CHAPTER II

THEORETICAL BACKGROUND

## 2.1 Chromatography

The term "chromatography" covers a variety of separation techniques based on a sample partitioning between a moving phase, which can be gas or liquid, and a stationary phase, a liquid or a solid. If the stationary phase is a solid, the method is know as adsorption chromatography; if a liquid as partition chromatography. All chromatographic separation techniques depend on the fact that substances to be separated distribute themselves between the mobile and the stationary phase in proportions vary from one substance to another. The manner in which the substances are distributed is most conveniently discussed by referring to the "sorption isotherm". The amount of a particular substance taken up by the stationary phase depends on the concentration in the mobile phase. The curve obtained by plotting the amount of sorbed per unit of absorbent against concentration in mobile phase, at constant temperature, is the sorption isotherm. The shape of the isotherm is one of the most important factor governing chromatographic behavior. The possible isotherm types are classified in Fig 2-1 by Giles et. al. (1) which are taken from liquid-solid system.

Sorbed isotherm

Band shape

(S)

(S) Concave

(L)

(L) Convex

(C)

(C) Linear

(H)

(H) Chemisorp-
tion

concentration of stationary phase

concentration of mobile phase
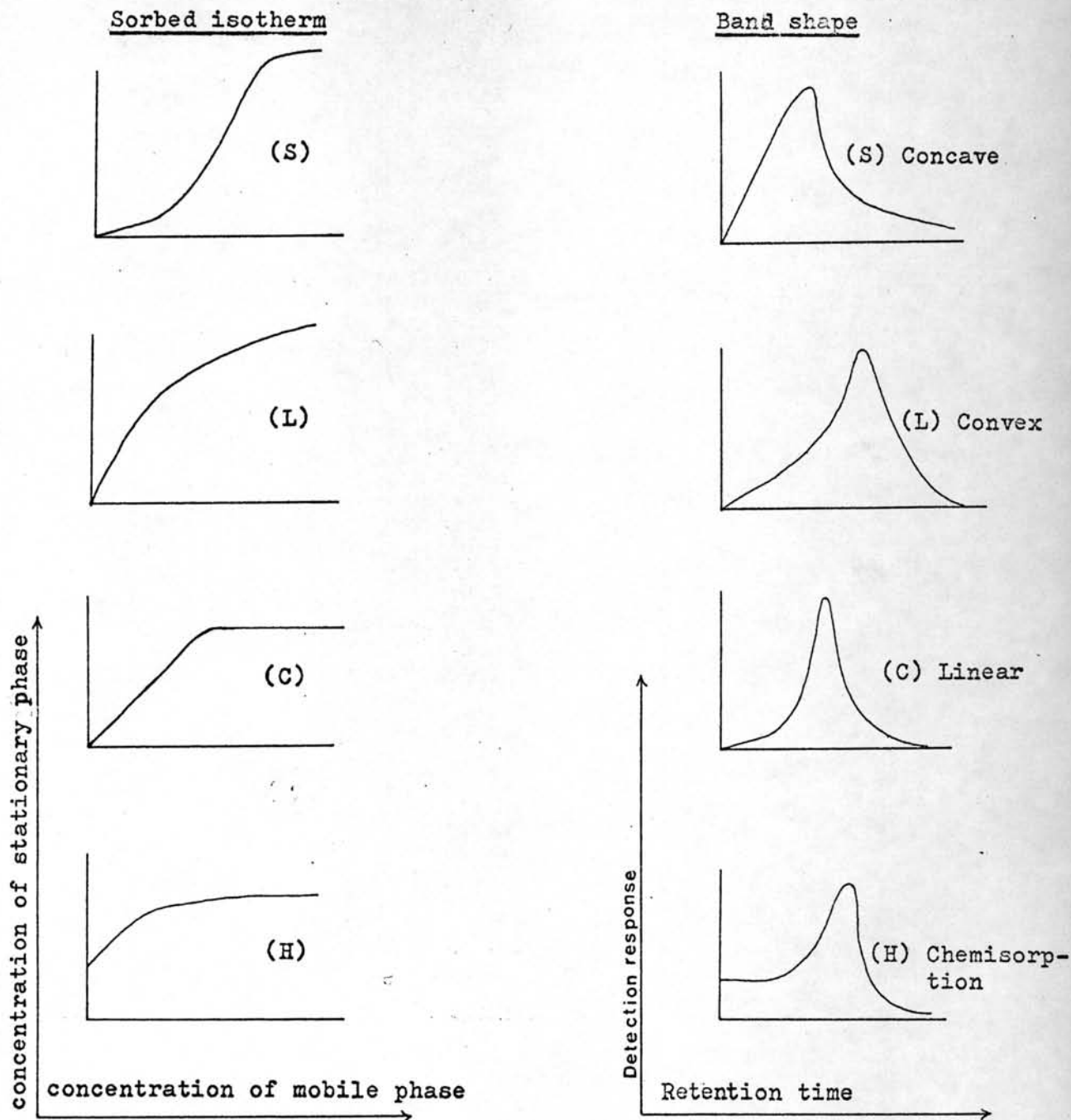
Detection response

Retention time

<u>Fig 2-1</u>    Curve of sorption isotherm and band shape

From general point of view, chromatography on paper or on supports treated with inorganic ion exchangers or with organic materials, may be classified as reversed phase partition chromatography. The organic compound fixed on the support constitutes the stationary phase, whilst the inorganic liquid used as eluant plays the part of the mobile phase. The essential requisites of a support are the ability to adsorb and hold a certain amount of organic solution as well as insolubility in both the organic and the inorganic phase, in addition to a high degree of chemical stability. Recent literature contains numerous examples of inert supports treated with organic liquid being used for inorganic separation. Such supports include silica gel, alumina and powdered polymers of styrene-divinylbenzene or polytrifluorochloro-ethylene. A solute will distribute itself between two liquid phases (stationary phase and mobile phase) according to its partition coefficient $\alpha$, which is a constant at a constant temperature for each of the solute.

Reagents such as tri (n-butyl) phosphate, different type of amines, etc, have been used quite frequently in extraction chromatography for the separation of elements. Chelating agents, with the exception of di (2-ethylhexyl) phosphoric acid, have been applied only rarely. These agents are mainly used dissolved in organic solvents, and after the evaporation of the solvents, the solid agent as well as the metal chelates formed can be precipitated on the column.

Dithizone or diphenylthiocarbazone, $S=C\begin{smallmatrix} NH-NH-C_6H_5 \\ N=N-C_6H_5 \end{smallmatrix}$, a
chelating agent, is a violet-black solid which dissolves in
most organic solvents, but there are only a few solvents, which
are suitable for dithizone to react with metal. Its solubili-
ties in chloroform and carbontetrachloride at room temperature
are approximately 2 g per 100 $cm^3$ and 0.05 g per 100 $cm^3$ respec-
tively. These two solvents are used almost exclusively in the
preparation of dithizone solutions for analytical purposes.
When a solution of dithizone in an immisible organic liquid is
shaken with an aqueous solution of a reacting heavy metal, an
internal complex dithizone is formed, which generally is so-
luble in the organic solvent and imparts a violet, red,
orange, or yellow colour, depending upon the metal involved.
Dithizone and the metal dithizonates are virtually insoluble
in neutral and acid aqueous solution, but some dithizonate
dissociated when their carbontetrachloride or chloroform so-
lutions are shaken with ditute acid. In basic aqueous solu-
tions, dithizone dissolves with a yellow colour to give the
alkali metal dithizonate, which may be considered to be com-
pletely dissociated.

Seventeen metals, Ag, Au, Bi, Cd, Co, Cu, Fe, Hg, In,
Mn, Ni, Pb, Pd, Pt, Sn, Tl and Zn, can form metal dithizonates.
Some metals can replace either one or both of the acidic
hydrogen of dithizone to form two different complexes. These
were called keto and enol forms by H. Fisher (2), but are
better named primary and secondary dithizonates. According
to Fischer, the hydrogen of the imide group of dithizone is

replaced by the metal to give the primary dithizonate and the hydrogen of the sulfhydryl group of the thiol form is replaced by the metal to give the secondary dithizonate.

Dithizonates of a univalent metal are given as follow:
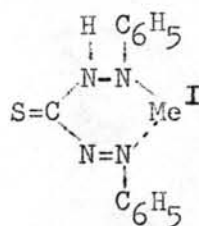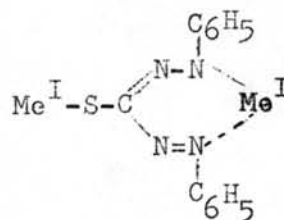
primary dithizonate                secondary dithizonate

In the present work dithizone dissolved in carbon-tetrachloride was used as liquid stationary phase and was fixed on an inert material, powder polymer of polytrifluoro-chloroethylene. The 17 metals which could form metal dithizonate complexes would be adsorbed on the column. The adsorption depends on the partition coefficient and the stability of the metal dithizonate complex. The eluting solution must have either one of the following properties: it can form complex with higher stability than the dithizonate complex, such as $\left[Zn(C_2O_4)_2\right]^{2-}$ has higher stability than zinc dithizonate complex $\left[Zn(HDz)_2\right]$ at pH 4; or it can decompose the metal dithizonate.

2.2  Theory of atomic absorption measurements

2.2.1  Emission and absorption of light

The emission and absorption of light are associated with the processes of transition of atoms from one steady state to another. For the case of steady state i and excited state k with energies of $E_i$ and $E_k$, when $E_k > E_i$, the $i \rightarrow k$ transition

results in the absorption of light, and the k ⟶ i transition results in the emission of light with a frequency

$$\nu_{ki} = \frac{\dot{E}_k - E_i}{h} \quad \dots\dots\dots\dots\dots(2.1)$$

where h is Plank's constant

According to Einstein's quantum theory of radiation, there may be three type of transition between levels i and k:

1. Emission transitions (k ⟶ i) from the excited state into a lower energy state, taking place spontaneously.

2. Absorption transitions (i ⟶ k) from a lower into a higher energy state, taking place in response to the action of external radiation of the same frequency $\nu_{ki}$

3. Emission transitions (k ⟶ i) from an excited state into a lower energy state stimulated by external radiation of the same frequency $\nu_{ki}$

The k ⟶ i emission transitions thus include two types of transition: spontaneous transition taking place without any external cause and transition stimulated by external radiation. The i ⟶ k absorption transitions are always stimulated by external radiation.

2.2.2 The concepts of the absorption line and absorption coefficient.

The absorption of light by the atoms of any elements can be observed by passing a beam of light from a light source with a continuous spectrum through a medium in which there are free

atoms of the element concerned. If an instrument with a high resolving power is used, it is easy to locate regions of lower intensity in the continuous spectrum corresponding to the energies of transition of the atoms from the lower to the higher energy state according to equation (2.1)

Atomic absorption follows an exponential law for the intensity I of transmitted light against absorbing volume length l; this law is similar to Lambert's law in molecular spectroscopy.

$$I = I_o e^{-k_\nu l} \qquad \dots\dots\dots\dots\dots(2.2)$$

Here $I_o$ is the intensity of the incident beam of light and $k_\nu$ is the absorption coefficient at the frequency $\nu$.

The absorption coefficient characterizes absorption lines, just as intensity characterizes emission lines. The laws for the distribution of absorption coefficients across absorption lines are similar to the laws for the distribution of intensity across emission lines. In emission spectroscopy, lines are considered strong or weak according to their integrated intensities; in absorption spectroscopy, similarly, they are classified according to their integrated absorption coefficients.

For the purpose of making measurements, a useful parameter is the absorbance A, defined as

$$A = \log (I_o/I) \qquad \dots\dots\dots\dots\dots(2.3)$$

Using equation (2.2) we get:

$$A = k_\nu \, l \, \log e$$
$$= 0.4343 \, k_\nu \, l \, .$$

It follows that absorbance is directly proportional to the absorption coefficient.

It was pointed out earlier that atomic absorption corresponds to transitions of atoms from lower to higher energy states. Naturally, therefore, the degree of absorption depends on the degree to which the lower level of a particular transition is populated. With thermodynamic equilibrium in a system, the level population is determined by Boltzman's law:

$$N_i = N_0 \, \frac{g_i}{g_0} \, e^{-\left(\frac{E_i}{kT}\right)} \qquad \ldots\ldots\ldots\ldots(2.4)$$

where $N_i$ is the number of atoms at the level with an energy $E_i$, $N_0$ is the number of atoms at the ground state with an energy $E_0 = 0$ and $g_i$ and $g_0$ are the statistical weights of the $i^{th}$ and ground state respectively. 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.

2.2.3   Sensitivity, detection limit, precision and accuracy
in atomic absorption spectrophotometry.

### 2.2.3.1   Sensitivity

Sensitivity is one of the analytical
characteristics which has been mostly discussed in
extolling the potentialities of the atomic absorption
method.  Absolute and relative sensitivity must be
distinguished.  Absolute sensitivity is a certain
minimum determinable amount of the element, reported in
weight units.  Relative sensitivity is the minimum
determinable concentration of the element in a given
matrix.  In atomic absorption, absolute sensitivities
are usually only reported when using the graphite
furnace or a hollow cathode absorption source.  For
these two devices the sensitivity depends, namely, on
the weight of the element and therefore, also on the
weight of the sample in the absorption medium.

For absorption flame photometry, a relative sen-
sitivity in aqueous solution is generally reported.
It is defined as the concentration, in solution, of
the element to be determined which will produce a
change, compared to pure solvent, of 1 % absorption, in
the optical transmission of the atomic vapour at the
wavelength of radiation used, i.e. a 0.0044 absorbance
unit.  This sensitivity depends on the atomic

absorption coefficient, on the absorption pathlength, the
atomization efficiency and the resulting concentration of
free atom in the flame gases. The concentration of free atoms
in the flame depends on the atomization yield and on the
degree of dissociation of a particular compound in the flame.

2.2.3.2 Detection limits

With most instruments less than one percent absorption
may be measured with good precision. Using scale expansion
the difference of the absorbed and unabsorbed signals may be
amplified only so much, until it is lost in the fluctuations
of the signal itself. To express this lowest detectable con-
centration the term detection limit is used. This is defined
as the concentration of an element in a solution which gives
a reading equal to twice the standard deviation of a series
of at least ten measurements near the blank level. In
practice an aqueous solution with concentration not more than
five time the detection limit was aspirated ten times, with
aspiration of the blank solution before and after each sample
aspiration. The ten "signals" were then calculated by sub-
tracting the mean of the adjacent blanks from the appropriate
readings, and the mean, $\bar{X}$ and standard deviation, S, of the
signals were then calculated. The detection limit was then
calculated according to the formula

$$D = 2 \times S \times \frac{C}{\bar{X}}$$

where D is the detection limit and C is the concentration of
the solution.

## 2.2.3.3 Precision

In flame atomic absorption analysis the relative standard deviation (R.S.D.) can frequently be as low as 0.5 %. Precision is affected by the general operating conditions including flame conditions, burner and lamp alignment and absorbance. Absorbances between 0.2 and 0.8, which is the approximate range for maximum precision, is the optimum range. Outside this range precision slowly decreases as absorbances become very high or very low. For relatively easy routine use a minimum concentration of five to ten times the detection limit is desirable. For all low values, repeated sample aspirations with alternative blank aspirations permit averaging of the signals. This procedure results in significant increase in precision when working near the detection limit (e.g. at concentrations 15 times the detection limit).

## 2.2.3.4 Accuracy

Atomic absorption measurements are made by

(1) Measurement of the absorbances of standards of known concentration

(2) Construction of a calibration curve showing the relationship between absorbance and concentration of the known standards.

(3) Measurement of the absorbance of the sample and deduction of the concentration from the calibration curve.

Factors which limit the accuracy of the determination are the

(1) accuracy of standards

(2) precision of measurement of the absorbance

(3) accuracy of interpolation between points on the calibration curve

(4) slope and linearity of the calibration curve

(5) satisfactory matching of standards and sample matrices.

Therefore if matrices of samples and standards are not well matched, erroneous results can be obtained. This effect is sometimes large and sometimes negligible depending on the particular element and matrix.

Accuracy could be improved by optimizing the instrument parameters to minimize noise and maximize precision, preparing standards of suitable concentration accurately, matching matrix components of the standards and the samples carefully, using the same technique to preconcentrate the standards where preconcentration techniques are used and repeating the analysis where practical.

The sensitivity and the detection limit of a number of elements for the Varian Techtron AA-5 are given in Table 2-1

Table 2-1    Sensitivity and detection limit   of some
elements for Varian Techtron AA-5

| Element | Sensitivity (ppm) | Detection limit (ppm) |
|---------|-------------------|-----------------------|
| Ag | 0.036 | 0.003 |
| Hg | 2.20 | 0.20 |
| Cu | 0.040 | 0.002 |
| Zn | 0.009 | 0.002 |
| Pb | 0.11 | 0.02 |
| Cd | 0.011 | 0.0006 |
| Co | 0.066 | 0.007 |

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