Title
In vitro comparison of calcium sucrose phosphate and calcium phosphopeptide for treatment of decalcifications

Author
Fernandes, Traci R.

Publication Date
1997

Peer reviewed|Thesis/dissertation

IN VITRO COMPARISON OF CALCIUM SUCROSE PHOSPHATE AND CALCIUM PHOSPHOPEPTIDE FOR TREATMENT OF DECALCIFICATION

by

TRACI R. FERNANDES, D.D.S.

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

ORAL BIOLOGY

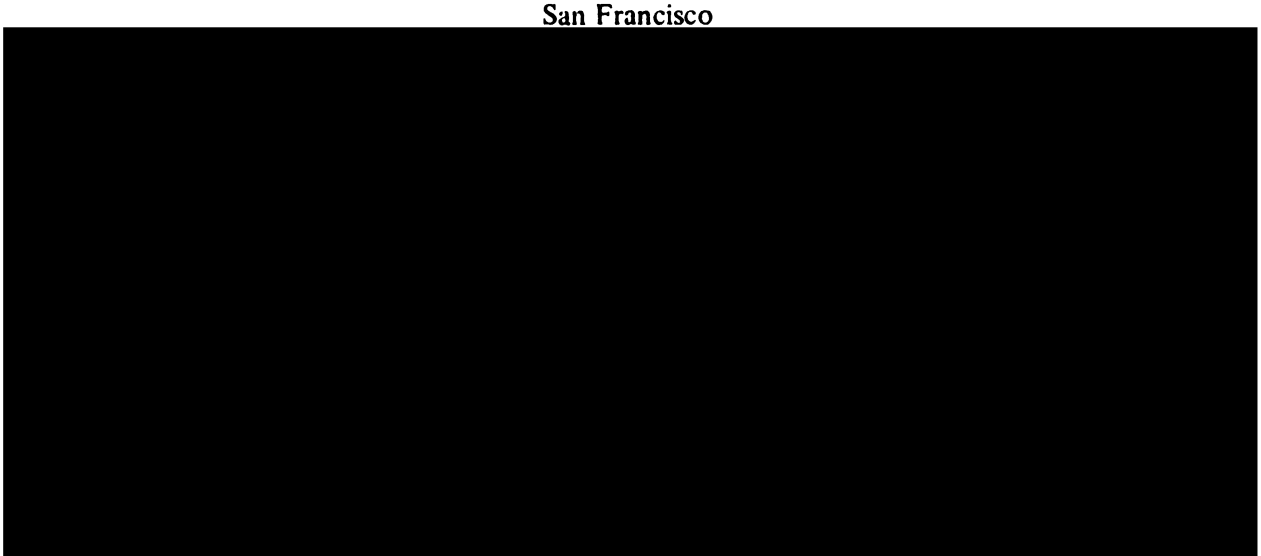
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



Date University Librarian

Degree Conferred: . . . . . .

With much love and gratitude,
to my parents, John and Nivia,
for their unending support and
encouragement.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people, who have contributed to the completion of this work.

Dr. John D. B. Featherstone, Interim Chair, Department of Restorative Dentistry and Department of Dental Public Health and Hygiene, Professor, Restorative Dentistry and Stomatology, and Director, Master's of Science program in Oral Biology, University of California, San Francisco, my advisor and mentor, for guiding and encouraging me in my research. It was a privelege to work with such a consummate dental researcher.

Dr. Joel M. White, Associate Professor, Department of Restorative Dentistry, University of California, San Francisco, for teaching me the practical aspects of optical experimentation and for his support.

Lauren Gee, statistician, Center for Knowledge Management, University of California, San Francisco, for her tireless efforts and friendship.

Dr. Pamela K. Den Besten, Associate Professor and Chair, Division of Pediatric Dentistry, and Dr. Grayson W. Marshall, Professor and Chair, Division of Biomaterials Science, Director, UCSF Dentist-Scientist Award and Integrated DDS-PhD Programs, both of the University of California, San Francisco, for serving on my thesis committee and providing me with their constructive criticism and advice during the preparation of this thesis.

The University of California, San Francisco, Orthodontic Division Faculty for the opportunity to pursue orthodontic training and preparing me for a career in dentistry.

The University of California, San Francisco, orthodontic residents for their friendship and thoughtful contributions to my education and research.

Eric Bitten and Clif Dunn, research assistants, for their assistance in the laboratory and for making the process enjoyable.

The oral surgeons in San Francisco and Tulare and their staffs for assisting in the collection of extracted teeth for this study. My gratitude also goes out to my mother, who spent many hours picking up collection jars from dental offices.

Finally, I wish to thank my family for believing in me and cheering me on along the way. They served as a wonderful source of motivation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

A. Introduction and Specific Aims

A.1. INTRODUCTION

Orthodontic appliances have repeatedly been implicated for increased incidence of decalcification.[1, 2] The white spot lesions (WSL's) that develop are the result of early arrested demineralization.[3, 4] While the condition may be stable, the tooth is esthetically and structurally compromised.[5]

Several studies have been done to establish protocols to effectively prevent the occurrence of WSL's.[1, 6] However, in cases of insufficient oral hygiene regimens and motivation on the part of the orthodontic patient, decalcification around orthodontic appliances remains prevalent. Due to the ability to transfer accountability to the orthodontic patient, in addition to the lack of easy and acceptable treatments, these enamel defects remain largely untreated. This can reflect negatively on the benefits of orthodontic treatment and poses a potential liability concern.

While prevention of decalcification should be the primary goal of the dentist and patient, effective treatments are warranted in cases where decalcification has not been circumvented. Treatments for decalcification have been established, but generally require removal of sound tooth structure to access the underlying opacity for remineralization. Ideally, a mechanism that allows optical and structural enhancement of the lesion without compromising tooth structure needs to be developed.

A.2. PURPOSE OF STUDY

The purpose of this study was to establish an effective, scientifically based treatment for dental enamel white spot lesions that conserves dental tissues and enhances demineralized enamel esthetically. Chemical products that are not commercially available in the United States were investigated for efficacy to quantitatively reverse the optical effects of demineralization. The ability of sodium hypochlorite to enhance the action of these chemical products was also investigated.

A.3. WORKING HYPOTHESIS

The hypothesis that was tested was that calcium sucrose phosphate (CSP) and calcium phosphopeptide (which will be referred to as "pentapeptide" or PP), in combination with sodium hypochlorite treatment, will allow for incorporation of minerals that will return demineralized enamel to a more "normal" color. (refer to sections B.5.2. and B.7.)

A.4. LONG TERM OBJECTIVES

Assuming that CSP and/or PP are found to be efficacious, an optimal treatment protocol (preliminary surface treatments, concentration, treatment time, repetition of treatments, etc.) will need to be established. The exact mechanism of action should also be explored and understood to further advance science and give light to other possible applications.

A.5. SPECIFIC AIMS OF PRESENT STUDY

Demineralization and remineralization of tooth enamel around orthodontic brackets has previously been studied *in vitro* and *in vivo* with similar results in

mineral profiles [7]. This ability to mimic demineralization allowed for an *in vitro* study to be employed in the testing of the hypothesis. A demineralization solution developed by White [8] was utilized to create subsurface caries-like lesions (also referred to as "white spot" lesions) on extracted human teeth. These artificial caries-like lesions were used as the basis for the experiments in the present study.

The determination of the efficacy of CSP and/or PP in "normalizing" the color of WSL's was achieved through the following specific aims:

a) Establishing the color effects of artificial enamel decalcification on extracted human teeth.

b) Establishing the color effects of bleach (5.25% sodium hypochlorite) on artificially demineralized and non-demineralized human enamel.

c) Documenting color changes of sound enamel treated with saliva, with and without prior surface bleach treatment.

d) Documenting color changes of sound enamel treated with CSP in conjunction with saliva, with and without prior surface bleach treatment.

e) Documenting color changes of sound enamel treated with PP in conjunction with saliva on sound enamel, with and without prior surface bleach treatment.

f) Documenting color changes of artificially created WSL's treated with saliva alone, with and without prior surface bleach treatment.

g) Documenting color changes of artificially created WSL's treated with CSP in conjunction with saliva, with and without surface bleach treatment.

h) Documenting color changes of artificially created WSL's treated with PP in conjunction with saliva, with and without surface bleach treatment.

B. BACKGROUND AND REVIEW OF THE LITERATURE

B.1. STATEMENT OF THE PROBLEM

Orthodontic treatment has been associated with an increase in enamel demineralization creating "white spot" lesions.[2, 6, 9] These lesions result due to increased plaque accumulation around the appliances,[10][11] as well as an altered microflora in orthodontic patients that is more cariogenic.[12, 13]

Regimens have been established that significantly decrease or eliminate the incidence of decalcification during orthodontic treatment. These regimens require the use of topical fluoride products in the form of rinses and gels. A 0.05% sodium fluoride rinse, in addition to twice daily use of an approved fluoride-containing dentrifice, is a recommended prevention protocol to eliminate the incidence of WSL's in orthodontic patients.[6]

While decalcification around orthodontic brackets is preventable, high levels of patient motivation and compliance are required throughout the course of orthodontic treatment. Given the length of treatment time and the need to closely monitor and encourage oral hygiene regimens with many patients, decalcification and resultant "white spot" lesions are still a common finding in orthodontic patients today.[1]

Many current treatment methods do not conserve the overlying sound enamel. (refer to section B.5.1.) Because the treatment is often considered more harmful than the condition, white spot lesions often remain untreated.

B.2. DECALCIFICATION-WHITE SPOT FORMATION

B.2.1. ENAMEL COMPOSITION AND STRUCTURE

Enamel is approximately 96% by weight mineral matter, mostly in the form of carbonated hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], with carbonate, HPO_4^{2-}, F^-, and other inclusions in the crystals.[14] The main inorganic consitituent of enamel, calcium, represents about 36% of enamel by weight. Phosphorous, which occurs as PO_4^{3-}, is approximately 18% by weight.[15] OH^- and carbonate are two other substantial components of enamel.

Enamel is composed of a patterned structure of enamel crystals.[16] Enamel crystals are hexagonal in shape and, when mature, measure approximately 35-40 nm in thickness.[17] These enamel crystals are much larger than apatite particles found in other mineralized tissues (e.g. bone).[16] The length of individual crystals vary and can be quite long, extending from the tooth surface to the dentinoenamel junction. There is sufficient space between individual crystals to allow for acid, phosphate, and fluoride flow. Proteins, lipids, and water envelope the individual crystals and the enamel prism as a whole, creating a prism sheath.[14] One enamel rod or prism is approximately 100 crystals across, measuring 4-5 μm in diameter.

Caries resistance of enamel is related to the maturity of the enamel, the size of the crystal, the strain and stresses within the crystal, and the alignment of enamel crystallites.[15] Fluoride ion substitution affects the physical and structural properties of the enamel crystal, improving crystallinity of enamel apatite.[18] The increases in fluoride levels in teeth that occurs with maturation, combined with a reduction in carbonate concentration in the surface enamel (carbonate is associated with increased enamel solubility), has been suggested to be the reason that mature

teeth are less susceptible to caries.[15] It is of interest, especially when discussing incipient carious lesions, to note that surface enamel is more dense and possesses a higher concentration of mineral salts than subsurface enamel, it is thus more resistant to the caries process.[19]

B.2.2. DEVELOPMENT OF THE WHITE SPOT LESION

Bacterial metabolism in dental plaque produces organic acids that attack the enamel crystals. Miller, in 1891, was the first to identify this as the mechanism of early caries that resulted in white spot lesions.[20]

Mineral loss from the outer tooth surface is the first step of caries attack resulting in surface softening of the outer enamel.[21, 22] Lactic and acetic acids are predominantly responsible for this surface penetration phenomenon.[23] While the surface layer becomes more porous, it may not be severely damaged. The lesion can in this way progress to deeper layers while maintaining a relatively intact surface. If the process continues, the mineral content will decrease to a critical level at which point cavitation will occur. The dissolution of the subsurface enamel crystals releases calcium and phosphate ions. Some of these ions diffuse out into the oral environment and are lost, while others redeposit at more superficial levels in the enamel structure. The resultant white spot lesion is caused by changes in the optical properties of the enamel due to subsurface demineralization.[3, 4]

After removal of orthodontic appliances, the white spot lesions will discontinue their progression because the cariogenic factors have been alleviated.[5] Some studies actually show regression and improved esthetics of the incipient caries lesion after the cariogenic challenge has ceased.[3, 5, 24] Since remineralization appears to be restricted to the surface layer,[25, 26] a theory for this gradual regression is surface abrasion.[5] Regardless of the mechanism for

this partial reversal, the remaining white scars, even of reduced degree, still represent an esthetic insult to the enamel.[5] (see figure 1)

B.2.3. HISTOLOGY OF THE WHITE SPOT LESION (WSL)

The fully formed white spot lesion has been characterized into four zones:[27] (see figure 2)

i) Translucent Zone

The most advanced front of the lesion, this zone was first referred to as the "light zone". In comparison to normal enamel, this region has a higher pore volume (1.0% vs. 0.1%, as measured by polarized light) and lower Ca/PO_4 ratio.[28] The crystal diameters are also reduced relative to sound enamel, measuring from 25 to 30 nm as compared to 35 to 40 nm for sound enamel.[17] This results in the irregularly larger intercrystalline micropores and interrod spaces reported by Orams, et. al.[29]

ii) Dark Zone

This zone, which is immediately below the translucent zone, is believed to be the result of remineralization [30] and consists of a reduced number of crystals of increased diameter (50-100 nm) [17]. While the apatite pattern is still apparent, there is a decreased area of normal enamel crystals and an increase in the number of short, irregularly shaped crystals of random orientation.[29] This zone is not present in all natural lesions.

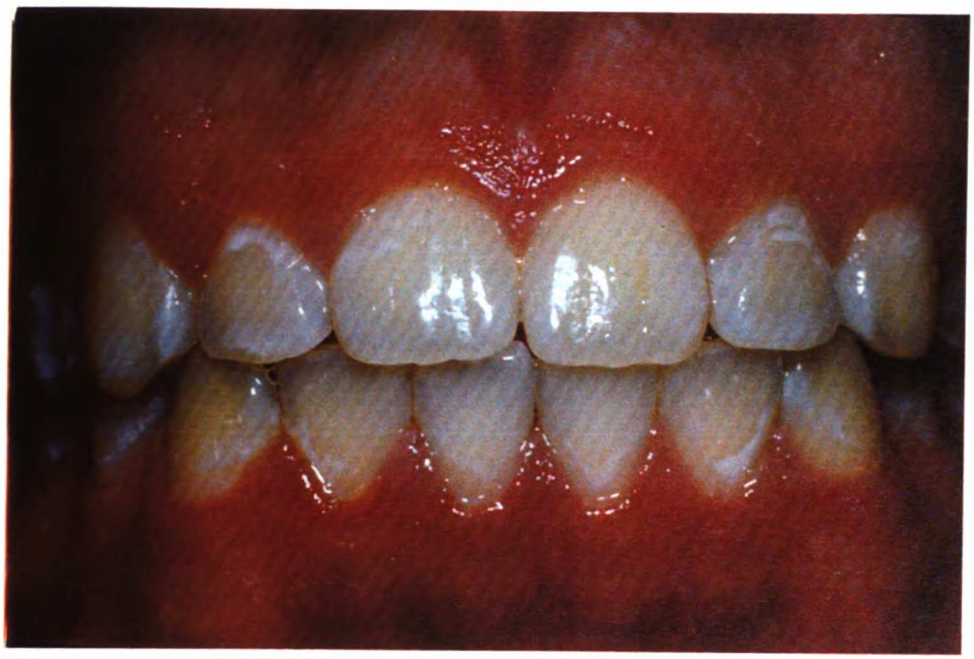


Figure 1: Intraoral photo shows marked decalcification along gingival portion of teeth after orthodontic treatment. These decalcifications are referred to as white spot lesions.

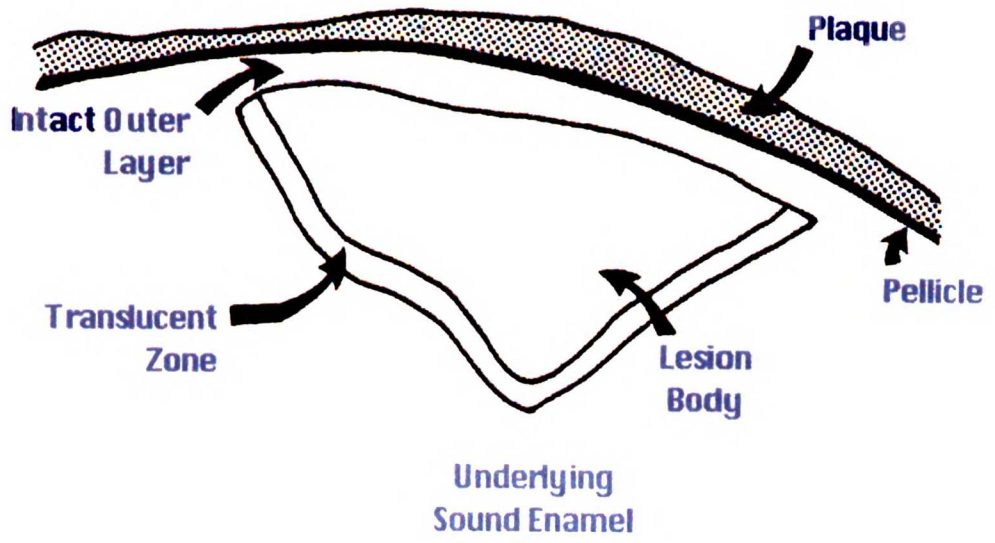


Figure 2: Diagrammatic representation of a white spot lesion separated into zones. The dark zone is not represented here since it is not always present. The enamel pellicle and plaque are seen at the enamel surface. (Adapted from J. D. B. Featherstone's Ph.D. thesis with permission)[31]

iii) Body of the lesion

This zone, which represents the majority of the white spot lesion, shows a loss of 30-70% of the mineral normally present in sound enamel. The inter- and intra-prismatic dissolution[32] results in decreased crystal diameters (10-30 nm)[17] with resultant spaces that are filled with unbound water, protein, and lipid.

iv) Surface zone

One of the defining characteristics of the advanced white spot lesion, this zone is a relatively intact outer layer covering the underlying lesion that maintains the original contour of the tooth surface.[33] While this region is initially demineralized, allowing for lesion progression to deeper regions of the enamel, dissolved minerals from the deeper layers redeposit at the surface and remineralize this zone.[33, 34] Crystal diameters in this zone range from 40-80 nm, which is larger than in sound enamel (35-40 nm).[25] Despite the increased pore volume (less than 5% as compared to 0.1% in sound enamel)[35] and mineral deficiency (a minimum of 8%),[34] it has been suggested that this zone still functions to protect the subsurface lesion from further demineralization.[34, 36]

B.3. ORTHODONTIC TREATMENT AND WHITE SPOT LESIONS

B.3.1. PREDISPOSING FACTORS TO WSL'S IN THE ORTHODONTIC PATIENT

Bands, brackets, and different orthodontic elements (e.g. elastics, plastic sleeves, springs) make oral hygiene more difficult and promote plaque retention.[10, 37, 38] (see figure 3) Although demineralization remains a risk with direct bonded brackets,[10, 13] these appliances may decrease the affected tooth surface and allow for more efficient oral hygiene as compared to bands.[39]

Zachrisson[40] has reported a lower incidence of WSL's with direct bonding as compared to that seen in fully banded patients. Orthodontic bands may pose additional plaque accumulation risks due to poor adaptation to tooth contours and the potential for cement wash-out.[37] Aside from the appliances themselves, other favored plaque accumulation sites include resin surfaces adjacent to bonded attachments, which provide a rough surface that allows oral microorganisms a greater chance to attach and grow.[41]

A more acidogenic oral environment has been correlated with orthodontic appliances,[42] increasing susceptibility to decalcification. The reason for this is a physical alteration of the microbial environment.[12, 13, 42] Bloom and Brown[42] found that orthodontic appliances resulted in significant increases in the facultative microbial population, including streptococci, lactobacilli, staphylococci, and yeast. Other studies have also reported on this increase of microorganisms, particularly *S. mutans*[12, 13, 43] and lactobacilli,[43, 44] These findings are consistent with the microflorae associated with enamel caries.[45]

Orthodontic appliances have also been associated with an increase in the concentration of carbohydrate in each milligram of plaque,[46] in addition to an increase in the volume of dental plaque and the number of bacteria. The carbohydrate increase is of note because it results in increased plaque tenacity, leading to ineffective saliva washing of enamel surfaces, decreased salivary neutralization of acids, and ultimately a more acidogenic plaque.[46]

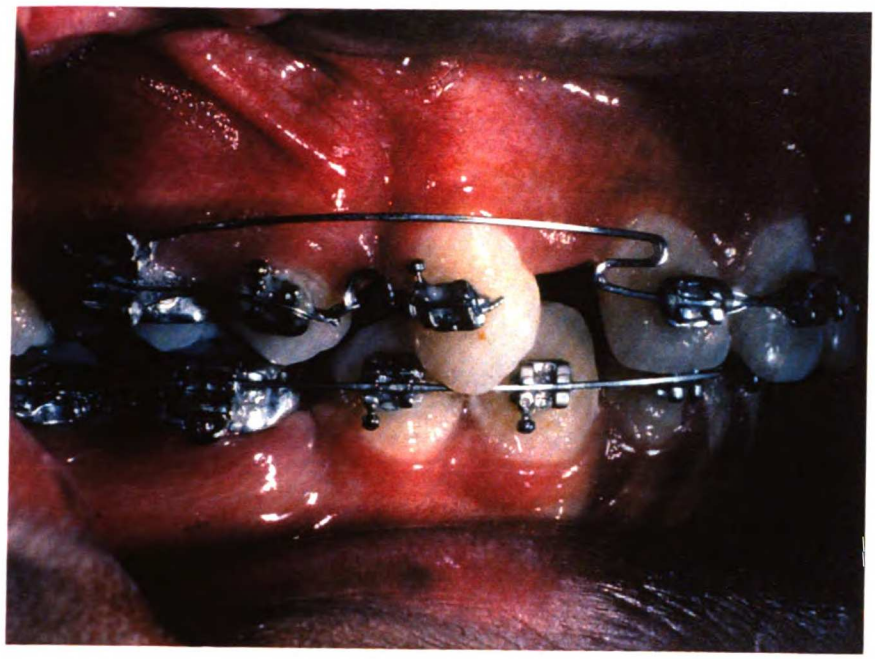


Figure 3: Intraoral photo depicting plaque accumulation around orthodontic appliances.

B.3.2. INCIDENCE OF WSL IN THE ORTHODONTIC PATIENT

Enamel opacities are not unique to orthodontic patients. Studies have indicated that well over half of individuals who have not had orthodontic treatment have enamel opacities.[47, 48] These opacities can be attributed to a variety of causes, 98 of which have been documented by Small and Murray.[49] For this reason, distinguishing the incidence of WSL's due to decalcification during orthodontic treatment has been an area of much research.

Estimates of WSL incidence among orthodontic patients vary. Regional water fluoridation,[19] patient education,[1] appliance types,[10, 50] and many other factors undoubtedly affect study findings and empirical reports. Studies have found that many patients, 50% according to one study,[2] experience a significant increase in the incidence of WSL's during orthodontic treatment. Gorelick[2] reported white spot lesions had the greatest incidence in the mandibular posterior and maxillary anterior teeth with orthodontic treatment, showing a 15 percent and 14 percent increase, respectively. Maxillary lateral incisors showed the greatest prevalence of WSL's, followed by mandibular canines, premolars, and molars.[1, 2] It was suggested from this pattern and other related findings that areas with the least accessibility to the free flow of saliva are the most at risk for enamel decalcification during orthodontic therapy. Susceptibility to decalcification is also generally more pronounced on the gingival portion of the tooth,[1] where higher plaque accumulation usually occurs.[37]

Given the increased propensity for plaque accumulation and an acidogenic environment in the orthodontic patient, it is not surprising that *in vivo* studies report measurable surface demineralization occurring within four weeks after appliance placement at the periphery of orthodontic brackets[6] and underneath ill-fitting bands.[45] Considering that the treatment time for orthodontic correction usually

ranges from 12-30 months, these studies indicate that demineralization in the orthodontic patient is a very rapid process.

Glatz and Featherstone[9] showed that demineralization was seen even with regular use of fluoride toothpaste (5-15% mineral loss to a depth of 75 μm immediately adjacent to the orthodontic brackets in 4 weeks). Demineralization was observed to an even greater extent in groups which used monofluorophosphate (MFP) paste (up to 36%, up to 120 μm depth).

Acid etching is routinely done prior to bonding of orthodontic brackets to enhance micromechanical retention. Investigations have determined that this procedure does not contribute to caries susceptibility in the orthodontic patient.[51] The etching effect has been found to be insignificant and the enamel returns to normal levels of mineralization within 24 hours.[52]

B.3.3. PREVENTION OF WSL DURING ORTHODONTIC TREATMENT

Efforts to incorporate fluoride release into orthodontic adhesives have been largely unsuccessful in preventing "white spot" lesions due to the limited time of release in comparison to the total orthodontic treatment time. One study of fluoride bonding agents found no fluoride detectable after 90 days with the highest concentrations within the first 24 hours.[53]

Lasers are not yet approved for the purpose of preventing caries, although some studies indicate that surface treatment of enamel with CO_2 lasers at specific operating parameters can inhibit caries-like progression from 10-85%.[54] Even if FDA approval is obtained for the purpose of decalcification prophylaxis, the lasers will be rather costly and, therefore, not widely accessible.

Regimens that show the greatest ability to reduce or completely eliminate the incidence of "white spot" lesions are sealants[40, 55] and fluoride therapies.[6, 56]

Sealants, in the absence of adequate oral hygiene and fluoride supplementation are insufficient protection against demineralization,[40] making them an inadequate "cure" to the problem of decalcification. Given the inefficiencies and inaccessibilty of other preventive modalities, fluoride therapies remain the most widely available, cost efficient, and highly effective for prevention of decalcification. Sodium fluoride (NaF) toothpaste (1000 ppm F as NaF) daily in combination with NaF mouthrinse (0.05 percent NaF) has been shown to give complete protection against demineralization *in vivo* and *in vitro* with a demineralization/remineralization cycling model with orthodontic brackets.[6, 7] A study by Øgaard and Rølla,[57] found significant retardation, but not complete elimination, of lesion development with daily use of a 0.2% NaF mouthrinse. Findings support the concept of frequent (daily) applications of relatively low concentration fluoride products for the elimination of caries. The presence of low concentrations of fluoride in saliva appears to effectively inhibit demineralization and enhance remineralization even in high caries susceptibility cases.[6, 7]

Patient compliance is a limiting factor for supplemental fluoride therapies and, thus, the problem of preventing "white spot" lesions remains. Non-compliant patients, orthodontic patients treated before the wide spread use of adjunctive fluoride therapies, and patients who have not received orthodontic treatment but have a high prevalence of WSL's, create a large population that would benefit from a conservative, predictable treatment protocol to reverse the esthetic and structural insult of the WSL.

B.4. ARTIFICIALLY CREATED DECALCIFICATION

Naturally occurring incipient caries lesions have been found to often be too variable in structure and too far advanced for reversal mechanism studies.[25] Because of this, in addition to the need for a large number of samples, several methods to simulate caries formation of varying degrees have been reported in the literature.[8, 58-62] For the purpose of this study, a method of artificial development of incipient caries lesions with a sound outer layer was desired.

White[8] developed a demineralization system that was easily reproducible, free of impurities such as mineral ions and fluoride, and had the versatility to create "life-like" early artificial carious lesions which were relatively fluoride free with good remineralization reactivity. The demineralization solution he devised utilizes lactic acid/calcium phosphate buffers containing 0.1-0.5% (w/w) polyacrylic acid (also known as Carbopol C907, MW = 450,000 daltons). The polyacrylic acid served as a surface dissolution inhibitor, similar to the function of the enamel pellicle. Calcium and phosphate are present at concentrations that produce 50% saturation with respect to hydroxyapatite. (refer to section C.2.2.1.)

B.5. TREATMENT OF WHITE SPOT LESIONS

B.5.1. CURRENT TREATMENT OF WHITE SPOT LESIONS

Currently, most "white spot" lesions remain untreated. In cases where such decalcifications are esthetically unacceptable to the patient or practitioner, several treatment modalities have been employed. Some of the treatments utilized to improve the esthetics of discolored teeth described in the literature include rubbing 18% hydrochloric acid into the tooth, both with and without heat,[63, 64] hydrogen peroxide with or without heat,[65-67] and techniques combining hydrochloric acid,

hydrogen peroxide and heat.[68] Other protocols that incorporate microabrasion or surface layer removal have also been reported.[64, 69, 70] It is of note that the majority of these treatments incorporate mechanical or chemical removal of the intact outer enamel layer. This is done as a means to allow for removal of the underlying affected tissue or to improve access to the underlying porous lesion, allowing more rapid subsurface remineralization through saliva exposure or some other chemical mechanism. As a means of reference, 30-40% phosphoric acid applied for one minute to teeth of an unstated mineral profile, reportedly etches enamel to a depth of 24-30 μm.[71]

The esthetic improvement related to the treatment was evaluated subjectively in the majority of these reports and little research has been done on the mechanisms by which these various treatments are "successful". Such methods of treatment are often at the expense of enamel material and can necessitate tooth isolation techniques. While the tooth isolation techniques are not desirable because of increased chair time required for the procedure, the removal of sound enamel is less acceptable given today's conservative approach toward removal of dental tissues. A less aggressive methodology needs to be developed that is equally or more effective at reversing the discoloration of white spot lesions.

B.5.2. POTENTIAL CHEMICAL THERAPIES FOR WHITE SPOT LESIONS

B.5.2.1. Calcium Sucrose Phosphate

B.5.2.1.1. Preparation and chemical composition

Calcium sucrose phosphate (CSP) was first prepared in Australia in 1960 [72]. CSP contains approximately 11% calcium, 9.5% organic phosphorous, and 2.5% inorganic phosphorous.[73] Preparation of the compound involves

phosphorylation of sucrose in the presence of calcium hydroxide.[73, 74] This results in a mixture of calcium sucrose phosphates and calcium phosphates, namely calcium sucrose mono- and di-phosphates and inorganic calcium orthophosphate, and disucrose monophosphate.[73] The compound is a fine, white powder that can be incorporated into foods, gels, and dentrifices without a discernible taste.[73]

B.5.2.1.2. Indications and efficacy

CSP was initially marketed as an anti-caries food additive under the trade name "Anticay".[73, 75-77] The premise during development was that free phosphates provided in the substance would incorporate into the dental plaque to produce a cariostatic effect.[73] Since its development, the efficacy of CSP as a caries inhibitor has been demonstrated in *in vitro* studies,[77-79] animal experiments,[80, 81] and clinical studies.[72, 82] The *in vitro* studies suggest various modes of anticaries potential, including the ability of the compound to reharden decalcified softened tooth enamel,[78] absorb into enamel surfaces,[79] and buffer the effect of acid in plaque.[77] While it is suspected that high concentrations of calcium and orthophosphate ions in CSP are responsible for the rapid remineralization of softened enamel, the mechanism remains unclear.[70] It has, however, been reported that calcium and phosphate ions in the presence of sugar phosphates remineralize softened tooth enamel in minutes, compared to the hours which are necessary for lower concentrations of calcium and phosphate ions without sugar phosphates.[78] Since remineralization has been related to Fluoran (CSP) paste, which contains less than 1 μg/g free fluoride,[83] it can be said that anticariogenic and remineralization capacities of CSP can not be attributed to any contribution from fluoride.

Evidence exists that many organic and inorganic phosphates reduce caries in experimental animals on cariogenic diets.[84] CSP has proven to be an effective source of such phosphates in animal anticariogenicity studies [80, 81]. Human clinical trials have demonstrated reduced caries incidence in children eating CSP containing foods (1% CSP by weight of the total carbohydrate content)[75, 85] with no untoward medical effects.[73, 86-89]

Since its introduction as an anti-caries agent, it has also found applications as a dentin desensitizing agent[78] and as a treatment agent for reversal of enamel discoloration associated with fluorosis[70, 90, 91] and demineralization around orthodontic brackets.[70] One study demonstrated, quantitatively, that CSP followed by immersion in artificial saliva, alone or in combination with a sodium hypochlorite pretreatment, can significantly improve the color (as measured by L*) of fluorotic lesions and white spot lesions *in vitro*.[70] In the same study, it was concluded using scanning electron microscopy (SEM) that CSP resulted in more normal light reflectance of fluorotic lesions due to the deposition of globular material between the enamel rods.[70] Such absorption of CSP into porous enamel surfaces was also evidenced in prior SEM studies.[79] The same mechanism of subsurface "fill", rather than actual remineralization, would be expected for the improvement of WSL color reflectance.[70]

Empirical and qualitative reports of improvement of discolored enamel with CSP have utilized varying treatment protocols. The first report in 1982, by Powell and Craig,[90] was an *in vivo* study of fluorosed teeth that utilized two 2-3 minute applications of 37% phosphoric acid for etching with polishing of the surface with pumice and glycerine being carried out before and after each phosphoric acid treatment, then a 4 minute 2% sodium fluoride solution treatment, followed by application of a thick layer of 40% CSP gel. Patients were instructed to allow the gel to dissolve. Use of a sodium monofluorophosphate or a sodium fluoride

dentrifrice at home was also advised. Myers and Lyons reported on a similar protocol, that only varied with a decrease in the time for each etch.[91]

The study by Den Besten and Giambro[70] demonstrated significantly improved esthetics of *in vitro* fluorotic and white spot lesions using 10% CSP followed by artificial saliva, with or without prior surface treatment with 5.25% sodium hypochlorite. The treatment effect, however, did appear to be enhanced by the sodium hypochlorite pretreatment. Normal (sound) enamel was not significantly affected by either treatment protocol in this study, which is a desireable result. The study also included a group of normal, fluorosed, and demineralized samples that were treated with hydrogen peroxide prior to CSP treatment and saliva immersion. The results for this group were not desirable. The samples all demonstrated increased L* values (appeared more white), including the sound enamel samples. This, in addition to experiments with hydrogen peroxide alone, indicated that hydrogen peroxide caused significant whitening that was not reversed with subsequent treatments, thereby excluding protocols containing hydrogen peroxide as viable treatment options.

Featherstone, et. al.,[83] measuring hardness profiles of artificial lesions treated with 10% CSP, found lesion depth reduction with rehardening to approximately 40 μm of the inner surface. CSP was found to be more efficacious at surface rehardening than the control paste (0.76% sodium monofluorophosphate) or saliva alone in this study.

B.5.2.2. Pentapeptides

B.5.2.2.1. *O*-phosphoserine [Ser(*P*)] sequences

Multiple *O*-phosphoserine [Ser(*P*)] sequences play important structural roles in many phosphoproteins.[92] Multiple phosphoseryl-containing sequences of proteins stabilize amorphous calcium phosphate, which is believed to influence the regulation of biomineralization, protein structure, and enzyme activity.[93-95]

Peptides containing the cluster sequence -Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-, the sequence found in casein, have been shown to be anticariogenic in various *in vivo* animal and *in vitro* human experiments.[96, 97] These findings may indicate a potential effectiveness of these pentapeptide chains preventing and, possibly, reversing demineralization of enamel.

Despite the understanding that Ser(*P*) sequences play important structural roles in phosphoproteins, the structure of the sequences and relationship between structure and function is yet unclear.[98] For the purpose of laboratory investigation to determine biochemical and structure-function relationships, synthetic means of creating Ser(*P*)-containing peptides were initiated in the 1950's and continued into the 1990's to find a predictable and efficient method.[92] After much research, findings at the University of Melbourne indicated that the use of Bos-Ser (PO_3 Ph_2)-OH in the Boc mode of peptide synthesis produces high yields of multiple Ser(*P*) residue peptides.[92]

In addition to triple Ser(*P*) sequences, Thr(P) clusters in pentapeptide configuration, although not generally found in calcium phosphate binding phosphoproteins, have also been shown to stabilize amorphous calcium phosphate at neutral and alkaline pH.[98, 99] A mechanism similar to that mentioned for multiple Ser(*P*) peptides is utilized for the synthesis of *O*-phosphothreonyl containing pepetides.[99]

Several natural acidic peptides have been reported to interact stongly with calcium ions and/or to promote mineralization. For example, the ability of the salivary phosphoprotein, statherin, to prevent spontaneous precipitation of calcium phosphate in saliva has been attributed to the hydrophilic *N*-terminal segment, Asp-Ser(*P*)-Ser(*P*)-Glu-Glu-.[94] Other Ser(*P*)-containing phosphoproteins, found in enamel, bone, and dentin, are believed to play a role in the nucleation and subsequent regulation of biomineralization of these tissues.[95] The multiple Ser(*P*)- segment, -Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-, is credited with the ability of casein to sequester large amounts of calcium, creating calcium phosphate interactions that lend structural integrity to the casein micelle.[100]

B.5.2.2.2. Topacal™

Pentapeptides (PP) have been incorporated into a commercial product marketed in Australia called Topacal™ (Nulite Systems International). This formulation contains a concentrated form of Calpep™ (calcium phosphopeptide, also referred to as CPP). Peptide CPP is isolated from milk casein as a complex of concentrated calcium and phosphate. It has been found that CPP is capable of binding with plaque, dentin, and enamel. This binding ability boosts local levels of calcium and phosphorous over 100%. Resultant surface layer buffering drives the surface reaction to produce more calcium hydroxyphosphate than was previously lost from acid attack.[101]

According to the product's informational flier,[101] Topacal™ consists of 54% distilled water, 40% Calpep™, 5% ethanol, 0.9% methyl paraben, and 0.1% food pigment by weight. It is marketed for occlusion of exposed dentin tubules, remineralization of enamel lesions, as an alternative to topical fluoride, and for the reduction of signs of fluorosis. Repeated applications are recommended for

increased benefit. Air drying of the tooth surface is advised before application. Topical fluoride can be applied following Topacal™ application. This allows for a beneficial reaction between fluoride and calcium hydroxyphosphate. As with in-office fluoride treatments, one hour without solid or liquid intake is recommended following treatment with Topacal™.

B.6. SALIVA'S REMINERALIZING EFFECT ON ENAMEL

Saliva has several structural features which influece its interaction in the oral environment and with dental enamel, in particular.[102] The ability of saliva to remineralize enamel is not clearly established in reported studies. Featherstone, [7] using artificial saliva with <.01mg/l fluoride added, stated that it was ineffective as a remineralizing solution. Conversely, ten Cate[103] found that the remineralizing capacity of saliva in the absence of concentrated fluoride agents is relatively fast. It has been concluded that WSL's, which are surface softened lesions, remineralize differently than subsurface lesions. For this reason, it has been recommended that WSL's should not be treated with concentrated fluoride agents because it will arrest the lesions and prevent complete repair. This is not the case for carious lesions developed in caries-susceptible areas like proximal surfaces and the gingival part of facial and lingual surfaces of posterior teeth. Lesions of this type should be treated topically with fluoride to prevent further lesion progression.[45, 57] Further investigation of the interaction of saliva with white spot lesions is needed, especially with respect to the effect on enamel optics.

B.7. THE EFFECT OF BLEACH ON ENAMEL

Previous studies have shown sodium hypochlorite (bleach) to effectively remove the surface organic layer and increase the penetration of mineral into carious

enamel lesions.[70, 104, 105] A study by Den Besten and Giambro also indicates that 5.25% sodium hypochlorite alone does not significantly alter L* (color gradient from white to black) in normal, fluorosed or WSL enamel samples *in vitro*.[70] (refer to section B.8.) This indicates that bleach is an acceptable treatment adjunctive in protocols for the reversal of white spot lesions.

B.8. ENAMEL OPTICS

B.8.1. OPTICS

B.8.1.1. Color Perception

Color is a form of radiant energy which is in the wavelength region detectable by the human eye. The eye is sensitive to light radiations from about 380 nm to 780 nm and capable of registering all colors from violet to red.[106] In the absence of light, color is non-existent. (see figure 4)

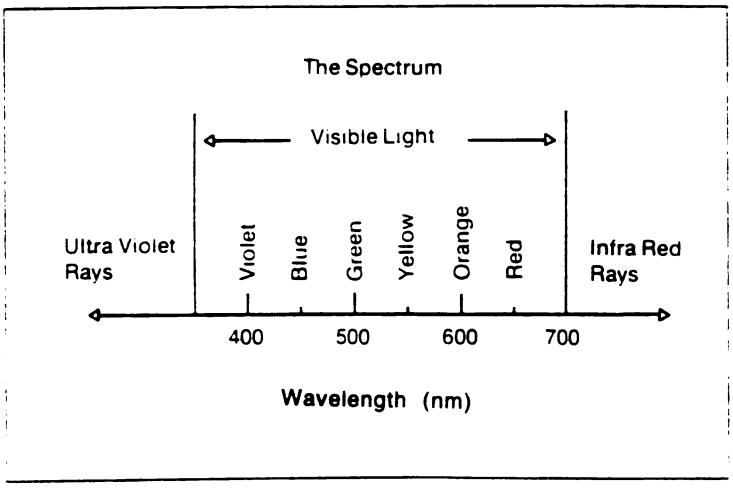


Figure 4: Diagram illustrates the wavelength correlations of colors in the visible light spectrum. (Reproduced from McLean with permission)[106]

According to McLean,[106] the color of a tooth seen by the observer depends upon:

a) the spectral energy distribution of the light source (e.g. daylight or artificial light)

b) spectral characteristics of the tooth in respect to absorption, reflection, and transmission

c) the sensitivity of the eye

d) conditions under which the tooth is being viewed (background, wet/dry, angle and intensity of illumination)

B.8.1.2. Three dimensions of color

There are three different dimensions which define color.[106] These will be explained here.

1) Hue: Hue is the type of color (e.g. red) experienced by the observer. It is possible to make nearly all colors by mixing of the three primary colors, which are red, green, and blue. The hue that is expressed is the result of the amount of absorption of the various color rays of the tooth dentin and enamel (e.g. grey-yellow, bluish-yellow).

2) Chroma: Chroma is the strength or purity of a color at any given value level. This dimension of color depends on the saturation of color or strength of hue, which is dependent on the thickness and translucency of the enamel and degree of calcification of the underlying dentin. When taken together with hue, these properties define the quality aspect of color

3) Value: Value (L*) is the total reflectance or luminance of an object. This is a photometric quality that is related to brightness or lightness. An object will appear white when all of the incident light is reflected. Conversely, when all

spectrum colors are absorbed an object appears black. Decalcified enamel appears white, and thus has an increased value or brightness relative to sound enamel.

B.8.1.3. CIE L*a*b*

The C.I.E. Colorimetric System (Commission Internationale d'Eclairage), which comprises standards and procedures of measurement, was established to numerically specify the color of a physically defined visual stimulus.[107] In the CIE System, the color of a material is specified by the intensity of the three primary colors reflected under standard conditions. The reflected tristimuli (red, green and blue) intensities are then compared with those from a white reference standard and the tristimulus values of the specimen are calculated.[106] Further calculations lead to a complete specification of the color of a material in terms of its dominant wavelength, percentage luminance or brightness, and its excitation or spectral purity.[106]

CIE 1976 (L*a*b*) relates tristimulus values to the uniform color space depicted in figure 5.[107] The L* function defines the lightness correlate, ranging from black to white. This is also referred to as value. The a* and b* coordinates combined represent chroma and hue. When b* is plotted against a*, the points resulting in the (a*, b*)-diagram are not uniquely related to chromaticity because their position depends on the value of L*.[108] The reliance of a* and b* on L* is more easily understood by looking at the diagram for the Munsell and Adam's coordinate systems. (see figure 6) In the Munsell color system, value is plotted on the vertical, hue is in the center in the horizontal plane, and chroma is the distance outward.[106]

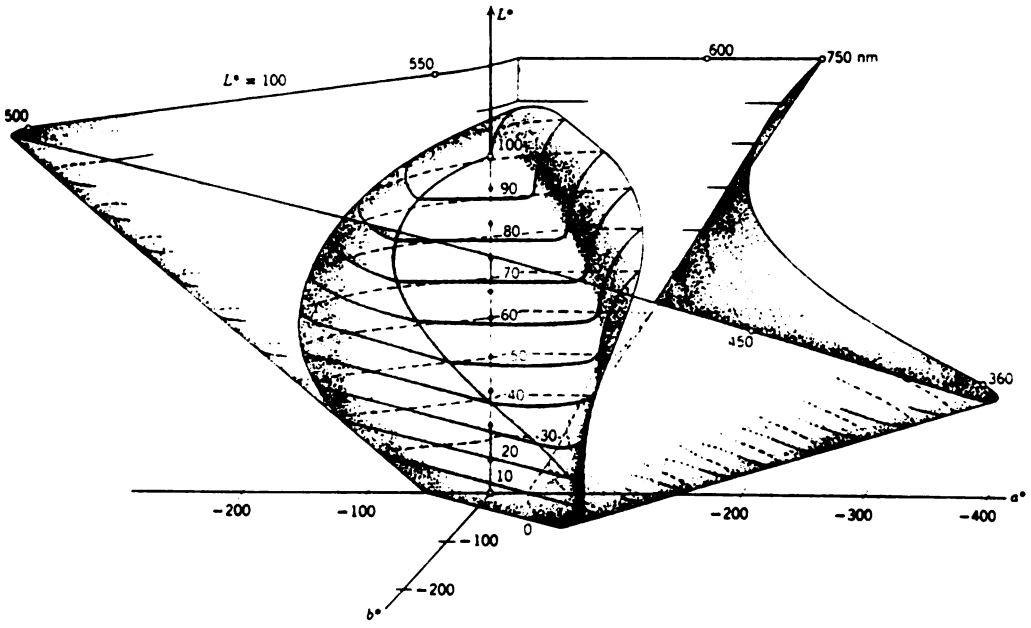


Figure 5: Diagrammatic representation of CIE 1976 (L*a*b*) color space with respect to standard illuminant D65. The colors of all object-color stimuli are contained in this area. (Reproduced from Wyszecki and Stiles with permission)[108]

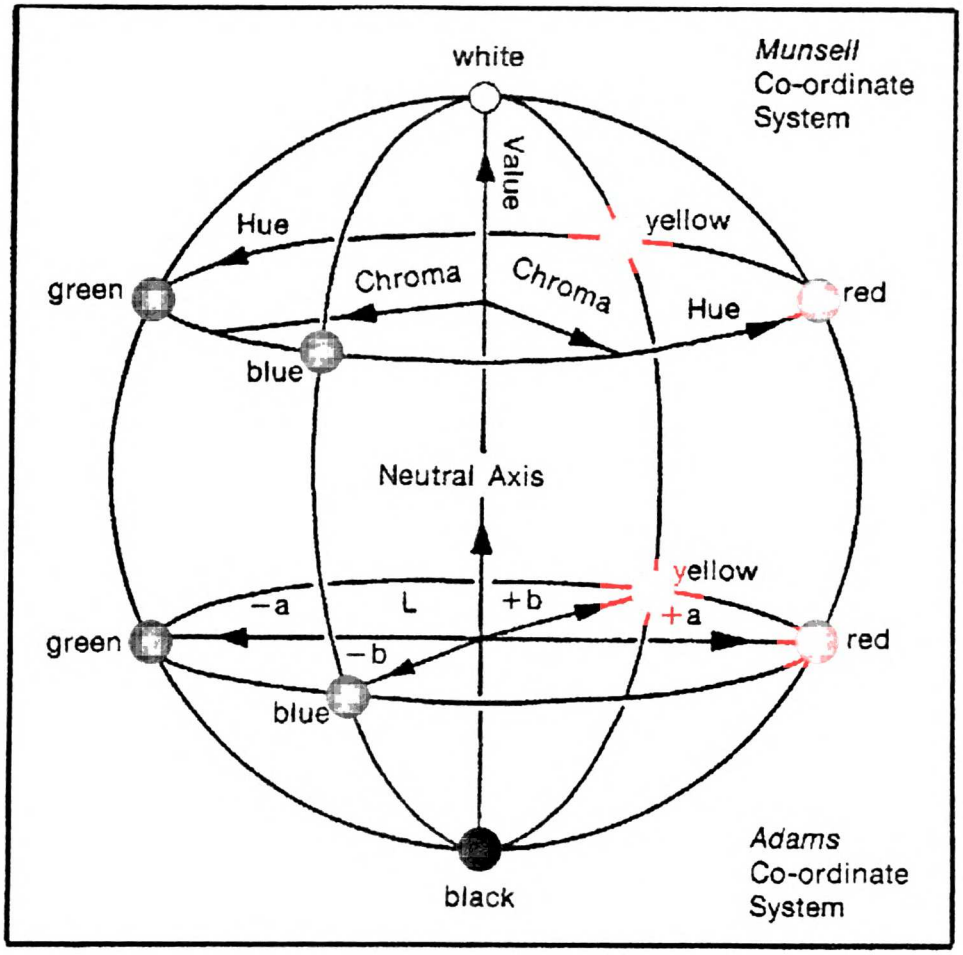


Figure 6: Diagrammatic representation of value, chroma, and hue in Munsell's coordinate system and their relation to L*a*b* in Adam's coordinate system, with L* along the vertical (neutral) axis. (Adapted from McLean with permission)[106]

CIE 1976 (L*a*b*) defines the quantities L*a*b* by the following equations:[107]

$$L^* = 116\,(Y/Y_n)^{1/3} - 16$$

$$a^* = 500\,[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}]$$

$$b^* = 200\,[(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}]$$

$$\text{with} \quad X/X_n,\ Y/Y_n,\ Z/Z_n > 0.01$$

X_n, Y_n, Z_n are the tristimulus values of the nominally white object-color stimulus that is the reference surface viewed with a standard illuminant (e.g. D_{65}).

Color difference formulas to obtain ΔL^*, Δa^*, and Δb^* values are simply the subtraction of the previous L*, a*, or b* value from the subsequent measurement for the same parameter. The total color difference, ΔE^*, between two color stimuli measured in L*a*b* is defined as:[108]

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

B.8.1.4. Color Measurement

While the human eye is generally regarded as the most sensitive and accurate perceiver of color, critical color perception varies from one individual to another and some individuals are unable to duplicate color perception with any reliability.[109]

To obtain correct color measurement a source of light that contains a full spectrum without excessive dominance of energy at any wavelength is required. If an object is viewed under a light source that has a greater energy in one or more specific wavelengths (color bands), then that color will be dominant. The blue end of the spectrum (450-500 nm) is slightly more dominant in average daylight.[106] (see figure 7)

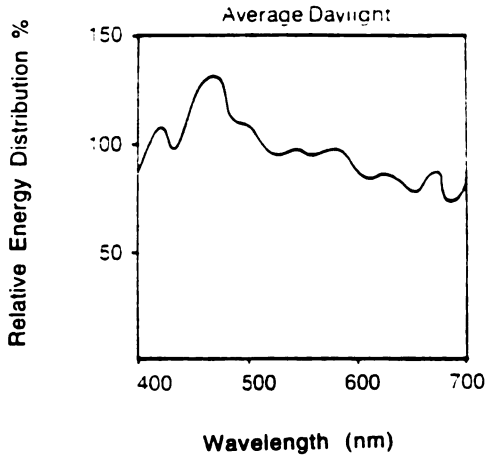


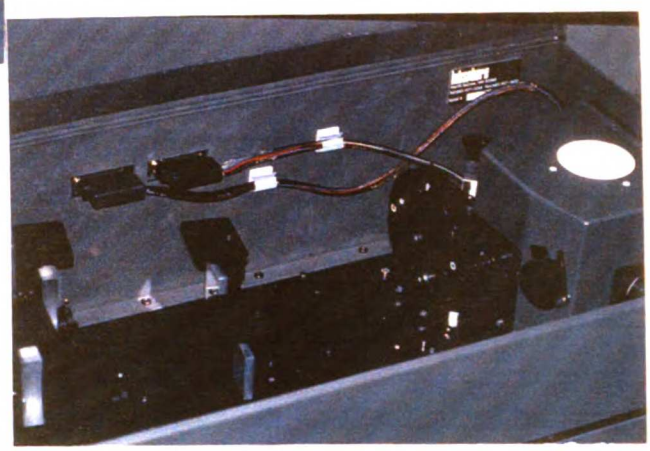
Figure 7: Diagram of the relative energy distribution of average daylight over the visible spectrum. (Adapted from McLean with permission)[106]

Regardless of the nature of the light source, color response results from either a reflected or a transmitted beam of white light or a portion of that beam.[106] Translucent objects, like enamel, both reflect and transmit some light. The amount of each is influenced by the reflection and refraction of light in the enamel rods.

For the present study, a spectrophotometer was utilized for color data collection. The spectrophotometer is a monochrometer that breaks up and measures different parts of light through the use of an integrating sphere. (see figure 8) The integrating sphere has two entry ports, which allows a reference beam to be transmitted at the same time as the beam that is directed at the tooth sample. This enables the detectors to calculate the difference in reflectance values between the reference (i.e. standard spectralon material, which is used throughout the integrating sphere) and the tooth sample. Absolute reflectance can then be converted to the uniform color scale, CIE L*a*b*, for measurement and analysis.

(a)

(b)



(c)

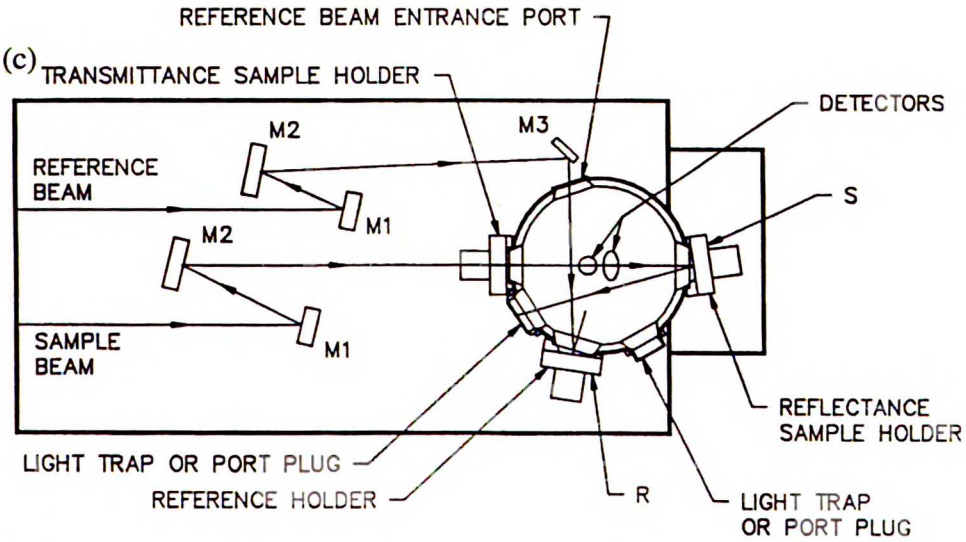


Figure 8: (a) Perkin Elmer Lamba 19 spectrophotometer (on right) and PC (computer on left) used to collect spectra, (b) Internal portion of spectrophotometer, (c) Diagrammatic representation of apparati and integrating sphere shown in (b) [M1= first mirror to direct beam, M2= second mirror, M3= third mirror, R= reference (spectralon plug), S= sample (tooth hemisection with mask exposing enamel window)]

B.8.2. PARAMETERS OF MINERALIZED ENAMEL

Enamel has a high surface area to volume ratio due to the small hydroxyapatite crystals and contains approximately 1% organic material of the total mass, which makes it very translucent. Enamel can transmit up to 70% light thru a 1 mm thick section, as opposed to dentin which generally will not transmit much more than 30%.[106]

According to previous studies on extracted teeth, L* values for normal enamel ranged from 68.1 to 70.5 when measured with a colorimeter.[70] The a* and b* measurements indicate that the color range for natural teeth lie in the yellow and red area.[110, 111]

B.8.3. PARAMETERS OF DEMINERALIZED ENAMEL

Decalcification can change the color, texture, and translucency of the tooth.[112] These changes have allowed for a variety of optical methods in the detection and quantification of early dental caries.[112-115]

Den Besten and Giambro[70] utilized a colorimeter to measure the L* parameter of color of naturally occurring white spot lesions associated with orthodontic treatment. As compared to sound (non-demineralized) enamel which gave L* values ranging from approximately 68-70, enamel with WSL's recorded L* values in the 81-83 range. This difference demonstrates that WSL's are significantly more white than sound enamel, which correlates with the visual assessment.

C. EXPERIMENTAL DESIGN AND METHODS

C.1. OUTLINE OF EXPERIMENTAL PROGRAM

The experimental program consisted of several parts to address the specific aims of this study. The main programmatic parts were:

- optical parameters of sound enamel
- optical consequences of sound enamel treated with saliva, with and without 5.25% sodium hypochlorite surface pretreatment
- optical consequences of sound enamel treated with CSP, with and without 5.25% sodium hypochlorite surface pretreatment
- optical consequences of sound enamel treated with PP, with and without 5.25% sodium hypochlorite surface pretreatment
- artificial decalcification to produce "white spot" lesions (refer to section C.2.2.1.)
- optical consequences of "white spot" lesions
- optical effects on "white spot" lesions treated with saliva, with and without 5.25% sodium hypochlorite surface treatment
- optical effects on "white spot" lesions treated with CSP, with and without 5.25% sodium hypochlorite surface treatment
- optical effects on "white spot" lesions treated with PP, with and without 5.25% sodium hypochlorite surface treatment
- interpretation of the results in terms of long range implications for treatment of "white spot" lesions with CSP and/or PP

C.2. EXPERIMENTAL DESIGN

C.2.1. TOOTH SAMPLES

C.2.1.1. Collection

Extracted human teeth were collected from oral surgery offices in San Francisco, Tulare, and Visalia, California. Jars containing double deionized water (DDW) with thymol crystals, which served as the storage media for the extracted tooth specimens, were provided to the offices by the investigator. Teeth were put into the DDW and thymol solution immediately following extraction without rinsing. Collectors were instructed not to clean or sterilize the teeth with any heat or chemicals to prevent alteration of proteins and/or optical properties of the tooth. The thymol in the storage solution served as an anti-fungal and anti-bacterial agent. Tooth samples were picked up by the investigator within 2 weeks of extraction.

C.2.1.2. Gamma Irradiation

A cesium (Cs^{137}) radiation source was used to sterilize the tooth samples at a dose above 173 krad. The samples were irradiated overnight submersed in the collection media. Following sterilization, the collection media was disposed of and replaced with fresh DDW and thymol.

Sterilization of teeth by gamma irradiation was reported by White, et. al.[116] to show no detectable changes in dentin structure, as measured by FTIR, UV/VIS/NIR, and permeability. Because enamel has less organic material than dentin, it has been assumed that the effects on enamel would be less pronounced and, therefore, negligible for the purposes of this study.

C.2.1.3. Inclusion and Exclusion Criteria

Molar teeth (including 3rd molars) which were non-carious and unrestored, with a minimum of 2/3 root formation, were considered for inclusion in the study. Inspection of the teeth under a stereoscope at 3X power in an air dried state was carried out. Samples were selected if a 4 mm high by 6 mm wide area could be delineated on the buccal or lingual surface that was free of discoloration, white spots, surface irregularities, and/or large craze lines.

C.2.1.4. Sample Preparation

Sterilized, selected tooth samples were sonicated and scrubbed with a toothbrush using an Ivory™ soap solution and rinsed in DDW. Teeth were then identified according to tooth type and categorized as having complete or incomplete root formation. Apices open greater than 1mm were defined as incomplete roots.

Root removal approximately 2 mm apical to the cemento-enamel junction (CEJ) was carried out using a 15 HC diamond wafering blade (Buehler, Lake Bluff, Ill.) on an Isomet low speed saw (Buehler, Lake Forest, Ill.). The crown was then hemisectioned into buccal and lingual halves using the same apparatus.

The pulp chamber and occlusal grooves were filled with a light-cured composite resin (Transbond XT®, 3M Unitek, Monrovia, California) to prevent introduction of nail varnish or acrylic into these areas during subsequent sample preparation. The tooth was then painted on the uncut surfaces with acid resistant nail varnish leaving a window approximately 4 mm high and 6 mm wide between the depth of the central groove and the CEJ. The sections were thereafter stored in individual vials with thymol crystals and a sterile gauze plug soaked with DDW to create an environment with 100% humidity similar to the oral cavity.

A mixture of superfine polymer acrylic and clear monomer (Great Lakes Orthodontics, Tonawanda, NY) was placed in a 35 X10 mm petri dish (Falcon® 1008, Becton Dickinson, Lincoln Park, New Jersey), indented with the tooth section, and hardened in a pressure pot to create a positioning jig. The window of the sample was positioned parallel to the bottom of the petri dish, slightly above the height of the petri dish rim. Such an orientation allowed the petri dish to rest firmly against the walls of the reflectance sample holder in the UV/VIS/NIR spectrophotometer apparatus with the unpainted window slightly protruding through the sample mask. (see figure 9)

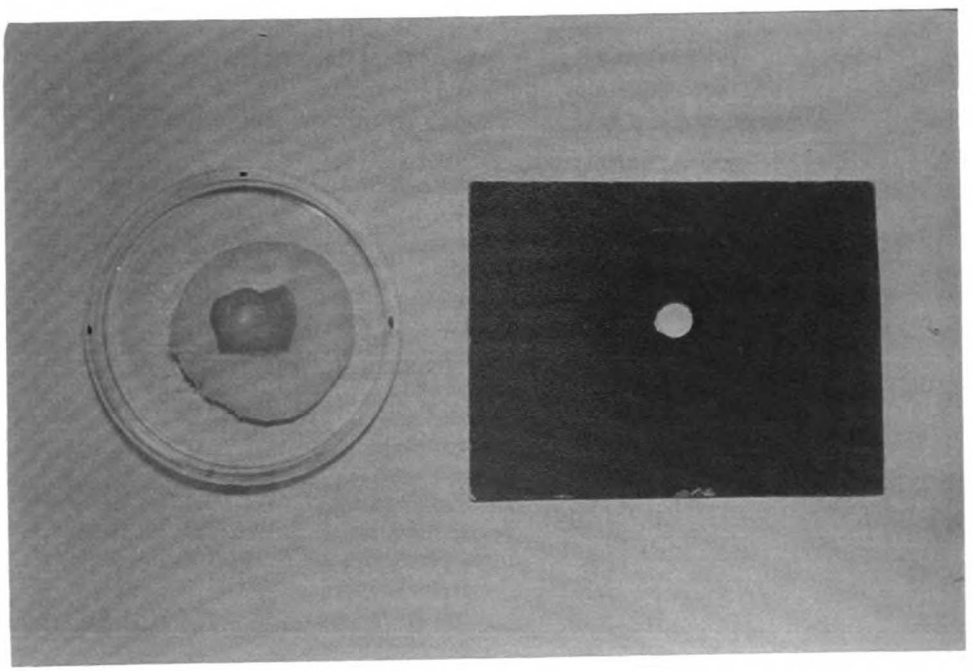


Figure 9: Hemisectioned tooth sample with 4 X 6 mm enamel window delineated with nail varnish. Each sample is custom fit in a clear acrylic jig in a petri dish. The three black markings along the edge of the petri dish align with a cross-grid on the back of the 4 mm diameter round black mask (at right) for reproducible positioning.

C.2.1.5. Sample Assignment

Nine (9) samples were assigned to each of twelve groups (refer to section C.2.2. below) for a total of 108 samples. The groups were as follows:

1) sound enamel treated with saliva only
2) sound enamel treated with CSP* and saliva
3) sound enamel treated with PP$^{\lambda}$ and saliva
4) sound enamel treated with NaOHCl$^{\delta}$, then saliva
5) sound enamel treated with NaOHCl, then CSP and saliva
6) sound enamel treated with NaOHCl, then PP and saliva
7) demineralized enamel treated with saliva only
8) demineralized enamel treated with CSP and saliva
9) demineralized enamel treated with PP and saliva
10) demineralized enamel treated with NaOHCl, then saliva
11) demineralized enamel treated with NaOHCl, then CSP and saliva
12) demineralized enamel treated with NaOHCl, then PP and saliva

* CSP = calcium sucrose phosphate (10%)
λ PP =pentapeptide (40%)
δ NaOHCl =sodium hypochlorite or "bleach" (5.25%)

Samples were originally randomly assigned to groups. Selected samples were reassigned to improve the balance between groups for tooth type, tooth section (i.e. buccal or lingual), and completion of root formation.

C.2.2. SAMPLE TREATMENTS

An flow diagram of the treatment groups is depicted in figure 10. The protocol for the individual treatment steps follows.

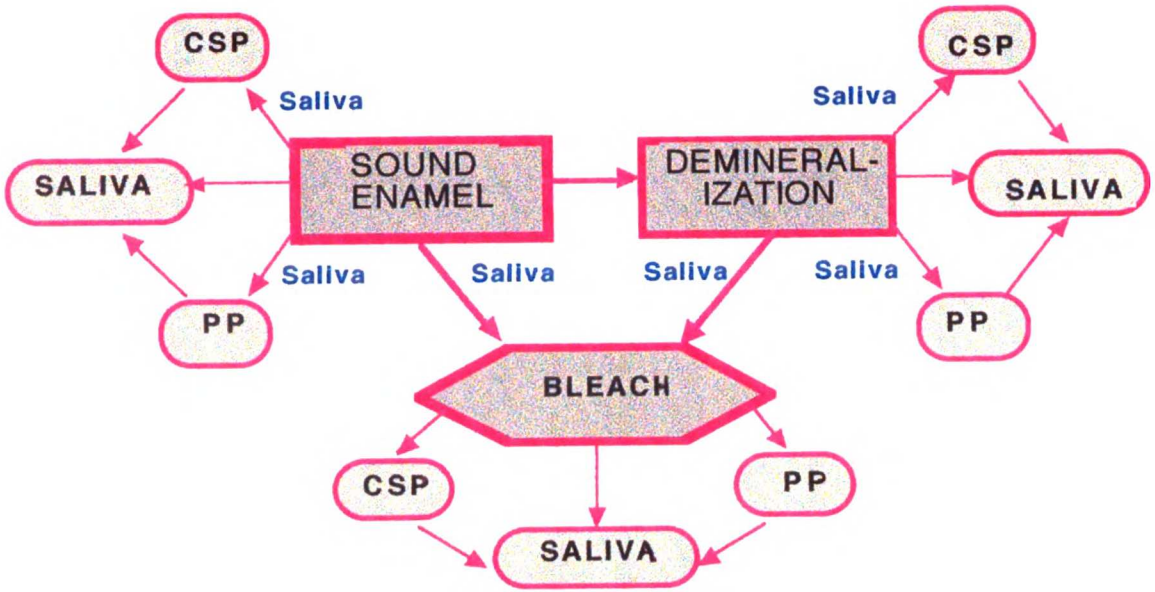


Figure 10: Study design represented as a flow diagram. All samples start as sound enamel and then follow one of twelve different treatment protocols. Saliva immersion of sound enamel samples and of demineralized samples is depicted by the word saliva written along the arrow to the next treatment stage. [CSP= calcium sucrose phosphate, PP= pentapeptide]

C.2.2.1. Demineralization Phase

Demineralization solution preparation

Subsurface caries-like lesions were created using a carbopol and hydroxylapatite (HAP) artificial caries demineralization solution developed by White[8]. The protocol to make 1 liter (L) of this solution involved adding approximately 700 mL of double deionized water (DDW) to a 1 L volumetric flask. 100 mL of 1.0 mol/L lactic acid stock solution, previously made and stored for at least one month, was then added. The storage time for the stock solution allows the polymers that are present in analytical grade concentrated lactic acid to be diluted and broken up. A pH meter (TIM 900 titration manager, TitraLab™, Copenhagen) was standardized using pH 4.00 ± 0.01 and pH 7.00 ± 0.01 (Fisher) certified buffer solutions. After standardization, the meter was used to monitor the adjustment of the pH of the lactic acid/DDW solution to 5.0 using 10% NaOH (Fisher). DDW was then added to the solution to made it up to 1 L. The solution was transferred to a beaker and 0.5g of hydroxyapatite (Calbiochem) was added. Stirring was carried out for ten minutes with a magnetic stir bar, followed by readjustment of the pH to 5.0 in the manner described previously. The solution was then covered and allowed to continue stirring overnight to ensure complete solution of the apatite up to the level of saturation. After stirring, the solution was filtered by vacuum on a Buchner funnel using #42 filter paper (Whatman Limited, England) to remove the excess apatite, leaving a solution saturated with respect to hydroxyapatite (HAP).

2.0g of Carbopol 907 (B. F. Goodrich Chemical Co.) was weighed out on an electronic balance (Mettler PM2000, Mettler-Toledo, Inc., Switzerland), then added to approximately 300 mL of DDW in a beaker while stirring. Stirring

continued for at least three hours to dissolve the carbopol, followed by readjustment of the pH to 5.0 with NaOH.

500 mL of the saturated HAP/lactate buffer was put in a 1 L beaker and approximately 300 mL of the carbopol solution was slowly added while stirring. The pH was once again adjusted to 5.0, with either 10% NaOH or 2% HCl. The stir bar was removed, the solution transferred to a volumetric flask, and made up to 1 L with DDW to produce a solution 50% saturated with respect to HAP. The solution was thoroughly mixed, the pH tested and readjusted, if necessary. The completed carbopol and HAP artificial demineralization solution was then transferred to a plastic reagent bottle and stored with added thymol crystals. Calcium analysis was performed on the solution. (refer to section D.2.1)

Demineralization of samples

Subsequent to baseline spectral measurements for color reflectance (refer to section C.2.3.), prepared samples were painted on the internal (cut) surfaces with nail varnish. The enamel window was cleaned by rubbing with moistened grade 2 pumice (0.776% Ca, 0.716% Mg, 1.599% Na, 1.752% K, 14.262% Al_2O_3, 69.610% SiO_2; Kerr Corp., Romulus, Michigan) on a cotton swab. The sample was then double rinsed with DDW and wiped with moist gauze. The samples were transferred to individual flat bottomed plastic vials and each immersed in 30 mL of the carbopol/HAP demineralization solution with the enamel window facing upward. The pH of the carbopol/HAP solution was tested. The samples were incubated at 37° C for 48 hours. After the first 24 hours, the samples were checked for proper positioning in the vial (window facing upward). The pH was again tested after incubation to ensure that the amount of demineralization had not affected the pH balance. The demineralized samples were rinsed with DDW, wiped with

gauze, and immersed in 5 mL of whole saliva. (refer to section C.2.2.2.) Color measurements were postponed until after saliva treatment.

C.2.2.2. Whole Saliva Phase--Pre-treatment

Whole Saliva Preparation

Saliva was collected from a single donor (TRF). The donor brushed with a fluoride dentrifice within 15 minutes of beginning saliva collection and thoroughly rinsed with water. Saliva production was stimulated by chewing on parafilm[s], producing approximately 1 mL per minute. The saliva was collected in a 50 mL centrifuge tube on ice, then centrifuged for 20 minutes at 3600 RPM. The supernatant was poured off and thymol crystals were added. The whole saliva was refrigerated no more than 24 hours before use to minimize protein degradation.

Saliva treatment of samples

Prior to immersion, the demineralized and sound enamel samples were first rinsed with DDW and wiped with gauze to remove any potential residue. The samples were then placed in 5 mL of whole saliva in a flat bottomed vial, with the enamel window facing upward, and incubated at 37° C for 24 hours. The samples were treated with saliva to produce a protein coating (i.e. artificial pellicle) that would mimic an *in vivo* situation. After incubation, the samples were brushed with an Ivory™ soap solution, rinsed with DDW, wiped with gauze, and returned to a 100% humidity environment. Color spectra over the visible light wavelengths were taken at this time. (refer to section C.2.3.)

C.2.2.3. Sodium Hypochlorite (NaOHCl) Phase--Surface Treatment

Samples were cleaned with moist pumice on a cotton swab (as described in section 2.2.1.), rinsed with DDW, and dried with compressed air. The enamel window was dabbed with a 5.25% NaOHCl impregnated cotton pellet. This treatment was carried out to remove the protein coating on the enamel surface. The bleach was allowed to sit on the tooth surface for 10 minutes prior to rinsing with DDW for 30 seconds. The samples were then returned to a 100% humidity environment. Color measurements (refer to section C.2.3.) were taken before proceeding to the next phase of the experiment.

C.2.2.4. Calcium Sucrose Phosphate (CSP) and Calcium Phosphopeptide (PP) Phase--Chemical Treatment

The teeth were rinsed with DDW and dried with compressed air prior to chemical treatment. Depending on the treatment the sample was assigned, either 10% CSP (*Fluoran*, Creighton Pharmaceuticals, Sidney, Australia) or PP (*Topacal™*, Nulite Systems International, Hornsby, Australia) was applied using a cotton pellet to provide complete coverage of the enamel window. The chemical was allowed to diffuse for 5 minutes. After such time, the excess chemical was wiped off with a cotton gauze, but was not rinsed. The sample was then immediately immersed in 5 mL of whole saliva in an individual vial. (refer to section C.2.2.5.) No optical data collection was carried out on these samples until after said saliva treatment was complete.

C.2.2.5. Whole Saliva Phase--Post-treatment

All samples were immersed individually in 5 mL of whole saliva as a final step to mimic oral exposure. Teeth that had chemical treatment were not rinsed or stored prior to saliva treatment to simulate an actual patient treatment protocol. The whole saliva was prepared as described in section C.2.2.2. The samples were incubated at 37° C for 24 hours in individual flat bottomed vials, with the enamel window facing upward. After incubation, the samples were brushed with an Ivory™ soap solution, rinsed with DDW, wiped with gauze, and returned to a 100% humidity environment. Final optical measurements were taken at this time. (refer to section 2.3.)

C.2.3. OPTICAL MEASUREMENTS AND DATA COLLECTION

C.2.3.1. Instrumentation and technique

A Lambda 19 model UV/VIS/NIR spectrometer and UVDM software (Perkin Elmer Corporation) were used to collect reflectance spectra in the visible light region of the spectrum (400-700 nm). The following parameters were used:

Ordinate	Mode:	Reflectance
	Scale Limit:	Max 100, Min 0
Abscissa	Range:	Max 700 nm, Min 400 nm
	Data Interval:	1 nm
	Split Sensitivity:	1nm/3
	Lamps:	D2-on, W-on
Instrument	Scan Speed:	480 nm/min
	Smooth:	10 nm

The instrument was turned on and the lamps allowed to stabilize for at least one hour before initializing the instrument. After initializing (irises open, lens in open position) and background correcting (irises closed to 1.5, lens in reflectance position, 4 mm diameter mask in sample holder port), a standard spectralon was scanned to obtain a 100% line, followed by 99% and 50% standard disks. The 99% and 50% standards were run again, but in the opposite order, at the end of each session to allow for evaluation of the instrument accuracy and stability.

Pecol software (Perkin Elmer Corporation) was used to convert the reflectance spectra to CIE L*a*b values (refer to section B.6.1.3. for definition) The following parameters were used:

Evaluation mode:	cLab	
Observer:	10	(what 10 standard observers would report for difference)
Illuminant:	D65	(equivalent to outside daylight)

UVDM software (Perkin Elmer Corporation) was utilized to convert the spectra to ASCII files for statistical analysis.

C.2.3.2. Sample optical data collection

All samples in all groups had a reflectance spectrum at baseline and after saliva and sodium hypochlorite treatments. (refer to sections C.2.2.2., C.2.2.3., and C.2.2.5.) Nail varnish was removed from the internal surfaces of the sample with acetone prior to spectra collection. To minimize reflectance variability due to desiccation, samples were dried with compressed air and then allowed to air dry for 1 hour prior to collecting reflectance spectra. (see section D.1.3.)

The tooth samples were mounted individually in a jig prepared for that specific sample. The jig was marked in three places to align with a cross-hair grid on the back of the mask in the sample port to enable reproducible positioning. (see figure 9) These sample orientation markings were done prior to collecting the baseline spectra.

One sample from each group was selected to have a second run on a separate day for each treatment phase. This was done to allow evaluation of reproducibility of technique (sample drying, sample and mask positioning, instrument set-up, etc.) and constancy of the spectrophotometer. (refer to section D.2.1.)

C.2.3.3. Statistics

The sample spectra were converted to L*a*b* values to evaluate color differences between treatment steps. Due to the multiple measurements for each sample, a repeated measures ANOVA was used to analyze the changes in L*a*b values after each treatment. ΔE was calculated using the equation $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Standard error measures were corrected for multiple comparisons using the Tukey-Kramer calculation. Findings were considered significant when $p<0.05$.

The independent variables in this study were the treatments and the sequence of the treatments. The dependent variables were the color spectra, which were converted to L*a*b* values.

Standards were run at the beginning and end of each spectra collection session to test for the constancy of the instrumentation. To analyze the variability of the standards both a paired t-test and a signed-rank test were carried out comparing each standard pre- and post- for a given session. The level of significance remained at $p<0.05$.

Repeatability was tested by running selected samples twice, on separate days, after each treatment step. A t-test was used to determine if the technique and instrumentation was repeatable.

D. Results

D.1. PILOT STUDIES

D.1.1. DEMINERALIZATION TIMES

Sound tooth samples were demineralized (according to the protocol outlined in Section C.2.2.1.) for either 24, 48 or 72 hours to determine the optimal amount of demineralization for this study. It was determined by visual assessment and spectral reflectance profiles that 24 hours of incubation in the demineralization solution followed by 24 hours of incubation in whole saliva produced an insufficient color change for the purposes of this study. Conversely, seventy-two hours of demineralization resulted in a marked color change, but was also found to produce changes in surface texture. The reflectance spectra were affected by the surface texture variation, making analysis of color changes more obscure. A demineralization time of 48 hours was determined to be best suited for the purpose of this study. It produced a color change detectable by the instrumentation without producing pronounced surface texture changes. (see figure 11)

D.1.2. SAMPLE POSITIONING FOR SPECTRA

In order to determine if sample positioning was an influential factor in the spectra and L*a*b* values obtained, two samples were placed in their individual jigs and rotated 360° in 90° increments. The resultant spectra and L*a*b* values

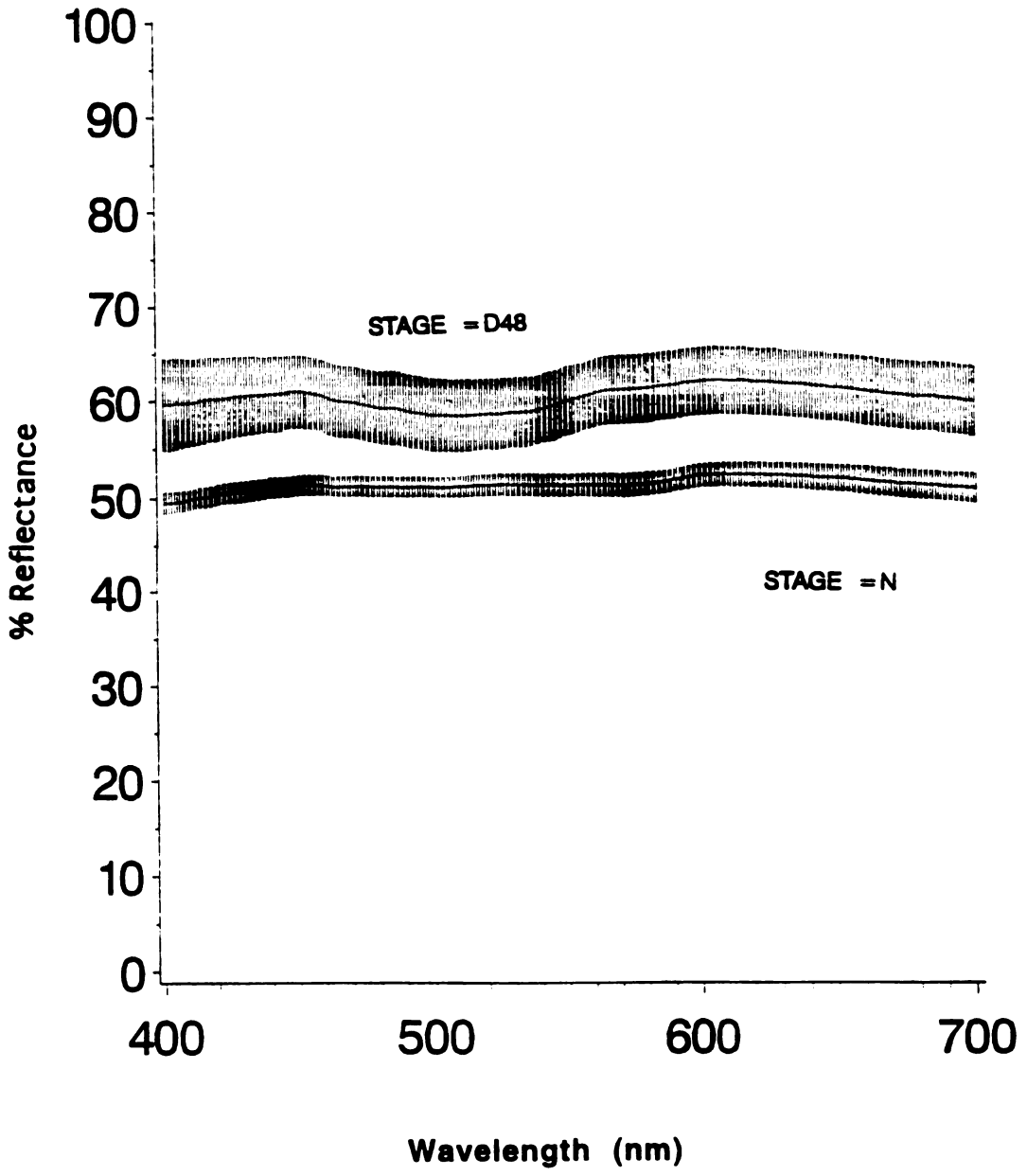


Figure 11: 95% confidence intervals from pilot studies for sound enamel (stage =N) and 48 hours of artificial demineralization (stage =D48) demonstrating no overlap in % reflectance over the visibile spectrum (400-700 nm).

were evaluated for changes with regard to position. It was observed that the spectral profiles and numerical values at 0° and 360°, which are identical positions, were the most similar. From this it was determined that sample positioning was an important factor that must be controlled throughout the experiment. (refer to section C.2.3.2)

D.1.3. SAMPLE DRYING TIMES FOR SPECTRA

It was observed that the percentage reflectance increased across the entire visible spectrum as the drying time of a sound enamel tooth sample increased. The slope of the overall spectral profile remained primarily the same, indicating that certain wavelength regions did not affect the magnitude of reflectance disproportionately. This finding is in apparent contradiction to an earlier study which found no change in L* for wet versus dry fluorosed tooth samples.[70] This may be due to fluorosed enamel responding differently to drying than sound or decalcified enamel, or may be contributed to the instrumentation used for measurement. It is, however, known that "whiteness" of WSL's increases with drying.[2]

In order to obtain predictable results, drying time trials were done to determine the time at which the reflectance spectra stabilized. A tooth sample was removed from a 100% humidity environment. Reflectance spectra were obtained after 2, 5, 10, 15, 20, 25, 30, and 40 minutes of air drying. The sample was left in the same position for each of the runs. The lid to the integrating sphere of the spectrophotometer was opened in between runs to allow for air drying. Observation of the time trial spectra demonstrated that the percentage reflection profiles largely stabilized after 30 minutes of air drying. (see figure 12) (refer to section C.2.3.2.)

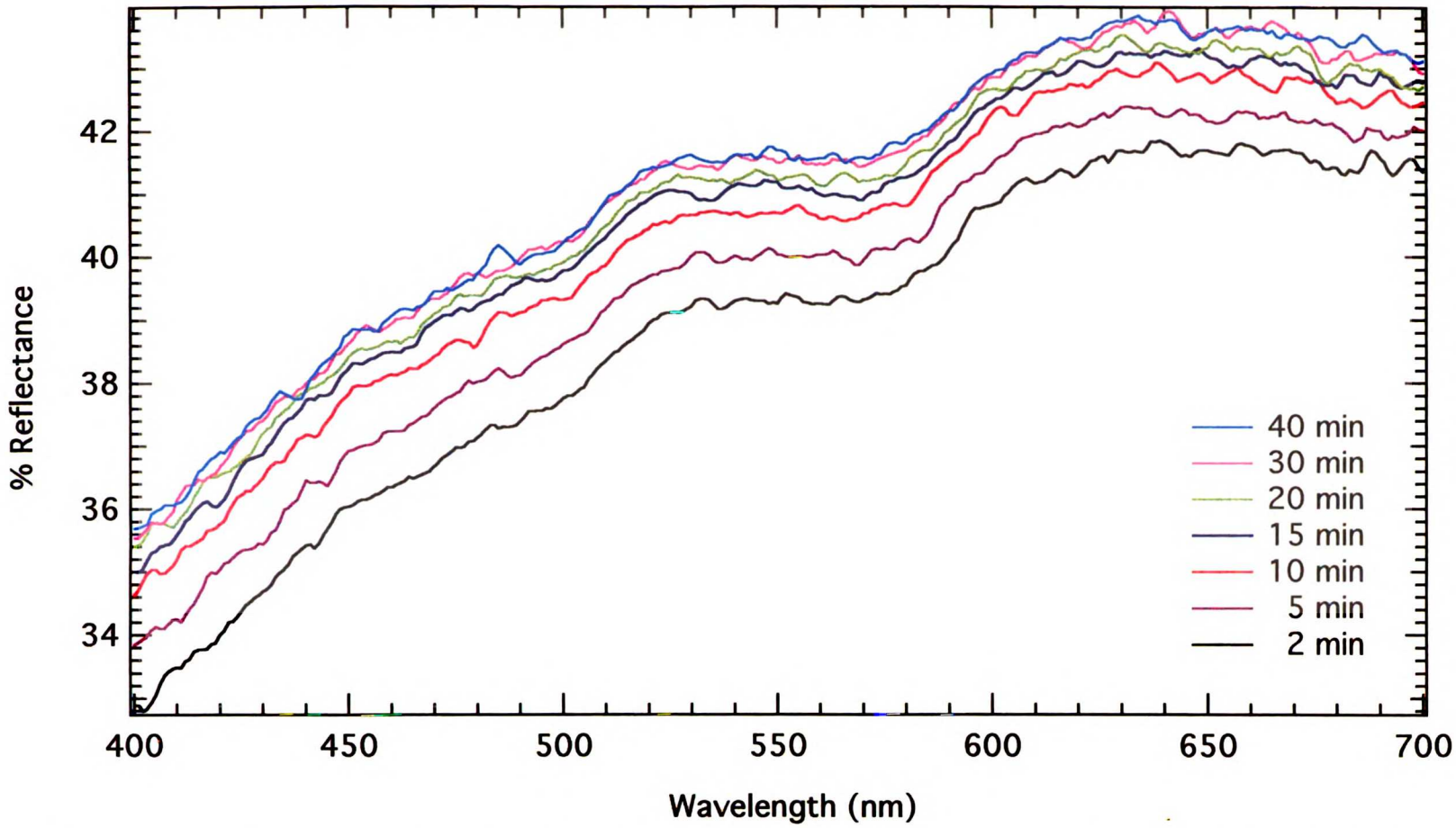


Figure 12: Spectra resulting from drying time trials on sound enamel. The same sample was used throughout the trial. Percentage reflectance is shown across the visible light spectrum (400-700 nm). Time designations represent amount of time the sample was dried. For clarity the 25 minute run was not included in the spectral overlays. The change in reflectance appears to stabilize after approximately thirty minutes.

D.1.4. SAMPLE SIZE DETERMINATION

Five samples were used in the pilot studies. Reflectance spectra were taken of the sound enamel and then again after every treatment stage. Treatments included artificial demineralization, followed by bleach or laser surface treatment, and finally calcium sucrose phosphate, pentapeptide, or saliva treatment. Power analysis, detecting differences at the 80% level with $p<0.05$, determined that a sample size of four was necessary to detect significant changes between samples when both the treatment stage and wavelength (400-700 nm in 1 nm increments) were considered. Using the same standards, it was calculated that a sample size of nine could distinguish significant differences when analyzing treatment stage only. Based on these calculations, nine samples were used for each group in each set.

D.2. MAIN STUDY

D.2.1. DEMINERALIZATION SOLUTION ANALYSIS

Calcium analysis was perfomed using gravimetric dilution. A Mettler Toledo balance was used for all measurements. Using a dilution factor (mL total/ mL solution), the demineralization solution was diluted 1:20 with 1000 ppm KCl. The KCl solution was used to reverse ionization of calcium under NO_2 flame conditions. Raw measurements for calcium concentration were converted to actual calcium concentration in ppm using the equation:

raw data (ppm) X dilution factor = actual [Ca] (in ppm)

Using this calculation it was determined that the demineralization solution contained 75.02 ppm Ca, which translates to 1.88 mmol/L.

Knowing that the demineralization solution protocol (refer to section B.4.) results in a 50:50 calcium and phosphate ratio in a saturated solution, the PO_4 concentration is calculated to be 1.12 mmol/L.

D.2.2. CONSTANCY OF INSTRUMENTATION

As previously stated, 99% and 50% standards with defined color parameters were run at the beginning and end of each spectra collection session to test the constancy of the equipment. The mean changes and standard deviations for each of the parameters, L*a*b*, are depicted in the table below. The numbers indicate that L* experienced the greatest change (-0.929 for the 50% standard, -0.911 for the 99% standard); however, the magnitude is still considerably smaller than the values required to determine significant differences for samples between treatment stages.

The pre and post data from each session were compared using both a paired t-test and a signed rank test, producing similar results. Despite the small magnitude of change, the consistency in direction, showed enough "drift" during the collection session to produce significant changes for all parameters for the 99% standard and all but b* for the 50% standard. Considering the magnitude of change required for sample treatments to demonstrate significance this small amount of instrument "drift", although demonstrating significance, is not of major concern. (see table 1)

D.2.3. REPRODUCIBILITY

In each of the 12 groups of nine samples, one sample was designated for reproducibility runs at each treatment stage. (refer to section C.2.3.2.) While ΔE did show a significant difference between the first run and the second run, this

Variability of Standards

Variable	N	Mean	Std Dev	Minimum	Maximum
SS1_L	17	105.4623529	3.0062072	100.0900000	110.5700000
SS1_A	17	-0.2158824	0.1582347	-0.5200000	0.0500000
SS1_B	17	1.1982353	0.5100519	0.3600000	1.8000000
99-1_L	17	106.4117647	2.9998442	100.5600000	111.3700000
99-1_A	17	-0.1670588	0.1713463	-0.4500000	0.1300000
99-1_B	17	0.9776471	0.5121588	0	1.6100000
99-2_L	16	105.5843750	2.9630772	100.0500000	110.4400000
99-2_A	16	-0.0950000	0.1633809	-0.3100000	0.1100000
99-2_B	16	1.0818750	0.5394963	0.0300000	1.8200000
50-1_L	17	85.2758824	3.1407066	79.0200000	90.2600000
50-1_A	17	-0.4441176	0.1823136	-0.6900000	-0.0900000
50-1_B	17	0.6476471	0.5011179	-0.3100000	1.2400000
50-2_L	17	84.3464706	2.7995981	78.7300000	88.3100000
50-2_A	17	-0.3841176	0.1798978	-0.6800000	-0.0400000
50-2_B	17	0.6670588	0.5142442	-0.2400000	1.4100000

						T-STATISTIC		WILCOXON	
VALUE	MEAN	SD	MEDIAN	MIN	MAX	STATISTIC	P-VALUE	STATISTIC	P-VALUE
L50: post-pre	-0.929	0.652	-0.720	-2.570	-0.030	-5.878	0.000	-76.50	0.000
A50: post-pre	0.060	0.096	0.050	-0.100	0.270	2.574	0.020	49.500	0.017
B50: post-pre	0.019	0.139	0.000	-0.370	0.180	0.575	0.573	20.500	0.304
L99: post-pre	-0.911	1.152	-0.790	-3.320	2.220	-3.164	0.006	-54.00	0.003
A99: post-pre	0.071	0.113	0.035	-0.110	0.270	2.511	0.024	43.000	0.024
B99: post-pre	0.116	0.217	0.055	-0.150	0.820	2.135	0.050	45.500	0.016

Table 1: Statistics for the variability of the standards (SS= standard spectralon, 99-1 represents the 99% standard run at beginning of session, 99-2 represents the 99% standard run at the end of the session, 50-1 represents the 50% standard run at the beginning of the session, 50-2 represents the 50% standard run at the end of the session; L=L*, A= a*, B= b*)

Sample Reproducibility Experiment

Measurement	Mean Difference Between Repetitions	Standard Error	df	T	Adjustment	Adjusted p-value
L (corrected)	-0.132	0.399	34	-0.33	Tukey-Kramer	0.7425
A (corrected)	0.221	0.129	34	1.71	Tukey-Kramer	0.0967
B (corrected)	-0.065	0.234	34	-0.28	Tukey-Kramer	0.7837
Delta E (from previous)	2.041	0.395	22	5.17	Tukey-Kramer	0.0000
Delta E (from initial)	0.859	0.522	22	1.65	Tukey-Kramer	0.1137

Table 2: Reproducibility statistics for samples which were rerun at each stage.

represents the accumulation of multiple small changes. When considering, L*a*b* as individual parameters, there were no significant differences between the original and repeat run. The p values for L*a*b* were 0.74, 0.10, and 0.78, respectively. (see table 2)

D.2.4. SET 1-SOUND ENAMEL PLUS CHEMICAL TREATMENT

This group consisted of three treatment groups of nine samples each.

Group 1: Sound enamel treated with saliva (24 hours).

Group 2: Sound enamel pretreated with saliva (24 hours), then treated with CSP (5 minutes) followed by saliva immersion (24 hours).

Group 3: Sound enamel pretreated with saliva (24 hours), then treated with PP (5 minutes) followed by saliva immersion (24 hours).

Reflectance spectra were taken at baseline (sound enamel) and again after the final treatment (saliva, CSP/saliva, PP/saliva). No data was collected after the saliva pretreatment for groups 2 and 3, with the assumption that the information from group 1 could be extrapolated to explain findings in these groups. It was further presupposed that the saliva would not create significant changes in the reflectance spectra. (see figure 13)

•Single Step Comparisons

Sound enamel to saliva treatment stage: (see figure 13) The only single step comparison available for this set is in group 1. No significant change in color parameters were found in this group for treatment of sound enamel with saliva. While this finding for L* is consistent with that of Den Besten and Giambro for sound enamel treated with artificial saliva,[70] they are inconsistent with those of

Set 2/group 1 (sound enamel to saliva pretreatment stage). It is likely that the small sample sizes contribute to this variation between groups receiving the same treatment.

•Multiple Step Comparisons

Sound enamel to either CSP or PP treatment stage: (see figure 13) CSP and PP groups (groups 2 and 3) had similar findings and will be discussed jointly. ΔL^* was significantly negative for these groups. This indicated that the enamel appeared less white. Unless this change can be attributed to the saliva pretreatment, this result is not entirely desirable since the treatment is intended to affect the color of only demineralized enamel, leaving sound enamel unchanged. Without data from the saliva pretreatment stage, it is difficult to interpret the influence of the CSP and PP beyond the contribution of the saliva pretreatment. This problem is accentuated by the fact that results in this and other sets for saliva's color effects on sound enamel conflict and are ambiguous.

Δb^* was found to be consistently negative for group 2 (CSP), but not group 3 (PP). The Δa^* was only weakly influenced with both treatment protocols.

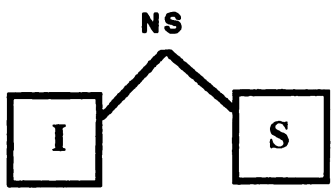
D.2.5. SET 2-SOUND ENAMEL WITH BLEACH PRETREATMENT AND CHEMICAL TREATMENT

This set was divided into three groups (nine samples each) as follows:

Group 1: Sound enamel pretreated with saliva (24 hours), surface treated with bleach (10 minutes) and then treated with saliva (24 hours).

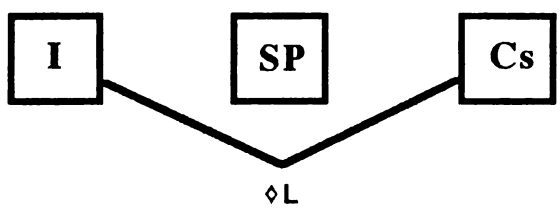
SET 1-SOUND ENAMEL

Group 1 Saliva only



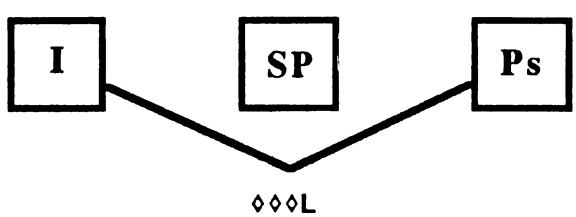
	ΔL*	Δa*	Δb*
I to S	-0.2	0.0	0.3

Group 2 CSP plus saliva



	ΔL*	Δa*	Δb*
I to Cs	-2.3	-0.3	-1.0

Group 3 PP plus saliva

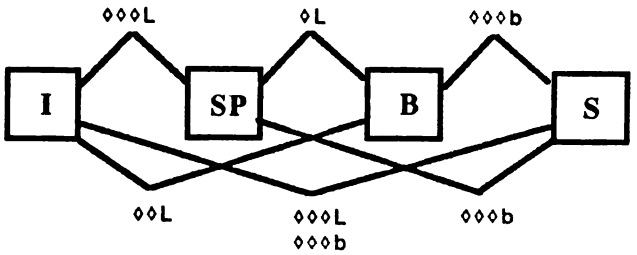


	ΔL*	Δa*	Δb*
I to Ps	-4.7	-0.1	-0.3

Figure 13: Sound enamel samples. The values for changes in L*a* b* are listed in the table on the right for set 1, groups 1, 2, and 3. Values in red represent significant findings. The level of significance is represented on the diagram to the left. (◊= $p<0.05$, ◊◊= $p<0.01$, ◊◊◊= $p<0.001$) [I= initial stage, S= saliva treatment, Cs= CSP treatment followed by saliva immersion, Ps= PP treatment followed by saliva immersion] Standard error values are shown in the appendix.

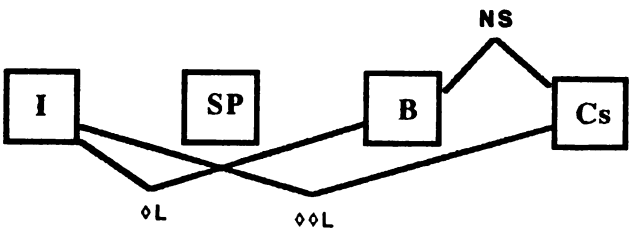
SET 2: SOUND ENAMEL, BLEACH TREATED

Group 1 Saliva only



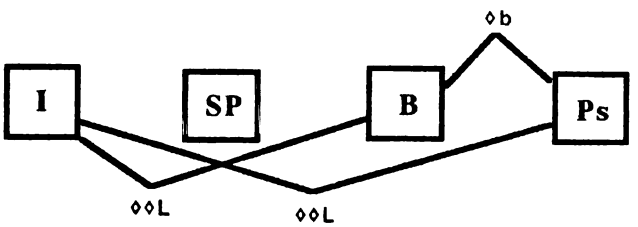
	ΔL*	Δa*	Δb*
I to SP	-5.7	0.0	0.7
SP to B	2.5	-0.3	-0.5
B to S	-0.7	-0.1	-1.7
I to B	-3.2	-0.3	0.3
I to S	-3.9	-0.4	-1.4
SP to S	1.8	-0.4	-2.2

Group 2 CSP plus saliva



	ΔL*	Δa*	Δb*
B to Cs	0.1	-0.1	-0.9
I to B	-4.2	-0.1	0.6
I to Cs	-4.1	-0.2	-0.3

Group 3 PP plus saliva



	ΔL*	Δa*	Δb*
B to Ps	0.4	0.1	-1.2
to B	-4.2	-0.5	0.0
to Ps	-3.8	-0.4	-1.2

Figure 14: Sound enamel samples with bleach surface treatment. The mean values for changes in L*a* b* are listed in the table on the right for set 2, groups 1, 2, and 3. Values in red represent significant findings. The level of significance is represented on the diagram to the left. (◊= $p<0.05$, ◊◊= $p<0.01$, ◊◊◊= $p<0.001$) Stages written in green represent treatment stages at which no data was collected. [I= initial stage, SP= saliva pretreatment, B= bleach surface treatment, S= saliva treatment, Cs= CSP treatment followed by saliva immersion, Ps= PP treatment followed by saliva immersion] Standard error values for the individual measures are shown in the appendix.

Group 2: Sound enamel pretreated with saliva (24 hours), surface treated with bleach (10 minutes), then treated with CSP (5 minutes) followed by saliva immersion (24 hours).

Group 3: Sound enamel pretreated with saliva (24 hours), surface treated with bleach (10 minutes), then treated with PP (5 minutes) followed by saliva immersion (24 hours).

Reflectance spectra were taken at baseline (sound enamel) and again after bleach surface treatment and final treatment (saliva, CSP/saliva, PP/saliva). As in Set 1, data was collected after the saliva pretreatment only for group 1, with the assumption that the information from Set 2/group 1 could be extrapolated to explain findings in the remaining groups, 2 and 3.

•Single Step Comparisons

Sound enamel to saliva pretreatment stage: Sound enamel pretreated with saliva demonstrated a significant negative change in L*. As previously discussed in section D.2.4., this finding is greatly magnified compared to that of Set 1/group 1 which displayed a minimal and insignificant change in L* when sound enamel was treated with saliva. This is inconsistent with Den Besten and Giambro's study, as well, which utilized artificial saliva.[70] No change was detected for a*. The Δb^* was consistently positive, however, not statistically significant.

Saliva pretreatment stage to bleach surface treatment stage: Data is only available for group 1 of this set. ΔL^* demonstrates a significantly positive change between these stages indicating that the tooth sample becomes more white. This is inconsistent with findings from a previous study of fluorosed, naturally demineralized, and sound enamel which show no significant change in L*.[70] (It is of note that the cited study did not include saliva pretreatment of the samples.)

The changes in a* and b* are consistently negative. These changes, however, are not statistically significant.

Bleach surface treatment to saliva treatment stage (Group 1): Minimal change was seen in L* and a* between these treatment stages. The change in b* was significantly negative.

Bleach surface treatment to CSP plus saliva treatment stage (Group 2): No significant changes were seen in any of the parameters for this group. The change in b*, however, demonstrated a consistently negative trend. These findings demonstrate similarity to group 1 of this set, indicating that CSP does not affect bleach treated sound enamel in a markedly different way than saliva alone.

Bleach surface treatment to PP plus saliva treatment stage (Group 3): In accordance with the findings in Set 1/group 1, L* and a* demonstrate minimal change, while b* was significantly negative. As in group 2, no effect can be attributed solely to PP from these findings.

•Multiple Step Comparisons

Initial to bleach surface treatment stage: When considering the groups individually, only the change in L* demonstrated significance, with a negative change between stages. While this finding seems to indicate that the enamel appears less white with bleach treatment, it must be considered that saliva pretreatment was performed between these stages, which produced a significant negative change in L*. Extrapolating from the data in group 1 would suggest that the negative influence of the saliva pretreatment was partially, but not completely, reversed by the bleach treatment. The saliva coated (pretreated) tooth represents the more "natural" situation of a protein pellicle; therefore, the effect of bleach on enamel should be considered more accurate from the saliva pretreated stage versus the initial.

Initial to saliva only treatment stage (Group 1): The overall treatment effect in this group demonstrated a negative trend for all three color parameters, with the change in L* and b* being significant.

Saliva pretreatment to saliva only treatment stage (Group 1): A positive change in L* (more white overall) and negative change for a* and b* were found. Only the change in b* was significant. The finding for L* is in contrast to that found for the initial to saliva only treatment stage for this group. This discrepancy is due to the significant effect of the saliva pretreatment on L*. Again, the findings from the saliva pretreatment stage should be considered to more accurately describe the "natural" situation for the reasons already cited.

Initial to CSP plus saliva treatment stage (Group 2): A significant negative change in L* was found, with little concomitant change in a* and b*. These findings are similar to Set 2/group 1 for L*, but do not elucidate the negative changes for a* and b* found in group 1. This may be an actual difference or simply due to the small sample size being insufficient to demonstrate stronger trends due to single or minimal aberrations. Overall, no comment can be made regarding the treatment effects of CSP plus saliva being markedly different than that of saliva alone.

Saliva pretreatment to CSP plus saliva treatment stage (Group 2): There is no data available for this group regarding the overall changes from the saliva pretreatment stage to the CSP plus saliva treatment stage.

Initial to PP plus saliva treatment group (Group 3): L*, a*, and b* all demonstrate a negative trend over these treatment stages. L* is the singularly significant change, which is the same as Set 2/group 2. This indicates that not only does PP not have a detectably different treatment effect from CSP, but it can not be clearly distinguished from saliva alone, either.

Saliva pretreatment to PP plus saliva treatment stage (Group 3): There is no data available for this group regarding the overall changes from the saliva pretreatment stage to the PP plus saliva treatment stage.

D.2.6. SET 3-DEMINERALIZED ENAMEL PLUS CHEMICAL TREATMENT

This group consisted of three treatment groups of nine samples each.

Group 1: Sound enamel artificially demineralized (24 hours) then immersed in saliva for surface remineralization (24 hours), then treated with saliva (24 hours).

Group 2: Sound enamel artificially demineralized (24 hours) and immersed in saliva for surface remineralization (24 hours), then treated with CSP (5 minutes) followed by saliva immersion (24 hours).

Group 3: Sound enamel artificially demineralized (24 hours) and immersed in saliva for surface remineralization (24 hours), then treated with PP (5 minutes) followed by saliva immersion (24 hours).

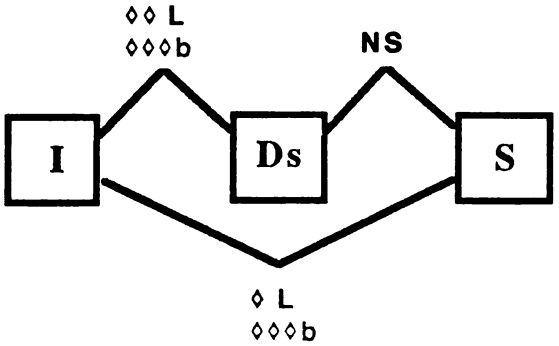
Reflectance spectra were taken at three time points: baseline (sound enamel), after the sample had been de- and re-mineralized (demineralization stage), and again after the final treatment (saliva, CSP/saliva, PP/saliva). (see figure 15)

•Single Step Comparisons

Initial to demineralization with saliva remineralization stage: When the groups are considered individually, not all of the color parameters demonstrate significant changes. When all of the demineralized samples in sets 3 and 4 are analyzed collectively, however, all the parameters are significantly affected (L*

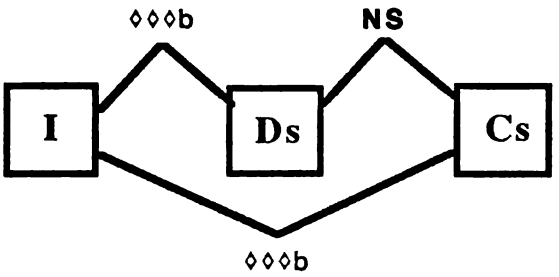
SET 3: DEMINERALIZED ENAMEL

Group 1 Saliva only



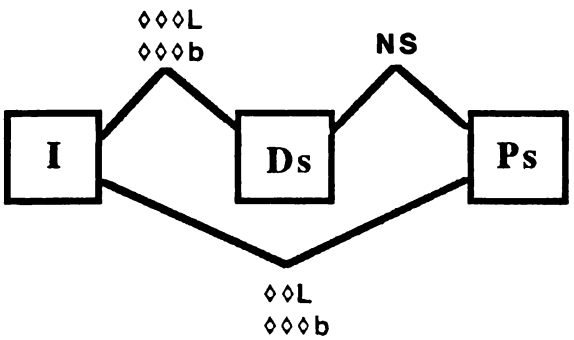
	ΔL*	Δa*	Δb*
I to Ds	4.1	-0.3	-5.5
Ds to S	-1.1	0.0	0.6
I to S	3.1	-0.3	-5.0

Group 2 CSP plus saliva



	ΔL*	Δa*	Δb*
I to Ds	2.7	-0.7	-4.9
Ds to Cs	-0.3	0.1	0.7
I to Cs	2.4	-0.5	-4.2

Group 3 PP plus saliva



	ΔL*	Δa*	Δb*
I to Ds	4.7	-0.5	-4.4
Ds to Ps	-1.3	-0.2	0.6
I to Ps	3.4	-0.8	-3.8

Figure 15: Demineralized enamel samples. The mean values for changes in L*a*b* are listed in the table on the right for set 3, group 1, group 2, and group 3. Values in red represent significant findings. The level of significance is represented on the diagram to the left. (◊= $p<0.05$, ◊◊= $p<0.01$, ◊◊◊= $p<0.001$, NS= not significant) [I= initial stage, Ds= demineralization followed by saliva immersion, S= saliva treatment, Cs= CSP treatment followe.d by saliva immersion, Ps= PP treatment followed by saliva immersion] Standard error values for the individual measures are presented in the appendix.

becomes more positive, a* and b* become more negative). This elucidates the problem of the small sample sizes used in this study failing to show significant changes that may in fact exist (i.e. Type II error in statistical terms).

Demineralization with saliva remineralization to saliva only treatment stage (Group 1): No significant changes were seen between these treatment stages. A negative trend (sample became less white) was seen for L* and a positive trend was seen for b*. Both of these trends showed a reversal of the changes brought about by demineralization, which indicates that saliva was partially reversing the color changes due to the initial demineralization. This finding was expected since saliva is well documented to assist in enamel remineralization.[57, 102]

Demineralization with saliva remineralization to CSP plus saliva treatment stage (Group 2): The only clear trend for this treatment group was a positive change for b*, which did indicate a reversal of the demineralization's effect on the b* color parameter. As in group 1, no findings were significant. No significant differences in color can be attributed to the CSP treatment, nor does CSP demonstrate any differences from saliva treatment alone.

Demineralization with saliva remineralization to PP plus saliva treatment stage (Group 3): The findings for group 3 correspond to those of group 1 (trend for negative ΔL^*, positive Δb^*). This again demonstrates some ability of the treatment regimen to reverse the color effects of demineralization on enamel. Any additive or subtractive contribution of PP beyond that of saliva alone is not distinguishable.

•Multiple Step Comparisons

Initial to saliva only treatment stage (Group 1): The findings are consistent with those seen for the single step comparison of the initial to the demineralized

stage for group 1 (significantly positive ΔL^*, trend towards negative Δa^*, significantly negative Δb^*). This indicates that the overall effect on the samples was greatly influenced by the demineralization with minimal reversal attributable to the saliva treatment.

Initial to CSP plus saliva treatment stage (Group 2): As in group 1, the findings in group 2 for this multiple step comparison conform to the group's single step comparison of the initial to the demineralized stage. Again, this indicates that the CSP plus saliva treatment had little effect on the demineralized enamel, leaving the enamel samples esthetically compromised.

Initial to PP plus saliva treatment stage (Group 3): In keeping with the pattern established for the overall treatment effect in set 3, these findings do not differ from those found after the demineralization step in this group. Overall, the enamel sample has undergone significant color differences from the initial state, indicating that the treatment to reverse demineralization was insufficient.

D.2.7. SET 4-DEMINERALIZED ENAMEL WITH BLEACH PRETREATMENT AND CHEMICAL TREATMENT

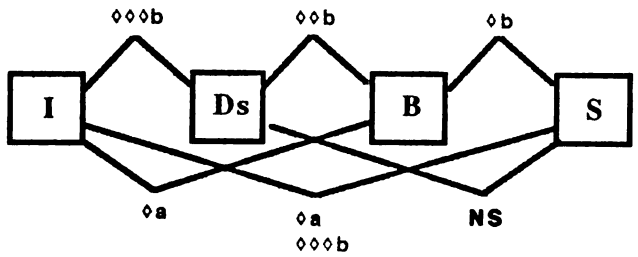
This group consisted of three treatment groups of nine samples each.

Group 1: Sound enamel artificially demineralized (24 hours) then immersed in saliva for surface remineralization (24 hours), surface treated with bleach (10 minutes), then treated with saliva (24 hours).

Group 2: Sound enamel artificially demineralized (24 hours) then immersed in saliva for surface remineralization (24 hours), surface treated with bleach (10 minutes), then treated with CSP (5 minutes) followed by saliva immersion (24 hours).

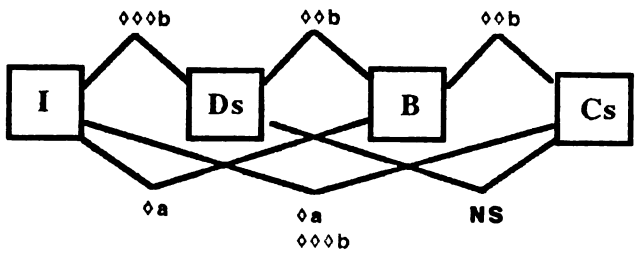
SET 4: DEMINERALIZED ENAMEL, BLEACH TREATED

Group 1 Saliva only



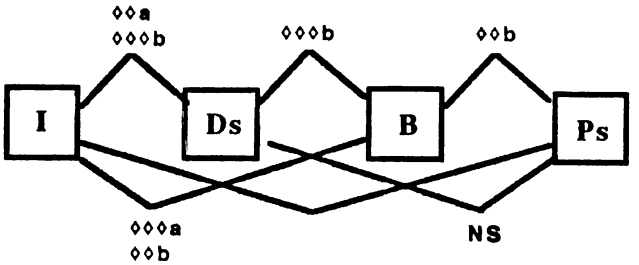
	ΔL*	Δa*	Δb*
I to Ds	2.6	-0.4	-4.3
Ds to B	0.4	-0.5	2.9
B to S	-2.8	0.0	-2.3
I to B	2.9	-0.9	-1.3
I to S	0.2	-0.9	-3.6
Ds to S	-2.4	-0.5	0.7

Group 2 CSP plus saliva



	ΔL*	Δa*	Δb*
I to Ds	1.3	-0.7	-3.6
Ds to B	-0.4	-0.5	2.3
B to Cs	-0.6	0.1	-2.0
I to B	1.0	-1.2	-1.3
I to Cs	0.4	-1.1	-3.2
Ds to Cs	-1.0	-0.4	0.3

Group 3 PP plus saliva



	ΔL*	Δa*	Δb*
I to Ds	2.2	-1.0	-4.9
Ds to B	0.5	-0.5	2.9
B to Ps	-1.4	0.3	-1.9
I to B	2.7	-1.4	-2.1
I to Ps	1.3	-1.1	-3.9
Ds to Ps	-0.8	-0.1	1.0

Figure 16: Demineralized enamel samples with bleach surface treatment. The mean values for changes in L*a* b* are listed in the table on the right for set 4, groups 1, 2, and 3. Values in red represent significant findings. The level of significance is represented on the diagram to the left. (◊= $p<0.05$, ◊◊= $p<0.01$, ◊◊◊= $p<0.001$) [I= initial stage, Ds= demineralization followed by saliva immersion, B= bleach surface treatment, S= saliva treatment, Cs= CSP treatment followed by saliva immersion, Ps= PP treatment followed by saliva immersion] Standard error values are shown in tables in the appendix.

Group 3: Sound enamel artificially demineralized (24 hours) then immersed in saliva for surface remineralization (24 hours), surface treated with bleach (10 minutes), then treated with PP (5 minutes) followed by saliva immersion (24 hours).

Reflectance spectra were taken at four time points: baseline (sound enamel), after the sample had been de- and re-mineralized, after bleach surface treatment, and again after the final treatment (saliva, CSP/saliva, PP/saliva). (see figure 16)

•Single step comparisons

Initial to demineralization with saliva remineralization stage: The direction of change for all three color parameters remains consistent amongst the groups and also amongst sets for demineralization. The statistical significance of the individual parameters varies between groups due to the small sample sizes. As stated previously for set 3, when the demineralized samples are grouped as a whole, all of the parameters are significantly changed, with the samples appearing more white.

Demineralization with saliva remineralization to bleach surface treatment stage: Groups 1, 2, and 3 showed a consistent negative trend for Δa^* and a statistically significant positive change in Δb^*. The effect on a* is additive to the effects of demineralization, while the effect on b* indicates some reversal of the color change associated with demineralization. The effect on L* was minimal, which is in contrast to the findings in set 2 with sound enamel that showed a statistically significant effect on L* from both the initial and saliva pretreatment stages. These contrasting findings would seem to indicate that bleach affects sound and demineralized enamel differently. This may be explained by the fact that the

demineralized samples are already whitened, so additional "whitening" with bleach may be inconsequential by comparison.

Bleach surface treatment to saliva only treatment stage (Group 1): Saliva treatment produces a trend for a negative ΔL^*, which indicates partial reversal of the demineralization color effects. A statistically significant negative Δb^* is also observed, which is additive to the demineralization effect on b*.

Bleach surface treatment to CSP plus saliva treatment stage (Group 2): In this group, only the difference in b* is statisticlly significant. This indicates a reversal of bleach's effect on the enamel color, but is not advantageous to correct the effect of demineralization on the enamel color.

Bleach surface treatment to PP plus saliva treatment stage (Group 3): L*, a*, and b* were changed in a negative, positive, and negative direction, respectively. Only Δb^* was a statistically significant difference, which is consistent with groups 1 and 2. The change in L* and a* demonstrated a partial reversal of the demineralization effects. The change in b* was additive to the demineralization change, which is undesirable.

•Multiple Step Comparisons

Initial to bleach surface treatment stage: ΔL^* was positive, Δa^* was negative, and Δb^* was negative over these treatment stages. In the individual groups, Δa^* is consistently statistically significant and Δb^* was statistically significant only for group 3. When the three groups (27 samples total) are combined, all three color parameters show highly significant modifications. The directions of change for the three parameters are the same as those for demineralized

enamel, indicating that the bleach did little to change the overall effect that demineralization had on the tooth.

Initial to saliva only treatment stage (Group 1): Both a* and b* are significantly altered in a negative direction. For a*, this appears to be due to the cumulative negative effect of demineralization and bleach treatment. For b*, it is due to the cumulative effect of demineralization and saliva treatment. This indicates that after the series of treatments, the samples were not returned to the original color due to alterations in two color parameters.

Initial to CSP plus saliva treatment stage (Group 2): As for group 1, the overall treatment result was a statistically significantly negative change in a* and b*.

Initial to PP plus saliva treatment stage (Group 3): Following the established pattern in groups 1 and 2, a* and b* showed statistically significant negative changes over the course of the treatments in group 3.

Demineralization to saliva only treatment stage (Group 1): A trend towards lower L* and a* values is seen between these multiple stages. Considering the effects of demineralization on enamel (I to Ds), it is observed that the effect on L* is partially reversed with saliva treatment, while the effect on a* is additive. Demineralization's effect on b* remains relatively unchanged overall.

Demineralization to CSP plus saliva treatment stage (Group 2): An aptitude towards reducing the L* value is seen for this group. As in group 1, this reversal of demineralization's effect on L* is only partial, but does indicate a desired treatment effect. It is not possible to attribute any real differences to CSP above those of saliva treatment alone based on the data.

Demineralization to PP plus saliva treatment stage (Group 3): The only clear tendency for this group was a negative change in b*, which is unique for set 4. L* did not show a clear trend due to a large standard error, which is attributable to an outlier in the data set. While the findings for this data group were slightly

varied from the other two groups in this set, they were minor and do not point to clear differences between treatments in color effects.

D.2.8. ΔE VALUES

Delta E's for each treatment stage from previous were significant for all sets of all groups. This indicates that changes in the individual parameters, as already described, resulted in changes that made the overall color significantly different after each treatment. This is a general overview and actual effects on the color parameters are best understood by taking the individual parameter changes into consideration.

D.2.9. CROSS GROUP COMPARISONS

D.2.9.1. Saliva Pretreatment

Although every group in sets 1 and 2 received saliva pretreatment, data is only available for Set 1/group 1 and Set 2/group 1. In Set 1/group 1, it is not termed "saliva pretreatment", but represents the same treatment (sound enamel treated with saliva). The data shows that, while the means of L* and b* show the same direction of change for both data sets, the standard errors in Set 1/group 1 make trends ambiguous. A reduction in the value (L*) is consistent with the remineralization associated with saliva.

D.2.9.2. Demineralization

As was discussed previously, the sample sizes for the individual groups did not always allow for significant changes to be seen with demineralization for all of the color parameters. When all demineralized samples were considered together (sets 3 and 4), L*, a*, and b* were all very significantly altered ($p<.0001$). The positive difference in L* is due to the chalky white appearance of the enamel. (see table 3)

Looking at whether DEMIN is different than initial

	N	Mean	SE	T Statistic	p-value
Change in L	54	2.93185	3.78805	5.6875	< 0.0001
Change in A	54	-0.60796	1.05607	-4.2304	0.0001
Change in B	54	-4.59296	1.75092	-19.2763	< 0.0001
Delta E	54	6.52742	2.39731	20.0085	< 0.0001

Table 3: This table shows the statistics for all demineralized samples from set 3 and 4 combined.

D.2.9.3. Bleach Surface Treatment

For sound enamel with a salivary pellicle (set 2), bleach significantly increased the L* value from the saliva pretreatment stage, making the tooth appear more white, and decreased the a* and b* values. The deviations in a* and b* were not statistically significant. The changes were opposite to those seen for saliva pretreatment (I to SP) for L* and b*. This reversal suggests that bleach removes the salivary pellicle and thereby changes the color of the tooth.

For demineralized enamel with a salivary pellicle (set 4), bleach has little effect on the L* value. This may be attributed to the tooth having reached the "outer limit" of the value (black to white) range with the demineralization process. There was a trend to reduce a* and a statistically significant increase in b*. The change in b* partially reversed the effect of demineralization.

The most notable contribution of the bleach treatment is the effect it had on subsequent treatments of demineralized enamel. The positive change of L* caused by the demineralization process was largely reversed for all three treatment groups when the surface had previously been treated with sodium hypochlorite. Such treatment effectiveness was not realized for the demineralized samples that did not receive bleach treatment.

D.2.9.4. Saliva Only Treatment

Saliva treatment of sound enamel (Set 1/group 1 and Set 2/group 1) gave varied results as discussed in the saliva pretreatment section. This is likely due to the fact that some of the samples used for this study were impacted teeth that had not had intraoral salivary exposure. Information regarding the eruption status of the samples was not available, so the data could not be analyzed to confirm this potential explanation.

Looking at whether change from initial due to BLEACH depends on intervening step

TTEST PROCEDURE

Variable: E_DELTA Delta E from initial

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	5.03014700	3.35058991	0.64482133	0.98939375	15.24682918	Unequal	0.8527	47.0	0.3982
SP	27	4.35519895	2.38550379	0.45909042	1.46471840	9.92095762	Equal	0.8527	52.0	0.3977

For H0: Variances are equal, F' = 1.97 DF = (26,26) Prob>F' = 0.0892

Variable: L_CHANGE Change in L (corrected) from initial

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	2.21000000	4.71040746	0.90651834	4.87000000	15.05000000	Unequal	5.7507	42.1	0.0001
SP	27	3.84222222	2.77811964	0.53464937	-9.62000000	1.38000000	Equal	5.7507	52.0	0.0000

For H0: Variances are equal, F' = 2.87 DF = (26,26) Prob>F' = 0.0091

Variable: A_CHANGE Change in A (corrected) from initial

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	1.18629630	1.17206638	0.22556428	-4.98000000	0.02000000	Unequal	3.2362	47.3	0.0022
SP	27	0.28555556	0.84731855	0.16306653	-2.73000000	0.27000000	Equal	3.2362	52.0	0.0021

For H0: Variances are equal, F' = 1.91 DF = (26,26) Prob>F' = 0.1042

Variable: B_CHANGE Change in B (corrected) from initial

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	1.54888889	2.21711929	0.42668481	-6.04000000	3.96000000	Unequal	-3.7956	40.1	0.0005
SP	27	0.29370370	1.20303510	0.23152421	-2.41000000	1.59000000	Equal	-3.7956	52.0	0.0004

For H0: Variances are equal, F' = 3.40 DF = (26,26) Prob>F' = 0.0027

Table 4: Statistics for bleach treatment effects from initial stage, considering intervening stage (either SP=saliva pretreatment or D= demineralization). Sample size is smaller for SP group because data was not collected for all samples that received SP.

Looking at whether effect of BLEACH changes depending on previous step

TTEST PROCEDURE

Variable: DELTA E Delta E from previous step

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	3.61761117	1.92634384	0.37072504	0.68293484	8.44194290	Unequal	1.2299	17.6	0.2349
SP	9	2.85389830	1.49445560	0.49815187	0.99609237	5.96591988	Equal	1.0819	34.0	0.2869

For HO: Variances are equal, F' = 1.66 DF = (26,8) Prob>F' = 0.4647

Variable: CHANGE L Change in L (corr) from previous step

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	0.18666667	2.05688338	0.39584739	-5.48000000	5.52000000	Unequal	-3.3165	16.1	0.0043
SP	9	2.51555556	1.74005108	0.58001703	-0.04000000	5.96000000	Equal	-3.0453	34.0	0.0045

For HO: Variances are equal, F' = 1.40 DF = (26,8) Prob>F' = 0.6483

Variable: CHANGE A Change in A (corr) from previous step

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	-0.47333333	0.73379939	0.14121976	-1.14000000	0.86000000	Unequal	-0.6119	15.0	0.5498
SP	9	-0.31222222	0.66666667	0.22222222	1.09000000	0.91000000	Equal	-0.5825	34.0	0.5641

For HO: Variances are equal, F' = 1.21 DF = (26,8) Prob>F' = 0.8240

Variable: CHANGE B Change in B (corr) from previous step

STEP1	N	Mean	Std Dev	Std Error	Minimum	Maximum	Variances	T	DF	Prob>|T|
D	27	2.71629630	2.14502671	0.41281058	-1.58000000	8.42000000	Unequal	6.8011	34.0	0.0001
SP	9	-0.47222222	0.66666667	0.22222222	-1.25000000	0.75000000	Equal	4.3521	34.0	0.0001

For HO: Variances are equal, F' = 10.35 DF = (26,8) Prob>F' = 0.0019

Table 5: Statistics for bleach treatment effects from previous stage (either SP=saliva pretreatment or D= demineralization). Sample size is smaller for SP group because data was not collected for all samples that received SP.

Demineralized enamel treated with only saliva (set 3) showed a tendency to decrease L* and increase b*. These findings mimic those found for saliva treatment/pretreatment of sound enamel.

Saliva treatment following bleach treatment for both sound (set 2) and demineralized enamel (set 4) samples significantly decreased b*, while the influence on L* tended to be negative. This decrease in L* is in accordance with the effect of saliva treatment/pretreatment on sound enamel and demineralized enamel without bleach surface treatment (sets 1 and 3, respectively). The significant change in b*, however, is unique to samples that received bleach treatment prior to the saliva treatment (sets 2 and 4). Changes in the b* parameter are difficult to define in the CIE L*a*b* system, thus no explanation of this finding is readily evident.

D.2.9.5. Calcium Sucrose Phosphate Treatment

CSP in conjunction with saliva treatment of sound enamel (set 1) demonstrates a significantly negative ΔL^*, as well as a tendency toward a negative Δb^*. Data is not available to determine the effect that CSP has on sound enamel with a salivary pellicle (i.e. following saliva pretreatment). It was presupposed that the data collected in Set 1/group 1 and Set 2/group 1 could be extrapolated for this purpose; however, the data was ambiguous. For this reason, the actual contribution of CSP to the mentioned changes in L* and b* for sound enamel can not be interpreted with any certainty.

Bleach treated sound enamel (set 2) did not experience any statistically significant changes in color with CSP plus saliva treatment. A negative trend for b* was noted. Overall, CSP showed no significant effect beyond the effect of saliva alone on sound enamel, with or without bleach treatment.

For demineralized, bleach treated enamel (set 4), treatment with CSP and saliva yielded similar results to set 2; however, the negative change for b* was statistically significant in this group.

Demineralized enamel (set 3) treated with CSP and saliva demonstrated no significant changes. The only change of note was a positive trend for Δb^*. This finding is similar to that for Set 3/group 1 with saliva treatment alone, indicating that the saliva rather than the CSP may be responsible for this observation. This positive trend is inconsistent with the color change noted in Set 2/group 2 and Set 4/group 2, both of which had bleach surface treatment prior to the CSP treatment. From this, it was gleaned that bleach surface treatment influences the effectiveness of subsequent treatments in reversing the effect of demineralization on b*. Although the bleach surface treatment itself partially reversed the effect of demineralization on b*, it influenced subsequent treatments to magnify the demineralization effects on b*. This phenomenon was not observed in demineralized samples that did not receive bleach treatment (set 3).

D.2.9.6. Pentapeptide Treatment

Sound enamel (set 1) treated with PP plus saliva produced a significantly negative ΔL^*. Data is not available to determine how much of this effect is attributable to the saliva pretreatment rather than the PP treatment; therefore, the actual contribution of PP to the mentioned changes in L* can not be interpreted with any certainty.

Bleach treated sound enamel (set 2) experienced a significant negative change in b* with CSP plus saliva treatment. A negative trend for b* was noted. This is analogous to the findings for saliva only treatment and CSP plus saliva

treatment in set 2. As with CSP, no treatment effect can be attributed to PP, since there was no detectable difference in treatment effect from that of saliva alone, with or without bleach.

PP plus saliva treatment of demineralized and bleach treated enamel (set 4) produced a statistically significant negative change in b*, similar to set 2 (bleach treated sound enamel). Additionally, a negative trend for ΔL^* and a positive trend for Δa^* were evident for set 4.

Demineralized enamel (set 3) treated with PP plus saliva produced no significant changes in color, but showed trends similar to saliva only treatment in set 3. These trends are a negative change in L* and positive change in b*. The trend for b* is in contrast to that seen for PP treatment of bleach treated samples (sound or demineralized). This reversal of Δb^* between bleach and non-bleach treated demineralized samples (sets 3 and 4) was seen for saliva only and CSP plus saliva treated samples as well. Interpretation of these findings indicates that no treatment effect can be attributed to PP, but a clear and consistent treatment effect can be attributed to bleach treatment of demineralized samples.

E. Discussion and Conclusions

The working hypothesis of this study was that CSP and PP, in combination with sodium hypochlorite treatment, would allow for incorporation of minerals that will return demineralized enamel to a more "normal" color. This treatment would be considered efficacious if it showed reversal of the effects of demineralization on the color parameters L*a*b* beyond those experienced with saliva alone. It was also important to determine if this treatment had any detrimental color effects on the sound enamel which would surround a treatable lesion.

Based on the data presented here the hypothesis can not be accepted. If CSP or PP had any affect it was largely masked by the subsequent saliva treatment. No data was taken between these treatments in the final stage because a natural situation was being simulated (i.e. the tooth bathed in intraoral saliva after treatment). For the purpose of determining efficacy of these products, however, it would be useful to collect data on the samples before the saliva treatment to isolate the effects of the CSP and PP.

Saliva, CSP, and PP consistently demonstrated tendencies toward reducing demineralization's effect on L* with or without bleach surface treatment; however, these reductions were never statistically significant. The small sample sizes, minimal treatment time and single dosage treatment may have been insufficient to detect any significant differences due to the treatments. Future studies should employ a greater nuber of samples and focus on the potential for increased optical benefit with multiple treatments.

Initial studies from which the sample sizes were determined proved to be of less variability than the groups in the main study due primarily to unforseen instrumental variations beyond the control of the investigator. The instrumentation difficulties resulted from the replacement of a burnt light source in the spectrophotometer midway in the experiment. All L*a*b* values collected beyond this timepoint were corrected to standard spectralon values before the bulb replacement.

Studies of saliva indicate that it can substantially remineralize portions of WSL's and partially return the color to that of sound enamel. A better understanding of saliva's effect on the optics of enamel, both sound and demineralized, and its interaction with other chemical treatments is needed to best capitalize on this "natural cure" for white spot lesions. The study design used in

this research should be altered to incorporate data collection before and after any saliva exposure in the protocol.

The effect the saliva, CSP, and PP treatments had on b* were dependent on whether or not bleach surface treatment had been performed. Due to the dependence of b* on L* and the complexity of the CIE L*a*b* color space, it is difficult to explain the actual effect these treatments had on the color of the tooth.

Bleach affected all parameters of color in a manner opposite to that of saliva pretreatment, indicating that bleach reverses the color by removing the salivary pellicle. Subsequent treatments appeared to be of greater magnitude, supporting the idea that sodium hypochlorite enhances the access for subsequent materials. Despite its effectiveness in removing the sallivary pellicle, bleach may not be the most ideal surface treatment. Bleach's shortcomings are related to its potential to influence the color of sound enamel surrounding the lesion to be treated and to it's corrosive nature, which necessitates isolation techniques (e. g. rubber dam) to protect the oral soft tissues. Other surface treatments, such as that obtained with a CO_2 laser, may also enhance subsequent treatment efficacy and should be explored.

While all three color parameters were considered equally in the mathematical analysis of treatment effects, subjective visual assessment of the samples indicated that the change in value or "brightness" (which corresponds to the L* parameter) had the greatest impact on the esthetics of the tooth. Even though the data does not indicate significant changes with the treatment protocol, distinct esthetic improvements were noted visually.

It is widely accepted that the sensitivity of the instrumentation available to measure color still does not match the perceptive abilities of the human eye. Clinical photography has been shown to be an inadequate method of monitoring the remineralization of WSL's.[117] The spectrophotometer used in this study has demonstrated an ability to register color changes with treatment, although it may not

be ideal. Chroma meters,[118] spectroradiometers,[119] and the optical caries monitor[115, 120] have been used with success in other studies regarding the enamel optics and may offer superior detection abilities. Future studies with CSP and PP may benefit from the incorporation of these alternate measurement devices.

Being that CSP and PP have been demonstrated not to have any untoward health effects, and in light of more advanced optical measurement systems that can be utilized intraorally, *in vivo* studies seem to be a logical and imminent realm for future research of these chemicals. This would allow for the determination of CSP and PP's efficacy in the optical reversal of naturally demineralized enamel.

F. Bibliography

1. Årtun J, Brobakken BO. (1986) Prevalence of carious white spots after orthodontic treatment with multibonded appliances. European Journal of Orthodontics, 8, 229-234.

2. Gorelick L, Geiger AM, Gwinnett AJ. (1982) Incidence of white spot formation after bonding and banding. American Journal of Orthodontics, 81, 93-98.

3. Fehr FR, Loe H, Theilade E. (1970) Experimental caries in man. Caries Research, 4, 131-148.

4. Darling AI. (1958) Studies of the early lesion of enamel caries. British Dental Journal, 105, 119-135.

5. Årtun J, Thylstrup A. (1986) A clinical and scanning electron microscopic study of surface changes of incipient caries lesions after debonding. Scandanavian Journal of Dental Research, 94.

6. O'Reilly MM, Featherstone JDB. (1987) Demineralization and remineralization around orthodontic appliances: An in vivo study. American Journal of Orthodontics and Dentofacial Orthopedics, 92, 33-40.

7. Featherstone JDB, O'Reilly MM, Shariati M, Brugler S. (1986) Enhancement of remineralisation in vitro and vivo. In: Factors Relating to Demineralisation and Remineralisation of the Teeth (ed. S Leach), pp 23-34. IRL Press Limited, Oxford, England.

8. White DJ. (1987) Use of synthetic polymer gels for artificial carious lesion preparation. Caries Research, 21, 228-242.

9. Glatz EGM, Featherstone JDB. (1985) Demineralization related to orthodontic bands and brackets-a clinical study. American Journal of Orthodontics, 87, 87.

10. Gwinnett AJ, Ceen RF. (1979) Plaque distribution on bonded brackets: A scanning microscope study. American Journal of Orthodontics, 75, 667-677.

11. Mizrahi E. (1982a) Enamel demineralization following orthodontic treatment. American Journal of Orthodontics, 82, 62-67.

12. Corbett JA, Brown LR, Keene HJ, Horton IM. (1981) Comparison of Streptococcus mutans concentrations in non-banded and banded orthodontic patients. Journal of Dental Research, 60, 1936-1942.

13. Mattingly JH, Sauer GJ, Yancey JM, Arnold RR. (1983) Enhancement of Streptococcus mutans colonization by direct bonded orthodontic appliances. Journal of Dental Research, 62, 1209-1211.

14. Featherstone JDB. (1990) An updated understanding of the mechanism of dental decay and its prevention. Nutrition Quarterly, 14, 5-11.

15. Nikkiforuk G. (1985) Understanding dental caries: etiology and mechanisms. Basic and clinical aspects. Karger, New York.

16. Eanes ED. (1979) Enamel apatite: chemistry, structure and properties. Journal of Dental Research, 58, 829-834.

17. Silverstone LM. (1983) Remineralization and enamel caries: significance of fluoride and effect on crystal diameters. In: Demineralization and Remineralization of the Teeth (eds. SA Leach, WM Edgar), pp 85-105. IRL Press Ltd., Oxford.

18. Frazier PD, Little MF, Casciani FS. (1967) X-ray diffraction analysis of human enamel containing different amounts of fluoride. Archives of Oral Biology, 12, 35-42.

19. Isaac S, Brudevold F, Smith FA, Gardner DE. (1967) Solubility rate and the natural fluoride content of surface and subsurface enamel. Journal of Dental Research, 37, 254-64.

20. Miller WJC. (1891) The chemo-parasitical theory of dental caries. Items of Interest, Vol. XIII, 364.

21. Arends J, ten Cate JM. (1981) Tooth enamel remineralization. J. Cryst. Growth, 53, 135-147.

22. Goldberg M, Arends J, Septier D, Jongebloed WL. (1981) Microchannels in the surface zone of artificially produced caries-like enamel lesions. J. Biol. Buccale, 9, 297-314.

23. Geddes DAM. (1972) The production of L(+) and D (-) lactic acid and volatile acids by human dental plaque and the effect of plaque buffering and acidic strength on pH. Archives of Oral Biology, 17, 537-545.

24. Backer Dirks O. (1966) Posteruptive changes in dental enamel. Journal of Dental Research, 45, 503-511.

25. Silverstone LM. (1977) Remineralization phenomena. Caries Research, 11 (Suppl 1), 59-84.

26. Silverstone LM. (1982) The effect of fluoride in the remineralization of enamel caries and caries-like lesions in vitro. Journal of Public Health Dentistry, 42, 42-53.

27. Gustafson G. (1957) The histopathology of caries of human enamel with special reference to the division of the carious lesion into zones. Acto Odontologica Scandinavica, 15, 13-55.

28. Johnson AR. (1975) The early carious lesion of enamel. Journal of Oral Pathology, 4, 128-157.

29. Orams HJ, Phakey PP, Rachinger WA, Zybert JJ. (1980) Ultrastructural changes in the translucent and dark zones of early enamel caries. Journal of Oral Pathology, 9, 54-61.

30. Joyston-Bechal S, Kidd EAM. (1980) Histopathological appearance of artifically produced caries like lesion of enamel treated with APF during lesion formation *in vitro*. Caries Research, 14, 45-49.

31. Featherstone JDB. (1977) Chemical aspects of dental caries. In: PhD Thesis. Vitoria University of Wellington, Wellington, New Zealand.

32. Featherstone JDB, Goodman P, McLean JD. (1979) Electron microscope study of defect zones in dental enamel. Journal of Ultrastructural Research, 67, 117-123.

33. Silverstone LM. (1968) The surface zone in caries and caries like lesions produced *in vitro*. British Dental Journal, 125, 145-157.

34. Featherstone JDB, Duncan JF, Cutress TW. (1978) Surface layer phenomena in in vitro early caries like lesions of human tooth enamel. Archives of Oral Biology, 23, 397-404.

35. Silverstone LM. (1973) Structure of carious enamel, including the early lesion. Oral Science Review, 3, 100-160.

36. Silverstone LM, Featherstone MJ. (1981) The effect of different remineralization regimes on lesion progression *in vitro*. Caries Research, 15, 198.

37. Sakamaki ST, Bahn AN. (1968) Effect of orthodontic banding on localized oral lactobacilli. Journal of Dental Research, 47, 275-279.

38. Shannon IL. (1981) Prevention of decalcification in orthodontic patients. Journal of Clinical Orthodontics, 15, 694-705.

39. Branett S, Servoss JM, Wolfson J. (1975) Practical methods of bonding, direct and indirect. Journal of Clinical Orthodontics, 9, 610-635.

40. Zachrisson BU. (1977) A post-treatment evaluation of direct bonding in orthodontics. American Journal of Orthodontics, 71, 173-189.

41. Weitman RT, Eames WB. (1975) Plaque accumulation on composite surfaces after various finishing procedures. Journal of the American Dental Association, 91, 101-106.

42. Bloom RH, Brown LR, Jr. (1969) Study of the effects of orthodontic appliances on the oral microbial flora. Oral Surgery, 17, 658-667.

43. Arneberg P, Øgaard B, Scheie A, Rølla G. (1984) Selection of streptococcus mutans and lactobacilli in an intraoral human caries model. Journal of Dental Research, 63, 1197-1200.

44. Adams RJ. (1967) The effects of fixed orthodontic appliances on the cariogenicity, quantity and microscopic morphology of oral lactobacilli. Journal of Oral Medicine, 22, 88-98.

45. Øgaard B, Rølla G, Arends J, ten Cate JM. (1988) Orthodontic appliances and enamel demineralization, part I: lesion development. American Journal of Orthodontics and Dentofacial Orthopedics, 94, 68-73.

46. Balenseifen JW, Madonia JV. (1970) Study of dental plaque in orthodontics patients. Journal of Dental Research, 49.

47. Mizrahi E. (1982b) Enamel opacities in primary and high school pupils. Journal of Dentistry, 11, 231-236.

48. Murray JJ, Shaw L. (1979) Classification and prevalence of enamel opacities in the human deciduous and permanent dentitions. Archives of Oral Biology, 24, 7-13.

49. Small BW, Murray JJ. (1978) Enamel opacities. Prevalence, classification and aetiological considerations. Journal of Dentistry, 6, 33-42.

50. Zachrisson BU, Brobakken BO. (1978) Clinical comparison of direct versus indirect bonding with different bracket types and adhesives. American Journal of Orthodontics, 74, 62-78.

51. Wei SHY. (1967) Remineralization of enamel and dentine: a review. Journal of Dentistry for Children, 34, 444-451.

52. Silverstone LM. (1977b) Fissure sealants: the susceptibility to dissolution of acid etched and subseequently abraded enamel *in vitro*. Caries Research, 11, 46-51.

53. Basdra EK, Huber H, Komposch G. (1996) Enamel surface alteration and enamel demineralization. American Journal of Orthodontics and Dentofacial Orthopedics, 109, 466-472.

54. Featherstone JD, Nelson DG. (1987) Laser effects on dental hard tissues. Advances in Dental Research, 1, 21-26.

55. Silverstone LM. (1975) The acid-etch technique: in vitro studies with special reference to the enamel surface and the enamel-resin interface. In: Proceedings of an International Symposium on the Acid-Etch Technique (eds. LM Silverstone, L Dogon), pp 13-39. North Central Publishing Company, St. Paul.

56. Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ. (1988) Reducing white spot lesions in orthodontic population with fluoride rinsing. American Journal of Orthodontics and Dentofacial Orthopedics, 101, 403-407.

57. Øgaard B, Rølla G, Arends J, ten Cate JM. (1988) Orthodontic appliances and enamel demineralization, part 2: prevention and treatment of lesions. American Journal of Orthodontics and Dentofacial Orthopedics, 94, 123-128.

58. Silverstone LM. (1980) Laboratory studies on the demineralization and remineralization of human enamel in relation to caries mechanisms. Australian Dental Journal, 25, 163-168.

59. Featherstone JDB, Holmen L, Thylstrup A, Fredebo L, Shariati M. (1985) Chemical and histological changes during development of artificial caries. Caries Research, 19, 1-10.

60. Holmen L, Thylstrup A, Featherstone JDB, Fredebo L, Shariati M. (1985) A scanning electron microscopic study of surface changes during development of artificial caries. Caries Research, 19, 11-21.

61. Arends J, Schuthof J, Jongebloed WG. (1980) Lesion depth and microhardness indentations on artificial white spot lesions. Caries Research, 14, 190-195.

62. Arends J, Christoffersen J. (1986) The nature of early carious lesions in dental enamel. Journal of Dental Research, 65, 2-11.

63. McCloskey RJ. (1984) A technique for removal of fluorosis stains. Journal of the American Dental Association, 109, 63-64.

64. Croll TP, Cavanaugh RR. (1986) Enamel color modification by controlled hydrochloric acid-pumice abrasion. I. Technique and examples. Quintessence International, 17, 81-87.

65. Ames JW. (1937) Removing stains from mottled enamel. American Dental Association Journal, 24, 1674-1677.

66. Younger HB. (1939*) Bleaching fluoride stain from mottled enamel. Texas Dental Journal, 57, 380-382.

67. Chandra S, Chawla TN. (1974) Clinical evaluation of heat method for bleaching of discolored mottled teeth. Journal of the Indian Dental Association, 46, 313-318.

68. Murrin JJ, Barkmeier WM. (1982) Chemical treatment of vital teeth with intrinsic stain. Ohio Dental Journal, 56, 6-10.

69. Chandra S, Chawla TN. (1975) Clinicl evaluation of the sandpaper disc method for removing fluorosis sains from teeth. Journal of the American Dental Association, 90, 1273-1276.

70. Den Besten P, Giambro N. (1995) Treatment of flourosed and white-spot human enamel with calcium sucrose phosphate in vitro. American Academy of Pediatric Dentistry, 17, 340-345.

71. Silverstone LM. (1974) Fissure sealants. Laboratory studies. Caries Research, 8, 2-26.

72. Wilson CJ. (1975) The effect of calcium sucrose phosphates chewing gum on caries incidence in children. 51, 521-525.

73. Company CSR. (1970) Anticay: a dental caries inhibitor, a review of research. Colonial Sugar Refining Company Limited, Sydney, Australia.

74. Curtin JH, Gagolski J. (1971) The preparation and chemistry of a calcium sucrose phosphate as calcium phosphate complex for use as a food additive for the reduction of dental caries. In: ANZAAS conference, Brisbane, Australia.

75. Harris R, Schamschula RG, Beveridge J, Gregory G. (1968) The cariostatic effect of calcium sucrose phosphate in a group of children aged 5-17 years. Australian Dental Journal, 13, 32-39.

76. Harris R, Schamschula RG, Gregory G, Roots M, Beveridge J. (1967) The cariostatic effect of calcium sucrose phosphate in a group of children aged 5-17 years. Preliminary report. Australian Dental Journal, 12, 105-113.

77. Clarke NG, Fanning EA. (1971) Plaque pH and calcium sucrose phosphate: a telemetric study. Australian Dental Journal, 16, 13-16.

78. Lilienthal B, Napper DH, Smythe BM. (1968) The hardening and softening of human tooth enamel. Australian Dental Journal, 13, 219-230.

79. Rogerson MJ. (1973) The role of calcium sucrose phosphate-calcium orthophosphate complex in the reduction of dental caries. Australian Dental Journal, 18, 160-166.

80. Lilienthal B, Bush E, Buckmaster M, Gregory G, Gagolski J, Smythe BM, Curtin JH, Napper DH. (1966) The cariostatic effect of carbohydrate phosphates in the diet. Australian Dental Journal, 11, 388-395.

81. Gustafson G, Stelling E, Mornstad H, Brunius F. (1973) The effect of the calcium sucrose phosphate preparation, Anticay® on dental caries in the golden hamster. Odontologisk Revy, 24, 325-334.

82. Company CSR. (1968) Review of a research project: anticay, a dental caries inhibitor. Colonial Sugar Refining Company Limited, Sydney, Australia.

83. Featherstone JDB, Cutress TW, Rodgers BE, Dennison PJ. (1982) Remineralization of artificial caries-like lesions in vivo by a self-administered mouthrinse or paste. Caries Research, 16, 235-242.

84. Nigel AE, Harris RS. (1964) The effects of phosphates on experimental dental caries: a literature review. Journal of Dental Research, 43, 1123.

85. Harris R, Schamschula RG, Beveridge J, Gregory G. (1969) The cariostatic effect of calcium sucrose phosphate in a group of children aged 5-17 years: Part IV. Australian Dental Journal, 14, 42-49.

86. Harris R, Roots M, Gregory G, Beveridge J. (1968) Calcium sucrose phosphate as a cariostatic agent in children aged 5-17 years: Part III. Dietary Factors. Australian Dental Journal, 13, 345-352.

87. Beveridge J, Harris R, Gregory G, Savage DCL. (1967) Prevention of dental caries: 1. Phosphates and dental caries. Medical Journal of Australia, 2, 54-57.

88. Beveridge J, Harris R, Gregory G, Savage DCL. (1968a) Prevention of dental caries: 2. Calcium sucrose phosphates. Medical Journal of Australia, 1, 120-123.

89. Beveridge J, Gregory G, Harris R, Schanschula RG, Savage DCL. (1968) Prevention of dental caries: 3. Clinical trial of calcium sucrose phosphates as a food additive. Medical Journal of Australia, 2, 256-260.

90. Powell KR, Craig GG. (1982) A simple technique for the aesthetic improvement of fluorotic-like lesions. Journal of Dentistry for Children, 49, 112-117.

91. Myers D, Lyon Jr. TC. (1986) Treatment of fluorosis or fluorosis-like lesions with calcium sucrose phosphate gel. Pediatric Dentistry, 8, 213-215.

92. Perich JW, Kelly DP, Reynolds EC. (1992) Efficient solution-phase synthesis of multiple O-phosphoseryl-contianing peptides related to casein and statherin. International Journal of Peptide Protein Research, 40, 81-88.

93. Reynolds EC, Riley PF, Adamson NJ. (1994) A selective precipitation purification procedure for multiple phophoseryl-containing peptides and methods for their identification. Analytic Biochemistry, 217, 277-284.

94. Hay DI, Moreno EC, Schlesinger DH. (1979) Phosphoprotein inhibitors of calcium phosphate precipitation from human salivary secretions. Inorganic Perspectives in Biol. Med., 2, 271-285.

95. Glimcher MJ. (1979) Phosphopeptides of enamel matrix. Journal of Dental Research, 58B, 790-806.

96. Reeves RE, Latour NG. (1958) Science, 128, 472.

97. Reynolds EC. (1987) The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. Journal of Dental Research, 66, 1120-1127.

98. Adamson NJ, Reynolds EC. (1995) High performance capillary electrophoresis of casein phosphopepetides containing 2-5 phosphoseryl residues: Relationship between absolute electrophoretic mobility and peptide charge and size. Electrophoresis, 16, 525-528.

99. Perich JW, Kelly DP, Reynolds EC. (1993) Efficient solution phase synthesis and use of multiple *O*-phosphothreonyl-containing peptides for calcium phosphate binding studies. International Journal of Peptide Protein, 41, 275-281.

100. Farrell HM. (1988) Chapter 9. In: Fundamentals of Dairy Chemistry (eds. NP Wong, R Jenness, M Keeney, EH Marth), pp 461-510. Van Nostrand Reinhold Company, New York.

101. International NS. (1996) Topacal™ technical product publication. Nulite Systems International Pty. Limited, Hornsby, Australia.

102. Lamkin MS, Oppenheim FG. (1993) Structural features of salivary function. Critical Reviews in Oral Biology and Medicine, 4, 251-259.

103. ten Cate JM, Arends J. (1978) Remineralization of artificial enamel lesions in vitro. II. Determination of activation energy and reaction order. Caries Research, 12, 213-222.

104. Robinson C, Kirkham J. (1990) The effect of fluoride on the developing mineralized tissues. Journal of Dental Research, 69, 685-691.

105. Christensen L, Larsen M, Fejerskov O. (1979) Effect of a mineralizing solution on sections of fluorosed human dental enamel *in vitro*. Caries Research, 13, 47-56.

106. McLean JW. (1979) The science and art of dental ceramics. Volume I: The nature of dental ceramics and their clinical use. Quintessence Publishing Co., Chicago.

107. C.I.E. (1978) Recommendations on uniform color spaces, color difference equations, and metric color terms. Bureau Cental de la CIE, Paris.

108. Wyszecki G, Stiles WS. (1982) Color Science: concepts and methods, quantitative data and formulae, 2nd edition. John Wiley and Sons, New York.

109. Culpepper WD. (1970) A comparison of shade-matching procedures. Journal of Prosthetic Dentistry, 24, 166.

110. Sproull RC. (1973a) Color matching in dentistry, Part I. The three dimensional nature of color. Journal of Prosthetic Dentistry, 29, 416-424.

111. Lemire PA, Burk B. (1975) Color in Dentistry. Ney Company, Bloomfield, CT.

112. Angmar-Manson B, ten Bosch JJ. (1987) Optical methods for the detection and quantification of caries. Advanced Dental Research, 1, 14-20.

113. Pine CM, ten Bosch JJ. (1996) Dynamics of and diagnostic methodsfor detecting small carious lesions. Caries Research, 30, 381-388.

114. Vaarkamp J, ten Bosch JJ, Verdonschot EH. (1995) Light propagation hrough teeth containing simulated caries lesions. Physical and Medical Biology, 40, 1375-1387.

115. Øgaard B, ten Bosch JJ. (1994) Regression of white spot enamel lesions. A new optical method for quantitative longitudinal evaluation *in vivo*. American Journal of Orthodontics and Dentofacial Orthopedics, 106, 238-242.

116. White JM, Goodis HE, Marshall SJ, Marshall GW. (1994) Sterilization of teeth by gamma radiation. Journal of Dental Research, 73, 1560-1567.

117. Linton JL. (1996) Quantitative measurements of remineralization of incipient caries. American Journal of Orthodontics and Dentofacial Orthopedics, 110, 590-597.

118. Giambro NJ, Prostak K, Den Besten PK. (1995) Characterization of fluorosed human enamel by color reflectance, ultrastructure, and elemental composition. Caries Research, 29, 251-257.

119. ten Bosch JJ, Coops JC. (1995) Tooth color and relectance as related to light scattering and enamel hardness. Journal of Dental Research, 74, 374-380.

120. Brinkman J, ten Bosch JJ, Borsboom PCF. (1988) Optical quantitation of natural caries in smooth surfaces of extracted teeth. Caries Research, 22, 257-262.

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 1 / Group 1	----------	----------	----------	----------	----------
saliva tx	Change in L from previous	-0.234	0.915	8	-0.26	Tukey-Kramer	0.8042
saliva tx	Change in A from previous	0.018	0.222	8	0.08	Tukey-Kramer	0.9382
saliva tx	Change in B from previous	0.272	0.434	8	0.63	Tukey-Kramer	0.5479
saliva tx	Delta E from previous	2.469	0.573	8	4.31		0.0026
saliva tx	Change in L from initial	-0.234	0.915	8	-0.26	Tukey-Kramer	0.8042
saliva tx	Change in A from initial	0.018	0.222	8	0.08	Tukey-Kramer	0.9382
saliva tx	Change in B from initial	0.272	0.434	8	0.63	Tukey-Kramer	0.5479
saliva tx	Delta E from initial	2.469	0.573	8	4.31		0.0026
----------	----------	Set 1 / Group 2	----------	----------	----------	----------	----------
CSP tx	Change in L from previous	-2.296	0.796	8	-2.89	Tukey-Kramer	0.0203
CSP tx	Change in A from previous	-0.342	0.455	8	-0.75	Tukey-Kramer	0.4731
CSP tx	Change in B from previous	-0.974	0.458	8	-2.13	Tukey-Kramer	0.0659
CSP tx	Delta E from previous	3.175	0.762	8	4.17		0.0031
CSP tx	Change in L from initial	-2.296	0.796	8	-2.89	Tukey-Kramer	0.0203
CSP tx	Change in A from initial	-0.342	0.455	8	-0.75	Tukey-Kramer	0.4731
CSP tx	Change in B from initial	-0.974	0.458	8	-2.13	Tukey-Kramer	0.0659
CSP tx	Delta E from initial	3.175	0.762	8	4.17		0.0031
----------	----------	Set 1 / Group 3	----------	----------	----------	----------	----------
P tx	Change in L from previous	-4.629	0.536	8	-8.63	Tukey-Kramer	0.0000
P tx	Change in A from previous	-0.120	0.168	8	-0.72	Tukey-Kramer	0.4943
P tx	Change in B from previous	-0.308	0.399	8	-0.77	Tukey-Kramer	0.4625
P tx	Delta E from previous	4.784	0.553	8	8.64		0.0000
P tx	Change in L from initial	-4.629	0.536	8	-8.63	Tukey-Kramer	0.0000
P tx	Change in A from initial	-0.120	0.168	8	-0.72	Tukey-Kramer	0.4943
P tx	Change in B from initial	-0.308	0.399	8	-0.77	Tukey-Kramer	0.4625
P tx	Delta E from initial	4.784	0.553	8	8.64		0.0000

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 2 / Group 1	----------	----------	----------	----------	----------
saliva pre-tx	Change in L from previous	-5.691	0.859	24	-6.63	Tukey-Kramer	0.0000
saliva pre-tx	Change in A from previous	0.027	0.240	24	0.11	Tukey-Kramer	0.9995
saliva pre-tx	Change in B from previous	0.729	0.288	24	2.53	Tukey-Kramer	0.0800
saliva pre-tx	Delta E from previous	5.887	0.604	24	9.75		0.0000
saliva pre-tx	Change in L from initial	-5.691	0.859	24	-6.63	Tukey-Kramer	0.0000
saliva pre-tx	Change in A from initial	0.027	0.240	24	0.11	Tukey-Kramer	0.9995
saliva pre-tx	Change in B from initial	0.729	0.288	24	2.53	Tukey-Kramer	0.0800
saliva pre-tx	Delta E from initial	5.887	0.767	24	7.67		0.0000
bleach tx	Change in L from previous	2.516	0.859	24	2.93	Tukey-Kramer	0.0345
bleach tx	Change in A from previous	-0.312	0.240	24	-1.30	Tukey-Kramer	0.5713
bleach tx	Change in B from previous	-0.472	0.288	24	-1.64	Tukey-Kramer	0.3758
bleach tx	Delta E from previous	2.854	0.604	24	4.73		0.0001
bleach tx	Change in L from initial	-3.176	0.859	24	-3.70	Tukey-Kramer	0.0058
bleach tx	Change in A from initial	-0.286	0.240	24	-1.19	Tukey-Kramer	0.6390
bleach tx	Change in B from initial	0.257	0.288	24	0.89	Tukey-Kramer	0.8091
bleach tx	Delta E from initial	3.736	0.767	24	4.87		0.0001
saliva tx	Change in L from previous	-0.682	0.859	24	-0.79	Tukey-Kramer	0.8562
saliva tx	Change in A from previous	-0.113	0.240	24	-0.47	Tukey-Kramer	0.9645
saliva tx	Change in B from previous	-1.689	0.288	24	-5.87	Tukey-Kramer	0.0000
saliva tx	Delta E from previous	2.783	0.604	24	4.61		0.0001
saliva tx	Change in L from initial	-3.858	0.859	24	-4.49	Tukey-Kramer	0.0008
saliva tx	Change in A from initial	-0.399	0.240	24	-1.66	Tukey-Kramer	0.3649
saliva tx	Change in B from initial	-1.432	0.288	24	-4.98	Tukey-Kramer	0.0002
saliva tx	Delta E from initial	4.418	0.767	24	5.76		0.0000

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 2 / Group 2	----------	----------	----------	----------	----------
bleach tx	Change in L from previous	-4.176	1.077	16	-3.88	Tukey-Kramer	0.0036
bleach tx	Change in A from previous	-0.063	0.215	16	-0.29	Tukey-Kramer	0.9535
bleach tx	Change in B from previous	0.590	0.384	16	1.54	Tukey-Kramer	0.3002
bleach tx	Delta E from previous	4.753	0.692	16	6.86		0.0000
bleach tx	Change in L from initial	-4.176	1.077	16	-3.88	Tukey-Kramer	0.0036
bleach tx	Change in A from initial	-0.063	0.215	16	-0.29	Tukey-Kramer	0.9535
bleach tx	Change in B from initial	0.590	0.384	16	1.54	Tukey-Kramer	0.3002
bleach tx	Delta E from initial	4.753	0.881	16	5.39		0.0001
CSP tx	Change in L from previous	0.096	1.077	16	0.09	Tukey-Kramer	0.9957
CSP tx	Change in A from previous	-0.113	0.215	16	-0.53	Tukey-Kramer	0.8595
CSP tx	Change in B from previous	-0.911	0.384	16	-2.38	Tukey-Kramer	0.0737
CSP tx	Delta E from previous	3.279	0.692	16	4.74		0.0002
CSP tx	Change in L from initial	-4.080	1.077	16	-3.79	Tukey-Kramer	0.0043
CSP tx	Change in A from initial	-0.177	0.215	16	-0.82	Tukey-Kramer	0.6957
CSP tx	Change in B from initial	-0.321	0.384	16	-0.84	Tukey-Kramer	0.6861
CSP tx	Delta E from initial	4.385	0.881	16	4.97		0.0001

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 2 / Group 3	----------	----------	----------	----------	----------
bleach tx	Change in L from previous	-4.176	0.955	16	-4.37	Tukey-Kramer	0.0013
bleach tx	Change in A from previous	-0.508	0.283	16	-1.79	Tukey-Kramer	0.2036
bleach tx	Change in B from previous	0.034	0.483	16	0.07	Tukey-Kramer	0.9972
bleach tx	Delta E from previous	4.576	0.739	16	6.19		0.0000
bleach tx	Change in L from initial	-4.176	0.955	16	-4.37	Tukey-Kramer	0.0013
bleach tx	Change in A from initial	-0.508	0.283	16	-1.79	Tukey-Kramer	0.2036
bleach tx	Change in B from initial	0.034	0.483	16	0.07	Tukey-Kramer	0.9972
bleach tx	Delta E from initial	4.576	0.919	16	4.98		0.0001
P tx	Change in L from previous	0.429	0.955	16	0.45	Tukey-Kramer	0.8956
P tx	Change in A from previous	0.109	0.283	16	0.38	Tukey-Kramer	0.9222
P tx	Change in B from previous	-1.244	0.483	16	-2.58	Tukey-Kramer	0.0505
P tx	Delta E from previous	2.576	0.739	16	3.48		0.0031
P tx	Change in L from initial	-3.747	0.955	16	-3.92	Tukey-Kramer	0.0033
P tx	Change in A from initial	-0.399	0.283	16	-1.41	Tukey-Kramer	0.3603
P tx	Change in B from initial	-1.210	0.483	16	-2.50	Tukey-Kramer	0.0579
P tx	Delta E from initial	4.678	0.919	16	5.09		0.0001

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 3 / Group 1	----------	----------	----------	----------	----------
demineralized	Change in L from previous	4.137	0.948	16	4.36	Tukey-Kramer	0.0013
demineralized	Change in A from previous	-0.318	0.289	16	-1.10	Tukey-Kramer	0.5278
demineralized	Change in B from previous	-5.513	0.334	16	-16.49	Tukey-Kramer	0.0000
demineralized	Delta E from previous	7.146	0.558	16	12.81		0.0000
demineralized	Change in L from initial	4.137	0.948	16	4.36	Tukey-Kramer	0.0013
demineralized	Change in A from initial	-0.318	0.289	16	-1.10	Tukey-Kramer	0.5278
demineralized	Change in B from initial	-5.513	0.334	16	-16.49	Tukey-Kramer	0.0000
demineralized	Delta E from initial	7.146	0.516	16	13.84		0.0000
saliva tx	Change in L from previous	-1.080	0.948	16	-1.14	Tukey-Kramer	0.5047
saliva tx	Change in A from previous	0.002	0.289	16	0.01	Tukey-Kramer	1.0000
saliva tx	Change in B from previous	0.558	0.334	16	1.67	Tukey-Kramer	0.2476
saliva tx	Delta E from previous	2.606	0.558	16	4.67		0.0003
saliva tx	Change in L from initial	3.057	0.948	16	3.22	Tukey-Kramer	0.0139
saliva tx	Change in A from initial	-0.316	0.289	16	-1.09	Tukey-Kramer	0.5323
saliva tx	Change in B from initial	-4.956	0.334	16	-14.82	Tukey-Kramer	0.0000
saliva tx	Delta E from initial	6.589	0.516	16	12.76		0.0000

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 3 / Group 2	----------	----------	----------	----------	----------
demineralized	Change in L from previous	2.692	1.444	16	1.86	Tukey-Kramer	0.1814
demineralized	Change in A from previous	-0.651	0.279	16	-2.33	Tukey	0.0801
demineralized	Change in B from previous	-4.847	0.426	16	-11.37	Tukey-Kramer	0.0000
demineralized	Delta E from previous	7.292	0.581	16	12.54		0.0000
demineralized	Change in L from initial	2.692	1.444	16	1.86	Tukey-Kramer	0.1814
demineralized	Change in A from initial	-0.651	0.279	16	-2.33	Tukey	0.0801
demineralized	Change in B from initial	-4.847	0.426	16	-11.37	Tukey-Kramer	0.0000
demineralized	Delta E from initial	7.292	0.819	16	8.91		0.0000
CSP tx	Change in L from previous	-0.302	1.444	16	-0.21	Tukey-Kramer	0.9762
CSP tx	Change in A from previous	0.113	0.279	16	0.41	Tukey	0.9138
CSP tx	Change in B from previous	0.669	0.426	16	1.57	Tukey-Kramer	0.2870
CSP tx	Delta E from previous	1.724	0.581	16	2.96		0.0091
CSP tx	Change in L from initial	2.390	1.444	16	1.65	Tukey-Kramer	0.2526
CSP tx	Change in A from initial	-0.538	0.279	16	-1.92	Tukey	0.1640
CSP tx	Change in B from initial	-4.178	0.426	16	-9.80	Tukey-Kramer	0.0000
CSP tx	Delta E from initial	6.640	0.819	16	8.11		0.0000

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 3 / Group 3	----------	----------	----------	----------	----------
demineralized	Change in L from previous	4.692	0.854	16	5.50	Tukey-Kramer	0.0001
demineralized	Change in A from previous	-0.540	0.299	16	-1.80	Tukey-Kramer	0.1996
demineralized	Change in B from previous	-4.402	0.534	16	-8.24	Tukey-Kramer	0.0000
demineralized	Delta E from previous	6.636	0.873	16	7.60		0.0000
demineralized	Change in L from initial	4.692	0.854	16	5.50	Tukey-Kramer	0.0001
demineralized	Change in A from initial	-0.540	0.299	16	-1.80	Tukey-Kramer	0.1996
demineralized	Change in B from initial	-4.402	0.534	16	-8.24	Tukey-Kramer	0.0000
demineralized	Delta E from initial	6.636	0.801	16	8.29		0.0000
P tx	Change in L from previous	-1.302	0.854	16	-1.53	Tukey-Kramer	0.3057
P tx	Change in A from previous	-0.220	0.299	16	-0.74	Tukey-Kramer	0.7465
P tx	Change in B from previous	0.558	0.534	16	1.04	Tukey-Kramer	0.5608
P tx	Delta E from previous	2.940	0.873	16	3.37		0.0039
P tx	Change in L from initial	3.390	0.854	16	3.97	Tukey-Kramer	0.0030
P tx	Change in A from initial	-0.760	0.299	16	-2.54	Tukey-Kramer	0.0540
P tx	Change in B from initial	-3.844	0.534	16	-7.20	Tukey-Kramer	0.0000
P tx	Delta E from initial	5.471	0.801	16	6.83		0.0000

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 4 / Group 1	----------	----------	----------	----------	----------
demineralized	Change in L from previous	2.570	1.466	24	1.75	Tukey-Kramer	0.3195
demineralized	Change in A from previous	-0.432	0.281	24	-1.54	Tukey-Kramer	0.4316
demineralized	Change in B from previous	-4.317	0.734	24	-5.88	Tukey-Kramer	0.0000
demineralized	Delta E from previous	7.165	0.779	24	9.20		0.0000
demineralized	Change in L from initial	2.570	1.466	24	1.75	Tukey-Kramer	0.3195
demineralized	Change in A from initial	-0.432	0.281	24	-1.54	Tukey-Kramer	0.4316
demineralized	Change in B from initial	-4.317	0.734	24	-5.88	Tukey-Kramer	0.0000
demineralized	Delta E from initial	7.165	1.056	24	6.78		0.0000
bleach tx	Change in L from previous	0.409	1.466	24	0.28	Tukey-Kramer	0.9922
bleach tx	Change in A from previous	-0.473	0.281	24	-1.68	Tukey-Kramer	0.3535
bleach tx	Change in B from previous	2.976	0.734	24	4.05	Tukey-Kramer	0.0024
bleach tx	Delta E from previous	3.625	0.779	24	4.66		0.0001
bleach tx	Change in L from initial	2.979	1.466	24	2.03	Tukey-Kramer	0.2043
bleach tx	Change in A from initial	-0.906	0.281	24	-3.22	Tukey-Kramer	0.0178
bleach tx	Change in B from initial	-1.341	0.734	24	-1.83	Tukey-Kramer	0.2861
bleach tx	Delta E from initial	5.821	1.056	24	5.51		0.0000
saliva tx	Change in L from previous	-2.807	1.466	24	-1.92	Tukey-Kramer	0.2485
saliva tx	Change in A from previous	0.009	0.281	24	0.03	Tukey-Kramer	1.0000
saliva tx	Change in B from previous	-2.306	0.734	24	-3.14	Tukey-Kramer	0.0215
saliva tx	Delta E from previous	4.317	0.779	24	5.54		0.0000
saliva tx	Change in L from initial	0.172	1.466	24	0.12	Tukey-Kramer	0.9994
saliva tx	Change in A from initial	-0.897	0.281	24	-3.19	Tukey-Kramer	0.0192
saliva tx	Change in B from initial	-3.647	0.734	24	-4.97	Tukey-Kramer	0.0002
saliva tx	Delta E from initial	6.720	1.056	24	6.36		0.0000

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
----------	----------	Set 4 / Group 2	----------	----------	----------	----------	----------
demineralized	Change in L from previous	1.343	0.907	24	1.48	Tukey-Kramer	0.4638
demineralized	Change in A from previous	-0.740	0.377	24	-1.96	Tukey-Kramer	0.2300
demineralized	Change in B from previous	-3.560	0.553	24	-6.44	Tukey-Kramer	0.0000
demineralized	Delta E from previous	4.501	0.595	24	7.57		0.0000
demineralized	Change in L from initial	1.343	0.907	24	1.48	Tukey-Kramer	0.4638
demineralized	Change in A from initial	-0.740	0.377	24	-1.96	Tukey-Kramer	0.2300
demineralized	Change in B from initial	-3.560	0.553	24	-6.44	Tukey-Kramer	0.0000
demineralized	Delta E from initial	4.501	0.724	24	6.22		0.0000
bleach tx	Change in L from previous	-0.369	0.907	24	-0.41	Tukey-Kramer	0.9767
bleach tx	Change in A from previous	-0.473	0.377	24	-1.26	Tukey-Kramer	0.5992
bleach tx	Change in B from previous	2.309	0.553	24	4.17	Tukey-Kramer	0.0018
bleach tx	Delta E from previous	3.692	0.595	24	6.21		0.0000
bleach tx	Change in L from initial	0.974	0.907	24	1.07	Tukey-Kramer	0.7079
bleach tx	Change in A from initial	-1.213	0.377	24	-3.22	Tukey-Kramer	0.0180
bleach tx	Change in B from initial	-1.251	0.553	24	-2.26	Tukey-Kramer	0.1355
bleach tx	Delta E from initial	4.210	0.724	24	5.81		0.0000
CSP tx	Change in L from previous	-0.584	0.907	24	-0.64	Tukey-Kramer	0.9164
CSP tx	Change in A from previous	0.120	0.377	24	0.32	Tukey-Kramer	0.9886
CSP tx	Change in B from previous	-1.972	0.553	24	-3.57	Tukey-Kramer	0.0080
CSP tx	Delta E from previous	2.549	0.595	24	4.29		0.0003
CSP tx	Change in L from initial	0.390	0.907	24	0.43	Tukey-Kramer	0.9727
CSP tx	Change in A from initial	-1.093	0.377	24	-2.90	Tukey-Kramer	0.0369
CSP tx	Change in B from initial	-3.223	0.553	24	-5.83	Tukey-Kramer	0.0000
CSP tx	Delta E from initial	4.557	0.724	24	6.29		0.0000

Looking at step-by-step changes

Treatment	Measurement	Mean	Standard Error	df	T	Adjustment	Adjusted p-value
Set 4 / Group 3							
demineralized	Change in L from previous	2.157	1.007	24	2.14	Tukey-Kramer	0.1688
demineralized	Change in A from previous	-0.967	0.259	24	-3.73	Tukey-Kramer	0.0053
demineralized	Change in B from previous	-4.919	0.494	24	-9.96	Tukey-Kramer	0.0000
demineralized	Delta E from previous	6.425	0.612	24	10.49		0.0000
demineralized	Change in L from initial	2.157	1.007	24	2.14	Tukey-Kramer	0.1688
demineralized	Change in A from initial	-0.967	0.259	24	-3.73	Tukey-Kramer	0.0053
demineralized	Change in B from initial	-4.919	0.494	24	-9.96	Tukey-Kramer	0.0000
demineralized	Delta E from initial	6.425	0.845	24	7.61		0.0000
bleach tx	Change in L from previous	0.520	1.007	24	0.52	Tukey-Kramer	0.9544
bleach tx	Change in A from previous	-0.473	0.259	24	-1.83	Tukey-Kramer	0.2850
bleach tx	Change in B from previous	2.864	0.494	24	5.80	Tukey-Kramer	0.0000
bleach tx	Delta E from previous	3.536	0.612	24	5.77		0.0000
bleach tx	Change in L from initial	2.677	1.007	24	2.66	Tukey-Kramer	0.0618
bleach tx	Change in A from initial	-1.440	0.259	24	-5.56	Tukey-Kramer	0.0001
bleach tx	Change in B from initial	-2.054	0.494	24	-4.16	Tukey-Kramer	0.0019
bleach tx	Delta E from initial	5.059	0.845	24	5.99		0.0000
P tx	Change in L from previous	-1.362	1.007	24	-1.35	Tukey-Kramer	0.5399
P tx	Change in A from previous	0.342	0.259	24	1.32	Tukey-Kramer	0.5584
P tx	Change in B from previous	-1.861	0.494	24	-3.77	Tukey-Kramer	0.0049
P tx	Delta E from previous	3.092	0.612	24	5.05		0.0000
P tx	Change in L from initial	1.314	1.007	24	1.31	Tukey-Kramer	0.5686
P tx	Change in A from initial	-1.098	0.259	24	-4.24	Tukey-Kramer	0.0015
P tx	Change in B from initial	-3.916	0.494	24	-7.93	Tukey-Kramer	0.0000
P tx	Delta E from initial	4.937	0.845	24	5.84		0.0000

Corrected Data

Sample	Process	Initial L	Initial A	Initial B	First Step L	First Step A	First Step B	First Step ΔE_i	Second Step L	Second Step A	Second Step B	Second Step ΔE_i	Second Step ΔE_p	Third Step L	Third Step A	Third Step B	Third Step ΔE_i	Third Step ΔE_p
SET=Set 1 / Group 1 PROCESS=SP+NB+S																		
111	SP+NB+S	74.91	-0.02	-0.86	.	.	.	.	.	.	.	.	.	75.12	0.22	1.19	2.07	2.07
112	SP+NB+S	75.91	0.98	3.14	.	.	.	.	.	.	.	.	.	72.12	2.22	4.19	4.12	4.12
113	SP+NB+S	75.91	0.98	3.14	.	.	.	.	.	.	.	.	.	76.12	0.22	2.19	1.23	1.23
114	SP+NB+S	80.91	0.98	6.14	.	.	.	.	.	.	.	.	.	75.12	1.22	4.19	6.11	6.11
115	SP+NB+S	72.91	-0.02	3.14	.	.	.	.	.	.	.	.	.	73.12	0.22	3.19	0.32	0.32
115r	SP+NB+S	73.58	-0.05	3.03	.	.	.	.	.	.	.	.	.	72.50	0.44	3.82	1.42	1.42
116	SP+NB+S	79.91	-0.02	3.14	.	.	.	.	.	.	.	.	.	82.12	0.22	4.19	2.46	2.46
121	SP+NB+S	80.91	-1.02	3.14	.	.	.	.	.	.	.	.	.	82.12	-0.78	2.19	1.56	1.56
122	SP+NB+S	78.91	0.98	5.14	.	.	.	.	.	.	.	.	.	80.12	0.22	6.19	1.77	1.77
123	SP+NB+S	70.91	0.98	3.14	.	.	.	.	.	.	.	.	.	73.12	0.22	4.19	2.56	2.56
SET=Set 1 / Group 2 PROCESS=SP+NB+C																		
131	SP+NB+C	72.58	-0.05	1.03	.	.	.	.	.	.	.	.	.	71.43	-0.48	1.03	1.23	1.23
132	SP+NB+C	77.58	0.95	4.03	.	.	.	.	.	.	.	.	.	75.43	0.52	3.03	2.41	2.41
133	SP+NB+C	73.91	-0.02	4.14	.	.	.	.	.	.	.	.	.	72.43	1.52	5.03	2.31	2.31
134	SP+NB+C	77.91	0.98	6.14	.	.	.	.	.	.	.	.	.	75.43	0.52	5.03	2.76	2.76
135	SP+NB+C	75.91	-0.02	6.14	.	.	.	.	.	.	.	.	.	74.43	0.52	6.03	1.58	1.58
135r	SP+NB+C	77.58	0.95	8.03	.	.	.	.	.	.	.	.	.	73.50	0.44	6.82	4.29	4.29
136	SP+NB+C	80.91	-0.02	7.14	.	.	.	.	.	.	.	.	.	73.43	-0.48	3.03	8.55	8.55
141	SP+NB+C	77.91	-1.02	6.14	.	.	.	.	.	.	.	.	.	79.43	-0.48	5.03	1.96	1.96
142	SP+NB+C	78.91	-0.02	3.14	.	.	.	.	.	.	.	.	.	75.43	-3.48	2.03	5.03	5.03
143	SP+NB+C	80.91	-0.02	3.14	.	.	.	.	.	.	.	.	.	78.43	-0.48	2.03	2.76	2.76
SET=Set 1 / Group 3 PROCESS=SP+NB+P																		
151	SP+NB+P	76.58	1.95	6.03	.	.	.	.	.	.	.	.	.	71.43	1.52	6.03	5.17	5.17
152	SP+NB+P	80.58	-0.05	4.03	.	.	.	.	.	.	.	.	.	75.43	0.52	3.03	5.28	5.28
153	SP+NB+P	65.91	0.98	3.14	.	.	.	.	.	.	.	.	.	64.43	0.52	4.03	1.79	1.79
154	SP+NB+P	77.91	0.98	7.14	.	.	.	.	.	.	.	.	.	71.43	0.52	4.03	7.20	7.20
155	SP+NB+P	80.91	0.98	4.14	.	.	.	.	.	.	.	.	.	75.43	0.52	4.03	5.50	5.50
155r	SP+NB+P	82.58	0.95	5.03	.	.	.	.	.	.	.	.	.	75.50	0.44	3.82	7.20	7.20
156	SP+NB+P	76.91	1.98	6.14	.	.	.	.	.	.	.	.	.	74.43	1.52	6.03	2.52	2.52
161	SP+NB+P	75.91	0.98	7.14	.	.	.	.	.	.	.	.	.	71.43	1.52	7.03	4.51	4.51
162	SP+NB+P	74.91	0.98	3.14	.	.	.	.	.	.	.	.	.	69.43	0.52	4.03	5.57	5.57
163	SP+NB+P	78.91	-1.02	4.14	.	.	.	.	.	.	.	.	.	73.43	-0.48	4.03	5.51	5.51
SET=Set 2 / Group 1 PROCESS=SP+B+S																		
211	SP+B+S	78.58	0.95	6.03	69.00	1.31	6.87	9.62	74.96	1.22	6.62	3.68	5.97	78.50	0.44	4.82	1.32	4.05
212	SP+B+S	73.58	0.95	5.03	68.00	1.31	5.87	5.65	68.96	1.22	5.62	4.67	1.00	68.50	1.44	4.82	5.11	0.95
213	SP+B+S	76.58	1.95	6.03	72.00	1.31	7.87	4.98	75.96	1.22	7.62	1.86	3.97	74.50	1.44	4.82	2.46	3.17
214	SP+B+S	81.58	0.95	5.03	80.00	0.31	5.87	1.90	81.96	-0.78	6.62	2.38	2.36	80.50	-0.56	3.82	2.22	3.17
215	SP+B+S	80.58	0.95	6.03	73.00	1.31	4.87	7.68	72.96	1.22	3.62	8.00	1.25	74.50	1.44	2.82	6.89	1.75
215r	SP+B+S	.	.	.	.	.	.	.	73.43	2.52	3.03	.	.	77.58	1.17	3.75	4.42	4.42
216	SP+B+S	80.58	-0.05	8.03	73.00	0.31	7.87	7.59	75.96	0.22	7.62	4.65	2.97	73.50	0.44	5.82	7.43	3.06
221	SP+B+S	76.58	1.95	5.03	70.00	1.31	5.87	6.66	71.96	0.22	5.62	4.97	2.26	71.50	0.44	3.82	5.44	1.87
222	SP+B+S	80.58	-0.05	1.03	76.00	-0.69	2.87	4.98	78.96	0.22	1.62	1.75	3.34	73.50	-0.56	-0.18	7.20	5.80
223	SP+B+S	78.58	1.95	5.03	75.00	3.31	5.87	3.92	76.96	2.22	4.62	1.69	2.57	77.50	1.44	3.82	1.70	1.24
SET=Set 2 / Group 2 PROCESS=SP+B+C																		
231	SP+B+C	74.58	-0.05	3.03	.	.	.	.	69.96	0.22	2.62	4.65	4.65	69.50	0.44	1.82	5.25	0.95
232	SP+B+C	70.58	-0.05	6.03	.	.	.	.	71.96	0.22	5.62	1.46	1.46	67.50	0.44	4.82	3.35	4.54
233	SP+B+C	73.58	0.95	3.03	.	.	.	.	66.96	1.22	3.62	6.65	6.65	70.50	0.44	2.82	3.13	3.71
234	SP+B+C	71.58	0.95	4.03	.	.	.	.	64.96	1.22	5.62	6.81	6.81	71.50	0.44	3.82	0.56	6.83
235	SP+B+C	79.58	1.95	4.03	.	.	.	.	76.96	0.22	5.62	3.52	3.52	76.50	0.44	3.82	3.44	1.87
235r	SP+B+C	78.66	1.95	4.10	.	.	.	.	76.43	0.52	5.03	2.81	2.81	79.58	1.17	3.75	1.26	3.46
236	SP+B+C	80.58	0.95	7.03	.	.	.	.	71.96	0.22	5.62	8.77	8.77	72.50	0.44	3.82	8.71	1.89
241	SP+B+C	75.58	0.95	3.03	.	.	.	.	73.96	1.22	4.62	2.29	2.29	71.50	1.44	4.82	4.48	2.48
242	SP+B+C	75.58	-0.05	2.03	.	.	.	.	70.96	0.22	2.62	4.67	4.67	66.50	0.44	2.82	9.13	4.47
243	SP+B+C	74.58	0.95	4.03	.	.	.	.	70.96	1.22	5.62	3.96	3.96	73.50	0.44	4.82	1.43	2.77
SET=Set 2 / Group 3 PROCESS=SP+B+P																		
251	SP+B+P	73.58	-0.05	0.03	.	.	.	.	67.96	0.22	1.62	5.85	5.85	69.50	0.44	0.82	6.18	1.75
252	SP+B+P	78.58	-0.05	4.03	.	.	.	.	76.96	-0.78	4.62	1.87	1.87	71.50	0.44	2.82	7.20	5.88
253	SP+B+P	75.58	2.95	2.03	.	.	.	.	71.96	0.22	1.62	4.55	4.55	73.50	0.44	-0.18	3.94	2.38
254	SP+B+P	68.58	1.95	3.03	.	.	.	.	67.96	2.22	4.62	1.73	1.73	69.50	1.44	4.82	2.08	1.74
255	SP+B+P	77.58	-0.05	7.03	.	.	.	.	67.96	0.22	4.62	9.92	9.92	69.50	0.44	2.82	9.12	2.38
255r	SP+B+P	76.66	-0.05	7.10	.	.	.	.	68.43	0.52	3.03	9.20	9.20	72.58	0.17	2.75	5.97	4.17
256	SP+B+P	80.58	0.95	8.03	.	.	.	.	72.96	0.22	7.62	7.67	7.67	73.50	0.44	4.82	7.79	2.86
261	SP+B+P	77.58	0.95	4.03	.	.	.	.	75.96	0.22	4.62	1.87	1.87	74.50	0.44	3.82	3.13	1.68
262	SP+B+P	76.58	0.95	7.03	.	.	.	.	73.96	0.22	5.62	3.06	3.06	75.50	0.44	4.82	2.51	1.75
263	SP+B+P	69.58	0.95	4.03	.	.	.	.	64.96	1.22	4.62	4.67	4.67	67.50	0.44	3.82	2.15	2.77

Corrected Data

Sample	Process	Initial L	Initial A	Initial B	First Step L	First Step A	First Step B	First Step ΔE_1	Second Step L	Second Step A	Second Step B	Second Step ΔE_1	Second Step ΔE_p	Third Step L	Third Step A	Third Step B	Third Step ΔE_1	Third Step ΔE_p
SET=Set 3 / Group 1 PROCESS=D+NB+S																		
311	D+NB+S	77.66	0.95	2.10	80.58	0.17	-2.25	5.30	.	.	.	.	.	80.05	0.19	-2.30	5.06	0.53
312	D+NB+S	79.66	0.95	7.10	81.58	1.17	-0.25	7.60	.	.	.	.	.	76.05	1.19	0.70	7.35	5.61
313	D+NB+S	69.66	1.95	4.10	75.58	1.17	-0.25	7.39	.	.	.	.	.	70.05	1.19	-1.30	5.47	5.63
314	D+NB+S	79.66	-0.05	5.10	83.58	0.17	-2.25	8.33	.	.	.	.	.	83.05	0.19	-0.30	6.38	2.02
315	D+NB+S	73.66	1.95	2.10	76.58	1.17	-3.25	6.14	.	.	.	.	.	76.05	1.19	-2.30	5.06	1.09
315r	D+NB+S	71.92	1.17	1.74	73.53	4.33	-3.72	6.51	.	.	.	.	.	78.12	1.26	-0.89	6.74	6.20
316	D+NB+S	75.66	-0.05	5.10	78.53	-0.67	0.28	5.64	.	.	.	.	.	79.05	0.19	0.70	5.56	1.09
321	D+NB+S	70.66	2.95	4.10	76.58	1.17	-0.25	7.56	.	.	.	.	.	75.05	1.19	0.70	5.82	1.80
322	D+NB+S	70.66	0.95	6.10	75.58	0.17	-0.25	8.07	.	.	.	.	.	80.05	0.19	0.70	10.86	4.57
323	D+NB+S	79.66	-0.05	5.10	85.58	2.17	-0.25	8.28	.	.	.	.	.	85.05	1.19	-0.30	7.73	1.12
SET=Set 3 / Group 2 PROCESS=D+NB+C																		
331	D+NB+C	68.66	0.95	3.10	74.58	-0.83	-3.25	8.86	.	.	.	.	.	72.05	0.19	-1.30	5.61	3.35
332	D+NB+C	80.66	0.95	1.10	82.58	1.17	-1.25	3.04	.	.	.	.	.	82.05	0.19	-1.30	2.88	1.12
333	D+NB+C	75.66	-0.05	2.10	81.58	1.17	-2.25	7.45	.	.	.	.	.	79.05	0.19	-1.30	4.81	2.87
334	D+NB+C	72.66	0.95	4.10	76.58	0.17	-0.25	5.91	.	.	.	.	.	77.05	1.19	0.70	5.56	1.47
335	D+NB+C	74.66	0.95	5.10	80.58	0.17	-0.25	8.02	.	.	.	.	.	82.05	0.19	0.70	8.63	1.75
335r	D+NB+C	79.92	2.17	6.74	78.53	0.33	-0.72	7.81	.	.	.	.	.	82.12	1.26	2.11	5.21	4.66
336	D+NB+C	75.66	-0.05	6.10	84.53	0.33	2.28	9.67	.	.	.	.	.	86.05	0.19	1.70	11.29	1.63
341	D+NB+C	85.66	0.95	6.10	79.58	0.17	-1.25	9.57	.	.	.	.	.	80.05	1.19	0.70	7.79	2.25
342	D+NB+C	88.66	1.95	5.10	83.58	0.17	-1.25	8.32	.	.	.	.	.	83.05	0.19	-1.30	8.69	0.53
343	D+NB+C	74.66	1.95	5.10	77.58	0.17	1.75	4.79	.	.	.	.	.	77.05	0.19	1.70	4.51	0.53
SET=Set 3 / Group 3 PROCESS=D+NB+P																		
351	D+NB+P	72.66	1.95	5.10	77.58	1.17	-0.25	7.31	.	.	.	.	.	76.05	1.19	0.70	5.61	1.80
352	D+NB+P	77.66	0.95	6.10	79.58	1.17	2.75	3.87	.	.	.	.	.	82.05	1.19	3.70	5.01	2.65
353	D+NB+P	78.66	0.95	2.10	82.58	0.17	-1.25	5.22	.	.	.	.	.	83.05	0.19	-1.30	5.60	0.47
354	D+NB+P	72.66	1.95	4.10	81.58	0.17	-0.25	10.08	.	.	.	.	.	75.05	0.19	2.70	3.28	7.17
355	D+NB+P	73.66	0.95	4.10	74.58	1.17	1.75	2.53	.	.	.	.	.	75.05	0.19	-0.30	4.68	2.32
355r	D+NB+P	77.92	1.17	6.74	71.53	0.33	-0.72	9.86	.	.	.	.	.	76.12	0.26	2.11	5.05	5.39
356	D+NB+P	79.66	-0.05	3.10	82.53	0.33	1.28	3.42	.	.	.	.	.	82.05	0.19	-0.30	4.16	1.66
361	D+NB+P	75.66	3.95	4.10	81.58	1.17	-1.25	8.45	.	.	.	.	.	83.05	1.19	-0.30	9.03	1.75
362	D+NB+P	77.66	0.95	5.10	85.58	1.17	-2.25	10.81	.	.	.	.	.	80.05	0.19	-0.30	5.95	5.95
363	D+NB+P	75.66	-0.05	7.10	80.58	0.17	0.75	8.04	.	.	.	.	.	78.05	0.19	1.70	5.91	2.70
SET=Set 4 / Group 1 PROCESS=D+B+S																		
411	D+B+S	77.12	0.22	-1.81	83.53	-0.67	-3.72	6.75	83.05	-0.81	-0.30	6.21	3.46	82.91	-0.69	-2.05	5.87	1.76
412	D+B+S	77.92	1.17	4.74	76.53	0.33	0.28	4.75	76.05	0.19	8.70	4.49	8.43	73.91	-0.69	2.95	4.77	6.20
413	D+B+S	82.92	1.17	8.74	83.53	0.33	4.28	4.58	83.05	1.19	7.70	1.05	3.56	75.91	1.31	4.95	7.97	7.65
414	D+B+S	86.92	3.17	6.74	84.53	0.33	0.28	7.45	87.05	0.19	4.70	3.61	5.09	82.91	0.31	2.95	6.21	4.50
415	D+B+S	84.92	0.17	7.74	85.53	1.33	2.28	5.62	85.05	0.19	4.70	3.04	2.72	81.91	0.31	3.95	4.84	3.23
415r	D+B+S	79.12	0.22	7.19	83.05	1.19	-0.30	8.51	85.12	0.26	6.11	6.10	6.80	81.25	0.34	2.71	4.96	5.15
416	D+B+S	78.92	0.17	5.74	79.53	0.33	-2.72	8.48	80.05	-0.81	-0.30	6.22	2.73	81.91	-0.69	-2.05	8.39	2.56
421	D+B+S	70.00	1.31	4.87	79.53	1.33	2.28	9.88	79.05	0.19	1.70	9.65	1.37	75.91	0.31	-0.05	7.75	3.60
422	D+B+S	70.00	0.31	1.87	82.53	0.33	-0.72	12.79	85.05	-0.81	-0.30	15.25	2.80	78.91	-0.69	-2.05	9.79	6.39
423	D+B+S	80.92	0.17	4.74	77.53	0.33	2.28	4.19	78.05	0.19	4.70	2.87	2.48	76.91	0.31	1.95	4.89	2.98
SET=Set 4 / Group 2 PROCESS=D+B+C																		
431	D+B+C	77.92	1.17	4.74	78.53	0.33	1.28	3.61	78.05	0.19	4.70	0.99	3.46	76.91	0.31	1.95	3.09	2.98
432	D+B+C	77.92	1.17	8.74	78.53	0.33	4.28	4.58	75.05	0.19	5.70	4.29	3.76	75.91	0.31	4.95	4.38	1.15
433	D+B+C	79.92	0.17	3.74	81.53	0.33	1.28	2.94	80.05	-0.81	2.70	1.43	2.35	79.91	0.31	1.95	1.80	1.36
434	D+B+C	84.92	0.17	7.74	85.53	0.33	2.28	5.50	80.05	0.19	8.70	4.96	8.44	81.91	-0.69	4.95	4.19	4.28
435	D+B+C	73.92	2.17	6.74	74.53	1.33	6.28	1.14	76.05	1.19	4.70	3.11	2.20	73.91	1.31	3.95	2.92	2.27
435r	D+B+C	71.12	2.22	7.19	73.05	2.19	5.70	2.44	77.12	1.26	5.11	6.42	4.22	75.25	1.34	2.71	6.16	3.06
436	D+B+C	78.92	4.17	2.74	81.53	0.33	-0.72	5.79	83.05	-0.81	4.70	6.76	5.74	80.91	-0.69	-0.05	5.95	5.21
441	D+B+C	79.92	2.17	6.74	80.53	1.33	2.28	4.58	81.05	0.19	3.70	3.80	1.89	79.91	1.31	1.95	4.87	2.37
442	D+B+C	70.12	0.22	0.19	76.53	-0.67	-2.72	7.10	79.05	0.19	-1.30	9.05	3.02	78.91	-0.69	-3.05	9.41	1.96
443	D+B+C	81.12	0.22	7.19	79.53	1.33	2.28	5.28	81.05	0.19	3.70	3.49	2.37	79.91	0.31	2.95	4.41	1.37
SET=Set 4 / Group 3 PROCESS=D+B+P																		
451	D+B+P	79.92	1.17	5.74	78.53	0.33	-0.72	6.66	78.05	1.19	4.70	2.14	5.51	78.91	1.31	1.95	3.92	2.88
452	D+B+P	81.92	0.17	4.74	81.53	0.33	0.28	4.48	81.05	-0.81	2.70	2.42	2.72	80.91	0.31	0.95	3.92	2.08
453	D+B+P	78.92	2.17	3.74	85.53	0.33	0.28	7.68	86.05	0.19	1.70	7.68	1.52	80.91	0.31	-1.05	5.51	5.83
454	D+B+P	73.00	1.31	6.87	82.53	0.33	4.28	9.92	83.05	0.19	4.70	10.34	0.68	80.91	0.31	3.95	8.49	2.27
455	D+B+P	81.92	2.17	6.74	81.53	1.33	1.28	5.54	87.05	0.19	3.70	6.28	6.13	82.91	0.31	1.95	5.23	4.50
455r	D+B+P	79.12	1.22	6.19	83.05	0.19	-1.30	8.52	84.12	1.26	4.11	5.42	5.62	81.25	0.34	-1.29	7.83	6.18
456	D+B+P	79.92	1.17	4.74	79.53	0.33	0.28	4.56	78.05	-0.81	3.70	2.92	3.90	79.91	0.31	1.95	2.92	2.79
461	D+B+P	79.92	1.17	7.74	80.53	0.33	2.28	5.56	79.05	1.19	5.70	2.22	3.82	78.91	0.31	3.95	4.02	1.96
462	D+B+P	73.92	2.17	6.74	78.53	0.33	-1.72	9.81	79.05	-0.81	0.70	8.47	2.73	76.91	0.31	-0.05	7.65	2.53
463	D+B+P	80.92	1.17	1.74	81.53	0.33	-1.72	3.61	83.05	-0.81	2.70	3.06	4.81	81.91	-0.69	-0.05	2.76	2.98