

**TEETH DEHYDRATION/ REHYDRATION AND THE EFFECT OF TIME
ON TOOTH SHADE SELECTION**

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ABSTRACT

Sama A. Suliman: Teeth Dehydration/Rehydration and the effect of time on tooth shade selection

(Under the direction of Harald H. Heymann)

Objective: The purpose of this clinical study is to estimate the time required for teeth to dehydrate and rehydrate and its relation to the accuracy of tooth shade selection.

Materials and Methods: Thirty-two participants were recruited, and color measurements were conducted using a spectrophotometer placed with a custom jig. Measurements were made at baseline, and then after isolation using a lip retractor at 1, 2, 3, 5, 7, 10 and 15 minute intervals to determine tooth dehydration time. After mouth rinsing for 5 minutes measurements were again made at the same interval time to determine tooth rehydration time. The values obtained were used to calculate CIEDE2000 values for color change between the baseline recordings and all intervals. The data were compared to the 50:50% perceptibility and acceptability thresholds and analyzed for the color change over time using ANOVA and Tukey test was used for multiple comparisons.

Result: There was a perceivable change in tooth color within the first minute of tooth dehydration ($P > 0.0001$). After the first minute, 87% of the teeth were beyond the ΔE_{00} perceptibility threshold (0.8), and 72% of the teeth were beyond the ΔE_{00} acceptability threshold (1.8). After 15 minutes of rehydration, 90% of the teeth were beyond the perceptibility threshold, and 65% were beyond the acceptability threshold. The original shade was not restored for the tested teeth even after 15 minutes of rehydration time. **Conclusions:** Shade selection procedures should be carried out within the first minute and before the teeth are exposed to dehydration by means of isolation. Teeth do not rehydrate within 15 minutes after rehydration.



“In the name of God, the Most Gracious, the Most Merciful”

To my parents, the reason why I am who I am

To my beloved family and loved ones

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LIST OF ABBREVIATIONS AND SYMBOLS

ΔE	Color differences
$^{\circ}\text{C}$	Degree of celsius
$^{\circ}\text{K}$	Kelvin
a^*	Red-green chromaticity
ANOVA	Analysis of variance
b^*	Yellow-blue chromaticity
C^*	Chroma
CI	Confidence interval
CIE	Commission International de l'Eclairage
CIELAB	Color system
CRI	Color rendering index
H^*	Hue
IRB	Institutional Review Boards
L^*	Lightness
LED	Light-emitting diode
ml	Millimeter
nm	Nanometer

RI	Refractive Index
S.D.	Standard deviation
TG	Toothguide 3D-Master
VC	Vitapan Classical

CHAPTER 1: REVIEW OF THE LITERATURE

1. Introduction

Tooth shade matching has proven to be a challenging task for the dental profession. The problem of color in dentistry was addressed a long time ago by Clark and he stated that “we as dentists are not educationally equipped to approach a color problem”.

Despite numerous advances in shade matching techniques in recent decades, based on current prospective and retrospective clinical studies, 50% of cemented ceramic crowns exhibit incorrect color matches.

Shade matching is a complex process where color determination, color communication and color reproduction all have to be successfully carried out. While all of these steps are equally important, chances of an esthetically pleasing color match become slim if the first step, color determination, is improperly performed. Tooth color can be determined by either a visual method or an instrumental method. The visual method is the most common and popular method used for the shade determination in the clinical practice, and is accomplished by matching the shade guide tabs to the natural tooth. Instrumental methods, use devices such as spectrophotometers, colorimeters and digital imaging to assess shade matching.

When color is measured, Delta E (ΔE) is usually identified. Delta E is the color difference, or the distance separating two points of color in the CIELAB system. When the color difference between two compared objects can be seen by 50% of the observers, it is a 50:50% perceptibility threshold. Similarly, a 50:50% acceptability threshold has been defined when a color difference is considered acceptable by 50% of the observers. A prospective multicenter study by Paravina *et al*,

concluded that the CIELAB 50:50% perceptibility threshold was observed when $\Delta E = 0.8$, whereas the 50:50% acceptability threshold was $\Delta E = 1.8$, under simulated clinical settings.

Color determination is an interplay where color perception and matching abilities need to meet under optimal conditions. Color perception occurs when light from a particular source is reflected by the object observed to the viewer. Hence, color perception is influenced by a triad of events which are: the light source, the optical properties of the object observed, the color matching ability of the observer, and the hydration state of the tooth itself.

Tooth dehydration makes teeth appear whiter due to increasing enamel opacity. The inter-prismatic spaces become filled with air instead of water so light can no longer be conducted from crystal to crystal. Loss of translucency due to dehydration, therefore, causes more reflection, which masks the underlying color of dentin, making the tooth appear lighter. Most of the dental procedures that are used in dental practice result in some dehydration of teeth. It is advised that shade selection of teeth involving restorative procedures be done at the beginning of the appointment, but little objective research exists to document this phenomenon in the dental literature. Thus, little is known in the literature about the dehydration/rehydration processes of the teeth and their relationship to the accuracy of the shade selection procedure. Therefore, the aim of this study was to estimate the time required for the teeth to dehydrate/ rehydrate and effect that process has on accurate shade selection.

2. Literature review

2.1. History of the Color in Dentistry

Tooth shade matching has proven to be a challenging task for the dental profession, and patients' expectations for esthetic restorations are justifiably high. Therefore accurate shade assessment is vital to generating consistently successful results. Despite numerous advances in shade matching techniques, prospective and retrospective clinical studies have documented high incidences of color incorrect matching with esthetic restorations.¹⁻⁵

Color was presented in 1611 as a three dimensional entity by Sigfried Forsius.^{1, 7} Since that time, there have been numerous systems invented and used to explain this tridimensional property. One of the color systems used worldwide with simplicity and flexibility is the Munsell Color Order System, which is the system of choice for color matching in dentistry. Clark⁶ in 1931, was the first one that addressed the problem of color matching between natural teeth and restoration materials and was the first attempt to organize dental colors based on Munsell color systems (Figure 1.1).

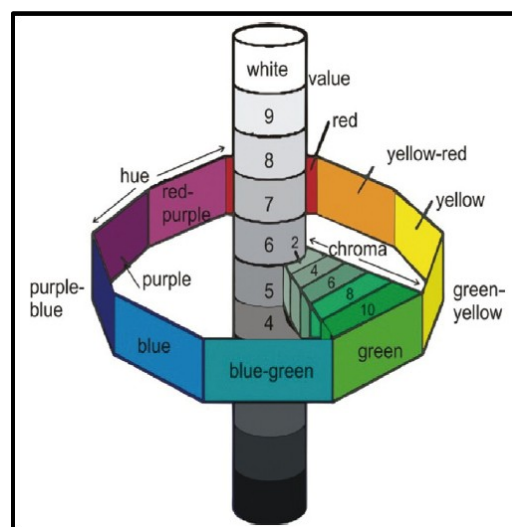


Figure 1.1: Munsell Color System.

The Munsell color system can be likened to a sphere or to a cylinder.^{8,9} In the cylinder, there are three dimensions of the color (*Hue, Chroma, and Value*). The dimension of *Hue* is the first dimension, the easiest to understand. In Munsell's words¹⁰, "it is that quality by which we distinguish one color family from another, as red from yellow, green from blue or purple." The *Hue* is represented by the ten colors arranged around the central axis. *Chroma*, "is the departure of a color sensation from that of white or gray; the intensity or saturation of a distinctive *Hue*; color intensity." *Chroma* describes the intensity of Hue in a color. In this diagram, *Chroma* is related to the spokes of the wheels; the colors are purest at the periphery and become progressively grayer as they approach the central achromatic *Value* axis.¹⁰

Value is that quality by which we distinguish a light color from a dark color. This characteristic is related to the achromatic (colorless) polar axis going through the Munsell color element. The nine *Value* levels are represented by the nine wheels in this system.¹⁰

Additionally, the Commission International de l'Eclairage (CIE, 1931) published the standards for color matching, but the absence of valid scientific evidence for color measurement did not allow significant improvements. In 1978 the CIE developed a new system three dimensional color system called CIE L*a*b* color space. The three coordinates that determine color in this nearly uniform space are the L* value (y-axis), which represents lightness, the a* value, which represents red-green chromaticity and the b* value which represents yellow-blue chromaticity.¹³ This system is the basis for transforming spectral energy data into meaningful color data, and calculate the difference between two colors using a formula that generates one number as a value for color differences which is called Delta E (ΔE) value.¹⁰

Delta E (ΔE) is the color difference, or the distance separating two points of color in the CIELAB system. It is defined by the following equation:^{2,11} $\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$

As noted earlier, when the color difference between two compared objects can be seen by 50% of the observers, it is a 50:50% perceptibility threshold. Similarly, a 50:50% acceptability threshold has been defined when a color difference is considered acceptable by 50% of the observers. A prospective multicenter study by Paravina *et al*, concluded that the CIELAB 50:50% perceptibility threshold was $\Delta E_{ab}=1.2$, whereas the 50:50% acceptability threshold was $\Delta E_{ab}=2.7$, under simulated clinical settings.¹²

Shade matching is a complex process where color determination, color communication and color reproduction all must be successfully carried out. As noted earlier, color determination in dentistry can be performed by two methods, either using a visual method or an instrumental method. The visual method is the most frequently used in dental practice by comparing a tooth's color to that of a shade guide. The first systematic shade guide was created by Clark based on visual assessment of human teeth, recorded in Munsell Hue, Value and Chroma, and it consisted of 60 ceramic tabs.¹³ Over the years, many shade assessment products appeared on the market. However, the shade matching guide that became very popular was the Vitapan Classical, (VC, Vita Zahnfabrik, Bad Sackingen, Germany) introduced in the mid-1950s.

The Vitapan classical shade guide consists of 16 tabs arranged into four groups based on Hue and within the groups according to increasing Chroma (also known as A, B, C, D arrangement). However there the history of complaints, primarily related to color range, distribution and user-friendliness has been significant.

Another shade guide that was developed is the Ivoclar- Vivadent Chromascop. The chromascop is arranged into five groups of four tabs based on hue (1= white, 2= light yellow, 3= dark yellow, 4= grey, 5= brown) and then further intragroup selections are made according to increasing chroma (from 10 to 40). This system differs from other shade guides by using three

digit numbering system. Hence, the tabs marked with group number (100 to 500), the lower the number, the less chromatic and lighter the tab in the respective group.

In late 1990s, there was a breakthrough in dental shade guides when the Toothguide 3D-Master (TG, Vita Zahnfabrik, Bad Säckingen, Germany) was introduced. As compared to the Vitapan classical version, it was found that the Toothguide matches natural teeth better, includes wider color range and has more uniform color distribution.¹⁴

This Product has improved the conventional shade matching by removing some of the subjectivity from shade tab based color determination. This system used six different levels of Values to create six groups of tabs, from 0 (the lightest) to 5 (the darkest). There are 3 Chroma levels, from 1 (the least chromatic) to 3 (the most chromatic) in each group (except in group 1 that has two chroma levels). Intermediate chroma levels (1.5 and 2.5) in groups 2, 3 and 4 are associated with hue variations – L (less red) and R (more red). In spite of these advantages, some dental professionals have difficulty in understanding the shade matching method and implementation of the Value chroma- hue concept. In addition, some users find a shade guide with 29 tabs is confusing.

A newer version of Toothguide, called the Linearguide 3D Master is a modification of the previous Toothguide. Linearguide contains exactly the same shade tabs, but shade matching is simplified and reduced to two steps. First, value selection (A dark-gray holder), containing only 6 middle tabs (0M2 to 5M2) is used. The small number of tabs with large color differences and the linear tab arrangement simplify group selection. Second, Chroma and Hue selection occur. A final selection based on Chroma and hue is made from the initial value group selected. Values from (0-5) in close approximation to each other and all on the same plane, allowing for quick and accurate evaluation against natural teeth. The Chroma guide of the selected value is then used to choose the

Chroma. This guide was found to generate more accurate shade matching results and is superior in a subjective evaluation compared to the Toothguide.¹⁴

There are a lot of interfaces which limit the use of shade guide systems and diminish the ability of a dentist to provide predictable color matching. Some of these limitations include the facts that, (1) shade guides are not constructed to exactly match natural teeth; (2) shade guides do not match other shade guides; (3) and there is inadequate control of the different batches of shade guides even from the same manufacturer.¹⁵

In order to minimize the high incidence of color mismatch when using a visual method, new instrumental methods for color determination were developed. There have been many electronic instruments available for shade determination using such technologies as: colorimetry, spectrophotometry and spectroradiometry.¹⁶

Colorimeters are relatively simple and low cost instruments. They measure the intensity of red, green, and blue light reflected from the object/tooth by using a filter. This technology is sometimes referred to as tristimulus value assessment. Colorimeters are not registering spectral reflectance, and can be less accurate than spectrophotometers. Moreover, aging of the filters used in colorimeters additionally can adversely affect accuracy.¹⁷

The spectrophotometer is a more complex instrument, and is the most accurate, useful and flexible instrument for color matching in dentistry. It measures and records the amount of visible radiant energy reflected or transmitted by an object/tooth one wavelength at a time for each Value, Chroma, and Hue, generating considerable amounts of data. The data obtained from spectrophotometers must be manipulated and translated into a form useful for dental professionals.¹⁸

The spectroradiometer is also used as an alternative color measuring instrument. The spectroradiometer was used not only for measuring the color of teeth, but also was used to determine the color of the shade guide tabs, and the translucency of various ceramics. It measures the spectral energy of a source. Spectrophotometer and spectroradiometer are reliable, and accurate instruments used for color matching. There is similarity between spectrophotometer and spectroradiometer measurements. A comparative study of spectrophotometer and spectroradiometer measurements in terms of translucency parameter of different restorative materials showed similar results which are highly correlated.^{19,20}

In spite of the first spectrophotometers being accurate, they were very complex instruments, bulkier, difficult to use and expensive. Recent advances in these electronic devices have resulted in cordless, small, portable, cost efficient, battery operated, contact-type spectrophotometers that provide enough shade information to help aid in the color analysis process.

The Vita Easyshade (Vita Zahnfabrik, Bad Säckingen, Germany) is one of the latest spectrophotometers available for clinical use that provides enough shade information to help aid in the color determination. It consists of two different sets of LEDs lights that measures the amount and spectral composition of light reflected from the tooth. The Vita Easyshade is the most accurate, fast and reliable device for shade determination of natural teeth and ceramic restorations. The results are highly correlated with standard shade systems VITA classical A1-D4, VITA Toothguide/Linearguide 3D Master. (Figure 1.2)



Figure 1.2: The Vita Easyshade Spectrophotometer.

Different measurement modes are possible with Vita Easyshade device: tooth single mode, tooth area mode (cervical, middle and incisal shades), restoration color verification (includes Lightness, Chroma and Hue comparisons) and shade tab mode (practice/training mode). It quantifies color by using the CIE $L^*a^*b^*$ system, and provides the difference in color Delta E (ΔE).

Color determination is an interplay where color perception and matching abilities need to meet the optimal esthetic results. Color perception occurs when light from a particular source is reflected by the object observed to the viewer. As light enters the eye it stimulates the neural sensors in the eye's retina to send a signal that is interpreted in the visual cortex of the brain. The reflected components of incident white light determine the color of an object.²¹ There are different

types of materials responding differently to the light. Transparent materials allow for the passage of light with little change. Translucent materials scatter, transmit and absorb light. Opaque materials reflect and absorb; however, they do not transmit light.

Natural teeth exhibit several characteristics besides color. The quality of translucency exists, which can be defined as the gradient between transparent and opaque, surface texture, and surface gloss.²² The translucent nature of tooth structure makes the color matching procedure more complex when compared to an opaque object. With less light returning to the eye. These characteristics of tooth structure affect the degree of light diffusion when striking a particular object. Hence, the accuracy of color perception depends on a triad of events: the light source, the optical properties of the object observed, and the color matching ability of the observer himself.¹ There are additional factors including the surrounding colors, the angle of observation, light and dark adaptation, and the size of the field of view, etc. However the most crucial factors are the light source, surface, and individual observer which are closely interrelated. A change of any one of them can result in a change in the perceived color. The light source is the most influential factor for shade selection.

The ideal light source is natural light, occurring around mid-day, for accurate color comparison, according to the recommendations of the Commission Internationale de l'Eclairage (CIE, 1971).²³ If the light source changes, then the light reflected from an object changes too. The Phenomenon of two objects appearing to match in color under one light condition but showing apparent differences under another light condition is called metamerism. Metamerism occurs frequently in dental practice, resulting to difficulty in shade matching. To minimize this metamerism phenomenon is by achieving the same spectral reflectance curves for two objects to be matched that will be always successfully matched regardless the light in which they are observed.²⁴ The absence of ideal light

conditions has led to the use of artificial lighting for color matching. A light source that is similar to standard daylight is ideal for shade matching due to the fact that the daylight have the basic qualities which are favorable for color development. These qualities are full color content, adequate intensity, and compatibility with human's eye. The light source quality is commonly described by color temperature, and Color rendering index (CRI) in which are all measured to reproduce the standard daylight.

Color temperature is defined as mean wavelength of the ambient light. The ideal color temperature for color rendering is 5,500 K. Color Rendering Index (CRI) is the measure of the completeness of the light spectrum and it refers to how a specific light source makes the color of an object in the environment appear to a person and also how the subtle variations in its shades and hues are replicated. The CRI of a light source is expressed as a number on a scale ranging from 0 to 100. The higher this number, the better is the ability of the lighting source to render the color details of an object accurately, and the eye ideally perceives color as it should be seen. Hence, the light source should be spectrally balanced in the visible range (370-780 nm), have a color rendering index of >90, so that the light source itself does not become a limiting factor.²³

Among optical properties of the object itself, there are multiple inherent optical properties of an object that interact with light and effect the perception of color. When light strikes object/tooth, four phenomena associated with the interactions of the tooth with the light flux can be described.²⁵

- 1) Specular transmission of the light through the tooth.
- 2) Specular reflection at the surface.
- 3) Diffuse light reflection at the surface.
- 4) Absorption and scattering of light within the dental tissues.

The translucency is the amount of incident light transmitted and scattered. Scattering makes the object opaque and is dependent on the size, shape, number of the scattering centers and refractive index.²¹ The color matching ability of the observer himself is subjective and influenced by external light conditions, experience, age, and fatigue of the human eye and physiological variables such as color blindness which all lead to inconsistencies in color matching ability.¹⁸

2.2. Tooth Dehydration / Rehydration Process

One of the most important aspect that affect the color measurement in dentistry is tooth hydration. Unfortunately, a paucity of studies exists in the literature related to this subject.

Moisture content in intact extracted teeth was first examined by Burnett and Zenewitz (1958).²⁶

They measured the changes in the moisture content of teeth. They found a 9.3 ± 0.37 percent of maximum moisture content of the teeth. Teeth were examined in a vacuum at 100°C with a constant weight for 24 hours. Rehydration was not completely observed even when placing the teeth in a moist environment at 37°C , because some moisture had been irreversibly lost during dehydration.

In 1980, a study conducted by Sorensen and Bonstein to measure the changes in hydration state on tooth color by using spectrophotometry by calculating ΔE for color change. In this study, they used 30 extracted lower molars and they immersed them in water at 37°C for four days. Later, they dehydrated the teeth at room temperature for 12 hours and rehydrated them for 48 hours. They measured every tooth at baseline, after 15 minutes, 30 minutes, 1 hour, and 12 hours for dehydration, and for rehydration they used the same intervals except that the last measurement was after 48 hours. The authors concluded that the L^* , a^* , and b^* values increased during dehydration, which means that teeth became lighter. Conversely, the color values decreased during rehydration

and teeth became darker.²⁷ Hence, tooth dehydration makes teeth appear lighter due to increasing enamel opacity. The inter-prism spaces become filled with air instead of water so light can no longer be transmitted from crystal to crystal.²⁴

As a consequence of tooth dehydration, translucency of dental enamel will be decreased according to study was done by Brodbelt et al. (1981), in which they examined the translucency of dental enamel by measuring hydration of the teeth with total transmittance of wavelength of the visible spectrum. In this study, the authors examined the wet and dehydrated enamel disks that collected from labial surfaces of extracted human maxillary central incisors by using a spectrophotometer for total transmission and color measurements. For dehydrated enamel, the authors used an air stream for a ten-second exposure to simulate clinical conditions. They repeated the procedure three times every 15 minutes and rehydrated the specimens for 48 hours in distilled water. Measurements with a spectrophotometer were performed and they found that the translucency of enamel was influenced by dehydration in (reduction in translucency) and translucency was restored with rehydration.²⁸ As a result of the replacement of the water around the enamel prisms by air, the light will be scattered, due to function of the difference in refractive indices of the two components.

The refractive index (RI) of dental enamel is approximately 1.7. Since the refractive index of refraction of water is 1.33 and that of air is 1.00, a larger difference and greater scattering of light are produced at an enamel-air interface. For that reason, dehydrated enamel has a lower translucency.²⁹

Furthermore, Russell et al. (2000)³⁰ conducted an *in vivo* study to determine the changes in color measurements of naturel teeth after dehydration and the time taken for tooth color to return to normal. In this clinical study, the authors used the CIELab system to evaluate L^* (Lightness), a^*

and b^* (chromaticity), as well as h^* which is the hue angle and C^* (*Chroma*). Fourteen subjects were divided into two groups of seven students. For the first group was, rubber dam was used for 15 minutes of teeth isolation and removed it and took three readings in total with an interval of 10 minutes. For the second group they dehydrated the teeth with polyvinylsiloxane impression for six minutes and three readings were taken as well. Color measurements were performed in both groups by a spectrophotometer with using a jig for positioning consistency of the measurement on a maxillary central incisor. The authors concluded that there were statistically significant changes in measurements of delta E and in L^* , a^* and b^* values. The largest changes noted in lightness values and with only relatively small changes noted in a^* and b^* for both rubber dam and impressions material group. Hence, teeth became brighter and less saturated after rubber dam application. The baseline values were regained after 30 min.³⁰

In a more recent study, Burki et al. (2012)³¹ investigated any change in tooth color resulting from dehydration and noted the time required for any change to return to baseline. The authors examined 20 subjects. For each subject a left or right maxillary central incisor was used with a positioning jig. The VITA Easyshade spectrophotometer was used for color measurement of the tested teeth. They collected a baseline spectrophotometric measurement, then the tested tooth was isolated with rubber dam and allowed to dehydrate. Spectrophotometric measurements of the test tooth during dehydration were obtained at 10 min intervals for 30 min. Then rubber dam was removed and subjects were instructed to drink a glass of water. Additional spectrophotometric measurements of the tested tooth were obtained at the same intervals for 30 min during rehydration. The authors stated that there were statistically significant changes in the shades of the teeth during dehydration. In which they found the test teeth became significantly lighter after just 10 min of dehydration. A perceivable color change had not returned to baseline shade within 30 min of

rehydration, unlike what was found in previous mention study done by Russel et al.³⁰ In this study, the baseline values were regained after 30 min of rehydration.

There are different factors that producing dehydration and rehydration of the teeth. For example: rubber dam, Optragates (which are a latex free lip and check retractor with elasticity and flexibility make it comfortable to wear and help the patient to keep their mouth open), or other isolation methods, like taking impression for dental procedures. Nowadays most of the clinicians are not using rubber dam since it is time consuming for rubber dam application, and in a typical clinical scenario, shade determination for ceramic restorations is usually not performed with a rubber dam in place. Retraction methods are more commonly used which provide relative levels of isolation by relieving the tooth of contact with wet soft tissues while still keeping the tooth within the confines of the oral cavity. For that reason, in this study the Optragate was used for isolation, which is very easy to use and fast application. Color determination therefore has to be carried out in controlled circumstances before the tooth dehydrates if a successful match is to be obtained.

In summary, Shade selection for direct and indirect restorations has always been a challenge for esthetic dentistry, especially with increasing patients' expectations. The success of the restorative treatment of esthetic teeth depends on the dentist's ability, skills, and on understanding the factors that play a role on shade selection procedure. One of the crucial factor that influence shade selection is dehydration/ rehydration process of the teeth. Little is known in the literature about the effect of the dehydration/rehydration process on the shade determination and the estimated time that is required for teeth to dehydrate/rehydrate.

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CHAPTER 2: MANUSCRIPT

1. Introduction

Shade selection for direct and indirect restorations has always been a challenging component of esthetic dentistry for clinicians.¹ Clark in 1931, was the first to address the problem of color in dentistry, and he stated that “we as dentists are not educationally equipped to approach a color problem”.² This statement applies to this day, despite numerous advances in shade matching techniques in recent decades. Based on current prospective and retrospective clinical studies, 50% of cemented ceramic crowns exhibits incorrect color matches.³⁻⁶

When color is measured, and color differences are identified, the CIELAB system is frequently used, which is based on the 1976 Commission Internationale de l'Eclairage (CIE) L*a*b* color space. The three coordinates that determine color in this nearly uniform space are the L* value (y-axis), which represents lightness, the a* value, which represents red-green chromaticity and the b* value which represents yellow-blue chromaticity. This system is the basis for transforming spectral energy data into meaningful color data.^{6,7} Recently, CIE published a new CIEDE2000 formula model which is extended to the CIE 1976 (L*a*b*) color-difference model with corrections for variation in color-difference perception dependent on Lightness, Chroma, Hue and Chroma-Hue interaction. Additionally, the CIE investigated the non-uniformity of the CIELAB space and developed this empirical correction to improve agreement between perceived visual color difference and numerical color difference.⁸

In the CIELAB system, Delta E (ΔE_{ab}) is the color difference, or the distance separating two points of color. It is defined by the following equation: $\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$.⁹ In the

CIEDE2000 system, Delta E (ΔE_{00}) is the total color-difference between two color samples with Lightness, Chroma and Hue differences. It is determined by the following equation⁸:

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C} \frac{\Delta H'}{k_H S_H}}$$

When the color difference between two compared objects can be seen by 50% of the observers, it is a 50:50% perceptibility threshold. Similarly, a 50:50% acceptability threshold has been defined when a color difference is considered acceptable by 50% of the observers. A prospective multicenter study by Paravina *et al*¹⁰ concluded that the CIELAB 50:50% perceptibility threshold was $\Delta E_{ab}=1.2$, whereas the 50:50% acceptability threshold was $\Delta E_{ab}=2.7$. The corresponding CIEDE2000 (ΔE_{00}) values were 0.8 and 1.8, respectively, for under simulated clinical settings.

Color determination is an interplay where color perception and matching abilities need to meet in the best of conditions. Color perception occurs when light from a particular source is reflected by the object and observed by the viewer. Hence, color perception is influenced by a triad of events: the light source, the optical properties of the object observed and the color matching ability of the observer himself.¹ The light source should be spectrally balanced in the visible range (370-780 nm), have a color temperature of approximately 5500°K and a color rendering index of >90, so that the light source itself does not become a limiting factor.¹¹ Among optical properties of the object itself, translucency and light scattering impact color perception. The translucency is the amount of incident light transmitted and scattered. Scattering makes the object opaque and is dependent on the size, shape, number of the scattering centers and refractive index.¹¹ The color matching ability of the operator is influenced by viewing conditions, color fatigue of the eye, age, experience and presence or absence of color blindness.⁷

Tooth dehydration makes teeth appear whiter due to increasing enamel opacity. The inter-prism spaces become filled with air instead of water so light can no longer scatter from crystal to crystal. Loss of translucency due to dehydration therefore causes more reflection which masks the underlying color of dentin, making the tooth appear lighter.^{6,11}

Color determination therefore must be carried out in controlled circumstances before the tooth dehydrates, if a successful match is to be obtained. Few clinical studies have examined how rapidly teeth dehydrate to a perceivable color difference. Russel *et al*¹² made measurements with a reflectance spectrophotometer on a single central incisor before and after 15 minutes of rubber dam isolation.¹³ The purpose of their study was to determine the changes in the ΔE after 15 minutes of rubber dam application and the time for tooth color to return to original values. Burki *et al*¹³ also conducted an in vivo study, to assess the effects of dehydration on tooth color. They isolated a central incisor with a rubber dam. They made measurements with a spectrophotometer before, and 10, 20 and 30 minutes after application. The levels of ΔE obtained after relatively short intervals of rubber dam isolation are well above the acceptability thresholds for shade incorrect match determined by Paravina *et al*¹⁰ ($\Delta E = 2.7$). Further studies are warranted to examine how fast perceptibility and acceptability thresholds of shade mismatch are reached.

In a typical clinical scenario, shade determination for ceramic restorations is usually not performed with a rubber dam in place. Retraction methods are commonly used which provide relative levels of isolation by relieving the tooth of contact with wet soft tissues while keeping the tooth within the confines of the oral cavity. However, for direct restorations, isolation is critical and a rubber dam is often used. Regardless of the means of isolation, the degree of dehydration that occurs and how rapidly its manifest is of great importance with regards to the timing of shade selection. It also is critical in ensuring an accurate color match of the final restoration to the tooth.

The purpose of the present study was to estimate the time required for teeth to dehydrate to perceivable and acceptable thresholds of color change using methods of relative isolation, and to determine the time required for the color change to return to normal level after isolation removal. The null hypothesis is that there is no perceivable difference between tooth shade before and after dehydration, and the time required to rehydrate the tooth is not proportional to the tooth's original shade.

2. Materials and Methods

2.1. Participant Selection:

Thirty-two participants were recruited for this clinical study that was conducted under approval of the Institutional Review Board (IRB) of the University of North Carolina at Chapel Hill (16-1620). These Participants were selected among students and staff members at the University of North Carolina at Chapel Hill based on the following criteria:

Inclusion criteria:

- Adult participants 18 – 45 years of age.
 - Participants have no adverse medical condition and are not on any medication.
- Unstimulated salivary flow was measured volumetrically to ensure that it is within normal limits (0.3-0.4 ml / minute).
- The tooth to be observed (central incisor) should be a sound natural tooth with no restorations, recent bleaching or severe discolorations, with a healthy periodontal condition.

Exclusion criteria:

- Participants have adverse medical condition and are on medication.

- Presence of a restoration.
- Presence of an orthodontic fixed appliance including the central incisors.

2.2. Instrument Used:

The VITA Easyshade spectrophotometer (Bad Säckingen, Germany) was used in this clinical study for color measurements. (Figure 1.2)



Figure 2.1: The Vita Easyshade Spectrophotometer.

2.3. Jig Preparation:

A custom-made jig was made for the central incisor of each participant from a clear silicone impression material (Affinity Crystal Clear, Clinician's Choice, CT, USA) to ensure consistency in the positioning of the spectrophotometer. The clear jig allowed visualization of the entire facial surface, with an access opening of 6 mm diameter, made by a disposable tissue punches, located in the middle third of the central incisor, to accommodate the Easyshade tip. (Figures 2.2, 2.3).



Figure 2.2: Custom Made Jig with Puncher.



Figure 2.3: Custom Made Jig with Spectrophotometer.

2.4. Color Measurements:

All participants were seated and reclined to a 45° position to the floor, to eliminate any shadowing effects from the nose/lips. Color measurements were conducted under controlled light settings (color temperature 5500°K and a Color Rendering Index (CRI) of 95) with neutral color surroundings. Lips were retracted by using an Optragate (Ivoclar Vivadent Inc., NY, USA). Optragate placement was done in 15 seconds, otherwise it was repeated allowing the patient to close, to ensure no pre-testing dehydration. The custom-made jig was seated in place, and measurements were made using the calibrated VITA Easyshade spectrophotometer determining L*, C*, H* values in accordance with the Commission Internationale de l'eclairage (CIEDE2000) system (Figure 2.4).



Figure 2.4: Positioning a Custom Made Jig with an Access Opening in the Middle Third of the Tested Tooth that Allowed Color Measurements with the Vita Easyshade.

All participants wore a nose clip during measurements to ensure that the same mode of breathing was used (mouth vs. nose breathing), ensuring the same air flow in all study subjects.

Dehydration time color measurements were performed according to the following time intervals:

0 (baseline), 2, 3, 5, 7, 10 and 15 minutes.

For rehydration time determination, the Optragate isolation device was removed from the mouth, and a full mouth rinse with water was done. Then participants were asked to close their mouth for 5 minutes. Color measurements to determine the extent of tooth rehydration were performed according to the same intervals that are mentioned in the dehydration phase of the study.

The L, C and H values obtained were used to calculate CIEDE2000 color differences (ΔE_{00}) between baseline and the other intervals for dehydration and rehydration. These values were compared to the perceptibility and acceptability thresholds as determined by Paravina *et al*¹⁰ to evaluate the time at which the clinicians have to determine a shade under relative levels of isolation before dehydration causes perceptible levels of color change which might adversely affect the shade selection processes.

2.5. Data Management and Statistical Analysis

The data were analyzed for color differences (ΔE_{00}) over time using an analysis of variance (ANOVA). Post-ANOVA contrasts were made between baseline and each time intervals (baseline and, 1, 2, 3, 5, 7, 10, 15 minutes) by using Tukey test with P values adjusted for multiple comparisons ($p < 0.0001$). Percentage changes between baseline and each time interval were assessed for color differences by using Clopper-Pearson (Exact) test with a 95% confidence interval. Additionally, this test was used to assess the proportion of the population that exceeded the ΔE_{00} perceptibility threshold of 0.8 or acceptability threshold of 1.8 at each time interval of dehydration and rehydration.

3. Results

The values for the mean, standard deviation, and 95% confidence interval of the color differences, expressed by ΔE_{00} at baseline, 1, 2, 3, 5, 7, 10, and 15 minutes time intervals of dehydration and rehydration can be seen in Table 1.2, Table 2.2 and Figure 2.5.

Table 2.1: Mean and Standard deviation of ΔE of dehydration.

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
D__Delta_E_1	3.9434	2.6156	3.0004	4.8864
D__Delta_E_2	4.0889	2.7937	3.0817	5.0961
D__Delta_E_3	4.5375	2.7596	3.5426	5.5325
D__Delta_E_5	4.5485	2.5411	3.6323	5.4646
D__Delta_E_7	4.7788	2.5717	3.8516	5.7060
D__Delta_E_10	4.8836	2.4819	3.9888	5.7784
D__Delta_E_15	5.1082	2.3880	4.2472	5.9691

Table 2.2: Mean and Standard deviation of ΔE of rehydration.

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
R_Delta_E_0	4.1351	2.6679	3.1732	5.0969
R_Delta_E_1	3.9832	2.2725	3.1639	4.8025
R_Delta_E_2	3.7674	2.5238	2.8574	4.6773
R_Delta_E_3	3.3529	1.9647	2.6446	4.0613
R_Delta_E_5	3.5969	2.0664	2.8519	4.3420
R_Delta_E_7	3.3841	2.5605	2.4610	4.3073
R_Delta_E_10	3.0599	2.0487	2.3212	3.7985
R_Delta_E_15	2.7279	2.1400	1.9563	3.4994

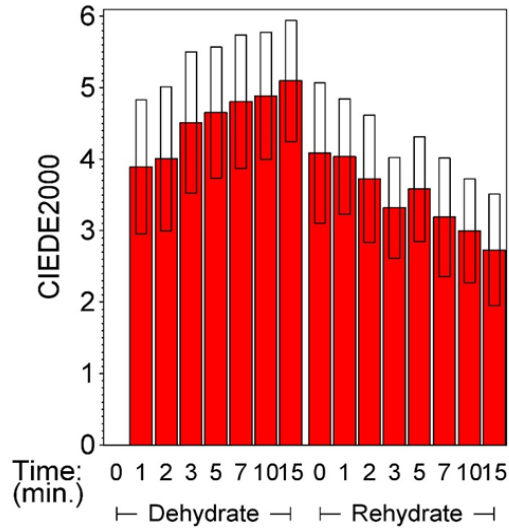


Figure 2.5: Changes in the (ΔE) of Dehydration and Rehydration.

Analysis of variance (ANOVA) indicated that there was statistically significant change in color over time in the mean ΔE_{00} for both dehydration and rehydration procedure ($P < 0.0001$). As time increases, the mean ΔE_{00} increases as well within the dehydration procedure. The values were compared to the 50:50% perceptibility and acceptability thresholds at ΔE_{00} of 0.8 and 1.8 respectively.¹⁰ Within the first minute there was a statistically significant change in ΔE_{00} and beyond the acceptability threshold.

Average change in L, C and H values are displayed in figures 2.6-2.8. Mean L value showed a significant spike within the first minute of dehydration, while it returned to its original value after 15 minutes of rehydration. Mean C value remained increasing until 15 minutes of dehydration, and didn't returned to its original value after 15 minutes rehydration. The mean H value remained decreasing until 15 minutes of dehydration, and also didn't returned to its original value after 15 minutes of rehydration. Therefore, continued increase in ΔE to 15 minutes is not only attributed to the change in L values, but also due to the changes in C and H values.

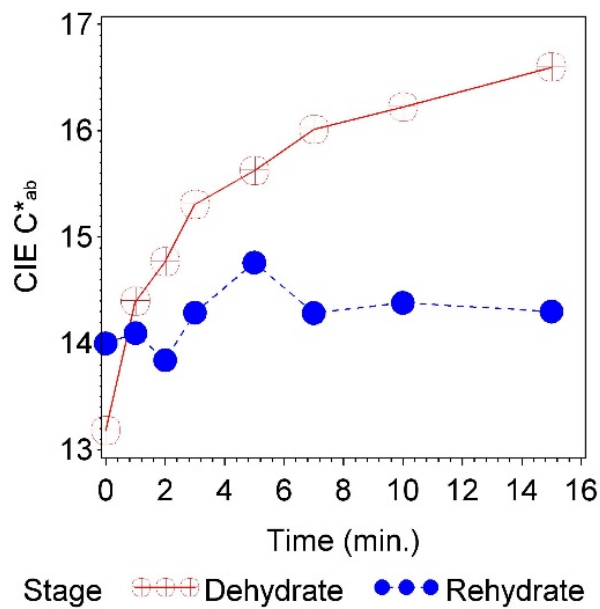


Figure 2.6: Mean Changes in L.

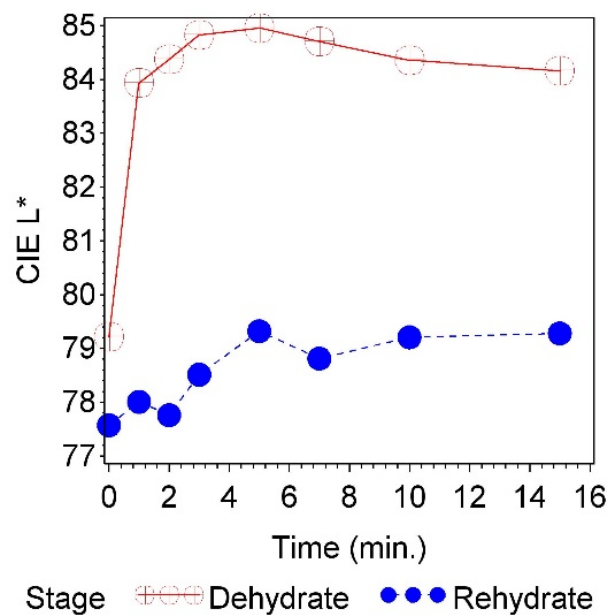


Figure 2.7: Mean Changes in C.

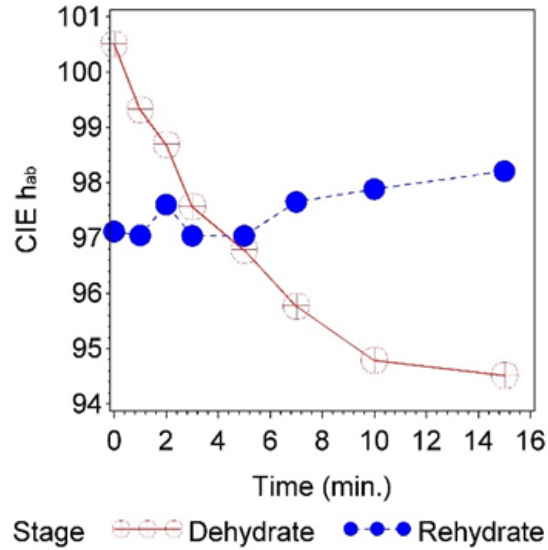


Figure 2.8: Mean Changes in H.

Clopper-Pearson (Exact) test with 95% confidence interval was used to assess the proportion of the population that exceeded the ΔE_{00} perceptibility threshold of 0.8 or the ΔE_{00} acceptability threshold of 1.8 at each time interval of dehydration and rehydration. 87% of teeth were beyond the ΔE_{00} perceptibility threshold (0.8) within the first minute of dehydration. While 90% of teeth were beyond the perceptibility threshold after 15 minutes of rehydration as shown in (Figure 2.9).

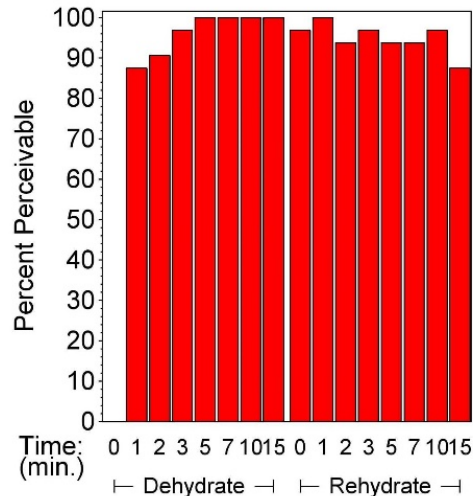


Figure 2.9: Percentage of Participants with Perceivable Color Changes during Dehydration and Rehydration.

Furthermore, 72% of the participants were beyond the acceptability threshold (1.8) with the first minute of dehydration. While 65% of the participants were beyond the acceptability threshold after 15 minutes of rehydration. Therefore, most of the teeth didn't returned to its original baseline shade values after 15 minutes of rehydration as shown in (Figure 2.10).

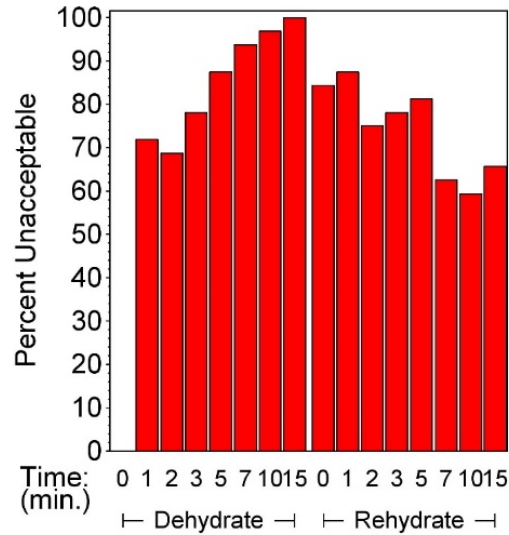


Figure 2.10: Percentage of Participants with Acceptable Color changes during Dehydration and Rehydration.

4. Discussion:

The present study revealed statistically significant and visually perceptible differences in tooth color measurements after dehydration and rehydration within the defined time intervals, rejecting the null hypothesis.

ΔE_{00} values were calculated to assess the changes in tooth color by using a hand-held spectrophotometer (VITA Easyshade). ΔE_{00} values were compared to Paravina *et al*¹⁰ study of 50: 50% ΔE_{00} perceptibly (0.8) and acceptability (1.8) thresholds. Within the first minute of dehydration there was a significant change in the ΔE_{00} (3.9) beyond the perceptibility threshold (87% of the participants) and acceptability threshold (72% of the participants). The ΔE_{00} remained

to increase constantly through the time intervals until 15 minutes ($\Delta E_{00} = 5.1$). Thereafter, participants were asked to rinse with water and close for 5 minutes to allow for teeth to rehydrate. ΔE_{00} measurements were calculated at the defined intervals. Tooth color did not return to its' original value after 15 minutes of rehydration, ΔE_{00} constantly decreasing from 4.1 to 2.7, with 65% of the participants beyond the acceptability threshold. Furthermore, the L (Lightness) returned to its original value after rehydration, while the C (Chroma) retained a higher value and a lower H (Hue) value after rehydration. Therefore, tooth color determination should be carried out prior to the start of any treatment, before the tooth dehydrates, for a successful color match to be achieved. When teeth dehydrate, and based on the results of this study, it would be clinically challenging to match the tooth's color within the allocated chair time due to the time it will require for the tooth to rehydrate and return to its original shade.

To understand the change in tooth color due to dehydration, the tooth's enamel and dentin have a defined refractive index (RI) when light passes through the tooth structure. Refractive index is the change in the light's direction when the transmitting medium changes. The RI of air is 1.00 and that of water is 1.33.¹⁴ When light passes through enamel (RI = 1.63) and then through dentin (RI = 1.54) the light refracts in a certain direction.¹⁵ When the tooth is moist, the inter-prism space is filled with saliva, and light will refract in a defined path. When the tooth dehydrates, the inter-prism space will be replaced with air, and the light will refract differently due to the difference in RI. The increased light refraction reduces the tooth's translucency and increases its luminosity, giving the tooth a whiter appearance. When the tooth is rehydrated, it may require a longer period of time, more than 15 minutes, for the saliva to refill the inter-prism spaces and restoring the original path of light refraction through the tooth, and finally restoring the tooth's original color.

Isolation is considered one of the pillars to the success of most restorative procedures. As the use of rubber dam is essential towards this success, it is unfortunately becoming an uncommon practice. Other methods of isolation have been introduced and offer relative isolation. The rationale for using the Optragate isolation device in the present study instead of the rubber dam, for documenting the dehydration process, is due to that rubber dam application can be time consuming and would have precluded timely measurements of the tooth dehydration process. According to a previous study, rubber dam application took an average time between 3–5 minutes.¹⁶ Consequently, the color measurement process will be adversely affected. In this study, a single operator (SS) was trained on the Optragate application in a timely manner (less than 30 seconds) to ensure consistency of the results.

In the literature, little is known about how long it takes for teeth to dehydrate/rehydrate and the effect time has on the shade selection process. A few clinical studies were found that measured the change in color with dehydration/rehydration effects on these changes. Russel *et al*¹², conducted an in vivo study to determine the changes in color measurements of naturel teeth in 7 subjects before and after isolation by using rubber dam for 15 minutes, also estimating the time taken for the tooth color to return to its original color. Color measurements were made by using a spectrophotometer with no mentioning of using a positioning jig. The authors used the CIELab system to evaluate L* (Lightness), a* (red – green) and b* (blue – yellow), as well as h (which is the hue angle) and C (Chroma). The authors concluded that teeth become brighter and less saturated after 15 minutes of rubber dam application, and in agreement with the present study. They also found that baseline values of the tooth's color were regained after 30 minutes of rehydration. Whereas the baseline values were not regained after 15 minutes of rehydration in the present study.

In a more recent study conducted by Burki *et al*¹³, color change due to dehydration was investigated in 20 subjects. For each subject a left or right maxillary central incisor was used with a positioning jig made for placement of the VITA Easyshade spectrophotometer. Baseline measurements were collected followed by rubber dam isolation. Measurements were obtained at 10-minute intervals for 30 minutes. The rubber dam was removed and subjects were instructed to drink a glass of water followed by additional color measurements for rehydration with same time intervals. They concluded significant changes in the color of teeth after 10 minutes of dehydration. Additionally, the tooth's original color had not returned to baseline color within 30 minutes of rehydration, contrasting Russel *et al*¹²'s finding and in agreement with the findings of the present study.

Deficiencies of the previously mentioned studies include long time intervals. Ten-minute intervals were used without the ability to specify exactly color change in a timely manner. Moreover, small sample sizes were used which may have significant effects on the outcome. A larger sample size, with greater diversity of age and gender, could reveal a more complete picture regarding the dehydration/rehydration patterns. Senior patients may have reduced salivary flow, enhancing the dehydration effects on color and time, also requiring longer time for the tooth to rehydrate and returning to its original color. Rubber dam isolation was used in both studies for the dehydration process. As specified earlier, the time required for rubber dam placement may adversely affect the results.

Ideally, longer time intervals for the rehydration color measurements would precisely predict when tooth rehydration would occur. This information is important for the clinician when missing the initial shade selection opportunity and would be suggested for further research. Another potential limitation of this study relates to the accuracy of the VITA Easyshade device. A positioning jig

was fabricated ensuring repeatability when measurements are collected. In addition, the mean of three measurements per interval was calculated ensuring reliability.

Within the limitations of this clinical study, the following conclusions may be made:

- Tooth dehydration within the first minute will lead to perceivable changes in the tooth's color, impacting the shade selection process.
- Teeth rehydration require more than 15 minutes to return to their original shade.
- Shade selection must be determined prior to the start of any restorative procedure.

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