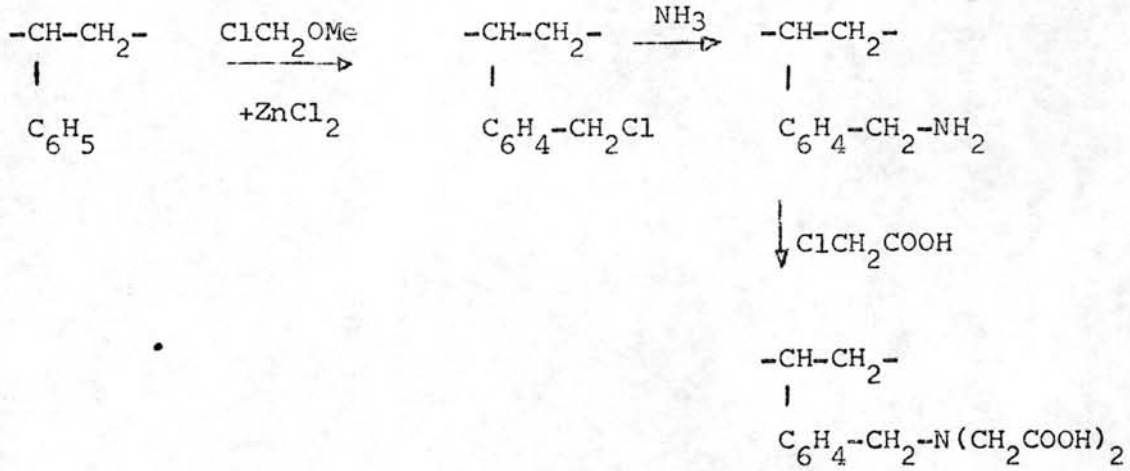
CHAPTER II

THEORY

2.1 Ion Exchange Chromatography (2)

In chromatography, two or more constituents of a sample are separated from each other more or less completely by virtue of differences in their distribution ratios between a stationary phase and a mobile phase in initimate contact with each other. Ion-exchange chromatography makes use of an ion exchange as the stationary phase; the solutes to be separated are taken up by the solid to different extents by virtue of ion exchange reactions.

Chelex-100 from Bio Rad Laboratories is a purified form of Dowex A-1 resin. In chelex-100, the group $-CH_2N-(CH_2COOH)_2$ is attached to the crosslinked polystyrene. It can be produced through a series of reactions (3) as follows:



The resin is stable under both acid and alkali conditions but changes its particle size very markedly as its counter-ion is changed. This swelling and contraction caused difficulty in maintaining a reasonable flow rate through columns. This effect is particularly obvious if larger particle size chelex-100 is used. Columns are packed freshly before use. The flow rate is not allowed to exceed 5 cm^3/min, since the exchange rate of the resin is rather slow.

Chelex-100 will not, however, remove metals held in organic and inorganic colloids which can be present even after ultra-filtration. Precautions must be taken to destroy such colloids prior to collection of the ions by the resin.

2.1.1 Equilibrium Distribution

The exchange of ions between a solid ion exchanging material and a solution is a typical reversible reaction. Suppose a solution containing the cations B^+ is shaken with a solid exchanger AR which contains the cation A^+, ions B^+ enter the exchanger, while ions A^+ take their place in the solution.

After a time, which may be range from a few minutes to several days depend primarily on the solid exchanger, no further change will be observed, and an equilibrium will have been established:

$$AR + B^+ \rightleftharpoons BR + A^+$$

If the ions B are doubly charged, the equilibrium will be represented by

$$2AR + B^{2+} \rightleftharpoons BR_2 + 2A^+$$

2.1.2 Selectivity Coefficient

To represent the final distribution of concentrations we write a selectivity coefficient, which for exchanges between ions of equal charge has the form:

$$E_A^B = \frac{(A^+)\ (\overline{B}^+)}{(B^+)\ (\overline{A}^+)} \qquad (2.1)$$

The symbols (A^+) and (B^+) indicate molar or molal concentration of ions in the solution. $\overline{A}^+$ or AR and $\overline{B}^+$ or BR indicate molar or molal concentration of ions bound on the resin.

For exchanges between singly and doubly charged ions, the selectivity coefficient becomes:

$$E_A^B = \frac{(A^+)^2\ (\overline{B}^+)}{(B^{2+})\ (\overline{A}^+)^2} \qquad (2.2)$$

here the concentration units do not cancel and it is essential to specify the units used.

To evaluate the ion exchange equilibrium properly, one must recognize that neither the solution nor the exchanger is ideal in the thermodynamic sense. The partial molal free energies are not linear functions of the logarithms of concentration, and this is specially true in the exchanger phase,

where the ions are much closer together than they are in the external solution. We can take this nonideality by introducing activity coefficients, as follows,

$$K_A^B = \frac{m_A \bar{m}_B}{m_B \bar{m}_A} \cdot \frac{r_A \bar{r}_B}{r_B \bar{r}_A} \qquad (2.3)$$

where m and r are mean molality and activity coefficient. K_A^B is the thermodynamic exchange constant. For a given pair of exchange ions, temperature and solvent, it is a true constant.

The selectivity coefficients of chelex-100 for some cations are given in Table 2.1.

Table 2.1 Selectivity Coefficient of Chelex-100 (4).

Cation	Log E	Cation	Log E
Hg	4.18	Zn	1.29
UO_2	2.69	Co	1.18
Cu	2.65	Mn	0.69
V	2.30	Ca	0.00
Pb	2.15	Ba	-0.17
Ni	1.67	Sr	-0.21
Cd	1.41	Mg	-0.32

2.1.3 Partition Ratio

From equation (2.1), the equilibrium constant for the system can be written as

$$K = \frac{a_A a_{BR}}{a_B a_{AR}} \qquad (2.4)$$

where a_A and a_B represent activities of the two ions in the aqueous solution, and a_{AR} and a_{BR} represent their activities on the solid-resin phase. The latter terms can be replaced by the products of the activity coefficient and the mole-fraction. That is

$$K = \frac{a_A X_{BR} f_{BR}}{a_B X_{AR} f_{AR}} \qquad (2.5)$$

where X_{BR} represents the mole fraction of the resin that is in the form of BR and fBR is an activity coefficient X_{AR} and f_{AR} are analogously defined. By rearranging equation 2.5, one obtains

$$\frac{a_A X_{BR}}{a_B X_{AR}} = K \cdot \frac{f_{AR}}{f_{BR}} = Kp \qquad (2.6)$$

where Kp is called the apparent, or practical equilibrium quotient.

It is important to note that the activity ratio f_{AR}/f_{BR} can not be directly measured by independent means, and the value of K is thus unknown. On the other hand, Kp can be determined experimentally by measuring the concentration

of ions on a resin and their activities in the solution in equilibrium with the resin. Such experiments show that Kp is not entirely independent of changes in ionic concentration on the resin or the activities of ions in the solution, particularly when the ions bear different charges. It has been found, however, that if one of the ions is in large excess, both in eluting solution and on the resin, Kp is relatively constant and independent of changes in concentration of the other ion; that is, when $a_A \gg a_B$, $X_{AR} \gg X_{BR}$ and a_A is fixed, Equation (2.6) becomes

$$\frac{X_{BR}}{a_B} = \frac{X_{AR}\ Kp}{a_A} \cong K_D \qquad (2.7)$$

where K_D is a partition ratio. The values of Kd represent the affinity of a given resin for some ion B relative to some other ion A. Where K_D is large, there is a strong tendency for the solid phase to retain ion B, where K_D is small, the reverse is obtained.

2.1.4 Electroselectivity

In the exchange of ions of equal charge, the ratio between the concentration of A and B does not change with dilution, aside from small effects due to nonideality. If the ions are of unequal charge, two effects are noted. First, the ion of higher charge is usually more strongly held by the exchanger, and second, the distribution shifts with dilution.

The more the solution is diluted, the more strongly the ion of higher charge is held by the resin, and vice versa.

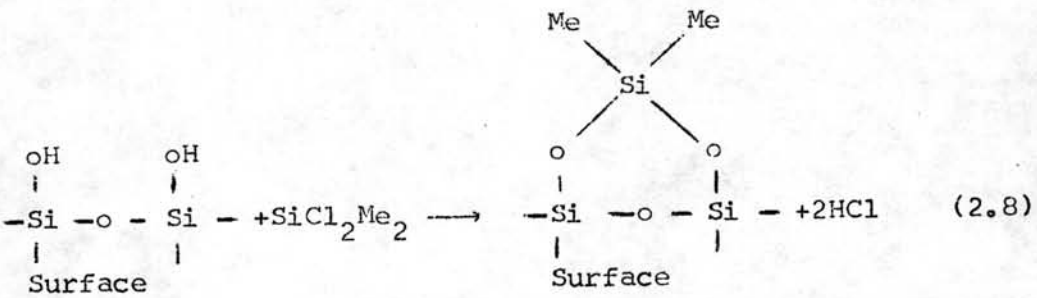
2.2 Reverse Phase Chromatography

The basic material from which solid supports are made is diatomite, which is also called diatomaceous earth, diatomaceous silica, or kieselguhr. Diatomite is composed of skeletons of single cell algae, or diatoms, which have been accumulated in large beds in different parts of the world. Each skeleton is made up of two flat, half cell walls connected by a band around their edges. Microscopic examination of the cell walls show that many regular holes or pores are present, having diameter of about 1μm. Owing to the large amount of fine structure, diatomite has an extremely high surface area.

Chromosorb W is prepared by partially fusing or calcining at 900°C a mixture of grey diatomite and a small amount of sodium carbonate used as a flux. This process converts a portion of the microamorphous silica to crystalline cristoballite, and the flux caused partial fusion of the particles to larger aggregates and, presumably, converts iron oxides to colorless sodium iron silicates.

Diatomite supports are not completely inert and some interaction exists with the solute molecules. The interaction may be observed as a peak tailing and in several cases the retention times of the solutes will be increased or,

as an extreme, they may be almost completely retained. It has been fairly well established that silanol (Si-OH) and siloxane (SiOSi) groups cover the surface of the diatomite supports which are essentially silaceous materials (5). The silanol groups, which act as proton donors, are more effective in forming hydrogen bonds than the siloxane groups, which act as an proton acceptors. The active sites of the support must be deactivated by special reaction. The support was allowed to react with dimethyl-dichloro-silane (DMCS) vapor, the product is chromosorb W-DMCS, then washed with methanol to remove the hydrochloric acid formed in the treatment. The use of DMCS to form a hydrophobic support was first reported by Howard and Martin (6). With two adjacent hydroxyl groups, the reaction probably proceeds as given in equation (2.8):



(2.8)

As with adsorption chromatography, the selection of the adsorbent will be influenced by the type of the sample that is to be separated. The quantity of material separated depends, other things being equal, on the amount of interchange of solute molecules between phases. It is desirable to select an adsorbent for which the volume of the adsorbed

phase is large. The separation efficiency increases with the decrease of grain size since the packing density increases. However, the pressure drop across the column (resistance to carrier flow) increases, there by increasing the retention time.

2.2.1 Ammonium Pyrrolidine Dithiocarbamate (7)

$CH_2(CH_2)_2 - CH_2NCSSNH_4$ Ammonium pyrrolidine-dithiocarbamate (APDC) forms water insoluble complexes with most metals except the alkalis and the alkaline earths. The complexes are adsorbed on the chromosorb W-DMCS, after which can be eluted with methyl isobutyl ketone [illegible] ethyl acetate, chloroform, etc. These complexes are stripped back with nitric acid before aspirated into the burner of the atomic absorption spectrophotometer. As this extractant is only slightly dissociated, the acidity of the aqueous phase changes only slightly after its addition, and the pH can be held constant by the addition of a small amount of buffer. This technique is primarily employed to concentrate a number of elements and to remove them from the undesirable matrix. For certain elements additional selectivity can be attained by the addition of a masking agent such as EDTA, tartrate, citrate or cyanide.

2.2.2 Practical Consideration

Long columns are impractical when it is necessary to extrude the adsorbent. With wide columns, it is more difficult

to obtain a uniform packing than with narrow columns. Apart from these considerations, it is desirable to use narrow columns so that the hold up of liquid per unit length of the column will be less and the sharpness of the breaks between the components, in terms of the volume of effluent, will be increased.

To avoid the formation of irregular shaped zones, the column should be packed uniformly. Usually, not much difficulty is experienced in packing with relatively coarse adsorbents, such as those with particles, ranging in size from 100 to 200 mesh (149 to 74 μm).

Wet methods are used to pack columns. The adsorbent and solvent are mixed to produce a slurry, which is poured into the tube and allowed to settle, and the solvent is permitted to drain off. The adsorbent must not be allowed to dry before use.

2.2.3 Rate of Flow

The rate of flow depends on the particle size of the adsorbent, the depth of packing, the viscosity of the liquid phase, and the pressure applied to the column. The rate of flow can influence the separation in two directions:

a. Since thermodynamic equilibrium is not established instantaneously, a high rate of flow increases the length of the column required to give one theoretical stage of separation.

b. The mixing caused by longitudinal diffusion will be more pronounced at very low flow rates, and this effect will tend to increase the length of column required to give one theoretical stage of separation. Actually, the effect of longitudinal diffusion does not seem to be very important at practical rates of flow, and one needs primarily to avoid operating at flow rates that are too high.

2.3 Atomic Absorption Spectrophotometer

2.3.1 Emission

Emission of light results from the spontaneous transition of an atom from a higher excited state (energy:E_1) to a state of lower energy (energy:E_2). The energy released by the atom, E_1-E_2 is wholly carried by the radiation or, more precisely, by a photon of frequency ν and energy $h\nu$. The principle of conservation of energy is expressed in this case.

$$\nu = \frac{E_1-E_2}{h} \qquad (2.9)$$

($h = 6.62 \times 10^{-34}$ in SI units or 6.62×10^{-27} in cgs units). Alternatively, the equation may be written in terms of wavelength

$$\lambda = \frac{C}{\nu} = \frac{hC}{E_1-E_2} \qquad (2.10)$$

where C is the velocity of light in vacuum. The wavelength of the radiation emitted is thus inversely proportional to the difference in energy levels involved.

Spectroscopists customarily use as an alternative unit, wavenumber ($\tilde{\nu}$), the inverse of wavelength.

$$\tilde{\nu} = \frac{1}{\lambda} = \frac{\nu}{C} = \frac{E_1 - E_2}{hC} \qquad (2.11)$$

The usual unit of wavenumber is the inverse centimeter (cm^{-1}) sometimes called Kayser.

2.3.2 Absorption

When a photon of frequency ν meets an atom in an energy state E_2, the atom may be able to absorb the photon and so raises its energy to $E_2+h\nu$. This, however, is only possible if this energy corresponds to one of the excited energy levels of that atom. If this condition is satisfied and E_1 is the excited state, we have

$$E_2 + h\nu = E_1$$

$$\nu = \frac{E_1 - E_2}{h} \qquad (2.12)$$

An atom can only absorb those radiations that it is able to emit.

2.3.3 Einstein Emission Coefficient

The probability of a spontaneous emission by transition between the levels E_1 and E_2 is defined as the fraction $d_{N_1 \longrightarrow 2}/N_1$ of the number of atoms in the upper level which drops spontaneously to the lower level E_2 in unit time.

If the number of atoms in the upper level at time t is N_1, the number $d_{N_1 \longrightarrow 2}$ passing the lower level in the

time interval (t, t+dt) can be expressed as

$$d_{N_{1\longrightarrow 2}} = AN_1 dt \qquad (2.13)$$

where A is a coefficient of proportionality called the spontaneous probability or Einstein Emission Coefficient.

The higher the probability of transition, the greater is the intensity of the emission line. The strongest lines correspond to the values of A of the order of 10^8 to 10^9 s^{-1}. (A must have the dimension of inverse time)

2.3.4 Einstein Absorption Coefficient

If N_2 atoms in the lower transition state are irradiated by a beam of frequency ν derived from equation (2.9) and of volume flux density $\rho(V)$ the number $d_{N_{2\longrightarrow 1}}$ of atoms that will adsorb the irradiating photons in time dt is proportional to N_2, $\rho(V)$ and dt, thus:

$$d_{N_{2\longrightarrow 1}} = \beta_{2,1} N_2 \rho(V) dt \qquad (2.14)$$

in which $\beta_{2,1}$ is a coefficient of proportionality known as the absorption coefficient or Einstein's Absorption Coefficient.

2.3.5 Selection Rule

The application of quantum mechanics makes it possible to calculate the Einstein coefficients A and B. The A and B coefficients for the electric dipole radiation are only a prior non-zero if the following conditions are met.

1. The levels involved must be of opposite parity.

2. The J number for the two levels may not differ by more than unity (while the restriction that a transition between two levels of J number zero is forbidden)

$$\Delta J = 0, \pm 1 \quad (0 \not\longrightarrow 0)$$

These are the general selection rules independent of the electron coupling.

2.3.6 Energy States of Neutral Atoms

Thermal equilibrium between the different energy levels of an atom in the flame is brought about by collisions with chemical species in the flame. The population ratio of two levels differing by ΔE is given by Boltzman's law:

$$\frac{N_2}{N_1} = \frac{g_2}{g_1} \exp(-\Delta E/KT) \qquad (2.15)$$

where

N_2 = no. of atoms per unit volume at the upper energy level.

N_1 = no. of atoms per unit volume at the lower energy level.

g_2 = 2J+1 where J is the total angular momentum number for the upper energy level (g is a statistical weight).

g_1 = 2J+1 for the lower energy level.

K = Boltzman's constant (1.38×10^{-16} erg/°K)

T = Absolute temperature (°K)

The excited level population is small in comparison with the ground state. Absorption is therefore greatest in lines resulting from transition from the ground state. These lines are called resonance lines in atomic absorption analysis.

2.4 Sensitivity, Detection Limit, Systematic Error, Precision and Accuracy

2.4.1 Sensitivity

Sensitivity is defined as the concentration of the element which produces a 1% absorption signal (.0044 absorbance) under optimum experimental conditions. This value is sometimes also referred to as the "perceptual sensitivity".

Sensitivity depends on the element measured, the chemical environment, and the instrumental parameters. After having fixed the operative conditions (chemical and physical) a run with synthetic solutions checks that the available sensitivity is adequate for the projected analysis.

2.4.2 Detection Limit

Detection limit is used to define the lowest detectable concentration of the element in the sample solution. This value is often also referred to as "fluctuation concentration limit". It may be defined as the concentration giving a signal equal to twice the background noise level.

$$D = 2S \times \frac{C}{X} \qquad (2.16)$$

D is the detection limit, C is the concentration of the solution, X is the mean and S is the standard deviation.

2.4.3 Systematic Error

The systematic error of an analytical method or, more precisely, of the result of an analysis is the difference between the true value and the mean of the measure is obtained by applying the method a large number of times. Errors of calibration and defects in the method of preparing samples are common sources of systematic error. Although every precaution may be taken in preparing calibration solutions, some unforseeable matrix effect may be present in a particular solution.

2.4.4 Precision

The precision of an analytical method is a measure of the closeness of agreement between successive experimental values obtained in a set of measurements of the same actual concentration. There are two components of precision : repeatability and reproducibility, both expressed in terms of standard deviation and coefficient of variance.

The standard deviation S is given by

$$S = \sqrt{\frac{\Sigma(x - x_o)^2}{n - 1}} \qquad (2.17)$$

where x_o = true (or mean) value.

x = individual measured values.

n = number of measurements.

The coefficient of variant V (Relative standard deviation RSD) is

$$\text{RSD} = V = \frac{S}{x_o} \times 100 \qquad (2.18)$$

Repeatability is restricted to the precision obtained in a particular laboratory working with a single instrument and the same set of reagents. Reproducibility relates to the same method but differing in the equipment and materials used.

2.4.5 Accuracy

This embraces precision, sensitivity and absence of systematic error. Accuracy is a measure of the deviation of an analysis from the correct value. Accuracy could be improved by optimizing the instrument parameters to minimize noise and maximize precision.