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The influence of water on the memory effect of the amylose tris(3,5-dimethylphenyl carbamate) stationary phase

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ABSTRACT

Acid/base modifiers are sometimes used as additives in normal phase elution on columns packed with CHIRALPAK® AD®. These modifiers affect enantioseparations in ways that are not yet fully understood for the lack of systematic studies. Shifts of the selectivity of certain pairs of enantiomers upon exposure of the column to these modifiers is amply documented. Furthermore, once the modifier has been removed from the mobile phase, the modified selectivity remains, which has been named the Memory Effect. After a column has been exposed to an eluent stream containing acidic/basic modifiers, this particular column no longer separates certain enantiomeric pairs with the same selectivity as a modifier naive column. This makes the transfer of developed methods from one to other CHIRALPAK AD columns difficult to predict, if the selectivity needs to be similar between the two columns. We selected four enantiomeric pairs for a systematic study of this Memory Effect. The selectivity of 4-chlorophenylalanine ethyl ester improves after a solution of ethanesulfonic acid (ESA) is percolated through the column. The selectivity of Propranolol and Tröger's base increases after a solution of Diiospropylamine is percolated through the column. The selectivity of Propranolol and Tröger's base enantiomers is inversely affected by percolation of the acid solution. The 4-chlorophenylalanine ethyl ester enantiomers is inversely affected by percolation of the base solution. In contrast, the selectivity of trans-stilbene oxide (TSO) is not affected by either modifier. Analytical studies of the stationary phase suggest that slow protonation/deprotonation of water molecules attached to the carbamate moiety may be responsible for the acid/base Memory Effect. To further the understanding of the effect of water on the Memory Effect, mobile phases – spiked with water (0.01–0.43%) – were used to measure changes in the Memory Effect. Finally, we showed that the influence of water on the Memory Effect can be minimized by percolating through the column a sufficiently concentrated solution of the appropriate base while using dried mobile phases.

1. Introduction

Two issues arise when normal phase separations of enantiomers are carried out on columns packed with CHIRALPAK AD as the stationary phase. Poor reproducibility of certain separations has been addressed by numerous groups. Three different conditions have been suspected to be the main contributors of the lack of reproducibility of normal phase analysis: the temperature [1], the silica type [2], and the water content [3–6] have all been identified as probable factors. The Acid/Base Memory Effect is the second issue that arises when using this stationary phase. When the column is percolated with either an acid or a base modifier, the chiral environment is changed significantly. After removing the acid/base modifier from the mobile phase, the selectivity of certain enantiomers remains influenced, as if the acid/base modifier were still in use. As a result of this change in the chiral environment, the selectivity of some enantiomeric pairs can be drastically affected. This residual modification of the selectivity for certain enantioseparations is called the Acid or Base Memory Effect.

In the pharmaceutical industry, multiple CHIRALPAK AD columns are needed when using different mobile phases and modifiers to analyze enantiomeric purity, in order to avoid variations in separation results [7]. To minimize the possible consequences of such variation, a laboratory will have one column for each type of mobile phase used: one column for normal phase, one column

Abbreviations: mAUs, milli Absorbance Units; mV, milliVolts; ESA, Ethanesulfonic Acid; DiPA, N,N-Diisopropylethylamine; TSO, *trans*-Stilbene Oxide; TFA, Trifluoroacetic Acid; CH_3C00H, Acetic Acid; DEA, Diethylamine; PTFE, Polytetrafluoroethylene; HPLC, High Performance Liquid Chromatography; 4CPEE, 4-Chlorophenylalanine Ethyl Ester; MP, mobile phase.

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for polar normal phase, and one for reversed phase. In addition to this, a laboratory will have one column for each type of modifier used: a column equilibrated with a mobile phase containing the acidic additives (Ethanesulfonic Acid – ESA, Trifluoroacetic Acid – TFA, Acetic Acid, etc.), a column equilibrated with a mobile phase containing the basic additives (Diethylamine – DEA, N,N-Diisopropylethylamine – DiPA, etc.), and a column equilibrated with mobile phases that do not receive any additives. For example, in the separation of 4-chlorophenylalanine ethyl ester, the suppression of the acidic portion of the amino acid by the use of an acidic mobile phase modifier is required for the separation to exist [8], yet once the column has been exposed to the acidic mobile phase modifier the stationary phase does not separate enantiomers that require basic mobile phase modifiers similarly to modifier naive columns [9]. This is a costly and cumbersome way for laboratories to remove the variations in separation results due to the Memory Effect. The goal of this work was to clarify the reasons for the additional columns required for acidic and basic modifiers and to propose possible remediations.

2. Experimental

2.1. Equipment

An HP 1100 (Agilent, Santa Clara, CA, US) was used to carry out all experiments and measurements reported here. A single isocratic pump and batches of prepared solutions were used, to eliminate possible variations of the alcohol concentration during individual tests. A column heater was used to control the separation temperature at 40 °C. An autosampler was used to allow for repetitive injections over extended runs of 56 h. A single wavelength detector was used because all enantiomeric pairs tested had excellent signal to noise ratio at 210 nm.

2.2. Chemicals

The mobile phase consisted of hexanes (a mixture of normal and branched hexanes) and Reagent Grade ethanol; both were received and used without further purification from Fisher Scientific (Pittsburgh, PA, USA). The 4-chlorophenylalanine ethyl ester (4CPEE), Ethanesulfonic Acid (ESA), tri-tert-butyl benzene (which was used to mark the column volume), Tröger's Base, and Propranolol Hydrochloride were obtained from Sigma–Aldrich (St. Louis, MO, USA). The *trans*-Stilbene Oxide (TSO) was received from Acros Organics (Geel, Belgium). The Diisopropylamine (DiPA) was obtained from Alfa Aesar (Ward Hill, MA, USA). All chemicals were not further purified prior to use.

2.3. Columns and stationary phase

The analytical 4.6×150 mm columns used for these studies were packed by Chiral Technologies (West Chester, PA, USA). The analytical columns had been used in previous studies of the Memory Effect but had not been exposed to any mobile phase or additive other than those which were documented in a previous publication [10,9]. In particular, the column was exposed to ESA, Ethanol, DiPA, and Hexanes as mobile phases and additives. The columns were also exposed to the enantiomeric pairs of TSO, 4CPEE, 4-chlorophenylalanine methyl ester, Ketoprofen, Propranolol, and Tröger's Base.

3. Procedure

The data was collected using two different time scales. In both methods, the concentrations of the racemic samples were approximately 1 mg/mL. The injection volume of each sample was adjusted to ensure that the integrated areas of the bands of each enantiomer was between 20,000 and 30,000 milli Absorbance Units times seconds (mAUs) – as measured by the Agilent Chemstation, in order to minimize the fluctuations of retention time for the particular pair for the enantiomers injected onto the column due to changes in the injected mass of enantiomer. Peak height, in milli Aborbance Units (mAU) or millivolts (mV), cannot be used due to the measurement of changing retention as the selectivity of the enantiomeric separation is influenced by the residual acidic mobile phase modifier. All separations were done with a 90:10 hexanes/ethanol mobile phase. Sample evaporation was controlled by using 200 μL inserts for the 1.8 mL High Performance Liquid Chromatography (HPLC) sample vials. Each vial was sealed with a Polytetrafluoroethylene (PTFE) septum and used only during one injection. Controlling the rate of sample evaporation was critical to insure identical masses of enantiomeric pairs would be injected onto the column over the 56 h of continuous operation. Once a sample vial has been punctured by the needle, even the smallest hole will allow the hexanes used to prepare the sample to begin to evaporate, changing the concentration of the injected sample. The column temperature was held constant at 40 °C. The temperature was controlled at 40 °C to eliminate instabilities caused by changes in the ambient temperature. Recognition in chiral chromatography is controlled by weak forces such as hydrogen bonding and dispersion forces, slight changes in temperature will influence the retention of enantiomeric pairs. By controlling the temperature well above room temperature any fluctuation in selectivity due to changes in ambient temperature can be minimize. When the columns had to be treated with an acid or a base modifier, a 100 μL injection of this modifier solution was made; the modifier concentration was approximately 10 mg/mL. For the ESA this solution was at the concentration of 10.8 mg/mL. For the DiPA, this solution was 10.6 mg/mL.

The main difference between the two time scales included the number of repeated sequences and the volume of mobile phase percolated through the column between repeated injections. The first time scale used 1 l of mobile phase and lasted 16 h while the second time scale used 4 l of mobile phase and required continuous operation for over 56 h.

The shorter time scale involved the periodic injection of racemic mixtures onto the column for 16 h. The injection sequence followed was: 4CPEE and then Ethanesulfonic Acid. 4CPEE was chosen due to the documented requirement of ESA to separate this enantiomeric pair [8,10]. The ESA solution was removed from the injection sequence after the 10th injection of the acidic solution had been completed. In all cases, the injections of the 4CPEE enantiomeric pair was repeated every 20–30 min, for the entire 16-h time period.

The second time scale consisted of a longer continuous run time (54 h was the minimum operation time) during which the injection sequence was interrupted to allow for additional time between repeated injections. For longer runs, the injection sequence followed was: 4CPEE, TSO, Propranolol, and then Tröger's Base followed by a period of percolating mobile phase through the column. Because all four racemic pairs were injected during these tests, the injection repeat rate of each pair was greater than 85 min before the same pair was again injected onto the column. The purpose of this additional time between injection was to determine if the Memory Effect was influenced by the racemic analytes themselves. In addition, as the longer run progressed the period of percolating the mobile phase was changed. Initially, the injection sequence was repeated every 103 min, until the end of the first liter of mobile phase (i.e., 650 column volumes). During the elution of this first liter of mobile phase, the acid modifier treatment was carried out. This treatment consisted of an 100 μL injection of the 10.6 mg/mL ESA solution following the Tröger's Base injection. During elution of the second liter of mobile phase, the injection cycle

was repeated every 85 min. The injection cycle was repeated every 116 min during the elution of the third liter of mobile phase. The remaining mobile phase was used with a repeat cycle of 133 min this was accomplished by increasing the mobile phase percolation time after the Tröger's Base injection. The internal column volume of the CHIRALPAK AD column used was 1.55 mL, as a result, the increases from 85 to 116 min and from 116 to 133 min allowed for 20, and then an additional 10 column volumes to pass through the column between repeated injections of the same enantiomeric pair.

Additionally, the short and long runs were designed to detect three indices of the effect of water on the separation of the enantiomers. First,the effect of water as a strong eluent should be detected in the selectivity of each enantiomeric pair. Water having the capacity to act either as an acid or as a base should have an explicit effect on the selectivity of these enantiomers. Second, the effect of water on the Memory Effect would be recognized as a change in the rate of decay in the selectivity. The selectivity of the 4CPEE enantiomers slowly decays after the stationary phase is no longer exposed to an acidic modifier like ESA as Ye et al. showed [7]. Third, since water is a factor in the instability of the reproducibility of retentions and resolutions in normal phase chromatography, the instability should be recognizable in the separation of the enantiomers.

The introduction of using TSO in the longer continuous injection sequence was to address the stability of the stationary phase's ability to separate a enantiomeric pair that does not require a mobile phase modifier. Two aspects of the introduction of water in the mobile phase can be measured. First, the influence of a strong polar mobile phase modifier would decrease the selectivity of the TSO enantiomeric separation. As the water content is increased the selectivity of the TSO enantiomers would decrease. Second, the equilibrium of the new mobile phase condition would be recognized by the stabilization of the TSO selectivity.

The purpose of the longer time continuous injection sequence was designed to measure the changes in selectivity, based on the changes in the mobile phase volume used between repeated injections. The longer time was also designed to measure the retention changes (if any) due to using extremely dried mobile phases instead of the reagent grade mobile phases. This last feature of the test was carried out by replacing the wet mobile phase with one that had been treated with molecular sieves (5A) from Supelco (St. Louis, MO, USA). This dried mobile phase test had two purposes: first, it would detect the difference between using reagent grade alcohol versus 100% ethanol; second, it would determine if using a dry solvent was sufficient to remove the influence of the additional water trapped in the stationary phase, which could be of great interest.

Removing the influence of the Memory Effect from a CHIRALPAK AD column is of great importance. An additional test was carried out in an attempt to remove the effect of the water introduced in the spiked mobile phase. In this test the column was treated with a base (diisopropylamine) to see if this additive was sufficient to remove the influence of water from the Memory Effect. The separation of Tröger's Base and Propranolol would be affected by this addition of base mobile phase modifier onto the stationary phase. DiPA was chosen as the base to act as a neutraliser of the acid influence from ESA. Two results could be expected, one would be based on direct neutralization of ESA, the other would be the neutralization of the residual acidic hydrogen attached to the polymer. If a direct neutralization of ESA occurred then an equal molar mass or more of DiPA would be required. On the other hand, if a residual acidic hydrogen where responsible for the separation of enantiomeric pairs, a much smaller amount of DiPA would be required.

Table 1
The change in selectivity measured during the 8 runs with changes in the water content of the mobile phase.

	% of water in MP	Rate of selectivity decay/h	Rate of selectivity decay/1000 column volumes
16 h runs			
1	No additional water	0.0105	0.272
2	0.1	0.00754	0.148
3	0.2	0.00599	0.155
4	0.3	0.00374	0.0965
56 h runs			
1	No additional water	0.0111	0.287
2	0.1	0.000222	0.0574
3	4.3	0.000918	0.0297
4	Dried	0.00276	0.0712

4. Results and discussion

Fig. 1 shows the results of the 16 h runs. The improvement in the selectivity of 4CPEE can due to the percolation of 10 injection of the 100 μL ESA solution can be seen during the first 4 h of the graph. The maximum selectivity for the 4CPEE enantiomeric pair was reached after the seventh acidic solution injection. This maximum selectivity can be seen as the plateau reached at a selectivity of 2.8. The selectivity of the enantiomers of the 4CPEE remained constant during the rapid injection sequences (Fig. 1 – dry mobile phase) after the acid equilibration, with a barely significant drop from 2.78 to 2.70 over 12 h, a loss of selectivity of only 0.0105 selectivity units per hour. Noticeably, however, the Memory Effect requires that ethanesulfonic acid be re-introduced onto the stationary only during the time when the dry mobile phase is used. As for the wetter mobile phase (Fig. 1 – ≃0.1% water, ≃0.2% water, and ≃0.3% water) the addition of ESA to the column has no effect. Stationary phases that have been exposed to water prior to treatment with acid modifiers are more susceptible to the acid modifier and the Acid Memory Effect.

The stability of the Memory Effect can be recognized by a decrease in the selectivity over time. In Table 1 the change in selectivity during the 16 h runs has been recorded. The introduction of water to the mobile phase percolating through the column slows the decay in selectivity. As a result, either water acts as a replacement for ESA or its presence stabilizes the mechanism of the Memory Effect. A strong but weakly acidic eluent seems unlikely to be able to mimic the effects of a strongly acidic mobile phase additive. On the other hand, the addition of water into a stationary phase already under the influence of the Acid Memory Effect would seem likely to affect selectivity if water (and hydrogen bonding) was the source of the Memory Effect's residual influence.

Fig. 2 illustrates the variations of the selectivity of 4CPEE during the longer runs. When water is removed from the mobile phase (Fig. 2 – dry mobile phase), the selectivity of the ester decays slowly and linearly after the stationary phase is saturated with the acid modifier. Even the addition of ≃0.01% water (Fig. 2 – 0.01% water) is sufficient to stabilize the Memory Effect. Similar to the 16 h runs (Fig. 1), the selectivity remains stable during the long run after the column has been saturated with water (see Fig. 2 – water saturated, ≃0.43% water). When the column is returned to a molecularly sieve dried mobile phase (Fig. 2 – dry MP (final)) the selectivity does not return to a value similar to the original selectivity (Table 2). Two conditions of the Memory Effect are still present after the four long runs: the influence on the separation selectivity and the selectivity decay have not returned to their original values. The 112 plus hours of exposure to the wet mobile phase have caused the column to retain the influence of the Acid Memory Effect. Even though ESA was injected on the column during the first 12 h of each run, only one injection of acid was required to reach the maximum selec-

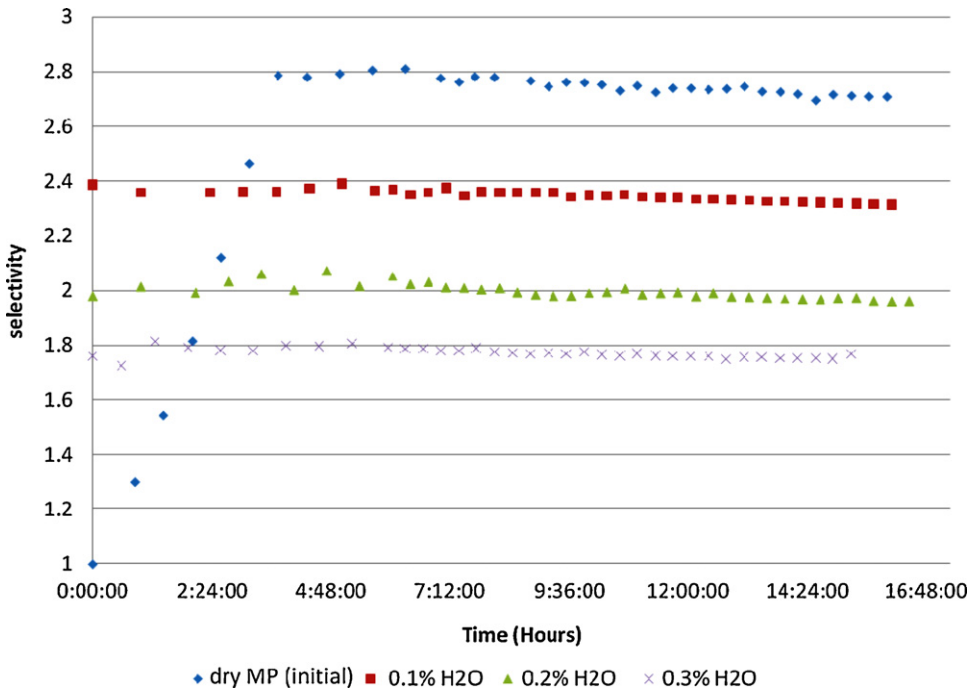

Fig. 1. Short run showing the effect of changing the water content in the mobile phase. The initial loading of the Acid Memory Effect can be seen in the first seven injections. Over the additional 12 h a slight loss of selectivity in the separation of 4CPEE can be detected. Even though injections of ESA were included in each sequence, only the dry MP indicates any change in separation capacity. Separation conditions: 90:10 Hexanes/Reagent Grade Alcohol, 1 mL/min, 40 °C, 210 nm UV detection.

tivity during the dried mobile phase run. The additional injections only improved the selectivity from 2.66 to 2.68. The linear decay of selectivity for the separation of 4CPEE was shown to extend for over 24,000 column volumes. To flush this amount of mobile phase – at 1 mL/min, would require over 16 days of continuous operation. Using dry 100% Ethanol does not have the capacity to remove the influence of water on the Acid Memory Effect once the conditions of the Memory Effect have been created.

The influence of water contaminating the mobile phase can be seen in Fig. 3. The ESA treatment was carried out during the first 10 injections of each sequence in the figure. The selectivity of the TSO enantiomers stabilizes during this same time period. A slight variance can be seen during this period of time, yet change in the selectivity of this enantiomeric pair shows that the equilibrium of the new mobile phase has taken place before the full ESA treatment has been completed. During the initial dry mobile phase the

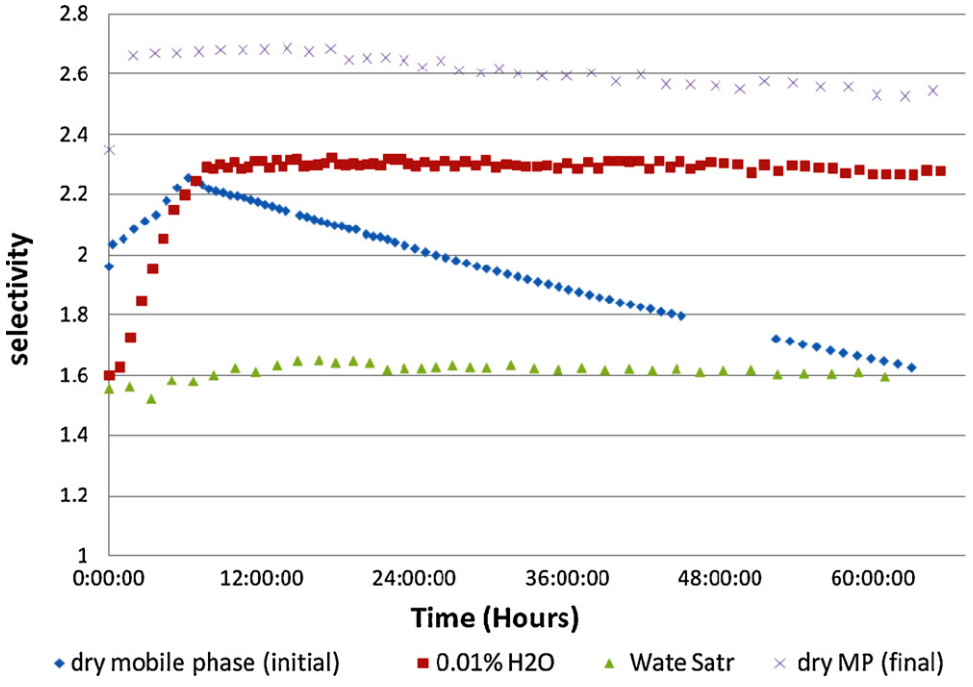

Fig. 2. Over 60 h the decrease in selectivity of the 4CPEE enantiomeric pair can be clearly seen in the long run with no additional water in the mobile phase. The introduction of ESA can be seen to improve the separation of this pair of molecules. The gap at 48 h was due to a sequence error, the mobile phase continued to elute from the column during this period of time. Separation conditions: 90:10 Hexanes/Reagent Grade Alcohol, 1 mL/min, 40 °C, 210 nm detection.

Table 2
The change in separation characteristics for the *trans*-Stilbene Oxide enantiomeric pair.

	trans-Stilbene Oxide				4-Chlorophenylalanine Ethyl Ester[a]			
% water	$k'1$	$k'2$	Selectivity	Resolution	$k'1$	$k'2$	Selectivity	Resolution
Initial conditions	0.872	2.65	3.05	5.34	2.285	4.397	1.925	2.74
0	0.806	2.45	3.03	5.89	2.35	5.24	2.23	3.29
0.01	0.797	2.33	2.92	5.78	2.08	4.77	2.28	3.39
0.43	0.635	1.54	2.42	4.38	1.82	3.02	1.64	2.43
Dried MP	0.847	2.63	3.10	6.20	1.95	5.24	2.68	4.05

[a] Maximum influence of the Acid Memory Effect.

selectivity begins at 2.99 and increase to 3.05 over the entire 56 h. Similarly, during the lowest water contamination the selectivity of the TSO enantiomers begins at 2.88 and increase to a maximum of 2.95. When using mobile phase that has been saturated with water the selectivity of this enantiomeric pair begins at 2.36 and reaches a maximum at 2.44. Only during the reintroduction of dried mobile phase does a decrease in selectivity occur. At the beginning of this sequence the selectivity is 3.18 and this number decreases until the TSO selectivity reaches 3.07. The variation in selectivity between the sequences can be attributed to the change in water concentration within the mobile phase. Yet the changes within the sequence, which is minimal, can be attributed to two possible phenomenons. First, as the equilibration of the new mobile phase is reached, the selectivity will fluctuate until the mobile phase is completely switched. Even though the difference in mobile phases is the variation in water, the slight change would be recognized by the stabilization in selectivity. This is seen during the first ten injections of each sequence. Second, the ESA treatment has significant influence in the selectivity of other enantiomeric pairs due to a change in the stationary phases chiral environment, the slight change in selectivity of the TSO enantiomeric pair during the first ten injections would not be unexpected. Both possible influences on the change in the selectivity of the TSO enantiomeric pair are not related to the Acid Memory Effect.

Using both Tröger's Base and 4CPEE to distinguish between the influences of wet mobile phases is illustrated in Fig. 4(a and b). Both graphs compare the selectivities obtained with an initially dry mobile phase (using reagent grade alcohol) and a mobile phase containing ≃0.43% water. Fig. 4(a) shows that even though water, as a strong eluent, does depress the selectivity of Tröger's Base, it does not cause an instability in the reproducibility of the selectivity. On the contrary, between the 31st and the 51st hours of the wet mobile phase long run the selectivity of Tröger's base does not change at all. Yet, with the dried mobile phase, the selectivity of Tröger's Base fluctuates significantly during this same time frame.

For 4CPEE (Fig. 4(b)) the results would also be surprising if water is expected to create instability in the selectivity. Instead, the figure shows that the stability of the Memory Effect clearly improves, allowing the continued separation of this racemic pair. The trendline for the data collected with the wet mobile phase shows that the Memory Effect can be extended upto 30,000 column volumes. If free silica/silanols/silanes were involved in the Memory Effect, the presence of additional water would certainly increase the instability of the separation of the ethyl ester, which is not the case. Therefore, uncapped silica/silanols/silanes are not involved in the mechanism of the Memory Effect, a conclusion consistent with the results of Zheng et al. [11].

The introduction of trace amounts of water into the mobile phase does reduce the selectivity and the resolution, as shown in Table 2. The initial conditions are those under which the analysis of TSO was done, when the columns were received from Chiral Technologies, Inc. The results with a mobile phase containing ≃0.3%

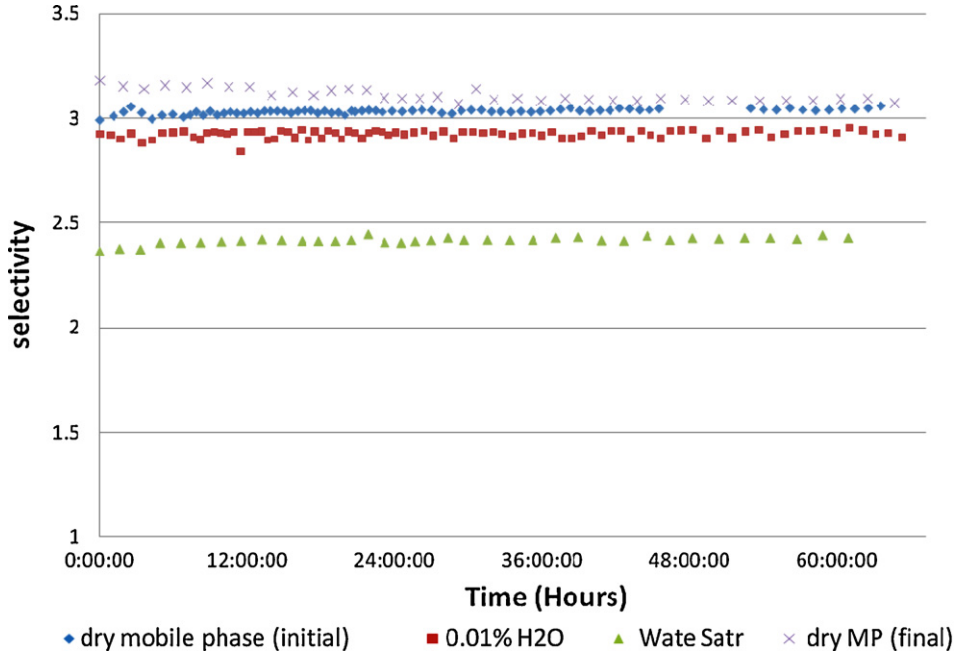

Fig. 3. Over 60 h the variation in the selectivity of the TSO enantiomeric pair due to the contamination of water in the mobile phase can be clearly seen in each of the long runs. The influence of the ESA is negligible, within the first 10 injections for each sequence the selectivity stabilizes. The gap at 48 h of the initial dry mobile phase test was due to a sequence error, the mobile phase continued to elute from the column during this period of time. Separation conditions: 90:10 Hexanes/Reagent Grade Alcohol, 1 mL/min, 40 °C, 210 nm detection.

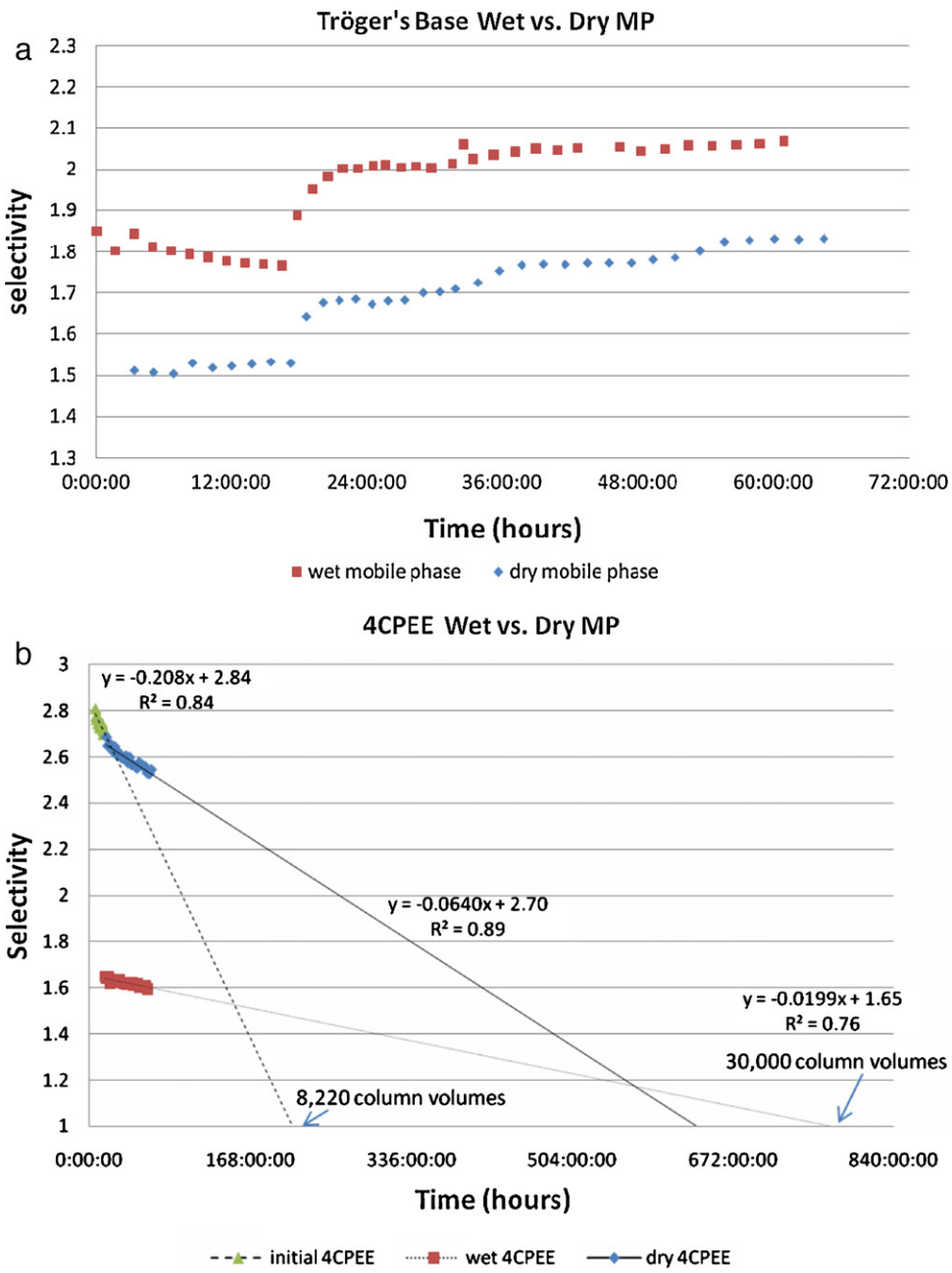

Fig. 4. (a) The influence of water on the selectivity of the Tröger's Base enantiomeric pair can be seen in this graph. The selectivity is improved and the plateaus seen in the dry mobile phase are minimized when compared with the wet mobile phase long run as well. The wet mobile phase contained additional 0.43% water; the dry mobile phase was treated with molecular sieves. The conditions are the same as Fig. 1. (b) The initial 4CPEE (green – dash line) was collected in the 16 h run, prior to the addition of any water into the stationary phase. The wet 4CPEE (red – dotted line) was collected as the maximum water content of the mobile phase (0.43%). Rinsing the column with pure 100% Ethanol dried with molecular sieves begins the removal of the Memory Effect (blue – solid line), but after 60 h, only a small portion of the Memory Effect had been eliminated. The conditions are the same as in Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

water are from the long runs included in this experimental section. The dried mobile phase was prepared using 100% ethanol. The overall selectivity of TSO is recovered after returning the column to dry mobile phase. The strength of the aqueous contaminated eluent is seen in the reduction of all four chromatographic characteristics: both retention factors, the selectivity, and the resolution. With neutral enantiomers, such as TSO, the addition of water into the mobile phase does influence the enantiomeric separation but the influence of water can be removed by flushing a dry mobile phase through the column. This is not the case with the enantiomeric pairs that are influenced by the Memory Effect. After the column has been exposed to water, the Memory Effect is more pronounced. As Fig. 4(b) indicates, the Memory Effect continues to influence the separation parameters of the 4CPEE enantiomers during the percolation of thousands of column volumes, even when the mobile phase is 100% ethanol dried on molecular sieves, as if the water were strongly bonded to the stationary phase.

Removing the Memory Effect after the column had been exposed to water is more difficult than if it had been eluted only under strictly normal phase conditions. As a result, flushing the column with dry mobile phase is no longer an option (Fig. 4(b)). The only possible method is the introduction of a base modifier. Fig. 5 shows the effects of adding DiPA to the stationary phase. Given the conditions under which this column has been exposed, only two

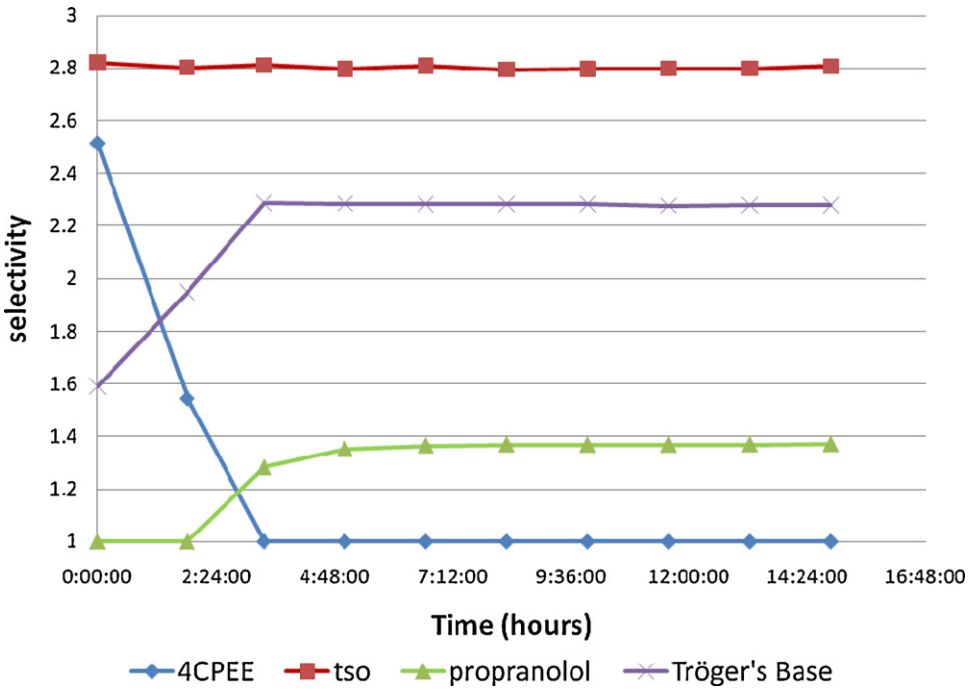

Fig. 5. Unlike the flushing of the column with dried mobile phase (Fig. 3(b) – dry mobile phase), the addition of DiPA removes the Memory Effect immediately. After only 2 100 μL injections of ~10 mg/mL of the base, the three enantiomeric pairs have returned to a stable selectivity.

injections of DiPA were needed to turn it to behave as effected by the Base Memory Effect. From the Base Memory Effect it had been documented earlier how to return a column to one behaving under acid/base native conditions [9]. Neutralisation of the Memory Effect required less than two injection of the DiPA solution, yet 10 injections of the of ESA solution were required to create the maximum separation with the Memory Effect. In this result it can be seen that a direct neutralisation did not occur. Instead, a weak base was able to neutralize a strong acid with the addition of a fraction of the molar mass. Neutralisation of the Memory Effect can be seen by two indicators in Fig. 5. First, the stabilization of the selectivity between enantiomeric pairs that require modifiers indicates that the Memory Effect is no longer influencing the separation of that pair. Second, the amount of base solution required for neutralisation can be measured – 2 injections of 100 μL at 10.8 mg/mL.

5. Conclusions

This systematic study of the Acid/Base Memory Effect observed with the CHIRALPAK AD stationary phase confirms the influence of water on Memory Effects. Water was suspected earlier to be one of the major contributors to the lack of reproducibility of column performance in normal phase chromatography [3–6]. Our work shows that the presence of water in the mobile phase actually extends the persistence and stabilization of the Memory Effect. This extension of the Memory Effect lasts for thousands of column volumes of dry mobile phase being percolated through this stationary phase.

We also show that once the column has been exposed to trace amounts of water in the mobile phase, the removal of the Memory Effect cannot be achieved merely by flushing the column with more, dry mobile phase. A base treatment must be performed on the column or the residual Acid Memory Effects will persist for more time than it takes to flush the column with 30,000 column volumes.

The exposure of the amylose tris(3,5-dimethylphenyl carbamate) stationary phase to trace amounts of water does influence the effects of acid mobile phase modifiers. In the past, the persistence of these modifier effects on the selectivity of enantiomeric pairs was avoided by using numerous separate columns, one for each possible class of modifiers used in the separation of racemic mixtures. Understanding that water is responsible for the persistence of the change in the chiral environment created by the acidic modifier can lead to understanding how to eliminate the requirement for the storage of additional columns in the laboratory. Two methods can be used to minimize the persistence of the Acid Memory Effect: always using dry solvents and to understand that this stationary phase can be quickly changed from acidic to basic conditions. In the first method, dry alcohol additives can be used by exposing the solvent to molecular sieves. Because they are hygroscopic, when Ethanol, Reagent grade Alcohol, Methanol, or Iso-propyl alcohol are exposed to atmospheric conditions, they absorb moisture from the environment. Minimizing stationary phase exposure to water in the mobile phase minimizes the persistence of the Acid Memory Effect. In the same time, acid modifiers can be neutralized by injecting a complimentary base modifer solution and percolating it through the column, and visa-versa. In this way the chiral environment inside the column can quickly be converted from its acidic to its basic nature. Since neutral molecules do not require a “neutral” chiral environment, concerns for the “modifier” native stationary phase conditions is unnecessary. One CHIRALPAK AD column can be used in either the acid or the base conditions if the column is treated with the appropriate mobile phases and the desired solutions of acid/base modifiers.

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