CHAPTER I
INTRODUCTION

In high-performance liquid chromatography (HPLC), columns or stationary phases are important compartments for chromatographic separations. The columns have been developed to give better separation, for example, to minimize residual silanols by encapping reversed-phase material and to maximize the exchange capacity of ion-exchange stationary phases. These steps have been taken in order to remove the deleterious presence of secondary retention mechanisms.

Considering stationary phase's chemical properties, the most commonly used chemical forces can be envisioned as a selectivity triangle [1], where hydrophobic, hydrophilic, and ionic interactions are positioned along the corners of the triangle, as shown in Figure 1.1.

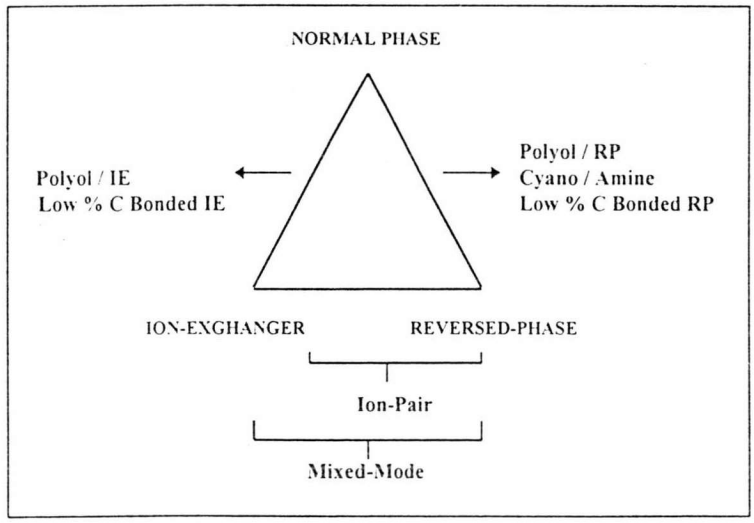

Figure 1.1 Triangle depicting the three major separation mechanisms: hydrophobic, hydrophilic, and ionic interactions. All bonded stationary phases typically have a ratio of at least two mechanisms [1].

The general method to separate discharged organic molecules is reversed-phase chromatography. The major retention mechanism of reversed-phase chromatography is the solvophobic or hydrophobic interaction that causes partitioning of the eluite between a polar mobile phase and a non-polar stationary phase. Ion-exchange chromatography is usually used to separate charge molecules or ions. The major retention mechanism of ion-exchange chromatography is the coulombic or electrostatic interaction between the eluite ion and eluent ion for the charged functional group in the stationary phase [1-3].

Recently, many chromatographers found out new ways to increase selectivity and better separation of complex compounds by using mixed-mode [1,4-5] and multimodal columns [6-10]. Mixed-mode columns were constructed by mixing discrete particles in a single column, retention of various compounds in high-performance liquid chromatography have been investigated. The better separation of mixed-ligands was shown and simultaneous mechanism could occur. The cobonding of ligand in liquid chromatography has the potential to produce hybrid stationary phases with nonlinear properties, which are distinctly different from those obtained either by column coupling or by the mixing of discrete particles, is defined as multimodal chromatography. Multimodal stationary phases are formed through the cobonding of various multifunctional ligands which are either synthesized in their final form, or functionalized *via* a second reaction. In multimodal chromatography, one interaction type predominates under a given set of mobile phase conditions, and a second interaction type predominates under a different set of conditions [6]. Multiple retention mechanisms are purposely employed to enhance selectivity which are useful for sample clean-up, peak confirmation, increased resolution and sample throughout, and to separate complex samples which have different properties (neutral, acid and base). This multimodal technique is frequently used for separating sample components with a wide range of capacity factor values. The different

separation modes can be conducted on a single column. The applications of these multimodal stationary phases are to separate complex mixtures of solutes having different properties, such as non-ionic and ionic compounds [7-11]. Besides, analysis of xenobiotics and their phase I and phase II metabolites could be separated on a multimodal column [12].

Phenylpropanolamine bonded-silica support is a multimodal stationary phase carrying both weak anion exchanger (secondary amino group) and hydrophobic functions. The retention mechanisms could be hydrophobic and ionic interaction depending on the pH of the mobile phase.

The structure of phenylpropanolamine-bonded silica support is shown in Figure 1.2. The hydrophobic site is the phenyl group. The ionic group is the secondary amino group which bahaves as an anion-exchanger [7].

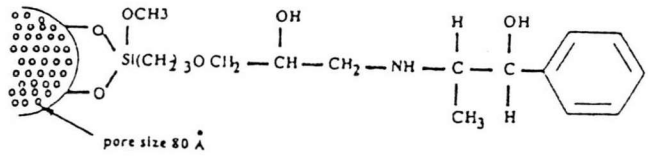

Figure 1.2 Structure of phenylpropanolamine-bonded silica particles.

In HPLC, the sample is dissolved in a solvent and introduced into the column. In general, the sample is preferably dissolved in a solvent identical with the mobile phase. However, it is occasionally necessary to use a different solvent in order to increase the solubility of the sample or to use a different eluite so as to obtain more favorable retention behavior. The solvents are characterized by their strength. A strong solvent can be defined as one that provides a small capacity factor (k′) for a given sample. The solvent strength is related to its polarity. As the solvent polarity is increased, the solvent strength increases in normal phase liquid chromatography (NPLC) and decreases in reversed-phase liquid chromatography (RPLC) [13].

Problem Difinition

The difference in polarity between sample solvent and mobile phase cause a significant problem in reversed-phase chromatography and ion-exchange chromatography. The deleterious effects of injecting samples of appreciable volume into liquid chromatographic systems, with a difference between sample solvent polarty and mobile phase polarity, have been well known [14-23]. Peak compression or peak deformation will occur for analytes coeluting with the system peak. Peak compression effects might give extreamly narrow peaks and increase theoretical plates, so this effect is of interest as a mean of improving the sensitivity in liquid chromatography [19]. Additionally, peak broading and peak spliting and decreasing of peak height occured when solvent was stronger than the mobile phase [20-22]. In ion-exchange chromatography, when the injection solvent was weaker than the mobile phase, the peak of the anion was higher than that obtained when mobile phase was used as the injection solvent. When the injection solvent was stronger than mobile phase, the peak broadening and the peak height were decreased [23]. An investigation of the effect of injection solvent could be performed by varying the solvent strength of the injection solvent both in ionicity and hydrophobicity.

Chromatographic behavior of five certain organic acids, namely phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid on phenylpropanolamine column (5 μm, 150 x 4.6 mm I.D.) in the pH range of 3.1 to 6.9 have already been investigated [7]. These acids were chosen in the study of the effect of solvent strength on the chromatographic behavior by using acetonitrile / 30 mM phosphate buffer (pH 3.1 - 6.9) 20:80 (v/v) as mobile phase (except L-ascorbic acid, for which 6% acetonitrile (v/v) was used). However, the effect of dissolving solvent on the chromatographic behavior of the acids in such column has not been explored. This thesis was interasively focused on the effect of solvent strength on the chromatographic

behavior of the certain organic acids on the multimodal stationary phase. The aims are to explore the dominant interaction between analytes and stationary phase at each eluent pH and to study the effect of the strength of dissolving solvent to the chromatograhic behavior of the acids.

Literature Reviews

Chromatographers have long investigated about chromatographic behavior of reversed-phase chromatography and ion-exchange chromatography within certained chromatographic conditions. Chromatographers have observed many related results concerning these modes in chromatography.

In 1973, J.H. Knox and J. Jurand studied application of high-speed liquid chromatography to the analysis of morphine, heroin, 6-(o-acetyl) morphine and methadone [24]. These compounds were analysed on Zipax SAX (strong anion exchanger), except methadone which was analysed on Zipax SAX (strong anion exchanger). In order to determine optimal conditions for their separation the effect of pH, ionic strength and the pressure on the capacity factor (k′) and the plate height (H) have been determined.

In 1975, J.H. Knox and A. Pryde modified adsorbents by chemically organic groups to form unimolecular layers on (a) a newly synthesized spherical silica gel and (b) Spherisorb Alumina A20Y [8]. They include three reversed-phase materials, two weak anion exchangers and a polar bonded cyano alumina. With one exception the bonded materials show performances which are good as, or better than those of unmodified adsorbents. The new material has been found to be particularly useful in the separation of compounds such as catecolamines, morphine alkaloids, tetracyclines, tricyclic antidepressants, vitamins, nucleotides, penicillin, barbiturates, and other polar substances of complex molecular structure. They gave excellent peak symmetry and high analytical speeds.

In 1976, P.A. Asmus, C.- E. Low and M. Novotny [9] investigated the separation mechanism of chromatographic packing which synthesized by reacting chrolodimethyl[4-(4-chrolomethylphenyl)buthyl]silane with small particle silica material and further modification of the bonded chrolomethyl residues with trimethylamine. It was shown that the ionic mechanism appears to be predominant but additional retention effects due to the organic matrix and residual silanol groups are still affected.

In 1977, C. Harvath , W. Melander and I. Molnar investigated the effect of solute ionisation on the retention of weak acids, bases, an ampholytes on octadecylsilica stationary phase both theoretically and experimentally methods [25]. It was shown that the retention was attributed to a reversible association of the solutes with the hydrocarboneceous ligand of the stationary phase. The observed depedence of the capacity factors on the ionic strength of the eluent and the hydrophobic surface of the solutes molecules showed good agreement with the theory.

In 1978, R. Eksteen and J.C. Kraak studied conditions for rapid separations of nucleobases and nucleosides by high-pressure anion exchange chromatography [26]. They investigated the retention behavior of nucleobases and their nucleosides in system consisting of water-ethanol mixtures with added electrolytes as mobile phase. The effect of pH, type and concentration of the counterion, ethanol content of the mobile phase and the temperature on the retention and column efficiency have been determined. They described a systematic investigation in which the possibilities of optimizing the separation of nucleobases and nucleosides with respect to selectivity and speed of analysis were explored more extensively, using a resin-type anion exchanger and isocratic elution by HPLC.

In 1982, S. Afrashtehfar and F.F.Conwell studied chromatographic retention mechanism of organic ions on a low-capacity ion-exchange adsorbent. They used a small electrical charge placed on the surface of non-ionic adsorbent by covalently bonding quarternary ammonium groups

[27]. A combination of two mechanisms has been found responsible for the greatly enhanced sorption of counter ions which are ion exchange and surface sorption. The latter is dependent on the electrical potential of the surface. The dependence of ionic strength of coion sorption is also predicted and the ability of adsorbed perchlorate ion to reverse the surface potential is demonstrated.

In 1983, J.B. Crowther, S.D. Fazio and R.A. Hartwick [28] separated oligonucleotides and other nucleic acid constituents on multifunctional stationary phases. Both octyl- and 3-chloropropyldimethylmonochlorosilane ligands were bonded, after which the chloro groups were converted into quaternary amines by nucleophilic substitution with benzyldimethylamine. It was shown that the resulting hydrophobic ion exchangers well suited for the separation of the oligonucleotides up to about 20 in chain length, improved separation being observed in comparison to existing ion-exchange or reversed-phase separation alone.

In 1987, G.B. Cox and R.W. Stout studied the retention mechanisms of basic compounds on silica under "pseudo-reversed phase" conditions. They investigated the mechanisms of retention of basic compounds on silica and C_8 reversed-phase packings [29]. Those were shown to be mainly ion exchange for compounds ionized under the conditions of elution and hydrophobic interaction for non-ionized materials. Evidence was obtained and shown that those interactions were with the siloxane bridges, not the silanol groups.

In 1989, N.E. Hoffman , S.- L. Pan and A.M. Rustum studied the distortion and multiplication of peaks that occurred when an eluite was injected dissolved in a solvent that was significantly stronger than the mobile phase in reversed-phase liquid chromatography [21]. They presented a qualitative interpretation of chromatographic phenomenon. It was found that the solvent strength and the volume injected affected peak shape

whereas the column length and diameter, particle size and type of reversed-phase did not affect general peak shapes.

In 1990, Nilsson, L.B. studied the possibilities of regulating peak compression effects [19]. The changes in capacity factor for the system peak relative to the retention of analytes were studied by varying the composition of the mobile phase. The parameters useful for altering the capacity factor ratio were found to be the ionic strength of the phosphate buffer and to some extent the pH, whereas the amount of acetonitrile and the concentration of the amine modifier gave negligible effects.

In 1991, J.J. Johnston, W.M. Draper and R.D. Stephens used simutaneous anion exchange and reversed-phase chromatography to separate xenobiotics and thier phase I and phase II metabolites by LC-MS compatible HPLC [12]. The retention of model compounds was manipulated by modifying the type, ionic strength, pH of the mobile phase, buffer and the type and percent of organic mobile phase modifier. By adjustment of chromatographic parameters, the test compounds were resolved in isocratic separations compatible with both TSP- and PB-LC-MS. It was shown that both reversed-phase and ion-exchange interactions are involved in chromatography of benzene and its metabolites.

In 1992, S. Wongyai investigated the effect of solvent strength on the chromatoghraphic behavior of drugs in reversed phase HPLC [22]. He found that the water content of the injected sample significantly influenced the chromatographic behavior of acidic , neutral and basic drugs on reversed-phase columns. The number of theoretical plates of each compound was distinctly dependent on the water concentration. Resolution of previously unresolved peaks could be achieved without change in retention time.

In the same year, retention of some simple organic anion in ion exchange HPLC were described by H.K. Lee and N.E. Hoffman [23]. The plots of capacity factor of organic anions *vs.* reciprocal of eluent ion contration showed good linearity. From the slope and y-intercept data to major retention mechanism was interpreted as ion exchange and reversed-phase interaction. The effect of acetonitrile fraction of the mobile phase, type and concentration of the eluent ion, and pH on the retention and selectivity were also investigated.

Later, they studied the effect of injection solvent on the peak height of simple organic anions on a styrene-divinylbenzene copolymeric anion exchanger [30]. It was found that when the injection solvent was stronger than the mobile phase, the peak was broadened. A taller peak was obtained when a weaker injection solvent is used. They also investigated the effects of volume injected and difference in strength of two solvents. Those results also indicated that standards for calibration should be dissolved in the same injection solvent that used for the analyte.

In 1993, P.J. Davis R.J. Ruane and I.D. Wilson [4] studied the chromatographic properties of a mixed-bed stationary phase combining reversed-phase and strong anion exchange properties. The simultaneous separation of ionized and unionized molecules has been examined using a range of test solutes. It was shown that the retention of ionisible molecules to be adjusted using pH whilst that of unchanged compounds was unaffected.

In 1994, S. Wongyai synthesized and characterized the phenyl-propanolamine bonded silica for multimode liquid chromatography of small molecules [7]. A multimodal silica support carrying weak anion exchange and hydrophobic functions. The retention of tested compounds was manipulated by modifying the pH and proportion of organic modifier in the mobile phase in isocratic mode. It was shown that the mechanism of interaction

was the same as that observed on mixed-bed column containing a mixture of reversed-phase and strong anion exchange particles.

Later, he separated L-(+)- ascorbic and D-(-)- isoascorbic acids by multimodal phenylpropanolamine-coated silica [31]. It was found that the ionic interaction predominantly when using an acidic mobile phase and the retention of the D-epimer was higher than the L-form. The retention order of the diastereoisomers was the same as that found in reversed-phase mode in which ionic interaction is predominant.

In 1995, M.-V. Ding, Y. Suzuki and H. Koizumi described elution behavior of anions and cations on the mixed-bed stationary phase of anion and cation exchange resins [32]. Organic and inorganic anions have shown the same elution behavior on the mixed-bed column as on a single anion exchange column. In contrast, different elution behaviors for cations have been observed between the mixed-bed column and a single cation exchange column. On the mixed-bed column, cations would be eluted by a mixed retention mechanism. The primary retention mechanism was ion exchange with eluent cation (H^+) for all cations. The ion exchange mechanism on the sites occupied by anion exchange groups made a greater contribution for monovalent cations. A partition mechanism between the stationary phase and mobile phase contributed to the separation of Mg^{2+} and Ca^{2+} by forming neutral compounds.

Purpose of the Study

The separation efficiency in high-performance liquid chromatography is affected by various of chromatograghic factors. The ability to understand and to control these factors is very important element. The purpose of this work is, therefore, to investigate the effect of solvent strength on a multimodal stationary phase which is multiple chromatographic mechanisms between reversed-phase and weak ion-exchange mechanism.

Where the predominant interaction is depended on the mobile phase conditions [6]. Five organic acids including phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid were chosen in order to studied the chromatographic behavior and important factors that affect it. The parameters studied are :

1. pH of the mobile phase, in a range of 2.5 to 6.5
2. The predominant interaction at any pH value
3. The solvent strength of the sample solution
4. The species distribution of the studied compounds within a pH range 2.5 - 6.5.