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Chaotropic Effect in Reversed-phase HPLC: A Review

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Abstract

Some inorganic anions such as BF_4^-, CF_3COO^-, ClO_4^-, and $H_2PO_4^-$, can affect the solubility of proteins and amino acids. Their effects were observed since 1888 by Fran Hofmeister. During the past decade, these ions have been used increasingly in reversed-phase high-performance liquid chromatography (HPLC), because of their abilities to improve the retention of basic substances in their protonated forms without changing a new column or mobile phase pH. Moreover, addition of these ions in mobile phase can improve theoretical plate number and tailing factor as well.

Key words: chaotropic, chaotropic agent, chaotropic effect

Content

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INTRODUCTION

Reversed-phase HPLC is an important tool for the determination of drug substances. A great majority of drugs including basic function groups and the behavior of basic compounds on reversed-phase HPLC is significantly interesting. However, there are some problems related to the separation of basic analytes by reversed-phase HPLC. For example, the elution of analyte in protonated forms may be closed to the void volume, or long analysis time may be needed for the basic analytes in neutral forms as well as the peak tailing. In addition, to suppress the basic analytes in their neutral forms, mobile phase pH must be adjusted two units above the analytes' pKa and this may be harmful to stationary phase materials[1]. These limitations can be solved by changing a new column, mobile phase pH, organic modifier, or by using "chaotropic agents".

Chaotropic agents

Chaotropic agents used in reversed-phase HPLC are usually small inorganic ions such as BF_4^-, CF_3COO^-, ClO_4^-, and $H_2PO_4^-$, with liophilic nature. Liophilic ions are characterized by significant delocalization of the charge, symmetry and spherical in shape, and absence in surfactant properties. The presence of these chaotropic ions in aqueous solution was found to disrupt the water structure or introduce chaos into structured ionic solution[2-3]. This effect was firstly observed by Franz Hofmeister[4]. The ability of chaotropic ions on the disruption of the solvation shell is arranged according to the Hofmeister series as follows,[5] $H_2PO_4^- < HCOO^- < CH_3SO_3^- < Cl^- < NO_3^- < CF_3COO^- < BF_4^- < ClO_4^- < PF_6^-$. From the left to the right, the chaotropicity increases with an increase in hydrophobicity, charge delocalization, symmetry, and overall electron density.

Mechanisms of action

Up to now the effect of chaotropic ions on the retention of basic analytes has been still unclear. However, three district processes may involve as the following[6].

1. Classic ion pairing involves the formation of essentially neutral ion pairs and their retention according to the reversed-phase mechanism.

2. In the chaotropic model, disrupt the analyte solvation shell and lead to an increase in its apparent hydrophobicity and retention.

3. Liophilic counteranions are adsorbed on the surface of the stationary phase, thus these introduce an electrostatic component into the general hydrophobic analyte retention mechanism.

All these three mechanisms probably exist but only one of them is dominating and it depends upon the eluent type, composition, and adsorbent surface properties.

Chaotropic and ion-pairing agents

Ionic additives used in reversed phase HPLC can be classified into two types [*i.e.*, ion pairing agents (amphiphilic ions) and chaotropic agents (small inorganic anions)]. These ionic additives have an effect on the retention of ionic or ionizable analytes. There are two types of ion-pairing agents (i.e., cationic and anionic ion-pairing agents). Ion-pairing agents could affect the retention of both acidic and basic compounds, while chatropic ions are used for improving the retention of only basic analytes[1]. Effects of liophilic ions (chaotropic ions) also depend on types of organic modifiers but independent for amphiphilic ions. Increasing retention of liophilic ions was seen from the increasing of acetonitrile concentration but not methanol. It was found that acetonitrile forms thick adsorbed layer on the surface of hydrophobic bonded phase, while methanol forms a classical monomolecular adsorbed layer. The thick adsorbed layer of acetonitrile acts as a pseudo-stationary phase and allows adsorption of chaotropic ions on this layer. The pseudo-stationary phase is suitable for ion accumulation, this creates an electrostatic potential on the stationary phase surface resulting to enhancement of the retention of protonated basic analytes. However, the increased retention with the increase in organic solvent for reversed-phase HPLC, is seen in low concentration of acetonitrile (0-20%). At high acetonitrile concentration, more than 25%, the retention of basic

analytes starts to decrease due to the normal effect of the increase of organic composition in the mobile phase[1].

Ion-pairing agent and ionizable analytes retention

Ion-pairing agents or amphiphilic ions are usually molecules with relatively long alkyl chain and have a charged group at one end. These substances are surfactants known as "soap chromatography" and possess highly localized charges. In the chromatographic system, these molecules are accumulated at the interface between the hydrophobic stationary phase and water/organic eluent. They are oriented at the interface so that the charged part of the molecule remains in the eluent and the hydrophobic part (alkyl chain) is adsorbed on the stationary phase surface. The interaction between hydrophobic part of ion-pairing agents and alkyl chain of bonded phase is irreversible. However, this interaction forms the charged surface and allows the accumulation or pairing of the opposite charge analyte ions. The retention of a charged analyte in ion-pairing mode depends on the adsorption of ion-pairing ions on the surface of the stationary phase. With the increase in the surface ions adsorbed, the retention of oppositely charged analytes increases, while similarly charged analytes as the ion-pairing reagent will elute faster[7]. The retention of adrenaline is in agreement with this observation[8]. The similar retention of adrenaline (basic analyte) is seen for different amphiphilic ions adsorbed on the surface of the reversed-phase material but the same surface concentration of those amphiphilic ions are adsorbed. Thus the retention is dependent on the surface charge density, which depends on the alkyl chain length of ion-pairing agents. The short chain is adsorbed more than long chain and eventually saturated.

Chaotropic ions and ionizable analytes retention

The interaction of chaotropic ions and hydrophobic stationary phase is reversible. The increase in chaotropic agents in the mobile phase leads to the increase in the retention of basic analytes. This effect is observed for only basic ions but not acidic or neutral species. With the increased chaotropicity following the Hofmeister series, the retention of basic analytes increases[1].

Improving tailing factor and peak symmetry by chaotropic agents

Some studies showed that the increasing of the chaotropic counter-anion concentration in the mobile phase for the separation of basic compounds led to an increase in the apparent efficiency of the system until the maximum plate number for the column is achieved. According to the study of Pan et. al[9]. on the effect of BF_4^- on the retention of three basic ophthalmic drugs, the efficiency for these drugs increases relatively fast when the concentration of counteranion BF_4^- was increased from 1 mM to 10 mM. Then, with a further increase in the counteranion concentration, the efficiency of the basic compounds increases slowly until it achieves the maximum column efficiency. Also with an increase of BF_4^- counteranion concentration, the tailing factor of basic compounds decreases and approaches the tailing factor of the neutral analytes. The profile of column efficiency in term of number of theoretical plate, N and tailing factor from the effect of counter-anion concentration are illustrated in Figure 1.

It has been shown that the PF_6^- counteranion has had the greatest effect on the improvement of the peak asymmetry at low concentrations compared to other chaotropic additives. At the highest concentration of counteranions (PF_6^-, ClO_4^-, BF_4^-), the number of plates for most of the basic compounds studied was similar to that of the neutral compound. In contrast, the neutral compound (here is phenols) showed that there was no significant change in retention and efficiency with increased counteranion concentration.

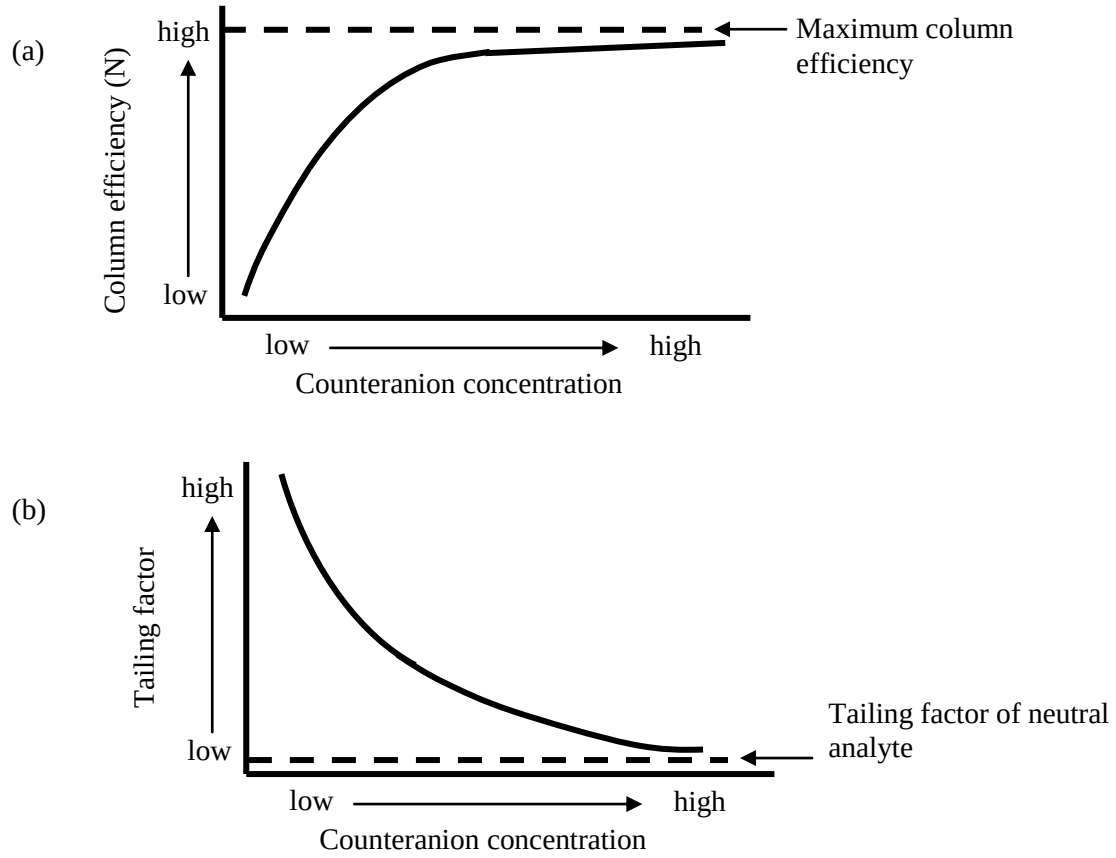

Figure 1. Profile of column efficiency in term of number of theoretical plate, N (a) and tailing factor (b) from the effect of counter-anion concentration.

One of the origins of peak tailing in chromatography can be attributed to overloading of adsorption sites[10-15]. From the studies of McCalley and others[10, 16-17] it has been shown that basic anlyte sample loading may also have an effect on peak efficiency. Thus, a decrease in sample loading has led to the improvement in the efficiency of basic compounds. However, it is sometimes necessary to inject large sample sizes to enable the detection of small impurities which consequently increase basic analyte tailing factor and decrease peak symmetry.

Since the analysis of basic compounds on ODS columns often suffers from broadening peaks and serious tailing which is caused by the secondary interaction between basic solutes and residual silanol groups[18]. Chaotropic additives can be added to the mobile phase to suppress secondary interactions with the stationary phase[1]. The adsorption of chaotropic counteranions in the adsorbed organic phase on top of the bonded phase can add an electrostatic component to the retention as well as suppressing some undesired secondary interactions leading to peak tailing of protonated basic compounds. The following trend which is the increase of basic analyte retention factor and decrease of tailing factor was found to be $PF_6^- > ClO_4^- \sim BF_4^- > H_2PO_4^-$.[9] For thermodynamic of analyte adsorption on the stationary phase, thermodynamic overload can occur when analyte concentration exceeds the linear region on the adsorption isotherm, and this isotherm curvature leads to right-angled peak[19-21]. The general adsorption isotherm is illustrated in Figure 2.

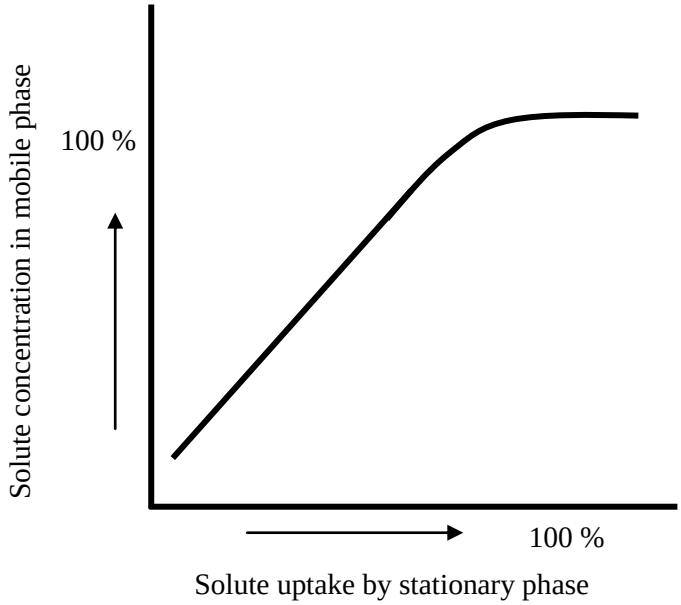

Figure 2. Adsorption isotherm

The greater the chaotropic counter-anion concentration, the higher the adsorption capacity and the straighter the analyte isotherm. This results in a shorter peak tailing. With an increase in counter-anion concentration at all analyte loadings, an increase in peak efficiency and a decrease in peak tailing can be achieved[9].

Applications of chaotropic agents

Chaotropic agents have been previously used in the field of biochemistry and life sciences for separation of macromolecules including proteins. During the past decade, the use of these agents to improve the separation of basic drugs and some alkaloids has been increasing. Clearly, the example could be seen from the studies of Hashem H. and Jira T[22]. They observed the effects of counteranions on the retention of beta-blocker. Beta-blockers are important substances of therapeutic value in the treatment of cardiovascular disorders and they can be used in the treatment of hypertention, angina pectoris, arrhythmia and congestive heart failure[23]. Although there were exceptions for some experiments, from the majority of studies they found that increasing of salt (counteranion) concentration led to the increasing of retention factor (k′). Two salts (i.e., perchlorate and dihydrogen phosphate) were examined in this study. The ability to increase the retention factor depends on salt types. They found that perchlorate anion was more efficient than dihydrogen phosphate anion, since anion solvation is related to the increasing of retention and the hydration of ions different from ions to ions. The anion that was least capable of being solvated leads to the greatest disruption of the analyte solvation and consequently the increase in analyte hydrophobicity. Dihydrogen phosphate anion in aqueous environment is highly solvated due to its hydrogen bonding capabilities. Perchlorate anion has four electron withdrawing oxygen atoms, which lead to delocalization of the charge density. By this property, perchlorate is lower solvated than dihydrogen phosphate anion. Piloz K. and Choma I[24]. presented the improvement of tetracyclines and flumequine separated with chaotropic effect. Four tetracyclines and flumequine could be separated well from each other on reversed-phase column with the mobile phase containing potassium perchlorate. Complete chromatogram could be achieved in 11 min.

Chaotropic agents can also be used to improve the separation of basic alkaloids[25-26]. Flieger J. studied the retention behavior of selected alkaloids from different classes and also tried to find the actual chaotropic mechanism on the retention of basic analytes. The author found that the addition of anionic salts to the mobile phase leads to the increase in retention, efficiency and separation selectivity of examined compounds. The order of their ability is in agreement with Hofmeister series. The important observation from his studies is ion-associated complex between chaotropic anion and basic analyte, which seem to be the most dominating mechanism apart from ion exchanging mechanism for the retention of basic ionizable substances.

Conclusion

In summary, chaotropic agents used as additives in mobile phase of HPLC in reversed-phase mode are small inorganic anions such as $H_2PO_4^-$, CF_3COO^-, BF_4^-, ClO_4^-, and PF_6^-. The use of these ions aims to improve the retention, separation efficiency and peak asymmetry of basic analytes. The separation of basic compound in reversed-phase HPLC usually suffers from some limitations. For example, the analyte's peak closes to unretained-compound, the mobile phase pH may be harmful to stationary phase material when the operation is done in the condition that forces the analyte into its neutral form. Moreover, the increasing load of basic analytes in order to increase analyte sensitivity can lead to a decrease in apparent peak efficiency and an increase in peak tailing. However, if an analysis must be performed at a relatively high sample load, the addition of a chaotropic additive may be employed to increase the apparent peak efficiency and symmetry. Much higher loading capacities could be obtained by operating columns with these mobile-phase additives without substantial deterioration in efficiency.

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