University of Alberta

A Comparative Surface Study of 3rd Molar Enamel from different Subjects.

by

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To Darrell, Fausto and Mateo

Abstract

Success of orthodontic treatment with fixed appliance relies on the ability to create a strong bond between the brackets and the enamel. Despite advances in dental material bond failure constitutes a major problem in the orthodontics practice. Anecdotal evidence from orthodontist points out that certain patients have a higher bond failure incidence than others. The purpose of this study was to identify surface characteristics of the enamel that may influence bond strength. Two by two millimeter enamel samples were obtained from the buccal aspects of third molars. The sections were then analyzed using X-Ray photoelectron spectroscopy (XPS) before and after 30 seconds etching with 37% phosphoric acid. Statistical significant differences were shown in surface enamel composition prior and after etching. Scanning electron Microscopy (SEM) and Krypton adsorption technique were performed to the etched samples. Enamel specific surface area was determined by applying the BET (Brunnauer, Emmmet and Teller) equation to the Krypton adsorption data. A statistical correlation between the grading system utilized here and the specific surface area could not be established. However, a linear regression analysis demonstrated that the ratio between Calcium, Carbonate and phosphate before and after etching may play a role in determining etched enamel specific surface area.

Further studies should focus on establishing systems that would allow predicting bond strength on individual patients. With this information orthodontist would take the adequate steps to prevent bond failure in these patients.

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List of Nomenclature

Accuracy: describes how close a measured value is to the true value. It depends upon the quality and calibration of the measuring device

Adhesion: The mechanism(s) that bonds two materials in close contact across an interface (Davidson, 1996).

There are three different mechanisms of adhesion in the dental field: (Nakabayashi & Pashley, 1998)

1. *Chemical Adhesion*: Based on primary valence forces: Covalent, ionic, or metallic bonds.
2. *Physical Adhesion*: Based on secondary valence forces: Van der Waals forces occurring at molecular dipoles, London dispersion forces at the interaction of induced dipoles and hydrogen bonds at the interaction of unshielded electron clouds.
3. *Mechanical adhesion*: Based on the penetration of one material into a different material at the microscopic level (Davidson 1996).

Adsorption: The accumulation of gases, liquids, or solutes on the surface of a solid or liquid. Adsorption is to be distinguished from absorption, a process in which atoms or molecules move into the bulk of a porus material. *Sorption* is a more general term that includes both adsorption and absorption. Adsorption can be divided into two broad classes: *physical absorption* and *chemisorption.*

Physical adsorption: equilibrium is very rapid in attainment and it is reversible. The adsorbate can be removed without changes by lowering the pressure. Physical absorption is usually

important only for gases below their critical temperature (vapours).

Chemisorption: Could be rapid or slow and may occur above or below the critical temperature of the adsorbate. Chemical specificity is higher and energy of adsorption is large enough to suggest that full chemical bonding has occurred. Chemisorbed gas is very difficult to be removed. If desorption occurs it is accompanied by chemical changes.

Desorption refers to the reverse of adsorption, and is a process in which molecules adsorbed on a surface are transferred back into a bulk phase.

Adsorbate: Molecules that have been adsorbed onto solid surfaces.

Adsorbent: a material having the capacity or tendency to adsorb another substance

Adsorption isotherm: The relationship between the gas pressure p and the amount w, in grams, of a gas or vapor taken up per gram of solid at a constant temperature.

Auger effect: A sample bombarded by electrons or x-rays will eject core electrons from a level E_x in atoms in a region of the sample up to 1 μm deep. The core hole is then filled by an internal process in the atom whereby an electron from a level E_y falls into the core hole with the energy balance taken by a **third electron** from a level E_y'. This last electron, called an **Auger electron** after **Pierre Auger** is then ejected from the atom with an energy E_a, given approximately by the equation: $E_a = E_y' + E_y - E_x$.

BET: Acronym for the Brunauer-Emmett-Teller equation which describes the isothermal formation of adsorbed multilayers as a function of Relative Pressure and from which the

Monolayer coverage can be found. This equation is mostly widely applied for adsorption of nitrogen at LN_2 temperature as the means to measure surface area in volumetric sorption instruments.

Hybridized dental hard tissue: When the surface and subsurface of dental hard tissue is demineralized, followed by infiltration and polymerization of monomers it forms a new structure: the hybrid layer.

Hybridization: In dental hard tissue it refers to the process of creating a hybrid layer.

Precision: Indicates the degree of reproducibility of a measurement. It depends on how well you make a measurement.

Resin tag: Infiltration of adhesive resin into the etched enamel surface.

Random error: Is the natural variation in multiple measurements where each measurement may be high or low to the true value and have no fixed or predictable pattern. The accuracy of each individual measurement will appear random.

Shirley- type background: In core-level XPS, a range of physically possible line profiles is possible and simple Gaussian or Lorentzian functions are very rarely adequate. Thus, Shirley backgrounds were introduced to remove as much asymmetry as possible from recorded data in a well-prescribed fashion, so that near symmetric synthetic models can be used to characterize the intensity under a peak. A Gaussian/Lorentzian line-shape is finite and, with the appropriate relative sensitivity factors (RSF), can be used to compare intensities from fitted peaks to those calculated from integration regions. Shirley backgrounds have the availability of well-

characterized RSF values coupled with a specific background and line-shapes.

Systematic Error: Measurements may display good precision but yet be very inaccurate. Often, you may find that your measurements are usually *consistently* too high or too low from the accurate value. The measurements are "systematically" wrong.

Takeoff angle: is defined as the angle between the surface normal and the axis of the analyzer lens. This allowed analysis of the elements in the top few atomic layers of the enamel surface.

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Chapter One: Introduction and Literature Review

1.1 Introduction

Orthodontic treatment with fixed appliances relies on the ability to bond composite material to enamel. Orthodontic brackets are bonded to the buccal or lingual surfaces of teeth by means of an acid-etch technique with composite adhesives. These brackets will then, mechanically, hold the arch wire against the tooth so that forces may be applied that will result in tooth movement. A strong and durable bond is therefore critical in fixed orthodontic treatment success.

Bond failure represents a major problem in everyday practice. Lost clinic time and lost treatment time present a major concern and the main source of added cost in treatment. Despite advances in dental materials, bond failure rates in orthodontics vary between 0.5% and 16%.[1, 2]

This concern has been addressed for many years with several studies looking at variables such as: bond adhesives,[3] brackets,[4, 5] etching time,[6] direct and indirect techniques,[7] etc. Since Buonocore's report in 1955 that acid etching of the enamel increases the bond strength, enamel has been the focus of extensive research.[8] The variations in both the quality and quantity of etched enamel have been shown in several publications.[9, 10] A correlation between bond failure and tooth type has also been established. In general, the posterior teeth (molars and premolars) show a higher percentage of bond failure.[1, 2, 7, 11-13] This has led to the speculation that enamel characteristics in different tooth types will contribute to different etch patterns and this, in turn, to different percentages of bond

failure.

One area that has received little attention is variations in enamel surface structure between patients. It has become anecdotally accepted that there are patients with higher bonding failure rates than others. Except for a brief mention in one publication[2] there has been no study looking at variance in enamel surface structure between patients and whether it is associated with bond failure.

The general purpose of this work was to examine *in vitro* the surface composition, of enamel from different subjects before and after etching and to establish if there is an association between any of the inorganic components and etched enamel surface area. This study was also designed to compare the more traditional method to evaluate enamel etched surface, Scanning Electron Microscopy (SEM), with the gas absorption technique that was customized to our samples.

1.2 Literature Review

1.2.1Dental Enamel

Enamel is the hardest and most mineralized tissue of the body. Mature enamel consists of 96% inorganic material that is comprised almost entirely of hydroxyapatite crystals.

The process of enamel matrix formation and calcification, known as amelogenesis, can be split into several phases.[14] Preameloblast cells are formed in the proliferative phase, and then differentiate in the presecretory phase to become ameloblasts. Ameloblasts will secrete enamel matrix proteins such as amelogenins and enamelins, which exist as a gel adjacent to the ameloblasts. As this gel is supersaturated in calcium and phosphate ions, these ions will interact with the proteins to form a carbonated hydroxyapatite precipitate almost immediately. Eventually the ameloblasts stop secreting proteins, leading to a maturation phase during which the apatite crystals grow and proteins are dissolved or resorbed. Therefore, once tooth formation is complete, no repair is possible.[15]

The microstructure of enamel consists of these aligned prisms or rods, organized perpendicular to the dentinoenamel junction, from which they extend towards the tooth surface.[14, 16, 17]

These rods resemble a keyhole-like structure with an average width of about 5 μm. Each rod consists of compacted hydroxyapatite crystals, arranged in bundles of approximately 1000 crystals[18], covered by a nanometre-thin layer of enamelin and oriented along the rod axis. The protein rich areas between rods, known as inter-rod enamel, result from the incoherence of combining crystals of different orientations in a given region of enamel[17, 19] (see figure 1.1).

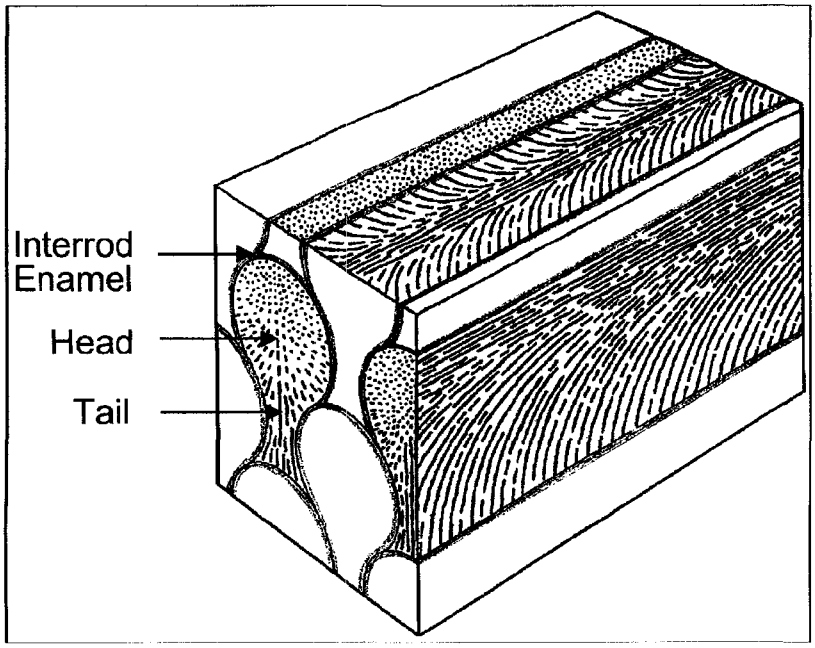

Figure 1.1: Schematic drawing of enamel microstructure. Keyhole-like rods of about 5 μm diametre aligned in parallel. Each rod is consisting of compactly packed hydroxyapatite crystals, arranged in bundles of approximately 1000 crystals. (From Habelitsz et al., 2001).[20]

The average size of the crystallites is about 30 nm thick by 60 nm wide and several microns long. (See above re: dentinoenamel junction).[14, 21]The mineral phase consists primarily of calcium hydroxyapatite $(Ca_5(PO_4)_3(OH))_2$. The remainder of the enamel is made up of 3% water and 1% organic matter including proteins and lipids. Pure hydroxyapatite (HA) (Figure. 1.2) does not occur on a macroscopic scale in biological systems. Enamel, dentine, cementum and bone are instead made up from a calcium-deficient and carbonate-containing apatite analogue. [22]

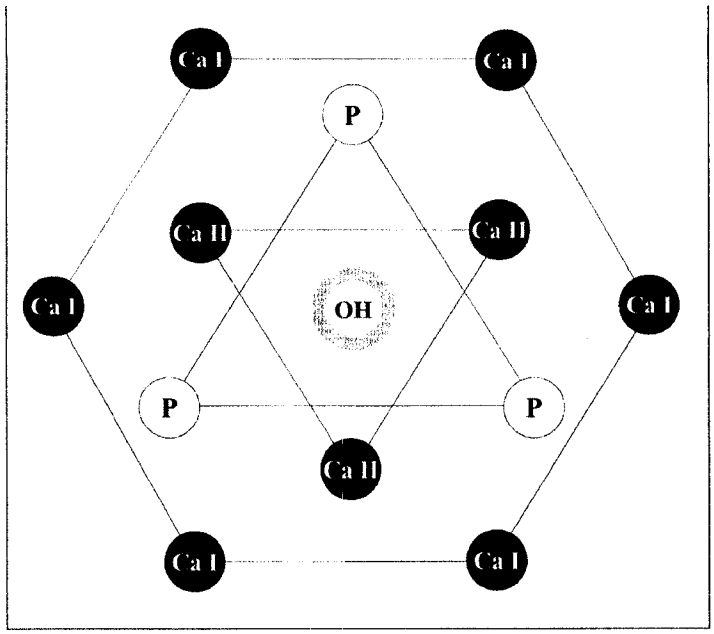

Figure 1.2: Crystal structure of hydroxyapatite. The overall planar hexagonal nature of the arrangement of calcium and phosphate ions around the central hydroxyl column can be appreciated in this diagram (from Robinson *et al.*, 2000).[23]

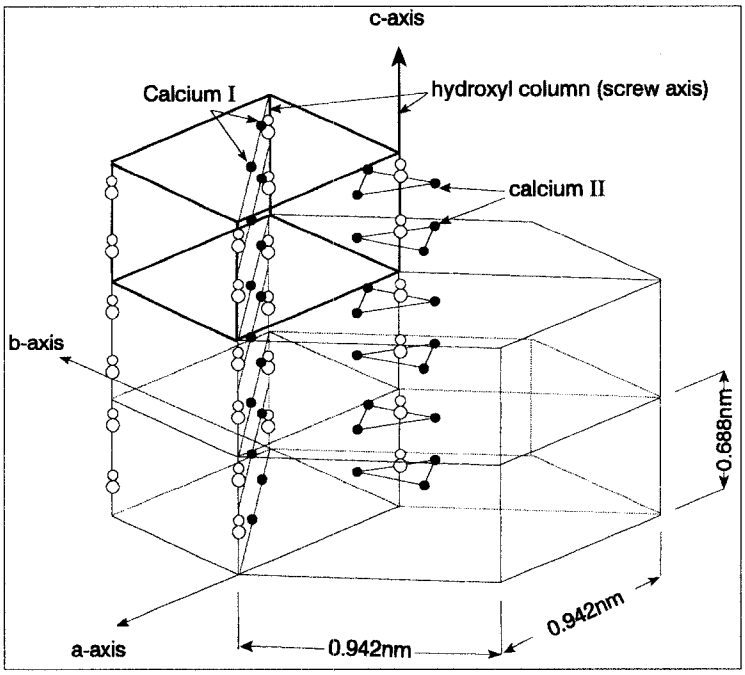

Figure 1.3: Crystal structure of hydroxyapatite: relationship between hexagonal unit structure shown in the previous picture (figure 1.2) and the rhomboidal crystallographic unit cell (shown in heavier lines). From Ichijo et al., 1992.[24]

Enamel also contains other trace metals. The trace elements existence in surface enamel is related to those elements that are incorporated into the apatite crystals during the mineralizing period and to those which diffuse into the tissue after completion of mineralization. Incorporation of additional phases such as carbonate, magnesium, sodium and fluoride into the apatite structure of enamel crystals results in changes in the physico-chemical and mechanical properties of enamel. For instance, a higher concentration of carbonate in crystal structure leads to a lower crystallinity and therefore, higher solubility of hard dental tissue. The effects (of a higher concentration of carbonate, specifically, or of additional phases in general) on the crystallite properties are: reduction in size, therefore increase in the surface area, change in morphology, from needle-like to rods to equiaxed crystals, depending on carbonate content, and increase in strain. On the other hand, fluoride substitution results in bigger crystals and fewer crystal defects, which, in turn, reduces enamel solubility. A study by Robinson *et al.* has shown a correlation between magnesium concentration and low density enamel and possibly between magnesium and protein.[25] Understanding the nature and mineral content of enamel is important when attempting to bond dental materials such as brackets in orthodontics. Because there is no turnover of enamel, trace elements to which the individual is exposed during the period of tooth development become incorporated and remain in the mineralized surface of the tooth. Some of these elements might reflect events that occurred during hard tissue formation where large amounts of ions are transported through the odontoblast or ameloblast layer. Further, some of these elements are probably exogenous in origin and accumulate principally by ionic exchange at the tooth surface.

Therefore, it is intuitive to speculate that enamel from different individuals will present unique characteristics.[26]

The mineral content as well as the concentration of enamel components changes from surface to interior. An inconsistent distribution of mineral and protein can occur near the dentinoenamel junction. Carbonate increases in concentration from the enamel surface towards the middle region with the increase slowing toward the amelodentinal junction.[27] Surface carbonate concentration decreases with age[28] and higher carbonate levels are found in abraded surfaces. Unerupted teeth have less carbonate in surface enamel, for which a feasible explanation may be a reduction in ameloblast metabolic activity "towards the end of their amelogenic path".[28]

There also seem to be differences between areas. Variations have been noted between cuspal, cervical, and fissure enamel. In the case of fluoride, Weatherell analyzed layers of ground or etched enamel from different surface areas showing a variable scatter of values,[29, 30] largely because fluoride concentrations varied from one part of the tooth surface to another.[31]

Besides having discrepancies in mineral content, hydroxyapatite is known to be an elastically anisotropic material. Spherical-indentation testing has exposed differences in mechanical properties between different enamel regions such differences may be due to variations in prism orientation.[32] Other researchers have hypothesized that local chemical

composition, i.e. degree of mineralization, could be a critical factor in explaining this characteristic.[33]

The anatomical surface of enamel influences the quality of etch pattern produced. Hence, acid–etch patterns will vary between tooth types and within the same tooth type between individuals: this is supported by several studies on the quality and quantity of etch patterns. Mattick and Hobson and Hobson *et al.*,[10, 34] found that anterior teeth had a greater proportion of "ideal" and "good" etches than posterior teeth.

The presence of a distinct "prismless" zone in the outermost part of the enamel was first reported several decades ago[35-37]. This layer averages 30μm in thickness and rarely extends over the entire surface, being most prominent over the gingival third. Boyde (1964) indicated that "the prismless area of the surface is widely held to possess caries-resistant characteristics".[38, 39] More recently, Whittaker[9] coincided with Boyde's assertion, stating that the amount of prismless enamel affects etching. He also suggested that prismless enamel is more common on posterior teeth.

Appreciating the microstructure of enamel and the carbonated hydroxyapatite in particular, is important in terms of our understanding how the tissue behaves when subjected to acid dissolution.

1.2.2 Enamel Etching

The acid-etch technique was introduced by Buonocore in 1955, who showed that certain

acids had the ability to modify the enamel surface.[8] Bowen (1963) later developed a composite resin that, in conjunction with the acid-etch procedure, became an efficient clinical procedure.[40] Gwinnet and Matsui and later Buonocore noticed the ability of hydrophobic dental resins to penetrate the subsurface microporosity created in etched enamel.[41, 42]

Buonocore's pioneering work introduced dentists to the idea of mechanical retention and chemical bonding.[8] These concepts have been further explored and today, several ways of bonding are known: 1) acid etching and the formation of resin tags; 2) bonding through the formation of a "hybrid layer"; 3) the creation of strongly bonded surface precipitates to which a resin can be chemically or mechanically bonded; and 4) chemical bonding to the inorganic or the organic components of tooth structure.[34]

The objectives of enamel etching are, in general, to clean the enamel, to remove the enamel smear layer and to increase roughness at a microscopic scale[43] by removing interprismatic and prismatic mineral crystals. Etching can also increase free energy of surface enamel[44] to allow monomer infiltration that will seal enamel surfaces, adding to the retention of resin composite restoration.[45] The diffusivity of the resin monomers is very important in the creation of resin-enamel hybrid layers. This new structure is neither resin nor tooth but a hybrid of the two. This hybrid layer, defined as resin –infiltrated enamel, dentin or cementum, presents different physical and chemical properties from those of the original tooth structure. For instance, high-quality hybrid layers can resist acid attack, as it has been confirmed by the clinical success of pit and fissure sealants on

enamel.[46, 47]

Acids in dentistry are utilized, in general, for two different functions. Polyalkenoic acids are used in glass-polyalkenoate cements due to a unique property of self-adherence to human calcified tissue.[48, 49] Other acids, such as phosphoric, citric and maleic, modify enamel and dentin surfaces as the first step of composite resin bonding to tooth tissue. In our studies we used phosphoric acid also known as orthophosphoric acid , a mineral (inorganic) acid having the chemical formula H_3PO_4. It may occur as a transparent solid or as a colorless liquid; it is soluble in water and alcohol. The molecular interaction of acids with HA is fundamental to the process of decalcification of or adhesion to mineralized tissues.

The mechanism of acidic interaction with HA was first identified in carboxylic acids. The adhesion and decalcification resulting from carboxyl acids interactions with HA was explained by the "Adhesion/Decalcification Concept" (AD concept) proposed by Yoshida *et al.* This process was found to involve two phases (Figure 1.4). In the first phase, carboxylic acids bond to the calcium of HA. Depending on the diffusion rate of the calcium–acid complexes into solution, the acid will, in the second phase, either remain attached to the HA surface with only limited decalcification involved or the calcium–acid complex will debond, resulting in a substantial decalcification effect.[48, 49]

Following this concept, it was then elucidated that the carboxyl groups of the acids form ionic bonds to Ca at the HA surface. This first phase may be determined largely by the pKa of the acids. At the same time, PO_4^{3-} and OH^- are extracted by H_3O^+ from the HA

surface and brought into solution. These processes occur with maintenance of electron neutrality at both the HA surface and in solution.[50]

In the same study, it was concluded that the AD concept that originally dictated the interaction of carboxylic acids with human hard tissues can be extended to inorganic acids and to all human hard tissues with varying HA crystallinity.[50, 51]

When the acid etching technique was first introduced, the proposed etching time was 30 seconds for 85% phosphoric acid.[8] It was later extended to 60 seconds but using 50% phosphoric acid, resulting in loss enamel tissue between 5 and 25 μm in depth[52, 53]. Fitzpatrick and Way found an average of 9.9 μm of enamel lost after 90 seconds of etching with 30% phosphoric acid.[54] A 7.5 μm average loss of enamel when 43% gel orthophosphoric acid was used and 6.5 μm with 37% liquid orthophosphoric, with 90 seconds of etching time.[55]

The depth of histologic change of the enamel, beyond the complete dissolution of the surface layer, can be estimated from determining the lengths of resin tags after sectioning the tooth longitudinally. The reported average depth of penetration ranges between 8 and 15 μm, with maximum tag lengths ranging up to 50 μm .[41, 42, 56-59]

By means of a scanning electron microscope, Moin and Dogon studied the effects of varying phosphoric acid concentrations on the enamel surfaces. The most consistently appropriate etching patterns were attained by the application of 30% to 40% phosphoric acid.[60] Conversely, Retief reported no significant difference in bond strength between 10%, 20%, 30%, 40%, 45%, and 50% concentrations of phosphoric acid.[61]

Adhesion/Decalcification Concept

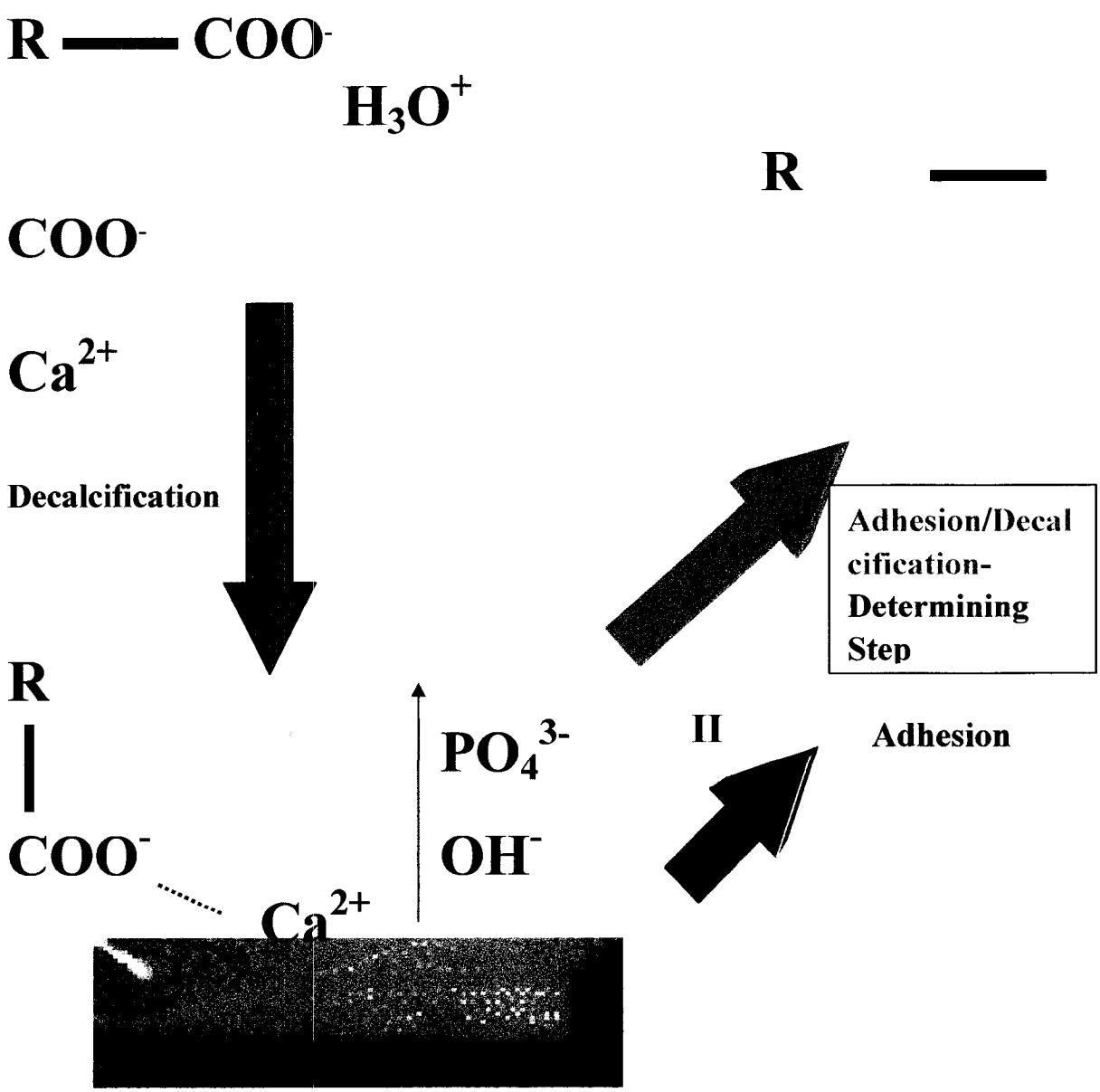

Figure 1.4 Schematic representation of the "Adhesion/Decalcification Concept" (after Yoshida et al., 2001).[51]

Most current clinical techniques use 37% phosphoric acid.

As stated earlier in this chapter, the existence of a surface aprismatic enamel layer has been well documented in the literature.[9, 37] Such a layer has been described as being less favorable to bonding *via* acid conditioning.[62] Exposure of the underlying prismatic enamel has been suggested prior to acid etching.[63]

A recent study showed that optimal bond strength to aprismatic enamel is achieved by increasing the time of acid etching.[64]

Knowledge of the variables influencing enamel bonding is essential when selecting the appropriate material and techniques.

1.2.3 Surface Scan Methods

Numerous techniques have been used for dental analysis. Atomic absorption spectrometry[65, 66], atomic absorption spectrophotometry[67, 68] and neutron activation analysis have been widely used.[69, 70] Other methods include spark source mass spectrometry,[71] optical emission spectrometry,[72] secondary ion mass spectrometry[73] inductively coupled plasma mass spectrometry,[74] differential pulse stripping voltammetry,[75] particle induced X-ray emission,[76] direct-current spectrum analysis[77] and X-ray fluorescence.[78] The range of elements to be determined as well as the method detection limit determined the technique of choice. For example, for single element analysis, the method used will aim to maximize accuracy and sensitivity.

Initial investigations on the enamel surface structure have encompassed light microscopy

applied to comparatively thick ground sections.[79-81] Scanning electron microscopy was later included as a potential technique that would allow various preparation methods and yield promising imaging results.[82, 83]

The typical techniques for the study of surfaces depend fundamentally on the interaction with photons (photo emission spectroscopy or PES, X-ray absorption fine structure, infrared (IR) spectroscopy); electrons (Auger electron spectroscopy or AES, electron energy loss spectroscopy, electron diffraction); ions (secondary ion mass spectroscopy, ion scattering spectrometry); or neutrons (neutron scattering).[15]

Still, the analysis of the inorganic elements of enamel is taxing. The hardness of the enamel makes the use of various techniques challenging. Additionally, several of the inorganic elements are present in very small quantities, making it very difficult to detect and quantify.

1.2.3.1 Scanning electron microscopy

The most common type of electron microscope is called the conventional scanning electron microscope (CSEM) or simply scanning electron microscopy (SEM). The SEM and its associated technique have a long record of use in the field of biomaterials. SEM [84-86]offers unique advantages over light microscopy such as high resolution and large depth of field.

SEM operates with an incident electron beam with an energy which can fluctuate from 1

to 100 kiloelectron volts (KeV), falling in the 20-40 KeV range most of the time. Imaging is commonly achieved by recognition of either the beam electrons, which are backscattered from the sample, or the secondary electrons generated on impaction of the primary beam with the specimen. There are two components in the detected beam: a high resolution one originating from a shallow sampling depth, and a low resolution one which maintains the lateral and the depth characteristics of the backscattered electrons.[87]

As a result, neither mechanism gives images that are solely representative of the surface structure.

The whole microscope column, including the sample chamber, operates under high vacuum ($<10^{-5}$ torr, 1 torr = 133 Pa) to prevent gas scattering of either the incident beam or the produced electrons. The presence of a vacuum, however, implies that samples must not contain any volatile species; they must be solid and dry. Samples that are hydrated in their native state (e.g. biological tissues and cells) must be dried or frozen prior to observation.

Almost all biological samples exhibit low conductivity. This presents a challenge as surface charges generated by the incident electron beam must be drained away to prevent distortion of the image. To overcome this phenomenon, specimens are coated with a thin layer of an electrically conductive material that dissipates the electrons and prevent the build-up of charge. Nevertheless, sample preparation can introduce artifacts and conductive coatings may prevent internal information by impeding the outgoing electron signals.[88] Furthermore, sample preparation implies that specimens do not preserve their

original microstructure.

To overcome the limitations of SEM, a second type of SEM called the environmental scanning electron microscope (ESEM) has been developed. The first commercial version of this product was made by the ElectroScan Corporation (Wilmington, MS, USA; later purchased by FEI/Philips Electron Optics) more than a decade ago. In recent years, ESEM has begun to make an impact across the diverse field of materials. The major advantage of ESEM is that hydrated and non-conducting samples, such as biological tissues, can be imaged without prior dehydration or conductive coating.[89-91]

Regardless of the limitations previously discussed, the SEM technique has been widely utilized in dentistry. SEM was used to explore the mechanical properties and microstructure of hypomineralized enamel[92] and the effect of etch duration on enamel surfaces[93].

The combination of SEM and X-ray microanalysis was employed to study hypomineralized permanent first molars, producing a direct correlation between analytical and morphological findings.[94] Enamel and dentin from human teeth previously irradiated by CO_2, were investigated by low vacuum scanning microscopy. Wet-SEM generated a compositional imaging based on atomic number.[95]

Scanning electron microscopy (SEM) has been the traditional method for studying the microscopic "surface structure" of tooth tissues in general.[96] Although SEM permits visualization of specimens; it is not a surface-specific technique in the exact sense.

1.1.3.2 X-ray Photoelectron Spectroscopy (XPS),

X-ray photoelectron spectroscopy (XPS), also referred to as electron spectroscopy for chemical analysis (ESCA), is a sensitive technique for investigating the elemental composition and the associated chemical bonding states of the near-surface region of a sample. It can extend to a depth of approximately 50 to 70 Angstroms. The technique relies on the emission of secondary electrons from the surface after the near surface atoms have been excited with X-rays. XPS provides the energy resolution needed to detect elemental peak position energy shifts due to chemical bond formation. It is this

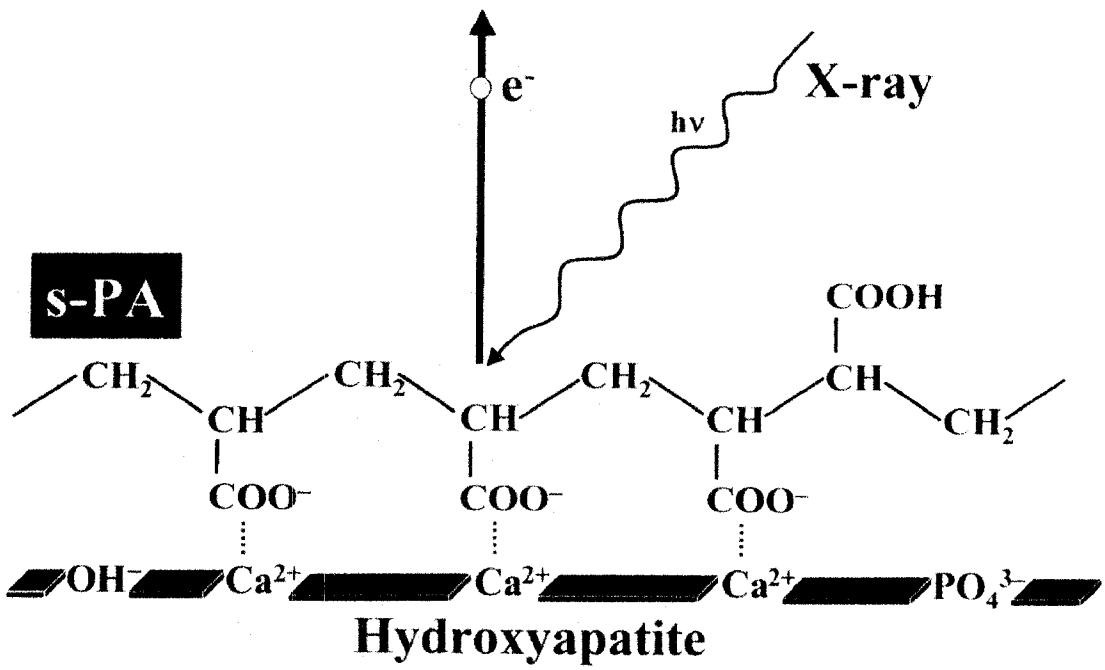

Figure 1.6 Schematic presentation showing the principle of XPS that involves the detection of ejected electrons (e^-) as a result of the impact of an x-ray with a certain kinetic energy (hv) on a monolayer-coated substrate.

elemental analysis technique that is unique in providing chemical state information of the detected elements, such as distinguishing between sulfate and sulfide forms of the element sulfur. Angle-resolved XPS can allow analysis of regions even closer to the sample surface. Relative atomic concentration percentages can be determined with a sensitivity of 0.1 to 1 atomic % for Li and heavier elements. Analysis is carried out in an ultrahigh vacuum system (~10^{-9} Torr or better).

XPS is the most widely used surface analysis technique because of its relative simplicity in use and data interpretation.

Figure 1.7: AXIS ULTRA XPS (University of Alberta, Surface Sciences)

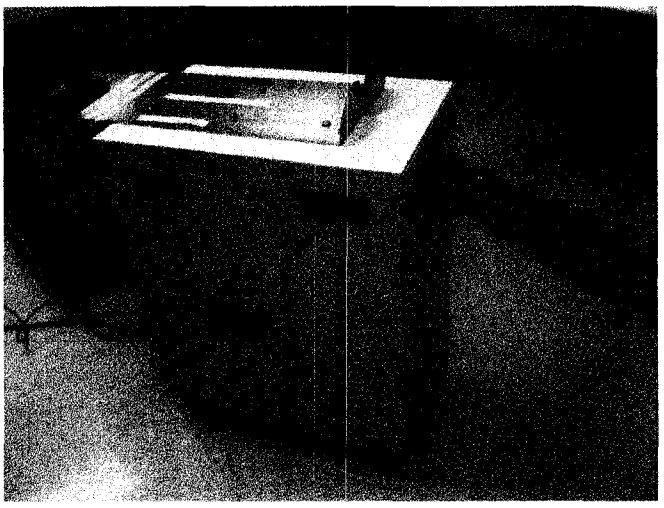

Figure 1.8: Ultra High Vacuum Transfer facility (University of Alberta, Surface Sciences)

The essential XPS procedure can be summarized as follows: X-ray photons (hv) from a monoenergetic beam are directed onto the sample. Photons are then absorbed by sample atoms; each absorption event results in the emission of a photoelectron. Electrons from all the orbitals of the atom with a binding energy (E_b) less than that of the X-ray energy are excited, although not all with equal probability. Therefore some peaks show more intensity in the spectra. The kinetic energy (KE) of the electron plus the energy required to remove it from its orbital to the spectrometer vacuum must equal the X-ray energy. If the X-ray energy is known and the kinetic energy is measured with the electron spectrometer, the binding energy of the electron can be calculated. Due to the short sampling depth of XPS, chemical information is acquired selectively from an ultra-thin layer at the substrate surface.[51, 97] In practical solid state experiments, a correction for the spectrometer work function, $Ø_s$, must also be applied, normally as part of the spectrometer calibration procedure. Thus, one obtains $E_b = hv - KE + Ø_s$.

Photoelectrons of different energies are separated and counted by retardation through a

focusing lens and deflection through an electrostatic analyzer onto an electron multiplier or channel plate. Although the measured binding energy (BE) is determined largely by the element and atomic orbital, it dependent as well on the chemical state and environment of the atom and can be affected by quite subtle initial and final-state relaxation effects. This technique is highly surface sensitive due to the low mean free path of the photoelectrons traveling to the surface of the solid. This unique characteristic requires ultra high vacuum (UHV) experimental conditions in order to allow the electrons to reach the analyzer. Electrons traveling from the sample surface towards the energy analyzer should encounter as few gas molecules as possible, to prevent them to be scattered and lost from the analysis.[97] An UHV environment has the added benefit that clean, model surfaces may be created and maintained throughout surface analysis.[15]

Since the atomic structure of each element is distinct from all the others, measurement of the positions of the electron lines allows identification of an element present at a sample surface. Elements adjacent to one another in the periodic chart produce electron lines which are well separated from one another so that no ambiguity exists in identification of adjacent elements. Carbon, nitrogen, oxygen and phosphorous are easily distinguishable [98] while hydrogen is not detected by XPS (See Figure 1.9).

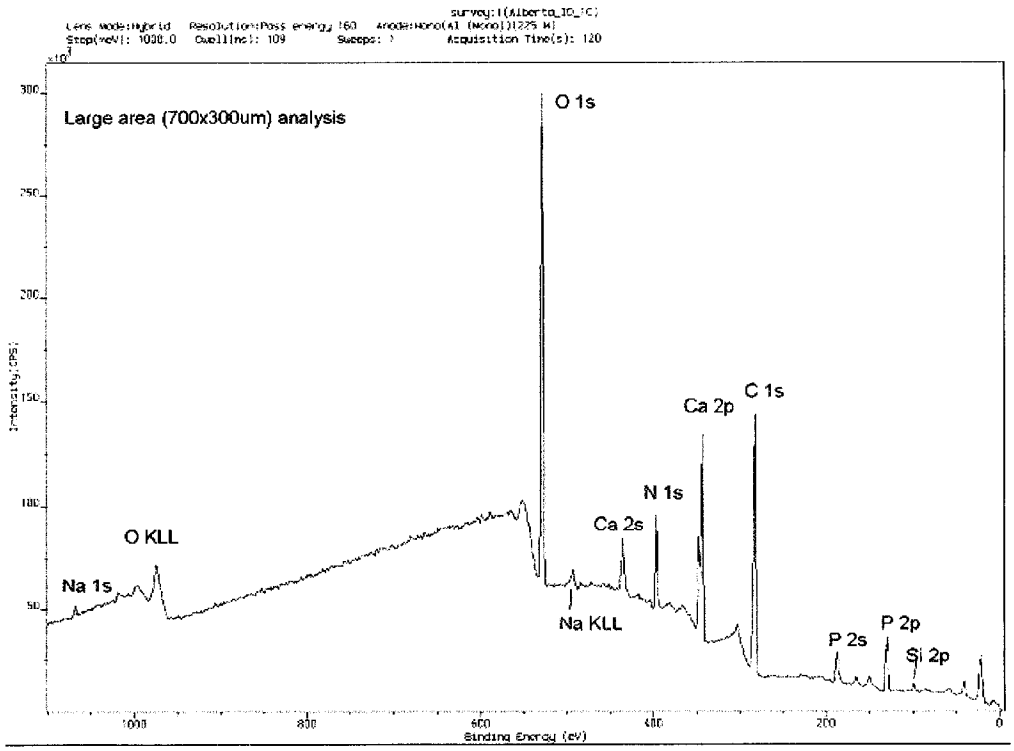

Figure 1.9: XPS survey spectrum for a dental enamel sample. It shows the presence of Ca, P, O, C. This has characteristics of typical XPS spectra obtained for all the dental enamel specimens.

XPS is a relatively non-destructive technique. No surface components are removed during XPS measurements. The soft X-ray source used for excitation avoids many of the problems associated with thermal degradation of sensitive materials. Sample composition remains constant during analysis.[97]

Unfortunately, XPS presents the same setback as the SEM reflecting information representing two components. High energy x-rays are used to excite electrons from the core-level orbital of the atoms. The kinetic energies (E_K) of the emitted photoelectrons are characteristic of the element and atomic orbital from which they originate, related to

the binding energy (E_B) through the relationship: $E_B = hv - E_K$. When a photoelectron is emitted from an atomic orbital, a "hole" is left behind. The core-hole state created by the XPS process is energetically unstable and may be filled by an electron from a higher orbital with the concurrent emission of a second Auger electron.[98]

Despite this setback, XPS has been widely used in the dental research field. The interaction of synthesized polyalkenoic acid with enamel and synthetic hydroxyapatite was elucidated by means of XPS.[99] It was also utilized to monitor the adsorption of active agents from six commercially available mouthrinses;[100] to provide evidence of chemical bonding of glass-polyalkenoate cement to enamel;[50] and to examine resin modified glass ionomer cements.[101] *Yoshioka et al.*[50] established adhesion/decalcification mechanisms of acid interactions with human hard tissues. Moreover, analysis of the chemical interaction of five carboxylic acids and two inorganic acids with enamel and two synthetic hydroxyapatite (HAp) powders was possible by using XPS.

In this study, a further high resolution spectrum was acquired for carbon, and subsequent data processing outlined any changes in the carbonate quantity.

1.2.4 BET Theory /Specific Surface Area

Gas adsorption at the clean surface of a solid is the most accepted method for determining the surface area. There are two adsorption categories: physical and chemical adsorption. In the case of physical adsorption, the interactions between the adsorbate and the adsorbent are electrostatic.[102, 103] Chemical adsorption involves specific forces, such as those that are operative in the formation of chemical bonds. Three phenomena may be

involved in physical adsorption: monomolecular adsorption, multimolecular adsorption and condensation in pores or capillaries. In a gas sorption experiment, the solid sample is heated and may either be degassed by vacuum force or exposed to inert gas to remove adsorbed foreign molecules. Controlled doses of an inert gas, such as nitrogen, krypton, or argon, are introduced and that gas is adsorbed. Krypton gas is used when the measured surface is expected to be less than 2 m^2/g, which typically is the case with pharmaceutical samples and natural organic materials. The samples are placed in a vacuum chamber at a constant and very low temperature, usually at the temperature of liquid nitrogen (-195.6 °C), and then subjected to a wide range of pressures in order to generate adsorption isotherms. The amounts of gas molecules adsorbed are determined by the variations in pressure due to the adsorption of the gas molecules by the material (the adsorbent). Various amounts of gas molecules will be adsorbed at different concentrations of the gas (the adsorbate). By knowing the area (σ) occupied by one adsorbate molecule, (for example, σ= 16.2 Å2 for nitrogen), and using an adsorption model, the total surface area of the material can be determined. The most well known and widely used is the BET model.[104]

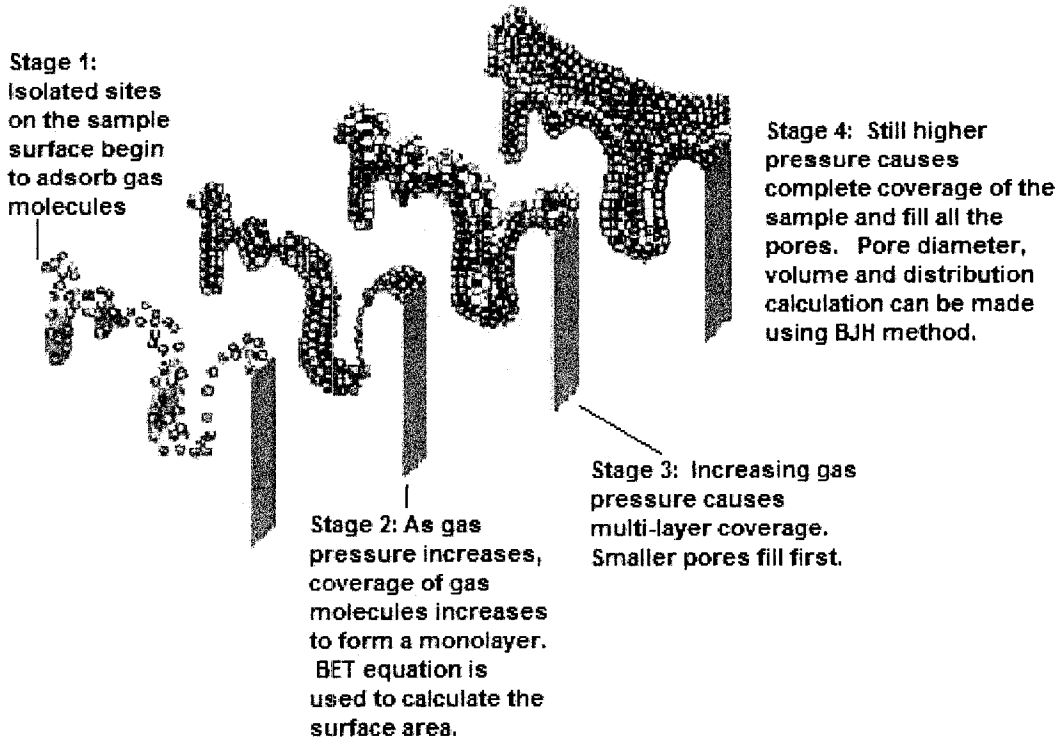

Figure 1.10: Schematic drawing representing the different stages in the progression of the gas absorption method. In the present study, only surface area was calculated (from Micromeritics® Analytical Service catalog, reprinted with permission).

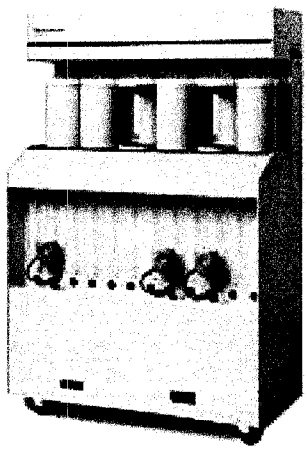

Figure 1.11: Micromeritics' ASAP 2420 Accelerated Surface Area and Porosimetry analyzer uses the gas sorption technique to generate data.

Adsorption is usually described through isotherms, that is, functions which connect the amount of adsorbate on the adsorbent with its pressure (if gas) or concentration (if liquid). The Langmuir isotherm, developed by Irving Langmuir in 1916,[106] describes the partitioning between gas phase and adsorbed species as a function of applied pressure.

This isotherm is based on three assumptions:

1. Adsorption cannot proceed beyond monolayer coverage.

2. All surface sites are equivalent and can accommodate, at most, one adsorbed atom.

3. The ability of a molecule to adsorb at a given site is independent of the occupation of neighboring sites.

The free gas and the adsorbed gas are in dynamic equilibrium, and the fractional coverage of the surface, θ, depends on the pressure, P, of the overlying gas. The variation of θ with pressure at a chosen temperature is called the *adsorption isotherm.*

There are 6 basic types of isotherms (Figure 1.12) and most solid samples should fall under one of these categories.[107] Type 1 occurs with microporous materials where adsorption takes place at extremely low pressures and then has multiple layers being formed. Micropore data reduction and Langmuir surface area are then typically calculated and reported under this category. Type 2 and 4 isotherms are indicative of either non-porous adsorbents or adsorbents having relatively large pores. B.E.T. surface area and BJH mesopore calculations are typically reported for these types of materials. Type 1, 2,

and 4 isotherms see an initial rise in the quantity adsorbed due to adsorbing molecules interacting with the most energetic regions of the solid surface. The quantity is much greater for microporous materials than for mesoporous materials. After the initial rise, the isotherm tends to flatten out as multiple layers of adsorbed gas are forming. This transition is where surface area calculations are determined. At the end of a Type 2 or 4 isotherms, there is an abrupt rise due to the adsorbing gas beginning a process of bulk condensation to a liquid. Mesopore information is calculated from this information using BJH method. Type 3 and 5 arise under conditions where adsorptive molecules have greater affinity for one another than they do for the sample material. Krypton surface area may be the only measurable characteristic for these types of materials. Type 6 isotherms are indicative of a nonporous solid with an almost completely uniform surface where multiple layers of adsorption are clearly visible. These isotherms are quite rare.

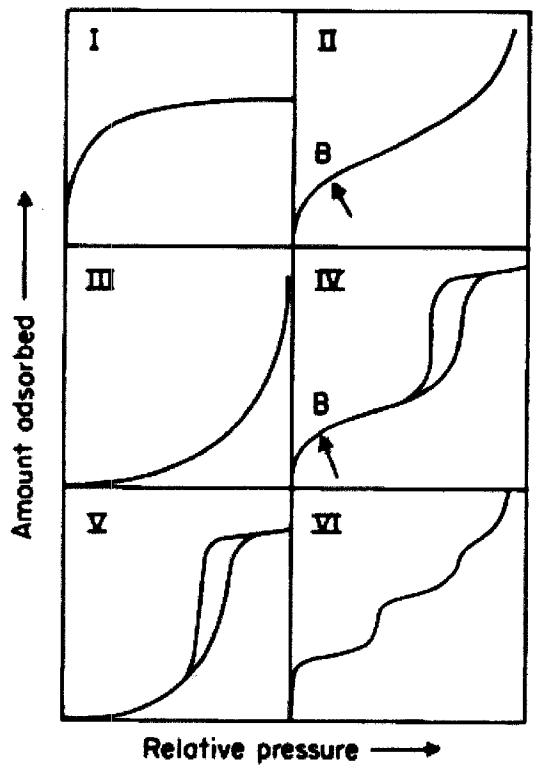

Fig.1.12 Types of Physisorption Isotherms

Pore Volume Distributions can be determined by either gas adsorption porosimetry (typically N_2, Ar or CO_2) or mercury intrusion porosimetry.[108] Gas porosimetry measures pores from 3.5 Angstroms to about 4000 Angstroms in diameter. Mercury porosimetry is applicable to pores from 30 Angstroms up to 900 micrometers in diameter.

To measure pore size by gas adsorption, isotherms are recorded from low pressures (approximately 0.00001 torr, minimum) to saturation pressure (approximately 760 torr). The pressure range is determined by the size range of the pores to be measured. Isotherms of microporous materials are measured over a pressure range of approximately 0.00001 torr to 0.1 torr. Isotherms of mesoporous materials are typically measured over a pressure range of 1 torr to approximately 760 torr. Once details of the isotherm curve are accurately expressed as a series of pressure vs. quantity adsorbed data pairs, a number of different methods (theories or models) can be applied to determine the pore size distribution.

Summary

Successful orthodontic treatment with fixed appliances depends on the ability to bond orthodontic brackets to enamel. The anatomical surface of etched enamel influences the bond strength. Characterization of the composition of surface enamel could help predict bond strength in different individuals. Surface composition of enamel could be examined by means of XPS. Specific enamel composition surface area could be determined through the application of the gas adsorption technique by the BET equation.

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Chapter Two: Research Paper 1

Surface Analysis of Etched Molar Enamel by Gas Adsorption

Key words: Enamel, SEM, etched, Gas adsorption, BET.

2.1 ABSTRACT

Much research has been devoted to studying etched enamel surfaces in an effort to improve bond strength. Scanning electron microscopy (SEM) has been the most commonly used technique to visually study etched enamel surfaces. The aim of this study was to introduce the gas adsorption technique as an option for examining the specific surface area of etched enamel. Secondly the study compared the results of gas adsorption and commonly used subjective classification of quality of etched enamel surface. Sixteen human third molars were used in this study. Enamel samples two by two millimeters were etched for 30 seconds with 37% phosphoric acid prior to viewing with SEM. Micrographs of etched enamel surfaces were graded according to the Galil and Wright classification. Type 4 etching was reported in 62.5% of our samples. Samples were then analyzed at Micromeritics Analytical Services (Norfolk, GA, USA). The total surface area of the etched samples was determined by the BET (Brunauer, Emmet and Teller) equation for gas (krypton) absorption. Pearson's Correlation Coefficient showed a lack of relationship between etch pattern and etched enamel surface specific area. Additional studies are recommended to determine the relationship between total surface area measured by gas adsorption techniques such as the one described here and bond strength. Furthermore factors which contribute to variability in etched enamel surface specific area

should be evaluated.

2.2 INTRODUCTION

The introduction of the acid-etch bonding technique in 1955[1] represents one of the most important advances in dentistry, and bonding to enamel has become a routine practice in many areas of dentistry. Phosphoric acid treatment, as described by Buonocore,[2] creates a porous enamel surface layer that, when penetrated by a low-viscosity resin bonding agent, enables the interlocking between composite resin and enamel. The retentive ability displayed by etched enamel for composite resin has historically been assumed to be a function of the increase in surface area due to etching and in the wettability of the etched enamel[3,4,5].

Much research has been devoted to the characterization of the physical and chemical properties of dental enamel in general, and the surface topography of acid-etched enamel in particular. The scanning electron microscope (SEM) has been widely used in dentistry to explore the surface and microstructure of enamel[6,7] as well as the effect of acid-etch on enamel surfaces[8-15]. Through the application of the SEM technique, a variation in quality and quantity of etched enamel was observed by Poole and Johnson in 1967[16], who are recognized as the first to classify etched enamel patterns. Silverstone in 1975[17], Brannstrom in 1978[18,19] and Gail and Wright in 1979[20,21] have further developed and/or modified the original Poole and Johnson classification. More recent studies have adopted one of these grading scales or have used their own modified version of them [9,10,13].

The qualitative nature of the data, along with the lack of consensus on which of the enamel etching grading scale should be universally adopted, makes comparisons between studies difficult. The adoption of a standardized classification is long overdue. Furthermore, these grading scales are subjective and at their best require reproducibility testing.

The absorption of gases in multimolecular layers, as a means of determining surface area of materials, was first described by Brunauer, Emmett and Teller in 1938[22]. Since then, the Brunauer-Emmett-Teller (BET) gas adsorption method has become the most widely used standard procedure for the determination of the surface areas of finely divided and porous materials[23]. The BET equation is used to determine the volume of gas needed to form a monolayer on the surface of a sample. A known volume of gas (adsorbate) is added to a solid material. At cryogenic temperatures, weak molecular attractive forces will cause the gas molecules to attach to the surface of the solid material. Gas (usually nitrogen) is added to the sample in controlled doses and the pressure in the sample container is measured after each dosing. A direct relationship exists between the pressure and the volume of gas in the sample container. The volume of gas adsorbed by the sample can be determined by measuring the reduced pressure due to the adsorption. This relationship is known as an adsorption isotherm[24,25]. The actual surface area can be calculated from knowledge of the size and number of the adsorbed gas molecules (see Figure 2.1).

The aim of this work was to address the limitations of a visual appraisal technique such

as SEM and identify a plausible alternative method to study etched enamel. Specifically, the present study was conducted to examine the gas adsorption technique as an alternative quantitative method to analyze etched enamel and to determine if a correlation exists between the BET enamel surface area and different grades of etchings as determined by visual examination.

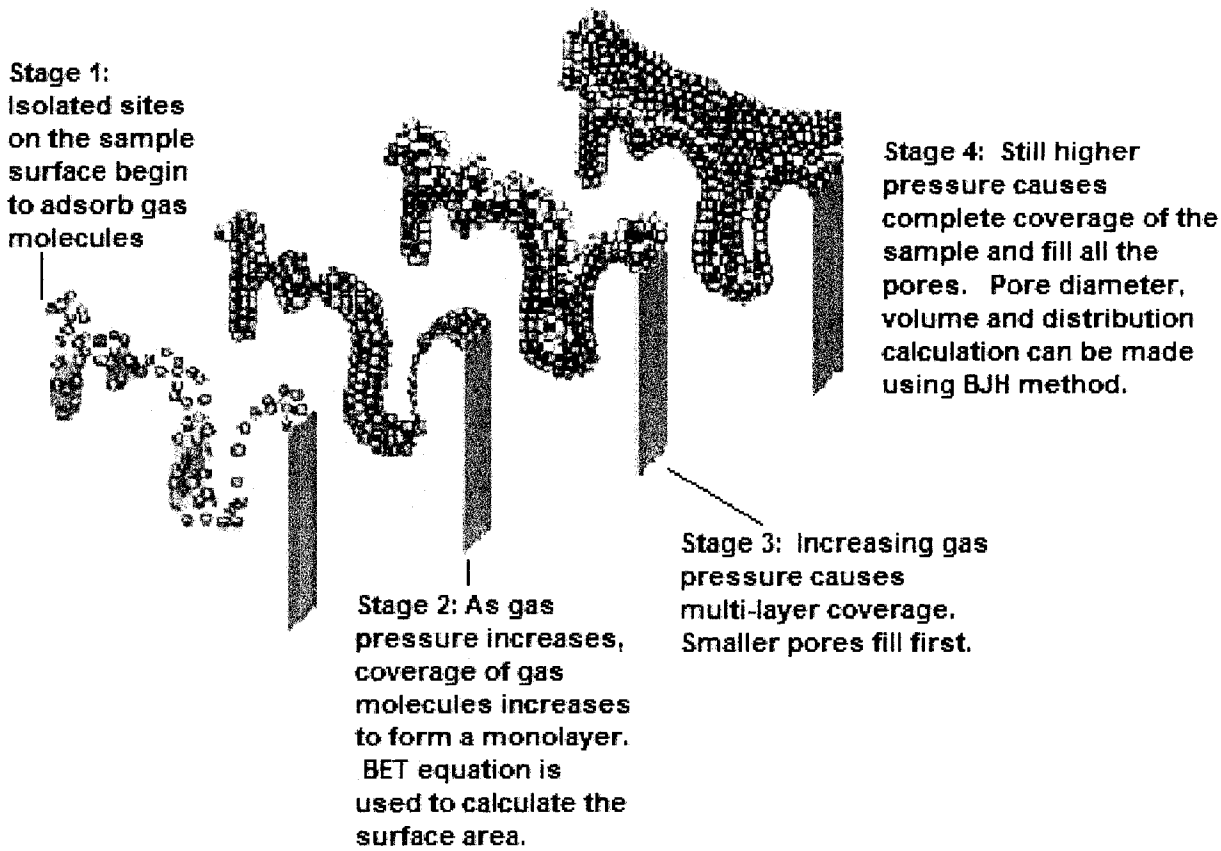


Figure 2.1: Schematic drawing representing the different stages in the progression of the gas absorption method. In the present study, only surface area was calculated (from Micromeritics® Analytical Service catalog, reprinted with permission)

2.3 MATERIAL &METHODS

2.3.1 Enamel Sample Preparation:

Sixteen unerupted human third molars extracted from individuals as part of their dental treatment were stored in a 0.1% thymol solution prior to use. Approximately five by two millimeter samples were obtained from the buccal aspects of the molars (from approximately the middle third of the surface). Dentin was removed from the samples. The intact enamel surfaces were etched for 30 seconds with 37% phosphoric acid. The etched surface was then rinsed with air and water for 5 seconds and air-dried. The treated samples were cut into two smaller samples of approximately two by two millimeter. They were randomly assigned to be analyzed by SEM or by gas absorption. The cutting was performed using a diamond disc (Brasseler Dental Instrumentation, Savannah, Georgia, USA). In order to seal the inner surface of each of the specimens, a layer of unfilled resin was applied and light cured for 10 seconds before the gas adsorption measurement.

2.3.2 Scanning Electron Microscopy:

The etched specimens were prepared for scanning electron microscopy (SEM) by sputter coating with gold to a thickness of 10 um. Viewing was carried out with a Hitachi SEM (model S-2700, Hitachi Ltd, Tokyo, Japan) operated at 10 kV. Photomicrographs were then submitted to two independent assessors (M.O and D.B) who scored the quality of

each etch pattern in a blind manner. Five types of etching patterns were used as diagnostic criteria according to Galil and Wright [20,21]:

- Type 1: Preferential dissolution of the prism cores, resulting in a honeycomb like appearance
- Type 2: Preferential dissolution of the prism peripheries, giving a cobblestone like appearance
- Type 3: A mixture of type 1 and type 2 patterns.
- Type 4: Pitted enamel surfaces as well as structures that look like unfinished puzzles, maps, or networks.
- Type 5: Flat, smooth surfaces.

After a 3-week interval, the assessments were repeated by the two evaluators (M.O three times and D.B one time). The values given to each enamel sample by the two assessors were then gathered and an intra-class correlation coefficient (ICC) was estimated.

2.3.4 Gas Adsorption Measurement:

Nitrogen gas is generally considered to be the most suitable adsorptive for surface area determination. Krypton gas is used when the measured surface is expected to be less than 5 m^2 as was the case with our enamel samples. Prepared samples were analyzed at Micromeritics Analytical services (Norfolk, GA, USA).

Briefly, enamel samples were degassed at 40 °C for 16 hours prior to their analysis. Samples were placed in a sample tube and heated under vacuum or flowing gas to remove contaminants on the surface of the samples. The sample weight was then obtained by

subtracting the weight of the empty sample tube from the combined weight of the degassed sample and sample tube. The sample tube was then placed in the analysis port of a 2420 Accelerated Area and Porosimetry System (Micromeritics, Norfolk, GA USA) for automatic analysis. The low surface area option was used. This option features a turbomolecular drag pump, which provides the high vacuum required for krypton analyses, and a 10 mmHg pressure transducer, which allows accurate, repeatable pressure resolution. The krypton adsorption isotherm was recorded at 120 K. The specific surface area (S_{BET}) was calculated according to the standard BET method [23, 24]. The BET equation is expressed by:

$$\frac{1}{W[(P_0/P) - 1]} = \frac{1}{W_m C} + \frac{(C-1)}{W_m C}\frac{P}{P_0}$$

Where W is the weight of krypton adsorbed at a given P/P_o (P the pressure and P_o the saturation vapor pressure), and W_m the weight of gas to obtain monolayer coverage and C a constant that is related to the heat of adsorption. A linear relationship between 1/W $[(P_o/P)-1]$ and P/P_o is required to obtain the quantity of krypton adsorbed. The above equation can be plotted as a straight line with $1 / v\,[(P_0 / P) - 1]$ on the y-axis and P / P_0 on the x-axis. This linear portion of the curve is restricted to a limited portion of the isotherm, generally between 0.05 and 0.30. The value of the slope and y-intercept of the line are used to determine the quantity of krypton adsorbed in the monolayer. This information is used then to calculate the total surface area S_{total} and specific surface area S by the following equation:

$$S_{total} = \frac{(v_m N s)}{V}$$

$$S = \frac{S_{total}}{a}$$

Where N is the Avogadro's number, *s* is the absorption cross-section, V molar volume of adsorbent gas and *a* weight of sample.

2.4 RESULTS

2.4.1 SEM Results

Scanning electron microscopic images of etched enamel surfaces were analyzed by two independent observers. The ICC showed intra-operator reliability of 0.84 and inter-operator reliability of 0.86. The SEM micrograph in Figure 2A represents the most prevalent etching pattern. This type of enamel etching was graded as type 4 and was found in 62.5% of our samples. Three enamel samples (19%) were graded as type 5 (Figure 2B), two (12.5%) as type 3 (Figure 2C), and just only one (6%) as type 1 or "ideal etch" (Figure 2D).

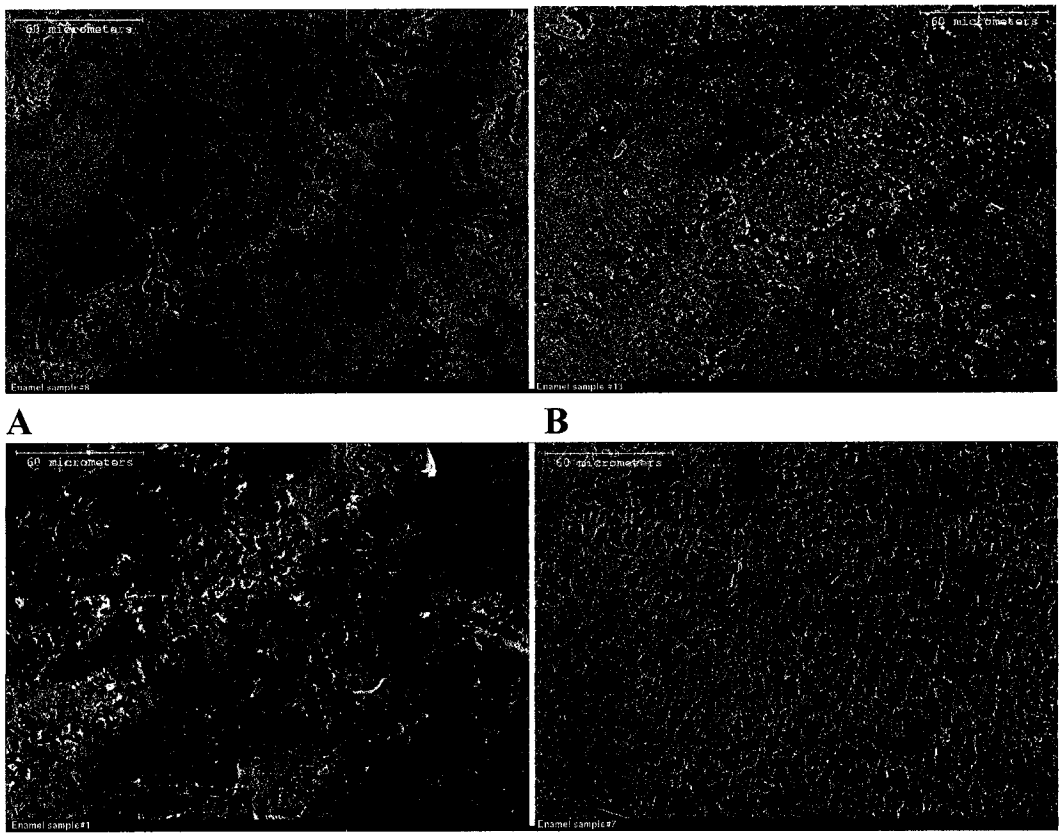
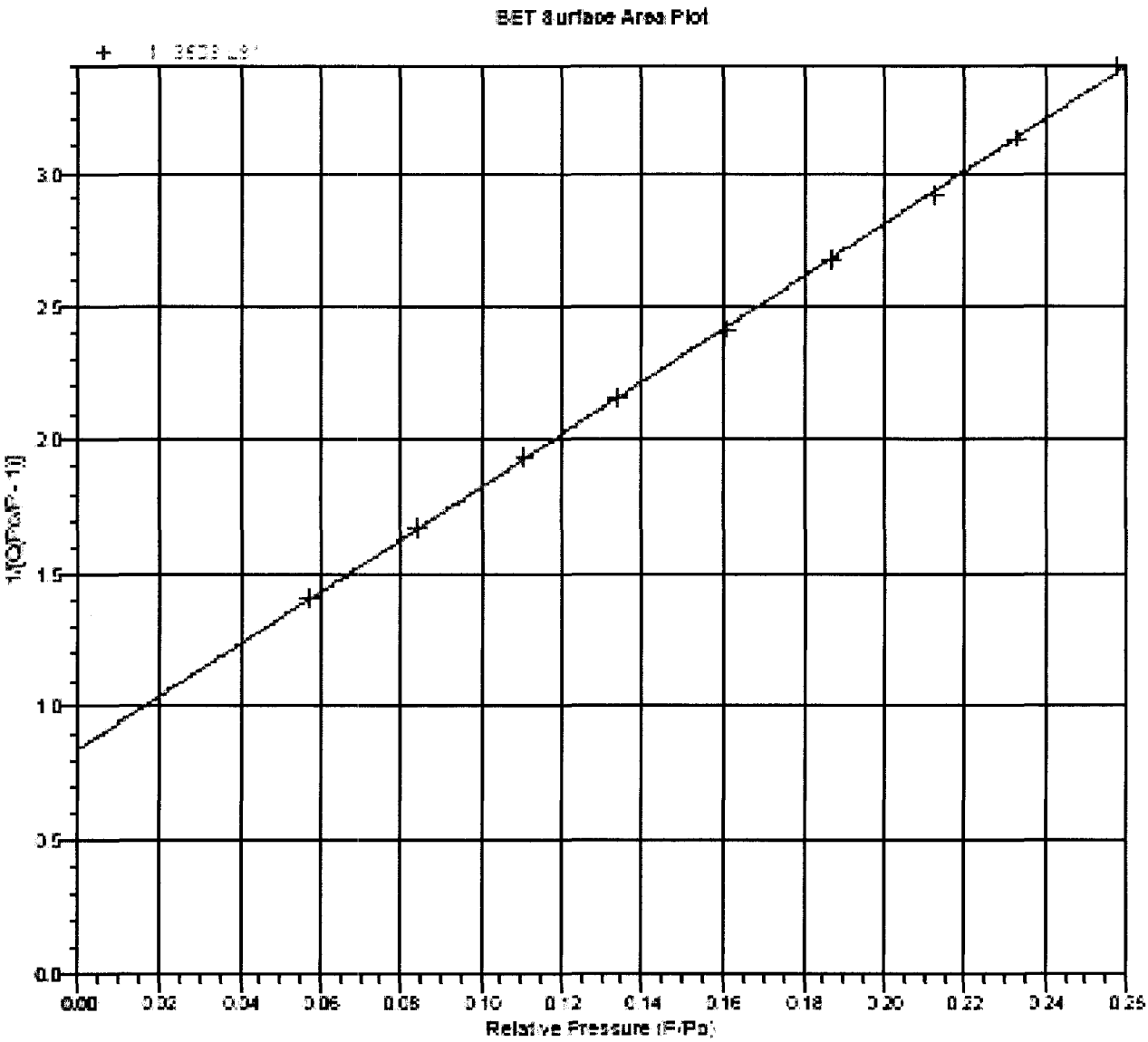


Figure 2.2: SEM micrographs showing: A) type 4 etch pattern, B) type 5 etch pattern, C) type 3 etch pattern and D) type 1 etch pattern or "ideal" etch

2.4.2 Krypton Adsorption Results

The estimation of specific surface areas S_{BET} took place using the traditional BET plots from the relevant adsorption isotherms (Figure 2.3A). The BET surface area is the multipoint surface area, calculated using 3 or more pressure points (Figure 2.3B). There were large surface area variations between individual enamel samples (Figure 2.4). The mean BET area was .359 m^2/g (SD .116).

A



B

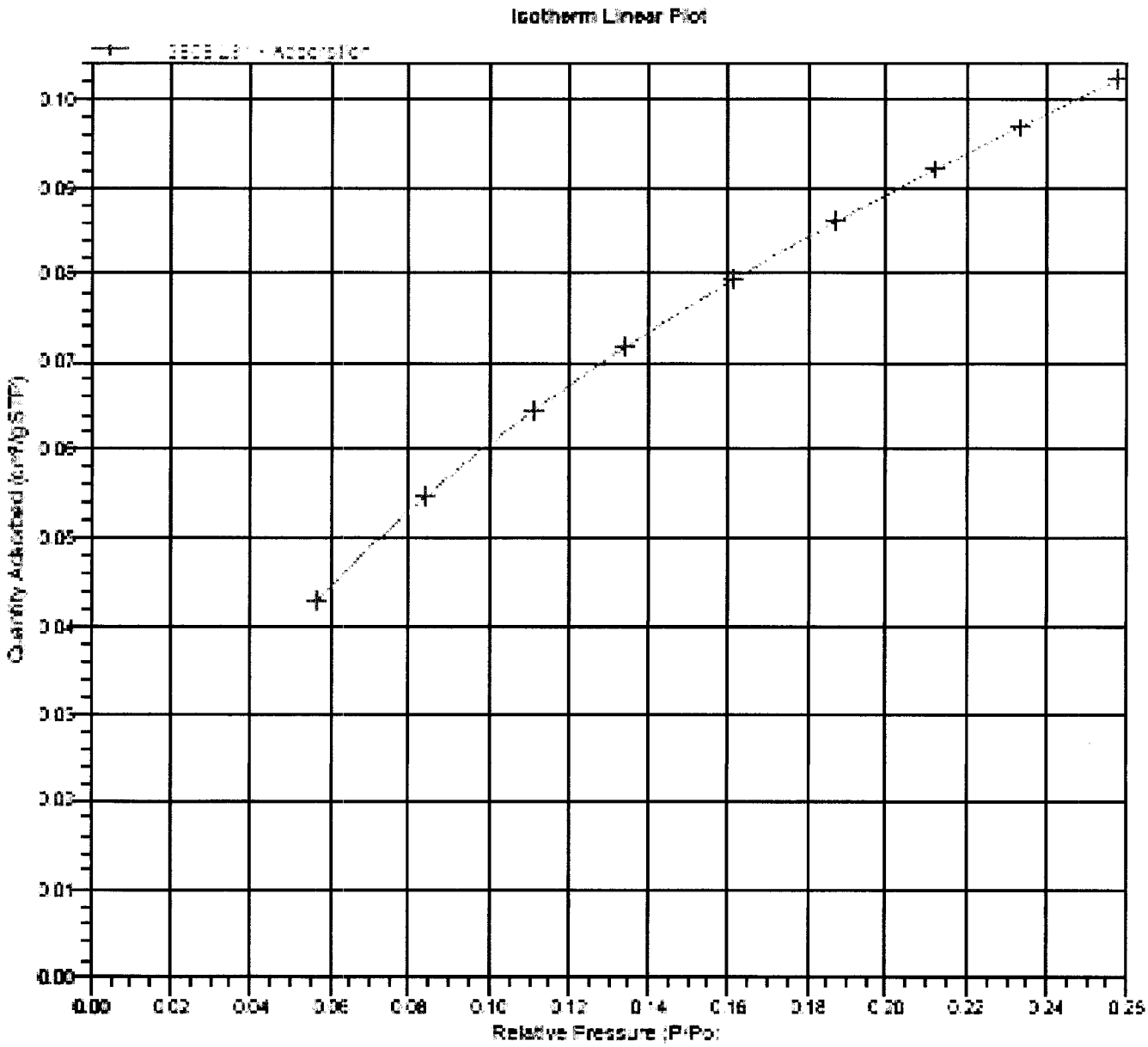


Figure 2.3: A) Krypton absorption isotherm of enamel sample #1, B) BET surface area plot for the same sample.

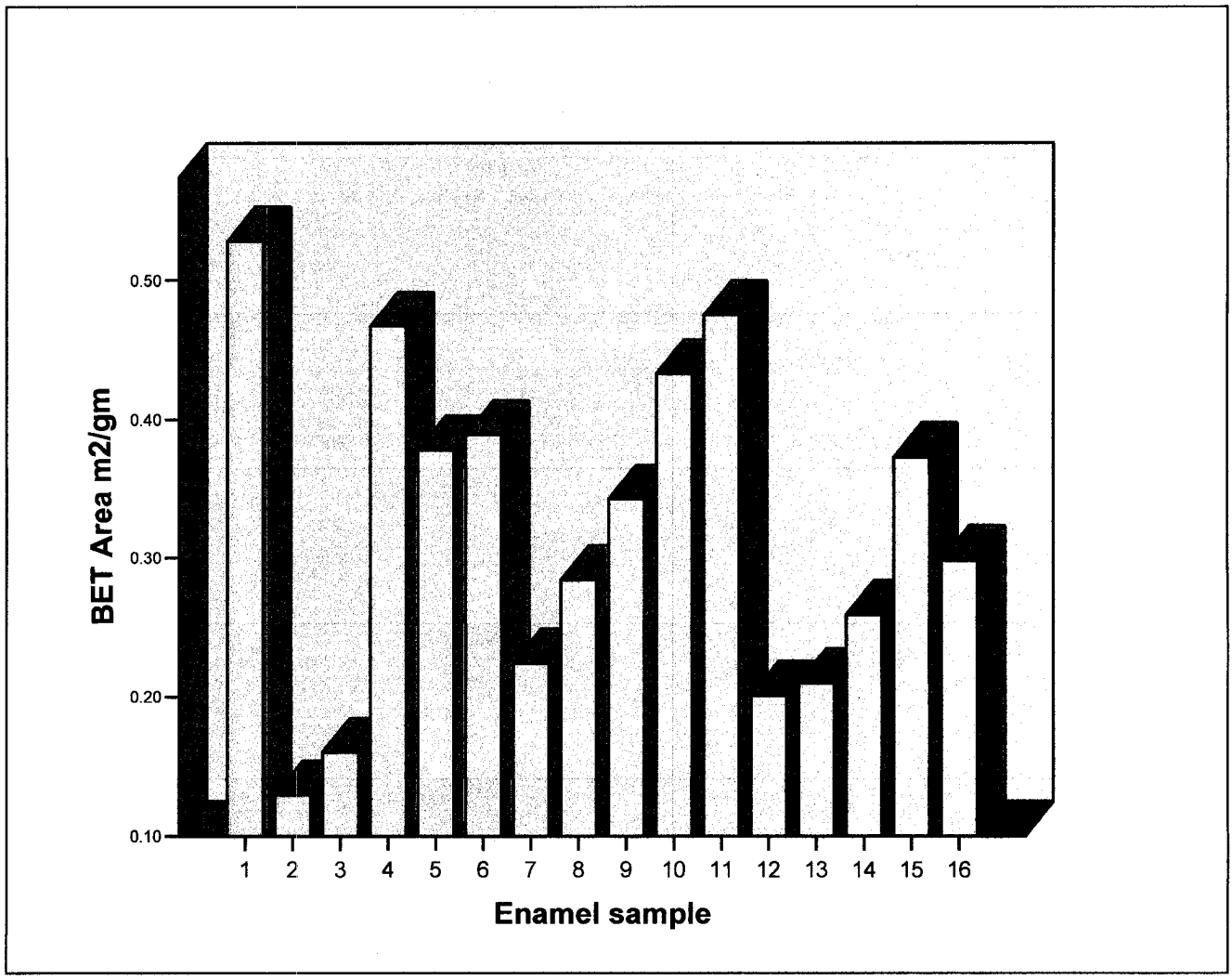


Figure 2.4: Surface area of individual enamel samples determined by krypton adsorption and calculated by BET equation.

Pearson correlation coefficient was performed to estimate a relationship between BET surface area and types of etched enamel. A lack of linear relationship between these two variables was demonstrated by a Pearson correlation of 0.079 (see Figure 2.5).

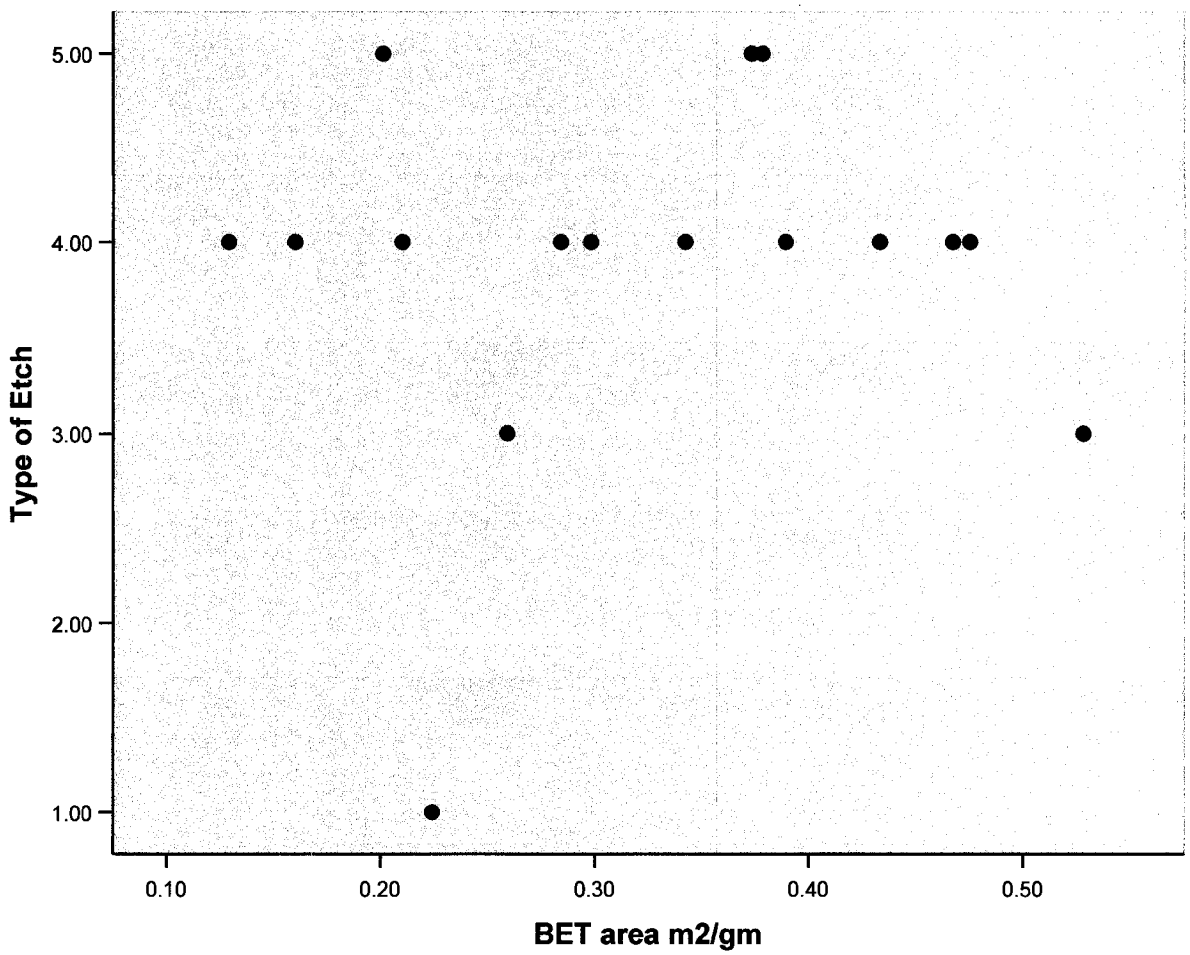


Figure 2.5: A Pearson correlation coefficient of r: 0.079 showed no correlation between BET surface area and type of etch. The above scatter plot confirms these results.

2.5 DISCUSSION

Scanning electron microscopy (SEM) has been the traditional method for studying the microscopic "surface structure" of tooth tissues in general[6-15]. SEM is not a surface-specific technique in the strictest sense. Images are generally generated by detection of either the electrons, which are backscattered from the sample, or the secondary electrons generated on impaction of the primary beam with the specimen. The detected beam thus has two components: one of high resolution originating from a shallow sampling depth, and one of low resolution which retains the lateral and the depth characteristics of the backscattered electrons. As a result, neither mechanism gives images that are solely representative of the surface structure. Another difficulty with the use of SEM in examining etched enamel is the lack of consensus on grading systems.

Several enamel etch type classifications have arisen since Poole and Johnson's original grading[16]. Silverstone's 1975 [17] basic etching pattern types are still widely used[26-28]. In type 1, the prism cores are preferentially removed, while the prism peripheries remain relatively intact. In type 2, the reverse pattern is observed. In type 3, areas of types 1 and 2 appear to coexist in the same regions. Brannstrom et al.[18,19] proposed a three point scale of surface roughness. Galil and Wright's[20] five point classification appears to have been adopted by the largest percentage of investigations[9,10,15]. Hobson et al. reported difficulty distinguishing between type 1 and type 2 enamel according to Galil and Wright and converted their five point scale to a four point scale[12].

In the present study, the Gail and Wright classification was utilized. The grading system from 1 to 5 is comprehensive and it seems to be the most widely accepted. However, and

albeit a high inter and intra-rater reliability score, both observers (D.B and M.O) reported difficulty grading the enamel micrographs. Several micrographs presented more than one type of etching. Gardner and Hobson[15] used histometric point sampling to address this issue and reported different etched types on the same sample.

In our study, both observers had agreed a priori to grade each sample according to the enamel type that was present in the largest quantities. This approach was taken as it was evident that many of the previous SEM studies did not use a systematic method to evaluate the etch patterns observed.

Our SEM studies showed only one sample as type I or an "ideal etch". These findings are in agreement with Mattick and Hobbson's report that only a small percentage of enamel surface area is etched ideally upon application of 37% phosphoric acid for 30 seconds[29].

Ten of our enamel samples were graded as type 4, meaning that 62.5% were poorly etched, or, in other words, displayed a "suboptimal etched pattern". Perdigao et al.[30] showed no correlation between the less well defined enamel etching patterns and shear bond strength, and Hobson and McCabe[13] inferred that high bond strength does not depend on an ideal etch pattern. A number of studies have concluded that there is no relationship between bond strength to acid etched enamel and etching conditions[31-34].

Moreover, two studies by Legler et al.[34,35] described a "poor" relationship between resin penetration depth and resin-enamel bond strength. Nakabayashi and Pashley[36] have hypothesized that resin-enamel bond strength depends on the cumulative cross-sectional area of the resin tags that infiltrate the etched enamel. The length of the tags has no effect on the cross-sectional area. The work of Nakabayashi and Pashley[36,37] demonstrated that exposure of enamel crystallites is more important than the display of "ideal" etch

patterns and that penetration of adhesive resins into porous enamel creates a new structure. This structure is part enamel and part resin and it is considered to be a hybrid layer[36, 38]. Hybridization is used routinely to achieve bonding to dentin.

These studies findings support the suggestion that enamel porosity is more important than a defined etch pattern. When phosphoric acid is applied to dental enamel surface, it creates microscopic pores by selective dissolution of the enamel[39]. It is assumed that an increase in porosity will result in an increase in surface area and that a large exposed area of enamel is optimal for the hybrid layer formation.

The need for methods that will measure etched enamel surface area as well as pore diameter and distribution is obvious. It is not possible to determine the surface areas of etched enamel by optical or electron microscopy because of the size and complexity of the pores. Porosity, pore volume-size distribution and specific surface area can be investigated by different methods such as mercury intrusion porosimetry and gas adsorption[42]. The two techniques are based on different physical interactions and cover specific ranges of pore size. Mercury porosimetry determines mesopores and macropores whereas gas adsorption covers the micropore range. Because of the predicted pore size of etched enamel, surface gas adsorption was used in this study.

Gas adsorption has been studied theoretically for most of the 21st century and the simplest of the resulting theories has provided the insight needed for most applications. Still, a literature search identified only two studies in dental enamel that utilized the gas absorption method[40,41]. Only one was performed on human dental enamel[41].

The specific surface area of each of our enamel samples is depicted in Figure 4. From

these results, it is evident that 3rd molars from different persons behave differently as a result of 30 seconds of exposure to 37% phosphoric acid. Our unpublished observations confirmed a diverse surface composition of third molar enamel from different individuals. That is, the atomic surface concentration percentages, as well as the ratio between components were different in all tested 3rd lower molars. This could explain the different behavior after etching.

A Pearson's correlation test did not show a relationship between BET surface area and enamel etch pattern. In view of the above discussion, these results are not surprising. We could speculate on different reasons for this lack of relationship, the most obvious being that "ideal" etch pattern does not imply a larger surface area and vice versa. Additional studies should be done measuring the specimens before and after etching by the gas adsorption technique.

In our opinion, it is of pivotal importance that etched enamel surfaces be analyzed in terms of specific surface area and porosity. We have displayed the techniques that are currently available to accomplish this type of analysis, which is in concert with the aim of this study - to illustrate the possibilities that gas adsorption analysis offer to researchers in different dental disciplines

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Chapter Three: Research Paper 2

Individual Variations in Surface Enamel Composition of Mandibular Third Molars

Key words: Enamel, surface analysis, X-ray photoelectron spectroscopy, etching

3.1 ABSTRACT

Acid etching increases bond strength by promoting microporosities on the enamel surface. Despite improvements in the acid-etch technique and dental materials, the rate of adhesive bond failure during orthodontic treatment remains high. Anecdotal evidence suggests variations in bond strength in different individuals. Thus, this study was conducted to comparatively analyze the composition of third molar enamel surfaces from different individuals before and after etching with 37% phosphoric acid.

Eleven lower third molars from different individuals were analyzed by X-ray photoelectron spectroscopy (XPS). Survey scans, detail scans and high-resolution XPS spectra were obtained from the 11 enamel samples. The concentration of the main matrix elements O, P, Ca, C and CO_3, and surface atomic ratios of Ca/P, Ca/CO_3, Ca/C, Ca/O, CO_3/P were investigated. Statistical analysis showed a difference between molars in the surface enamel concentration and ratio of these elements before and after etching.

These findings may explain, in part, variability in bond failures among patients despite the best efforts of the dental practitioner to avoid failures.

3.2 INTRODUCTION

The acid etching technique was first described in 1955 [1]. It entails discrete etching of the enamel to facilitate selective dissolution of prism cores or peripheries, resulting in microporosities into which resin can flow and can be polymerized to form a mechanical bond to the enamel. Since Buonocore's report that acid etching of enamel increases the bond strength, enamel has been the focus of extensive research [1]. Variations in both the quality and quantity of etched enamel have been shown in several publications. [2-5]

The extent of surface etching and demineralization of enamel was assumed to be dependent on the type of acid, the etching time, and the concentration of the etchant [6-9]. Yet, a correlation between bond failure and tooth type was recognized with the posterior teeth (molars and premolars) showing a higher percentage of bond failure [10-15]. All of this research has led to the speculation that enamel characteristics in different tooth types will contribute to different etch patterns and this, in turn, to diverse percentages of bond failure. Indeed, Hobson et al observed a significant difference in acid-etch patterns in different tooth types[16] implying the need to limit etching studies to a specific tooth category. Yet, a study of nine human mandibular first pre-molars concluded that enamel organization is directly correlated to individual teeth. In other words, gross morphology and enamel characteristics are pertinent to a particular tooth, rather than being unique to tooth type.[17]

The chemical variations in enamel composition have been historically investigated by dissecting the enamel into a series of contiguous samples from the surface to the dentinoenamel junction. [18-20] The discrepancy between surface enamel content and deeper

enamel composition has been documented [21]. While the chemical composition variations from surface to inner enamel are important in other areas of dentistry, they are not critical in orthodontics where brackets are bonded to the enamel surface. On the other hand, restorative dentistry, for example, requires optimal bonding to both surface and subsurface enamel to obtain sufficient clinical restoration longevity, which is typically much more significant than the 18 to 24 months typically required of an orthodontic bond. For the analysis of hyperfine layers of solid samples, it is necessary to apply surface-sensitive analytical techniques like X-ray photoelectron spectroscopy (XPS). Due to the XPS sampling depth of just a few nm, chemical information is acquired selectively from an ultra-thin layer at the enamel surface. This technique has been shown to be a powerful research tool for studying surfaces and surface changes in dentistry. [22-24]

One area that has received little attention is variations in enamel surface structure between patients. It has become anecdotally accepted that there are patients with higher bonding failure rates than others. Except for a brief mention in one publication [25], there has been no study examining variance in enamel surface structure between patients and whether it is associated with bond failure. This study was, therefore, devised to identify variations in surface enamel composition in mandibular molars from different individuals before and after etching with 37% phosphoric acid. The first hypothesis to be tested is that there is no difference in the measured changes in surface enamel composition that occur with acid etching between the same tooth type (mandibular molars) from different individuals. The second hypothesis to be tested is that there is no difference in the ratios of surface enamel components prior to and after etching.

3.3 MATERIALS & METHODS

3.3.1 Enamel sample preparation

Eleven extracted sound impacted human mandibular 3rd molars were selected for this study. These molars were extracted from individuals who gave informed consent and for whom the treatment (extractions) had been planned. Confidentiality of the patients was protected and all procedures were approved by the Health Research Ethics Board of the University of Alberta. After extraction, the teeth were stored separately in 0.1% thymol solution to prevent their dehydration and to prevent bacterial and fungal growth in the storage media.[26]. Two by two millimeter samples were obtained from the buccal aspects of the molars: the cutting was performed using a diamond bur (Brasseler Dental Instrumentation, Savannah, Georgia, U.S.A.). The dentinal layer was completely removed from the cut sections with a football shaped diamond bur (Brasseler Dental Instrumentation, Savannah, Georgia, U.S.A.). After XPS testing, the intact enamel surfaces were etched for 30 seconds with 37% phosphoric acid. The etched surface was then rinsed with air and water for 5 seconds and air-dried prior to being tested again in XPS adsorption.

3.3.2 X-ray photoelectron spectroscopy

The XPS scans were acquired using an AXIS ULTRA XPS (Kratos Analytical, Manchester, U.K.), which employed monochromatic AlKα x-rays (hv = 15 eV) as well as charge neutralization. The x-ray gun was operated at 210 W. The XPS data were acquired at photodetector takeoff angles of 55°. All samples had a survey spectrum (0-1000 keV) as well as high resolution spectra of carbon. The survey scan was performed with pass

energy of 160 eV while 20 eV was used for the high resolution spectra. The binding energies of the photoelectrons of insulated samples were calibrated by assuming that the adventitious carbon (C 1s) has a binding energy of 284.6 eV. The data were recorded digitally by Kratos XPS Casa software on a Sun computer system. During the data acquisition, the pressure in the sample chamber did not exceed 2.5×10^{-9} Torr.

Specimens were allowed to degas under high vacuum (10^{-10} torr) for at least 48 hours and then exposed to the incident AlKα beam for alignment and signal optimization for approximately 1 minute prior to analysis. Survey scans and high resolution scans of the C 1s, Ca 2p, O 1s, N 1s and P 2p regions were recorded. The high resolution spectra were labeled with their respective abbreviated elemental names, followed by the related quantum number of the ejected photoelectron. The C 1s spectrum was used to analyze the percentage of carbon present as carbonate. Four chemical states of carbon were fitted to each C 1s peak envelope. The components of the first state (at 285eV) are C-C/C-H. The next carbon species corresponds to ether carbon C-O, followed by CO_2^- and carbonate carbon (CO_3^{-2}), which has the highest binding energy in the spectrum.

The analysis of the spectra was performed following a Shirley-type background subtraction where the individual photoemission features are fitted with representative Gaussian distributions using least-squares optimization. The peak positions, amplitudes, and full widths at half maximum parameters are obtained from the Gaussian distribution analysis. The peak areas correspond to the total area with respect to the background subtraction. The atomic concentrations were calculated using the algorithm and sensitivity factors contained in Kratos Analytical Software.

3.4 RESULTS

Survey scans, detail scans and high-resolution XPS spectra were obtained from the 11 enamel samples.

3.4.1 Composition:

A representative survey XPS spectrum from dental enamel is shown in Figure 3.1. The peaks are labeled with the abbreviated elemental name, followed by the quantum number of the ejected photoelectron. Thus, the main peaks present are labeled as Ca (2s and 2p), P 2s, O 1s, C 1s and N 1s, with other smaller peaks present as well, which were not considered in this study. For instance, in a few samples, Mg, K and F were observed with atomic percentages between 0 -1%. The area under the peaks allows quantification of the relative amounts of each element present, while the shape and position of the peaks represent the chemical state of each element.

The high resolution scan of carbon showed four different peaks, representing carbon at different binding energies. The separation between these peaks allows differentiation of these four carbon species (CH; -CO; $-CO_2$; $-CO_3$). Total carbon concentration and $-CO_3$ concentration (along with the concentrations of Ca, P, O and Na) were great enough to allow analysis. XPS results are presented as atomic mass concentration percentage normalized to 100. In table 3.1, we have included carbonate (CO_3) and total carbon concentration (C) thus; the sum of all components included in the table is exceeding 100. The ratio of each element was obtained by dividing the atomic mass concentration percentage of each element after etching by the atomic mass concentration percentage

before etching. Their coefficient of variation was attained by dividing the standard deviation of each element by the mean atomic mass concentration (table 3.2).

Calcium atomic concentration increased an average of 2.5 fold after etching. The highest increase observed for calcium was 4.59 fold (enamel sample number 3) and the lowest 1.58 fold (sample number 7). Carbon decreased an average of 0.39 fold and carbonate ($-CO_3$) 0.50 fold with only one sample increasing 1.74 fold. On average, phosphorus increased almost 3 times (2.93) and oxygen 1.68 (table 3.2).

A one-sample T-test was performed with hypothesized parameter one and a significance level $\alpha=0.05$. All five variables showed a statistically significant difference: Ca ($p<0.001$), C ($p<0.001$), CO_3 ($p=0.006$), P ($p=0.001$), O ($p<0.001$).

The ratios between components were obtained by dividing the atomic mass concentration of calcium by each of the other components and the atomic mass concentration of carbonate by phosphate. The mean, standard deviation and coefficient of variation was calculated for each ratio. Please refer to table 3.3.

A paired sample t-test was performed to compare the ratios before and after etching.

The difference before and after etching was significant for the following ratios at a significance level $\alpha=0.05$: Ca/C ($p<.000$); CO_3/P ($p=.001$); Ca/O ($p<.000$); and Ca/CO_3 ($p=.012$).

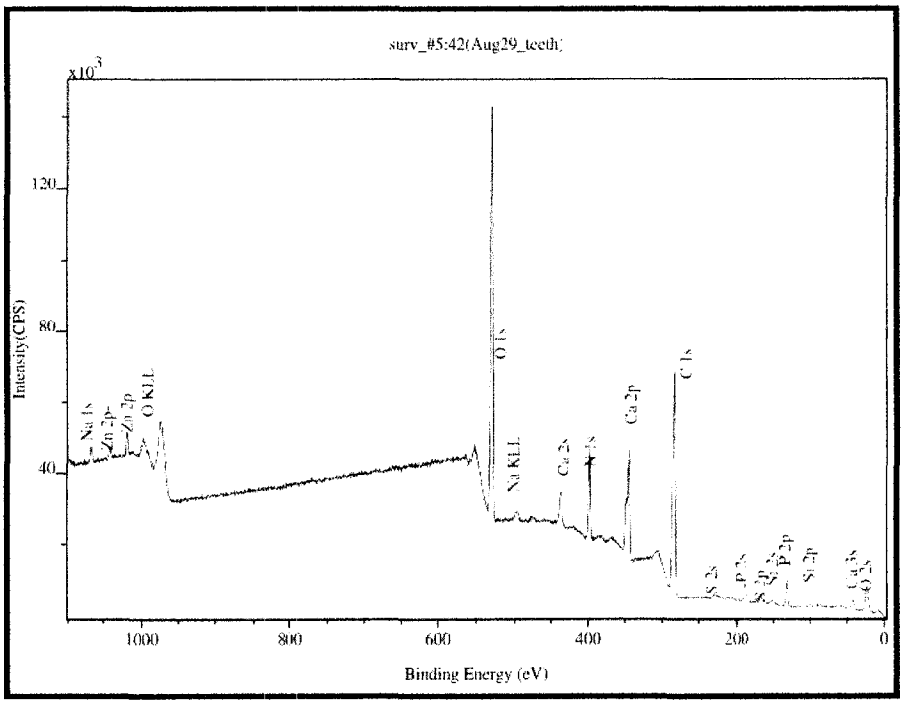

Figure 3.1 Representative survey XPS spectrum from dental enamel before etching

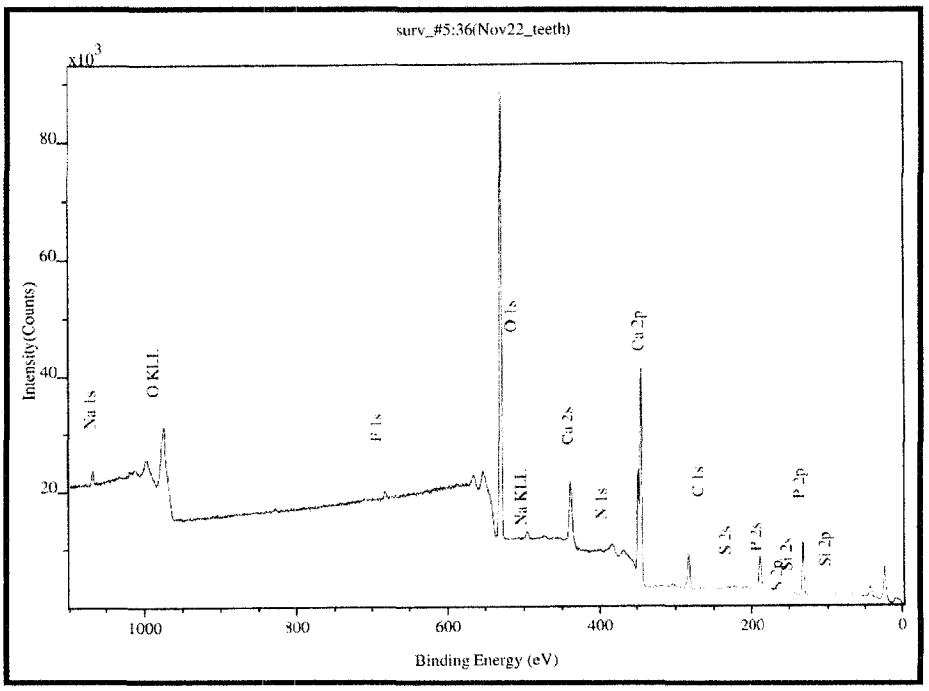

Figure 3.2 Representative survey XPS spectrum from same specimen after etching

Enamel Specimen #	Ca Before	Ca After	C Before	C After	CO_3 Before	CO_3 After	P Before	P After	O Before	O After
1	3.91	8.63	60.49	41.42	10.07	6.88	4.73	6.97	23.38	36.3
2	4.77	13	55.25	16.03	14.87	0.95	2.81	10.4	27.02	55.55
3	2.88	13.21	56.89	23.1	14.17	1.09	1.71	10.01	23.35	49.37
4	5.14	14.53	53.23	19.77	12.2	3.08	2.95	9.94	28.79	51.53
5	3.4	11.38	58.79	25.56	14.4	11.24	2.03	9.56	24.94	48.17
6	6.4	14.31	54.89	21.8	18.85	7.8	4.18	9.83	32.51	50.56
7	8.63	13.65	46.62	20.27	12.71	22.15	5.81	9.31	37.61	50.65
8	5.47	11.48	57.41	23.23	20.09	12.65	3.59	9.19	32.04	47.99
9	8.7	14.7	46.36	17.17	20.38	3.06	5.78	10.71	38.3	52.53
10	6.82	14.76	53.62	14.14	13.56	2.12	4.48	10.9	33.24	54.91
11	7.22	14.57	50.93	11.26	8.3	4.12	4.99	11.57	35.43	65.93

Table 3.1: Atomic mass concentration percentage (AMC) for calcium (Ca), carbon (C), carbonate (CO_3), phosphorus (P) and oxygen (O) before and after etching.

* Please note that since CO_3 and C are included in this table, the total sum of the components is exceeding 100%.

Enamel Specimen #	Ca Ratio*	C Ratio*	CO_3 Ratio*	P Ratio*	O Ratio*
1	2.21	.68	.68	1.47	1.55
2	2.73	.29	.06	3.70	2.06
3	4.59	.41	.08	5.85	2.11
4	2.83	.37	.25	3.37	1.79
5	3.35	.43	.78	4.71	1.93
6	2.24	.40	.41	2.35	1.56
7	1.58	.43	1.74	1.60	1.35
8	2.10	.40	.63	2.56	1.50
9	1.69	.37	.15	1.85	1.37
10	2.16	.26	.16	2.43	1.65
11	2.02	.22	.50	2.32	1.61
Mean (StdD)	2.50 (.86)	.39 (.12)	.50 (.48)	2.93 (1.36)	1.68 (.26)
Coefficient of Variation(C.V)	34%	31%	98%	47%	15%

Table 3.2: Differences in atomic mass concentration percentages (AMC) for calcium(Ca), carbon (C), carbonate ($CO_{3)}$, phosphorus (P) and oxygen (O) as a result of etching (as measured with XPS in 11 enamel specimens).

* A difference of 1 implies no change in AMC; <1 implies a decrease in AMC; >1 implies an increase in AMC.

Enamel Specimen	Ca/C Before	Ca/C After	CO_3/P Before	CO_3/P After	Ca/CO_3 Before	Ca/CO_3 After	Ca/P Before	Ca/P After	Ca/O Before	Ca/O After
1	0.06	0.21	2.13	0.99	0.39	1.25	0.83	1.24	0.17	0.24
2	0.09	0.81	5.29	0.09	0.32	13.68	1.70	1.25	0.18	0.23
3	0.05	0.57	8.29	0.11	0.20	12.12	1.68	1.32	0.12	0.27
4	0.10	0.73	4.14	0.31	0.42	4.72	1.74	1.46	0.18	0.28
5	0.06	0.45	7.09	1.18	0.24	1.01	1.67	1.19	0.14	0.24
6	0.12	0.66	4.51	0.79	0.34	1.83	1.53	1.46	0.20	0.28
7	0.19	0.67	2.19	2.38	0.68	0.62	1.49	1.47	0.23	0.27
8	0.10	0.49	5.60	1.38	0.27	0.91	1.52	1.25	0.17	0.24
9	0.19	0.86	3.53	0.29	0.43	4.80	1.51	1.37	0.23	0.28
10	0.13	1.04	3.03	0.19	0.50	6.96	1.52	1.35	0.21	0.27
11	0.14	1.29	1.66	0.36	0.87	3.54	1.45	1.26	0.20	0.26
Mean (StDev)	0.11 (0.05)	0.71 (0.30)	4.31 (2.12)	0.73 (0.71)	0.42 (0.20)	4.86 (4.55)	1.51 (0.25)	1.33 (0.10)	0.18 (0.03)	0.26 (0.02)
Coefficient Variation	45%	42%	49%	97%	48%	94%	17%	7%	17%	8%

Table 3.3: Atomic mass concentration percentage of the ratios of Ca/C, Ca/O, Ca/P, Ca/CO_3, and CO_3/P before and after etching.

3.5 DISCUSSION

The selection of elements (C, $C0_3$, O, P Ca) analyzed in this study was based on the assumption that they may play a role during mineralization of dental enamel and thus determine the surface composition of mature enamel. Carbon is a constituent of enamel in the form of carbonate. During enamel maturation, as mineral concentration increases,

the carbon concentration decreases.[27] Calcium concentration correlates directly with enamel mineralization as does phosphorus. There are several studies showing an inverse correlation between magnesium and calcium/phosphorus as well as carbonates and calcium/phosphorus. Hypomineralized and thus more caries-susceptible enamel has been linked with high magnesium and carbonate content. [28, 29]

Pure hydroxyapatite (HA) does not occur in biological systems such as enamel. Instead, enamel is made of a calcium-deficient and carbonate-containing apatite analogue[30]. The primary difference between carbonated apatite as opposed to hydroxyapatite is that carbonate (CO_3^{2-}) in the former replaces phosphate (PO_4^{3-}) in the latter. The substitution of PO_4^{3-}, a tetrahedral structure, with CO_3^{2-}, a planar structure, disrupts the crystal structure and weakens chemical bonds, leading to a more acid soluble mineral[31]. At a given pH, the solubility of hydroxyapatite is approximately an order of magnitude below that of carbonated apatite[32], meaning that demineralization will occur much easier.

In the present study, we observed a calcium atomic concentration percentage increase of 2.5 fold on average following the etching procedure. The increase in Ca could be explained through the known mechanism of acidic interaction with HA. In the first phase of this process, carboxylic acids bond to the calcium of HA; in the second phase, Ca either remains attached to the HA surface with only limited decalcification involved or the calcium–acid complex will separate resulting in a substantial decalcification effect.[33, 34] Based on this mechanism, a Ca decrease should be expected upon a considerable decalcification, whereas an increase in Ca concentration would suggest a limited

decalcification. The purpose of acid etching in orthodontics is to create a morphologically porous layer and increase the surface free energy that will result in the low-viscosity fluid resin penetrating the enamel surface. Nakabayashi and Pashley[38] have hypothesized that resin-enamel bond strength depends on the cumulative cross-sectional area of the resin tags that infiltrate the etched enamel. Exposure of enamel crystallites is more important than the length of the tags. Consequently, for orthodontics purposes only limited decalcification should be required; an increase in calcium concentration should be desirable after etching.

All five enamel components were analyzed individually. The most important observation was the great variation between samples. For instance, Ca atomic concentration in one sample increased 4.59 fold after etching and in another sample, only 1.58 fold. Total carbon concentration decreased an average of 0.39 and carbonate ($-CO_3$) alone decreased an average of 0.50. This observation of Carbon upon etching are in agreement with published data showing that the earliest histological changes in enamel demineralization are associated with the removal of magnesium- carbonate-rich mineral. [39] Thus, if a limited decalcification takes place in the enamel surface, one should expect to see a increase in Ca content and a decrease in Carbon content.

Variations in carbonate concentrations between samples were obvious; in one sample it increased 1.74 fold. On average, phosphorus increased almost 3 times (2.93): the highest increase was 4.71 and the lowest, 1.47 (see table 1.2). Variations between samples were considerable different for all five elements analyzed.

The data presented was normalized to 100 and it does not represent an absolute value. In line with previous publications using XPS, we also analyzed the atomic mass concentration percentage as ratios: Ca/C, Ca/O, Ca/CO_3, Ca/P and CO_3/P.

Pure hydroxyapatite has a Ca/P ratio of 1.67. Due to the impurities present in enamel, the Ca/P ratio has historically been found to be in the range of 1.48 ± 0.09.

In our study, ratios of unetched enamel samples varied from .82 to 1.74. Such great variation agrees with previously reported data in a study involving untreated enamel surfaces[35]. A decrease in this ratio usually signals a decrease in strength of the enamel surface.[27] Thus, an enamel specimen with a Ca/P of .82, approximately half of the reported ratio, would theoretically required less time to etch.

As stated earlier, when carbonate substitutes for phosphate in HA, it disrupts the crystal structure and weakens chemical bonds, leading to a more acid soluble mineral[31]. It follows that hypomineralized and thus more acid-susceptible enamel has been linked with high magnesium and carbonate content. In our study, calcium/carbon ratios varied from .05 to .19 before etching and .29 to 1.29 after etching, indicating a increase in calcium atomic mass concentration percentage and a decrease in Carbon atomic mass concentration percentage (see table 3.3). Individual variability, as shown here, must be taken into account when considering possible relationships between enamel chemistry and etching.

A statistical analysis was performed comparing ratios before and after etching. The results for Ca/C, Ca/O, Ca/CO_3 and CO_3/P were significant while those for Ca/P were not

significant (see table 3.3). This is not surprising as it is well established that etching alters the composition of surface enamel. Our results demonstrate considerable variation in the chemical composition of enamel hydroxyapatite, while showing a relatively stable Ca/P ratio. Our observations are in agreement with those of Robinson et al.[36] and Weatherell et al[29]. They have previously shown that the concentrations of Ca and P vary (both in the deeper parts of enamel and also at the surface) while the Ca/P ratio is relatively constant. Quantitative examination of superficial demineralized enamel and in vivo remineralized enamel by others indicated only minor differences in the Ca/P.[37]

More studies should be performed to analyze enamel composition from the perspective that only a limited decalcification may be required to achieve a successful bonding in orthodontics.

Probably the most important finding to emerge from this work concerns the heterogeneity of enamel, which, even within a single type of tooth, seems to be characteristic of this tissue. With each refinement of the chemical and physical analytical methods used in studying enamel, the degree of heterogeneity becomes more pronounced.

Thus, explanations for the variable clinical results observed in enamel etching and bond failure may now take into account the possible influences of physical, structural and chemical differences.

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Chapter Four: Research Paper 3

Relationship between surface composition and surface area in etched enamel.

Key words: Enamel, XPS, BET, Gas adsorption, composition, surface, etch

4.1 ABSTRACT

Successful orthodontic treatment with fixed appliances depends on the ability to bond orthodontic brackets to enamel. It is well accepted that surface characteristics of etched enamel can affect bond strength. Thus, characterization of the surface of enamel could aid in predicting bond strength in different individuals. In this study, eleven enamel samples taken from the buccal side of lower third molars were etched with 37% phosphoric acid. Their surface composition was analyzed by means of X-ray photoelectron spectroscopy (XPS) before and after etching. The atomic surface composition percentages of Calcium (Ca), Phosphorus (P), Carbon (C), Carbonate (CO_3) and Oxygen (O) were measured and quantified as ratios before and after etching. Specific surface enamel area was calculated by the gas adsorption method by function of the BET equation. Multiple regression analysis showed that the model comprised of Ca, P and CO_3 could explain 50 % of the variance in BET area.

4.2 INTRODUCTION

The acid-etch technique was pioneered by Buonocore[1] who showed that certain acids had the ability to modify the enamel surface. However, it wasn't until Bowen[2] developed a composite resin that, in conjunction with the acid-etch procedure, became an efficient

clinical procedure. Dentists were then introduced to the idea of mechanical retention and chemical bonding. Phosphoric acid treatment creates a porous enamel surface layer that is penetrated by a low-viscosity resin-bonding agent. The inflow of the bonding agent into the porous zone results in the formation of resin tags and micromechanical retention to etched enamel is established.

Notwithstanding advances in dental materials, bonding to enamel remains dependent on etching. Thus, great effort has been allocated to elucidate the critical factors that would determine the quality or quantity of surface enamel etching. A number of studies have examined alternative acids and chelating agents, as well as variations in concentrations and times.[3-12] The use of phosphoric acid at 37% for 30 seconds has been the standard practice for enamel etching in pre-molars. However, a 1985 paper reported teeth that etched poorly even with 60 seconds of acid application.[12]

A significant difference in acid-etch patterns achieved on different tooth types was reported by Hobson et al,[13] implying the need to limit etching studies to a specific tooth type. Nonetheless, a study of nine human mandibular first pre-molars concluded that enamel organization is directly correlated to the variations in gross morphology of a particular tooth, rather than being unique to a tooth type.[14] In a previous study, we had demonstrated a significant difference in surface composition of enamel involving the same tooth type (mandibular molars) from different individuals by means of the X-ray photoelectron spectroscopy.[15, 16] This difference was observed prior to and after 30 seconds of etching with 37% phosphoric acid.

Clinically, it has become anecdotally accepted that there are patients with higher bonding failure rates than others. It is possible that the surface characteristics of the enamel of certain teeth in these patients may play a role in such failures. One of the critical factors in such cases could be surface enamel composition. This study was therefore designed to identify one or more surface enamel components that could determine the specific surface area after etching. The surface enamel specific area was determined by the gas adsorption method.[16] The hypothesis to be tested is that there is no correlation between surface enamel components and BET area.

4.3 MATERIALS & METHODS

4.3.1 Enamel sample preparation

Eleven extracted sound human mandibular 3rd molars were selected for this study. These molars were extracted from individuals as part of their planned dental treatment and all of them were impacted. The procedures used for collecting teeth were approved by the Health Research Ethics Board of the University of Alberta and all individuals gave informed consent. After extraction, the teeth were stored separately in 0.1% thymol solution. This solution was used to prevent dehydration of teeth collected immediately after extraction and to prevent bacterial and fungal growth in the storage media.[17] Two by two millimeter samples were obtained from the buccal aspects of the molars, including approximately the middle third of the surface. The cutting was performed using a diamond disc (Brasseler Dental Instrumentation, Savannah, Georgia, U.S.A.). The

dentinal layer was completely removed from the sections with a football shaped diamond bur (Brasseler Dental Instrumentation, Savannah, Georgia, U.S.A.). After XPS testing, the intact enamel surfaces were etched for 30 seconds with 37% phosphoric acid. The etched surface was then rinsed with air and water for 5 seconds and air-dried prior to being tested again with XPS adsorption.

4.3.2 Sample size calculation:

In the milieu of multiple regressions, sample size can be approached from no less than four different angles: (a) power for the overall fit of the model, (b) power for a specific predictor, (c) precision of the estimate for the overall fit of the model, and (d) precision of the estimate for a specific predictor.[30]

In our studies we calculated the sample sized using the correlation coefficient. Although there are basic differences between regression and correlation, a set of data that have a statistically significant regression coefficient (slope), would also yield a statistically significant correlation coefficient.

A power of .90 and alpha of 0.05 was assumed to calculate the sample size. Calcium ratio was identified as probably the most important variable in predicting etched enamel specific surface area. We had also anticipated a large positive correlation between these two variables. For a correlation of .8 the recommended sample size was 12 (see correlation table in the appendix).

In terms of presenting reporting results and measurements, the use of three significant figures for the BET specific area measurement was selected, accepting the uncertainty of the last digit. The accepted convention is that only one uncertain digit is to be reported for

a measurement.

4.3.4 X-ray photoelectron spectroscopy

The XPS scans were acquired using an AXIS ULTRA XPS (Kratos Analytical, Manchester, U.K.), which employed monochromatic AlKα x-rays (hv = 15 eV) as well as charge neutralization. The x-ray gun was operated at 210 W. The XPS data were acquired at photodetector takeoff angles of 55°. All samples had a survey spectrum (0-1000 keV) as well as high resolution spectra of carbon. The survey scan was performed with pass energy of 160 eV, while 20 eV was used for the high resolution spectra. The binding energies of the photoelectrons of insulated samples were calibrated by assuming that the adventitious carbon (C 1s) has a binding energy of 284.6 eV. The data were recorded digitally by Kratos XPS Casa software on a Sun computer system. During the data acquisition, the pressure in the sample chamber did not exceed 2.5×10^{-9} Torr. Specimens were allowed to degas under high vacuum (10^{-10} torr) for at least 48 hours and were then exposed to the incident AlKα beam for alignment and signal optimization for approximately 1 minute prior to analysis. Survey scans and high resolution scans of the C 1s, Ca 2p, O 1s, N 1s and P 2p regions were recorded. The C 1s spectrum was used to analyze the percentage of carbon present as carbonate. Four chemical states of carbon were fitted to each C 1s peak envelope. The analysis of the spectra was performed following a Shirley-type background subtraction, the individual photoemission features were fitted with representative Gaussian distributions using least-squares optimization. The peak positions, amplitudes, and full width at half maximum parameters were obtained from the Gaussian distribution analysis. The peak areas correspond to the total

area with respect to the background subtraction.

The atomic concentrations were calculated using the algorithm and sensitivity factors contained in the Kratos Analytical Software.

4.3.5 Gas Adsorption Measurement

Nitrogen gas is generally considered to be the most suitable adsorptive for surface area determination. Krypton gas is used when the measured surface is expected to be less than 5 m^2/g as was the case with our enamel samples. Prepared samples were analyzed at Micromeritics Analytical Services (Norfolk, GA, U.S.A.). Briefly, enamel samples were degassed at 40 °C for 16 hours prior to their analysis. Samples were placed in a sample tube and heated under vacuum or flowing gas to remove contaminants on the surface of the samples. The sample weight was then obtained by subtracting the weight of the empty sample tube from the combined weight of the degassed sample and sample tube. The sample tube was then placed in the analysis port of a 2420 Accelerated Area and Porosimetry System (Micromeritics, Norfolk, GA U.S.A.) for automatic analysis. The low surface area option was used. This option features a turbomolecular drag pump, which provides the high vacuum required for krypton analysis, and a 10 mmHg pressure transducer, which allows accurate, repeatable pressure resolution. The krypton adsorption isotherm was recorded at 120 K. The specific surface area (S_{BET}) was calculated according to the standard BET method.[18] The BET equation is expressed by:

$$\frac{1}{W[(P_0/P)-1]} = \frac{1}{W_m C} + \frac{(C-1)}{W_m C}\frac{P}{P_0}$$

where W is the weight of krypton adsorbed at a given P/P_0 (P the pressure and P_0 the saturation vapor pressure), and W_m the weight of gas to obtain monolayer coverage and C a constant that is related to the heat of adsorption. A linear relationship between 1/W $[(P_0/P)-1]$ and P/P_0 is required to obtain the quantity of krypton adsorbed. The above equation can be plotted as a straight line with $1 / v\ [(P_0 / P) - 1]$ on the y-axis and P / P_0 on the x-axis. This linear portion of the curve is restricted to a limited portion of the isotherm, generally between values of 0.05 and 0.30. The value of the slope and y-intercept of the line are used to determine the quantity of krypton adsorbed in the monolayer. This information is used then to calculate the total surface area S_{total} and specific surface area S by the following equation:

$$S_{total} = \frac{(v_m N s)}{V}$$

$$S = \frac{S_{total}}{a}$$

where N is the Avogadro's number, s is the absorption cross-section, V molar volume of adsorbent gas and a weight of sample.

4.4 RESULTS

Figure 4.1 shows BET specific surface for the etched enamel specimens expressed as m^2/g.

The results of the surface analysis composition before and after etching are summarized in Table 4.1, which summarizes the amount of change of each surface enamel component that occurred with etching. That is the atomic mass concentration percentage after etching divided by the atomic mass concentration percentage before etching.

The XPS spectra for the untreated and etched enamel specimens are shown in Figure 4. 2 A and 4.2 B, respectively. Multiple regression was conducted to determine the best linear combination of the ratios of Ca, C, CO_3, P and O before and after etching for predicting enamel surface area. The correlation matrix indicated large correlations between C and CO_2. High correlations among predictors indicate the likelihood of problems with multicollinearity. These highly correlated predictors affect the Tolerance (Tolerance =1/VIF) values and the significance values of beta. To deal with this problem we eliminated the variables that were highly correlated. The combination of Ca, CO_3, and P predicted enamel surface area, F (4,29) =4.294 (p= 0.051) with two variables contributing to the prediction: Ca (p=0.015) and P (p=0.022). However, all of the variables need to be included to obtain these results, since the overall F value was computed with all the variables in the equation. The beta weights, presented in Table 4.2, suggest that the difference in Ca before and after etching contribute the most in predicting the BET area. The adjusted *R* squared value was .50. This indicates that 50% of the variance in BET area was explained by this model.

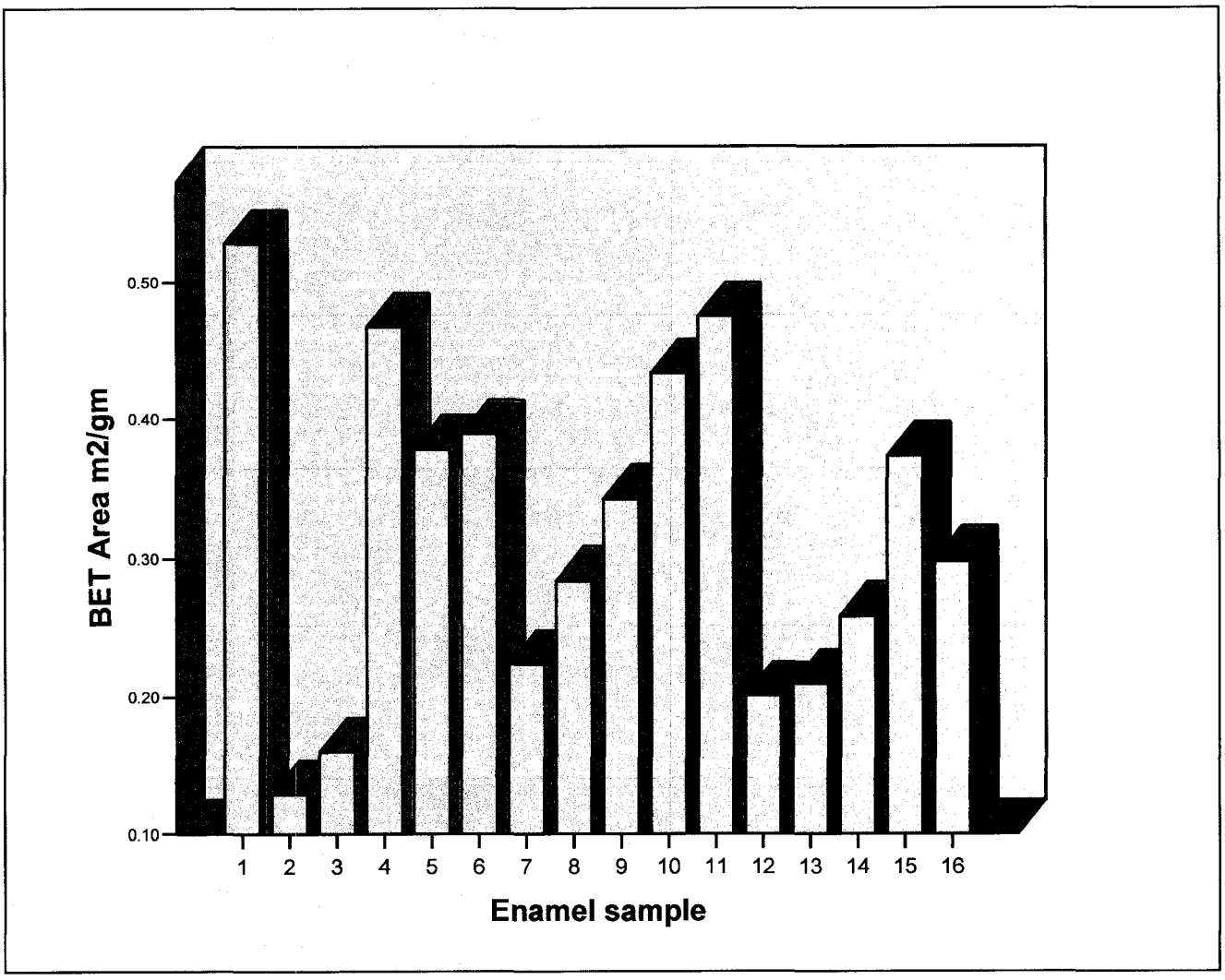
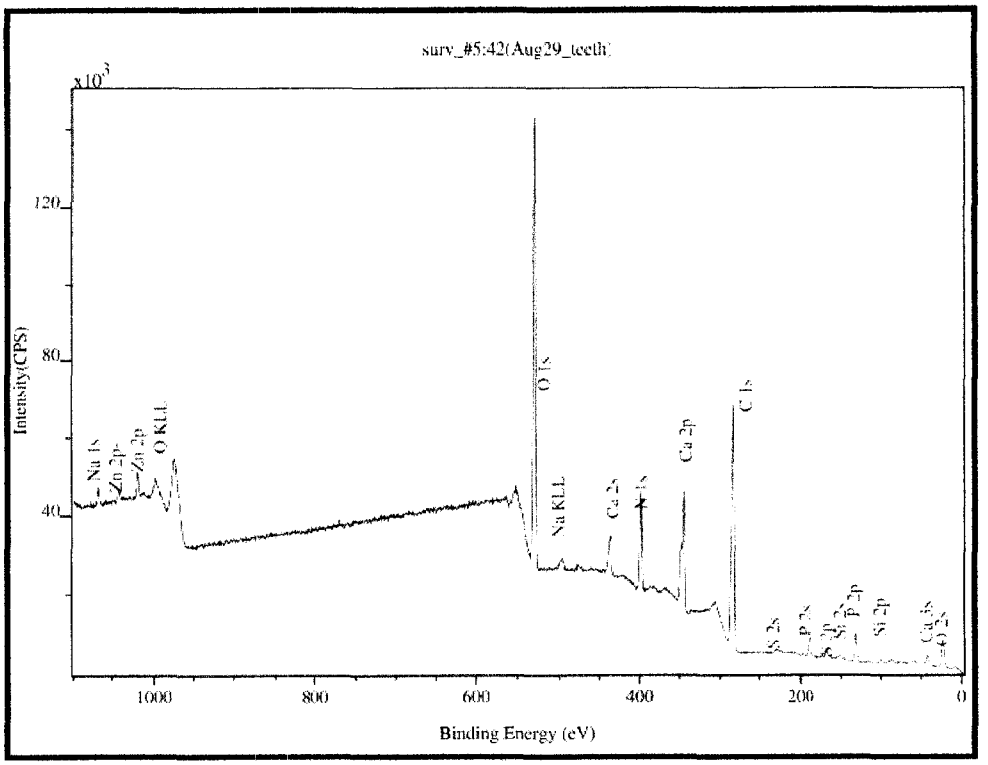


Figure 4.1: Surface area of individual enamel samples determined by krypton adsorption and calculated by BET equation

Enamel Specimen #	Ca Ratio	C Ratio	CO_3 Ratio	P Ratio	O Ratio	BET Specific Surface Area m^2/g
1	2.21	.68	.68	1.47	1.55	.528
2	2.73	.29	.06	3.70	2.06	.160
3	4.59	.41	.08	5.85	2.11	.467
4	2.83	.37	.25	3.37	1.79	.389
5	3.35	.43	.78	4.71	1.93	.284
6	2.24	.40	.41	2.35	1.56	.342
7	1.58	.43	1.74	1.60	1.35	.433
8	2.10	.40	.63	2.56	1.50	.475
9	1.69	.37	.15	1.85	1.37	.201
10	2.16	.26	.16	2.43	1.65	.373
11	2.02	.22	.50	2.32	1.61	.298
Mean (StDev)	2.50 (.86)	.39 (.12)	.50 (.48)	2.93 (1.36)	1.68 (.26)	.359 (.116)
CV (Coefficient variation)	34%	31%	96%	46%	15%	32%

Table 4.1: Differences in atomic mass concentration percentage (AMC) for calcium(Ca), carbon (C), carbonate (CO_3), phosphorus (P) and oxygen (O) as a result of etching (as measured with XPS in 11 enamel specimens) and surface area for each enamel specimen expressed in m^2/g (determined by gas adsorption measurement) after etching. Ratio was obtained by dividing the AMC after etching by the AMC before etching. Coefficient of variation (CV) equals standard deviation (SD) over mean times 100.

A



B

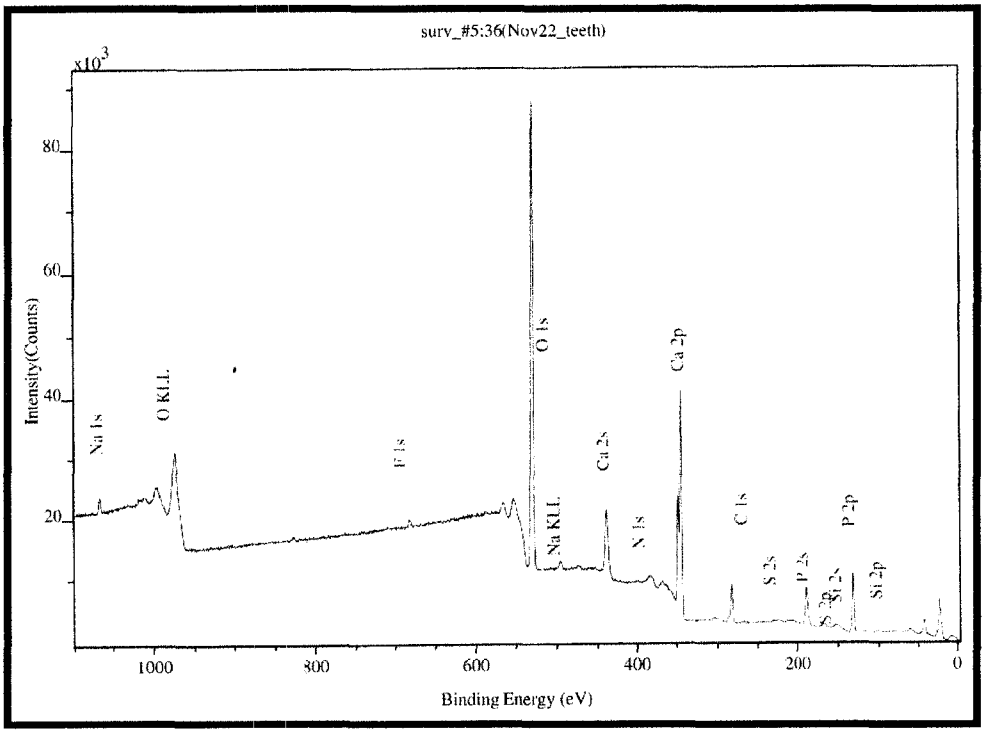


Figure 4.2: A) Representative survey XPS spectrum from dental enamel before etching.

B) Representative survey XPS spectrum from same specimen after etching.

Model	Estimated Coefficients	
	B	P-value
Ca Difference	.326	. 015
CO_3 Difference	.112	.098
P Difference	-.188	.022
Constant	0.04	

Table 4.2: Beta (β) Weights, suggesting the difference in calcium before and after etching contribute the most in predicting the BET area. P-values show that only Ca and P are significantly contributing to the equation. However, all the variables were included in the equation.

4.5 DISCUSSION

When phosphoric acid is applied to the enamel surface, selective dissolution of the surface occurs, opening up microscopic pores, thereby increasing the surface area. In a previous study, we introduced the gas absorption method as an alternative means of measuring specific surface enamel area.[16] The BET equation was used to determine our etched samples' specific area with the assumption that a larger BET area means a larger exposed surface area of the enamel. This assumption is in line with Nakabayashi and Pashley's work[19] proposing that exposure of enamel crystallites is more important than the display of "ideal" etch patterns. They hypothesized that resin-enamel bond strength depends on the cumulative cross-sectional area of the resin tags that infiltrate the etched

enamel. The length of the tags has no effect on the cross-sectional area. In figure 1, we can see the specific surface area of each of our enamel samples. From these results, it is evident that 3rd lower molars from different individuals behave differently as a result of 30 seconds of exposure to 37% phosphoric acid.

XPS is a highly discriminating and specific method of surface analysis. Due to its sampling depth of just a few nanometers, chemical information is acquired selectively from an ultra-thin layer at the enamel surface. XPS has been widely used in the dental research field .[20-22] We have previously used this method to identify differences in lower 3rd molar surface enamel composition from different individuals.[15]

The XPS spectra for the untreated enamel surface (figure 4.2) show peaks indicating the presence O, Ca, C and P. Figure 3 shows the same enamel specimen after treatment with 37% phosphoric acid.

Linear regression analysis showed that a linear regression model of differences in Ca, CO_3, and P before and after etching could significantly predict BET area. Only Ca and P were significant whereas CO_3 was no significant. However all three variables needed to be included to obtained these results. Our results are in agreement with previous work that showed dissolution of enamel to be a function of several factors including calcium and phosphate concentration. Others factors noted by these workers were degree of saturation with respect to hydroxyapatite (DS_{HA}), pH, and acid concentration and type.[23-25] Since the same acid at the same concetration was used in our samples, these last two variables were controlled. Our results suggest that the ratio of Ca, P and CO_3 before and

after etching could explain fifty percent of the varience in etched enamel surface area. The ratio of the five studied components (Ca, C, CO_3 , P and O) could explain seventy one percent of the variance. In terms of the remaining thirty percent, other surface enamel components such as magnesium may play a role.The earliest histological changes in enamel demineralization are associated with the removal of magnesium- carbonate-rich mineral. [29]

As a consequence of developmental factors and the effects of exposure in the mouth, the composition of enamel is rather variable, between and within teeth.[26]

Several workers have shown that the concentration gradient of calcium across normal enamel varies considerably and it is rarely uniform.[27, 28] We have reported the same observation.[15] The particular difficulty of understanding the physico-chemical mechanisms of enamel demineralization arises from the natural variability of enamel mineral.[24]

To the best of our knowledge, this is the first study correlating surface enamel components with the etched enamel surface area. More studies need to be performed prior to applying any results to the clinical setting. Probably the most significant outcome from this work relates to the awareness of the different techniques available to study enamel in relation to etching.

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Chapter Five: Discussion and Recommendations

The clinical success of resin bonds to acid-etch enamel as a result of etching[1] has opened new frontiers in dentistry allowing dental treatments that were previously impossible with conventional techniques. It has made a significant impact on the practice of orthodontics. In orthodontics, treatment with fixed appliances relies on the ability to bond composite material to enamel. The acid–etch technique entails the application of a mild acid on the enamel surface to facilitate selective dissolution of prism cores or peripheries resulting in microporosities into which resin can flow and can be polymerized to form a mechanical bond to the enamel. Therefore, the demineralization of dental enamel is of clinical relevance in dentistry and in orthodontics, in particular.

The present work has been undertaken to understand the critical factors that may determine the quality and quantity of surface enamel etching. A better appreciation of the characteristics of surface enamel and the etching process, in general, would assist in developing or improving dental materials and/ or techniques.

Our samples consisted of 16 third molars from different individual collected at one oral surgery office. The oral surgery staff was provided with several plastic containers and 1% thymol solution. They were instructed to collect only third molars that were impacted and place molars from each individual in different containers. Information in regards of sex, age and ethnicity of the patients was withheld to comply with Health Research Ethics Board of the University of Alberta.

One lower molar was chosen from each patient. In a few cases, left and right lower molars from a particular patient were missing considerable enamel tissue as a result of the

surgical procedure itself, In these cases, (five in total) one upper molar was used instead of lower molar. Our first research paper's objective was to examine the gas adsorption technique as an alternative quantitative method to analyze etched enamel. Thus, it was not imperative to distinguish between upper and lower third molars within our sample size. In this study all sixteen specimens were included (eleven lower and five upper third molars). Research papers two and three's objectives are intimately related to the differences in surface enamel composition in different individuals; including upper and lower third molars would have added a confounding variable. Thus, only lower third molars (eleven specimens) were used for these two studies.

Enamel is the hardest biological substance in the human body and is a crystalline structured material consisting of both a mineral and an organic phase. The prevailing mineral phase (96%) consist primarily of calcium hydroxyapatite $(Ca_5(PO_4)_3(OH))_2$. The remainder of the enamel is made up of 3% water and 1% organic matter including proteins and lipids.

The chemical composition of enamel was analyzed by means of x-ray photoelectron spectroscopy (XPS). Our results showed considerable variation in the chemical compositions of the enamel hydroxyapatite, while the Ca/P ratio remained relatively stable. Our observations are in line with previous publications[2, 3] showing that although the concentrations of Ca and P varied, both in the deeper parts of enamel and also at the surface, the Ca/P ratio was relatively constant. The most significant finding to emerge

from this segment of our study concerns the heterogeneity of enamel. Differences in composition between our specimens were evident and statistically significant, before and after etching. Retrospectively, the XPS protocol should have included control samples (for example, enamel specimens that were water rinse and air dried without the acid-etch step).

We have also presented a method based on adsorption of gases that can be used to determine human enamel specific surface area through the BET equation. Surface area can be evaluated by generating the conditions required to adsorb a monolayer of gas molecules onto a sample. If this process is extended and gas is allowed to condense in the pores, the specimen's fine pores structure can be determine. Evaluation of the adsorption and desorption of gases can reveal information about the pore size, pore area, pore distribution and pore shape. In this study, we focused specifically on the surface area of our enamel samples. Recent studies have proposed that resin-enamel bond strength is the result of the cumulative cross-sectional area of the resin tags that infiltrate the etched enamel surface.[4] Increasing the length of the pore does not increase the cumulative cross-sectional area. Contrary to what was historically thought, there is a lack of correlation between depth of etch and enamel-resin bond strength. Thus, knowledge of pore shape and distribution could aid in predicting bond strength.

To the best of our knowledge, this is the first time that this technique is used to evaluate enamel surface area after etching. Henceforth, an increased number of studies will

improve the validity and reliability of this method, thereby making it possible to better understand the biological mechanisms behind the variations in etched enamel.

The third and last part of this work consisted in identifying one or more surface enamel components that could determine the etched enamel specific surface area, determined by the gas adsorption method. Liner regression analysis showed that a linear regression model comprised of Ca, CO_3, and P (before and after etching ratios) could significantly predict BET area. Nonetheless, Ca and P were significant whereas CO_3 was not. Our results are in agreement with previous work that showed dissolution of enamel to be a function of several factors including calcium and phosphate concentration. In retrospect, a very useful approach could have been measuring enamel BET specific surface area before etching and comparing it with BET measurements after etching. Such information could be interrelated not only with surface enamel composition but different types of etching agents as well as different concentrations. If a combined approach of Scanning Electron Microscopy and BET specific surface area were to be undertaken again, environmental SEM would be recommended.

In terms of the specific area measurements, the precision of the gas adsorption technique in measuring dental enamel surface was not determined. Please note that precision refers to how closely individual measurements agree with each other whereas accuracy refers to how closely a measured value agrees with the correct value. Our research protocol was designed to compare enamel surface specific area from third molars from different individuals. Hence precision was important. Precision leads to the concept of significant figures. The number of significant figures deals with precision only and it is directly

linked to a measurement. In layman's term: if a person needed only a rough estimate, two significant figures should be satisfactory, otherwise one should use three significant figures or better yet four significant figures. Micromeritics® gas adsorption summary reported the BET surface area using 4 significant figures. When taking readings using digital equipment, the readout will often give an unjustified confidence in the precision of your readings. Since our values range between .5828 and .1286 this was an unreasonable display of precision.

While it is possible that Micromeritics' ASAP 2420 Accelerated Surface Area and Porosimetry analyzer can read to this level of precision, in theory, our experimental setup is not allowing for this level of precision.

We have chosen to use 3 significant figures for the BET specific area measurement accepting the uncertainty of the last digit.[18]

In terms of the accuracy of the BET measurements, it depends upon the quality and calibration of the measuring device. By working with a company such as Micromeritics® that has obtained ISO 9001 - 2000 certification, (please refer to appendix) we are assuming the highest standard in accuracy.

There are several ways to make a reasonable estimate of the random error in a particular measurement. The best way is to make a series of measurements of a given number of samples and calculate the mean and the standard deviation. In our particular case, the cost of each BET analysis was high. Therefore, it was preferred to increase the number of samples to be analyzed than to repeat measurements on the same sample. It would be highly recommended for the next study to perform repeated measurements on the same

specimen. Control samples, that are samples that would follow the same protocol except the acid etching step, should be included as well.

When studying the enamel surfaces of erupted teeth, it should be realized that all surfaces that have been exposed to the oral environment are covered by microbial deposits of highly varying thickness and composition. It is therefore difficult to interpret structural findings in such surfaces. The enamel specimens used in this study were from sound molars that were not in contact with the oral milieu: hence, the aforementioned issue does not apply. Moreover, once a tooth has been formed, the overall patterns of chemical variations in the enamel seem more or less fixed. It is also been shown that there is a discrepancy between surface enamel content and deeper enamel composition. [5, 6 7 8] Theuns[9] showed that enamel solubility also increased towards the dentinoenamel junction.

Thus, if enamel is worn through attrition, the new tooth surfaces reveal chemical characteristics typical of interior enamel. Such worn surfaces appear, for instance, to be as soft as the corresponding interior region of teeth,[10] with similar low density and high carbonate concentration[11].

This is a point that should be taken into account when bonding brackets to older patients or when selecting teeth for in-vitro studies

Much research has been done to study the composition of enamel. Notwithstanding its prominent role in dentistry, dental enamel has also been studied for other purposes. Trace element content of the mineralized dental enamel reflects the biological environment during the time of tooth development and the oral environment associated with the erupted tooth.[12] Because of this unique characteristic, dental analysis has been used to examine nutritional status,[13, 14] environmental pollution,[15, 16] and geographical variation in trace element exposure.[14, 17] As a final consideration, it is my opinion that this work is a small contribution in the quest towards understanding dental enamel. The gas adsorption method as a means of measuring enamel surface should be validated and there are opportunities for improvement in almost all of our protocols. The significance of this study relies on presenting the dental enamel as a heterogeneous tissue unique to every single tooth.

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APPENDIX A

		Sample Size Calculation using Correlation Coefficient					
		H0: rho=0	Ha: rho=rhoA (not equal zero)				
		Power=.90 and alpha=0.05					
	rhoA	zetaA	n		Z(beta)	Z(alpha)	
	0.1	0.100335348	1047		1.28155	1.959964	
	0.2	0.202732554	258.7				
	0.3	0.309519604	112.7				
	0.4	0.42364893	61.54				
	0.5	**0.549306144**	37.82				
	0.6	**0.693147181**	**24.87**				
	0.7	0.867300528	16.97				
	0.8	1.098612289	11.71				
	0.9	1.47221949	7.848				
	-0.1	-0.10033535	1047				
	-0.2	-0.20273255	258.7				
	-0.3	-0.3095196	112.7				
	-0.4	-0.42364893	61.54				
	-0.5	-0.54930614	37.82				
	-0.6	-0.69314718	24.87				
	-0.7	-0.86730053	16.97				
	-0.8	-1.09861229	11.71				
	-0.9	-1.47221949	7.848				