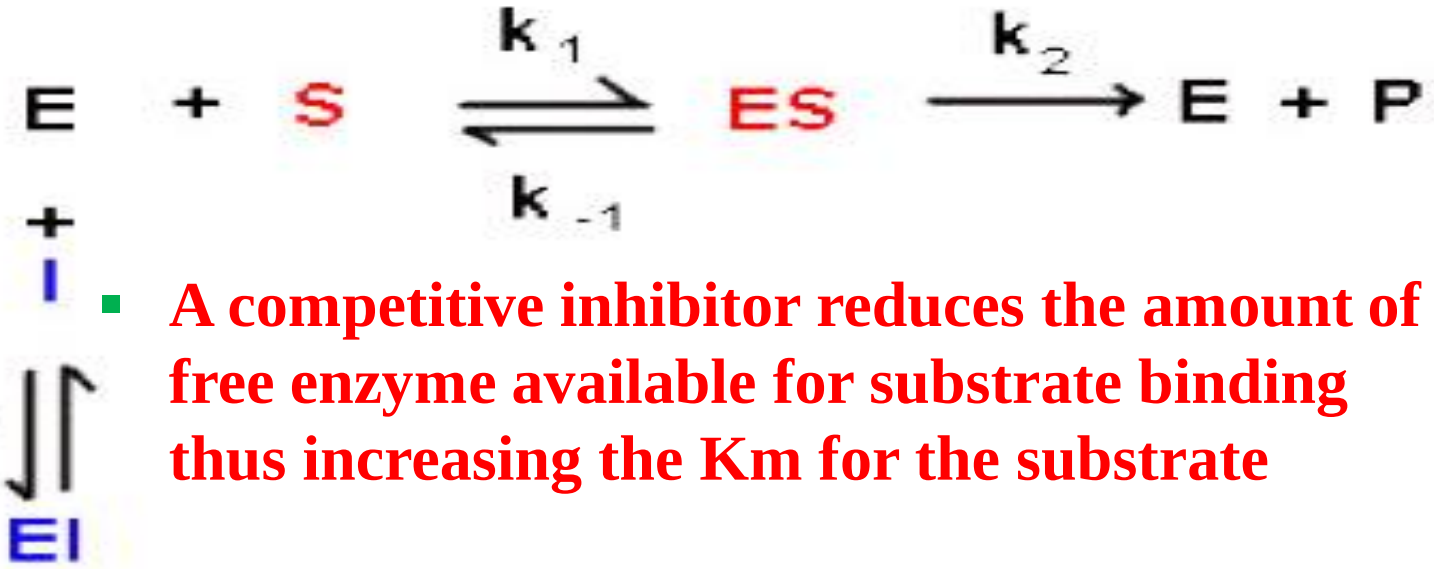
Competitive Inhibition

- Competitive inhibitor competes with a substrate for the enzyme - substrate binding site

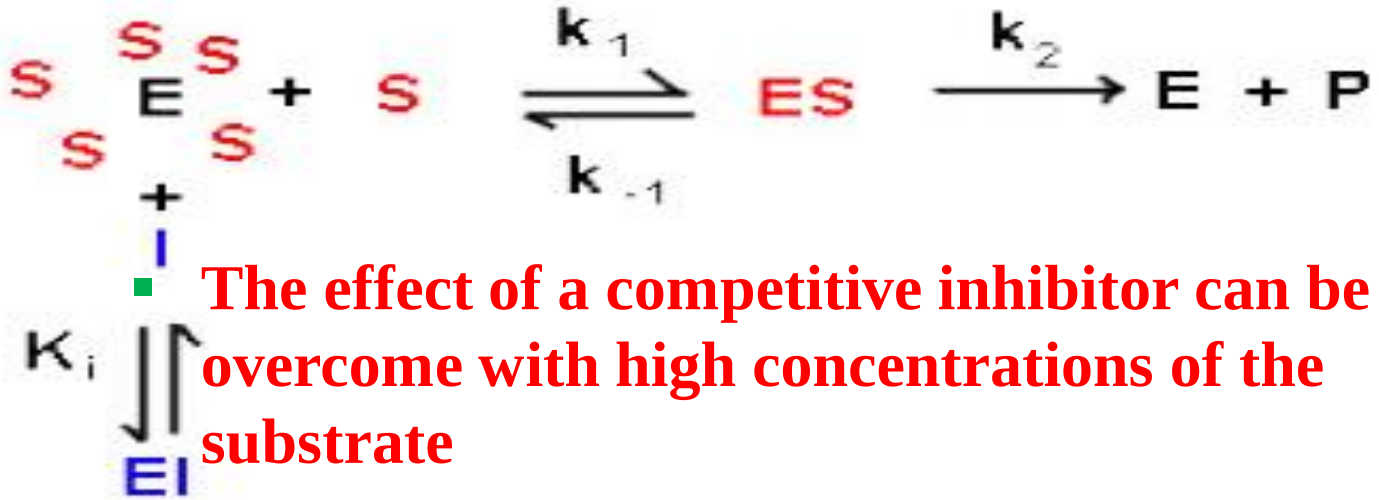
Malonate is a competitive inhibitor of succinate for succinate dehydrogenase



Competitive Inhibition

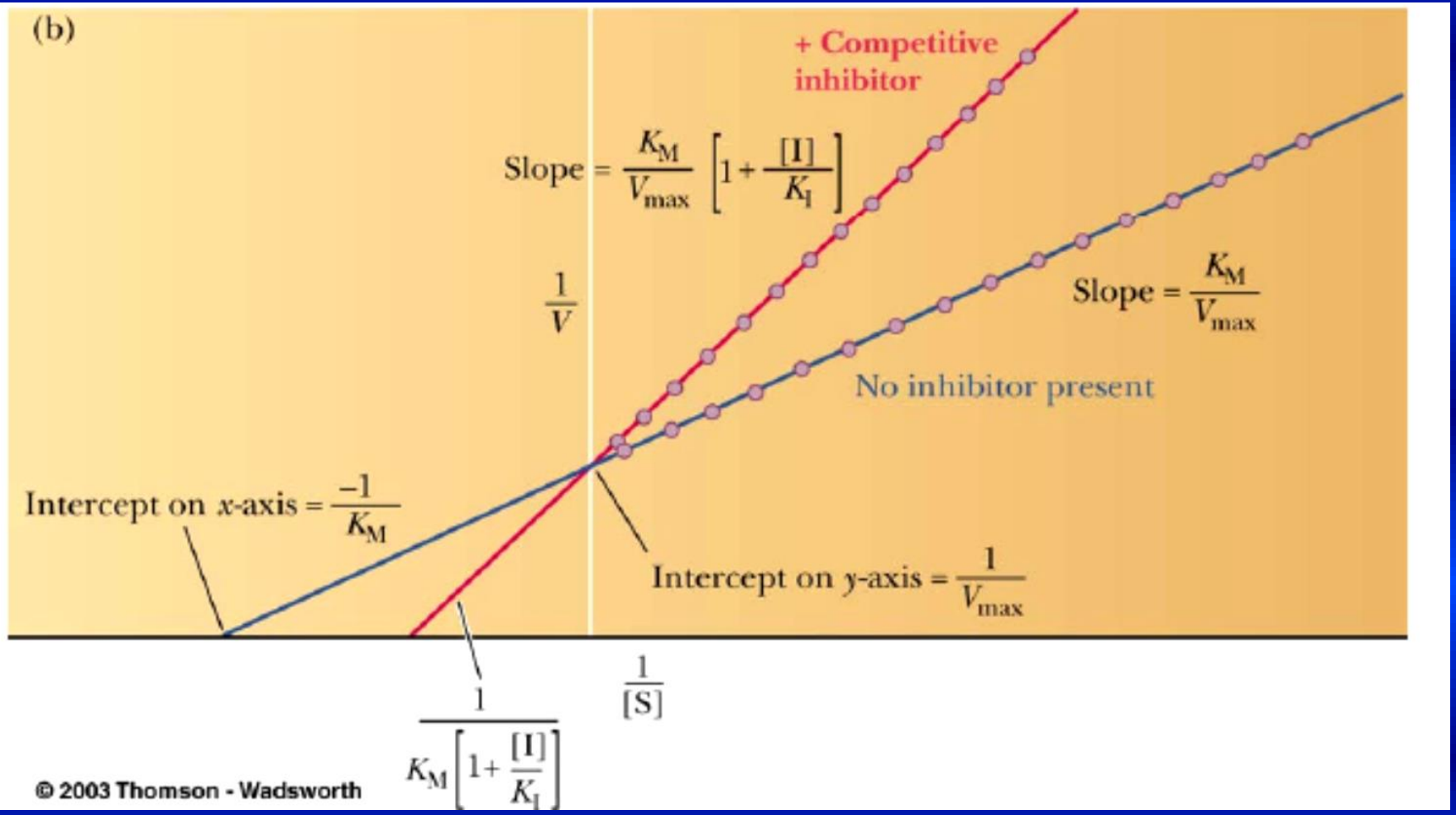


- A competitive inhibitor reduces the amount of free enzyme available for substrate binding thus increasing the Km for the substrate



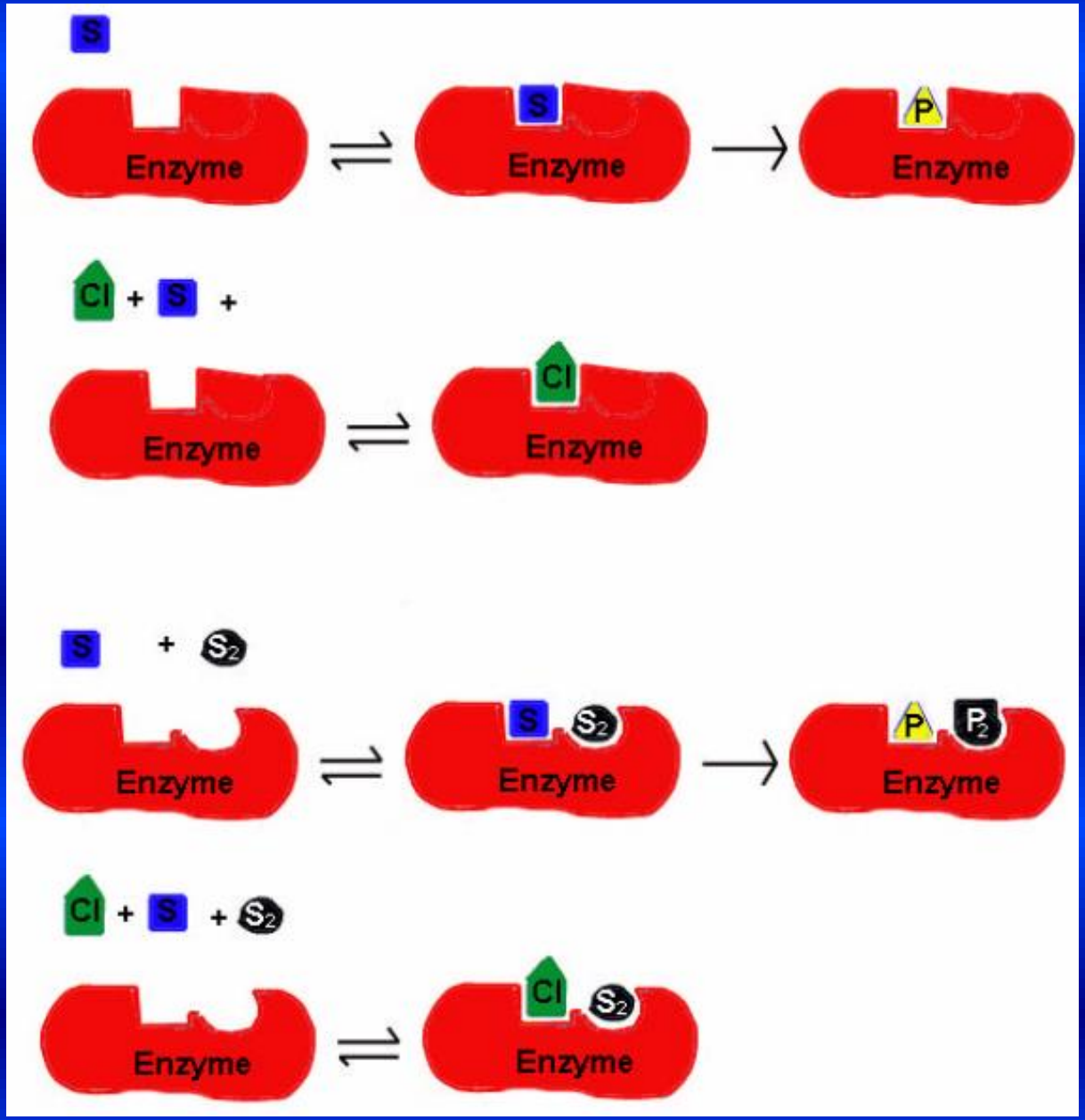
- The effect of a competitive inhibitor can be overcome with high concentrations of the substrate

Competitive Inhibition



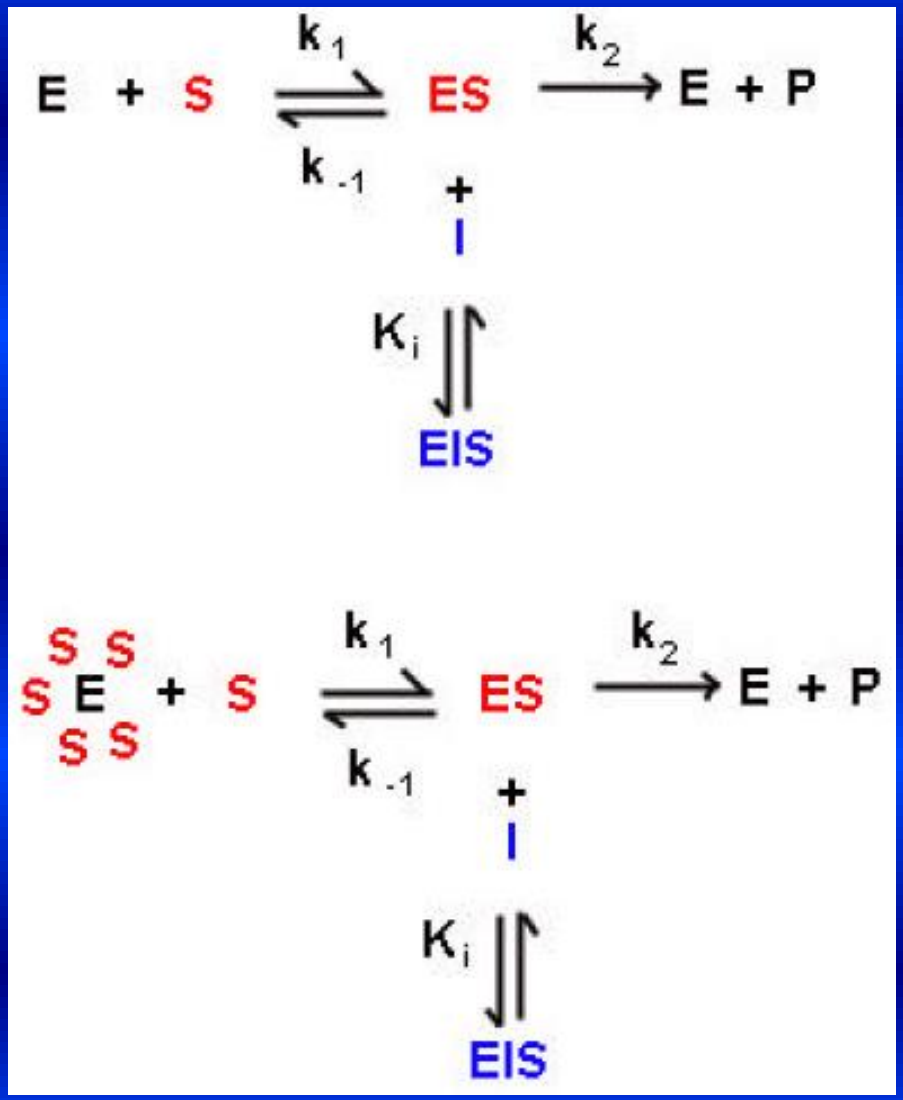
Competitive Inhibition

- Unimolecular Reaction
- Bimolecular Reaction

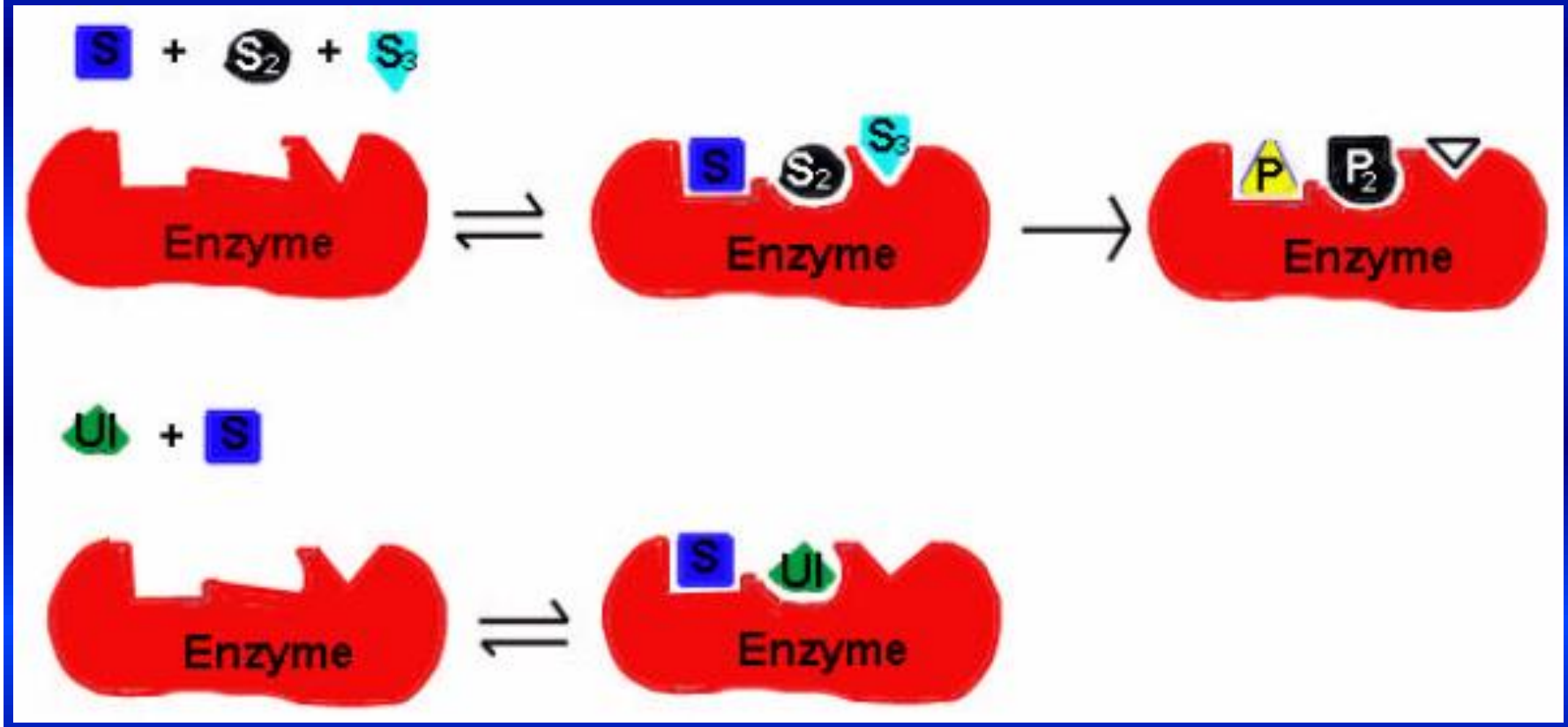


Uncompetitive Inhibition

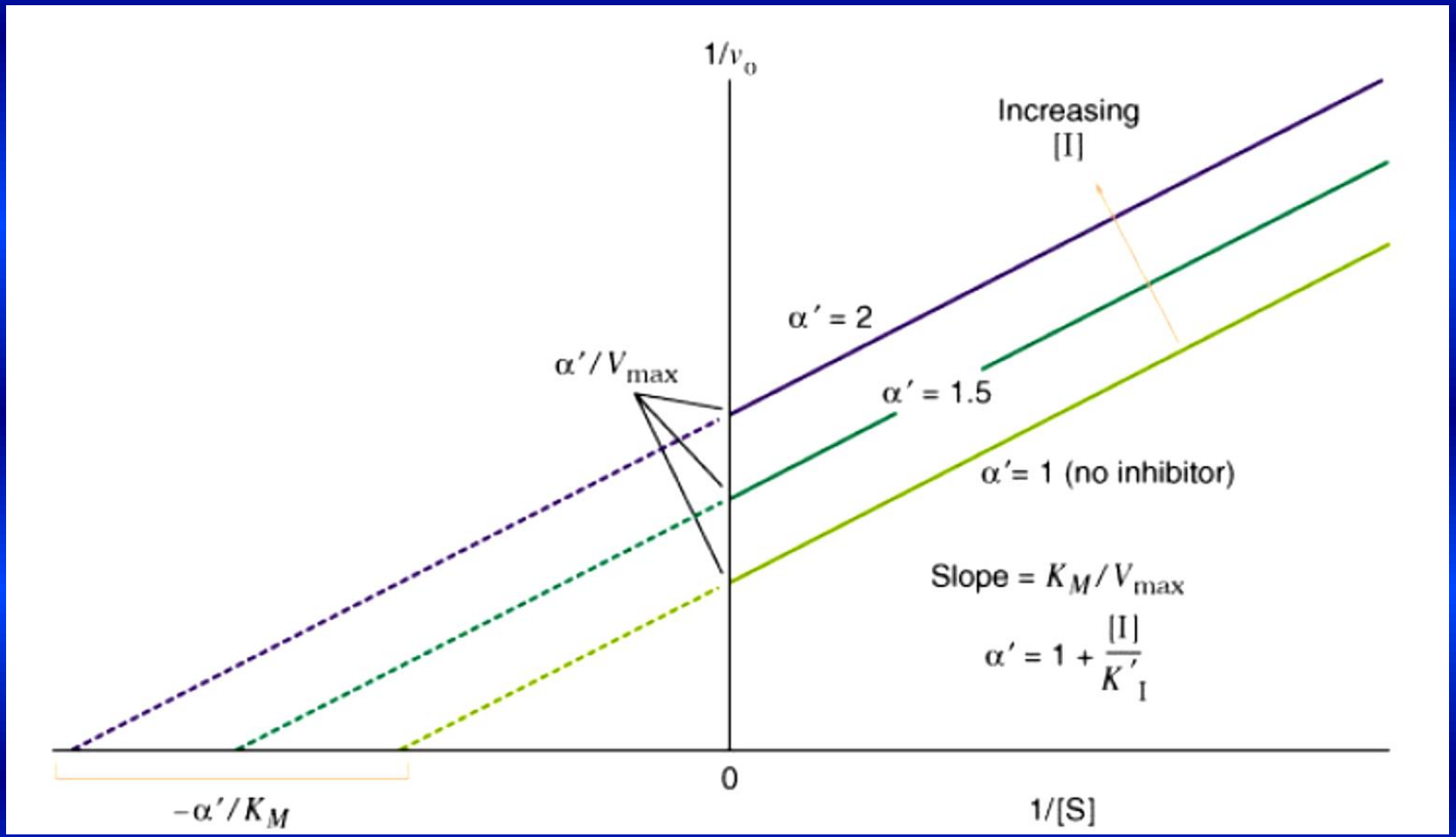
- An uncompetitive inhibitor binds to the enzyme substrate complex but not to free enzyme
- The result is a decrease in Vmax and Km
- The effect of an uncompetitive inhibitor can not be overcome by high concentrations of the substrate



Uncompetitive Inhibition

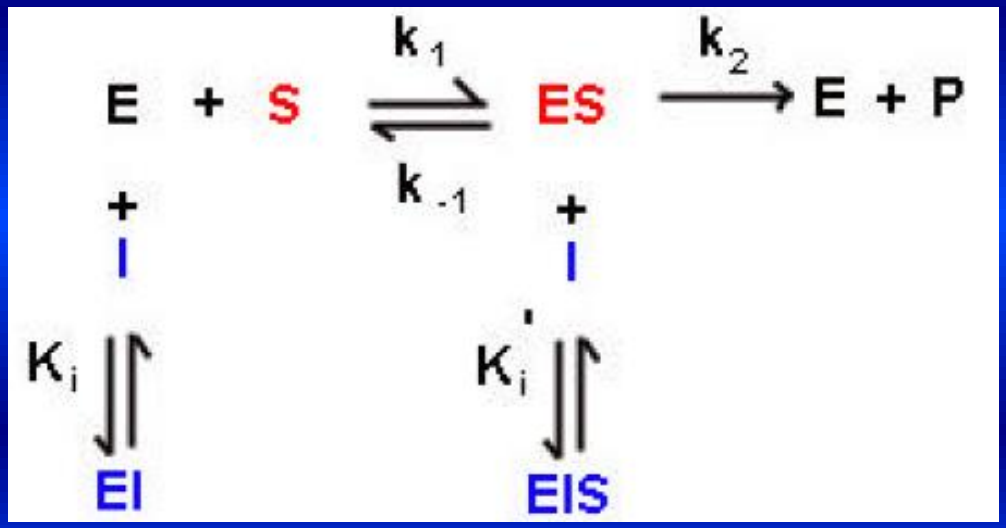


Uncompetitive

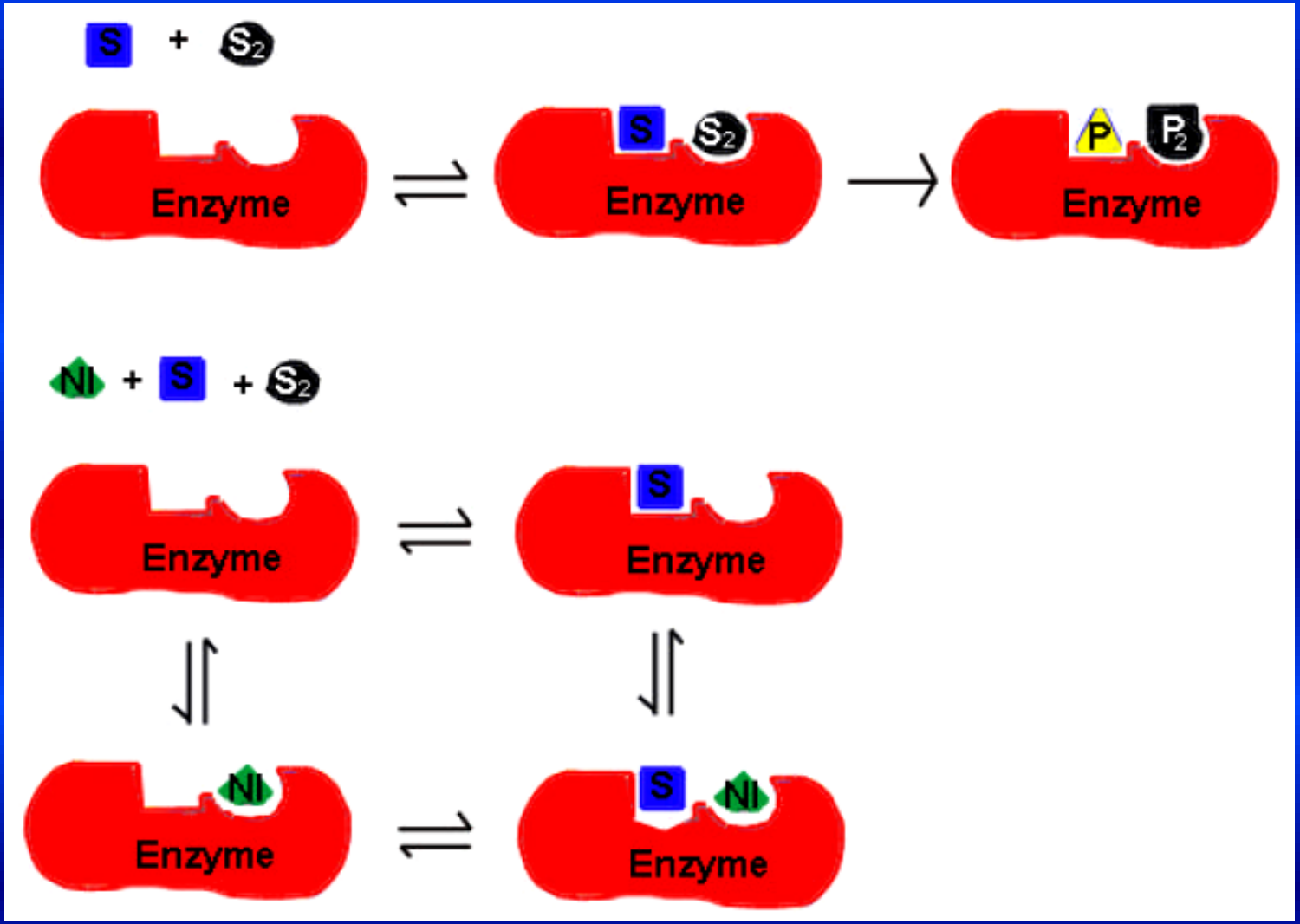


Mixed or Non-Competitive Inhibition

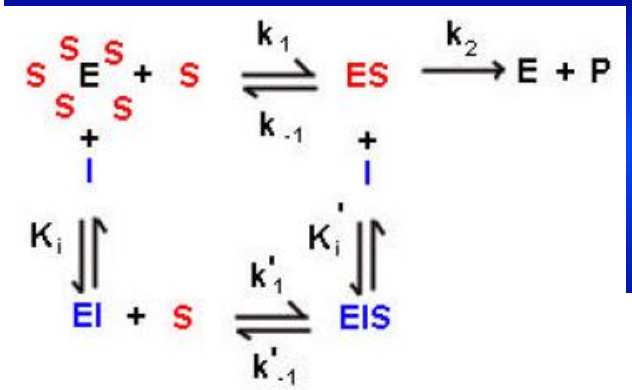
- The inhibitor can bind to both free enzyme and the ES complex
- The affinity of the inhibitor to the two complexes might be different
  - If binding of inhibitor changes the affinity for the substrate, Km will be changed and called mixed inhibition
  - If only Vmax affected called Non-competitive inhibitor



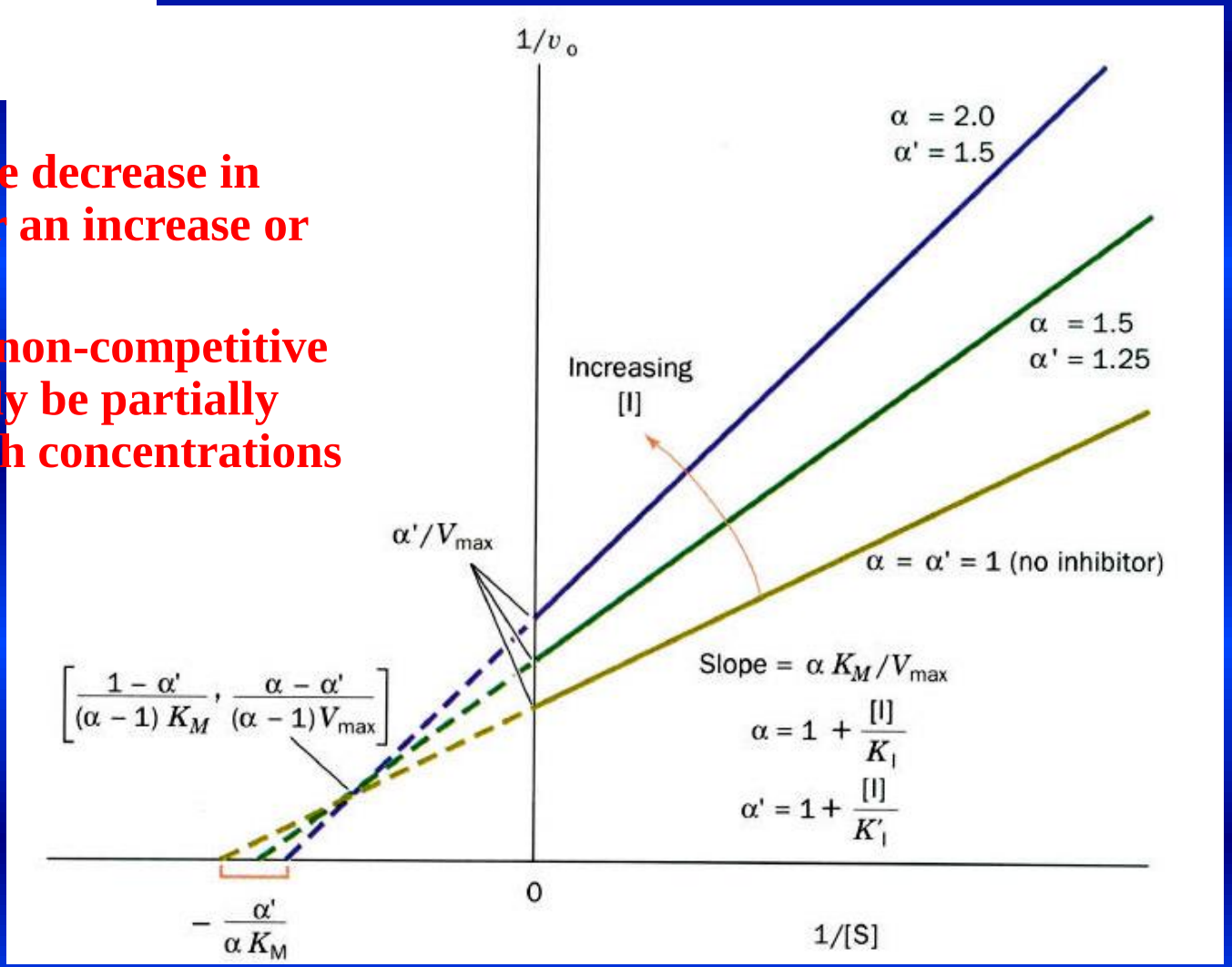
Mixed Inhibition



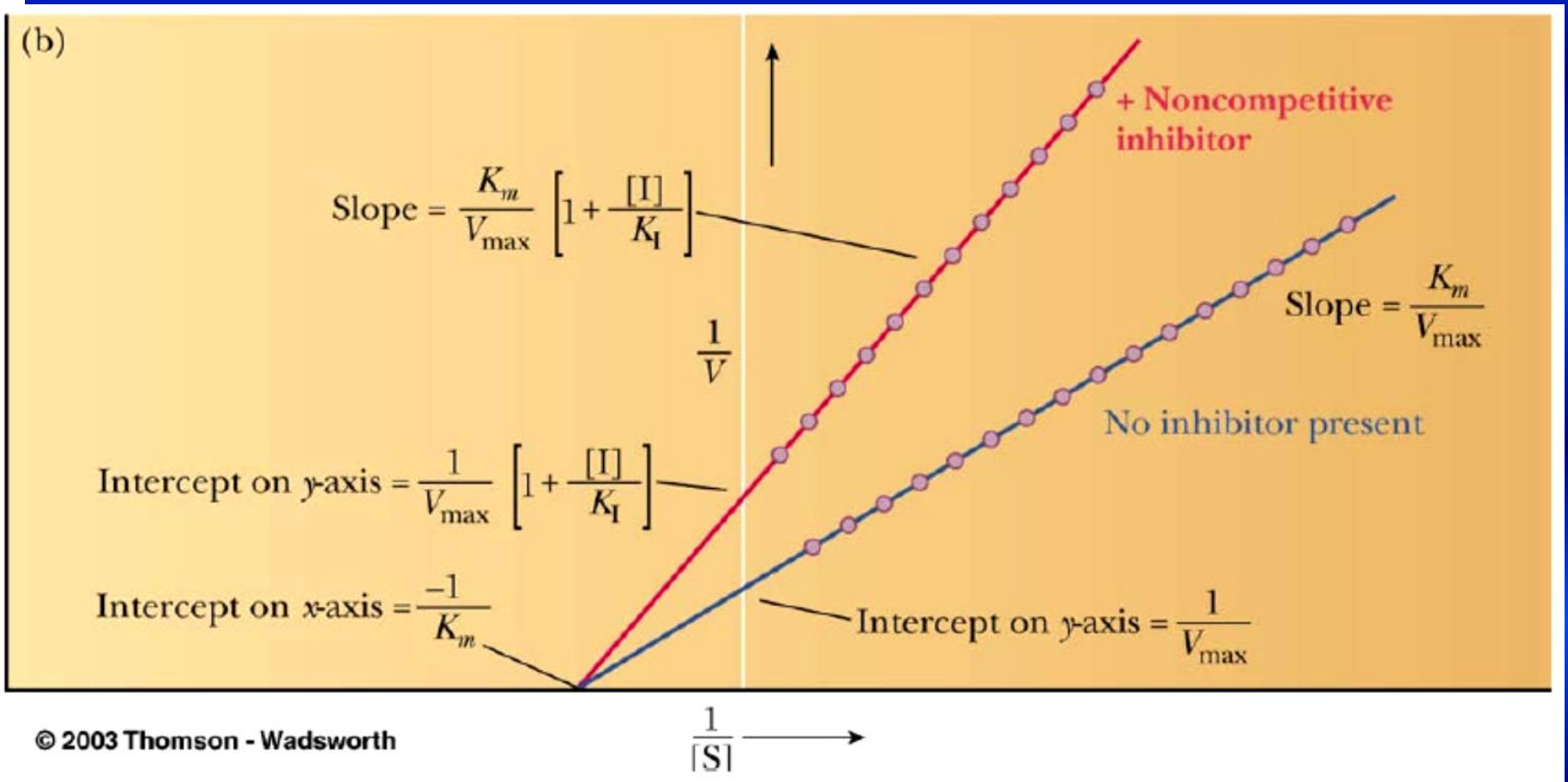
Mixed Inhibition



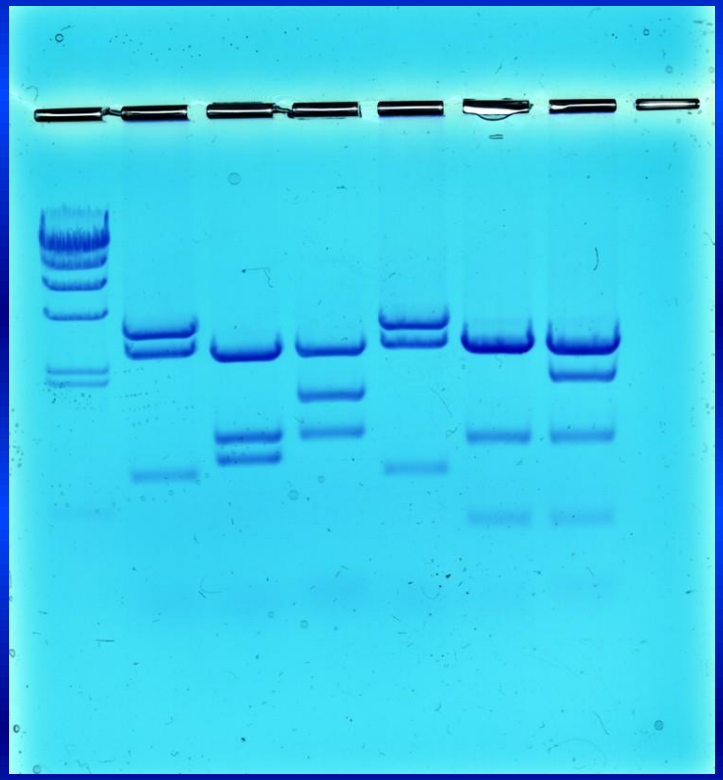
- The result will be decrease in Vmax and either an increase or decrease in Km
- The effect of an non-competitive inhibitor can only be partially overcome by high concentrations of the substrate



Non-Competitive



Gel Electrophoresis



What is Gel Electrophoresis?

Gel electrophoresis separates molecules on the basis **of their charge and size**. The charged macromolecules migrate across a span of gel because they are placed in an electrical field. The gel acts as a sieve to to retard the passage of molecules according to their size and shape.

The negatively charged particles move toward the positive electrode while the the positive charge particles move toward the negative electrode.

How does electrophoresis work?

- The gel is made from agar/polyacrilamide
- negative molecules is
  - DNA
  - RNA
  - Protein/Enzim
- Molecules sort based on
  - Charge
  - Size
  - shape

CHROMATOGRAPHY

Chromatography basically involves the separation of mixtures due to differences in the distribution coefficient (equilibrium distribution) of sample components between 2 different phases.

One of these phases is a mobile phase and the other is a stationary phase.

Distribution Coefficient (Equilibrium Distribution

Definition:

$$\frac{\text{Concentration of component A in stationary phase}}{\text{Concentration of component A in mobile phase}}$$

Different affinity of these 2 components to stationary phase causes the separation.

Kinds of Chromatography

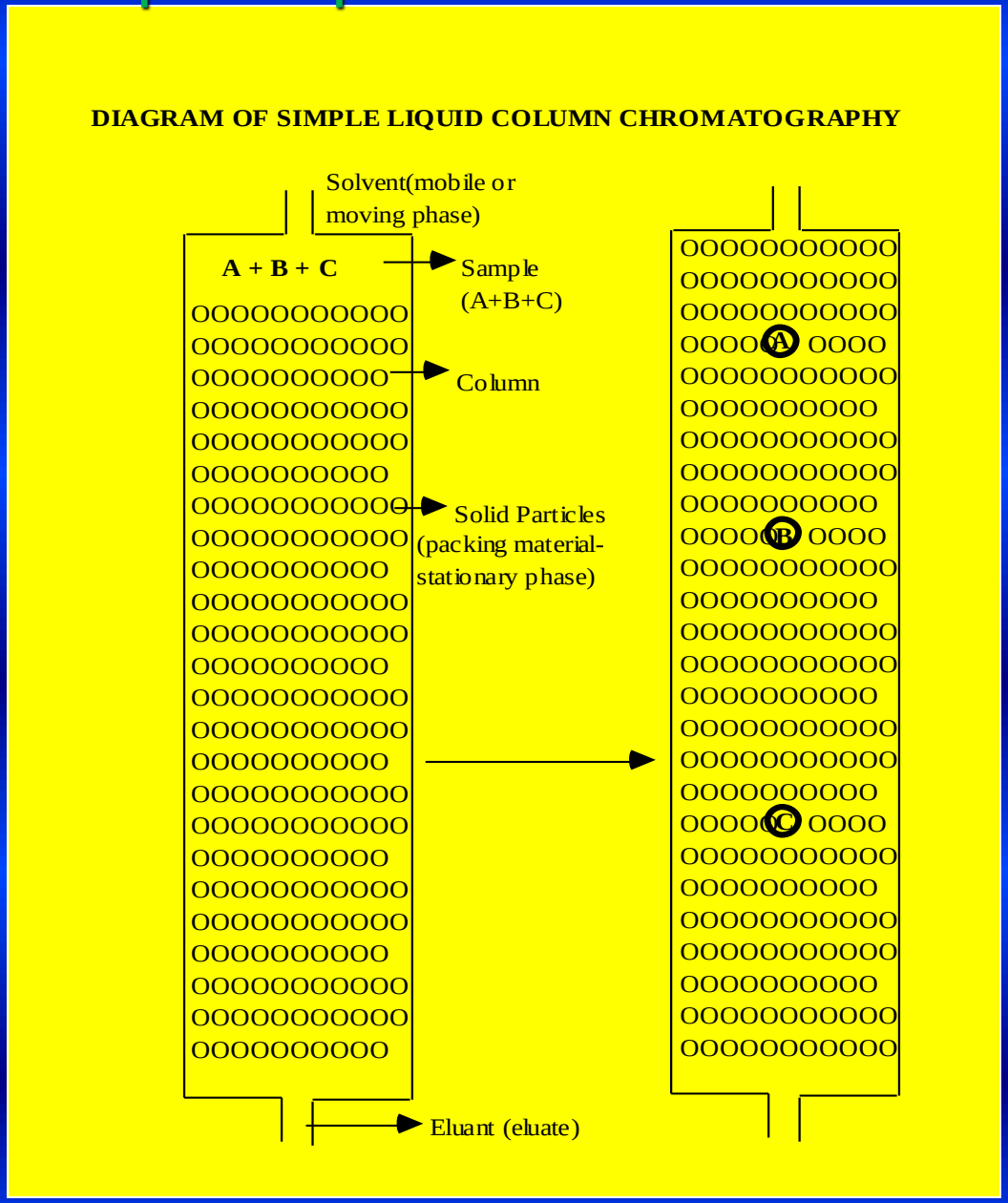
1. Liquid Column Chromatography
2. Gas Liquid Chromatography
3. Thin-layer Chromatography

LIQUID COLUMN CHROMATOGRAPHY

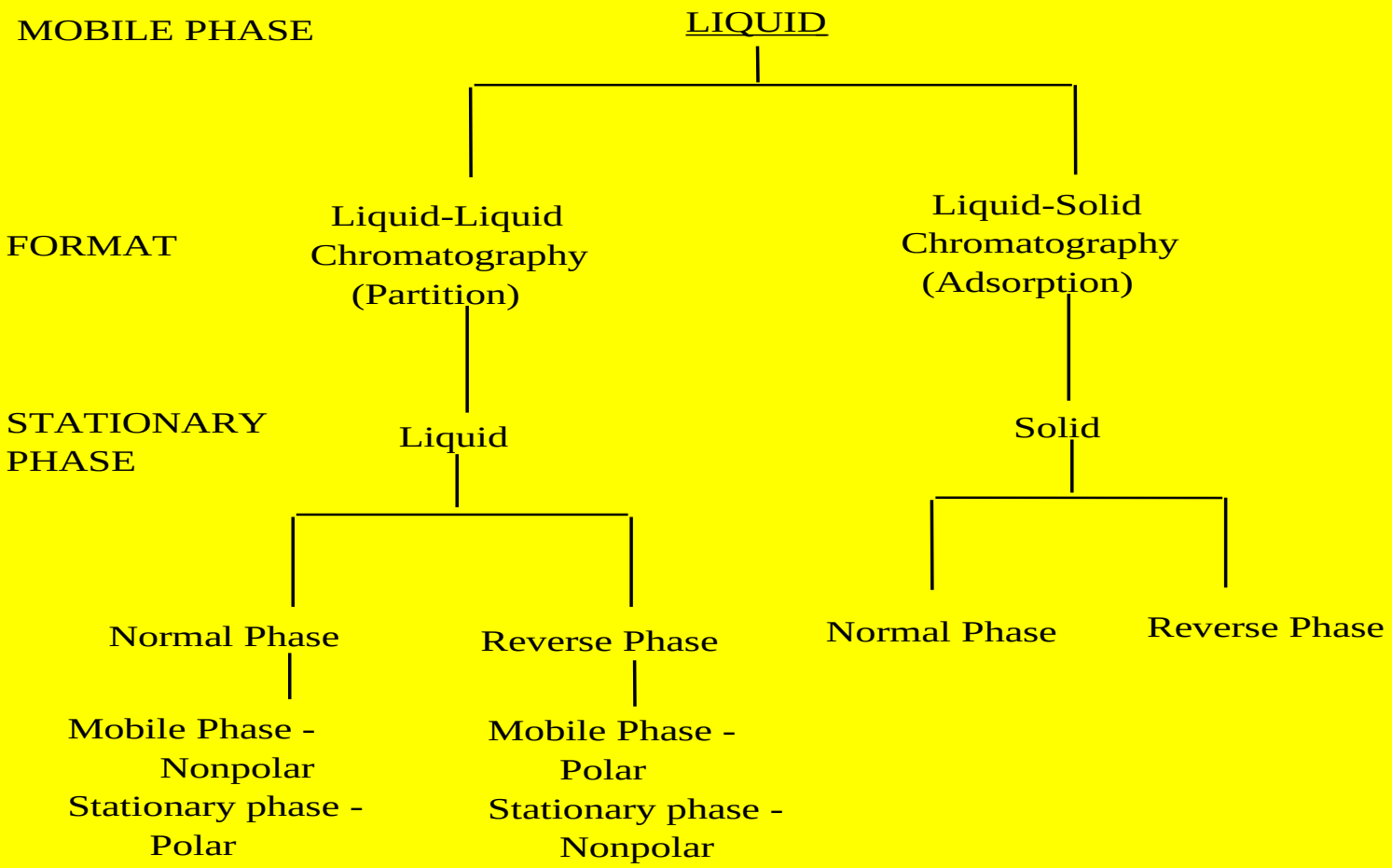
A sample mixture is passed through a column packed with solid particles which may or may not be coated with another liquid.

With the proper solvents, packing conditions, some components in the sample will travel the column more slowly than others resulting in the desired separation.

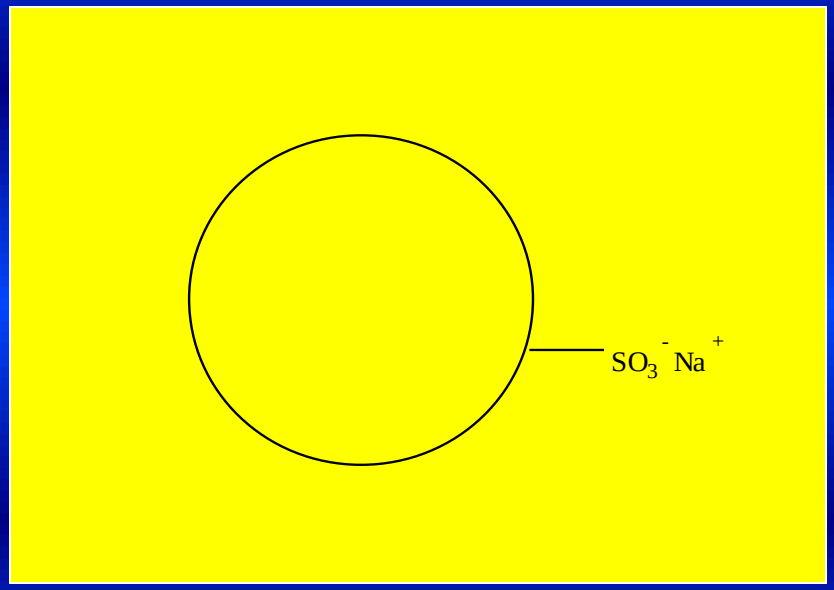
Diagram of Simple Liquid Column Chromatography



Types of Chromatography

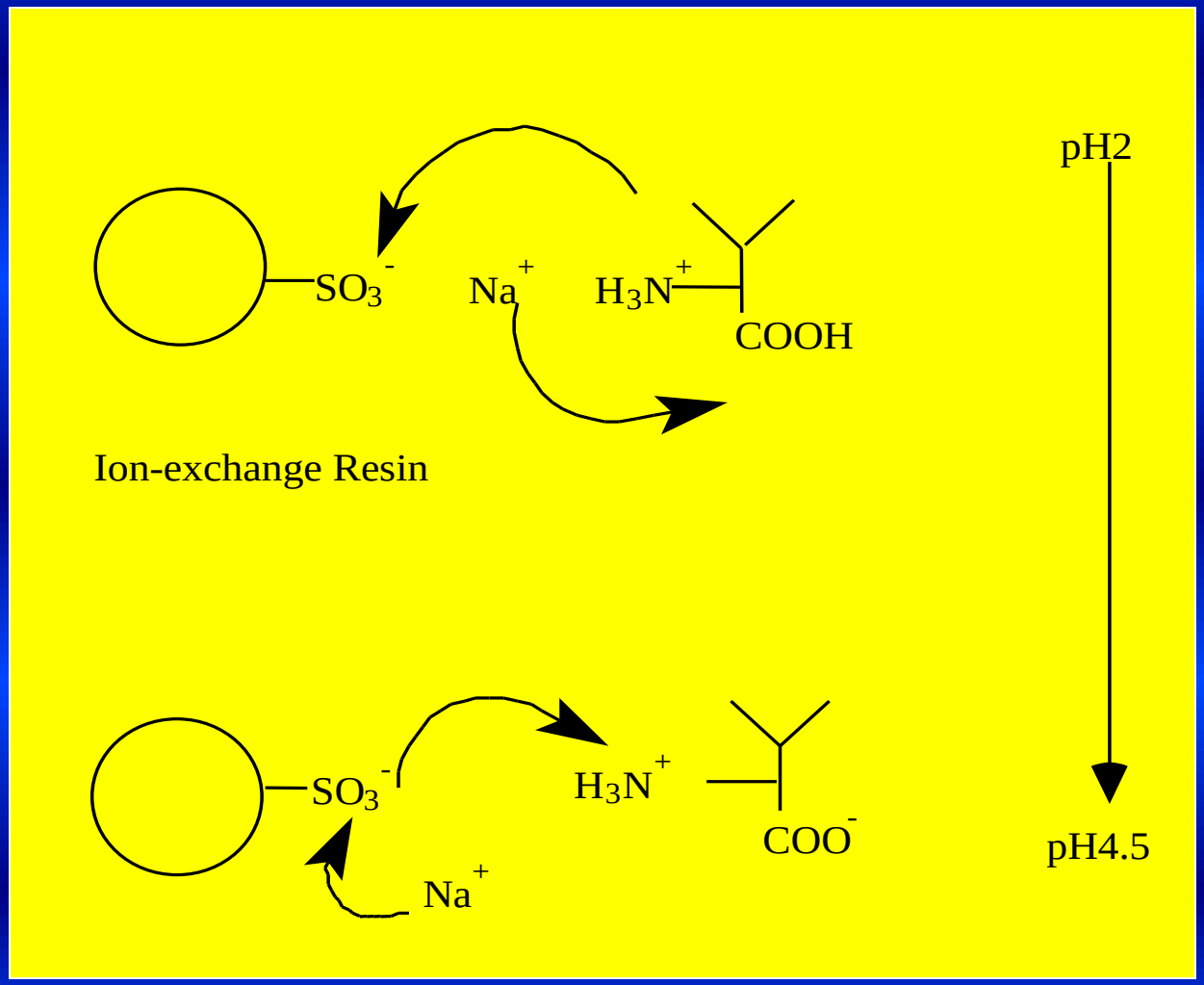


ION-EXCHANGE CHROMATOGRAPHY

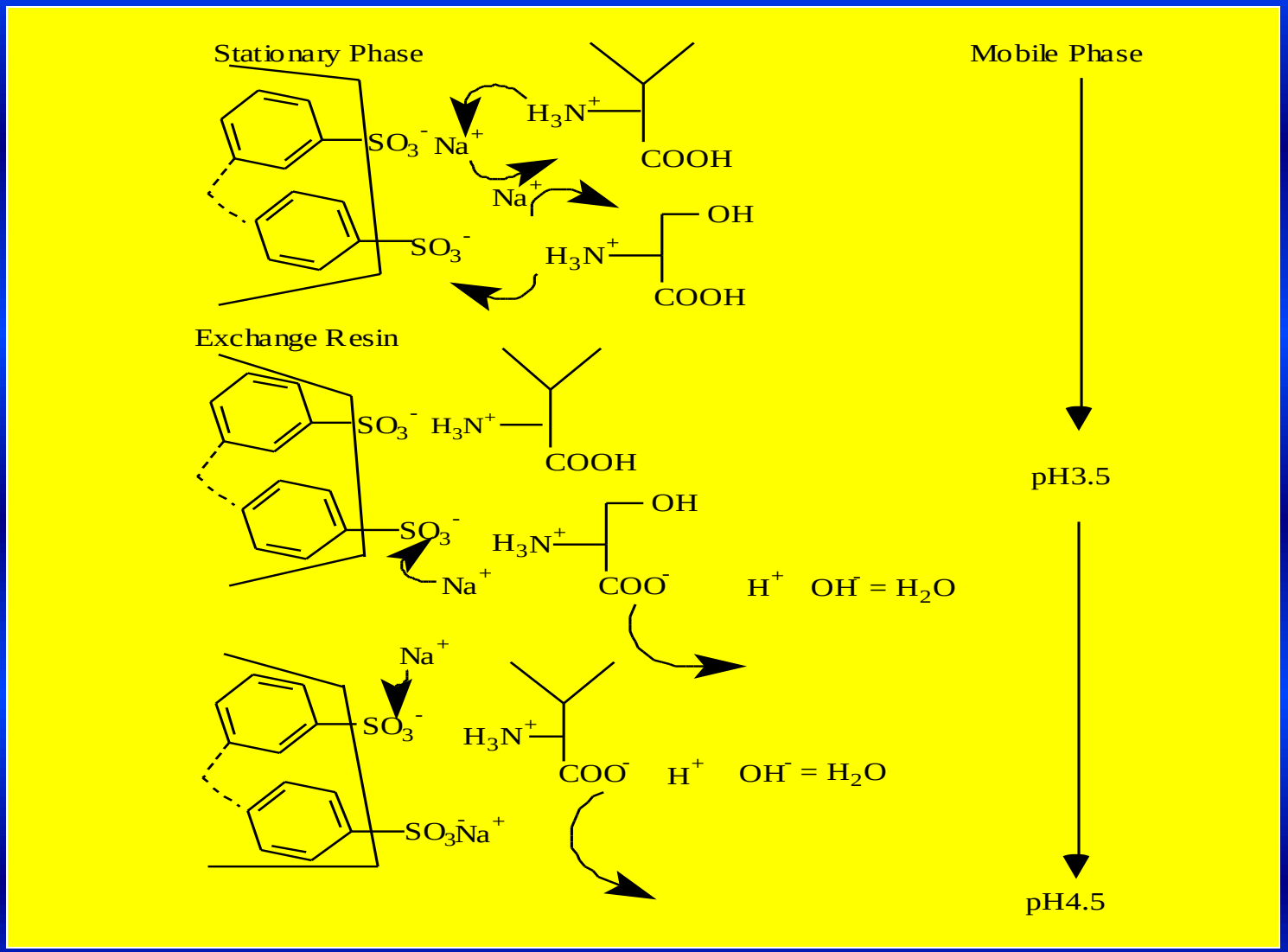


Separation in Ion-exchange Chromatography is based on the competition of different ionic compounds of the sample for the active sites on the ion-exchange resin (column-packing).

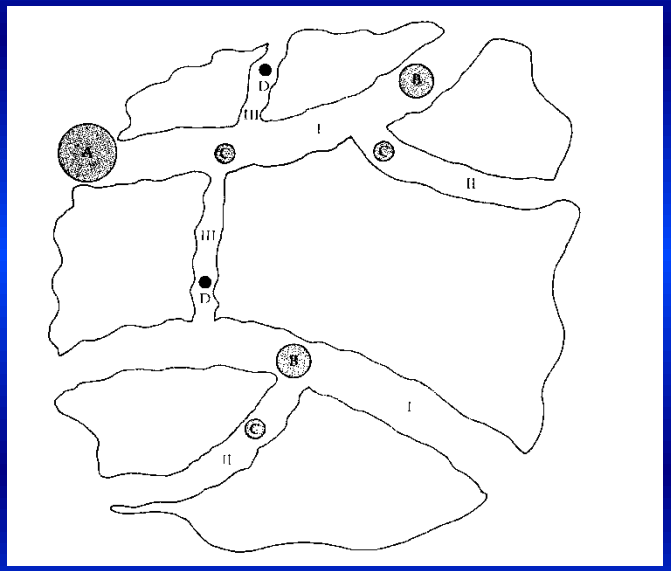
MECHANISM OF ION-EXCHANGE CHROMATOGRAPHY OF AMINO ACIDS



Chromatography of Amino Acids



GEL-PERMEATION CHROMATOGRAPHY



Gel-Permeation Chromatography is a mechanical sorting of molecules based on the size of the molecules in solution.
Small molecules are able to permeate more pores and are, therefore, retained longer than large molecules.

SOLVENTS

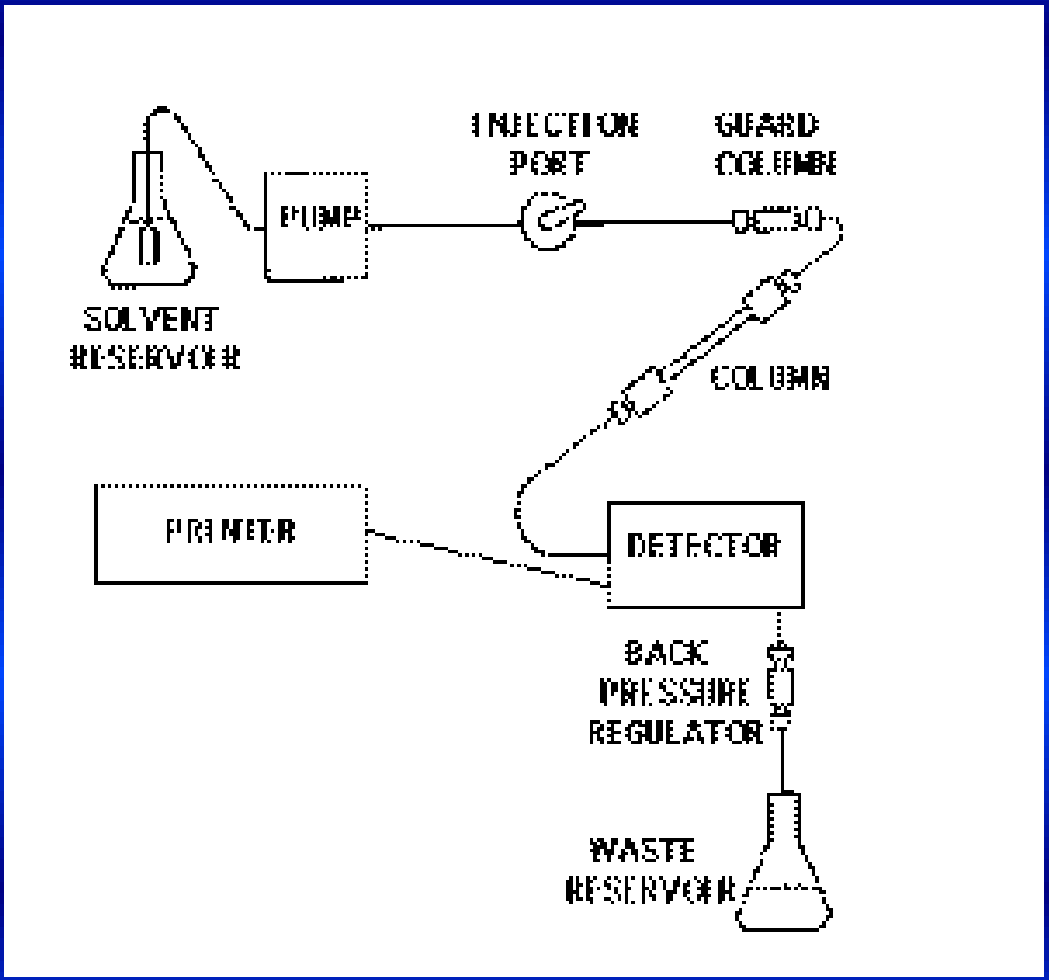
Polar Solvents

Water > Methanol > Acetonitrile > Ethanol > Oxydipropionitrile

Non-polar Solvents

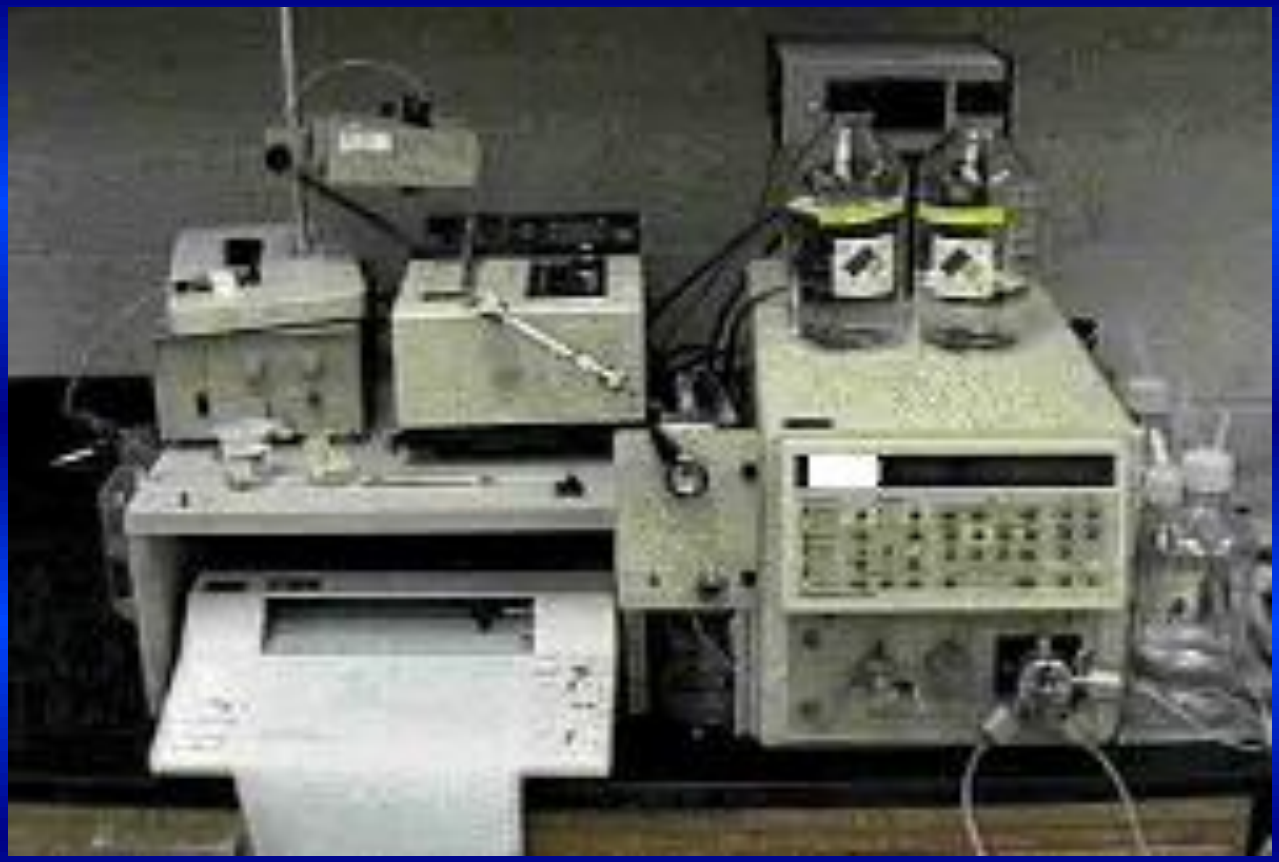
N-Decane > N-Hexane > N-Pentane > Cyclohexane

Schematic Diagram of Liquid Chromatography

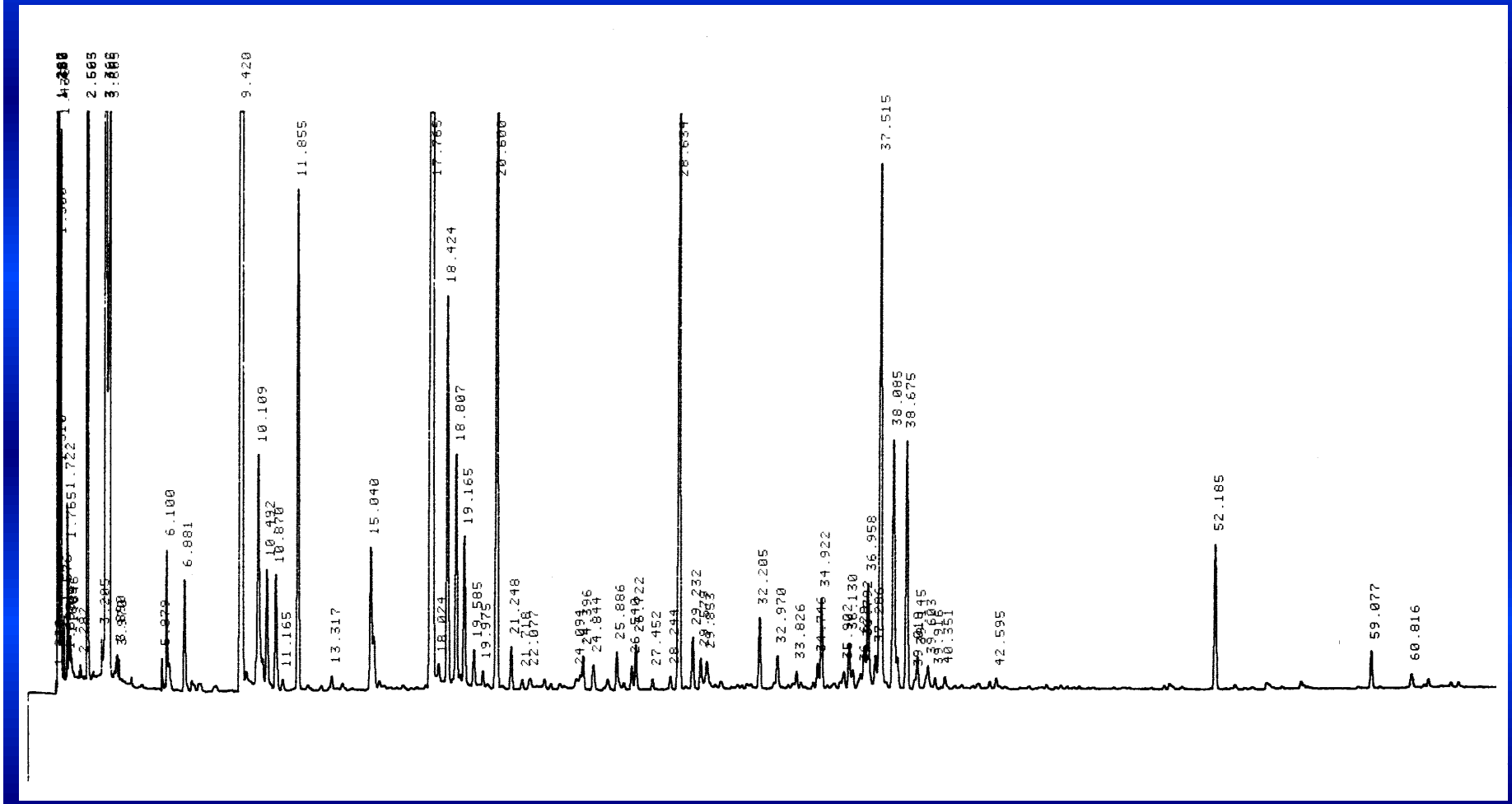


High Performance Liquid Chromatography

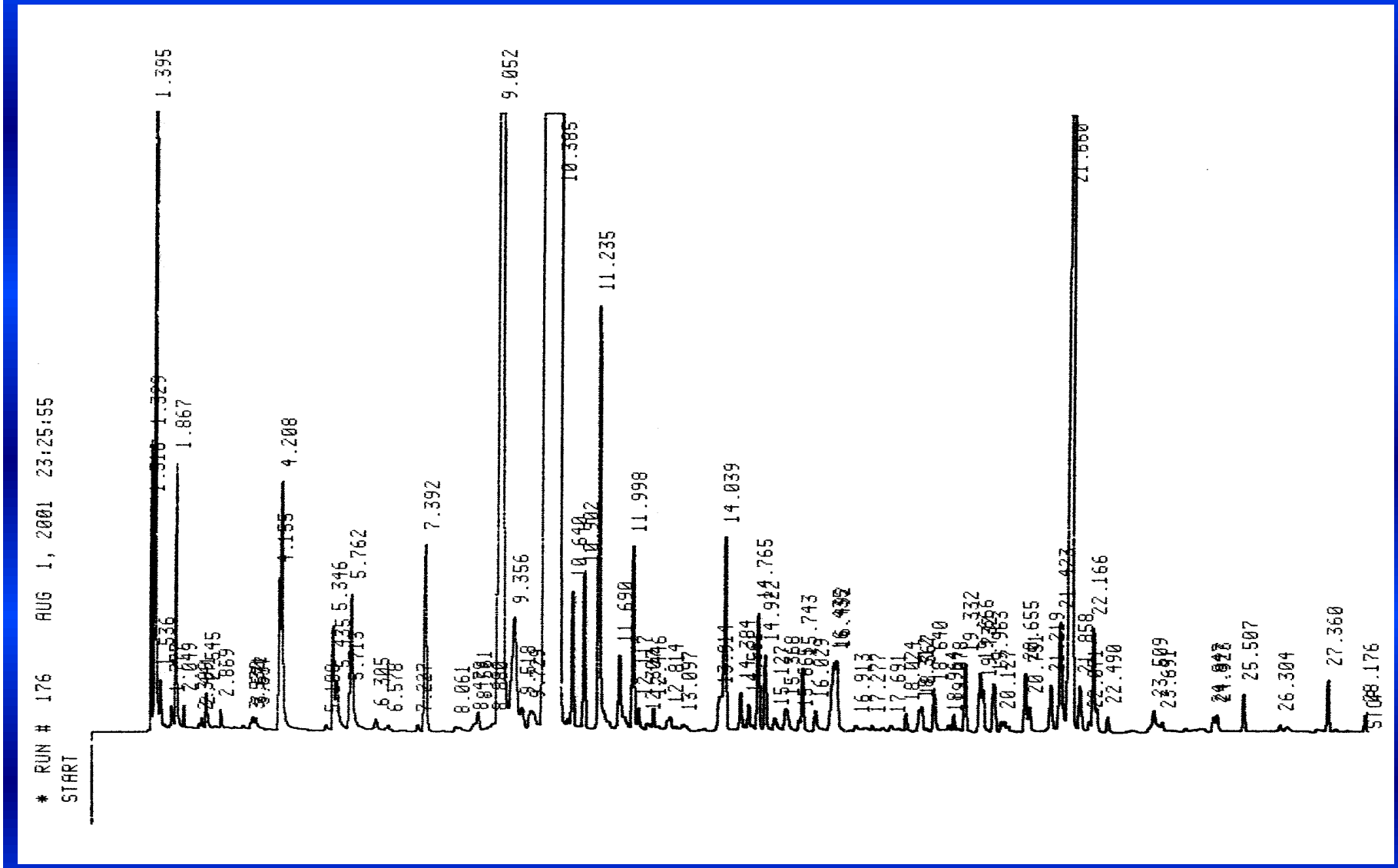
High Performance Liquid Chromatography



Chromatogram of Organic Compounds from Fermented Cabbage

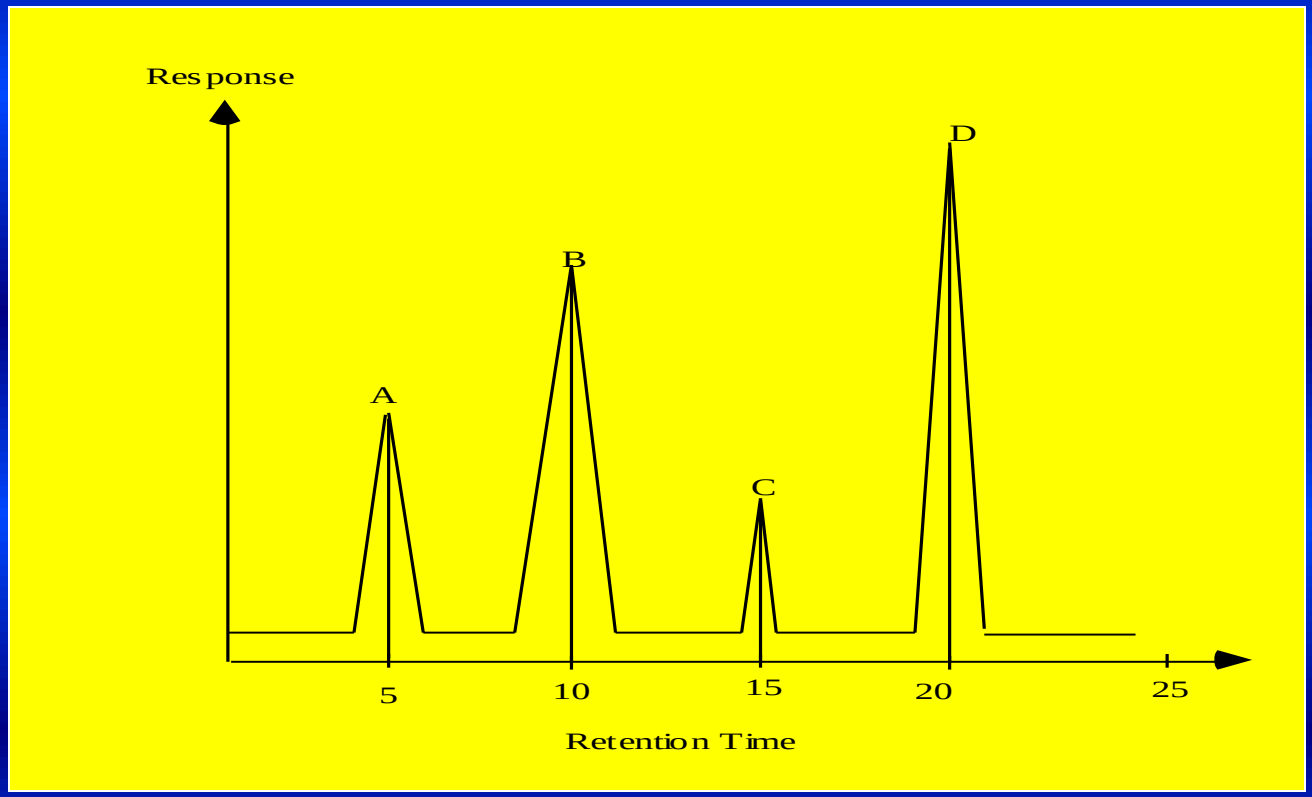


Chromatogram of Orange Juice Compounds



Retention Time

Time required for the sample to travel from the injection port through the column to the detector.



THANK YOU !

Tugas :

1. Isolasi dan karakterisasi enzim ()
2. Kromatografi enzim ()
3. Kloning DNA bakteri ()
4. Enzim restriksi ()

- Tugas : kumpulkan via email : sumardi_bio@yahoo.co.id
- Terakhir tanggal : 15 Nopember 2017
- Presentasi (kira2 : 10 slide), Membuat PPt
- Membuat makalah, ada teori dasar, dari 1 jurnal bhs inggris (ada latar belakang, metode, hasil, diskusi)
- makalah 5-7 halaman spasi 2, boleh ada gambar dan tabel.

Ujian lesan senin