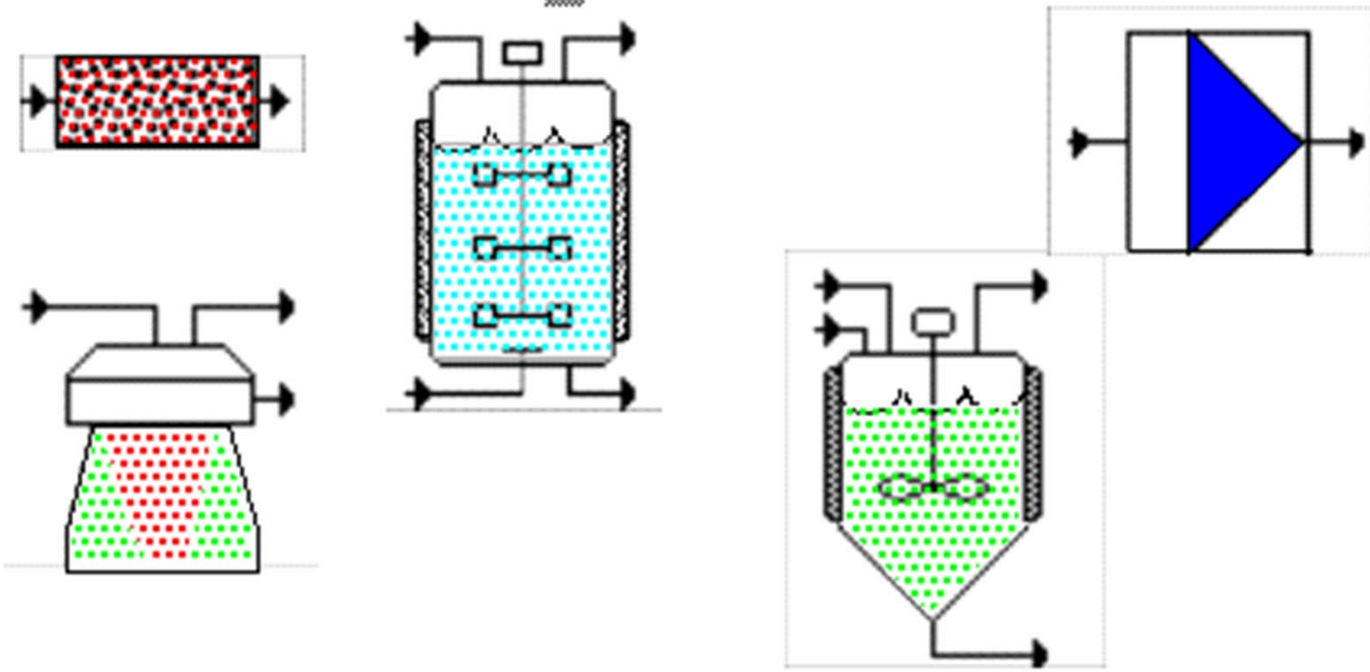
PRECIPITATION



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- Removal of insolubles(Filtration, Centrifugation)
- Isolation of products(Extraction, Supercritical Fluid Extraction, Adsorption)
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- Polishing(Crystallization, Drying)

1. Basic Concepts

- PRECIPITATION

- 생물학적 물질를 정제하는데 가장 간편하고 직접적인 방법
- 난용성 화합물이나 염을 형성시키는 물질을 첨가한다든지, 유기용매를 적당량 첨가함으로써 침전시켜 분리/정제
- 단백질 또는 항생제의 회수에 사용

에탄올 용액으로부터 항생제를 침전시키기 위해 용매(nonsolvent)를 첨가한다든지 단백질을 침전시키기 위해 ammonium sulfate 첨가

2. Precipitation with a Nonsolvent

- 저온에서 (-5) 용매를 첨가하면 용액의 용해도가 감소
- 사용 용매가 물과 섞이는 경우에만 효과적
- 평형상태에서 침전물의 chemical potential

$$\mu_i(\text{solids}) = \mu_i(\text{solutions})$$

$$= \mu_i^0(\text{solutions}) + RT\ln y_i$$

y_i : concentration of solute i in the solution

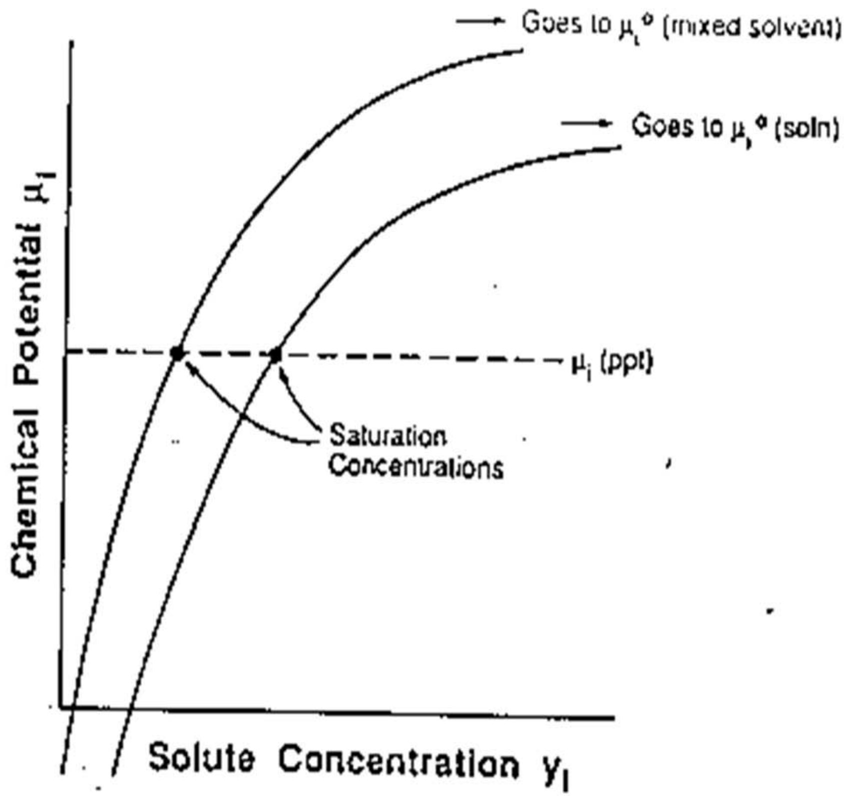


Figure 8.1-1. Precipitation in a mixed solvent. Adding a second solvent will often increase the solute's chemical potential and hence reduce its solubility. Such nonsolvents often have an effect which is highly dependent on pH.

- Nonsolvent

1) 항생제(Moderate MW)

The feed is commonly a solution in a solvent like ethanol or t-butanol, and the nonsolvent is water

2) 단백질(High MW)

The feed is usually a aqueous solution at the isoelectric pH.

The nonsolvent is a water miscible organic solvent like ethanol and acetone.

- Heuristics

1. Precipitation at low temperature increases yield and reduces denaturation.
2. Precipitation is best at ionic strengths 0.05-0.2 M . Higher ionic strengths require excess solvent and more dilute solutions yield a finely divided precipitate which is difficult to filter
3. High molecular weight solutes require less solvent to initiate precipitation.

$$\left(\begin{array}{c}\text{Volume \%}\\ \text{required for}\\ \text{precipitation}\end{array}\right) = 1.8 - 0.12\ln\left(\begin{array}{c}\text{solute}\\ \text{molecular}\\ \text{weight}\end{array}\right)$$

4. The solubility of one protein is usually decreased by the presence of other proteins.
5. Precipitated solute which will not redissolve is probably denatured. It produces further purifications.

3. Precipitation with Salts

-염석(Salting out) : 단백질의 분리와 분별에 널리 사용.

염은 황산 암모늄 과 황산 나트륨이 일반적으로 사용

- 분리 효율성이 높고 경제적이며, 단백질의 변성이 거의 일어나지 않음

ex 1) 염석에 의한 침전

Solubility in Water $K = [K^+][NO_3^-]$

K : solubility product

Chemical Potentials

$\mu_i(\text{solids}) = \mu_i(\text{solutions})$

$= \mu_i^0(\text{solutions}) + RT\ln[i] + z_i \mathcal{F} \Phi$

[i] : concentration of species i

z_i : ionic charge Φ : electrostatic potential

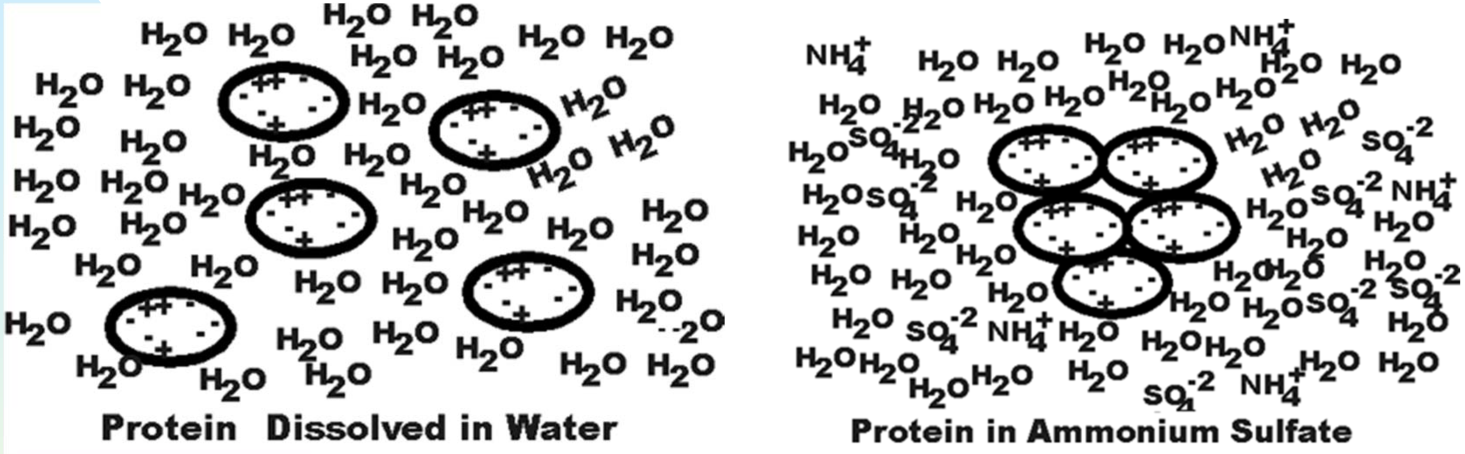
$$\ln K = \frac{\mu_{K+}(\text{solids}) - \mu_{K+}^{0}(\text{solution}) + \mu_{NO3-}(\text{solids}) - \mu_{NO3-}^{0}(\text{solution})}{RT}$$

solution of potassium nitrate which is slightly below saturation

↓ add sodium nitrate

potassium nitrate preciptation

ex 2): salting out protein



- 염을 첨가하면 용액의 염 농도는 증가하며 단백질의 용해도는 현저하 게 감소하여 침전
- K(solubility product) = [protein]$[NH_4^+]^n[SO_4^{2-}]^m$
- 침전 단백질로부터 $(NH_4)_2SO_4$ 분리가 어려움

C. Choosing Salts

- Anions are effective in following order:

  citrate › PO_4^{3-} › SO_4^{2-} › $CH3COO^-$ › Cl^- › NO_3^-

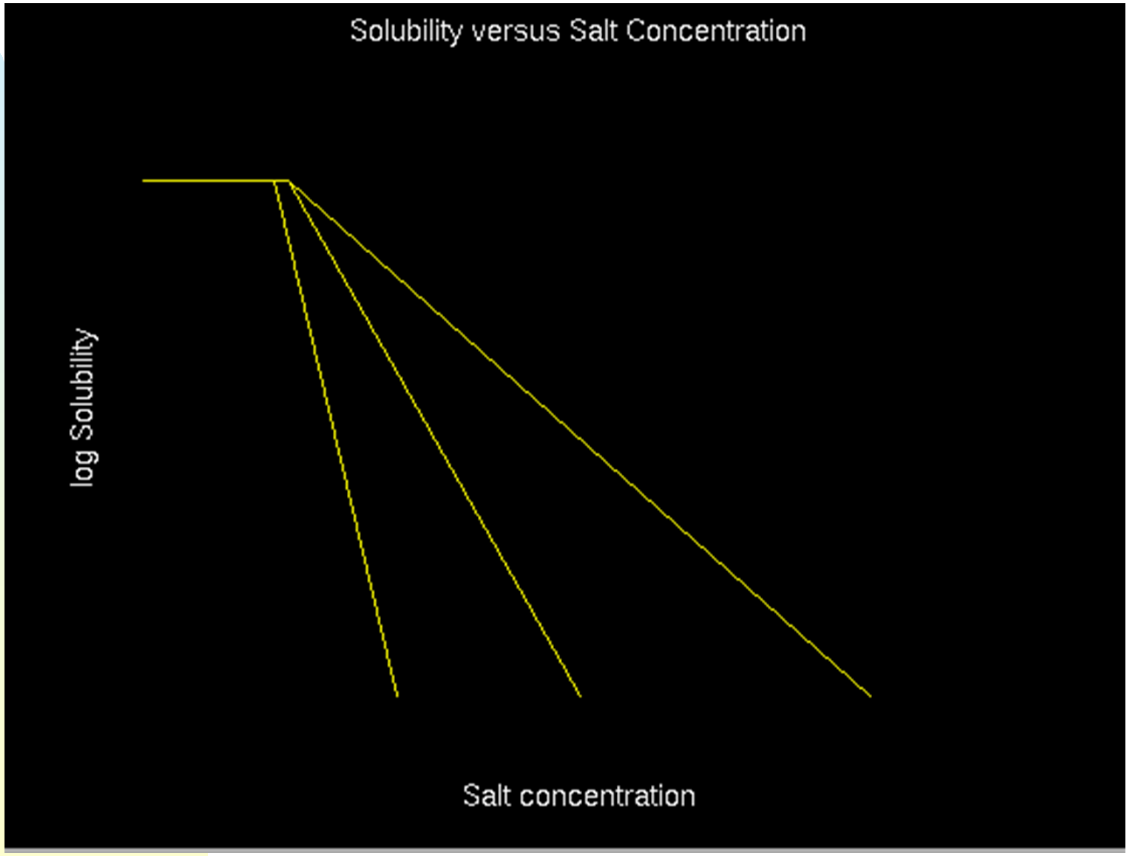
- Cations are effective in following order :

  $NH4^+$ › K^+ › Na^+

- Choose a salt that is cheap, for you will use a lot of it.
- Choose a salt so that the density of the precipitate is different than the density of solution. Doing so is necessary for separation by centrifugation
- Add a solid salt rather then a salt solution to minimize dilution.
- Interpolate between experiments using an equation like

  ln[protein solubility] = A - m[salt concentration]

A: constant(function of protein, pH and Temp.)
m : constant(function pH, mixing and salt)



4. Precipitation with Temperature

- Temp. increase $\longrightarrow$ selectively denaturation $\longrightarrow$ precipitation

  * Desired properties (enzymatic activity) can be destroyed

- Assumption : Denaturation follows first order chemical kinetics with an Arrhenius temperature dependence

$$\frac{d[P]}{dt} = -k[P]$$

[P] : dissolved protein concentration

k : rate constant

$$k = k_0 e^{-E/RT}$$

k_0 : characteristic constant

E : activation energy of the denaturatuion ex 3) Purification of erythrocyte enzymes with chloroform

- This solvent does not alter these enzymes, but it denatures and precipitates hemoglobin, which is the most concentrated protein in solution.

- To purify erythrocyte enzymes, we need only to shake an aqueous solution of these solutes with chloroform and then centrifuge to remove the precipitated hemoglobin.

5. Large Scale Precipitations

- Large Scale (Process Scale-up) : 톤이나 킬로그램까지 제조 하는 process를 의미한다기 보다는, 기존의 실험 방식보다 10배 정도 이상의 process 의미

- Idealization

1) Initial mixing : 용질을 포함한 원료를 nonsolvent 나 염에 혼합
2) Nucleation : 핵심이 생성되고 침전이 시작
3) Diffusion limited growth : 확산에 의해 침전물 성장
4) Flow influenced growth : mixing하여 성장을 촉진
5) Flocculation : 콜로이드 입자는 더 큰 flocs 으로 집합
6) Centrifugation : flocs 분리

- Initial Mixing

혼합이 아무리 격렬하다 하더라도, 순식간에 균일한 (homogeneous)용액을 만들지는 못한다.
균질성을 이루는데 필요한 시간 은

$$t = l^2/(4D)$$

l : 혼합으로 야기된 turbulent eddies의 평균 크기이며
D : 용질의 확산계수

$$l = \left(\frac{\rho\,\nu}{P/V}\right)^{1/4}$$

ρ : solution 's density
ν : kinematic viscosity
(P/V) : power per volume of the stirring

- Nucleation

핵심생성은 작은 입자가 나타나고 성장하기 시작하는 과정

Inorganic system : 핵심생성은 느릴 수 있으며 오랫동안 과포화가 상태가 지속 될 수 있음 (비,눈)

Colloidal system : 순간적임

- Diffusion Limited Growth

$$\frac{dy_i}{dt} = -ky_i^2$$

y_i : concentration of solute particles

k : rate constant(l/mol sec)

$$k = 8\pi DdN$$

D : diffusion coefficient

d : solute diameter

N : Avogadro's number

- Diffusion Limited Growth

$$\frac{1}{y} = \frac{1}{y_{i0}} + kt$$

y_{i0} : initial solute concentration

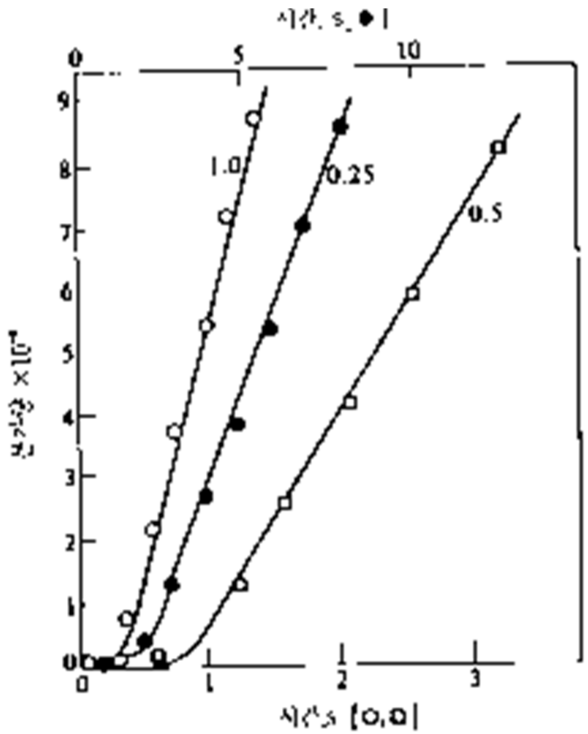
$$y_i \overline{M} = y_{i0} \overline{M}_0$$

$\overline{M}_0$: initial solute molecular weight

$$\overline{M} = \overline{M}_0 (1 + y_{i0} kt)$$

$\overline{M}$: average molecular weight

* k 결정



0.008M $CaCl_2$의 존재하에서 형성된 3가지 농도의 a-카세인 침전

- Flow Influenced growth

Growth of large particle : 교반에 의한 입자들의 충돌에 의해 성장

$$\frac{dy_i}{dt} = -ky_i^2$$

$$k = \frac{2}{3} aNd^3 \left(\frac{P/V}{\rho\ v}\right)^{1/2}$$

a : sticking coefficient

다음과 같이 입자의 일정한 부피 분율을 가정

$$\o = \left(1/6\Pi d^3\right) y_i \overline{M}$$

$$\frac{y_i}{y_{i0}} = \exp\left[- \left(4/\Pi a\o \left(\frac{P/V}{\rho\ v}\right)^{1/2}\right) t\right]$$

- Flocculation

- 침전물을 회수하기 위한 원심분리 전 플럭이 형성되고 안정화시키는 단계
- 숙성단계(aging)

6. Conclusions

- 침전은 항생제나 단백질을 농축하고 정제시키는 간단한 방법

– 항생제는 second solvent 첨가, 단백질은 염(ammonium sulfate)
을 첨가,

– Large scale의 침전을 설계하기 위하여 process에 대한 속도 제한
단계를 결정하기 위한 small scale의 실험이 필요