THE EFFECT OF WATER CONCENTRATION ON THE RATE AND EXPANSION OF DEMINERALIZED DENTIN MATRIX

by

F. John Harmon, D.D.S.

Submitted to the Faculty of the School of Graduate Studies
of the Medical College of Georgia in partial fulfillment
of the Requirements for the Degree of Master of Science

May
2000

THE EFFECT OF WATER CONCENTRATION ON THE

RATE AND EXPANSION OF DEMINERALIZED DENTIN MATRIX

This thesis is submitted by F. John Harmon, D.D.S., and has been examined and approved by an appointed committee of the faculty of the School of Graduate Studies of the Medical College of Georgia.

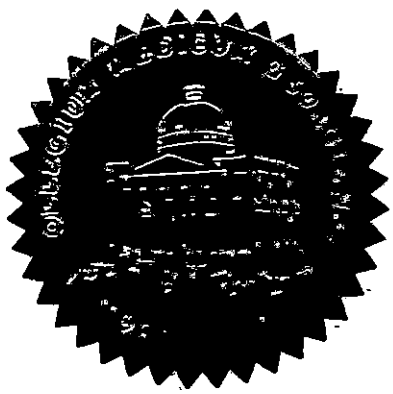
The signatures below verify the fact that all required changes have been incorporated and that the thesis has received final approval with reference to content, form and accuracy of presentation.

This thesis is therefore submitted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology and Maxillofacial Pathology.

5/1/00
Date

[redacted]
Major Advisor

[redacted]
Department Chairperson



[redacted]
Dean, School of Graduate Studies

F. JOHN HARMON, D.D.S.
The Effect of Water Concentration on the Rate and Expansion of Demineralized Dentin Matrix
(Under the direction of DAVID H. PASHLEY, D.M.D., Ph.D.)

The purpose of this study was to test the rate and expansion of air-dried, collapsed, demineralized dentin matrix using three different water-HEMA solutions. Solutions of 10, 25 and 50% water (by weight) were formulated with corresponding amounts of HEMA. Eighteen dentin specimens (0.2 mm thick) were demineralized in 17% EDTA for 5 days, yielding type I collagen disks approximately 200 μm thick.

A linear variable differential transformer (LVDT) was used to measure vertical dimensional changes in the demineralized dentin specimens. The specimens were air-dried with compressed air, then one of the three test solutions was applied, and the rate and amount of expansion was measured.

Repeated measures ANOVA with least squares means comparison test revealed a significant ($p < 0.0001$) relationship between water concentration and both the rate and amount of re-expansion of dried, demineralized dentin. Hansen's solubility parameter for hydrogen bonding forces was used to express the ability of the different solutions to interact with the demineralized dentin matrix.

INDEX WORDS: Water-HEMA, Demineralized Dentin Matrix, Solubility Parameter

ACKNOWLEDGMENTS

I would like to express my sincere thanks and gratitude to my research committee for their help in assisting me complete this Master's thesis. Thanks to their guidance, support and encouragement, this work has been successful. To Dr. David Pashley, my major advisor, for helping me remain focused and always having a kind word. And to Dr. James Borke, Dr. Frederick Liewehr and Dr. Frederick Rueggeberg for their desire to help me make the thesis better.

I would also like to thank the many other people who have contributed to this project during the past two years. To Dr. Carl Russell and Ms. Kelli Agee for their help with the statistical analysis. To Dr. Yi Zhang and Dr. Rachel Terada for their assistance in Dr. Pashley's laboratory. To Mrs. Anita Smith for introducing me to the LVDT. And to Mrs. Debbie Nunley, Mrs. Wanda Yarbrough, Mrs. Janet Ergle, Mrs. Petra Lockwood, Mr. Don Mettenburg, and Ms. Cindy Williams for their support in Dr. Rueggeberg's laboratory.

I would also like to thank my endodontic mentors, Dr. Lesley West, Dr. Frederick Liewehr and Dr. William Patton, for allowing me the time away from the residency to complete this project.

And finally, to my parents, Anne and Donald Harmon, for providing me with the opportunity to excel.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. INTRODUCTION

A. Statement of the Problem

Dentin bonding is used in many areas of clinical dentistry, including restoration of teeth with resin composite, and with resin luting cements in the placement of crowns, inlays, veneers, and posts. In order to obtain the highest bond strength to dentin, the moist bonding technique is recommended (Kanca, 1992a, 1992b, 1996; Gwinnett, 1992, 1994). However, several dentin bonding systems available today still recommend a dry bonding technique, where acid-etched dentin is air-dried prior to the application of primer. Dry bonding results in a decreased bond strength compared with bonding to moist dentin (Gwinnett, 1992).

Adhesive bonding techniques involve acid-etching enamel and dentin with a phosphoric acid gel or solution. Acid-etching removes the smear layer and the mineral phase from superficial dentin, leaving behind an intact, exposed collagen fibrillar network on the surface of the mineralized dentin base. After acid-etching, rinsing, and moistening with water, interfibrillar spaces are created between the collagen fibrils that allow the dentin primer (a solution of monomers) to diffuse into the network. A critical point in the moist bonding technique is maintenance of the space between collagen fibrils in an expanded state after mineralized dentin has been removed. If water that replaced the mineral phase between collagen fibrils is evaporated before the monomer diffuses in to replace it, the network quickly collapses, resulting in a decrease in the interfibrillar space

and permeability loss of the resin monomer (Pashley *et al.*, 1993). Once the primer monomer diffuses into the interfibrillar spaces and polymerizes, a dentin hybrid layer is created, composed of polymerized resin, dentin collagen fibrils, and residual mineral particles (Van Meerbeek, 1993).

Neither the rate nor the degree of expansion of dried dentin collagen fibrils following primer application, nor the optimal primer composition to maximize re-expansion of air-dried acid-etched dentin has been studied. This inadequacy results from lack of quantitative techniques to measure expansion of this microscopic layer (*ca.* 5-10 μm).

The purpose of this study was to quantitatively measure the rate and expansion of demineralized dentin matrix over time, using three solutions with varying water-HEMA concentrations, and 100% water. This knowledge will facilitate development of optimal primer formulations that insure full expansion of the collapsed collagen matrix (in minimal time) before monomer is added and polymerized.

We hypothesized that the expansion rate of collapsed, demineralized dentin matrix is proportional to the water concentration of the model primer solutions. Higher water concentration should result in greater and quicker expansion of collapsed, demineralized dentin matrix.

The specific aims to test the hypothesis are (1) to develop a quantitative method to measure shrinkage and expansion of demineralized dentin collagen in real-time, and (2) to measure the rate of expansion of air-dried demineralized dentin as a function of water concentration using three water-HEMA solutions, and 100% water, and to express this relationship mathematically.

B. Review of Related Literature:

The success of the moist bonding technique (Kanca, 1992a, 1992b, 1996; Gwinnett, 1992, 1994) is thought to be due to preventing collapse of the collagen fibril network of demineralized dentin matrix after acid-etching (Sugizaki, 1991; Pashley *et al.*, 1993, 1994; Inokoshi *et al.*, 1997). However, attempts to measure the degree of collagen collapse suffered from measurement artifacts. For example, the high vacuum required for scanning electron microscope (SEM) observations causes profound dentin shrinkage (Sugizaki, 1991). Critical-point drying techniques reduce, but do not prevent shrinkage of demineralized dentin in SEM studies (Carvalho *et al.*, 1996a). Many clinicians dry acid-etched preparations to evaluate the degree of enamel etching. Air-drying causes a rapid collapse of the demineralized dentin matrix. However, the network re-expands when rehydrated with water, thereby restoring the interfibrillar spaces between collagen fibers, allowing optimal bond strength of resins to dentin (Gwinnett, 1994). Presumably, this re-expansion could be accomplished using aqueous solutions of water-soluble monomers of various concentrations.

Dentin collagen matrix shrinkage has been measured by different means with varying degrees of success; however, the expansion rate of demineralized dentin matrix has not been measured. Sugizaki (1991) covered a dentin reference zone with varnish and acid-etched the uncovered dentin with 40% phosphoric acid for 40 seconds prior to rinsing and drying the area. He then bonded resin to the surface and sectioned the specimen for SEM examination to observe the amount of linear shrinkage relative to the reference height of the masked mineralized dentin control. Most specimens exhibited 40-70% vertical shrinkage of the etched-bonded zone. This technique did not permit the rates

of shrinkage nor the expansion to be measured over time, but only presented the final outcome. Uno and Finger (1996) used an optical microscope with the fine focus adjustment (Z-axis) calibrated in microns to measure the depth of dentin demineralization by various acidic conditioners and different etching times. They reported a good correlation between optical and SEM measurements, but neither method provided information about the rates or expansion of collagen shrinkage over time. Atomic force microscopy (AFM) was used to measure the shrinkage rate of dentin surfaces during acid-etching (Kinney et al., 1993; Marshall *et al.*, 1997). However, AFM cannot measure changes in height greater than 600-800 nm (0.6-0.8 μm). This instrument is insufficient to measure changes in dentin during air-drying after acid-etching with 37% phosphoric acid for 15 seconds. After acid-etching for 15 seconds then air-drying, Uno and Finger (1996) measured approximately 5-10 μm shrinkage.

The expansion rate of collapsed collagen matrix is important clinically because the adhesive materials should be applied only when the primer has fully expanded the collapsed network. Premature application of adhesive may not permit optimal diffusion of resin monomer into the collagen fiber network, thereby decreasing the final bond strength of the restoration. To be effective clinically, collagen expansion must occur within a clinically acceptable time-limit so treatment time is minimized.

Solubility Parameters. In 1949, Hildebrand proposed the term *solubility parameter* (δ) to describe or predict the possible molecular interactions in a solution. The solubility parameter is the square root of the cohesive energy density of the solution with units of $(J/cm^3)^{1/2}$. Cohesive energy density (expressed as energy per unit volume or

J/cm^3), is the amount of energy associated with all of the molecular interactions in a solution.

The wetting characteristics of a monomer acting on a polymer may be expressed in terms of the Hildebrand solubility parameter (δ) and polarity (p) of the resin (Asmussen and Uno, 1993). The surface of a polymer is softened when liquids having the same polarity (defined as the total intermolecular interactions due to dipole-dipole attractions) and solubility parameter as the polymer are applied. Polarity and solubility parameters are used extensively in polymer chemistry to predict the interaction of different polymers by using glues of appropriate solubility parameters to swell the polymer surface, loosening the polymer chains and allowing the glue to penetrate.

Asmussen *et al.*, (1991, 1993) were the first to suggest that the mechanism of action of many primers used in adhesive dentistry was the wetting and perhaps "softening" of collagen fibrils. The ability of a series of primer formulations to improve bond strength was tested, and the authors inferred that the solubility of a given polymer (collagen) in a given solvent is favored if the solubility parameters of the polymer and solvent are similar.

Miller (1995) and Miller *et al.*, (1998) found that primers with low or high solubility parameters were unable to interact with a collagen surface. Their research also indicated that primers having approximately the same solubility parameters as collagen should produce good interactions. However, rather than use the single Hildebrand solubility parameter, Miller (1995) and Miller *et al.*, (1998) chose to use Hansen's (1969) triple-component solubility parameter. Hansen (1969) developed an extension of the Hildebrand solubility parameter for use in polymer-liquid interactions, where he divided

the Hildebrand solubility parameter into three components that together estimate the total molecular force interactions. The Hansen solubility parameter describes the interaction of a polymer and a solvent as the sum of contributions due to dispersive forces (δ_d), polar forces (δ_p) and hydrogen bonding forces (δ_h). The Hansen solubility parameter is described by the equation $\delta_{total} = \delta_d + \delta_p + \delta_h$.

The dispersive force contribution (δ_d) is from interactions between non-polar molecules and the attraction is due to van der Waals forces (very weak forces). The polar force contribution (δ_p) is due to dipole-to-dipole interactions between polar molecules. Dipole-to-dipole interactions are caused by the attraction of the positive end of one polar molecule to the negative end of another polar molecule. Dipole-to-dipole interactions are stronger than attractions between non-polar molecules, (*i.e.* polar forces are stronger than van der Waals forces). The contribution due to hydrogen bonding (δ_h) is caused by molecules that hydrogen bond with each other. Hydrogen bonding is an especially strong type of dipole-to-dipole attraction, where one hydrogen atom serves as a bridge between two electronegative atoms, holding one atom by a covalent bond, and the other by purely electrostatic forces.

Miller *et al.* (1998) suggested that δ_h values of primer components could be used to rank or compare primers according to their ability to form or break hydrogen bonds. Water has the highest δ_h value of any solvent (37.3 $(J/cm^3)^{1/2}$), and readily breaks interfibrillar hydrogen bonds and re-expands shrunken collagen matrices. However, since water cannot polymerize to form a dentin hybrid layer, a primer must be added to it. Hydroxyethyl methacrylate (HEMA), is a water-miscible polymerizable monomer that

has a relatively low δ_h, 16.1 $(J/cm^3)^{½}$. As more water is added to HEMA, the δ_h value of the mixture increases.

Asmussen and Uno (1993) hypothesized that, in each step of a dentin-bonding procedure, the next reagent in the procedure should have approximately the same solubility parameter and polarity as the surface left by the preceding step. Dentin conditioning (acid-etching), the first step of the dentin bonding procedure, leaves behind exposed dentin collagen fibrils (a semi-solid biopolymer). The aim of conditioning is to expose dentin collagen fibrils and facilitate monomer penetration into the exposed matrix in order to achieve micro-mechanical retention. Marshall *et al.* (1999) suggested that when the demineralized dentin matrix is air-dried, collagen fibrils touch and form interfibrillar hydrogen bonds. For a primer to be effective in re-expanding the collapsed matrix, it must be able to break the interfibrillar hydrogen bonds formed during drying.

This study evaluated the relationship between the water concentration of three different water-HEMA mixtures and their ability to re-expand shrunken, demineralized dentin matrix. Since there is a relationship between water concentration and δ_h, there may also be a high correlation between δ_h of model primers and their ability to expand shrunken dentin matrix.

II. MATERIALS AND METHODS

Extracted, unerupted human third molars were obtained from oral surgery clinics in Augusta, GA and stored at 4° C in isotonic saline with 0.2% sodium azide (to inhibit bacterial growth). The teeth were sectioned at right angles to their long axes, through the mid-crown region to remove occlusal enamel. A second parallel section was made above the pulp chamber to produce dentin disks approximately 0.2 mm thick. Each disk was then demineralized in 17% EDTA for five days at 25° C with constant stirring to remove the mineralized dentin and enamel. This process yields a demineralized dentin disk composed primarily of type I collagen, approximately 9 x 7 x 0.2 mm.

The instrument used to measure dentin shrinkage and expansion was the linear variable differential transformer (LVDT) portion of a thermal mechanical analyzer (TMA), (TA Instruments, New Castle, DE). The TMA was modified by removing the oven, thereby exposing the underlying contact probe. Changes in vertical displacement were recorded by a computer attached to the modified TMA instrument (Fig. 1).

The contact probe was a quartz rod 3 mm in diameter, stepped down to a final flat tip diameter of 0.89 mm. A 500 mg weight was applied to the weight pan during all procedures. The weight of the probe and empty pan was 3.09 g. Due to the presence of a spring in the apparatus, the actual vertical weight on the disk was only 0.22 g.

The LVDT had a sensitivity of ± 0.1 μm over a linear range of 300 μm. Calibration of the unit was verified by vertically displacing the contact probe using a

micrometer accurate to ± 0.5 μm (Model No. 331 711 30, Mitutoyo, Tokyo, Japan). The correlation coefficient of the LVDT micrometer values compared to the direct micrometer values was very high (0.99), as was the R^2 value (Fig. 2).

The TMA was operated isothermally at 22 ± 2° C at an ambient relative humidity of 40-65%, as measured with a laboratory hygrometer (Thermo-Hygrometer, Hanna Instruments, Woonsoclat, RI). During testing, the dentin specimen was fixed to the bottom of a Plexiglas well (volume 4.7 mL) with a viscous cyanoacrylate cement (Zap-It, Dental Ventures of America, Corona, CA) to prevent movement.

Each experiment began with the contact probe supported by wet, fully expanded, demineralized dentin matrix, with the initial height serving as baseline for the subsequent expansion of the collapsed demineralized dentin. Beginning with fully expanded, wet dentin matrix, dry, compressed air (0% relative humidity) was blown on the surface at a 45° angle from a distance of 3 cm at a rate of 500 mL/sec. The rate of matrix collapse was continuously measured with the LVDT and recorded. Once shrinkage was complete (indicated by a plateau in the curve), the collagen disk was treated with 100% water (Solution A, positive control) and re-expanded to the original height. After a steady baseline was obtained, dry compressed air was again applied to shrink the demineralized dentin matrix to the same plateau, after which one of three test solutions (10, 25 and 50 weight percent water) was added and the rate and degree of expansion of the collapsed matrix was measured over time. Each solution was randomly tested six times on each specimen, and a total of eighteen specimens were used in the experiment.

Table I lists the sequence of solution application. This application matrix was used to prevent an "order effect" of the solutions, which could occur if the water-HEMA

solutions were tested consecutively (*i.e.* testing solution B six times, followed by C six times, followed by D six times).

Table I

Randomized Solution Application Order

Group	Solution Order	Solution*
1	B C D	
2	C D B	B = 10% H_2O-90% HEMA
3	D B C	C = 25% H_2O-75% HEMA
4	B D C	D = 50% H_2O-50% HEMA
5	D C B	
6	C B D	

*All values are weight percent.

The nonaqueous solvent used to vary water concentration was 2-hydroxyethyl methacrylate (HEMA). This compound is a water-miscible, but water-free monomer commonly used as a primer component in many commercially available dentin bonding systems.

Figure 1. *The modified TMA instrument used to measure changes in the rate and expansion of dried, demineralized dentin matrix.*

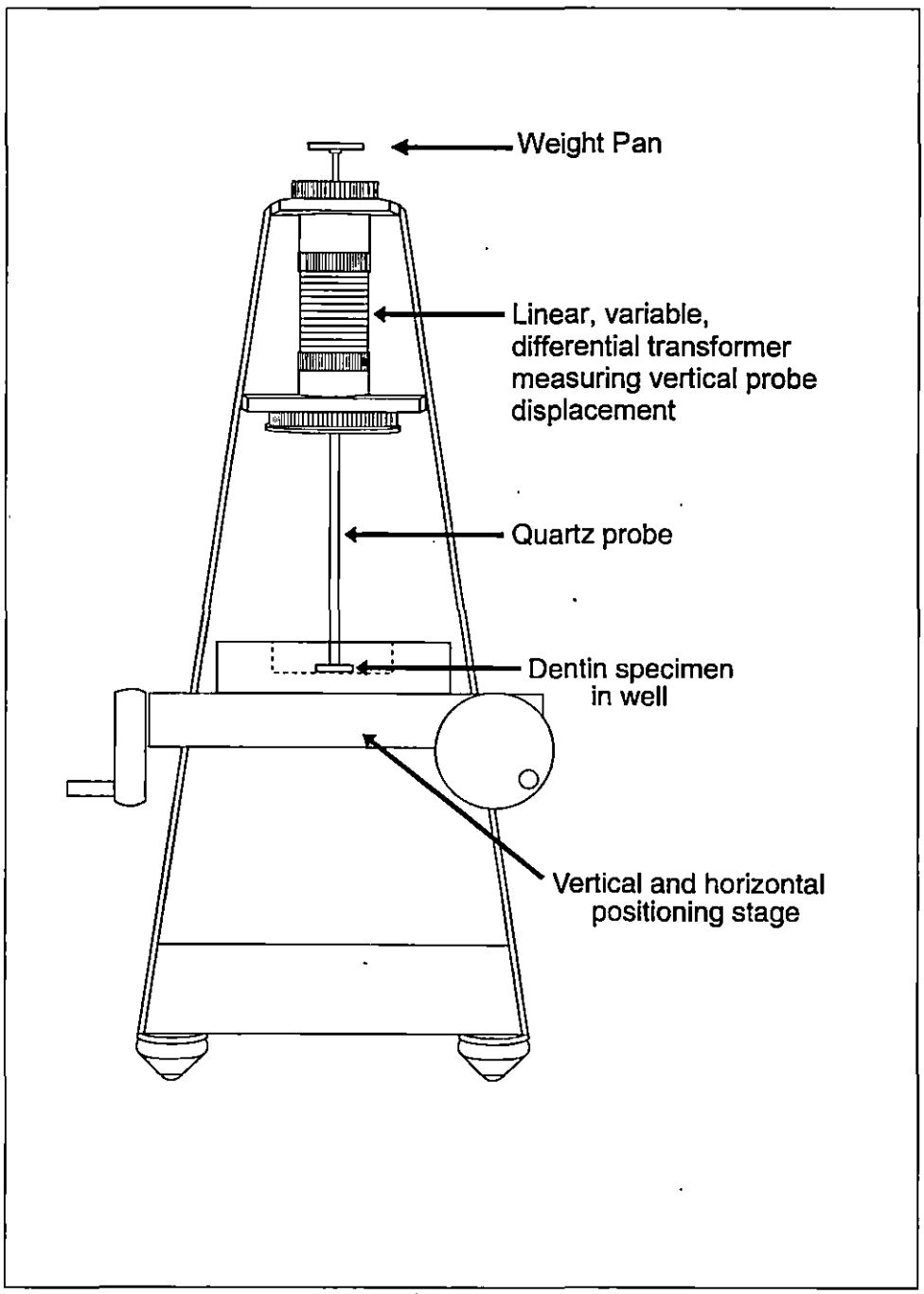

Weight Pan
Linear, variable, differential transformer measuring vertical probe displacement
Quartz probe
Dentin specimen in well
Vertical and horizontal positioning stage

Figure 2. *LVDT calibration correlation of measured mechanical probe displacement with calculated LVDT probe displacement.*

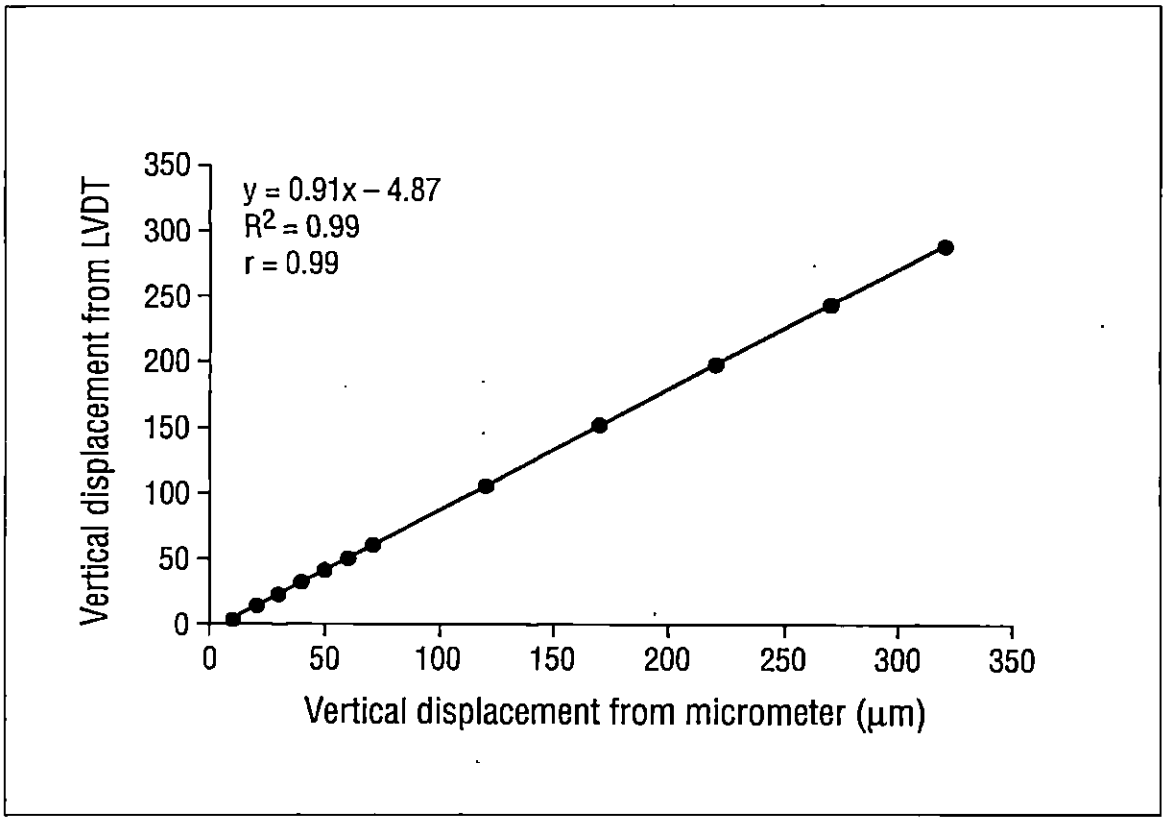

y = 0.91x – 4.87
R2 = 0.99
r = 0.99
Vertical displacement from LVDT
Vertical displacement from micrometer (μm)
0
50
100
150
200
250
300
350

Table II shows water-HEMA concentrations used in the study, expressed both in weight percent water and moles per liter.

Table II

Composition of Water-HEMA Solutions

Solution	Water %	Moles/L	HEMA %	Moles/L	Molar Ratio HEMA/H_2O
A	100	55.56	0	0	0.00
B	10	5.56	90	6.92	1.24
C	25	13.89	75	5.76	0.41
D	50	27.78	50	3.85	0.14

Percentages are weight/weight. Molecular weight of HEMA = 130.1 g/mole.
HEMA/H_2O = molar ratio.

Each water-HEMA solution was applied with a glass pipette, using 3 to 4 drops (150-200 μL) to completely cover the demineralized dentin disk. The test solution was applied carefully to avoid touching the contact probe resting on the dentin surface.

After adding the test solution, dentin collagen expansion was followed for 20 minutes, or until a steady-state plateau was reached. The specimen was then thoroughly rinsed with three, 4.7 mL washes of distilled water. Water not only extracted any residual HEMA, but also re-expanded the matrix. During the washing process, the specimen was left in the chamber in its original position. This method ensured that the same specimen area was tested each time, and that the degree of re-expansion was followed to ensure that the specimen returned to its original, expanded height. This process verified that the previous treatment had not altered the expansion ability of the matrix. Suction (Rico Suction, Burlington, NC) was used to remove the water after each rinse cycle. After rinsing, the specimen was prepared for another test cycle. This procedure was repeated until all the test solutions were applied. Each specimen required approximately 16 hours

of data collection in order to test all of the groups shown in Table I. Controls consisted of 100% water (Solution A) to re-expand the air-dried dentin.

Types of Data Generated. The data generated were a continuous measurement of contact probe vertical displacement with respect to time. A total of 323 tests were performed on eighteen dentin specimens (323 tests for solution A, 107 tests for solution B, 108 tests for solution C, and 108 tests for solution D).

Calculation of expansion rate for each test was based on steady-state plateau values. The expansion rate was approximated by finding the time in minutes required to reach 50% of maximal expansion, divided into the height, in microns, of the 50% expansion value. This rate was also expressed as a percentage of the expansion rate produced by 100% water.

The total re-expansion height produced by each primer solution was expressed in both absolute (microns) and in relative (%) terms. Relative expansion was calculated as the height produced by a primer solution, relative to the height produced by 100% water in that specimen. Expressing the data in this manner decreased the variability among specimens as determined by the coefficient of variation (i.e. the standard deviation divided by the mean).

Method of Data Analysis. A repeated measures analysis of variance was used to evaluate differences in the rate and extent of expansion as a function of water concentration (moles/L). A least squares means comparison test was then used to compare the data after completing the ANOVA using statistical software (Statistical Analysis Software, Version 6.12, SAS Institute, Cary, NC).

Regression analysis was used to examine the linear relationship between the rate and height of expansion of dried, demineralized dentin matrix and water concentration (moles/L) of the primer solutions. Similar regression analysis was performed to describe the relationship between the final specimen expansion and the solubility parameter for hydrogen bonding forces (Miller *et al.*, 1998). Least square means, correlation coefficients, and R^2 values were also calculated.

Comparison of primer hydrogen bonding capacity. We hypothesized that, as interfibrillar space decreased and collagen fibrils touched, associations were developed via weak forces (hydrogen bonding, Van der Waals forces, etc.) that tended to stiffen the collapsed matrix and prevented expansion. Primers, especially those containing polar molecules, that can break interfibrillar H-bonds may expand the matrix and create interfibrillar spaces for resin infiltration (Nakabayashi and Pashley, 1998). In the current study, HEMA, a relatively poor H-bonding substance, was used to adjust water concentration (a very strong H-bonding substance). Because we mixed two solvents of widely differing hydrogen bonding tendencies (water and HEMA), we chose the solubility parameter model to express their relative H-bonding capacities.

Solubility parameters. The solubility parameter is a quantity characterizing the intermolecular forces in a liquid, a solid, or between a liquid and a solid. The square of the solubility parameter is the cohesive energy density of a material. If two solutions have similar chemical structures, their cohesive energy densities will be approximately equal, and they should mix or be "soluble" in each other. Thus, miscibility can be predicted if cohesive energies or solubility parameters of the liquids are known. Similarly, the ability of a solvent to soften or partially solubilize the surface of a solid polymer can be

predicted from their relative cohesive energy densities. For a solvent to soften a solid surface, the solvent must first wet and spread on the surface. Thus, solubility parameters can also predict the ability of a solvent to wet a surface (Gardon, 1963).

Solubility parameters for many substances can be calculated by summing the contributions due to each functional group in the molecule (van Krevelen, 1990). This process has been done for various water-HEMA mixtures and for collagen at various stages of hydration (Miller, personal communication).

III. RESULTS

Figure 3 shows a graph of height change of a demineralized disk during dehydration with compressed air, its re-expansion with 100% water (Solution A), followed by a second cycle of air-dehydration, and then re-expansion by solutions B, C and D (10, 25 and 50 weight percent H_2O, respectively). The first dehydration cycle provided information about the degree to which that particular specimen could shrink when dehydrated (note that it reached a "shrinkage plateau" at - 100 μm).

The original height and the dehydrated height established the dimensional boundaries for that particular specimen. The first shrinkage produced by air-drying gave a negative slope of -54.5 ± 17.1 μm/minute (mean ± SD, N = 270), and a change in height of -103.4 ± 33.5 μm (Table III). When water was added to re-expand the shrunken matrix, the matrix expanded at a much faster rate (+203.1 ± 91.0 μm/minute) than the original rate of shrinkage ($p < 0.001$), and reached a height that was slightly, but significantly ($p = 0.018$), lower (+94.2 μm vs. -103.4 μm), than the original height.

When the second shrinkage was induced by air-drying in preparation for the use of the experimental primers, the rate of shrinkage was -49.5 ± 17.4 μm/minute, a value that was slightly, but significantly ($p = 0.012$), lower than the original shrinkage rate. The change in matrix height (-97.3 ± 23.6 μm, Table III) was not different from the first shrinkage ($p = 0.759$), however.

Figure 3. *Expansion of dried, demineralized dentin by water (A) and by model primers B, C and D.*

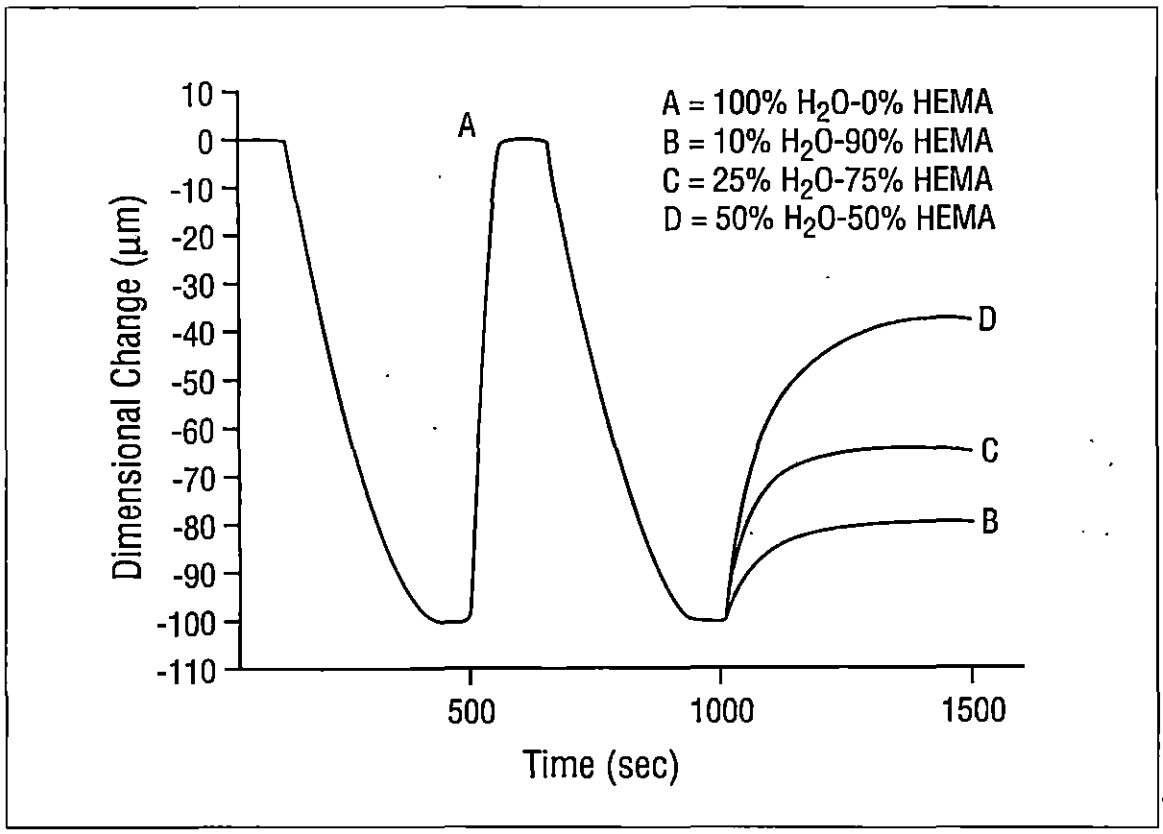

A = 100% H_2O-0% HEMA
B = 10% H_2O-90% HEMA
C = 25% H_2O-75% HEMA
D = 50% H_2O-50% HEMA
Dimensional Change (µm)
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
A
D
C
B
500
1000
1500
Time (sec)

Table III

Shrinkage vs. Re-expansion Produced Only by Air-drying and Water Application

Procedure	Change in Height (μm)	Slope (μm/min)
First Shrinkage	-103.4 ± 33.5 (270)[a]	-54.5 ± 17.1 (270)[b]
First Re-expansion	+94.2 ± 22.2 (270)[b]	+203.1 ± 91.0 (270)[c]
Second Shrinkage	-97.3 ± 23.6 (269)[a]	-49.5 ± 17.4 (269)[d]

Similar superscript letters identify equivalent groups ($p > 0.05$). Numbers in parentheses represent total observations made on 18 specimens.

When primer B was added (10% water-90% HEMA), the dry, demineralized, dentin expanded slowly, relative to the rate using 100% water (Solution A, Positive Control), and to a lesser degree (approximately 20% of control height). The expansion reached a plateau after approximately 20 minutes (Fig. 3). After washing the specimen and chamber with three, 4.7 mL rinses, the test cycle was repeated with one of the other primers chosen at random. Figure 3 indicates that the model primer containing 25% water-75% HEMA (line C) expanded faster than the primer containing 10% water-90% HEMA (line B), but only reached approximately 35% of the fully expanded control height (Solution A). After rinsing with three water rinses, the third model primer (50% water-50% HEMA; line D) produced an even faster expansion rate with the height reaching 62% of control.

A summary of the test results of all eighteen specimens is shown in Table IV. Shown are the specimen dimensions and the maximum shrinkage measured for each specimen during air-drying phase of the experiment. Each dentin specimen is unique, and therefore, individual variation was expected among specimens.

Table IV

Specimen Dimensions and Total Shrinkage

Specimen Number	Size (mm)	Original Thickness (μm)	Absolute Shrinkage (μm)	Percent Shrinkage
1	10 x 5	160	54	34
2	13 x 6	190	99	52
3	10 x 5	200	76	38
4	11 x 5	200	96	48
5	12 x 5	200	77	39
6	13 x 4	200	82	41
7	8 x 5	180	96	53
8	9 x 7	200	134	67
9	8 x 8	320	197	62
10	9 x 7	180	64	35
11	9 x 8	220	90	41
12	12 x 7	200	122	61
13	9 x 7	220	141	64
14	10 x 7	220	123	56
15	11 x 7	220	133	60
16	8 x 7	200	121	61
17	9 x 7	180	94	52
18	8 x 7	200	130	65
	Mean ± SD	205 ± 33	107 ± 34	52 ± 11

These completely demineralized specimens ranged in thickness from 180-320 μm (205 ± 33 μm, mean ± SD, N = 18). Thickness was measured with a digital micrometer (Model No. 331 711 30, Mitutoyo, Tokyo, Japan) before and after demineralization. Shrinkage varied from 34% to 67% (52 ± 11 μm, mean ± SD, N = 18).

The average rates and degrees of re-expansion of the eighteen specimens induced by the three model primers are shown in Table V. With each of the three solutions, complete, 100% re-expansion did not occur, no matter how long the solution was left in contact with the specimen. Solution B expanded the dried matrix 20 ± 5% of control, solution C produced 35 ± 4% expansion, and solution D produced 62 ± 6% expansion

relative to control. The results are listed as the rate and height of expansion for the different water concentrations (moles/L) in both absolute and percent values (Table V).

Table V

Water Molar Concentration, and Rate and Height of Expansion

Weight Percent H_2O	Water Concentration (moles/L)	Collagen Expansion Rate (μm/min)* Absolute	Percent†	Collagen Expansion Height (μm) Absolute	Percent†
B (10)	5.6	6.94 ± 5.46^a	4 ± 4^a (107)‡	18.71 ± 5.98^a	20 ± 5^a (107)‡
C (25)	13.9	14.22 ± 4.65^b	9 ± 5^b (109)‡	32.49 ± 7.75^b	35 ± 4^b (109)‡
D (50)	27.8	18.74 ± 6.22^c	12 ± 7^c (107)‡	58.94 ± 13.38^c	62 ± 6^c (107)‡
A (100‡)	55.6	203.14 ± 6.22^d	100 (270)‡	94.18 ± 22.24^d	100 (270)‡

*Values are mean ± SD, N = 18. †Percent of water-treated controls. ‡Values in parentheses are the number of measurements made with each solution. Groups identified by similar superscript letters are equivalent ($p > 0.05$).

Figure 4 is the regression analysis of expansion rate of dried, demineralized dentin matrix and the three model primers and control solution expressed in the molar water concentration. Although there was some dispersion of the data points, the correlation coefficient ($r = 0.95$) was significant ($p < 0.001$), with an R^2 value of = 0.90. A similar regression analysis was done on the final matrix expansion height versus the molar water concentration of the test solutions. In this case, the correlation coefficient increased to $r = 0.91$ and the R^2 value was 0.83 (Fig. 5).

Figure 4*. Molar water concentration of model primers vs.*

expansion rate of demineralized dentin matrix.

A, B, C and D refer to the primer designations listed in Table II.

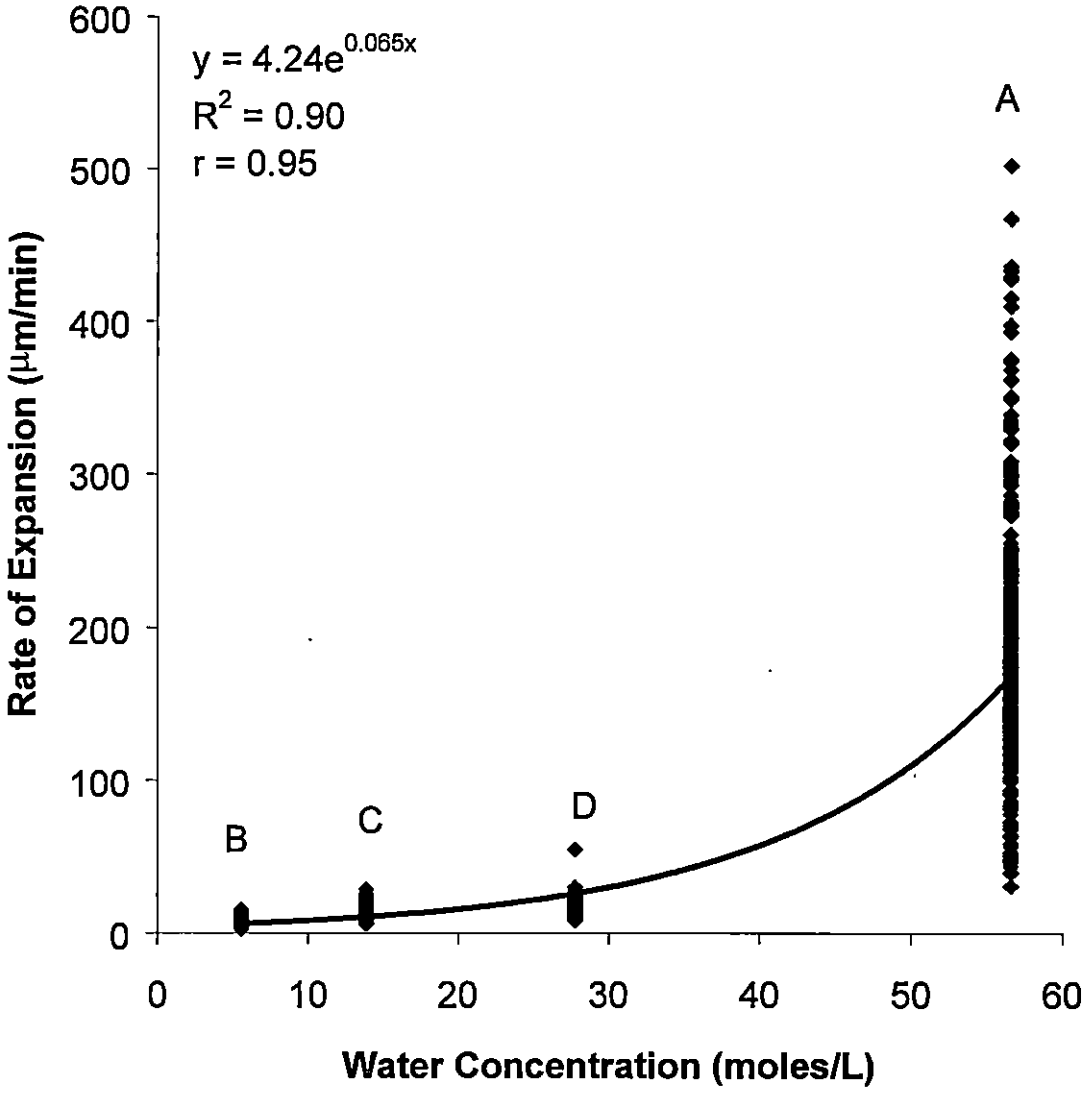
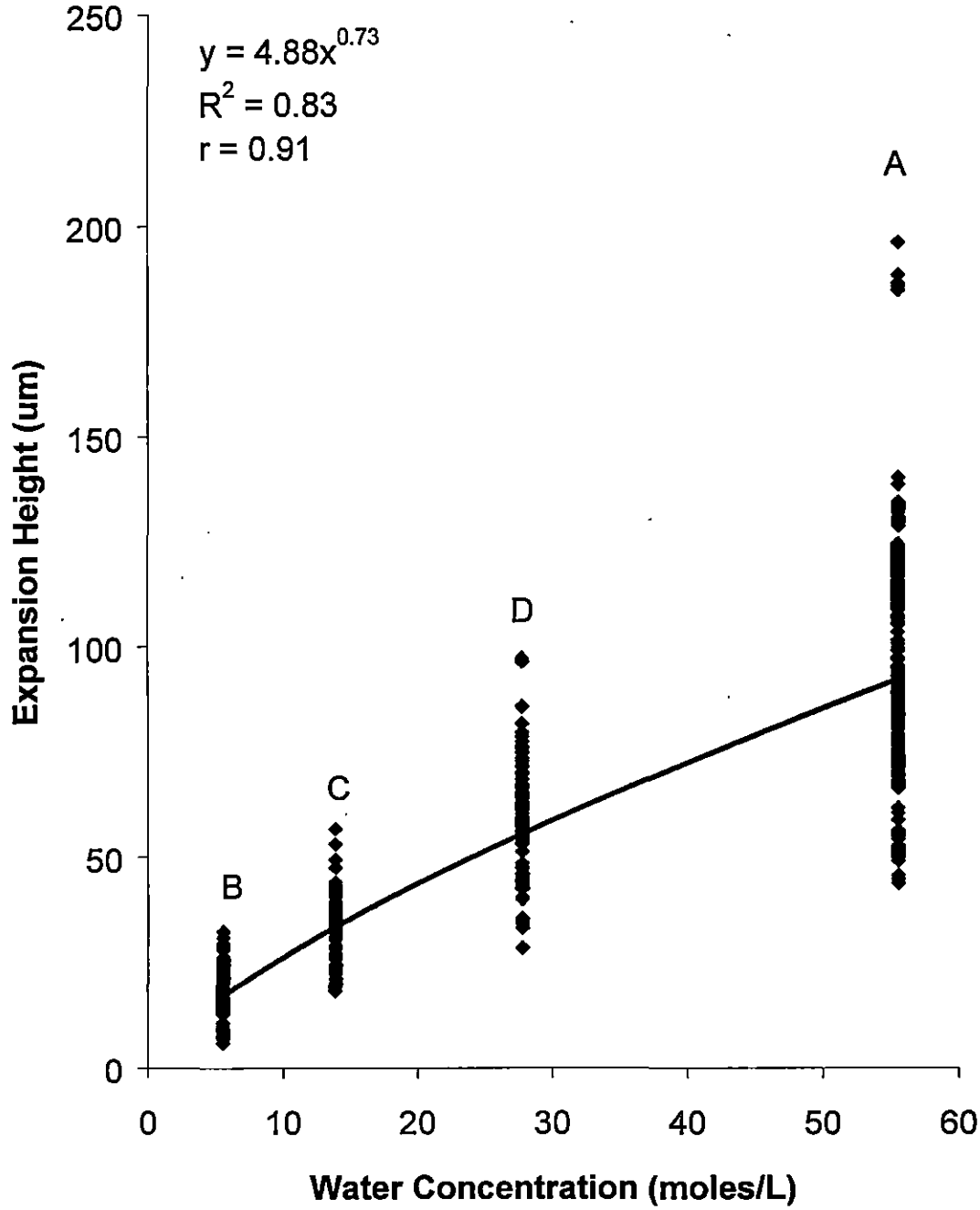

y = 4.24e^0.065x
R^2 = 0.90
r = 0.95
A
B
C
D
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Rate of Expansion (μm/min)
Water Concentration (moles/L)

***Figure 5**. Molar water concentration of model primers vs. expansion absolute height of dried, demineralized dentin matrix.*

A, B, C and D refer to the primer designations listed in Table II.


y = 4.88x^0.73
R^2 = 0.83
r = 0.91
Expansion Height (um)
Water Concentration (moles/L)
250
200
150
100
50
0
0
10
20
30
40
50
60
A
B
C
D

IV. DISCUSSION

Mature dentin is approximately 70 weight % inorganic material, 20 weight % organic material, and 10 weight % water (located primarily in the dentin tubules, but also adsorbed on the surface of the mineral or in interstices between crystals). By volume, dentin is 45% inorganic, 33% organic and 22% water (Ten Cate, 1998). The inorganic component consists mainly of hydroxyapatite, while the organic phase is about 90% type I collagen with fractional amounts of proteoglycans and phosphoproteins (Ten Cate, 1998). After demineralization, the dentin matrix is about 33% collagen and 67% water (Carvalho *et al.*, 1996b).

Type I collagen found in dentin is a triple helix configuration made up of three polypeptide subunits known as α-chains; each chain consists of 1000 amino acids, and is wound in a left-handed helix. The three α-chains are coiled around each other in a right-handed superhelix which is stabilized by inter-chain hydrogen bonds (extrahelical telopeptides). In order for the three α-chains to wind into a triple helix, they must have the smallest amino acid, glycine, at every third position along the chain. Each of the three chains, therefore, has the repeating structure Gly-Xaa-Yaa, where Xaa and Yaa may be any amino acid, but are frequently the imino acids: proline and hydroxyproline (Kadler *et al.*, 1996). Both proline and hydroxyproline are rigid, cyclic imino acids that limit the rotation of the polypeptide backbone and thus contribute to the stability of the triple helix. Imino acids do not have primary amino groups. Instead, they are secondary amines

that limit the rotation of the polypeptide backbone, and thus contribute to the stability of the triple helix. The telopeptide region of collagen, which does not have a repeating Gly-Xaa-Yaa structure and does not adopt a triple-helical conformation, accounts for 2% of the molecule and is critical for fibril formation. In this configuration, type I collagen is a rigid, fibrous biopolymer of identical or similar subunits.

Type I collagen molecules self-assemble into *D*-periodic cross-striated fibrils, where $D = 67$ nm, the characteristic axial periodicity of collagen (Kadler *et al.*, 1996). The fibril forming individual collagen molecules consist of an uninterrupted triple helix 300 nm (0.3 μm) in length by 1.5 nm in diameter. During fibril formation, the molecules self-aggregate (Nimni, 1991). After aggregation, the molecules begin slowly developing covalent cross-links, especially in mineralized tissue collagen matrices, making them much tougher and more able to resist acids than soft tissue collagens (Veis and Schlueter, 1964; Knot and Bailey, 1998).

Collagen fibrils range from 20 to 200 nm in diameter (averaging about 100 nm in dentin), with cross-striations every 67 nm. They are made of aggregations of collagen molecules that overlap with each other by approximately 25% of their length. Collagen fibrils can grow by association into larger collagen fibers. Individual collagen fibers vary in diameter from less than 1 μm to about 12 μm, and are composed of collagen fibrils. The fibers, which are usually arranged in bundles, undergo some branching and are of indefinite length (Kastelic *et al.*, 1978).

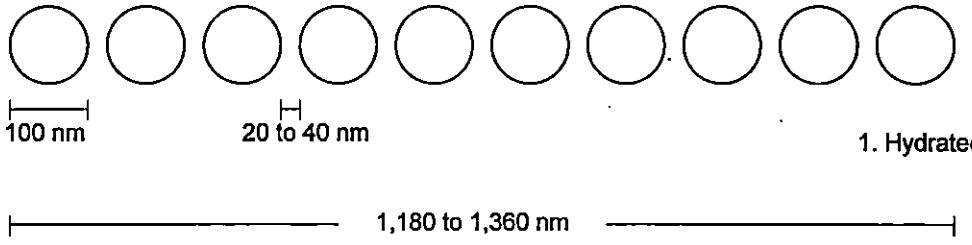
Collagen Fibril Shrinkage. Wet, demineralized dentin matrix consists of a network of collagen fibrils about 100 nm in diameter, that are separated by 20-40 nm interfibrillar spaces (Fig. 6). During drying, collagen fibrils pass through two stages; first

there is interfibrillar water loss, followed by intrafibrillar water loss (Frantzl and Daxer, 1993).

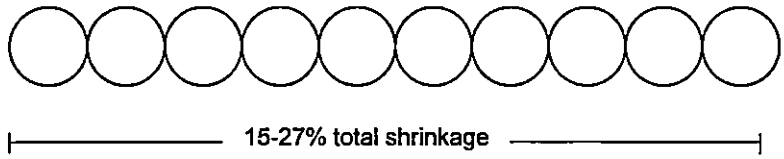
Shown in Fig. 6-2 are ten fibrils, each 100 nm in diameter for a total bundle width of 1000 nm (1 μm). If the distance between each fibril is 20 nm, then the total interfibrillar space is 180 nm (9 x 20 nm). If this value is added to the 1000 nm total bundle fibril width, the total distance is 1,180 nm (Fig. 6-1). If the interfibrillar spaces were 40 nm wide, then the total linear interfibrillar contribution would be 360 nm (9 x 40 nm). When added to the total fibril width of 1,000 nm, a total width of 1,360 nm is derived (Pashley, unpublished observations).

If drying decreases interfibrillar distance in demineralized dentin, then the collagen mesh would shrink 15% if the spaces are 20 nm wide ([180 ÷ 1,180] x 100 = 15%). If the spaces were 40 nm wide, the matrix would shrink 26.5% ([360 ÷ 1,360] x 100 = 26.5%) as shown in Fig. 6-2. But wet, demineralized dentin collagen shrinks approximately 50% (Table IV). Therefore, either the interfibrillar spaces are larger than 40 nm, the collagen fibrils themselves also shrink (Fig. 6-3.), or both phenomena occur.

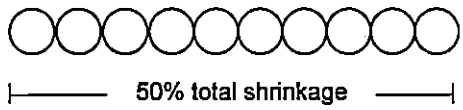
***Figure 6**. Inter- and intrafibrillar collagen fibril shrinkage.*



1. Hydrated collagen fibrils



2. Interfibrillar Shrinkage

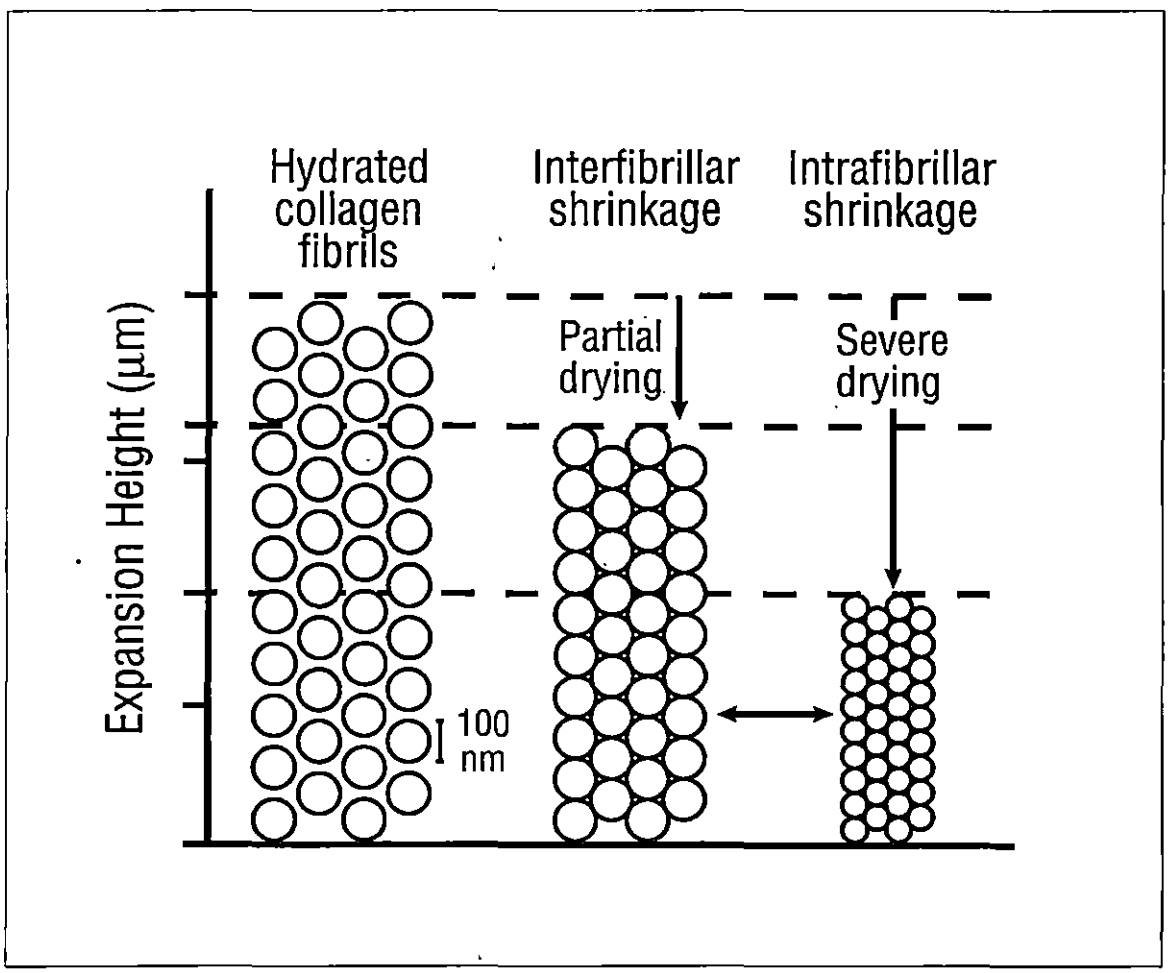


3. Intrafibrillar Shrinkage

There are at least two very different possible explanations for the varying degrees of expansion caused by the three model primers in this experiment. The first is a physical explanation based on changes in the modulus of elasticity of collagen. The modulus of elasticity of wet dentin matrix is about 7-8 MPa, whereas air-dried collagen has a modulus of almost 300 MPa (Maciel *et al.*, 1996). During air-drying, water evaporates from interfibrillar spaces first, and then from within the collagen fibrils themselves (intrafibrillar spaces). As interfibrillar space decreases, collagen fibrils approximate (Fig. 7) sufficiently to form interfibrillar hydrogen bonds. It is hydrogen bond formation that is thought to collapse the collagen matrix causing shrinkage and making the fibrils stiff after air-drying. When 100% water is added, these molecules rapidly diffuse into the collagen fibrils, where they compete with interfibrillar, interpeptide hydrogen bonds (H-bonds). That is, water molecules want to form clusters of water molecules around the very chemical groups in collagen that form interpeptide hydrogen bonds. Since there is much more water than collagen (on a molar basis), the interfibrillar hydrogen bonds break as water molecules form H-bond clusters around the carboxyl and imino groups within collagen. As water molecules cluster around these groups, the collagen stiffness falls from 300 to 8 MPa (Maciel *et al.*, 1996), and the collagen fibrils begin to separate again. Interfibrillar spaces open and fill with water. This ability of water to break hydrogen bonds is responsible for the large differences in stiffness between wet versus dry hair, wool, cotton and paper, and collagen (Barton, 1991).

The partial or incomplete expansion produced by the 10% water-90% HEMA solution may be due to having too little water available to successfully break all of the intrinsic hydrogen bonds within the dried collagen. Thus, the stiffness of collagen

Figure 7. *Schematic of inter- and intrafibrillar collagen fibril shrinkage.*


Hydrated collagen fibrils
Interfibrillar shrinkage
Intrafibrillar shrinkage
Partial drying
Severe drying
Expansion Height (µm)
100 nm

remains high enough to prevent it from re-expanding. The presence of HEMA in this model serves only to change the water concentration; it has no effect on the collagen or water.

The model primer containing 25% water-75% HEMA re-expands more because its higher water concentration breaks more hydrogen bonds between adjacent collagen fibrils, thereby lowering its elastic modulus and allowing more expansion of the collapsed fibril matrix. Similarly, the primer containing 50% water-50% HEMA expands the fibril matrix even more, but not as much as 100% water.

An alternative explanation for the partial expansion of dried demineralized dentin by the model primers is based on the view that the collagen biopolymer is similar to a synthetic solid polymer (Asmussen *et al.*, 1991). The collagen surface can be softened by certain solvents having the same fractional polarity (defined as the fraction of the total intermolecular interactions that are due to dipole-dipole attractions) and solubility parameter (δ_s defined as the square root of the cohesive energy density) as the polymer itself (Asmussen and Uno, 1993). Indeed, these authors presented three-dimensional graphs of bond strength, solubility parameter, and fractional polarity showing the highest bond strengths of a wide variety of resin formulations occurred at unique combinations of solubility parameters and fractional polarities.

However, Assmusen and Uno's (1993) use of an average solubility parameter, while a useful first approximation, failed to consider specific hydrogen bonding forces. That is, there are combinations of polymer (p) and solvents (s) for which $\delta_p = \delta_s$, but yet the polymer and solvent do not show natural solubility. Mutual solubility only occurs if the degree of hydrogen bonding in the polymer and the solvents are equal. This problem

led Hansen (1967, 1969) to modify the solubility parameter concept to include the constituent forces d, p and h for the contribution to δ due to dispersion forces (δ_d), polar forces (δ_p), and hydrogen bonding (δ_h), respectively.

Any solvent miscible with water (e.g. methanol, ethanol, acetone, HEMA) mixes because of mutual hydrogen bonding. Methyl methacrylate has no terminal hydroxyl group so it can not mix (*i.e.* hydrogen bond) with water and is considered immiscible. Hydroxyethyl methacrylate, by having a primary hydroxyl group, is very water miscible because it easily hydrogen bonds to water. Water-HEMA mixtures, when viewed from a solubility parameter perspective, hydrogen bond with each other. For instance, in a 10% water-90% HEMA mixture, the molar concentrations of the two substances are almost equal (Table II). Thus one mole of water can H-bond with one mole of HEMA, and there is little water available to interact with collagen molecules that H-bonded with each other when they became closely approximated by air-drying. The next highest water concentration (25% water-75% HEMA), would have more moles of water than of HEMA (Table II), and hence would have more water available to compete with interfibrillar H-bonds between collagen molecules. This increased water content would permit breakage of some H-bonds between collagen fibrils, but not enough to obtain a soft, compliant, fully-expanded collagen network. Fifty percent water-50% HEMA (weight percent) provides more water for H-bond interaction, resulting in more expansion. Each of these solutions has different solubility parameters (Table VI). Table VI lists the three Hansen solubility parameters of water-HEMA mixtures ranging from 100% water-0% HEMA, to 0% water-100% HEMA. Also listed are the solubility parameters of the three model primers that were used in this study (B, C and D). Note that the solubility parameter for

hydrogen bonding forces (δ_h) varies from a low of 16.1 in 100% HEMA to 37.3 $(J/cm^3)^{½}$ in 100% water.

Table VI

Solubility Parameters of Water-HEMA Mixtures

Test Solution	Water (wt%)	HEMA (wt%)	Molar Ratio HEMA/H_2O	Solubility Parameters* $(J/cm^3)^{½}$ δ_p	δ_h	δ_d
A	100	0	0.00	27.3	37.3	12.2
	90	10	0.02	25.6	35.3	12.5
	80	20	0.03	23.9	33.2	12.9
	70	30	0.06	22.2	31.1	13.2
	60	40	0.09	20.5	29.0	13.6
D	50	50	0.14	18.7	26.9	13.9
	40	60	0.21	17.0	24.8	14.3
	30	70	0.32	15.2	22.6	14.6
C	25	75	0.41	14.3	21.6	14.8
	20	80	0.55	13.4	20.5	15.0
B	10	90	1.24	11.6	18.3	15.3
	0	100	0.00	9.8	16.1	16.7

*Solubility parameters for polar forces (δ_p), hydrogen bonding (δ_h), and dispersion forces (δ_d), from Miller (personal communication).

Miller *et al.* 1998, calculated these same three solubility parameters for collagen (Table VII). Note that the solubility parameters for collagen are highly dependent on the state of hydration. Since the solubility parameters of a solution are related to a number of important physical properties, such as the ability of these solutions to wet a substrate, it becomes valuable to estimate the solubility parameters of demineralized dentin in wet versus dry conditions. Miller (1995) has done this in his thesis. His table of solubility parameters for collagen are shown in Table VII. Note that the δ_h values vary from a low of 15.2 in dry collagen to a high of 32-34 $(J/cm^3)^{½}$ in 20-30% collagen hydrated with 80-70% water, respectively.

Table VII

Solubility Parameters for Wet and Dry Collagen

Collagen State	Water (wt%)	Polypeptide (wt%)	Solubility Parameters* $(J/cm^3)^{1/2}$ δ_p	δ_h	δ_d
	100	0	27.3	37.3	12.2
	90	10	26.5	35.7	12.6
	80	20	25.6	34.0	13.1
Wet	70	30	24.7	32.2	13.6
	60	40	23.7	30.2	14.2
	50	50	22.7	28.2	14.7
	40	60	21.6	26.0	15.3
	30	70	20.3	23.6	16.0
	20	80	19.0	21.0	16.7
	10	90	17.6	18.2	17.5
Dry	0	100	16.1	15.2	18.3

*Solubility parameters for polar forces (δ_p), hydrogen bonding (δ_h), and dispersion forces (δ_d), from Miller (unpublished observations).

In dentistry, unlike medicine, the state of hydration of collagen can be altered physically by air-drying, or chemically by the use of water miscible, but water-free, solvents (Maciel *et al.*, 1996). That is, when bonding systems dissolved in water-free but water-miscible solvents are applied to demineralized dentin, it is very likely that the collagen water content rapidly approaches that of the solvent.

According to Asmussen and Uno (1993), when solvents of differing composition are applied to fully hydrated dentin, they will "dissolve" in the collagen when the differences between their solubility parameters and that of collagen are smallest. If the collagen is dried first, its solubility parameters change (Table VII), and so would the ideal primer for causing its "solution/swelling." Theoretically, the opposite should also be true. If we begin with fully hydrated dentin and suspend it in a solvent system (*e.g.* water-HEMA mixture) that has a different set of solubility parameters, the collagen should "shrink" as water that was H-bonding with collagen begins to H-bond with HEMA. This

action would permit peptide-peptide H-bonding in collagen to occur, causing further shrinkage.

Although the true water content of air-dried, demineralized dentin is not known, it is likely that, regardless of its original concentration, the collagen would rapidly equilibrate with the water concentration of whatever primer is applied on it. This equilibration would change the collagen solubility parameters and hence, how it would interact with primers.

Asmussen and Uno (1993) reported that there was a positive relationship between resin-dentin bond strength and the solubility parameter and fractional polarity of adhesive resins. They concluded that the optimum values for these variables were important for wetting and penetrating the surface, and perhaps for collagen "softening," which in turn, leads to high bond strength. In the present thesis, bond strength was not measured. Rather, the degree of expansion of dried, demineralized dentin model primers was measured. The relationship between the degree of expansion or collapse of demineralized dentin and resin-dentin bond strength is not clear. Gwinnett (1994) showed that moist dentin (i.e. expanded) produced much higher bond strengths than dry dentin (i.e. collapsed), but the bond strengths between these two extremes is unknown. Most HEMA-containing bonding systems use 35-45% HEMA, 55-65% water (wt%), although the relationship between bond strength and HEMA concentration gives a rather flat bell-shaped curve (Munksgaard and Asmussen, 1984). Our results indicate that any solution with a water concentration less than 100% will not fully expand demineralized dentin. It is clear that none of the water-HEMA mixtures used in this study permitted full

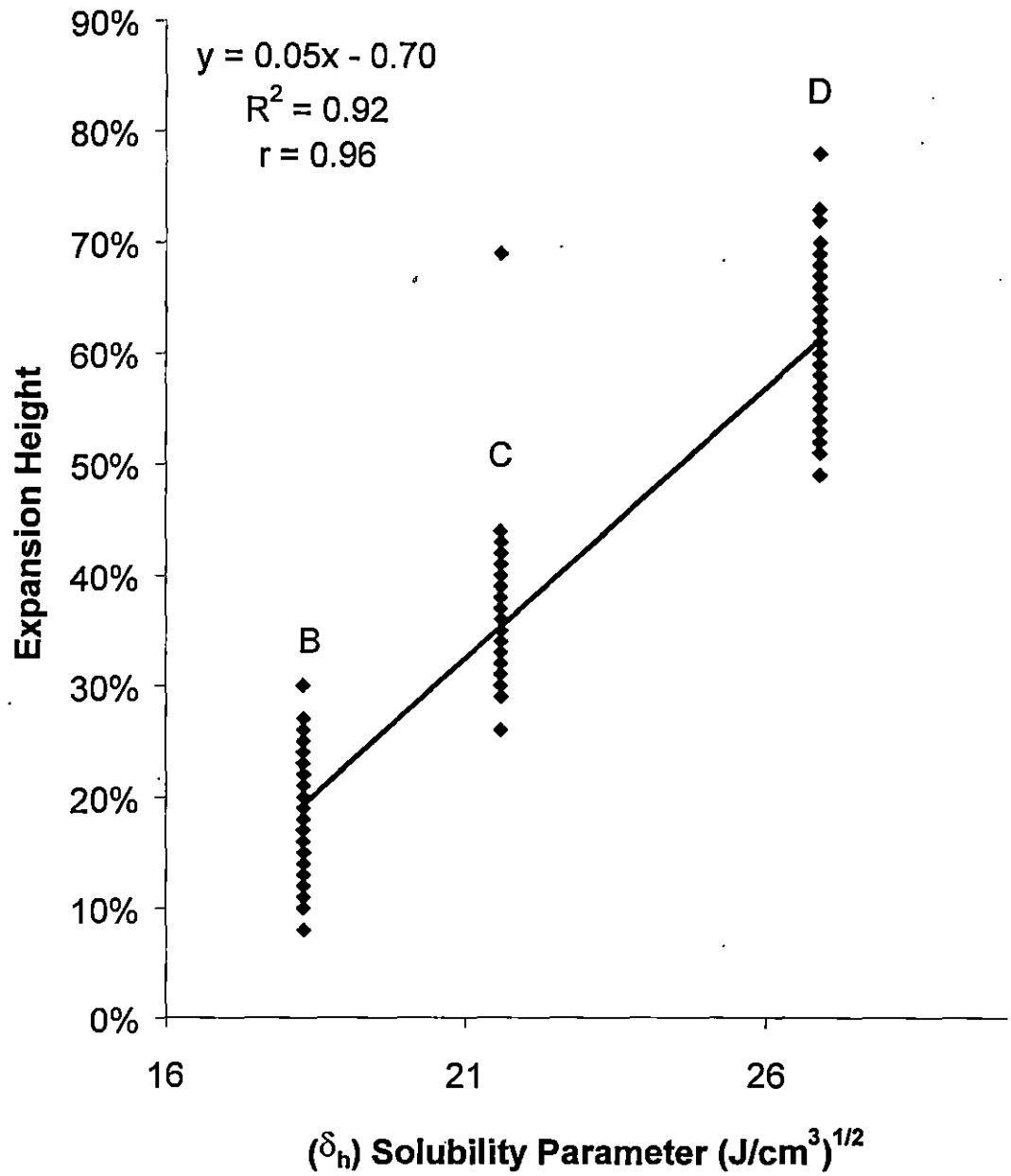
expansion of the demineralized matrix. Further research is necessary to determine if the 60% expansion does, indeed, produce optimal bond strengths.

The linear relationship between the rate and extent of expansion of dried demineralized dentin as a function of the water concentration of the model primers (Figs. 4 and 5) is probably due to the very strong influence of water on the solubility parameter for hydrogen bonding (see Table VI). Substitution of the δ_h values for the water concentration of the model primers gives a significant linear relationship of percent expansion height of the matrix vs. δ_h (Fig. 8). The correlation ($r = 0.96$) was highly significant ($p < 0.001$) as was the R^2 value (0.92).

Although a similar relationship exists between expansion height and δ_p, we believe that it is fortuitous due to the fact that both δ_h and δ_p increase in water-HEMA primers (Table VI), as water concentration increases. Studies of the swelling pressure of dried, demineralized dentin matrix in solvents of widely different δ_h and δ_p (Pashley, unpublished observations), have shown the swelling pressure correlates better with δ_h ($R^2 = 0.75$) than with δ_p ($R^2 = 0.27$). Thus, it is likely that δ_h is the critical variable among the Hansen triple component solubility parameters. This hypothesis will be tested in the future by comparing the extent of expansion produced by a series of model primers with increasing δ_h vs. constant δ_h and increasing δ_p.

The use of an LVDT contact probe on demineralized dentin disks provides very useful quantitative information that will provide new insight into how adhesive monomers interact with the dentin matrix. This methodology should lead to improvements in resin-dentin bonding.

***Figure 8**. Linear relationship between percent expansion height of dried, demineralized dentin and the H-bonding solubility parameter (δ_h) of the three model primers.*


y = 0.05x - 0.70
R2 = 0.92
r = 0.96
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Expansion Height
B
C
D
16
21
26
(δh) Solubility Parameter (J/cm3)1/2

V. SUMMARY

The purpose of this study was to test the rate and expansion of air-dried, collapsed demineralized dentin matrix using three different water-HEMA solutions. Solutions of 10% water-90% HEMA, 25% water-75% HEMA and 50% water-50% HEMA (by weight), and 100% water were evaluated. Eighteen dentin specimens (0.2 mm thick) were demineralized in 17% EDTA for 5 days, yielding type I collagen disks approximately 200 μm thick.

An LVDT from a modified TMA (with the oven removed) was used to measure vertical dimensional changes of the dentin specimens. Specimens were air-dried with compressed air, then one of three model primers was applied and the rate and amount of expansion was measured over time compared to 100% water.

Three hundred twenty-three experiments were conducted and the results were analyzed using a repeated measures ANOVA, followed by a least squares means comparison test. Analysis revealed a significant ($p < 0.0001$) relationship between water concentration and both the rate and amount of re-expansion of dried, demineralized dentin matrix. Dentin matrix expansion with the 10% water-90% HEMA solution was 20 ± 5% of the re-hydrated, water control value; with 25% water-75% HEMA it was 35 ± 4%; and with 50% water-50% HEMA it was 62 ± 6% (Table VIII).

A similar significant relationship was found between the extent of expansion of demineralized dentin and Hansen's solubility parameter for hydrogen bonding forces

(δ_h). The results are consistent with the ability of water to break interpeptide hydrogen bonds by preferentially producing H-bonds with water. The extent to which this interaction can occur is proportional to the molar water concentration of the primer.

Table VIII

Expansion Rate and Height of Solutions B, C, and D.

Weight Percent H_2O	Water Concentration (moles/L)	Collagen Expansion Rate (μm/min)* Absolute	Collagen Expansion Rate (μm/min)* Percent†	Collagen Expansion Height (μm) Absolute	Collagen Expansion Height (μm) Percent†
B (10)	5.6	6.94 ± 5.46^a	4 ± 4^a (107)‡	18.71 ± 5.98^a	20 ± 5^a (107)‡
C (25)	13.9	14.22 ± 4.65^b	9 ± 5^b (109)‡	32.49 ± 7.75^b	35 ± 4^b (109)‡
D (50)	27.8	18.74 ± 6.22^c	12 ± 7^c (107)‡	58.94 ± 13.38^c	62 ± 6^c (107)‡

*Values are mean ± SD, N = 18. †Percent of water-treated controls. ‡Values in parentheses are the number of measurements made with each solution. Groups identified by similar superscript letters are equivalent ($p > 0.05$).

VI. REFERENCES

Asmussen E, Hansen EK, Peutzfeldt A (1991). Influence of the solubility parameter of intermediary resin on the effectiveness of the Gluma bonding system. J Dent Res 70:1290-1293.

Asmussen E, Hansen EK (1993). Dentine Bonding Systems In: Direct Posterior Filling Materials and Dentine Bonding. Edited by G. Vanherle, M. Degrange, G. Willems, Van der Poorten n.v. Printers, pp. 33-47.

Asmussen E, Uno S (1993). Solubility Parameters, Fractional Polarities, and Bond Strengths of Some Intermediary Resins Used in Dentin Bonding. J Dent Res 72:558-565.

Barton AFM. Handbook of Solubility Parmaeters and other Cohesion Parameters. CRC Press, Inc., Boca Raton, 1991, pg. 249.

Carvalho RM, Yoshiyama M, Brewer PD and Pashley DH (1996a). Dimensional changes of demineralized human dentin during preparation for scanning electron microscopy. Arch Oral Biol 41:379-386.

Carvalho RM, Yoshiyama M, Pashley EL, Pashley DH (1996b). *In vitro* study on the dimensional changes of human dentine after demineralization. Arch Oral Biol 41:369-377.

Frantzl P, Daxer A (1993). Structural transformation of collagen fibrils in corneal stroma during drying. An X-ray scattering study. Biophys J 64:1210-1214.

Gardon JL (1963). Relationship between cohesive energy densities of polymers and Zisman's critical surface tensions. J Phys Chem 67:1935-1936.

Gwinnett AJ (1992). Moist versus dry dentin: Its effect on shear bond strength. Am J Dent 5:127-129.

Gwinnett AJ (1994). Dentin bond strength after air drying and re-wetting. Am J Dent 7:144-148.

Hansen CM (1967). Three Dimensional Solubility Parameter and Solvent Diffusion Coefficient. Importance in Surface Coating Formulation. Doctoral Dissertation, Danish Technical Press, Copenhagen.

Hansen CM (1969). The universality of the solubility parameter. Ind. Eng Chem Prod Res Dev 8:2-11.

Inokoshi S, Harada N, Nakaoki Y, Urabe I, Sano H, Yamada T and Tagami J (1997). Quality of bonding agents: Adhesion mechanism, In: Factors Influencing the Quality of Composite Restorations, Theory and Practice, edited by Dondi dall'Orologio, G and Prati C, Ariesdue S, Publishers, Carimate (Cano) It, pp. 17-34.

Kastelic J, Galeski A, Baer E (1978). The multi-composite structure of tendon. Connect Tiss Res 6:11-23.

Kadler KE, Holmes DF, Trotter JA, Chapman JA (1996). Collagen fibril formation. Biochem J 316:1-11.

Kanca J (1992a). Effect of resin primer solvents and surface wetness on resin composite bond strength to dentin. Am J Dent 5:213-215.

Kanca J (1992b). Resin bonding to wet substrate. I. Bonding to dentin. Quint Int 23:39-41.

Kanca J (1996). Wet bonding: Effect of drying time and distance. Am J Dent 9:273-276.

Kinney JH, Balooch M, Marshall GW, Marshall SJ (1993). Atomic force microscope study of dimensional changes in dentine during drying. Arch Oral Biol 38:1003-1007.

Knott L, Bailey AJ (1998). Collagen cross-links in mineralized tissues: A review of their chemistry, function and clinical relevance. Bone 22:181-187.

Maciel K, Carvalho RM, Ringle RD, Preston CD, Russell CM, Pashley DH (1996). The effects of acetone ethanol, HEMA and air on the stiffness of human demineralized dentin. J Dent Res 75:1851-1858.

Marshall GW, Inai N, Wu-Magidi I-C, Balooch M, Kinney JH, Tagami J, Marshall SJ (1997). Dentin demineralization: Effects of dentin depth, pH and different acids. Dent Mater 13:338-343.

Miller RG (1995). Adhesive bonding to dentin with isocyanate copolymers. Ph.D dissertation, University of Missouri, Kansas City.

Miller RG, Bowles QC, Chappelow CC, Erick JD (1998). Application of solubility parameter theory to dentin-bonding systems and adhesive strength correlations. J Biomed Mater Res. 41: 237-243.

Munksgaard EC, Asmussen E (1984). Bond strength between dentin and restorative resins mediated by mixtures of HEMA and glutaraldehyde. J Dent Res 63:1087-1089.

Nimni ME (1991). Collagen Structure and Function. Encyclopedia of Human Biology, Volume 2, Academic Press, Inc. pp. 559-574.

Nakabayashi N, Pashley DH. Hybridization of Dental Hard Tissues, Quintessence Publishers, Tokyo, 1998, pg. 48.

Pashley DH, Ciucchi B, Sano H and Homer JA (1993). Permeability of dentin to adhesive resins. Quint Int 24:618-631.

Pashley DH, Ciucchi B, Sano H (1994). Dentin as a bonding substrate. Dtsch Zahn 49:760-763.

Sugizaki J (1991). The effect of the various primers on the dentin adhesion of resin composites. Jpn J Conserv Dent 34:220-265.

Ten Cate AR (1998). Oral Histology: Development, Structure, and Function. 5th Ed. St. Louis: Mosby-Year Book, 1998:150-196.

Uno S, Finger WJ (1996). Effects of acidic conditioners and dentin demineralization and dimensions of hybrid layers. J Dent 24:211-216.

Van der Graaf ER, Ten Bosch JJ (1993). Changes in dimensions and weight of human dentin after different drying procedures and during subsequent rehydration. Arch Oral Biol 38:97-99.

Van Meerbeek B, Dhem A, Goret-Nicaise M, Braem M, Lambrechts P, Vanherle G (1993). Comparative SEM and TEM examination of the ultrastructure of the resin-dentin interfusion zone. J Dent Res 72:495-501.

Van Krevelen DW (1990). Cohesive Properties and Solubility, Chapter 7, In: Properties of Polymers. Their correlation with chemical structure; their numerical estimation and prediction from additive group contributions, 3rd ed. Elsevier, Amsterdam, pp. 189-225.

Veis A, Schlueter RJ (1964). The macromolecular organization of dentin matrix collagen. I. Characterization of dentin collagen. Biochem 3(11):1650-1657.

APPENDIX

Shrinkage and expansion of demineralized dentin on a mineralized base. Eighteen trials were also performed on one specimen with a mineralized base (to more closely simulate conditions found in clinical practice), using three different test solutions of 5% water-95% HEMA, 10% water-90% HEMA and 25% water-75% HEMA. The specimen was etched for 15 seconds with aqueous 37% phosphoric acid, rinsed with water, and then luted into the Plexiglas well with sticky wax.

In a technique similar to that described previously, a baseline reading was established on the wet, mineralized dentin for two minutes. Compressed air was then blown on the surface at a 45° angle from a distance of 3 cm, and the rate of shrinkage recorded on the TMA computer. Once shrinkage was complete and a steady-state achieved, the mineralized disk was treated with one of three test solutions (5%, 10% and 25% H_2O) and the expansion was measured over time (results shown in Table IX).

Table IX

Rate and Height of Expansion of Collapsed Demineralized Dentin Matrix on a Mineralized Base

Solution (% H_2O)	Concentration (moles/L)	Expansion Rate (μm/min) Absolute	Expansion Height (μm) Absolute	Percent†
5	2.28	3.88 ± 0.40 (6)[a]	5.49 ± 0.81 (6)[a]	43.81%
10	5.56	8.19 ± 1.75 (6)[b]	8.43 ± 1.07 (6)[b]	64.50%
25	13.89	13.17 ± 2.01 (6)[c]	11.07 ± 0.83 (6)[c]	89.37%
100	55.56	-	12.32 ± 0.94 (6)[c]	100.0%

Numbers in parentheses are the number of observations made on a single specimen. Different superscript letters indicate significant differences ($p > 0.05$).
† Percent of water-treated controls.

In this experiment however, in addition to shrinkage and expansion of the collagen network, there was also warpage of the mineralized base (data not shown). Van der Graaf and Ten Bosch (1993) reported that dehydration of mineralized dentin produced 1.4-2.0% volumetric shrinkage. This is sufficient to warp or twist the specimen. This warpage can cause the contact probe to move up or down ± 5-10 μm depending on where the probe is with respect to the twisting of the specimen. This obviously introduces large errors in attempts to measure changes in the thickness or height of the demineralized surface zone. Therefore, the percent expansion is greater compared to the percent expansion of demineralized dentin collagen alone. Figure 9 presents a composite graph illustrating the typical expansion of the dried, collapsed demineralized matrix on a mineralized dentin disk base, following application of the three different test solutions.

The use of completely demineralized specimens (compared to the use of demineralized dentin matrix attached to a mineralized base), was selected after a preliminary study showed that dentin disks with a mineralized base exhibited excessive warpage when air-dried (i.e. in addition to the demineralized dentin matrix shrinking with air-drying, the mineralized base also warped). Application of 35% phosphoric acid gel to the surface of mineralized dentin for 15-30 seconds will demineralize the dentin surface to an approximate depth of 5-10 μm, and leave behind a mineralized base. This application closely simulates conditions found in clinical practice, but yielded ambiguous results, because it was difficult to determine how much change occurred in the mineralized versus the demineralized dentin. However, the results of three trials of the three primers on one specimen is shown in Figure 9 for the purpose of comparison. The water concentrations of the primers used on the mineralized specimen were: model

primer A = 5% water-95% HEMA; primer B = 10% water-90% HEMA, and primer C = 20% water-80% HEMA. Although there were quantitative differences in the slopes and heights of expansion due to the different water concentrations, the results were qualitatively similar. That is, the heights of the re-expansions were all proportional to the water concentration of the primers, and they all reached steady-state heights that were far below the control heights.

Figure 10 shows a regression analysis of the expansion rate of dried, acid-etched dentin on a mineralized base. A mineralized dentin disk was fixed to the bottom of the well and its surface acid-etched with 37% phosphoric acid liquid for 15 seconds and then rinsed with water. After shrinking the demineralized matrix (as shown in Fig. 9), the three model primers were applied randomly and the rate of expansion followed. The regression analysis revealed a significant correlation coefficient ($r = 0.93$ and $p < 0.001$) and an R^2 value of 0.85.

***Figure 9**. Expansion of demineralized dentin on a mineralized base by model primers.*

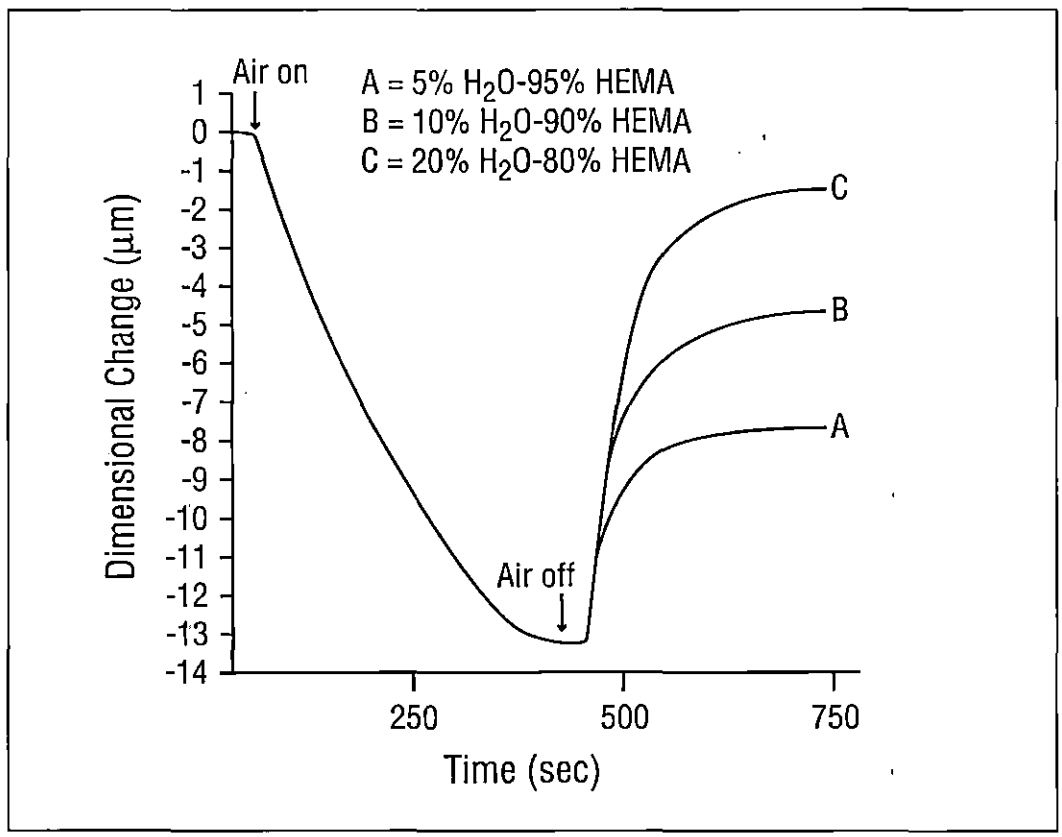
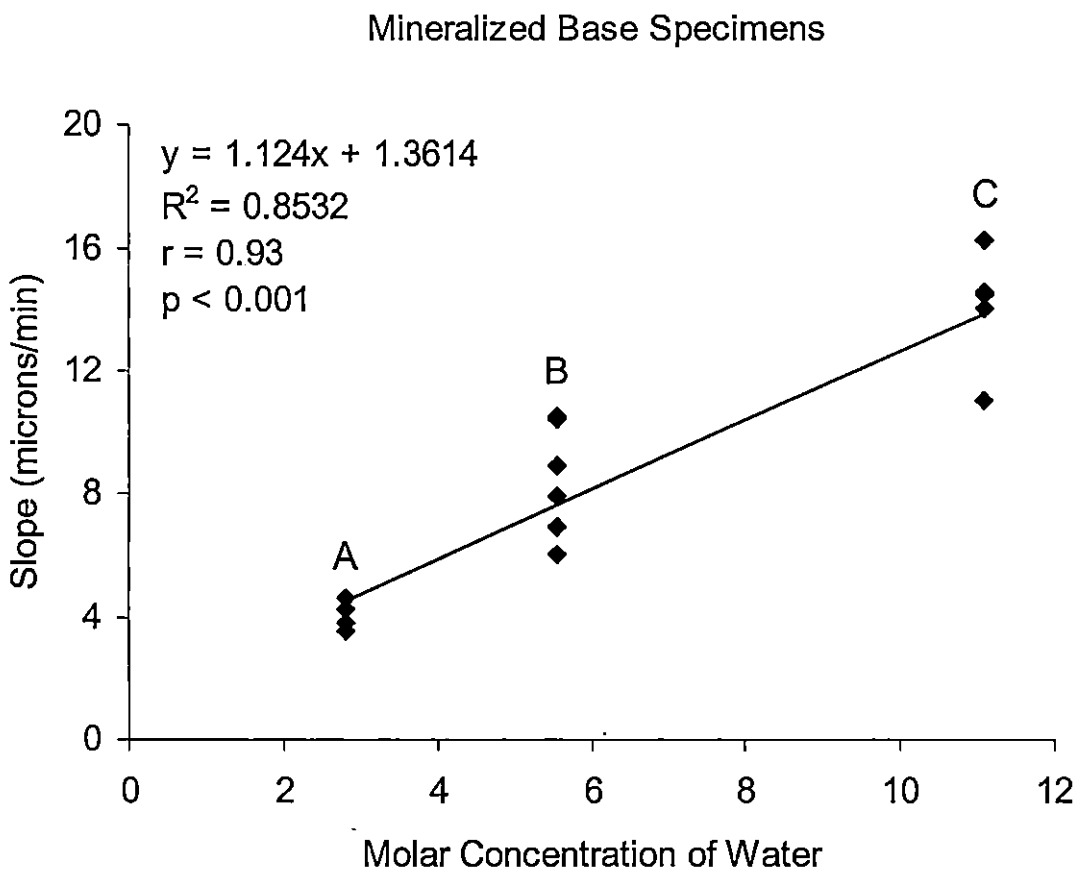

Air on
A = 5% H_2O-95% HEMA
B = 10% H_2O-90% HEMA
C = 20% H_2O-80% HEMA
Dimensional Change (µm)
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
C
B
A
Air off
250
500
750
Time (sec)

Figure 10. *Regression analysis of the rate of expansion of mineralized base specimens.*


Mineralized Base Specimens
y = 1.124x + 1.3614
R2 = 0.8532
r = 0.93
p < 0.001
A
B
C
Slope (microns/min)
Molar Concentration of Water
0
4
8
12
16
20
0
2
4
6
8
10
12