Elution Chromatography

Kinetic Analysis
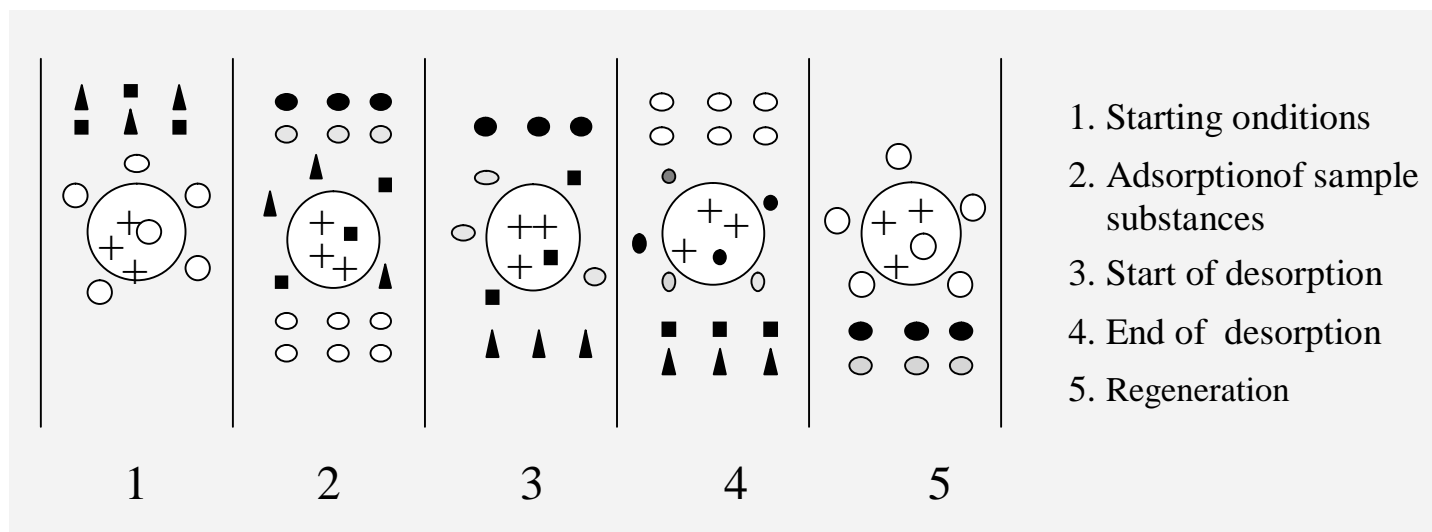### Scaling up Chromatography

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Bio process lab.

Kinetic Analysis

• Introduction

- The column contain equilibrium stages
- The concentration profile is the result of diffusion and chemical reaction
- The actual diffusion and chemical reaction between solute and packing(5 step)

1. Starting onditions
2. Adsorptionof sample substances
3. Start of desorption
4. End of desorption
5. Regeneration

– To see the effects of diffusion and reaction, a solute pulse flowing into a packed column
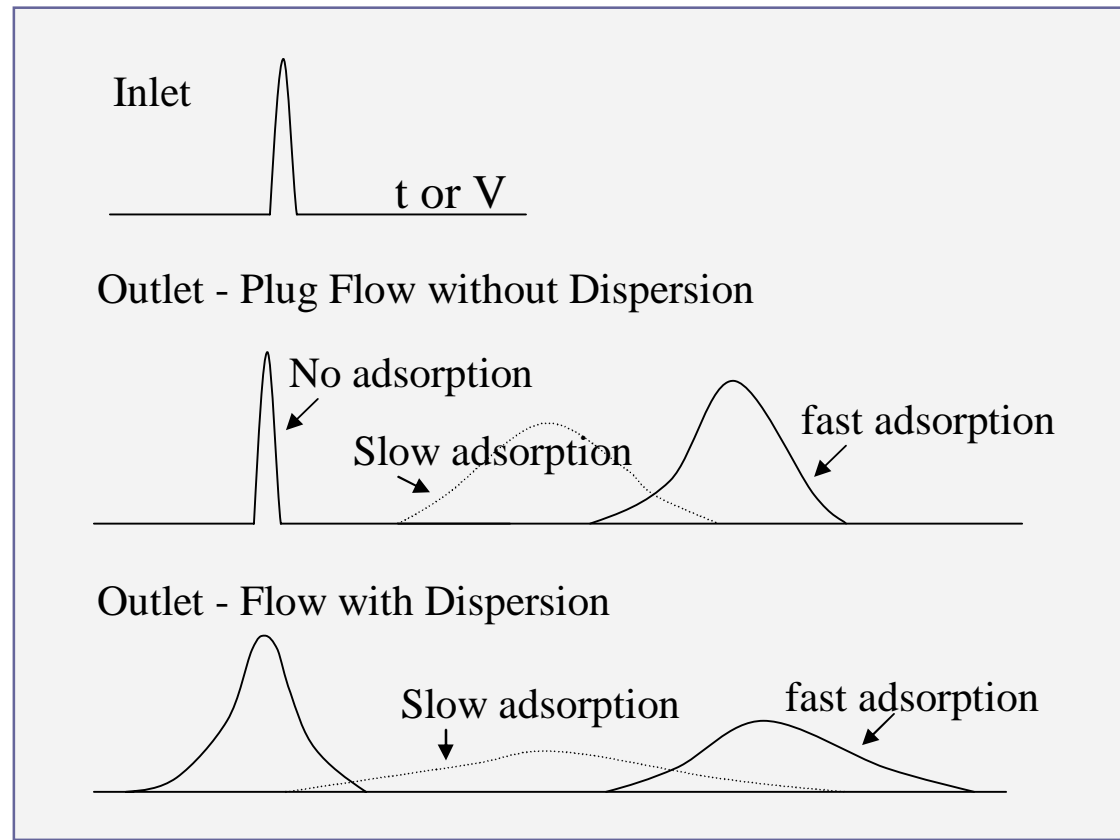

Figure 1. Modeling elution chromatography with rate processes

- **A quantitative approximation**

Mass balance equation

$$\varepsilon \frac{\partial y}{\partial t} + (1 - \varepsilon) \frac{\partial q}{\partial t} = E \frac{\partial^2 y}{\partial z^2} - \upsilon \frac{\partial y}{\partial z}$$

y, q = concentration of the solutes in
      mobile and stationary phase
z = column length
t = time
υ = linear velicity of the mobile
      phase
ε = void fraction
E = apparent axial dispersion
      coefficient

- In many cases

$$(1 - \varepsilon) \frac{\partial q}{\partial t} = -\upsilon \frac{\partial y}{\partial z}$$

- initial condition

$$t = 0, \quad z = 0, \quad y = \left(\frac{M}{A} \right) \delta(z)$$
$$\text{all } z, \quad q = 0$$

- to solve these equation

- if mass transfer is controlling

$$(1 - \varepsilon) \frac{\partial q}{\partial t} = ka(y - y*)$$

K = mass transfer coefficient
a = packing area per bed volume
y* = concentration in solution at equilibrium

- if diffusion within pores is rate controlling

$$(1 - \varepsilon)\frac{\partial q}{\partial t} = \sqrt{\frac{D}{t'}}\, a(y - y*)$$

D = effective diffuwion coefficient
t' = some characteristic time

- if reversible chemical reaction is rate controlling

$$(1 - \varepsilon)\frac{\partial q}{\partial t} = ky = k'q$$

k, k' = forward and reverse rate constants of this reaction

$$l = \int_0^\lambda dz = \left[\frac{\upsilon}{ka}\right]\int_{y0}^y \frac{dy}{y - y*}$$

Number of transfer units(**NTU**)

Height of a transfer unit(**HTU**)

$$\therefore \quad l = HTU \bullet NTU$$

$$y = y_0 \exp\left(-\left(\frac{t/t_0 - 1}{\sqrt{2\sigma^2}}\right)^2\right)$$

$$= y_0 \exp\left(-\left(\frac{t/t_0 - 1}{\sqrt{2/NTU}}\right)^2\right)$$

$$NTU = \frac{1}{\sigma^2} = \frac{l}{HTU} = \frac{kal}{\upsilon}$$

The concentration at the peak

$$\int_{-\infty}^{\infty} yHdt = M$$

The result

$$y_0 = \frac{M / Ht_0}{\sqrt{2\pi(NTU)}} = \frac{M}{[\varepsilon + (1-\varepsilon)K]V_B}\sqrt{\frac{kal}{v}} = \frac{M / A}{[\varepsilon + (1-\varepsilon)K]}\sqrt{\frac{ka}{lv}}$$

,

$$y = \left[\frac{M / A}{[\varepsilon + (1-\varepsilon)K]}\sqrt{\frac{ka}{lv}}\right]\exp\left(-\frac{kal}{2v}\left(\frac{t}{t_o}-1\right)^2\right)$$

$$y = \left[\frac{M / A}{[\varepsilon + (1-\varepsilon)K]}\sqrt{\frac{ka}{lv}}\right]\exp\left(-\frac{kal}{2v}\left(\frac{V}{V_o}-1\right)^2\right)$$

- ## **Example**

  ‣ **Aspartame Isomer Separation**

	Peak Time t_0 (min)	Peak Spread $t_0\sigma$ (min)
d aspartame	62	3
l aspartame	71	6

  column length : 25cm
  diameter : 0.41cm
  particle diameter : 45×10^{-4} cm
  particle volume fraction : 0.62

  Problem : Find the apparent rate constant k for this separation and compare these rate constants with those expected form the mass transfer correlation

  $$\frac{k}{\upsilon} = 1.17 \left(\frac{d\,\upsilon}{\nu} \right)^{-0.42} \left(\frac{\nu}{D} \right)^{-0.67}$$

  d : packing diameter
  υ : solvent velocity
  ν : kinematic viscosity
  D : diffusion coefficient
  $\quad 0.7 \times 10^{-5}$ cm^2/sec

Solution

$$\frac{y}{y_0} = \exp\left(-\left(\frac{t/t_0 - 1}{\sqrt{2/NTU}}\right)^2\right)$$

$$= \exp\left(-\frac{(t - t_0)^2}{2t_0^2/NTU}\right)$$

For the d aspartame

$$\frac{t_0}{\sqrt{NTU}} = \text{Peak spread}$$

$$\frac{62\ \min}{\sqrt{NTU}} = 3\ \min, \quad NTU = 427$$

for the l aspartame, $NTU = 140$

$$k = NTU\left(\frac{\upsilon}{al}\right)$$

The velocity under the conditions given

$$\upsilon = \frac{H}{((\pi/4)d^2)}$$

$$= \frac{2.0\,cm^3/60\ \sec}{[\pi/4(0.41\,cm)^2]}$$

$$= 0.25\ cm/\sec$$

Therefore

$$a = 6(1\text{-}\varepsilon)/d$$

for the d aspartame

$$k = \frac{427\ (0.25\,cm/\sec)}{[6(0.62)/45\times10^{-4}\,cm\,]25\,cm}$$

$$= 5.2\times10^{-3}\,cm/\sec$$

for the l aspartame,

$$k = 1.7\times10^{-3}\ cm/sec$$

The values for the mass transfer coefficient

$$k = 1.17 \left(0.25 \frac{cm}{\sec} \right) \left[\frac{0.01 \; cm^2 / \sec}{45 \times 10^{-4} \; (0.25 \; cm / \sec)} \right]^{0.42}$$

$$\times \left[\frac{0.7 \times 10^{-5} \; cm^2 / \sec}{0.01 \; cm^2 / \sec} \right]^{0.67} = 5.6 \times 10^{-3} \; cm / \sec$$

▶ The Effects of Axial Dispersion

Problem : For slow (laminar) flow in a long thin tube, the concentration
profile of a pulse injected at t = 0 and z = 0 is given by

$$y = \frac{M / (\pi / 4) d^2}{\sqrt{4 E t \pi}} \exp \left(- \frac{(z - t \upsilon)^2}{4 E t} \right)$$

E : dispersion coefficient, $E = \upsilon^2 d^2 / 192 D$
D : solute's diffusion coefficient
υ : velocity,   t : time
z : tube length, $z = l = \upsilon t_0$
d : diameter

find NTU

Solution

rewriting

$$z = l = \upsilon t_0$$

$$y = \frac{M/(\pi/4)d^2}{\sqrt{4Et\pi}} \exp\left(-\frac{t\upsilon^2}{4Et}\left(\frac{t}{t_0}-1\right)^2\right)$$

Using the expression for E

$$y = 4.98\frac{M}{d^3}\sqrt{\frac{D}{l\upsilon}} \exp\left(-\frac{48Dl}{d^2\upsilon}\left(\frac{t}{t_0}-1\right)^2\right)$$

$$NTU = \frac{96\,Dl}{d^2\upsilon}$$

Scaling up Chromatography

- ## Introduction

    - At the larger scale, we want a bigger capacity but with the same yield and purity

    - To increase the capacity, we are able to increase the solute concentration in the feed and the flow through the column

    $$y = y_0 \exp\left(-\left(\frac{V}{V_0} - 1\right)^2 \Big/ 2\sigma^2\right)$$

    - parameter : y_0, V/V_0, σ

• Changes in the standard deviation

$$\sigma^2 = \frac{\upsilon}{kal}$$

- it is a function of the velocity, column
  length, rate constant
- d : sphere's diameter

rate constant k :

- solute   packing

- if the controlling step by diffusion
  and fast reaction within the particles
$$k \propto \frac{1}{d} \implies \sigma^2 \propto \frac{d^2\upsilon}{l}$$

- if the controlling step by mass transfer
  between the bulk and the particle
$$k \propto \left(\frac{\upsilon}{d}\right)^{1/2} \implies \sigma \propto \frac{\upsilon^{1/2}d^{3/2}}{l}$$

• it may also change because of dispersion

$$\sigma = \sqrt{\frac{2E}{l\upsilon}} \propto d\sqrt{\frac{\upsilon}{lD}}$$

Table 7.5-1 Changes in the Standard Deviation

Controlling step	The quantity σ^2 is Proportional to	Remarks
Internal diffusion and reaction	$\dfrac{d^2 v}{l}$	Probably the most common case
External mass transfer	$\dfrac{v^{1/2} d^{3/2}}{l}$	Supported by the most complete analysis
External(Taylor) dispersion	$\dfrac{d^2 v}{Dl}$	Likely to become more important at large scale
Axial diffusion	$\dfrac{D}{lv}$	Rarely important
Column of actual equilibrium stages	$\dfrac{1}{l}$	Assumes the number of stages N is proportional to the length

• Scale up the separation

‣ Keep the ratio of packing diameter to column diameter

  – preserve the character of the flow in the packed bed
  – use larger, cheaper packing

‣ Fix d and increase both υ and l at constant (υ/l)

  – at this case, the pressure drop can increase dramatically
  – because of small constant d, the pressure drop is already high

‣ use short fat columns

  – υ and l used in the small scale separation
  – increased capacity is due to their greater cross sectional area

- ## **Example**

  ### ▸ **Fumarase Chromatography**

  10g of the enzyme fumarase are being purified in an ion exchange column. At a velocity of 30cm/hr, the peak in concentration exits the column in 93min and the standard deviation of this peak is given as 12min

  (a) how long must we purify for a 90% yield

  $$\frac{y}{y_0} = \exp\left(-\left(\frac{t/t_0 - 1}{\sqrt{2\sigma^2}}\right)^2\right) = \exp\left(-\frac{(t - 93\,\text{min})^2}{2(12\,\text{min})^2}\right)$$

  Thus $\sigma = 0.129$,

  $$yield = \frac{1}{2} + erf\,\frac{t - t_B}{\sqrt{2}\,t_B\,\sigma} \qquad 0.9 = 0.5 + erf\,\frac{t - 93}{\sqrt{2}\,93(0.129)}$$

  $$t = 115 \text{ min}$$

  We must wait 115/93(-1.24) times longer then the peak time to get a 90% yield

(b) If we in crease the flow to 60cm/hr, how long must we run for this same yield if the process is controlled by diffusion and reaction?

$t_0 = 46.5 \text{min}$

from Table 7.5-1

$$\sigma = 0.129 \left[\frac{60}{30} \right]^{1/2} = 0.182$$

$$0.9 = 0.5 + erf \frac{t - 46.5}{\sqrt{2}\,46.5(0.182)}$$

$t = 61.8 \text{ min}$

We must wait 61.8/46.5(=1.33) times the peak time to get a 90% yield

(c) How long must we wait if the process is controlled by mass transfer

If the process is controlled by mass transfer,

$t_0 = 46.5 \text{mim}$

$\sigma \; \alpha \; \upsilon^{1/4}$

$$\sigma = 0.129 \left[\frac{60}{30} \right]^{1/4} = 0.153$$

$$0.9 = 0.5 + erf \frac{t - 46.5}{\sqrt{2}\,46.5(0.153)}$$

$t = 59.4 \text{ min}$

We must wait only 1.28 times the peak time for a 90% yield

(d) How long must we wait if Taylor dispersion controls?

If Taylor dispersion is rate controlling

$$\sigma \alpha \, \upsilon^{1/2}$$

The results are exactly the same as part(b)

(e) How long must we wait if the column actually contains equilibrium stages

Thus $\sigma = 0.129$

$$\frac{t}{46.5 \, \text{min}} = \frac{115 \, \text{min}}{93 \, \text{min}}$$

Thus t is 57.5min, 1.24 times the time for the peak

▶ **Transferrin Desalting**

A dilute feed in which 80% of the total solute is transferrin and 20 % behaves like sodium chloride is to be desalted on a dextran gel column. Operation the column at 10cm/hr gives the following results:

	Peak Time t_0 (min)	$t_0\sigma$ (min)
Void voumne	27	-
Transfferrin	41	4
Salts	88	4

Problem : What is the maximum velocity and the time which will give a 99%
yield of thransferrin which is 98% pure

Solution :

$$\sigma = \frac{t_0\sigma}{t_0} = a\,v^{1/2}$$

$$\sigma(transferri\,n) = \frac{4\,min}{41\,min} = a\left(\frac{10cm}{hr}\right)^{1/2}$$

$$a = 0.0309\,\sqrt{hr\,/\,cm}$$

$$\sigma(salt) = \frac{4\,min}{88\,min} = a\left(\frac{10cm}{hr}\right)^{1/2}$$

$$a = 0.0144\,\sqrt{hr\,/\,cm}$$

salt   yield   ,

$$purity\ of\ i = \frac{y_0(i)\,yield(i)}{\Sigma_j\,y_0(i)\,yield(i)}$$

$$0.02 = \frac{(yield\ \ salt\,)(0.2)}{0.99(0.8)}$$

$$yield\ \ salt = 0.08(8\%)$$

$$0.99 = \frac{1}{2}\left[1 + erf\,\frac{vt\,/((10cm/\,hr)41min) - 1}{\sqrt{2}\,0.0309\sqrt{hr\,/\,cm}\sqrt{v}}\right]$$

$$0.08 = \frac{1}{2}\left[1 + erf\,\frac{vt\,/((10cm/\,hr)88min) - 1}{\sqrt{2}\cdot 0.0144\sqrt{hr\,/\,cm}\sqrt{v}}\right]$$

$$v = 93cm/\,hr$$

$$t = 7.4\,min$$