Influence of variation in mobile phase pH and solute pK_a with the change of organic modifier fraction on QSRRs of hydrophobicity and RP-HPLC retention of weakly acidic compounds

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ABSTRACT

The variation in mobile phase pH and ionizable solute dissociation constant (pK_a) with the change of organic modifier fraction in hydroorganic mobile phase has seemingly been a troublesome problem in studies and applications of reversed phase high performance liquid chromatography (RP-HPLC). Most of the early studies regarding the RP-HPLC of acid–base compounds have to measure the actual pH of the mixed mobile phase rigorously, sometimes bringing difficulties in the practices of liquid chromatographic separation. In this paper, the effect of this variation on the apparent n-octanol/water partition coefficient (K_{ow}'') and the related quantitative structure–retention relationship (QSRR) of log K_{ow}'' vs. log k_w, the logarithm of retention factor of analytes in neat aqueous mobile phases, was investigated for weakly acidic compounds. This QSRR is commonly used as a classical method for K_{ow} measurement by RP-HPLC. The theoretical and experimental derivation revealed that the variation in mobile phase pH and solute pK_a will not affect the QSRRs of acidic compounds. This conclusion is proved to be suitable for various types of ion-suppressors, i.e., strong acid (perchloric acid), weak acid (acetic acid) and buffer salt (potassium dihydrogen phosphate/phosphoric acid, PBS). The QSRRs of log K_{ow}'' vs. log k_w were modeled by 11 substituted benzoic acids using different types of ion-suppressors in a binary methanol-water mobile phase to confirm our deduction. Although different types of ion-suppressor all can be used as mobile phase pH modifiers, the QSRR model obtained by using perchloric acid as the ion-suppressor was found to have the best result, and the slightly inferior QSRRs were obtained by using acetic acid or PBS as the ion-suppressor.

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1. Introduction

Hydrophobicity, generally expressed by n-octanol/water partition coefficient (K_{ow}), constitutes an important physicochemical parameter conventionally used in quantitative structure–retention relationship (QSRR) studies for various bioactive compounds including pharmaceuticals and natural products [1–5]. The relationship between log Kow and log k_w, the logarithm of retention factor (k) of analyte obtained by extrapolating to neat aqueous mobile phase in reversed-phase high performance liquid chromatography (RP-HPLC),

is usually adopted for Kow determination, which is also known as Collander equation [6–9]. In general, Collander equation is limited to neutral solutes. In fact, most biomedical molecules are more or less dissociated, therefore, buffers including acids and bases are added into the mobile phase to suppress the dissociation of compounds with acid–base properties, which results in improved chromatographic retention and peak shape in RP-HPLC [10–12]. However, the dissociation is completely suppressed only when the pH of mobile phase was adjusted to at least 2 pH units lower than dissociation constant (pK_a) of the solute, which means that very strong acidity of mobile phase is necessary for those compounds with small pK_a value (e.g., p$K_a \leq 4$), decreasing the life of chromatographic columns as well as apparatus. The apparent n-octanol/water partition coefficient (K_{ow}'') has been proposed to correct K_{ow} so as to describe the hydrophobicity of ionizable solutes more precisely. Moreover, we have reported the RP-HPLC retention behavior of carboxylic acids and phenols by using acetic or perchloric acid as the ion-suppressor in binary hydroorganic mobile phase, as well as the QSRRs of hydrophobicity and retention for these weak acids. A better linear

relationship relating $\log K_{ow}''$ with $\log k_w$ than that relating $\log K_{ow}$ with $\log k_w$ has been established and applied successfully to K_{ow} measurement and retention prediction of weakly acidic compounds in our previous studies [13–16].

In RP-HPLC procedure, the retention of an ionizable compound depends on its hydrophobicity and ionization degree, which in turn depends on mobile phase pH and analyte pK_a. It is generally acknowledged that both mobile phase pH and solute pK_a vary with the change of organic modifier fraction in the mobile phase [17]. Therefore, precise measurement and control of pH and pK_a are essential to correct analysis results. The IUPAC has endorsed rules and procedures for pH standardization of electrode systems in water and in binary aqueous-organic solvent mixtures commonly used as mobile phases in RP-HPLC [18–20]. On the basis of IUPAC recommendations, three different pH scales are usually employed in pH measurement of mobile phase in RP-HPLC. The most common is the aqueous pH scale ($_w^w$pH), which is obtained by calibrating the electrode system with aqueous buffers (w), and mobile phase pH is measured in aqueous fraction (w) before mixing it with the organic modifier [21]. If the electrode system is calibrated with aqueous buffers, but mobile phase pH is measured after mixing the aqueous fraction with the organic modifier, the pH scale in the mobile phase solvent (s) relative to water (w) as standard-state solvent is obtained as $_w^s$pH. If the electrode system is calibrated with buffers prepared by mobile phase solvent (s), and pH is measured in the same mobile phase solvent (s), the pH scale is obtained as $_s^s$pH. $_w^s$pH or $_s^s$pH scale is recommended to express true mobile phase acidity, because they clearly represent variation of the mobile phase pH with the change of the organic modifier content. However, working in the $_s^s$pH scale requires preparation and maintenance of the standard mixed solvents, most of which are not commercially available, thus $_w^s$pH is usually chosen to describe mobile phase pH in practice. Similarly, the symbols s and w used for pH scales can also be extended to solutes pK_a scales, and accordingly there are three different pK_a scales, i.e., $_w^w pK_a$, $_w^s pK_a$ or $_s^s pK_a$, in dissociation characterization of analytes in RP-HPLC mobile phase [22].

Subirats et al. [17] pointed out that the variation of mobile phase pH and solute pK_a resulting from the change of organic modifier fraction in the mobile phase affect the retention of acid–base compounds on RP-HPLC. They established the precise relationships between true mobile phase pH and organic modifier fraction, which are very useful to estimate the actual pH variation of mobile phase with the change of the organic modifier content when the measurement of mobile phase pH is not easy to operate, i.e., in the case of highly automated liquid chromatographic experiments where different mobile phase components from independent reservoirs are pumped into and mixed within the apparatus. They also derived a number of models that relate the retention of an acid–base compound with its pK_a and the organic modifier fraction in the mobile phase. These models are greatly helpful in predicting the proper mobile phase composition in which the differences on ionization degree between analytes with similar aqueous pK_a values are significant, thereupon improving the selectivity of RP-HPLC for acid–base compounds and explaining the relative separation mechanism, as well as avoiding fruitless experimental time and reagent consuming.

Since the retention times of acid–base compounds are affected by mobile phase pH and solute pK_a, the influence of the variation in pH and pK_a seems involuntarily necessary to be considered when studying the QSRRs of $\log K_{ow}''$ vs. $\log k_w$ for weakly ionizable compounds. However, in our previous studies on the QSRRs of $\log K_{ow}''$ vs. $\log k_w$ for acidic compounds, direct adoption of $_w^w$pH and $_w^w pK_a$ always gave satisfactory results. This phenomenon has attracted our attention on the reason behind.

In definition, the k_w value of a weak acid at a certain mobile phase pH is the weighted average of retention factors of all the neutral and ionic species formed in the neat aqueous fraction of mobile phase [23]. Accurate pH value of the neat aqueous fraction of mobile phase refers to $_w^w$pH. At a specific $_w^w$pH, the ratios of all the neutral and ionic species of the weak acid and their contributions to k_w are invariable. Therefore, k_w value is definitely independent of the organic modifier fraction in the mobile phase. The main purpose of this paper is to find out whether K_{ow}'', as well as the related QSRRs of $\log K_{ow}''$ vs. $\log k_w$ for weakly acidic compounds are influenced by the variation in $_w^w$pH or $_s^s$pH of mobile phase and $_w^s pK_a$ or $_s^s pK_a$ of acidic solute arising from the change of organic modifier fraction. In addition, the QSRRs of $\log K_{ow}''$ vs. $\log k_w$ modeling by 11 substituted benzoic acids were compared by using three types of ion-suppressors, i.e., strong acid, weak acid and buffer salt, and the role of different ion-suppressors on the retention mechanism was preliminarily investigated.

2. Theoretical basis

An acid–base equilibrium for a monoprotic weak acid in its diluted aqueous solution ruled by $_w^w pK_a$ is described as

$$HA = H^+ + A^- \tag{1}$$

with $_w^w K_a = a_{(H^+)} \times a_{(A^-)}/a_{(HA)}$. Due to the diluted solution of the studied system, the activities of species can be replaced by concentrations

$$_w^w K_a = \frac{c_{(H^+)} \times c_{(A^-)}}{c_{(HA)}} \tag{2}$$

When the aqueous solution is diluted with an organic modifier, the concentrations of species existing in the solution are changed. Given the addition of the organic modifier affects all the species to the same degree [24], the concentrations of species change from $c_{(H^+)}$, $c_{(A^-)}$ and $c_{(HA)}$ to $c_{(H^+)}\varphi_{H_2O}$, $c_{(A^-)}\varphi_{H_2O}$ and $c_{(HA)}\varphi_{H_2O}$, respectively, where φ_{H_2O} is the volume fraction of water in the mobile phase, thereby $_s^s pK_a$ is represented as

$$_s^s K_a = \frac{c_{(H^+)} \times \varphi_{H_2O} \times c_{(A^-)} \times \varphi_{H_2O}}{c_{(HA)} \times \varphi_{H_2O}} = \frac{c_{(H^+)} \times c_{(A^-)} \times \varphi_{H_2O}}{c_{(HA)}}$$
$$= _w^w K_a \times \varphi_{H_2O} \tag{3}$$

The difference between $_s^s pK_a$ and $_w^w pK_a$, ΔpK_a, is obtained

$$\Delta pK_a = _s^s pK_a - _w^w pK_a = -\log\varphi_{H_2O} \tag{4}$$

In general, buffers used as ion-suppressors may be strong or weak acids or bases. If an aqueous buffering solution is prepared from a strong monoprotic acid, e.g., perchloric acid, as Espinosa et al. discussed [25], the difference between $_s^s$pH and $_w^w$pH of the solution, i.e., ΔpH is

$$\Delta pH = _s^s pH - _w^w pH = -\log\varphi_{H_2O} \tag{5}$$

The equalities $\Delta pH = _s^s pH - _w^w pH = _s^s pK_a - _w^w pK_a = \Delta pK_a$ are obtained by comparing Eq. (4) with Eq. (5), which can be alternated to

$$_s^s pH - _s^s pK_a = _w^w pH - _w^w pK_a \tag{6}$$

As Subirats et al. proposed [17], $_w^s pH - _s^s pH = _w^s pK_a - _s^s pK_a = \delta$, where δ is a constant that depends only on the organic modifier used. These equalities can be transformed to

$$_s^s pH - _s^s pK_a = _w^s pH - _w^s pK_a \tag{7}$$

By comparing Eq. (6) with Eq. (7), the following equalities are obtained

$$_s^s pH - _s^s pK_a = _w^s pH - _w^s pK_a = _w^w pH - _w^w pK_a \tag{8}$$