1996

Scanning electron microscopy study of etched capillary surfaces

Mira Kaczmarek
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**SCANNING ELECTRON MICROSCOPY STUDY
OF ETCHED CAPILLARY SURFACES**

A Thesis

Presented to

the Faculty of the Department of Chemistry

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Mira Kaczmarek

August, 1996

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ABSTRACT

SCANNING ELECTRON MICROSCOPY STUDY OF ETCHED CAPILLARY SURFACES.

by Mira Kaczmarek

The chemical etching process is used to physically roughen and consequently increase an inner surface of a capillary column in order to improve coating efficiency of the stationary phases in liquid and electroseparation techniques. The inner surface of a fused silica capillary column treated with an ammonium hydrogen difluoride solution under various conditions of temperature, time, and concentration of the etching reagent is studied using a scanning electron microscope. The topography of the etched surfaces changed considerably as the conditions of chemical etching are varied. The etching performed at elevated temperature produced a mildly roughened and homogenous layer of increased surface area. The optimized roughened surface of the inner capillary is compared to the bare and untreated surface. Electron dispersive spectroscopy is employed to penetrate the surface of the inner wall of the fused capillary to determine the composition of the etched surface. The etched surfaces of the inner wall of a fused silica capillary column characterized in this report are dramatically different when compared with previous studies of other types of capillary columns.

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Most importantly, the work is dedicated to my family...my thoughts are always with my mom and two most wonderful daughters Dominika and Kamila, whose love fulfill every moment of my life.

Mira Kaczmarek

2/4/96

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1. Introduction.

1.1. Development of capillary column separation.

Capillary column separation techniques are one of the most vital tools for characterization of an array of organic and inorganic materials in the field of chemistry, electronics, medicine, pharmaceuticals and the environment (1). The open tubular column (OTC), today known as and preferentially called a capillary column, is at the heart of the separation technique in which the solute species are separated based on differential interaction between the stationary and mobile phases (2). The OTCs which resulted in a dramatic increase in separation power were introduced by M.J.E. Golay in 1957 and were primarily developed for gas chromatography (3, 4). In the late 1970s, with the development of a highly inert and durable glass material of fused silica, capillary columns were adopted to high performance liquid chromatography (HPLC) (5, 6). The OTC provided several advantages to liquid chromatography such as high efficiency, high resolution, low sample consumption, and a short analysis time (7, 8). In the last few years, capillary columns have been used extensively in electroseparation methods such as a capillary zone electrophoresis (CZE), high performance capillary electrophoresis (HPCE), and capillary electrochromatography (CEC) (8, 9, 10, 11). EC is a developing analytical technique in which the mobile phase fluid

is driven through a microbore column containing a stationary phase by electroosmosis using an electric field. The technique has the potential to serve as an ideal bridge for connecting liquid chromatography and electroseparation such as micro-high performance liquid chromatography and capillary zone electrophoresis. Thus, EC results in a combination of the high efficiency in CZE with the wide range of applications and selectivity in micro-HPLC. The technique is used for unique and diverse separations of neutral and ionized molecules, small organic species, large molecules such as oligonucleotides, sugars, peptide, and proteins (12, 13, 14). In EC solvent transport is achieved by electroosmotic flow (EOF) that occurs at the electrical double layer of the solid-liquid interface, and it is generated uniformly along the length of the capillary. CEC is a hybrid separation method of HPLC and HPCE that results from the combined effect of partitioning and electrophoresis. The biggest advantage of using EOF is that the column efficiency is increased as a consequence of a more efficient plug-like flow versus a less efficient parabolic flow profile (9). The inherent separating power and wide application of capillary columns in a variety of separation techniques makes understanding of the physical and surface chemistry governing their preparation and properties very important.

1.2. Capillary column.

In general, the capillary column is a tube of circular cross-section consisting of three regions: an outer polyimide coating, the capillary column wall itself, and an inner layer of a stationary phase as illustrated in Figure 1.

Figure 1. Schematic representation of a section of the capillary column (not to scale).

The polyimide coating is layered onto the column wall while manufacturing of the column, and it serves two purposes: (i) to fill any flaws in the tubing; (ii) to provide a water proof barrier. Both of the functions add to the strength and the inertness of the column. The thermal exposure barrier of the external coating is around 400 ° C, and above that temperature the column becomes harder and very brittle (14). The polyimide film thickness is typically in the 0.5-1.5 μm range for highly efficient multipurpose capillary columns used in gas chromatography (15, 16). The internal layer of the column is a thin film of a stationary phase coated onto the inner wall of the capillary tubing. The stationary phase is characterized by the thickness, uniformity, and chemical nature which varies depending on the separation application. Wide-bore capillary columns of 0.53 mm internal diameter or higher are coated with the stationary phase of up to 5 μm film thickness (17). The thickness of the stationary phase is very important and it is optimized in order to obtain an appropriate sample loading onto the column. Furthermore, this film must maintain its integrity and remain stable without forming droplets when the temperature of the column is varied (18). The typical stationary phase is a polysiloxane polymer such as phenylsilane or cyanoethylsilane. These polar phases are chemically bonded onto the glass surface modified with an alkaline solution. In gas chromatography, the chemical bonding of the stationary phase to the column wall is very important since the stationary phase layered onto the glass surface, on a macroscopic scale, is never a

uniform film causing decreased column separation efficiency (8, 19). These chemically bonded stationary phases on the glass surface of the capillary tube exhibit the essential properties of low volatility, good thermal stability, and chemical inertness, thus providing good separation efficiency. Pesek et al. (9) reported a silica based octadecene phase synthesized via a hydride intermediate for tetracycline separation using a high performance capillary electrophoresis technique. It is essential that the stationary phases are deposited as smooth, thin, and homogenous in order to achieve a high separation efficiency in any type of capillary column. Therefore the characterization and preparation of the inner capillary wall is as important as the stationary phase itself, and it is the main objective of this study.

1.2.1. The wall of a capillary column.

In early studies various materials have been used for the fabrication of a capillary column wall such as the borosilicate (Pyrex), quartz, plastic, and stainless steel. Lately, fused silica has essentially replaced these materials due to its superior physical and chemical properties. Fused silica has a high melting point, a low coefficient of thermal expansion, and a high resistance to chemical attack. The ideal material for capillary column construction exhibits low adsorption activity. Most importantly, fused silica surface can be modified physically and chemically in order to accommodate a selective stationary film, and to make it more

inactive toward organic species, therefore decreasing peak tailing and improving separation power (18). Additionally, fused silica capillary tubing possesses excellent mechanical properties of extremely high flexibility due to its thin wall, and superior optical properties which are utilized in detector systems in high performance capillary electrophoresis and electrochromatography (16, 20, 7, 21, 22).

Chemically, fused silica is a silicon dioxide obtained from a natural quartz mineral rock which is fabricated at extremely high temperature and vacuum in the presence of a flame or an electrical field. The silicon dioxide fabricated under these conditions is composed of a highly cross-linked, three dimensional structure of tetrahedral arrangements of four oxygen atoms bonded to each silicon atom. The amorphous material has a very low surface area, and it is almost entirely dehydroxylated (20). Furthermore, the bulk silica material contains less than 1 ppm of metallic species as reflected in overall column inertness and durability. Capillary tubing with an internal diameter of 20-300 μm or greater than 300 μm are fabricated for capillary electrophoresis separations and liquid chromatography applications, respectively (5, 8, 11, 23, 10, 16).

1.2.1.1. Inner surface of capillary column wall.

There are several basic and crucial requirements for the inner surface of tubular columns: (i) no or negligible adsorption of the solute molecules which cause peak tailing or irreversible adsorption, especially when polar solutes are separated on non-polar stationary liquid films; (ii) no catalytic activity of the surface which may cause decomposition of solutes and/or stationary phases especially at elevated temperature; (iii) suitable homogeneity and geometry in term of roughness of the support layer, to generate stable, uniform films of the stationary liquid (24). Thus, the actual surface composition and the structure of the inner wall are of more importance than the bulk properties in order to obtain efficient separation.

The inner wall surface of fused silica possesses an extremely low number of hydroxyls, around 0.21 groups per square nanometer, which results in consistently lower adsorptivity as compared with other glass surfaces. This could also account for the increased difficulty observed in coating some stationary phases on fused silica (25). The hydroxyl groups attached to the surface silicon atoms are called silanols. These hydroxyl groups are ionized at a pH higher than 2, and this carry a strong negative charge at neutral pH and higher so that simple proteins in capillary zone electrophoresis show coulombic interaction with the surface. The resulting adsorption causes band spreading and peak tailing, as well

as denaturation of the proteins. Wang et al. (23) summarized that the basic interaction between proteins and the capillary surface are very complex as a result of various mechanisms such as electrostatic attraction, hydrophobic interaction, and hydrogen bonding, all contributing to the magnitude of adsorption. Presumably, the silanol groups on the fused silica surface exist in several chemical states depending on the extent to which they are hydrogen-bonded to other silanol species and surface bound water. Those which are not perturbed or involved in any interactions are described as free silanols. The actual existence and the relative quantity of these bonded and free silanols are strongly dependent on the thermal and chemical history of the material. The surface of the silica can easily adsorb or desorb water at room and elevated temperatures, respectively. The water can be readily removed by heating up to 400 ° C . Thereafter the dehydration is irreversible and is complete at above 800 ° C (18, 26).

An inner surface of a glass capillary column is often subjected to physical and/or chemical modification in order to promote coating of the stationary phase. Adsorption involves changing the nature of the glass surface by chemical modification of active hydroxyl groups, followed by increasing or decreasing of the critical surface tension which is a measure of the wettability of the solid surface. For the best protein separations the hydroxyl active sites are simultaneously eliminated and utilized in the surface modification by silation

and hydrosilation reactions in order to chemically bond the stationary phase for electrochromatography (9). It is reported that a high silanol concentration is advantageous for chemical surface modification; the stability and the effectiveness of the bonding increase by increasing the number of bonds of the coating to the surface (11, 14). It has also been established that during the silanization only highly acidic surface silanols react; the less acidic vicinal groups remain unreacted and can be deprotonated at higher pH. The vicinal silanols are defined as hydroxyl groups which are attached to adjacent silicon atoms. The unreacted silanols can be shielded by the umbrella of neighboring groups that have not undergone reaction, and are usually less exposed (27). This reduces their likelihood for interaction, not only with the silation reagent, but also with solute molecules, lessening column activity.

Physical modifications encompass all process for increasing the specific surface area including etching with inorganic acids and bases, or gases such as hydrogen chloride and hydrogen fluoride. The latter species is the focus of this study and it is discussed thoroughly in the following chapters. Overall, both chemical and physical modification processes are used to produce a roughened surface, and consequently enhance its wettability by a wider variety of liquid phases.

1.2.1.2. Surface wettability.

In order to achieve a high separation efficiency in gas chromatography, a uniform and homogenous film of a stationary phase is applied to the inner wall of the fused silica capillary column. The film must maintain its integrity and not rearrange to form droplets as the temperature varies. The droplets formation are due to cohesive forces of the liquid greater than the adhesive or wetting forces between the liquid phase and the glass surface. This causes the liquid stationary phase to contract into beads or globules producing a thick and non uniform coating which decreases column efficiency (28). The wetting properties are described by a thermodynamic function which expresses the tendency of a liquid drop to spread on a solid surface. A liquid droplet such as that of the stationary phase placed onto a solid surface may spread to cover the surface or it may remain as a stable drop as illustrated in the Figure 2. The angle formed between the tangent to the liquid drop and the solid surface is defined as the contact angle. The solid and liquid are defined to have good wetting properties for small theta angles, where the liquid freely spreads over the solid surface. Studies of the wettability of the capillary inner surface by a stationary phase are based on the measurement of the cosine of the angle theta which is graphed against the surface tension of the tested liquid. Theta contact angle measurement is also used to evaluate the thermal stability of the surface layer. A high value of the contact angle is an indication of good wettability of the liquid by the solid and refers to

the surface well covered, and consequently good thermal stability. The correlation of the forces between the liquid and solid surface is also expressed as the surface tension at the boundary phase of a liquid drop at rest on the solid. The critical surface tension is defined as the value of the liquid surface tension above which liquids show a finite angle on a given surface. The best surface coating is obtained when liquids have a surface tension less than the tension of the solid, the contact angle is zero and there is complete wetting of the solid by liquid.

Figure 2. Schematic representation of (a) non wettable and (b) wettable surfaces.

The fused silica surface in a capillary column possesses a high critical surface tension of around 75 dyne/cm based on the contact angle measurement.

However, most of the typical liquid stationary phases have a surface tension of around 20-50 dyne/cm. Thus by definition, such an incompatibility of the surface tension will result in a non wettable surface and the coating of the liquid layer is obstructed. It is reported, that adsorbed water converts a high surface tension to a low surface tension, and the drying process has an inverse effect. Surprisingly, a humidity of around 0.6 % effects surface changes, therefore the conditions of the surface tension measurements have to be well defined. Additionally, acid leached silica in soft glass has a much lower surface tension of around 40-50 dynes/cm (29).

The increase of the wetting capabilities is very closely correlated with the roughening of the surface; a more roughened material has better wetting properties. The principle of increased wettability is that the stationary phase coated onto a roughened surface fills all the holes on the glass surface so that on the macroscopic level it appears that the contact angle the liquid makes with the surface has been decreased. In reality the same contact angle exists but not on macroscopic level. In general, the surface of a liquid of similar composition as the solid surface exhibits good wetting properties, and the liquid spreads freely over the surface. The inner surface of a capillary column has been determined to have adequate wetting properties as well as a high surface area for even

turn essential for performing separations in capillary columns of the smallest internal diameter.

1.3. Capillary column preparation.

One of the primary reasons for treatment of the inner surface of the capillary column wall prior to the stationary phase coating is chemical and/or physical modification to enhance the surface wettability and consequently increase the surface area available for coating.

1.3.1. Acid cleaning and leaching.

Typically the first step for surface modification is an acid leaching of the column by gaseous or liquid hydrochloric acid in order to eliminate contamination from fabrication and dust particles. It has been shown, that the column treated this way exhibits: (i) better deactivation by thermal silation; (ii) a higher degree of inertness toward sensitive compounds. Thus, the acid leached columns are highly efficient, well deactivated, and thermally stable (28, 30). Furthermore, hydrochloric acid leaching produces no discernible surface roughening and the surface is free of damage when compared with sodium hydroxide or phosphoric acid techniques. It is suspected that the roughening may have occurred on a molecular level. The rate of acid leaching and contamination extraction are

proportional to the square root of the time; thus longer leaching time produces a silica surface of greater depth and purity. On the other hand, this longer leaching time may cause more carbon contamination from the leaching solution.

Therefore the optimal acid treatment time varies from 12-48 hours.

Acid leaching of soft glass such as soda-lime increases the density of the surface hydroxyls. These groups are utilized as an intermediate in the deactivation methods such as silation, in which the hydroxyl groups are replaced by inert silyl-ether groups (methychlorosilane) (30).

1.3.2. Surface etching.

In the case of the silica matrix, the etching process is defined as breaking up the silicon-oxygen network and dissolving it in the solution. The etching process differs from the leaching treatment which in turn is defined as a selective removal of portions of the glass material without substantial destruction of the silicon-oxygen bonding network (31). The freshly drawn capillary wall has a relatively smooth surface with a ratio of internal surface area to its apparent surface area slightly larger than unity (28). Thus roughening of the inner surface of the capillary is performed in order to enhance the wettability to compensate for losses in surface energy and an increase in the surface area. As explained before, a liquid spreads better on a roughened surface than a smooth one because

a surface covered by liquid drops releases more energy due to interfacial forces. Therefore more energy is released as a consequence of the increased surface area under the liquid drop. The correlation of the surface roughness and the previously described contact angle is defined as a roughness factor. The roughness factor is a ratio of the cosines of the apparent theta angles or a ratio of the microscopic and macroscopic surface areas respectively for the rough and smooth surfaces. The value of the roughness factor equals unity for a smooth surface and is greater than unity for roughened surfaces in which the apparent angle increases. A typical surface roughness factor of 5 to 7 is obtained for soft glass surfaces (11).

It is reported that an untreated glass surface is difficult to coat. Non-etched glass typically coated with a liquid methylsilane stationary phase produces a very uneven layer with a poor coating efficiency of only 50 % (19). Various roughening methods have been explored for glass capillary columns such as treatment with aqueous solutions of hydrofluoric acid, sodium chloride, or deposition of barium carbonate. These methods are limited to soft glass capillary with a sodium composition matrix and have a lot of disadvantages such as irregularity in the structural formation or leaching of the surface matrix by certain liquid phases (11, 19, 32). Additionally, these modification techniques increase the surface roughness significantly to coat a uniform thin layer of a polymeric film. One of the most successful roughening techniques is the

treatment of the inner surface of the capillary column with a high concentration of gaseous hydrogen fluoride. Onuska et al. (15) used an etching method with ammonium hydrogen difluoride in wide-bore capillary columns. The intent of the method is to eliminate the drawbacks of non-uniformity and the safety considerations associated with the commonly used hydrogen fluoride method. At elevated temperature, ammonium hydrogen difluoride dissociates to form gaseous hydrogen fluoride and ammonia which in turn dissolves the silica surface producing silicon tetrafluoride. Upon cooling, the silica is redeposited on the etched surface in the form of whiskers-resembling a needle-like structure. The optimum factors influencing the crystal growth as well as uniform coverage of the surface by whiskers is established. It is reported that around a 1000-fold increase of the surface is obtained by whisker production. Wide-bore Pyrex capillary columns prepared and deactivated in this manner are found to be neutral, with no adverse adsorption properties. On the other hand Sandra et al. (1) reported that in spite of the surface increase achieved by growing whiskers, a decrease in separation efficiency is observed due to lack of an adequate method to cover/deactivate the whiskers. Additionally, the extremely high degree of surface roughening by whiskers formation causes non-uniformity in the coating of the liquid stationary phase. This result is supported by another study in which hydrogen fluoride etching is used but the formation of too rough a layer composed of very active whiskers is avoided by dynamic removal of silicon tetrafluoride (24). While extensive testing of etching has been done on

borosilicate and soda lime capillary columns, less data and characterization have been reported for the chemical etching of fused silica.

In contrast, numerous studies can be found in the semiconductor field where fluoride media are routinely used for cleaning and etching of various silica material such as quartz. However, it is important to know that fused silica is an amorphous form of silicon dioxide unlike quartz. Therefore the behavior of fused silica is expected to be different from that of quartz, or even of an amorphous material such as borosilicate or soda lime glass (20, 33, 34, 35, 36).

In a review by Lee et al. (21) it is mentioned that gaseous hydrogen fluoride etching applied to fused silica produced holes rather than any outgrowths such as whiskers or dendrites. Pesek et al. (9) reported that application of the method used by Onuska to a small-bore fused capillary produces an increase in surface area and radial extensions. In this way the etched capillary surface provides a compatible geometry to facilitate solute/bonded phase interactions to promote the degree of retention required for conversion of electrophoresis into the electrochromatography technique.

1.4. Characterization of the capillary column.

1.4.1. SEM study.

Scanning electron microscopy (SEM) is a technique well suited for characterization of the inner surface of a capillary column in order to determine surface roughness as well as to monitor the formation of silica based dendrites or whiskers (5, 32).

1.4.1.1. The principle of SEM analysis.

SEM is used to obtain both qualitative and quantitative information about the morphology or composition of the sample features in order to optimize the correlation of microscopic with chromatographic data. The technique provides reproducible characterization of all image features such as particle size, inclusions, and the internal pores of a solid structure. In principle, SEM analysis should extract the maximum amount of information obtained per feature while minimizing both the complexity and the time required for analysis.

In SEM, an electron beam is passed through an evacuated chamber and then focused by electromagnetic lenses onto the sample surface. The beam is rastered over the specimen and then synchronized with the beam of a cathode ray tube

(CRT). Upon electron impingement of the sample, the inelastically scattered secondary electrons are emitted from the sample and collected by a scintillator. The signal produced is then used to modulate the brightness of the cathode ray tube to form the CRT display screen. A difference in secondary emission results from changes in the surface topography. The backscattered (elastically) electrons are collected to form the image and the subsequent contrast results from compositional difference. Today, highly magnified SEM imaging is accomplished by a completely computerized controlled manipulation of the electron beam that is rastering over the sample surface. Highly evolved cameras are employed to record the images on the display screen and print the micrograph pictures (37, 38, 39).

1.4.1.2. SEM of modified inner capillary surface.

The true nature of the rough surface of the inner capillary column was investigated by employing SEM instrumentation in 1975 (21). Since then SEM has been extensively used for the elucidation of the planar and three dimensional morphology of modified surfaces and for the correlation of this information with other techniques such as ESCA, Auger electron spectroscopy, and chromatographic analysis, which, in turn, provides valuable insight for the determination of appropriate surface treatment (30, 40).

SEM instrumentation is routinely employed to determine whether acid leaching has any effect on the gross structure of the inner column surface. The analysis provides rapid determination of the morphology of the inside wall without the inner surface undergoing any discernible physical or chemical roughening. SEM used to investigate soft glass capillaries demonstrated that the etching of the column with basic solution decreased the inner wall diameter by producing a new porous layer (25).

Onuska et al. (15, 41) used SEM to characterize various deactivated inner surfaces. The SEM micrographs illustrated that the glass capillary: (i) treated with hydrochloric acid as the leaching reagent is pitted on the external surface; and (ii) treated with barium carbonate as the deactivation roughening etchant is covered with crystal on the internal surface. Furthermore, the inner surface of the capillary treated with hydrogen fluoride gas and then examined by SEM demonstrated the presence of whiskers, the filamentary silica species formed by dissolution and deposition of silica on the inner capillary wall. The SEM study illustrated the importance of investigating the growth and extent of silica whiskers as well as their rate of formation and distribution throughout the inside wall that produces a uniform layer on the inner surface of the glass capillary. Pesek et al. (9) implied that thermal etching of the inner surface of a fused silica capillary produced radial extensions, which uniformly were improved with an increase of the etching temperature. Additionally, column

inspection over the entire length using SEM demonstrated adequate homogeneity of a newly developed surface layer, once the etching conditions are controlled.

Modern SEMs employ an electron dispersive spectroscopy (EDS) portion of the unit for qualitative characterization of various solid surfaces. A characteristic X-ray from each element is generated when the electron beam is positioned onto the image feature. Those elements above sodium in the Periodic Table are readily detected by energy dispersive spectroscopy. The relative intensities of the selected elements in the X-ray spectrum are calculated and used by the software-based analyzer system to assign each feature to a particular category, thus a determination of the sample composition is easily obtained (42).

1.4.2. BET characterization of the inner surface of fused silica column.

The fused silica capillary column is chemically etched to roughen the inner surface of column wall in order to improve the coating of the stationary phase. Onuska et al. (15) reported that the roughened surface of the inner wall of a borosilicate capillary is increased approximately 1000-fold when compared with a bare and untreated inner surface. There is no indication of the method or the technique used for surface measurement evaluation. Additionally, no other study of surface measurement of the capillary column is found. The

measurement of surface area is routinely performed following a classical method developed by Brunauer, Emmet, and Teller, which is simply referred to as the BET method. In numerous Catalytica studies, the surface areas of various materials are investigated using fully automated BET instrumentation.

The surface area measurement method involves first the elimination of the preadsorbed gases and vapors from the solid surface in the outgas stage of the BET instrument. The outgassed material is then transferred to the analysis section of the unit and cooled to the boiling point of liquid nitrogen, which in turn, serves as the adsorbate gas. The solid surface is then incrementally exposed to higher partial pressures of the nitrogen adsorbate gas until the entire surface is covered with a monolayer of nitrogen gas. The extent of the surface of the solid is calculated by multiplying the number of molecules of the adsorbed gas required for this monolayer times the area covered by each adsorbed gas molecule. The sample pressure is recorded as a fraction of the saturation pressure; it is defined as a relative pressure. The volume of gas adsorbed (condensed) by the solid sample is determined and expressed as a function of the relative pressure. The BET surface area is determined by extrapolation of these two measured parameters. Typically, five point measurements are generated to construct the adsorption isotherm (43, 44, 45).

1.5. The aims of the study.

The modification of the capillary inner surface by physical roughening is conducted in order to increase the surface wettability and increase the surface area for better adaptation (better bonding) of the stationary phase. Pesek et al. (9) proposed that a capillary column pretreated in this way demonstrates improved separation of proteins in electrochromatography. Therefore in this study, the chemical etching of a fused silica capillary column is performed analogously to the method previously used by Onuska et al. (15) and adopted by Pesek et al. (9). Onuska used ammonium hydrogen difluoride at elevated temperature to produce the whisker features on the inner surface of Pyrex capillaries. The uniform and adequately roughened surface exhibited no adsorption effects and good separation efficiency. The reported optimum of the factors influencing uniform coverage and the conditions controlling the filament growth provided a good background of chemical etching by fluoride media so that the SEM technique can be adopted for characterization of the inner surface. Pesek et al. (9) adopted this etching technique for modifications of narrow bore fused silica capillaries. The inner wall of the column treated with ammonium hydrogen difluoride for 3 hours at 400 ° C displayed an increase of the silica surface area by formation of uniform and homogeneously distributed radial extensions. Furthermore, the etched capillary was modified by the silanization/hydrosilation

method leading to direct silicon-carbon bonds on the surface in order to obtain enhanced stability in electrochromatography.

In this study, the influence of temperature, time of chemical etching as well as the concentration of the etchant are investigated since they affect the roughening process as previously described. The column etching reproducibility and repeatability of the roughening process are closely evaluated since these two aspects are essential in preparation of highly efficient separation columns.

The characteristics of the inside of the fused silica capillary column are observed using a scanning electron microscope, and the conditions of the etched surface are investigated by studying the micrographs. New surface wall composition is confirmed using energy dispersive spectroscopy (EDS) and inductively coupled plasma atomic emission spectroscopy (ICPAES). The surface area of the inner wall of the fused silica capillary is measured using the physisorption analysis by BET. Overall the inner surface of the fused silica capillary column is discussed based on a correlation of SEM micrographs, BET surface area measurements, as well as an optical microscope and visual observations. The established and optimized conditions of column treatment as well as the characterization of the etched inner surface can be correlated with the chromatographic performance of selected capillaries providing valuable insights for the determination of the

appropriate glass surface to produce a capillary column of superior separation efficiency for electrochromatography.

2. Experimental.

All experimental procedures are the modifications of the reported chemical etching of fused silica capillary tubing by Pesek at el.(9) and of the borosilicate glass capillary by Onuska at et.(15). Some of the innovative experimental details were learned from discussions with chemists at Catalytica Incorporated.

2.1. Reagents and consumable materials.

The ammonium hydrogen difluoride solid (catalog number-22,482-0) of 98 % purity, a melting point of 125 ° C, and a decomposition temperature of 1570° C, was obtained from the Aldrich, Milwaukee. The sodium silicate ($\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ catalog number-Sx0750), which was used initially for sealing of the glass capillary, was obtained from EM, New York. The high purity HPLC grade methanol and acetone solvents were purchased from Baxter Diagnostic Incorporated, Illinois (the catalog numbers are respectively CAS67-56-1 and CAS 67-64-1). The 37 % wt/v concentrated nitric acid was obtained from J.T. Baxter, Philipsburgh, New York (catalog number 9535-33). Throughout the experimental part deionized water of 18 mega ohms from the in-house supply was used for rinsing of the

inside of the fused silica column. A total of 20 meters capillary column of 0.530 mm internal diameter used for the whole study was obtained from J&W (PN 160-2530). The fused silica capillary column was untreated and not deactivated.

2.2. Equipment

2.2.1. Chemical etching setup.

2.2.1.1. Capillary cleaning and etching setup.

The static acid cleaning and chemical etching were performed using the apparatus schematically represented in Figure 3. The capillary column was filled with the appropriate solution from a 10 ml syringe connected with tygon tubing at one end. The other end of the capillary tubing was extended by an additional twenty centimeters of tygon tubing into a waste reservoir. The cleaning and etching solutions were injected into the capillary column, which was positioned in the horizontal plane. Approximately 5 ml of cleaning or etching solution were used to rinse out the column. Typically 90 % of the capillary column volume was effectively filled with the solution. The filled column pieces were stored in a flat Pyrex beaker. It was observed that the filled capillary columns placed onto a clean cotton Kimwipe or positioned vertically for a second were empty due to gravity

flow of the solution. At this point, each column was inspected under light for the presence of the leaching or etching reagent.

Figure 3. Schematic representation of the apparatus for static cleaning and chemical etching (not to scale).

2.2.1.2. Capillary rinsing and drying setup.

Two independent gas chromatography column rinsing kits were used respectively for capillary tube rinsing and drying. The schematic representation

of the capillary rinsing kit is illustrated in Figure 4. The setup consisted of a

Figure 4. Schematic representation of the apparatus for rinsing and drying of the capillary tubing (not to scale).

10 ml vial with a screw cap and a Teflon lid with a small opening for the Teflon "tee" adaptor. The horizontal opening of the "tee" connection was used for the

Teflon fitting for inert/dry gas. The in-house nitrogen and helium were supplied at about 20 psi pressure for column rinsing and drying, respectively. At the top of the "tee", a swagelock fitting was used as a connection for a short tygon tube and a short piece of capillary column.

2.2.2. The methane-oxygen torch setup.

High pressure methane and oxygen gases were used to operate the flame torch. The primary flame was methane. The oxygen gas was slowly introduced into the methane flame until a cone of blue was observed. This high temperature flame was required to seal the ends of the treated fused silica capillary column.

2.2.3. Conventional and microwave furnaces.

A conventional muffle furnace programmable up to 1600 ° C was used for the thermal treatment of the capillary column. In some of the preliminary experiments, the MAS 300 microwave furnace from CEM Corporation, North Carolina, was used. The microwave unit was programmed for 100 % power, representing 900 watts at appropriate temperature and time conditions.

2.2.4. Minor key accessories.

Stainless steel tweezers and vice-bench pliers were essential tools throughout the experimental operation with the capillary columns. Some of the capillary column sections were as short as 2-3 mm. The vice-bench pliers were used to open the capillary column lengthwise. Glass beakers and 50 ml glass test tubes were used, respectively, for short and long term storage of the capillary columns. 100 ml volumetric Nalgene flasks were used for chemical reagent storage. Additionally, a razor blade and a metric ruler were used for cutting and measuring the capillary tubing.

2.3. Instrumentation.

2.3.1. Microscopy setup.

2.3.1.1. SEM-EDS.

An Amray model 1820I scanning electron microscope (SEM) was used for the characterization of the internal wall of the fused silica capillary column. The SEM consists of three separate modules which are the electron optical console, the electronic and display console, and the energy dispersive x-ray spectrometer

Figure 5. Schematic representation of the SEM-EDS system

(EDS) system as schematically illustrated in Figure 5. The optical console consists of the electron optical column, specimen chamber and stage, as well as vacuum and vibration isolation systems. The instrument was operated at 20 kV, 40 mA emission current, and at a working distance of 10-30 mm. The sample support was tilted from 10-40 ° towards the incident beam for the examination of the inner surface of the capillary from a cross-sectional cut. The intact capillaries were adjusted to the horizontal plane parallel to the electron beam.

2.3.1.2. Sputtering system.

The sputtering system was used to gold plate the capillary column in order to make the glass a conductive material. The sections of the capillary column used for SEM study were obtained: (i) by breaking with bench-vice pliers in order to open the capillary column lengthwise; (ii) by cutting with a razor blade to obtain the cross-sectional capillary view as depicted in Figure 6. Double adhesive carbon tape was placed on the SEM sample holder (stub). The cut portions of the capillary column were mounted onto the carbon tape. The residual portions of the capillary column were blown away with compressed air. The SEM stub with the sample were located in the vacuum chamber of the sputtering system and the gold was plated onto the material for 30-60 seconds.

2.3.1.3. Optical microscope.

The light microscopes were used for the primary inspection of the capillary column. Both of the systems employed a computerized camera to take the color images. The capillary tygon tubing was checked for any fractures using 20 to 60 times magnification.

Figure 6. Capillary column opened by a lengthwise cut (a) and a cross-sectional cut (b).

2.3.2. Surface area analyzer.

The BET automated system from Micromeritics (Model ASAP 2000) was used to determine the surface area of the inner wall of the fused silica column. Between 20 to 40 sections of capillary column, each of 10 cm length, at a total sample weight of 500 to 1000 mg were loaded into the glass BET sample tube. The sample tube was sealed and connected to the external manifold for gas flow. The material was outgassed for 1 hour at 200 ° C in order to remove preadsorbed gases and vapors from the surface of the solid. During the analysis, the tubes with the samples were immersed in liquid nitrogen for cooling to the boiling point of the adsorbate gas. The system was evacuated to 10^{-5} torr of pressure. The BET surface area was determined based on a measurement of five adsorption points. The capillary columns from non-destructive BET analyses were used for further experiments.

2.4. Procedures.

2.4.1. Preparation of the etching reagent.

1, 3, and 5 g of ammonium hydrogen difluoride solid was weighed out into a volumetric Nalgene flask and diluted with methanol solvent to 100 ml total volume to prepare respectively 1, 3, and 5 % wt/v of the etching solution. The

prepared solution was sonicated for one hour in an ultrasonic bath. The etching solution was cooled down to room temperature. The 5 % w/v was a saturated solution and contained a substantial amount of undissolved solid. Except for reproducibility tests, the etching solution was not filtered in most of the experiments. A fresh etching solution was prepared prior to each of the chemical treatments.

2.4.2. Column preparation.

2.4.2.1. Inspection and cutting of the capillary column.

The capillary column tubing was inspected under the optical microscope for any external damage. The fractured pieces of the tubing were removed. The rest of the capillary tubing was cut into 10 cm pieces. The residual column pieces from the cutting were blown away with a nitrogen stream. The column tubing for the reproducibility experiment was cut to about 50 cm in order to fit into the muffle furnace.

2.4.2.2. Thermal acid treatment.

A 10 cm capillary column was filled with a concentrated hydrochloric acid from a 5 ml syringe. Typically 80-90 % of the column volume was filled. Placement of

the column in the horizontal position was essential to prevent the acid from dripping out from the end of the capillary. Both ends of the capillary tubing were sealed by using a high temperature methane-oxygen flame. The columns were closed and bent at the temperature of the glass melt; here some of the HCl was lost and consumed by the flame. The sealed capillary pieces contained on average 60-80 % of volume filled with the acid. The columns placed in a glass beaker were transferred into the muffle furnace. The muffle oven was programmed for 24 hours at 80 ° C. The columns were typically heated overnight for 12-16 hours. After that, the columns were opened by cutting off the sealed ends. On average, around 7-9 cm of the column tubing length was left. The opened tubing was washed with water using the rinse kit as described in section 2.2.1.2. Approximately 10 ml of water and acetone were used for rinsing the column. The rinsed columns were transferred to the drying assembly (Figure 4) and purged with helium for five minutes at 20 psi.

2.4.2.3. Chemical etching

The acid-cleaned and helium dried sections of the capillary columns were filled with ammonium hydrogen difluoride etching reagent using the previously described procedure for thermal acid treatment (section 2.2.1.1.). The static chemical etching was done by filling the capillary with ammonium hydrogen difluoride and allowing it to sit at room temperature for one hour. After that the

methanol solvent was removed by uniform flow of helium gas at around 20 psi. Upon gas purging through the column, a milky-white discoloration of the inner column wall was observed throughout the whole length of the tubing, except for the first 5 mm at the capillary ends. The discoloration indicated that a chemical reaction had already taken place at room temperature. The portions of tubing with no milky discoloration were marked and defined as unsuccessfully etched capillary parts. The columns with deposited ammonium hydrogen difluoride were then sealed with the flame and placed into the flat beaker. Various conditions of the etching process such as temperature, time, and concentration of etching reagent, were used:

- (i) temperatures of 100, 200, 300, 350, 400, 500° C as well as room temperature;
- (ii) time of 1, 3, 6, 15, 24, 72 hours;
- (iii) 1, 3, 5 % w/v of ammonium hydrogen difluoride in methanol.

Subsequently, the capillary was opened. The short sections (5-8 cm) of capillary tubing were rinsed with around 10 ml of methanol and dried with helium gas.

2.4.3. The reproducibility test.

Three 50 cm long capillary tube pieces were used for the reproducibility test. These longer pieces of the capillary column were treated with the respectively increased volumes of ca. 10 ml of cleaning, etching, and rinsing solutions.

The capillary drying procedure was prolonged to about 15 minutes per each column section. The capillary columns were filled with concentrated HCl solution using a 10 ml syringe as described before. The columns were flame sealed and heated overnight at 80 ° C. After that, the columns were opened and rinsed with about 20 ml of water followed by 20 ml of acetone solvent. The drying of the capillary column was repeated for 15 minutes with helium at 20 psi. The cleaned columns were filled with ammonium hydrogen difluoride using a 10 ml syringe, sealed with the flame, and then placed into the oven for 3 hours at 350 ° C. After heating, the columns were treated with about 10 ml of methanol and dried again with helium gas for 15 minutes. Each column was divided into the five equal sections respectively labeled as reproducibility test A, B, C, D, and E. Each of these sections was divided again into the beginning, middle, and end and respectively labeled such as reproducibility test Ab, Am, Ae, etc. This procedure allowed a very thorough examination of the etched capillary.

2.4.4. The repeatability test.

A short column of 10 cm was acid-treated and etched at 350° C for 3 hours as described in section 2.4.2.3. The prepared capillary was opened lengthwise and by a cross-sectional cut and inspected under the SEM. The surface modification process was repeated on at least three different dates in order to test its repeatability.

3. Results and discussion.

Our work have shown that the fused silica capillary column is both inherently straight and remarkably flexible. For example, bending of the capillary tubing into a small circle of around 1-2 cm diameter did not cause any visible external damage to the column. Therefore, in spite of its fragile appearance, the capillary column is extremely durable.

Other work has shown that the fluoride media have been well known as a cleaning, etching, and dissolution solvents of silica. Etching with ammonium hydrogen difluoride is one of the most frequent techniques used in semiconductor applications, where lapped surfaces of quartz are chemically polished. The adsorption of hydrogen and ammonium positive ions onto the surface of quartz changes the electronic configuration around the silicon which makes the final dissolution by hydrogen fluoride (46). The mechanism of quartz dissolution is based on electron motion along the Si-O link. The quartz dissolution as a function of temperature has a exponential behavior, and the dissolution is a thermally activated Arrhenius process. An increase in temperature subsequently increases the quartz dissolution rate.

In spite of the vast popularity of fused silica, the etching of this material is poorly explored (7). Pesek et al. (9) reported that at elevated temperature the

original surface of the fused silica capillary is dissolved by the etching agent and then precipitated onto the wall in rather large particles, producing a more uniform surface layer at higher temperature and longer times (34). Onuska et al. (15) reported that the silica surface of a Pyrex capillary column is dissolved by reaction with gaseous hydrogen fluoride producing silicon tetrafluoride, which, in turn, is deposited back on the etched surface in the form of crystalline whiskers of silicon oxide.

The whiskers represent filamentary crystals with a near perfect structure. Hydrogen fluoride gas is produced by the dissociation of ammonium hydrogen difluoride at elevated temperature to form gaseous hydrogen fluoride and ammonia. The actual chemistry occurring on the inner surface of both a borosilicate and a fused silica capillary is very complex due to vapor-liquid-solid phase reaction, and it is unknown (15, 40).

In review of whiskers technology (47), it is reported that the driving force of the whiskers growth arises from recrystallization and a decrease in supersaturation conditions, respectively, for metallic and metallic oxide species such as gold, copper oxide, and aluminum oxide. For oxide whisker growth, controlled supersaturation conditions are a prime consideration because there is good evidence that the degree of supersaturation determines the prevailing growth in vapor phase whiskers. Low supersaturation is

required for whisker growth whereas medium supersaturation supports bulk growth; and intermediate supersaturation is evidenced by the growth of dendrites and/or platelets. At higher supersaturation, powders are formed by homogenous nucleation in the gas phase. Additionally, it is pointed out that the development of the necessary supersaturation gradient is entirely dependent upon the prevailing temperature gradient, which leads to inherent control difficulties in multiple or complex reaction systems. In a simple case, such as etching the capillary in a conventional oven, the temperature gradient approach becomes more difficult and less controllable due to the substantial volume of the oven chamber dimensions. Isothermal growth conditions circumvent this problem, but require other means of supersaturation control. The employment of a steady-flow air system in the oven reduces the influence of the temperature gradient. Furthermore, it is suggested that the vapor-liquid-solid mechanism occurs in two steps. First, there is a deposition from the vapor directly on to a liquid solution in a vapor-liquid system. The second step occurs in a liquid-solid system and the deposition is precipitated from the supersaturated liquid solution at the liquid-solid interface. For a faceted (smooth) solid-liquid interface, the vapor-liquid-solid mechanism produces unidirectional crystal growth perpendicular to the solid-liquid phase. The temperature gradient zone arises because of continuous melting and freezing of solvent at hotter or colder reactions of the

liquid zone. A temperature gradient results in deposition of the larger crystals on colder zones and smaller crystals or no crystals on hotter zones.

There are a few important aspects for optimizing the surface roughening of the inner surface of the capillary column. An increase in surface roughening results in a compatible topography for better surface wetting and increases the column capacity factors. This is extremely important in capillary columns with a very small internal diameter where a useful capacity factor with mobile phases of realistic composition should be achieved (19).

3.1. Light microscope observation.

The fused silica capillary column was inspected under lower magnification using the light microscope. Figure 7 and Figure 8 are, respectively, the photographs of the fused silica capillary column with and without the external polyimide coating. The polymeric coating was burned off with a flame. In each photograph a color background was applied in order to enhance the contrast of the capillary sections, such as the inner openness or the capillary wall itself. From the photographs, it is obvious that the walls of the capillary tubing are very thin, which makes the column extremely flexible.

Figure 7. Fused silica capillary column without the polyimide coating magnified using an optical microscope: (a) 30x; (b) 47.3x.

Figure 8. Fused silica capillary column with the polyimide coating magnified under optical microscope: (a) 47.3x; (b) 65.5x.

At the beginning of experimental work with the capillary column, it was learned that poorly etched capillaries had an uneven distribution of hydrochloric acid or etching solution. This contributed to unsuccessful column treatment. Figure 9 portrays a poor distribution of hydrochloric acid in the fused silica capillary column. The three light spots along the capillary columns are the droplets of unevenly distributed hydrochloric acid.

Figure 9. Uneven distribution of hydrochloric acid in fused silica capillary tubing magnified (8x) under optical microscope.

3.2. SEM study of the etching process.

A great portion of the study is effectively illustrated by micrograph images obtained from the scanning electron microscope. An average of five SEM micrographs were taken per test, and about 80 chemical etching processes were conducted.

One of the most surprising and important aspects of the SEM study is the apparent ease with which the images of rough objects can be interpreted by a newcomer in the field. It was learned that the inner surface of the fused silica capillary column can be easily observed in different perspective at magnifications of 300, 600, 1000, 3000, 6000, and 11000 times. In a major part of the SEM study these magnifications were used in order to perform comparisons of the surfaces.

In SEM analysis, the electron beam enters the specimen chamber and impinges the specimen at a single point location. The interaction volume of both elastic and inelastic scattering produces detectable signals and by measuring its magnitude, detection of the specimens local topography is possible (39). The elastic (high angle) and inelastic (low angle) interactions are, respectively, the electrons scattered from the positive nucleus and negative electron of an atom of the specimen (38). In SEM, in order to cover a

representative area of the sample, the electron beam is moved throughout the inner surface of the fused silica wall. The information obtained from SEM consists of both scan location in X-Y space and the corresponding set of intensities from the detector (Figure 10).

Figure 10. The principle information displayed on the CRT by image scanning, where the magnification is defined as a ratio of the length of the area scanned on the CRT over area on the scanned sample.

As it is presented in the figure above, the SEM image consists of a two-dimensional reconstruction of the information derived from the samples, the information from a three-dimensional volume of space is effectively projected onto the two dimensional plane. The information contained in the image such as inclusion or globular features, conveys the proper shape of the analyzed feature: the synchronous sample-CRT is contrived to maintain the geometric relationship of any arbitrary chosen set of points on the specimen and on the CRT. Thus, as an example, a triangle on the sample maintains its shape regardless of the CRT magnification. However, there are some factors that influence changes in a final image. First, imperfections such as broken-off capillary parts in the scan can lead to image distortion. Second, the perceive size of feature differs as a result of the magnification. Additionally, it is important to know that the image does not rotate as the magnification is changed. Figure 11a portrays a surface with rough topography having circled features that are incrementally magnified, respectively, in Figures 11 b, c, d. A relative rotation of the image is obtained when changing the working distance. The working distance is defined as the distance between the final lens pole piece of the specimen to the uppermost portion of the sample. A high magnification is obtained by minimizing the working distance.

All micrographs obtained from the SEM study show a difference in the chemical etching of the fused silica with regard to reagent concentration,

Figure 11. SEM magnification of the crystalline structures on the inner wall of fused silica: (a) 300x; (b) 600x; (c) 995x; (d) 3020x.

time, and temperature. The influence of chemical etching on the inner surface of the fused silica capillary column is discussed in the following chapters.

3.2.1. An inner surface of a bare capillary column.

The bare, untreated, non deactivated inner surface of the fused silica capillary column is illustrated in Figures 12 and 13, respectively, for the lengthwise cut (l.c.) and the cross-sectional (c.c.). The incompatible inner surface of the bare fused capillary with respect to both the wetting and the geometrical properties is illustrated as a very smooth surface. Some of the dust particles are observable as the broken-off pieces of glass tubing, which are distributed throughout the whole length of the capillary column. The crack in the surface is easily observable as the consequence of lengthwise cracking from bench-vice grips.

3.2.2. Acid treated surface of the inner wall of capillary column.

The surface of the inner wall, after overnight treatment with concentrated hydrochloric acid at an elevated temperature of 80 ° C does not indicate any discernible topographic features or roughening. This is in good agreement with the previously reported data (21). The acid treated surface resembles the

Figure 12. The bare, untreated, and not deactivated inner surface of fused silica capillary column. Inner surface opened by a lengthwise cut and magnified: (a) 300 x; (b) 1000x; (c) 4729x.

Figure 13. The bare, untreated, and not deactivated inner surface of fused silica capillary column. Inner surface opened by a cross-sectional cut and magnified 300x.

Figure 14. Inner surface of fused silica column after hydrochloric acid at 80 °C overnight treatment prepared by a cross-sectional cut and magnified 1000x.

Figure 15. Inner surface of fused silica column after hydrochloric acid treatment at 80 ° C overnight (lengthwise cut): (a) 300x; (b) 1020x; (c)4600x.

Figure 16. Inner surface of fused silica column after hydrochloric acid treatment at 300 °C for 3 hours (lengthwise cut): (a) 2060 x; (b) 3740x; (c) 8850x; (d) 18700x.

image of a smooth surface, i.e. that of a bare capillary. Each is shown in Figure 14 and Figure 15, for the column opened by a cross-sectional cut and a lengthwise cut, respectively. The dust particles are presumably removed however the pieces of the broken capillary still remain on the surface. Since the majority of the etching process was conducted at elevated temperature and prolonged time, the acid treated surface was heated for 3 hours at 300 ° C, in order to determine whether reaction conditions rendered any change. The micrographs in Figure 16 are the images of the acid surface treated at the 300 ° C for 3 hours. Figure 16 a and b and Figure 16 c and d are the images of the acid treated surfaces washed with water, and water-acetone, respectively. There is no discernible topography evident in any of the images of the capillary inner surface investigated after the hydrothermal acid treatments. Based on the surface study of the inner wall before etching or deactivation, it was reported that the surface thermally treated with hydrochloric acid causes an increase in polarity of the stationary phase, if the residual chlorides are not effectively removed (22).

3.2.3. The effect of temperature on chemical etching on the inner surface.

The etching processes conducted at room and at elevated temperatures are discussed on an individual basis. In all temperature experiments, the inner

surfaces of fused capillary were etched with 5 % w/v ammonium hydrogen difluoride, according to the procedure described in section 1.3.2.

3.2.3.1. Room temperature etching.

It appears that the etching process occurs at room temperature after a one hour treatment. Upon removal of methanol solvent from the etchant matrix, a “milky” discoloration of the inside of fused silica capillary was observed. The “milky” or “foggy” discolorations are typical for etching of various types of glass surfaces, such as silica or quartz with fluoride media. Fused capillary treated with pure methanol solvent without the etching reagent did not contribute to the milky discoloration on the inside of the capillary tubing.

A series of micrographs from room temperature etching conducted at different etching times are illustrated in Figures 17-20. The micrographs at lower magnification in Figure 17 a and b portray the surface as being sparsely covered with particulates of irregular elongated shape with sufficiently intensive enough edges and no interior structure. They also reveal very dark spots resembling the pitted or uncovered surfaces. The size of the features is measured by comparing the elongation to the micrograph scale and it varies from 1-5 μm ; the radii for the pitted holes (dark area) are approximately 10 μm .

Figure 17. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at RT. Inner surface opened by a lengthwise cut and magnified: (a) 300x; (b) 1000x; (c) 3000x; (d) 6000x.

In SEM images, the topographic contrast arises because backscattering and secondary electron generation depend on the angle of incidence between the beam and the sample. The angle of the incidence varies because of the roughness (topography) of the capillary, resulting in the contrast formation, which, in turn is related to the physical shape of the material. The gray scale of the SEM images varies from a light whitish-gray to a blackish-gray level and corresponds to the full range of input voltage from the SEM amplification system. In such a display, the minimum contrast level in the input signal that the eye can perceive is about 5%. Values of the natural contrast that fall below the 5% cannot be distinguished by the observer in such an image (39). The images in the high dynamic range of the contrast span are easily interpreted. The lightest and the darkest colors on the grayish scale represent respectively the top and the bottom of the features as well as the highest and the lowest points on the surface.

High magnification of the crystalline features is obtained by decreasing the working angle of the specimen with respect to the detector system position. Blurred pictures are obtained beyond a certain magnification, as shown in Figure 18. No more information can be gained from the image. The profile reveals, the irregular shape and size of the crystal with a rough surface at the base. The heights of the features are approximately 1-2 μm . However, the

Figure 18. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at RT. Inner surface opened by a cross-sectional cut and magnified 15000x.

distance measurement in such image can be meaningful only if the local angle tilt on the surface is known. For a highly tilted surface, the magnification perpendicular to the tilt axis is less than that parallel to the tilt axis by a factor of a cosine of the tilt angle. Therefore, the backscatter and the secondary electrons are collected using a commonly tilted angle of 30-45 degree toward the scintillator-photomultiplier detector system (39).

The inner surface of the capillary column treated with hydrochloric acid at 80° C, rinsed with water and acetone, and etched with ammonium hydroxide difluoride overnight at room temperature is illustrated in Figure 19.

The treated surface is more evenly covered with crystalline features of sharp contours and has more body structure than that resulting from a short etching time of one hour. Another etching done overnight indicated the deposition of different shape features. Figure 20 illustrates a surface treated with hydrochloric acid at room temperature, and rinsed with acetone only followed by etching with ammonium hydrogen difluoride overnight at room temperature. The morphology of the surface is dramatically different from the surface obtained previously. It resembles "a carpet of the pebbles." The reason for different shapes of the species is unknown, however, it is clear that room temperature etching for one hour or overnight is not controlled well enough to produce the same topography of the inner surface layer.

These observations cannot be compared with any other finding due to the lack of extensive study of inner surface of capillary columns, especially that of surfaces of fused silica columns. The images of inner surfaces prepared by cross-sectional and lengthwise cuts differ upon increase in surface roughening. This is due to the electrons impinging on inner surface of capillary column under different angles, resulting in various electron interaction volumes within the solid matter.

Figure 19. Capillary column treated with concentrated HCl at room temperature, rinsed with water and acetone, and etched with saturated ammonium hydrogen difluoride overnight at RT. Inner surface opened by a lengthwise cut and magnified: (a) 300x; (b) 1000x.

Figure 20. Capillary column treated with concentrated HCl at 80 °C, rinsed with acetone, and etched with saturated ammonium hydrogen difluoride overnight at RT. Inner surface opened by a lengthwise cut and magnified: (a) 1030x; (b) 3040x.

In a lengthwise cut with no tilted surface, the tendency for the forward scattering causes most of the electrons to propagate down into the wall of capillary column. In a cross-sectional cut with a surface tilted with respect to detector, the electron propagation is more closely directed to the surface, and the electron interaction is consequently reduced. A simply analogy is a person sitting in an airplane flying over mountains at different times of day and observing various surface topographies of the land as the angles of the sun varies with respect to the earth's horizon. At sunset or sunrise, with the sun slightly above the horizon, the passenger observes a three-dimensional topography of mountains, and the land appears as a rough surface. At noon, with a sun at its zenith, and the sun irradiation perpendicular to the horizon, the passenger perceives a two-dimensional structure of mountains, and the land resembles a flat surface. Figure 21 schematically illustrates the electrons impinging on various surfaces, the electrons interaction volume with the surface matrix, and the SEM images for a capillary column prepared by cross-sectional and lengthwise cut, respectively.

3.1.1.2. Elevated temperature etching.

SEM images obtained from etching of an inner surface with 5 % w/v of ammonium hydrogen difluoride at 100, 200, 300, 350, 400, 500 ° C are reviewed in separate sections. The etching time at each temperature is approximately

Figure 21. A schematic representation of electron beam-solid interaction and SEM images for the capillary column prepared by (a) lengthwise cut and (b) cross-sectional cut (not to scale).

one hour. The images of the surface etched using microwave radiation and conventional heating are compared. All etched surfaces are examined with respect of surface roughness, increase of surface area, wetting properties, and surface compatibility for coating with a stationary phase. It is important to report that capillary column samples for SEM study are prepared in duplicate, and various parts of each capillary are investigated in order to extract the maximum information about the etching process.

(i) Etching at 100 °C.

The micrographs of the inner surface treated with fluoride etchant at 100 °C for one hour are shown in the Figure 22 and 23, respectively, for a capillary column prepared by cross-sectional and lengthwise cuts. The inner surface opened by cross-sectional cut is viewed as a moderately rough surface and resembles grayish-white compacted clouds. Under closer magnification of 3000 times, a similar surface topography is elucidated in the background of image obtained from the lengthwise cut as pointed out in Figure 23 c. The inner surface opened by lengthwise cut has analogous topography to the one obtained at room temperature. Under increased magnification, the crystalline structures are composed of large spikes which appear to originate from an elongated backbone. The features resemble the branches of a pine tree. These pine tree branch-like features are pointed out in various directions. The

Figure 22. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 100 ° C. Inner surface opened by a cross-sectional cut and magnified: (a) 302x; (b) 1000x; (c) 3000x.

Figure 23. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 100 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 302x; (b) 1000x; (c) 3000x.

features are frequently superimposed on each other and are densely distributed throughout whole length of capillary inner wall. They vary in size from 20-30 μm . These measurement determinations deviate slightly from a real size of crystalline structures. The local tilt angle of crystalline features with respect to the inner surface of capillary column is unknown, and it is rather difficult to determine at this point. Only the object which ideally lies in planes perpendicular to the optical axis are reconstructed with minimum distortion. The surfaces which are tilted relative to the normal scan plane are shortened in the reconstruction. The dimensions of features obtained by SEM determinations are used for comparisons with other structures only. Further magnifications of pine tree branch like features in Figure 23 b and c indicated that the side crystalline outgrowths are composed of smaller globular or irregular shape structures "glued" together.

(ii) Etching at 200 °C temperature.

Pesek et al. (9) reported that etching at 200 °C temperature contributed to formation of large spikes protruding from inner wall of fused silica. The mechanical study by vibration of long pieces of capillary tubing determined that the surface with large particulates exhibited poor mechanical stability, and it is easily damaged. SEM micrographs of the surface etched at 200 °C are presented in Figures 24 and 25. Under low magnification, the inner surface

Figure 24. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 200 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 300x; (b) 1000x; (c) 3000x, (d) 6000x.

Figure 24e. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 200 ° C. Inner surface opened by a lengthwise cut and magnified 15000x.

Figure 25. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 200 ° C. Inner surface opened by a cross-sectional cut and magnified 6000x.

has a similar topography to the pine branch-like features as the surfaces obtained by etching at room and elevated (100 °C) temperatures. Under increased magnification, the pine tree branch-like features have short needle outgrows, and many of them are actually broken off the main backbone as shown in Figure 24 b and c. Furthermore, the needle-like features of irregular shape are very close with respect to each other. It is suspected that the increase of the etching temperature cause dissolving of these crystalline features. Under intense magnification of approximately 15000 times, the background topography under the crystalline features appear to have some irregular shape structures smaller than 1 μm . The profile of the inner surface opened by a cross-sectional cut is the SEM displayed in Figure 25.

(iii) Etching at 300 ° C.

The topography of the inner wall has dramatically changed after column treatment with fluoride etchant at 300 ° C. The surface morphology obtained under these conditions is illustrated in Figure 26. The etched surface is of a mild roughness, and it resembles a series of sand dunes. Presumably, the etching at this temperature causes considerable melting of the crystalline features to form a relatively flat, two dimensional topography. This porous layer of inner column wall appears to be very uniform and homogeneous with good properties for coating of a liquid stationary phase.

Interestingly, micrographs presented in Figure 27 are the images of the capillary with no milky discoloration observed upon removal of methanol from the etchant matrix. The inner surface of the capillary is portrayed as a fluffy layer with a diminutive type of dendrite like features. As previously reported, the milky discoloration is an excellent indication of effective etching inside the capillary tube.

(iv) Etching at 350 ° C.

The inner surface obtained by etching of the capillary column at 350 ° C is analogous with the one obtained from etching at 300 ° C. The micrographs of uniform and homogenous surface of very mild roughness are presented further on, in the reproducibility study (Section 2.4.3). The inner surface formed under these conditions appears to be adequate for coating of a stationary phase due the following reasons: (i) a considerable increase of a surface area; consequently, (ii) considerable increase of wettability; (iii) good uniformity and homogeneity; and (iv) a mild degree of roughening when compared with a surface composed of crystalline features. All of these contribute to improvement in the coating of a stationary phase and the development of a highly efficient capillary column.

Figure 26. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 300 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 600x; (b) 3020x.

Figure 27. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 300 ° C. Inner surface opened by a cross-sectional cut and magnified: (a) 131x; (b) 300x; (c) 600x.

The images of the inner surface from static etching performed in an unsealed capillary column are presented in Figure 28. Presumably, under these conditions, a gaseous hydrogen fluoride produced by dissociation of ammonium hydrogen difluoride escapes from the capillary, and a considerably diminished dissolution of silica surface is attained. In the dynamic etching process employed by Onuska et al. (15) the fluoride etching media is continuously supplied. Thus the concentration of gaseous hydrogen fluoride is sufficient for dissolution of silica and formation of whiskers on the inner surface of the glass capillary. Interestingly, in another study by Sandra et al. (32) the fluoride etchant is continuously supplied to form a silica tetrafluoride species, which, in turn is continuously removed in order to avoid the crystallization of extremely active whiskers.

(v) 400 ° C temperature

A mild roughened layer on the inner wall of the capillary column is produced by fluoride etching at 400 ° C. The inner surfaces exposed by cross-sectional and lengthwise cuts of capillary tubing are presented in Figure 29 and Figure 30, respectively. As previously emphasized, the images of inner

Figure 28. Unsealed capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 350 °C. Inner surface opened by a lengthwise cut and magnified: (a) 1140x; (b) 3020x.

surface topography vary for the capillary opened by different cuts due to electrons impinging on different surfaces; various tilt angles of the sample with respect to the detector result in the formation of different images of the same surface. Furthermore, the effective magnification in all micrographs of the inner surface of a circular capillary varies depending on the angle of a feature relative to the normal scan plane. In an image of a faceted surface and a rough feature the effective magnification varies from place to place in the image. For a highly tilted surface the magnification perpendicular to the tilt axis is less than the magnification parallel to the tilt axis by a factor equal to the cosine of the tilt angle (39). Measurements of any feature dimensions in such an image is only meaningful if the local tilt angle of the surface is known. Tilting of the particular inner surface of the capillary by 1-5 degrees with respect to the SEM detector result in changes of the structure measurement. In this study, all dimensions elucidated from SEM analysis are averaged out and reported as the appropriated ranges of the micrometer scale.

Figure 29. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 400 ° C. Inner surface opened by a cross-sectional cut and magnified: (a) 1140x; (b) 3020x and slightly tilted.

Figure 30. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 400 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 300x; (b) 605 x; (c) different spot under 600x.

(vi) Etching at 500 ° C.

A further increase of the etching temperature is not considered as an important step in the modification of inner surface by roughening for two reasons. First, the capillary column is largely damaged due to degradation of the external polyimide coating, and the column tubing becomes brittle. Second, the inner surface produced under those conditions is similar to the one obtained at 300-400 ° C as shown in Figure 31a-c. However, under increased magnification, the inner surface appears to be more heterogeneous and less uniform on a microscopic scale as illustrated in Figure 31 d and e. The image taken of the capillary column prepared by a cross-sectional cut (Figure 32) indicated formation of larger radial roughening when compared with the lower temperature etching at 300 ° C. On the other hand, as it is mentioned before the local diminutive angle of tilt is unknown therefore the comparison could be inadequate.

(vii) Etching using a microwave heating.

A very mild surface roughening is produced by etching of fused silica capillary using microwave heating. The micrographs in Figure 33 and 34 taken under a low magnification are of poor quality. Both images of the inner surface appear as blurry dark and light spots. More roughening is perceived by

Figure 31. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 500 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 302x; (b) 600x;(c) 1010x;

Figure 32. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 500 ° C. Inner surface opened by a cross-sectional cut and magnified (a) 990x; (b) 3000x.

Figure 33. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 400 ° C using microwave heating. Inner surface opened by: (a) a lengthwise cut and magnified 300x; (b) a lengthwise cut and magnified 300x-different spot; (c) a cross-sectional cut and magnified 300x.

Figure 34. Capillary column etched with saturated ammonium hydrogen difluoride for 1 hour at 500 ° C using microwave heating: (a), (b) different spots of inner surface opened by a lengthwise cut and magnified 300 x; (c) inner surface opened by a cross-sectional cut and magnified 300x.

viewing of the inner surface prepared by a cross-sectional cut of the capillary column as illustrated in Figures 34 c. The sponge like surface without any visible radial extensions appears to be very homogenous. The diminutive roughening produced by etching using microwave heating could be an ideal surface for coating.

3.2.4. Effect of various times on chemical etching.

Onuska et al. (15) reported that the growth rate as well as the whiskers shape on the inner wall of a borosilicate capillary are dependent on the etching time. Shorter etching times of approximately one hour produced smaller silica crystals distributed sparsely throughout the whole length of the column. Longer etching times, from 2-3 hours, produced the largest whisker growth with a maximum surface coverage. Heating beyond this period resulted in globular terminations and shorter whiskers. It is believed, that the longer times of etching and consequently heating cause dissolution of whisker crystals in the vapor phase resulting in a decrease of whisker dimensions. The formation and dissolution of the inner silica surface of the capillary column is based on a very complex and unexplored vapor-liquid-solid mechanism. Neither the processes nor their mechanisms are elaborated in this study.

The influence of heating time on the etching process is also based on the examination of the inner surface topography as determined by scanning electron microscopy. For that purpose the capillary column was treated with saturated ammonium hydrogen difluoride (5 % wt/v) at 300 ° C for various etching times. Upon examination of the inner surface shown in Figure 35 a and b, a white deposition of large silica structures is detected around 20 minutes of etching time. The silica features vary in size with an average length of approximately 10 μm as compared with the SEM scale in Figure 35 c. The silica structures are arranged in no particulate direction throughout the whole length of capillary inner wall. In the Figure 35 d-e, the deposition appears to be of a fluffy character which is typical for many silica species. Under increased magnification of 5000-15000 times as shown in Figure 35 e and f, some surface features are observed below the fluffy deposition. The surface topography has dramatically changed after one hour of etching as shown previously in Figures 26 (section 3.2.3.2.). The etched surface layer of the capillary prepared by a lengthwise cut resembles the topography of beach sand. A very similar topography results from extended heating for three to six hours. The irregular extensions vary in size from 5 to 20 μm for etching at 300 to 400 ° C (Figure 36 and 37 respectively). The extensions decrease considerably in size with prolonged heating from fifteen to seventy two hours as shown in Figures 38 and 39, respectively.

Figure 35. Capillary column etched with saturated ammonium hydrogen difluoride for 20 minutes at 300 ° C. Inner surface opened by a cross-sectional cut and magnified: (a) 600x; (b) 1000x; (c) 2040x.

Figure 35. Capillary column etched with saturated ammonium hydrogen difluoride for 20 minutes at 300 ° C. Inner surface opened by a cross-sectional cut and magnified: (d) 3180x; (e) 5050x; (f) 15000x.

In the first case, the extensions are not observable under low magnification of 600 times. This magnification is sufficient to observe the features obtained from three hours etching. The average size of the surface feature is less than 5 μm . In the second case, the surface topography starts to appear upon relatively high magnification of 3000 times. The average feature size is estimated to be less than 2 μm . It is inferred that the latter extended etching time decreases the size of features as a consequence of either progressive silica melting upon prolonged heating or dissolving in the vapor phase.

An increase of the etching time at room temperature up to 24 hours causes the formation of larger crystalline features with a better coverage of the inner surface of the fused silica capillary as shown in Figure 19 (Section 3.2.3.1.). The etching of fused silica surface at room temperature is presumably based on a liquid-solid phase mechanism.

Figure 36. Capillary column etched with saturated ammonium hydrogen difluoride for 3 hours at 300 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 304x; (b) 605x; (c) 1010x.

Figure 37. Capillary column etched with saturated ammonium hydrogen difluoride for 3 hours at 400 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 615x; (b) 2000x; (c) the surface slightly tilted under 1010x magnification.

Figure 38. Capillary column etched with saturated ammonium hydrogen difluoride for 15 hour at 300 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 1090x; (b) 3000x.

Figure 39. Capillary column etched with saturated ammonium hydrogen difluoride for 72 hours at 300 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 605x; (b) 1000x; (c) 3000x; (d) 5000x.

3.2.5. Effect of various concentrations of the etching reagent.

It is known that the crystal growth of filamentary silica whiskers is directly dependent upon the concentration of the etching solution (15). Therefore, the inner wall of the fused capillary was treated with a different concentration of etchant solution for 3 hours at 300 ° C. The etching using the lowest concentration of 1 % ammonium hydrogen difluoride produced a topography hardly visible even upon a very high magnification as presented in Figures 40 a and b. The surface morphology is not clear. The three-dimensional surface topography could be elucidated based on the contrasts of lighter and darker grayish patterns. The surface topography obtained after treatment with 3 % etchant is presented in Figures 41 a and b. Under this magnification, the inner surface appears to have a very uniform and homogenous structure. The roughen surface magnified 9000 and 12000 times as shown in the Figures 41 c and d resemble the previously observed topography with considerably shorter extensions. The extensions are arranged in a uniform pattern presumably as a consequence of the small dimensions. On average, the size of these extensions is less than 1 μm . The surface view with an angle of around 30 degrees and prepared from the cross-sectional cut indicated that the roughening is very superficial with a depth of less than 0.5 μm when

Figure 40. Capillary column etched with 1% w/v ammonium hydrogen difluoride for 3 hours at 300 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 6900x; (b) 9700x.

Figure 41. Capillary column etched with 3 % w/v ammonium hydrogen difluoride for 3 hours at 300 ° C. Inner surface opened by a lengthwise cut and magnified: (a) 1010x; (b) 2100x; (c) 9450x (d) 12000x.

Figure 42. Capillary column etched with 3% w/v ammonium hydrogen difluoride for 3 hours at 300 ° C. Inner surface opened by a cross-sectional cut and magnified: (a) 4860x; (b) 10200x.

compared to the microscale in Figures 42 a and b. An increase of the etchant concentration up to 5 % w/v produced the previously described topographies and are shown in Figure 26 (Section 3.1.1.2.). Extensions of a larger size are produced and the roughening is considerably more extensive when compared with the 3 % w/v etchant roughening.

3.2.6. Reproducibility of the chemical etching.

Some images from the initial reproducibility etching test at 300 ° C are shown in Figures 43 a-c. The inner surface topography varies throughout the whole length of each capillary as well as with respect to the capillary tubing position itself in the muffle furnace. The inner surface layer composed of crystalline structures are observed in the capillaries placed close to wall of the muffle furnace as well as on the respective ends of each capillary column.

The inner surface of the capillary column placed ideally in the center of the oven is composed of a mildly roughen surface. Under infinitesimal viewing angles, the surface resembles a low hill topography. It is speculated that the large dimensions of the muffle chamber as compared to the microscopic dimension of the column tubing has a substantial temperature gradient.

Figure 43. Inner surface of the same fused capillary column treated with 5 % w/v etchant for 3 hours at 300 ° C. Capillary surface prepared by a lengthwise cut: (a) mild topography (1000x); (b) sponge like topography (1310x); (c) crystalline feature (1000x).

Under these temperature conditions, the etching is not well controlled in order to produce an adequately homogenous and uniform surface.

Another reproducibility test was conducted by etching of the inner surface at 350° C. The three 50 cm long capillary columns were treated with saturated etching reagent for 2.5 hours. After the etching process, each of the capillary columns was carefully prepared as described in section 3.2.6. A schematic representation of the capillary tubing prepared for SEM analysis is presented in Figure 44.

Figure 44. Schematic representation of the capillary cut for the reproducibility test (not to scale).

This setup allowed for a very thorough inspection of the etched surface. Pieces of the same capillary column were prepared by a lengthwise cut and

positioned on one SEM sample stub as shown in Figure 45. The images of the surface topography for one 50 cm column section are presented in Figures 46-50, respectively. The inner surfaces were observed under low and high magnifications, of 600 and 6000 times, respectively. Identical images of the inner surface are observed throughout the whole length of the tested capillaries.

It is known that carbon contamination contributes to the erratic result in the reproducibility test for the Pyrex capillary column (18). However the ubiquitous nature of carbon in the lab environment makes it essentially impossible to exclude carbon contamination.

Figure 45. Typical setup of one capillary column prepared by a lengthwise cut for SEM analysis.

Figure 46. Capillary column etched with saturated ammonium hydrogen difluoride for 2.5 hours at 350 ° C. Inner surface opened by a lengthwise cut and magnified 600x: (a) Ab; (b) Am; (c) Ae sections.

Figure 47. Capillary column etched with saturated ammonium hydrogen difluoride for 2.5 hours at 350 ° C. Inner surface opened by a lengthwise cut and magnified 600x: (a) Bb; (b) Bm; (c) Be sections.

Figure 48. Capillary column etched with saturated ammonium hydrogen difluoride for 2.5 hours at 350 ° C. Inner surface opened by a lengthwise cut and magnified 600x: (a) Cb; (b) Cm; (c) Ce sections.

Figure 49. Capillary column etched with saturated ammonium hydrogen difluoride for 2.5 hours at 350 ° C. Inner surface opened by a lengthwise cut and magnified 600x: (a) Db; (b) Dm; (c) De sections.

Figure 50. Capillary column etched with saturated ammonium hydrogen difluoride for 2.5 hours at 350 ° C. Inner surface opened by a lengthwise cut and magnified 600x: (a) Eb; (b) Em; (c) Ee sections.

3.2.7. Repeatability of etching process.

Initially, the etching process was repeated at 300 ° C for 3 hours which gave a series of various topography surfaces as elaborated in Section 3.2.6. and illustrated in Figure 43. Presumably, the different surfaces are produced as a result of a considerable temperature gradient in the muffle chamber. The etching process at this temperature can be considered as not very well controlled. Etching with saturated ammonium hydrogen difluoride at 350 ° and 400 ° C for 3 hours repeated on different occasions appears to produce the same mildly roughen surface as the ones in Figures 35 and 36 (Section 3.1.1.2.). Under these conditions the etching is well controlled, and it can be easily reproduced.

3.3. Electron dispersive spectroscopic analysis.

Electron dispersive x-ray spectroscopic analysis (EDS) are used in order to confirm the composition of the crystalline features and the roughened surface under it. For x-ray determinations, small pieces of the fused silica capillary column are coated with a thin layer of carbon by evaporation in order to increase the thermal and electrical conductivity. The carbon coating is used to minimize heat damage as well as specimen charging both of which could

result in erroneous x-ray analysis. The light atomic carbon species do not absorb x-rays, thus can not be determined by this analytical technique. The capillary column samples coated with a more conductive layer such as gold contribute to better image quality. However, the gold species is known to cause spectral interference as well as could produce artifacts, and are not suitable in quantitative determinations. In the EDS analysis, the sample distance with the respect to the detector defined as a viewing height is setup in the 25 to 28 mm range in order to improve the detection of an x-ray signal.

The SEM-EDX micrographs of the crystalline structures deposited on the roughened surface are presented in Figures 51 a and b, respectively.

As previously emphasized, during the etching process at 300 °C for 3 hours a temperature gradient is developed across the inner wall of the capillary column which in turn combined with the concentration gradient are the primary reasons for the formation of various surface topographies. The x-ray analysis demonstrates that the crystalline features as well as the etched rough surface under it are primarily composed of a silicon, oxygen, and fluoride. The nitrogen presence is very difficult to determine due to superimposition and spectral interference from the oxygen and fluoride signals.

The main advantage of the EDS spectroscopic analysis is the capability of acquiring the data by “spot” analysis for a rapid qualitative evaluation of the

sample surface. In the analysis, the 20 kV energy beam was position exactly on the desired spot of the crystalline feature or the roughen surface. These spots are circled in Figures 51 a and b, respectively. The x-ray spectra are presented in the form of Gaussian peaks as shown in Figure 52 a and b, respectively. The quantitative x-ray spot analysis performed under a magnification of 2520 times indicated that the content of the fluoride and oxygen is slightly higher on the crystalline features than the surface under it. The presence of the aluminum in the fused silica spectrum is due to the signal obtained from the SEM sample holder. The EDS findings are in good agreement with other data obtained from SEM observations and elemental analysis. The capillary column with a characteristic crystalline topography of “branches of a pine tree” treated dynamically with copious volumes of water or methanol solvents and examined using SEM analysis reveled good stability of the inner surface layer. This confirmed that the crystalline layer is made of silica which is not soluble in either of the solvents. Additionally, both solvents collected after washing of the capillary column and analyzed by inductively coupled plasma atomic emission spectroscopy confirmed that both solutions contained less than 100 ppb of silicon species, the detection limit for these analyses.

Figure 51. The crystalline structure (a) deposited on the roughened surface (b) after etching of the capillary with 5 % w/v ammonium hydrogen difluoride at 300 ° C for 3 hours.

00 C 3hrs 5 10kV reproducibility test
avg 2520x
not on periodic

00 C 3hrs 5 10kV reproducibility test
avg 2520x

Figure 52. EDS spectra of crystalline structures (a) deposited on the roughened surface (b).

3.4. Surface area measurement.

Onuska et al. (15) reported that a 1000-fold increase of the surface area is observed upon etching of the inner surface of a capillary column. Thus, in view of complexity of the real solid-gas interface and of the different mechanisms that may contribute to physisorption in fused silica capillaries, surface area measurement by standard BET appeared to be both the simplest and the fastest method. The measurements of the surface area performed for the bare-untreated and roughened capillaries are reported in Table 1.

The BET analysis have indicated that the measurements of surface area for etched capillary column are not reproducible. Additionally, the surface area of non etched capillary column is greater than the one of etched capillary, which is contradictory with expectations. It appears that the surface area of fused silica capillary column is too low to be correctly measured by the BET technique at this point. It is known that a very low (less than 3 m²/g) surface area measurements could be improved by using krypton as an adsorbed gas: krypton has much lower saturation pressure compared to nitrogen and, therefore, the difference between the adsorbed and the non-adsorbed gas can be calculated more accurately. Additionally, in order to achieve low surface area measurements considerably longer pieces of etched capillary column are required (at least 10 meters). Kohr et al. (11) stated the inner surface area of capillaries for CE with a usual length of less than 100 cm and an I.D. between

50 and 100 μm is on the order of a few square centimeters, hence the established method cannot be used. It is suspected that up to a 1000-fold surface area increase reported by Onuska et al. (15) is based on either : (i) visual examination of the surface topography obtained from SEM micrographs; or (ii) the measurement of the concentration of the surface hydroxyl before and after column treatment.

Table 1. Surface area of bare and etched fused silica capillary column.

Name of the capillary	temp ($^{\circ}\text{C}$)	time (hrs)	conc. (% wt/V)	capillary wt (g)	surface area (m^2/g)
non etched, bare capillary	N/A	N/A	N/A	0.5	1.51
capillary etched	300	3	5	0.06	8.23
capillary etched	300	3	5	0.18	0.71

3.5. Investigation of the best roughened surface.

The etching of fused silica capillary column using 3 % w/v at 350 $^{\circ}\text{C}$ for 3 hours appear to produce the most suitable inner surface topography for the coating of a stationary phase. The surface layer is roughened on a diminutive

microscale, and the physical roughening seems to be superficial. The roughening using these particular conditions does not contribute to changes of inner column diameter. It can be easily understood from the micrographs presented in Figure 42 that the etching method should increase the surface area, and consequently increase the wettability of the inner surface.

From an Energy Scanning Chemical Analysis study, it is reported that the inner surface of a soft glass capillary column is substantially contaminated with carbon from the environment. This erratic carbon contamination is suspected to cause many problems of irreproducibility observed in previously reported capillary column preparation. Columns purged with oxygen during drawing contain less than 10 % carbon and they have to be carefully handled afterward in order to maintain this state (18).

4. Conclusion.

The study presented here established that hydrogen fluoride etching of the inner surface of the fused capillary column produces several types of surfaces depending on the reaction conditions.

(i) Etching from room temperature up to about 300 °C produces a surface of very rough topography. The inner surface is covered very densely with crystalline silica structures, which, in turn resemble the branches of a pine tree and are pointed out in various directions. It is suspected that this topography contributes to the substantial increase of the surface area and good wetting properties. On the contrary, this surface in spite of the microscopic dimensions could be too rough and/or too thick; thus the coated stationary phase will remain as a rough surface and the internal diameter of the capillary column will be decreased, both contributing to an overall decrease in column efficiency.

(ii) Etching at elevated temperature from 350 to 400 °C produces a very uniform and homogenous surface with minimal roughening when compared with the surface composed of large crystalline features. The surface appears to have a moderate increase in surface area and consequently possesses intermediate wetting properties. Additionally, the height of the

roughened surface layer is small enough (especially for 3 % w/v etching at 350 °C for 3 hours), that on the microscopic scale it appears smooth and does not change the inner diameter of the capillary column. The surface features have a size of a few tenths of a micron. End-on view of these etched capillaries confirmed that the roughening is very superficial. This is very desirable as it will not result in a thick layer of stagnant mobile phase and the associated band broadening.

(iii) Etching at temperatures higher than 400 ° C, produces an inner surface of a mild roughness and poorer uniformity. Under these conditions, the etching has to be conducted in a nitrogen atmosphere to prevent the decomposition of the polyimide coating.

Most important, it has been determined that the SEM technique is a very attractive and effective tool in microanalysis for fast and explicit characterization of etched surfaces. Furthermore a newly developed roughened surface on the inner wall of the capillary column should facilitate improved wetting and consequently spreading of a chromatographic stationary phase. On the other hand, this new surface on the column wall has to be adequately deactivated for further use in chromatographic analysis. Otherwise, a highly active surface may cause the breakdown of organic molecules in the mobile phase, consequently contributing to the decrease in

the separation power. The results presented in this study are of preliminary importance for further column modification/deactivation studies for GC, capillary electrochromatography and supercritical fluid chromatography to develop superior chromatographic columns.

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