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Review

Chromatographic silanol activity test procedures: the quest for a universal test

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Abstract

Reversed-phase chromatography is the most used and the most studied method of modern liquid chromatography. There is yet no ideal support available for preparing reversed-phase stationary phases, but the vast majority have historically been and are still prepared on microparticulate silica. The silica surface has a number of properties which make it attractive for derivatization, including easily controlled particle size and porosity and mechanical stability. There are several types of surface silanols which have their own unique properties that affect both chemical derivatization reactions and adsorptive interactions with solutes. The relative distribution of these different types of silanols may affect the characteristics of silica-based stationary phases more than the absolute number of surface silanol groups. The relative importance of each of these different types of silanols has not yet been unambiguously established. Free or isolated silanols, internally hydrogen-bonded vicinal silanols, and geminal silanols all have been implicated as the primary reaction and adsorption sites. There are many different synthetic schemes that have been used to block the remaining silanols, and ''deactivated'' phases are very popular. Unfortunately, there is still no universally agreed upon method to measure the accessibility or interaction of these silanols with solute molecules. Many tests have been proposed, focusing mainly on chromatographic probe molecules, but different tests run on the same column will often show different interactions. We will briefly review the surface chemistry of silica and focus on the multitude of tests that have been proposed. Our focal point will be silanol activity test; other aspects of column performance will not be included. Where possible, comparisons among the methods will be made.

Keywords: Silanol activity; Stationary phases, LC; Silica; Reviews

Contents

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1. Introduction

The vast majority of separations for biomedical, pharmaceutical, and environmental analyses are performed using high-performance liquid chromatography (HPLC). The most widely used method of modern liquid chromatography (LC) is reversed-phase chromatography. In reversed-phase LC the separation mechanism is dependent upon interactions between analyte components, the mobile phase and the surface of the packing. In spite of recent advances in the investigation of alternative packings such as zirconia, alumina, titania and polymer-based packings, microparticulate silica continues to be the most commonly used [1]. The silica surface remains the most dominant packing due to its versatility, high column efficiency, mechanical stability and easily controlled particle size and porosity. Chromatographic silica can be found in both spherically and irregularly shaped particles. Spherical particles are used for analytical procedures due to the ease and reproducibility with which the particles can be packed into efficient columns [2]. Although superior to the other available packings, silica is by no means an ''ideal'' support for reversed-phase LC, especially in the analysis of basic substances. Studies have shown that the broad and tailing peaks, increased retention, column-to-column irreproducibility, and peak shape irreproducibility found in the analysis of basic samples were due to problems in the underlying silica and not in the bonded phase [3].

The silica surface contains both silanols (Si–OH) and siloxanes (Si–O–Si). Siloxanes are hydrophobic and have been shown to have very little to do with solute retention [4]. Silanol groups are considered to be the strong adsorption sites and are hydrophilic in nature. Hydrated silica surfaces have a layer of silanol groups which can be removed by full hydroxylation to give a maximum surface concentration of about 8 $\mu mol/m^2$ [2]. There are three types of silanols found on the surface of amorphous silica with porous structures: geminal, vicinal and isolated (Fig. 1). The identification and concentration of the different types of silanols can be determined by a number of spectroscopic techniques such as ^{29}Si cross-polarization magic-angle-spinning nuclear magnetic resonance (^{29}Si-CP-MAS-NMR), proton-spin-counting solid-state NMR, and diffuse-reflectance infrared Fourier transform spectroscopy (DRIFT) [5]. The three types of silanols have different adsorption activities and a number of studies have been performed in order to determine which silanol group dominates as the primary reaction and adsorption site, yet no definitive answer has been provided [6–11]. Silanols present on the surface serve as attachments for the alkyl groups used in bonded phases. However, due to steric hindrance, a problem arises when the maximum concentration of C_8 or C_{18} ligands is less than the density of the silanols found on chromatographic grade silica [12]. This results in residual silanols within the bonded phase. The unreacted silanol groups are weakly acidic, and their presence causes difficulty when analyzing basic compounds such as those found in biomedical, pharmaceutical, and environmental compounds. Similarly, the presence of metals in contaminated silicas also causes silanols to become highly acidic due to the activation of surface silanol groups. Recently developed ''new generation'' silicas which are less acidic and highly purified are being manufactured and have been found to give better separations for basic materials [13,14].

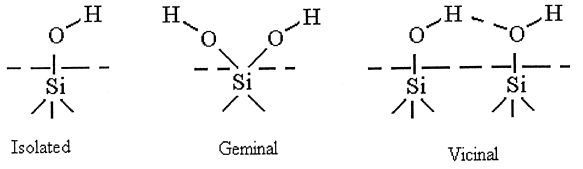

Fig. 1. Various types of silanols.

A number of studies have been conducted in order to devise ways to block, eliminate, or decrease the number of remaining silanols present [1,8,9,12, 15,16]. One of the first procedures which attempted to remove the effects of the residual silanols was endcapping. Relatively small reagents were used to react with the remaining silanol groups. It was found that after extensive bonding with the most common endcapping reagents the silanol concentrations were reduced but not completely eliminated [3]. Other techniques included the use of mobile phase additives, silica pre-treatment procedures, and the use of reactive silanes [16].

The differences in retention among columns from different manufacturers as well as those differences among columns from the same manufacturer but

from different lots have caused a surge in development of characterization tests. Numerous tests have been developed for the characterization of reversed-phase material through elemental analysis, physical measurements, the evaluation of chromatographic data using statistical methods, spectroscopic analysis and chromatographic evaluation. However, currently there is no universally accepted method of measurement available to determine the residual silanol activity toward solute molecules in reversed-phase LC, although several chromatographic tests have been proposed.

In this paper we will briefly discuss the minimal qualifications which a sufficient column test should provide and review a number of chromatographic approaches which have been proposed to characterize columns according to their silanol activity. Our focal point will be silanol activity tests, other aspects of column performance will not be included. We will also discuss the comparisons which have been made for the different tests.

2. Chromatographic test procedure characteristics

With more than 100 different stationary phases available from different column manufacturers, it can be difficult for analysts to select an appropriate column for a particular separation. Chromatographers also have difficulty reproducing results from the literature due to the lack of classification systems for columns. For example, the United States Pharmacopeia's column classification system groups all of the C_{18} packings commonly used in reversed-phase LC (3–10 μm) into one category [17]. Characterization of chromatographic properties allows for the classification of columns into groups based on similar retention behaviors. This would allow for greater ease in column selection and method adjustment due to variability. A sufficient column probe should be fast, simple and nondestructive. According to Boguz test mixtures should consider the efficiency of the column for neutral, basic, and acidic solutes and the resolution of close eluting compounds in the test mixture [18]. In some cases probe compounds are selected which are similar to the analytes because they react with the reversed-phase support in a similar manner. However, a more general approach is suggested because individual tests must be contrived for each type of separation. Stationary phase properties have an enormous influence on retention; therefore test mixtures should include a variety of organic compounds with different functional groups [19]. This allows for the consideration of the chemical properties of the analytes as well as the column properties. Compounds used in test mixtures should also be easily accessible in order to promote universal usage and acceptance. Test conditions for chromatographic characterizations should be close to the actual chromatographic conditions to which the samples will be exposed.

The following chromatographic tests have been proposed to assess the level of silanol activity as well as determine the effects of this activity on analyte components. The multitude of tests can be classified into two groups: those run under isocratic elution conditions and those run under gradient elution conditions (see Table 1).

3. Isocratic elution test procedures

The majority of the chromatographic tests proposed for silanol activity characterization have used isocratic conditions. Although many separations are possible only using gradient elution there are many advantages to using isocratic elution. For example, gradient elutions are more time consuming due to column equilibration times. The use of isocratic elutions allows for columns to be tested in a more timely manner which promotes a more expedient column selection process. Also gradient elutions cannot be carried out with certain HPLC detectors, thus limiting the range of solutes as possible test compounds. Generally, chromatographs run under gradient elution conditions are more prone to have problems within the baseline than those run under isocratic conditions [2].

A chromatographic test probe was proposed by Daldrup and Kardel for the selection of reversed-phase HPLC columns for clinical and forensic toxicological screening [20]. Three compounds were chosen after the evaluation of possible test solutes. Diphenhydramine and diazepam were selected to reflect the residual silanol activity and resolution,

Table 1
Various silanol activity test procedures

Author(s)	Solute(s)	Mobile phase	Si–OH activity indicator
Daldrup and Kardel [20]	Diazepam, diphenhydramine, MPPH	ACN–phosphate buffer (pH 2.3)	Columns which eluted diphenhydramine before MPPH and had RRT values 1.5–1.6 deemed suitable for forensic and clinical screening of drugs
Sadek and Carr [21]	DMDPC	MeOH–cyclam	Smaller *R* values indicative of less active silanols
Goldberg [22]	1-Dimethyl phthalate and diethyl phthalate, 2-phthalate, theophylline and caffeine	(1) MeOH–water (65:55) (2) ACN–acetate buffer (20:80)	RRT value variation Retention times
Walters [23]	1-DETA and anthracene, 2-nitrobenzene and benzene	(A) ACN (B) *n*-heptane (C) ACN–water (65:35)	(1) k' DETA/k' anthracene (2) k' nitrobenzene
Kimata et al. [24]	Caffeine and theophylline	(1) MeOH–water (20:80) (2) MeOH–buffer (pH 2.7 and 7.6)	Retention of caffeine good measurement of number of residual silanols
Verzele and Dewaele [25]	Acetylacetone, 1-nitronaphthalene, naphthalene	MeOH–water(60:40) with 5% sodium acetate	Ratio of retention values for naphthalene and 1-nitronaphthalene>1.4 for minimal silanol activity. Acetylacetone elutes symmetrically if no trace metal
Engelhardt et al. [26]	Toluene, ethylbenzene, aniline, toluidine (or phenylaniline) isomers, phenol *N*,*N*-dimethylaniline, and benzoic acid ethyl ester	(1) MeOH–water (49:51, w/w) (2) MeOH–1 m*M* phosphate buffer (pH 7)	Asymmetry values and elution order of basic solutes, aniline and phenol
Mant and Hodges [27]	Four polypeptide standards	(A) 10 m*M* sodium perchlorate (pH 7) (B) 50% ACN with 50 m*M* sodium perchlorate linear A–B gradient	Retention of polypeptides
Eymann [30]	Benzyl amine, 2-(4-methyoxyphenol)ethylamine, *N*-naphthylethylene-1,2-diamine	(1) 65% ACN (2) 40% ACN–water and H_2SO_4 (3) 40% ACN–water and buffer (pH 7) (4) Water	Retention of amines
Mutton [31]	Pyridine, benzyl amine, benzyl alcohol, *N*-acetylprocainamide·HCl, phenol, 4-nitrobenzoic acid, phenyl ether, 4-chlorocinnamic acid, 2-hydroxy-5-methylbenzaldehyde	(1) 0.1% phosphoric acid (2) 95% ACN and phosphoric acid	Retention time, peak width and peak asymmetry variations

respectively. Diazepam's relative retention value was perceived to depend on the degree of effective surface concentration of bonded alkyl groups. 5-(*p*-Methylphenyl)-5-phenylhydantoin (MPPH) was used as a reference substance to calculate relative retention times (RRTs) and theoretical plate numbers. The mobile phase consisted of acetonitrile (ACN)–phosphate buffer (30:70, w/w) with a of pH 2.3. Packings were divided into two subgroups: those in which dihydramine eluted after diazepam and those in which diphenhydramine eluted before MPPH. In the first case the diphenhydramine peak was strongly tailed, however, the second set of results showed symmetrical peaks for the elution of diphenhydramine. Columns which fell in the later category were deemed more suitable for clinical and forensic toxicological screening of polar and neutral drugs due to the perceived presence of fewer or less active silanol groups. According to the study, the carbon content needed to be fairly high in

order to run toxicological drugs in one isocratic run. It was determined that columns which possessed an RRT of 1.5–1.6 were seen as suitable stationary phases for the type of work to be done. We caution against the use of carbon content as a descriptor of the column's suitability for certain types of analysis; when reported alone the carbon content values are uninterpretable.

Sadek and Carr developed a chromatographic test for silanophilic interaction based on 5,14-dimethyl-7,12-diphenyl-1,4,8,11-tetraazacylotetradecane (DMDPC) as a marker compound [21]. DMDPC is a tetraaza, which characteristically creates silanol–amine interactions (Fig. 2). The purpose of the study performed was to characterize the efficiency of 1,4,8,11-tetraazacyclotetradecane (cylam) as a silanol blocker. The retention of DMDPC was found to be heavily dependent on silanophilic interactions. DMDPC showed severe peak tailing and demonstrated increased retention due to the nitrogen–silanol interactions. Isocratic elution conditions were used with MeOH as the mobile phase and small amounts of cyclam were added in the mobile phase in varying amounts. The retention was evaluated by monitoring the capacity factors (k') of DMDPC and chrysene in a defined ratio:

$$R = \frac{k' \text{ of DMDPC} - k' \text{ of chrysene}}{k' \text{of chrysene}}$$

It was found that the smaller the R value, the less important the silanophilic interactions became in the overall solute retention. However, for a test procedure to be efficient the determination of residual silanol activity should not be based on the retention of a single compound. Interactions between compounds in the analyte solution play a role in the retention as well.

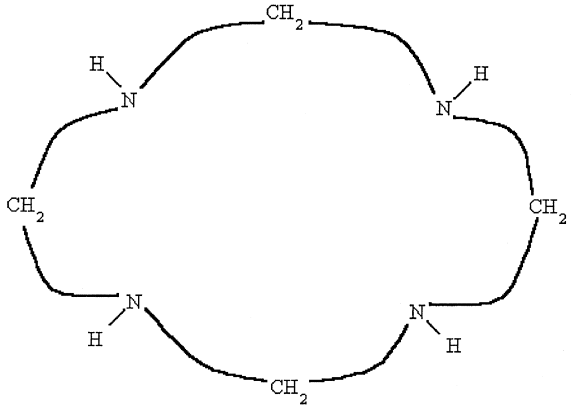

Fig. 2. General cyclic tetraaza structure.

Goldberg proposed a series of test mixtures designed to analyze the chromatographic interaction of certain classes of compounds with a selected column [22]. There were five different tests proposed; one each for polar, acidic and basic compounds and two test mixtures contained nonpolar compounds. Retention data was used in order to calculate selectivity and capacity factor values.

Parameters related to the efficiency of the column were not evaluated. The compounds that comprised test two and test four were polar and basic compounds, respectively. They were used to detect interactions with residual surface silanol groups. The polar compounds dimethyl phthalate and diethyl phthalate were run in a mobile phase of MeOH–water (65:35) mixture at ambient temperature. The basic compounds theophylline and caffeine were run at 37°C in a ACN–acetate buffer (20:80) mixture. It was found that the pattern of relative retention values was altered when comparing the results from the polar and nonpolar groups. This variation was credited to the compounds' interactions with residual underivatized silanol groups. It is important to note that the polar compounds were evaluated at a pH where silanols are suppressed. It was also found that the packings which have relatively low retention of the nonpolar and semi-polar compounds have much higher values for caffeine. Although a classification system cannot be created using the test procedure, the test does reveal significant differences in the columns.

Walters presented a column classification scheme which was based on hydrophobic and silanophilic interactions [23]. The hydrophobic interactions were determined by the capacity factor ratio of anthracene and benzene and the silanophilic interactions were detected by the capacity factor ratio of *N*,*N*-diethyltoluamide and anthracene. The efficiency of the columns was also examined. There were three mobile phases used: mobile phase A which was composed of acetonitrile, mobile phase B which included dry *n*-heptane, and mobile phase C which consisted

of ACN–water (65:35, v/v). The three test solutions were composed of *N*,*N*-diethyl-*m*-toluamide (DETA) and anthracene, nitrobenzene and benzene, and uracil, benzene, toluene and anthracene, respectively. There were two test procedures developed for the determination of residual silanols. The first and preferred method as described earlier was based on the capacity factor ratio of DETA and anthracene. It was assumed that the retention of DETA was sensitive towards silanol activity while the retention of anthracene was assumed to be determined solely by hydrophobic interactions. However, the capacity factor of anthrance is also affected by shape selectivity. This test will also show a convoluted sum of the two interactions. A second test was based on the capacity factor of nitrobenzene after passing 50 ml of ACN, methylene chloride and mobile phase in sequence through the column. The second test was discarded due to the timely equilibration and conditioning requirements for the column, however a linear relationship was found between the two methods.

Kimata et al. developed a test procedure for the characterization of silica C_{18} packing material [24]. Solutes used in the test mixture were easily obtained if not already present in a well equipped analytical laboratory. The isocratic gradient contained either MeOH–water (20:80) or MeOH–buffer (40:60, pH 7.6 or 2.4). Hydrophobic properties were examined by alkylbenzenes with various alkyl groups. The shape selectivity was examined by triphenylene and *ortho*-terphenyl while caffeine and theophylline were used to test the hydrogen bonding ability of the stationary phase. Alkyl amines with pK_a values greater than 9 were used to examine the contribution to the silica surface from exchange sites. The retention of the polar amines was normalized by the use of phenol and benzyl alcohol to conceal any differences in the hydrophobic properties of the columns. It was found that the retention of caffeine was a good measurement of the number of residual silanols. Separation factors were used to attempt to cancel the effect of hydrophobic interaction as much as possible. It was concluded that the procedure was sufficient for the estimation of the extent of trimethylsilyation for stationary phases. The method was deemed as suitable for the characterization of surface coverings, types of silanes, amounts of silanol present, and the amount of ion-exchange sites.

Verzele and Dewaele proposed a method of evaluation for HPLC column packing materials [25]. The test mixture consists of acetylacetone, 1-nitronaphthalene and naphthalene. The isocratic mobile phase consisted of MeOH–water (60:40) with 0.5% sodium acetate. Naphthalene was used to show the kinetic parameters of the column and column deterioration. 1-Nitronaphthalene was used to show the degree of residual silanol activity or degree of deactivation by endcapping. The ratio of retention values for naphthalene and 1-nitronaphthalene for columns which were properly endcapped was found to be 1.4 or higher. The acetylacetone was found to elute as a symmetric peak on silica surfaces which were totally free of metal. The test was found to be a suitable procedure for the indication of trace metal presence.

One of the most prominent tests to date was proposed by Engelhardt et al. [26]. This method was developed in order to provide a universal test method which could be used to compare the various selectivities of reversed-phase stationary phases. The isocratic elution system consisted of either MeOH–water (49:51, w/w) or MeOH–1 m*M* phosphate buffer (pH 7), however the unbuffered eluent was used most often. Engelhardt suggests that the influence on retention when using a buffer should not be noticeable, however, he found this to be untrue in his study. It should be noted that the use of mobile phase without a buffer restricts the range of compounds that can be test components based on their pK_a values. In most cases buffered eluents are used in routine analysis in industry and the use of unbuffered mobile phases would produce an environment which is dissimilar to that of the analyte. The test mixture contained toluene and ethylbenzene, which was used to monitor the hydrophobic properties; aniline, toluidine isomers and *N*,*N*-dimethylaniline which were chosen to monitor silanophilic interactions; and phenol and benzoic acid ethylester to check for polar interactions. The toluidine isomers were interchangeable with phenylaniline isomers. Both sets of isomers were dissimilar only by their pK_a values; this was presumed to show that separation was based on silanophilic interactions only, with no contribution from hydrophobic interaction.

Silanophilic interactions were determined by the asymmetry value and elution order of the basic solutes, aniline and phenol. It was found that silanophilic interactions could be deemed as negligible when aniline was eluted before phenol. Interactions were also considered negligible when the isomers were not separated and were eluted with symmetric peaks. It was also proposed that the asymmetry value at 10% of the *p*-ethylaniline peak could be used to characterize silanophilic properties. Three classes of columns were established according to this classification: columns which had asymmetry values less than two, those with asymmetry values between two and four, and those which had values greater than four. When evaluating the columns for residual silanol effects the peak asymmetry of the basic samples was found to reveal the largest differences. It was suggested that the relative retention values of basic samples should be consistent regardless of whether or not the eluent contained buffer. It was noted that the application of buffers and neutral salts disguised retention contributions and resulted in symmetrical peak shapes and reduced retention. Numerous studies were carried out evaluating the effects of mobile phase composition and temperature on retention. Optimum conditions were selected at 40°C and mobile phase compounds were prepared by weight. According to Engelhardt et al. columns were classified as ''good'' if aniline eluted before phenol, the ratio of peak asymmetry values for aniline and phenol was less than 1.3, the isomers were hardly separated and the ratio of the k' values was less than 1.3, and *N*,*N*-dimethylaniline (DMA) eluted before toluene.

4. Gradient elution test procedures

Gradient elution is suggested for the separation of large molecules and samples with capacity factor ranges whose optimum could not be maintained with an isocratic method [2]. In practice it is usually suggested that method development begin under gradient elution conditions because it separates samples faster, detects early or late eluting low concentration components more frequently, and it gives an estimation of the retention range of the analytes. Most of the basic samples encountered in environmental, pharmaceutical, and biomedical analysis require gradient elution conditions for the most efficient separation. The main advantage of using gradient elution in chromatographic testing is that it provides an atmosphere which will be more similar to the conditions under which the actual sample will be run.

Mant and Hodges developed a silanol activity test which utilized four polypeptide standards [27]. The peptides were used to monitor the residual silanol activity over a pH range of 2.0 to 7.0 on both commercial columns and columns prepared in the laboratory. The peptides contained 1 to 4 basic lysine residues without possessing any acidic residues. There were not any changes in the range of net charge, $+1$ to $+4$, over the entire pH range. Guo et al. had previously determined that the optimum resolution of peptides is achieved when 15 to 40% of the organic solvent in the gradient is present [28]. Mobile phase compositions were composed with the intention of creating significant yet controllable ionic interaction of the standards with the stationary phase. Solvent A was 10 m*M* sodium perchlorate (pH 7.0), and solvent B was 50% ACN with 50 m*M* sodium perchlorate. A linear A–B gradient was run. It was found that the general application of the standards in the above system was sufficient to monitor wide ranges of silanol activity. A series of gradient systems with varying pH values and organic modifiers were tested before the final gradient system was picked. Variation of the mobile phase pH was responsible for variation in selectivity. It was concluded that this test was capable of determining activity of well deactivated columns [29].

Eymann proposed a testing procedure which could be utilized for the determination of hydrophobic and silanophilic interactions as well as trace metal impurities on the silica surface [30]. There were four different eluents: water, ACN–water (65:35), ACN–water (40:60) and 10 ml 1 *M* H_2SO_4 and ACN–water (40:60) and 10 ml 1 *M* pH 7 buffer. Gradient elutions were run as follows:

t_0	A = 90%	B = 0%	C (or D) = 10%
t_{20}	A = 0%	B = 90%	C (or D) = 10%

There were four different test mixtures evaluated.

Test mixtures 1–4 contained neutral compounds, amines, chelate forming compounds and acids, respectively. The amine test mixture was used to determine silanophilic interactions. The mixture contained benzylamine, 2-(4-methyoxyphenyl)ethylamine, and *N*-naphthylethylene-1,2-diamine. It was found that when testing reversed-phase columns elution peaks at pH 3 were more important when determining the major differences in the silanophilic retention characteristics of the columns. The chelate forming test mixture contained 2,2′-bipyridine, 2,3-dihydroxynaphthalene and a pharmaceutical research compound. This test mixture was used to detect different metal ion species on the silica surface. Overall, it was found that the test was sufficient for the determination of silanophilic and trace metal interaction detection. However, one of the problems with this test is that there was not an internal reference compound included in each test mixture. The relative retention times of different types of compounds would result in a better tool for classification.

Mutton reported a test method which was designed to comparatively study a number of recently developed reversed-phase packings [31]. The test mixture was comprised of pyridine, benzylamine, *N*-acetylprocainamide·HCl, benzyl alcohol, phenol, 4-nitrobenzoic acid, 2-hydroxy-5-methylbenzaldehyde, 4-chlorocinnamic acid and phenyl ether. Several mobile phase compositions were tested and the final system consisted of solvent A, 0.1% (v/v) phosphoric acid in water, and solvent B, 95% (v/v) acetonitrile and 0.1% (v/v) phosphoric acid in water. The gradients used were: B=0% (2 min) to 100% over either 20 or 40 min, then held at 100% for 10 min. The solution was then returned to B=0% over 2 min. Three commercial columns were evaluated using this mixture. The results led to the determination of a single column (Intersil) as superior for the analysis of a specific set of pharmaceutical compounds. McCalley reached the same conclusion in a study published after this work was completed [32].

5. Comparisons of test procedures

The literature is full of various chromatographic testing procedures. Although we have not included tests which employ statistical methods, spectroscopic analysis, physical measurements, elemental analysis, and those which do not specifically address silanol interactions, the multitude of chromatographic test procedures is still apparent. The influx of the different tests has caused some researchers to begin to compare the procedures with one another. One of the most recent comparative studies was performed by Claessens et al. [33] who compared the Engelhardt et al. [26], Tanaka (Kimata et al. [24]), Galushko [34], and Walters tests [23]. Claessens et al. examined both the hydrophobicity and silanol activity tests. It was found that while the hydrophobicity tests seemed to produce interchangeable data, the results of the silanol activity tests varied significantly. Five different tests were compared in the silanol activity evaluations, each of the tests previously mentioned and a ‘‘modified’’ Engelhardt test. The modification to the test was the addition of a buffer to the mobile phase. Although the Tanaka test does not include a silanol test specifically, Claessens compared the hydrogen bonding capacity information from that test to the silanol activity results of the other four tests. It was found that the results from the tests were inconsistent. Columns which would be ranked as having low silanol activity with one testing procedure would be ranked as having a high silanol activity by another method. It was also found that the modified Engelhardt test and the unbuffered test were poorly correlated. Some correlation was found between the Galushko and Tanaka tests which lead to the indication of a problem in nomenclature because the Tanaka test is supposed to detect hydrogen bonding activity not silanol activity. It was also determined that column classifications which are based on silanol activity values are dependent upon which test procedure is employed.

6. Conclusion

The chromatographic test procedures for residual silanol activity in reversed-phase LC columns have been reviewed. Although a number of these test procedures exist, there is not one universally accepted procedure to date. Before a test of this nature can be produced a number of discrepancies have to

be addressed. Many of the debates deal with matters related to ensuring the test procedure environment is similar to the environment the sample will be exposed to. For example, whether or not to test in buffered or unbuffered mobile phases, which pH to run, and to what degree do the organic modifiers affect the pK_a values of the analyte and the silanols. Once these matters have been settled a more accurate, universal method can be produced. A test of this nature would be highly beneficial to chromatographers as well as the biomedical and pharmaceutical industries. It would aid column manufacturers in batch and column reproducibility. The development of such a test procedure would also enable researchers to select columns more appropriately and with greater confidence. As we move into the new millennium, the quest for a universal silanol activity test method continues.

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