# CHAPTER II

# THEORY

## Basic Theory of Headspace Analysis (5,11,37-40)

Headspace analysis (HSA) is an indirect method for the determination of volatile components in liquid or solid by gas chromatography analysis of the vapor phase which is in thermodynamic equilibrium with the sample to be analysed in a closed system.This is a gas extraction technique which is based on the distribution of a substance in two immiscible phase , i.e., gas-liquid or gas-solid. When a substance is added into a two phases system , it will distribute itself in the two phase in a definite manner. The equilibrium distribution of substance between the different phase can be expressed by the distribution law.

$$\frac{C_{i,1}}{C_{i,2}} = K = \text{distribution coefficient} \qquad (2.1)$$

where $C_{i,1}$ is the concentration of solute i in the phase 1

$C_{i,2}$ is the concentration of solute i in the phase 2

K is the distribution coefficient or distribution constant

In gas-liquid system of HSA , the phase 1 is the liquid phase and the phase 2 is the gas phase. Therefore the gas-liquid distribution coefficient can be expressed as the following .

$$K = \frac{C_{i,1}}{C_{i,2}} \qquad (2.2)$$

where $C_{i,l}$ and $C_{i,g}$ are the equilibrium concentrations of the component i in liquid phase and gas phase , respectively.

K is the gas-liquid distribution coefficient.

The expression of the distribution law is valid only for ideal system as can be seen from a thermodynamic derivation of the law. From the studied system , it is the thermodynamic closed system. Then, when it reaches to the equilibrium, the chemical ($\mu$) of solute between two phase are equal. Therefore

$$\mu_{i,l} = \mu_{i,g} \qquad (2.3)$$

where $\mu_{i,l}$ and $\mu_{i,g}$ are the chemical potential of solute i in liquid phase and gas phase, respectively.

The chemical potential of any solute in solution can be expressed as

$$\mu_i = \mu^{\circ}_i + RT \ln a \qquad (2.4)$$

where $\mu^{o}_{i}$ is the chemical potential of solute i in a specific reference state and it is a constant independent of the composition but it is dependent of the temperature and pressure of system.

R is the gas constant.

T is the absolute temperature.

a is the activity of solute in solution.

By substituting the chemical potential expressed in equation (2.4) into equation (2.3) , it gives

$$\mu^{\circ}_{i,l} + RT \ln (a_{i,l}) = \mu^{o}_{i,g} + RT \ln (a_{i,g}) \qquad (2.5)$$

or

$$\ln(\frac{a_{i,l}}{a_{i,g}}) = \frac{\mu^{o}_{i,g} - \mu^{o}_{i,l}}{RT} \qquad (2.6)$$

The right hand side of this expression is a constant , therefore the ratio of a actively of solute i ($a_i$) in different phase also must be constant.

$$\frac{a_{i,l}}{a_{i,g}} = P = \text{Partition coefficient} \qquad (2.7)$$

The activity of any solute in solution can be written as (41)

$$a = \gamma C \qquad (2.8)$$

where $\gamma$ is the respective activity coefficient.

C is the concentration of solute in solution.

However , an approximation holds true for dilute solution and ideal behavior , $\gamma$ is equal to 1 and equation (2.7) can be change to the original form as equation (2.1).

$$\frac{C_{i,1}}{C_{i,2}} = K = \text{distribution coefficient}$$

Since headspace analysis depends on equilibrium existing between the liquid or solid phase and the vapor phase that is injected into the gas chromatograph (GC). The parameter measured in headspace analysis i.e. , the peak height (h) or the peak area (A) are the proportional to the partial vapor pressure ($p_i$) of the volatile component i in the headspace. Then , it can be expressed as the following equation: (42)

$$A \text{ or } h = f\, p_i \qquad (2.9)$$

where f is the detector response factor.

The partial pressure of the volatile solute i above the solution depends on the concentration of component i in the vapor phase ($C_{i,g}$) , peak area (or peak height) can be shown to the concentration form, is directly proportional to the concentration of component i in the vapor phase.

$$A = rC_{i,g} \qquad (2.10)$$

where $C_{i,g}$ is the concentration of the component i in vapor phase which introduces into gas chromatograph.

r is the proportional constant.

In addition , according to Raonlt's and Dalton's laws the partial vapor pressure ($P_i$) is generally expressed as (43)

$$P_i = \gamma_l P^{\circ}_i X_{i,l} = PX_{i,g} \qquad (2.11)$$

where $X_{i,g}$ and $X_{i,l}$ are the mole fraction of component i in gas and liquid phase, respectively.

$P^{\circ}_i$ is the vapor pressure of pure liquid component i

P is total vapor pressure.

$\gamma$ is the activity coefficient.

Generally , for diluted solutions the activity coefficient can be assumed to be a constant (the concentrations less than or equal to 1% or 10,000 ppm) (44). With constant activity coefficient , Raoult 's law ($\gamma_l P^{\circ}_i$) can be simplified to Henry's law , which stated that the vapor pressure of the pure component i is given by

$$P_i = HX_{i,l} = PX_{i,g} \qquad (2.12)$$

where H is the Henry's constant and is the product of $\gamma_i$ and $P^{\circ}_i$.

In such a relationship with the distribution coefficient , K (45).

$$K = \frac{X_{i,l}}{X_{i,g}} = \frac{P}{H} \qquad (2.13)$$

This expression shows that the Henry' s constant (H) is inversely proportional to the distribution coefficient (K) and is used frequently in practical thermodynamic computations while the distribution coefficients are more convenient for analytical applications.

The aim of the analysis is to determine the initial concentration of analysed sample in solution. Under such conditions the formula acquired a special value satisfying this requirement, which is derived in the following manner.

The equilibrium condition for any component between liquid sample and headspace gas , the relationship is given by Vitenberg (46) can be identified from a mass balance

$$C^{\circ}_l V_l = C_l V_l + C_g V_g \qquad (2.14)$$

where $C^{\circ}_{l}$ is the initial concentration of solute in liquid sample before equilibration.

$C_l$ and $C_g$ are the concentrations of solute in liquid phase and gas phase after equilibration,respectively.

$V_l$ and $V_g$ are the volume of the liquid phase and gas phase , respectively.

And according to the distribution law in equation (2.2), $C_l = KC_g$, thus

$$C^{\circ}_{l}V_l = C_lV_l + C_gV_g \qquad (2.15)$$

or

$$C^{\circ}_{l} = C_g (K + \frac{V_g}{V_l}) \qquad (2.16)$$

This formula is the principle of headspace analysis and it also forms the basic of the more useful methods of measuring distribution coefficients. Therefore , if the initial concentration $C^{\circ}_{l}$ , the volume of the liquid phase ($V_l$), the volume of gas phase ($V_g$) by and the concentration after equilibrium($C_g$) , which can be determined from headspace by gas chromatograph , are known , the distribution coefficient (K) of the solute can be easily calculated or measured with this equation.

## Sensitivity of Headspace Analysis Technique.

The basis parameters determining the sensitivity of headspace analysis are the value of distribution coefficient (K) and the relationship of the two phase volumes in equilibrium closed container. In reality , the sensitivity (S) is defined as the ratio of signal (47). Thus , the sensitivity of the HSA method can be expressed as a following

$$S = \frac{A}{C^{o}_{l}} \qquad (2.17)$$

where A is the peak area of gas chromatographic analysis.

$C^{o}_{l}$ is the initial concentration of component.

Since the peak area depends on the compound (m) which is introduced into the chromatographic column and the detector response factor (f). Therefore, it can be written as following (48)

$$A = fm = fC_g v_g \qquad (2.18)$$

where f is the detector response factor

m is the mass of the component

$C_g$ is the concentration of solute in gas after equilibration

$v_g$ is the volume of sample of equilibrium headspace gas introduce into the column or injection volume.

If the values of A from equation (2.18) and $C^{\circ}_{i}$ from equation (2.16) are substituted into equation (2.17), then it would yield as

$$S = \frac{fv_g}{(K + \frac{V_g}{V_l})} \qquad (2.19)$$

This equation shows the relationship of the sensitivity of headspace analysis with the nature of the analyte (or detector response factor) , the injection volume, the ratio of two phases and the distribution coefficient.

## Method of Increasing the Analytical Sensitivity of Headspace Analysis Technique.

1. Temperature

The distribution coefficient is related to the temperature and vapor pressure by the following equation (11,37)

$$K = \frac{RT d_L}{\gamma_i P^{o}_{i} M_L} \qquad (2.20)$$

where $d_L$ is the density of liquid phase.

$M_L$ is the molar mass of the liquid phase.

$P^{\circ}_i$ is the vapor pressure of pure component i .

This equation shows that the distribution coefficient (K) is directly proportional to the temperature , and

$$\frac{dP}{dT} = \frac{P\Delta H_{vap}}{RT^2} \qquad (2.21)$$

where $\Delta H_{vap}$ is the molar heat of vaporisation , which is the change in enthalpy accompanying the transfer of 1 mol of component i from a solution into the gas phase.

This equation (2.21) is known as Clausius-Clapeyron equation which is shown the relationship between vapor pressure and temperature (5). Since P , $\Delta H_{vap}$ and $RT^2$ are positive , hence the right hand side of equation (2.21) is positive. Therefore , the vapor pressure (P) will be increase when the temperature (T) is increased. When the vapor pressure of component i is increased , the solubility of the component i in an aqueous solution would be reached. Thus, this result is the enhancement of the extraction of the solute into the gas phase.

Moreover , the enhancement of the sensitivity in headspace technique can be achieved by lowering the distribution coefficient (K) can be seen from equation (2.19).

However , increasing the temperature of system would be enhancing the vapor pressure of component i that can be seen from the Clausius-Clapeyron equation and the distribution coefficient is inversely proportional to the vapor pressure of pure component as shown in equation (2.20). Thus , increasing the temperature would lower the distribution coefficient , therefore it would be enhancing the sensitivity of the headspace analysis.

However , enhancing the sensitivity of headspace analysis technique by increasing the temperature is of limited experimental application owing to the rise of bursting the container or of losing the components as a result of chemical interaction with the material used as a septum (49).

2. Phase Ratio (50)

Equation (2.19) indicates that the sensitivity of the analysis (S) increases with the decreasing of the phase ratio ($V_g/V_l$). However , reducing the ratio $V_g/V_l$ to a minimum value some what increases the error in the determination of the initial concentration of the substance in the solution can be seen from equation (2.22) which is the differential form the equation (2.16).

$$\frac{\Delta C^o_l}{C^o_l} = \frac{\Delta C_g}{C_g} + \frac{\Delta K}{K}\left(\frac{K}{K+\frac{V_g}{V_l}}\right) \qquad (2.22)$$

This equation shows that the increase of the ratio $V_g/V_l$ lowers the contribution of the error in the determination of K to the total error of an analysis

(especially in the favorable cases with low K). Therefore , if the conditions of gas-chromatographic analysis ensure sufficient sensitivity for the determination of $C_g$ , the ratio of $V_g/V_l$ should be increased to the limits allowed by the system used to establish the distribution coefficient (K).

3. Injection Volume

The sensitivity of headspace analysis technique can be increased by increasing the injection volume as shown in equation (2.19) and Halasz (51) indicated the same result by deriving equation which was shown the relationship between the peak area (A) and the concentration integral with time ($\int$ Cdt).

$$A = \gamma \int Cdt = \gamma \int \frac{F_1}{F_1 + F_2} dt \qquad (2.23)$$

where $\gamma$ is a factor of proportionality and C is the concentration of the sample in the carrier gas of gas chromatograph which depends on the flow rates of the sample ($F_1$ ) and that of carrier gas ($F_2$).

In quantitative evaluation of analysis , this holds true for constant flow rate of gas mixture ($F_1+F_2$) can be seen from equation (2.24)

$$A = \frac{\gamma}{F_1 + F_2} \int F_1 \, dt \qquad (2.24)$$

The integral , $\int F_1 dt$ , is the quantity of the sample injected m expressed in the number of molar or recalculated , in mass units and the constant values of ( $\gamma$/ $F_1+F_2$) may be combined as follows

$$A = fm \qquad (2.25)$$

where A is the peak area on a chromatogram

f is the detector response factor

m is the total mass of sample introduced into the chromatographic column.

Equation (2.25) can be rewritten in the from of the concentration and injection volume of the gas phase as

$$A = fC_gV_g \qquad (2.26)$$

where $C_g$ is the equilibrium concentration of solute in gas phase.

$V_g$ is the injection volume.

It is evident from equation (2.26) that the peak area can be increased by increasing volume. Therefore , the sensitivity (S) which is proportional to the peak area on a chromatogram as shown in equation (2.17) would be increased in the same of injection volume.

4. Salting Out Effect

Another way of increasing the sensitivity of the headspace analysis technique is the increase of the activity coefficient ($\gamma_l$). It can be achieved by the addition of an electrolyte such as sodium chloride , sodium sulfate , ammonium chloride , etc. into the solution. This technique is known as " salting out effect " . In general , the addition of soluble salt to an aqueous solution of an organic compound decreases the solubility of that compound according to Setschenow's equation (52).

$$\log S = \log S_o - k_s M \qquad (2.27)$$

where $S$ is the solubility of organic compound in the salt solution .

$S_o$ is the solubility of organic compound in pure water.

$M$ is the molarity of the salt.

$k_s$ is a constant called a salting out constant whose value depends on the organic compound and on the nature of the salt.

The physicochemical basis of salting out is rather complex , one factor is that the high concentration of salt may remove water of hydration from the organic species , thus their solubility in water are reduced and their partial vapor pressure are increased (53) . The result of this is the enhancement of soluble salt into the solution.

## Pharmaceutical Formulation (54-57)

The medications are important to the people. In generally, the medications are effective and provide the patient with convenience of handling.The dosage forms have many types i.e., tablets, syrup, parental and suppositories. From a pharmaceutic standpoint, solid dosage forms are generally more stable than liquid counterparts and thus are prefered for poorly stable drugs. Therefore, the tablets are most frequently used.

## Pharmaceutical Tablets

Tablets are solid dosage forms of medicinal substance usually prepared with the aid of suitable pharmaceutical adjuncts. Different tablets may vary in size, shape, weight, hardness, thickness, disintegration characteristics and in other aspects, depending upon the intended use of the tablets and their method of manufacture. The majority of tables are used in the oral administration of drugs and many of these tablets are prepared with colorants, flavorants and coating of various types(54). But the same classes of components in adding to the active ingredients, which are one or more agents functioning as (1) a diluent, (2) a binder or an adhesive, (3) a disintegrant and (4) a lubricant. All nondrug components of a formula are termed excipients.

1. Diluents

Diluents are fillers designed to make up the required bulk of the tablet when the drug dosage itself is inadequate to produce this bulk. The dose of some drugs is sufficiently high that no filler is required (i.e., aspirin and certain antibiotics).

Tablet formulations may contain a diluent for secondary reasons: to provide better tablet properties such as improved cohesion to permit use of direct compression manufacturing, or promote flow.

Regardless of why a diluent is selected, diluents and all other tablet excipients must meet certain criteria in the formulation. These include the following:

1. They must be nontoxic and acceptable to the regulatory agencies in all countries where the product is to be marketed.

2. They must be commercially available in an acceptable grade in all countries where the product is to be manufactured.

3. Their cost must be acceptably low.

4. They must not becontraindicated by themselves (i.e., sucrose) or because of a component (i.e., sodium) in any segment of the population.

5. They must be physiologically inert.

6. They must be physically and chemically stable by themselves and in combination with the drug(s) and other tablet components.

7. They must be free of any unacceptable microbiology "load."

8. They must be color-compatible (not produce any off-color appearance).

9. If the drug product is also classified as a food, (certain vitamin products), the diluent and other excipients must be approved direct food additives.

10. They must have no deleterious effect on the bioavailability of the drug(s) in the product.

2. Binders and Adhesives

These materials are added either dry or in liquid from during wet granulation to form granules or to promote cohesive compacts for directly compressed tablets. These materials are much more effective when they are added as solutions in the preparation of granulations than when they are added dry to a direct compression formula.

Modified natural polymers, such as the alginates and cellulose derivatives (methylcellulose, hydroxypropyl methylcellulose and hydroxypropyl cellulose), are common binders and adhesives. Used dry for direct compression, they have some binder capabilities, while aqueous solutions have adhesive properties. Hydroxypropyl cellulose may also used as an alcohol solution to provide an anhydrous adhesive. Ethylcellulose may be used only as an alcoholic solution, and it may be expected to drugs in the resulting tablets when wet granulation is employed. Polyvinylpyrolidone is a synthetic polymer that may be used as an adhesive in either an aqueous solution or alcohol. It also has some capabilities as a dry binder.

3. Disintegrates

A disintegrant is added to most tablet formulations to facilitate a breakup or disintegration of the tablet when it contacts water in the gastrointestinal tract. Disintegrants may function by drawing water into the tablet, swelling, and causing the tablet to burst apart. Such tablet fragmentation may be critical to the subsequent dissolution of the drug and to the attainment of satisfactory drug bioavailability. Starch USP and various starch derivatives are the most common disintegrating

agents. They also have the lowest cost. Starch is typically used in a concentration range of 5 to 20 % of tablet weight.

4. Lubricants, Antiadherents and Glidants

These three classes of materials are typically described together because they have overlapping functions. A material that is primarily described as an antiadherent is typically also a lubricant, with some glidant properties as well. The differentiation between this terms is as follows: Lubricants are intended to reduce the friction during tablet ejection between the walls of the tablet and the walls of the die cavity which the tablet was formed. Antiadherents have the purpose of reducing sticking or adhesion of any of the tablet granulation or powder to the faces of the punches or to the die wall. Glidants are intended to promote flow of the tablet granulation or powder materials by reducing friction between the particles.

5. Colors, Flavors and Sweeteners

The use of colors and dyes in tablet making has served three purposed over the years disquising of off-color drugs, product identification, and production of a more elegant product. With the continual decertification of many synthetic dyes, pharmaceutical manufacturers are becoming quite concerned as to how future tablet for mulations will be colored. The availability of natural vegetable colors is limited, and these colors are often unstable. Two forms of color have typically been used in tablet preparation.

Flavors are usually limited to chewable tablets or other tablets intended to dissolve in the mount. In general, flavors that are water-soluble have found little acceptance in tablet making because of their poor stability. Flavor oils are added to tablet granuations in solvents, are dispersed on clays and other absorbents, or are emulsified in aqueous granulating agents. Various dry flavors for use in pharmaceutical products are also available from flavor suppliers. Usually, the maximum amount of oil that can be added to a granulation without influencing its tabulating characteristics is 0.5 to 0.75 %.

The use of sweeteners is primarily limited to chewable tablets. various sugars used as tablet excipients have been described earlier. Mannitol is reportedly about 72% as sweet as sucrose. Unit recently, saccharin was the only about 500 times sweeter thansucrose. Its major disadvantages are that it has a better aftertaste and has been reported to be carcinogenic. A new article sweetener that is expected to largely replace saccharin is aspartame. The primary disadvantage of aspartame is its lack of stability in the presence of moisture. When aspartame is used in a formulation i.e., a chewable tablet with hygroscopic components, it will be necessary to determine its stability under conditions in which the product can adsorb atmospheric moisture.

## Orally Ingested Tablets (54,57-62)

Well over 90% of the tablets manufactured today are ingested orally. Orally ingested tablets are designed to be swallowed intact, with the exception of chewable tablets. For example the tablets ingested orally i.e., Sugar- and Chocolate-Coated tablets and Film-coated tablets.

1. Sugar- and Chocolate-Coated Tablets.

Chocolate-coated tablets are nearly a thing of the past. They are too easily mistaken for candy by children. Sugar-coated tablets suffer the same disadventage. Their primary historical role was to produce an elegant., glossy, easy-to-swallow tablet dosage form. Also, they permit separation of incompatible ingredients between coating and core, and this fact has been widely utilized in preparing many multivitamin mineral combinations. The process as originally developed was time-consuming and required skilled coating artisans to be conducted properly. Earlier sugar coating s typically doubled tablet weight. Today, water-soluble polymers are often incorporated in the sugar solution, automated-spray coating equipment is employed, and high-drying-efficiency sidevented coating pans are used. The result is that the coatings are more elastic and mechanically stable, coat weight may be 50 % or less of the core weight, and the process may be completed in a day or less.

2. Film-Coated Tablets

Film-coated tablets were developed as an alternative procedure to the preparation of coated tablets in which drug was not required in the coating. The initial film-coating compositions employed one or more polymers , which usually included a plasticizer for the polymer and possibly a surfactant to facilitate spreading, all delivered to the tablets in solution from an organic solvent. The film-coating process was an attractive tablet coating method since it permitted the completion of the tablet coating operation in a period of one or two hours. An airless spray coating procedure was typically employed for such film-coating compositions, using either conventional coating pans or sidevented equipment. During the decade of

the 1970s, several factors began to make solvent-based film coating less attractive. These factors were the increase in cost of the organic solvents, OSHA restrictions on working exposure to solvent vapor discharge to the atmosphere. As a result of these influences, many companies have now converted their earlier film-coating process to a totally aqueous-based procedure. Polymers such as hydroxypropyl cellulose and hydroxypropyl methylcellulose, which are dissolved in water with an appropriate plasticizer, are now widely used to produce immediate-release film coatings. The recent development of a colloidal dispersion of ethylcellulose in water also makes it possible to produce slow or controlled-release film coatings without the use of organic solvents. A 30% ethylcellulose dispersion is marketed under the trade name Aquacoat by the FMC Corporation.

Film-coated tablets offer a number of advantages over sugar-coated tablets. These advantages include better mechanical strength of the coating based on the elasticity and flexibility of the polymer coating, little increase in tablet weight, the ability to retain debased markings on a tablet through the thin film coating, the avoidance of sugar, which is contraindicated in the diets of a significant segment of the population, and the employment of a process that may be contineous, or that readily lends itself to automation . The primary disadvantage of film coating compared with sugar coating is that it is difficult to produce film-coated tablets that match the physical appearance and elegance of the sugar-coated product. Film coating in the future will assume increasing importance as a means of controlling drug delivery release rates from both tablets and bead particles as well as from drug crystals. Film-coated tablets, which are basically tasteless, also offer the advantage over sugar-coated tablets of being less likely to be mistaken for candy.