

Summer Scholar Report

Characterization of a Zwitterionic C18 Stationary Phase Used in Liquid Chromatography

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Introduction

Reversed-phase liquid chromatography (RPLC) has long been the most widely used of all liquid chromatographic methods [1, 2]. The most common RPLC stationary phases are built on silica supports and derivatized with octadecane (C_{18}). With C_{18} phases, the bulk of the retention of a solute is due to a partition mechanism where the solute can completely dissolve in both the mobile and stationary phases [3]. However, some basic solutes do not perform adequately with traditional C_{18} stationary phases, exhibiting tailing peak shapes. These poor peak shapes are typically caused by the solute interacting with residual silanols on the surface of the silica through an adsorption mechanism [4-6]. For this reason, basic solutes are generally separated on RPLC stationary phases under carefully controlled conditions [7]. It has been found that at neutral pH, basic solutes exhibit severe peak tailing, yet at basic pH, the peak shape improves dramatically [8]. Symmetric peak shapes are required in a chromatographic separation for adequate resolution and quantitation. These factors are especially important when running problematical basic compounds, which make up a large proportion of pharmaceuticals. As such, pharmaceutical research and industry have much to benefit from an improvement of chromatographic peak shape.

Figure 1: Diagram of the zwitterionic stationary phase Obelisc R. Figure adapted from Sielc Obelisc web material. (http://www.sielc.com/Products_Obelisc.html, accessed Sep 2010)



A stationary phase new to the market, Obelisc R, has been manufactured and marketed by Sielc for the improved separation of polar and basic solutes in liquid chromatography (LC). The Obelisc R stationary phase is based on a zwitterionic chain chemistry, where a cationic group near the support is connected by a hydrophobic chain to an anionic group (Figure 1). The Obelisc R phase is advertised as a replacement for phases with a variety of different retention mechanisms including: reversed phase, ion exchange, and ion exclusion. However, the combined retention mechanism for this new zwitterionic stationary phase has yet to be characterized. In this research, evaluation of the reten-

tion and peak shape (tailing factor) of neutral and basic solutes is performed to provide a deeper understanding of the retention process for a zwitterionic stationary phase. A comparison of the Obelisc R zwitterionic phase to a traditional C_{18} phase using methanol and methanol-water mobile phases is performed.

Experimental Methods

An HPLC system (Shimadzu) consisting of a pump (flow rate of 1.00 mL/min), a UV-Vis detector (210 nm), and a Rheodyne injection valve (10 μ L loop) was used for all experiments. An Econosil C_{18} column (4.6 x 250 mm, 10 μ m particle size) and a Sielc Obelisc R column (4.6 x 100 mm, 5 μ m particle size) were used for the investigation. Six different isocratic mobile phases were employed, from 100% methanol (Alfa Aesar) to 75% methanol – 25% unbuffered Mega Ohm H_2O (Millipore). The pH of the water was measured at pH 9.0.

Solutions of uracil, amitriptyline, propranolol, nortriptyline, propranolol (Sigma), toluene (Fisher Scientific), ethylbenzene, benzene (Alfa Aesar), and quinizarin (Aldrich) were prepared in HPLC grade methanol (Alfa Aesar) with concentrations ranging from 10^{-2} to 10^{-4} M. Uracil was used as a nonretained marker. The solutes in this study are commonly used in stationary phase characterization and provide a method to directly compare phases characterized outside of this study [9]. The structures and designation of these solutes as neutral hydrocarbons or basic solutes are displayed in Figure 2.

Data Analysis

Each peak was baseline subtracted using a second derivative zero and subsequently fit with one to two asymmetric double sigmoidal (ADS) functions, using commercially available

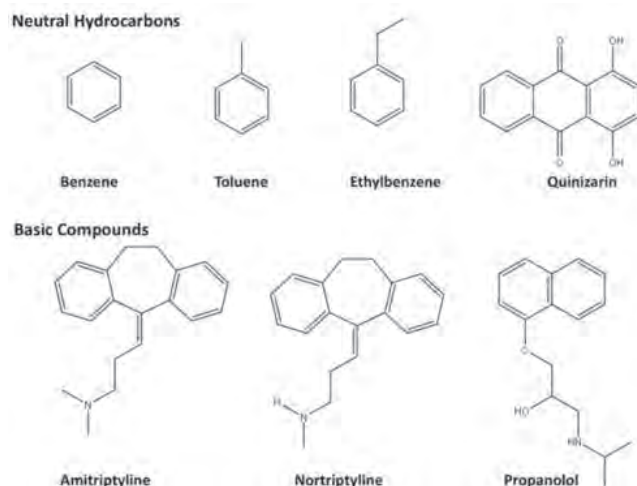


Figure 2: Structures of neutral and basic solutes used in this study.

software (PeakFit 4.2, Systat Software Inc.). The number of ADS functions was chosen in order to minimize residuals for the fit. Statistical moments were calculated from the fitted peak, using commercially available software (Microsoft Excel). In the statistical moment method, the moments may be calculated directly from the zone profiles, without *a priori* assumption of peak shape. The first moment (M_1) represents the mean retention time and is calculated by

$$M_1 = \frac{\int C(t)t dt}{\int C(t)dt}$$

where $C(t)$ is the solute concentration as a function of time (t). The retention factor is determined by

$$k = \frac{M_{1,t_r} - M_{1,t_0}}{M_{1,t_0}}$$

where M_{1,t_0} and M_{1,t_r} are the first moment for the solute and the nonretained marker, respectively. The retention factor provides a measure of the solute's affinity towards the stationary phase and can be used as a direct comparison between two stationary phases [9]. The US Pharmacopeia (USP) peak tailing factor (T_f) is a measure of peak symmetry and is useful when discussing peak asymmetry [10]. T_f is calculated using

$$T_f = \frac{a + b}{2a}$$

where a and b are the front and back half-widths at 5% of the peak height. $T_f = 1.0$ for a symmetric peak; $T_f > 1.0$ for a tailing peak; $T_f < 1.0$ for a fronting peak.

Results & Discussion

Traditional C_{18} Stationary Phase

Neutral Hydrocarbons

The retention factor (k) provides a measure of the solute's affinity for the stationary phase. A small retention factor indicates only a small degree of interaction of the solute with the stationary phase, while a larger retention factor indicates more interaction with the stationary phase material. The retention factors of the hydrocarbons separated on the C_{18} phase with pure methanol mobile phase are summarized in *Table 1*. Quinizarin, a common metal chelating agent, is the most retained solute. The remaining hydrocarbons have similar k values, near 1, showing low retention on C_{18} with pure methanol mobile phase. The influence of the water content on the retention factor is shown in *Figure 3*. As water percent increases from 0 – 25 %, retention factors

	C18	Zwitterion
Benzene	0.28	-0.01
Toluene	0.32	-0.01
Ethylbenzene	0.51	-0.01
Quinizarin	1.11	0.09
Amitriptyline	-0.19	-0.24
Nortriptyline	-0.18	-0.18
Propranolol	-0.26	-0.29

Table 1 – Retention factors (k) in pure methanol.

increase by a factor of 3 to 10. Overall, the hydrocarbons become slightly more retained on the C_{18} phase as the polarity of the mobile phase increases.

The tailing factor (T_f) of a solute provides a measure of the peak shape. The magnitude of the tailing factor hints at the rates at which the solute enters and exits the stationary phase. For instance, if a peak shape shows tailing, some of the solute molecules are remaining in the stationary phase longer than the average, indicating an increased interaction with a “slow” site within the stationary phase. In silica materials, this “slow” site can be residual silanols in the support. Thus, the tailing factor provides an indication of the kinetics the solute entering and exiting the stationary phase. The tailing factors of the hydrocarbons in pure methanol can be seen in *Table 2*. The tailing factors for the neutral hydrocarbons are slightly greater than 1 on the C_{18} phase, indicat-

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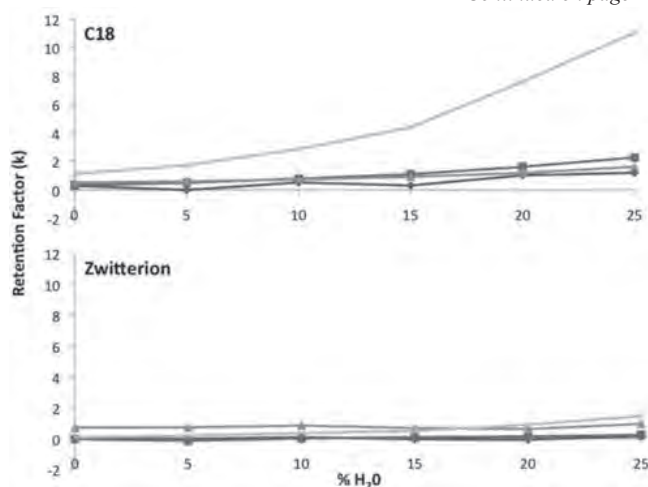


Figure 3: Retention factors (k) of the neutral hydrocarbon solutes (benzene, toluene, ethylbenzene, and quinizarin) plotted as a function of mobile phase composition.

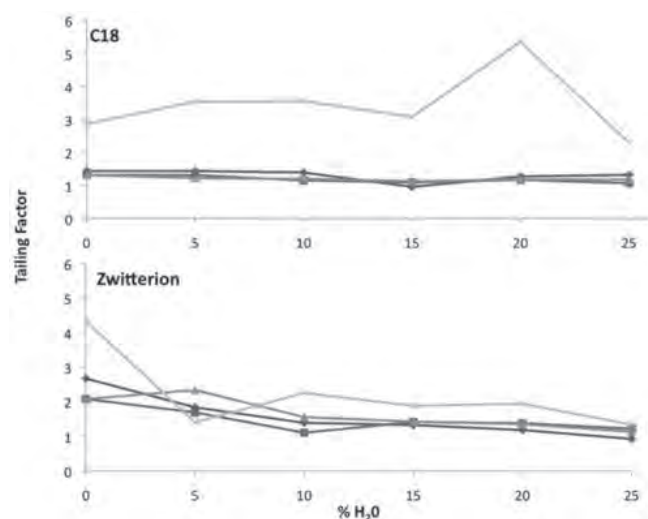


Figure 4: Tailing factors (T_f) of the neutral hydrocarbon solutes (benzene, toluene, ethylbenzene, and quinizarin) plotted as a function of mobile phase composition.

Summer Scholar

Continued from page 9

ing a slight tailing peak shape. Most of the neutral hydrocarbons have similar tailing factors and thus peak shape. However, quinizarin has a more tailing peak shape, indicating quinizarin has a stronger interaction with “slow” sites in the phase. Quinizarin is a common metal chelating agent and thus may be interacting with metals dissolved in the stationary phase support. The effect of the mobile phase water content on tailing factor is shown in *Figure 4*. The tailing factors remain fairly constant for the neutral hydrocarbons as a function of mobile phase water content. Thus, the polarity of the mobile phase does not affect peak shape of hydrocarbons on the C₁₈ stationary phase.

Basic solutes

All basic solutes have negative *k* values, indicating they elute before the nonretained marker, uracil (*Table 1*). This could indicate that the solutes are ionic in nature. As water percent increases from 0 – 25 %, retention factors remain constant and consistently low (*Figure 5*). Thus, the retention of basic compounds is not controlled by the amount of water in the mobile phase. Future work with the mobile phase pH will examine this retention effect.

The tailing factors for the bases are slightly less than 1 on the C₁₈ phase, indicating a fronting peak shape (*Figure 6*). The basic compounds have similar tailing factors and thus peak shape. The effect of the mobile phase water content on tailing factor is shown in *Figure 6*. The tailing factors remain fairly constant for the basic compounds as a function of mobile phase water content. The fronting peak shape is interesting, as it is not typically expected for basic solutes under reversed phase conditions. However, the pH here is unbuffered at 9.0. Thus, it will be interesting to see the difference in peak shape as a function of pH in future studies.

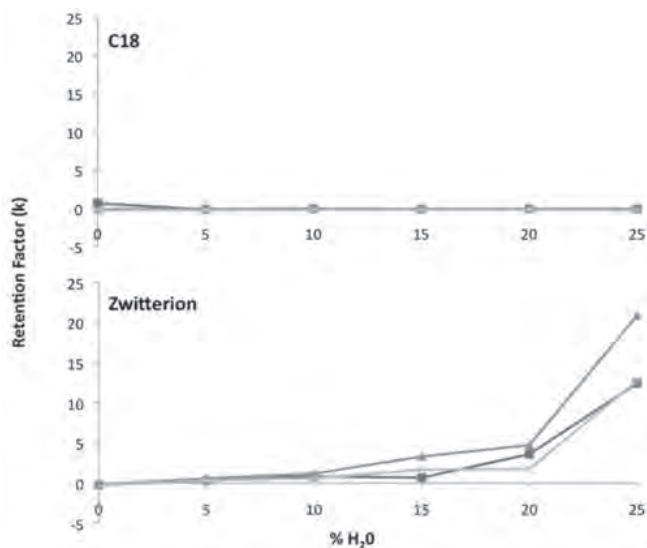


Figure 5: Retention factors (*k*) of the basic solutes (amitriptyline, nortriptyline, and propanolol) plotted as a function of mobile phase composition.

	C18	Zwitterion
Benzene	1.44	2.67
Toluene	1.31	2.07
Ethylbenzene	1.31	2.07
Quinizarin	2.86	4.33
Amitriptyline	0.71	0.90
Nortriptyline	0.70	0.93
Propanolol	0.82	0.92

Table 2 – Tailing factors (*T_p*) in pure methanol.

Zwitterionic Stationary Phase

Neutral Hydrocarbons

The retention factors are small and slightly negative for almost all hydrocarbons on the zwitterionic phase (*Table 1*). The negative retention factor indicates the solute elutes earlier than the nonretained marker. In comparison, the retention factors are much smaller for almost all solutes on the zwitterionic phase than on the C₁₈ phase. As water content increases from 0 – 25 %, retention factors remain fairly constant, with only a slight increase (*Figure 3*). This is an interesting difference between the C₁₈ and the zwitterionic phases, showing water may not play the same role in retention as it does in the traditional C₁₈ material.

The tailing factors for the neutral hydrocarbons are greater than 1 on the zwitterionic phase, indicating a tailing peak shape (*Table 2*). Thus, the neutral hydrocarbons have an interaction with some type of “slow” site in the zwitterionic phase. Overall, the neutral hydrocarbons have a more symmetric peak shape on the C₁₈ phase than on the zwitterionic phase. However, more research would be needed to identify the nature of the “slow” site since the stationary phase chemistry is quite different from traditional C₁₈ derivatization on silica. The tailing factors for the neutral hydro-

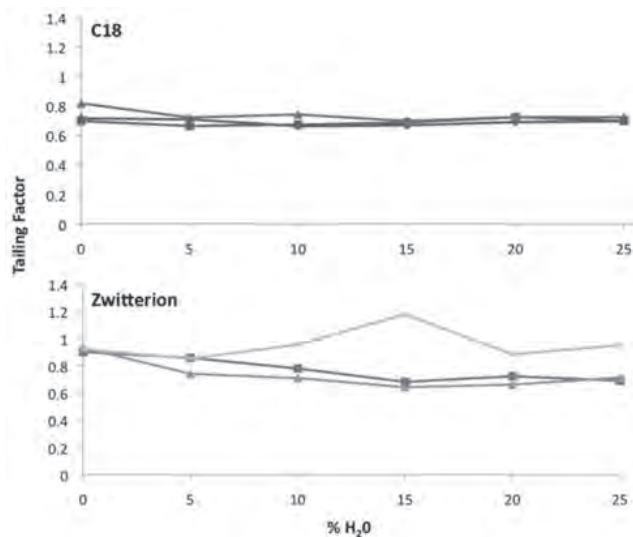


Figure 6: Tailing factors (*T_p*) of the basic solutes (amitriptyline, nortriptyline, and propanolol) plotted as a function of mobile phase composition.

carbons decrease as a function of mobile phase water content (Figure 4). Thus, the polarity of the mobile phase positively influences the peak shape of hydrocarbons on the zwitterionic stationary phase.

Basic solutes

The retention factors are negative for all basic compounds, showing no retention on the zwitterionic material (Table 1). The basic solutes are less retained than the hydrocarbons on the zwitterionic phase. Retention for the basic solutes is similar for both stationary phases, indicating no change in retention for the two phases with pure methanol mobile phase. Retention factors increase drastically as a function of water content (Figure 5). As water content increases from 0 – 25 %, retention factors increase by a factor of 12 to 20. While only a small difference in retention was observed for the neutral hydrocarbons, there is a distinct difference observed for the basic solutes. In addition, this shows that water may play a critical role in the retention mechanism for the zwitterionic material. Future studies will continue to investigate this effect as a function of pH and solute type.

The tailing factors for the basic compounds are slightly less than one on the zwitterionic phase, indicating a slightly fronting peak shape (Table 2). All the basic compounds have similar tailing factors and thus peak shape. Interestingly, peak shapes are slightly more symmetric (less fronting) on the zwitterionic phase compared to the C18 phase for the basic solutes. The tailing factors for the basic solutes slightly decrease as a function of mobile phase water content (Figure 6). Thus, the polarity of the mobile phase does

have a large effect on the peak shape of the basic solutes.

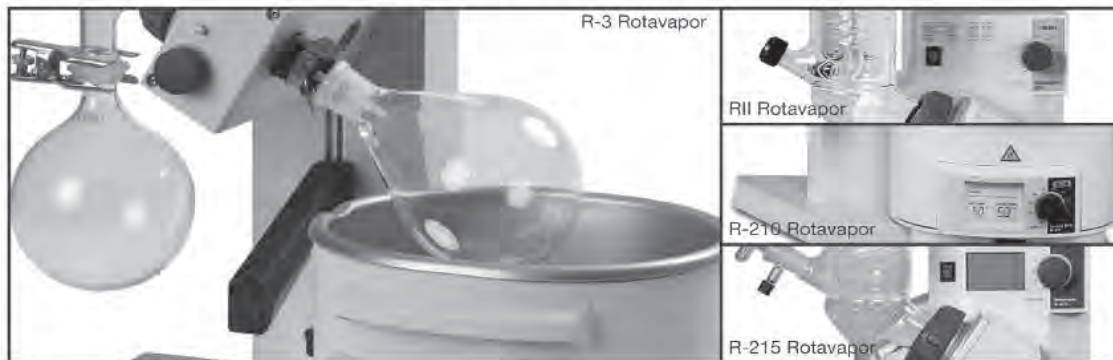
Conclusion

This study is among the first to compare and characterize the Obelisc R stationary phase with the traditional C₁₈ stationary phase used in RPLC. In this study, the retention factor and tailing factor of neutral hydrocarbon and basic solutes have been systematically evaluated. Overall, neutral hydrocarbons are retained and basic solutes are not retained by the C₁₈ stationary phase. Neutral hydrocarbons show a slightly increasing trend in retention, while basic compounds show an even smaller increase in retention as percent water in the mobile phase is increased. In general, all neutral solutes are less retained on the zwitterionic phase than on the C₁₈ stationary phase. Basic solutes have similar retention (negative retention factors) in pure methanol. Interesting differences are observed for the two phases as a function of water content in the mobile phase. In general, the C₁₈ phase has more symmetric peak shapes for the neutral hydrocarbons in pure methanol. However, as the water content in the mobile phase is increased, the peak shapes for the zwitterionic phase improved for the neutral hydrocarbons. In contrast, the zwitterionic phase has more symmetric peak shapes for the basic solutes in pure methanol. The water content in the mobile phase has no influence on the peak shape of basic solutes on either phase. Based on these results, Obelisc R shows promise in RPLC for separation of basic solutes and neutral hydrocarbons with a polar mobile phase. Future research involving pH of the mobile phase

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will provide more insight into the retention mechanism of this novel stationary phase.

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