Journal of Oral Research

ORIGINAL ARTICLE

Measuring color change of tooth enamel by *in vitro* remineralization of white spot lesion.

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Receipt: 10/12/2015 **Revised:** 10/20/2015
Acceptance: 11/11/2015 **Online:** 11/11/2015

Abstract: Objective colour determination is based on calculating the colorimetric distance (ΔE) within a colour space. So far, the most used colour space in dentistry is CIE L*a*b (Comission Internationale de l´Éclairage). CIE L*C*h* has been recently developed, showing a better correlation with the perception of the human eye. Objective: To determine the ability of an *in vitro* remineralisation substance to blend the colour of white spot lesions (WSL) with sound enamel, determining ΔE by using the CIE L*C*h* colour space. Methods: *In vitro* WSL was generated by immersing 10 samples obtained from human third molars in a demineralization solution for 72h. Amorphous calcium phosphate stabilized by casein phosphopeptide (CPP-ACP) was then applied for 60 days while maintaining the samples in artificial saliva at 37ºC. To evaluate the colour of enamel, images were taken from the samples placed in specifically designed silicone moulds after generating the WSL (pre-stage) and after remineralisation by scanning, applying the colorimetric distance equation (ΔE*CMC) according to the Colour Measurement Committee. Results: Treatment with CPP-ACP caused a significant ΔE decrease with respect to the pre-stage ($p<0.001$), while the analysis of parameters that make up the colour showed a reduction in the difference of hue (ΔH) ($p<0.001$) and brightness (ΔL) ($p<0.01$) after applying CPP-ACP. Discussion: CPP-ACP penetrated to the depth of the white spot lesion, making its appearance similar to that of the sound enamel, probably because of the formation of different mineral phases than that of the original structure, although pores were not completely filled.

Keywords: *White spot lesion, Enamel remineralisation, Enamel colour measure.*
DOI: *10.17126/joralres.2015.072.*

Cite as: *Tolcachir B, Crohare L & Gallará RV. Measuring color change of tooth enamel by in vitro remineralization of white spot lesion. J Oral Res 2015; 4(6): 371-377.*

INTRODUCTION.

The white spot lesion (WSL) or incipient carious lesion is the first clinical manifestation of the disease and affects only the tooth enamel. It appears clinically as an opaque, whitish area with noticeable loss of translucency particularly visible after drying of the area, or even in the presence of moisture, depending on the progress of the lesión[1]. It is an optical phenomenon due to absorption and refraction of light in the affected area, as a result of the ultrastructural changes that occur in different areas of the lesion, but especially as a result of increased porosity and mineral loss.

The therapeutic approach to these lesions, from the philosophy of minimal intervention, is mainly based on the control of risk factors that are causing this disease and the application of remineralising substances with the aim of reversing the emerging carious lesion[2]. One of the main aspects taken into account when different remineralising substances are studied is their ability to blend the colour of the affected area with the appearance of the surrounding sound enamel.

Tooth shade determination can be made objectively by

J Oral Res 2015; 4(6): 371-377. **DOI:***10.17126/joralres.2015.072*

instrumental methods as colorimeters, spectrophotometers and digital cameras supported by image analysis software. Each of these methods has a limitation, whether the need for expensive instruments, in the case of the first two, or difficulty of reproducibility in the case of digital cameras.

For the objective determination of the difference between two perceived colours, mathematical equations are used to calculate the colorimetric distance (ΔE). Colorimetric distance is defined as the value that represents the distance between the positions of two colours within the colour space. So far the most frequently colour space used to calculate the colour of natural teeth and restorative materials has been the standard established by the Comission Internationale de l'Éclairage in 1976 (CIE L*a*b*)[3,4].

Later, the Comission Internationale de l'Éclairage developed the CIE L*C*h* colour space based on the previous standard. The new colour space had a better correspondence with the perception of the human eye, and while today this space is widely used in the textile industry and art, it has just been recently included in the assessment of the natural colour of teeth[5]. Until the publication of this paper, there were no studies using this colour space to measure colour changes caused by remineralising agents in WSL areas with respect to sound enamel.

CIE L*C*h* is based on a polar coordinate system, where L * (brightness) corresponds to the vertical axis, ranging from black (0%) to white (100%) going through all shades of grey. The values of C* chroma (colour intensity or saturation) are represented as horizontal plane radii measured from the central axis of brightness, with values ranging from 0% to 100%. As for the values of h*, they correspond to hues or shades (perceived colour) and are represented by angles expressed in degrees ranging from 0° (inclusive) to 360º (excluded) rotating around the vertical axis L*[6] (Fig. 1).

In this *in vitro* study, the ability of a remineralising substance to blend the colour of WSL with surrounding sound enamel is evaluated using the CIE L*C* h* colour space.

MATERIALS AND METHODS.

Sample preparation

This study was approved by the Institutional Ethics Committee on Health Research of the School of Dentistry at Universidad Nacional de Córdoba, Project ODO-CIEIS Nº 87.

Five retained third molars extracted according to the dentist's specific indications were used. The inclusion criterion involved these teeth being free of cavities, white spots or hypoplasia. Teeth

Figure 1. Schematic representation of the CIE L* C* h* colour-space system.

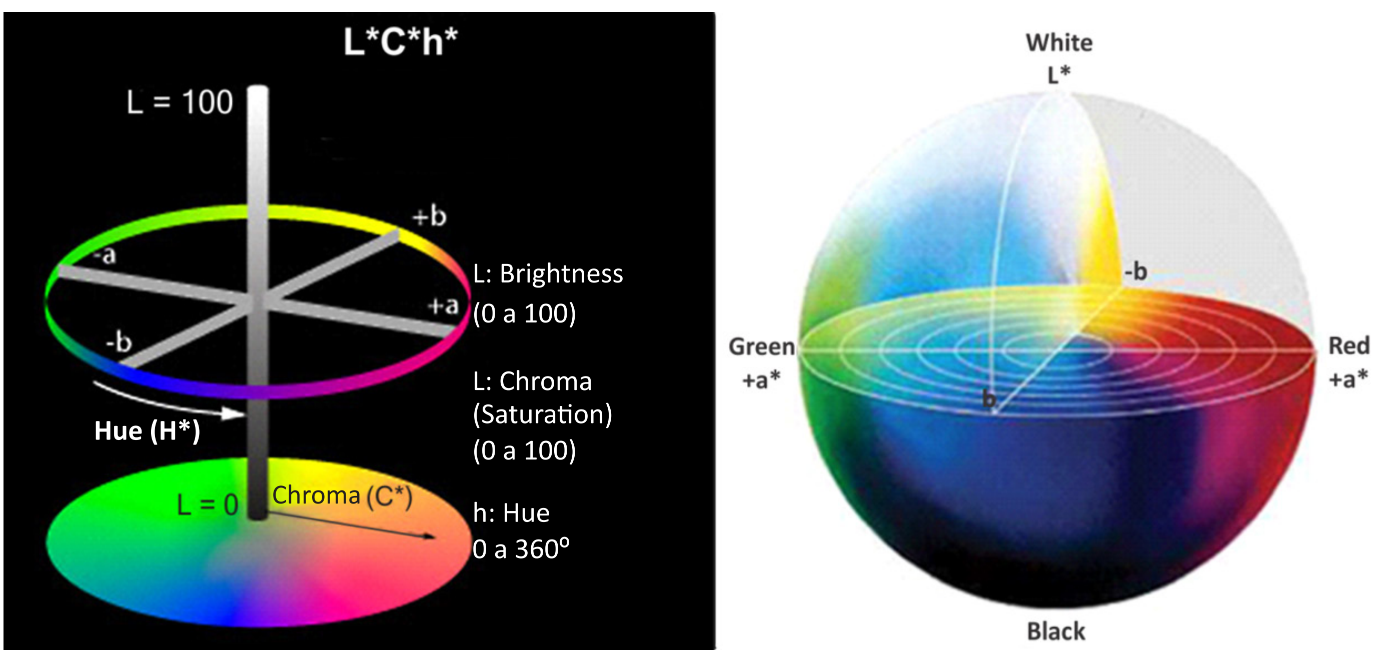
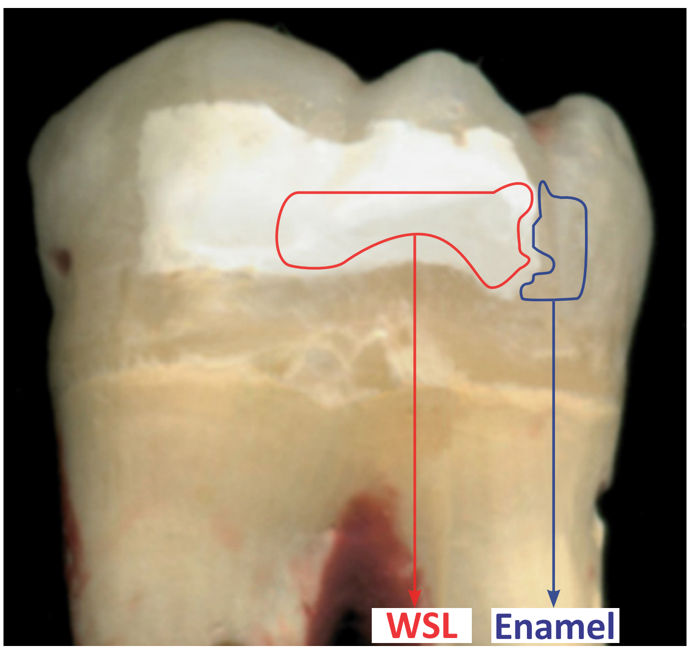


Figure 2. Images of the silicone mould where the sample can be repositioned on successive occasions.

Figure 3. WSL area in red and sound enamel area in blue. The same approach was used for pre- and post-treatment stages of the same sample.



were properly washed with distilled water and sectioned in mesial-distal direction, thereby obtaining a vestibular half and lingual or palatal half, making up a total sample of 10 segments (n=10). Each half was covered with acid-resistant nail polish (Colorama, Maybelline, New York, USA) exposing only a window of 2x6mm in the centre.

Then teeth were immersed in a demineralising solution with pH 4.5 for 72h as proposed by Rooij and Nancollas[7], with a change of solution at 48h to produce the WSL. After that time, they were properly washed with double-distilled water, the nail polish was removed and the presence of the WSL was verified visually. The samples were stored in distilled water at 4ºC.

Mi Paste® (GC Corporation, Japan), which contains amorphous calcium phosphate stabilized by casein phosphopeptides (CPP-ACP) as active ingredient, was used to promote remineralisation. The product was applied daily on the white spot lesion using a microbrush during 3 minutes for 60 days. Throughout the experimental period, samples were stored at 37ºC in artificial saliva (Farmacia & Laboratorio Vip, Argentina) with complete renewal every 24 hours.

Measuring colour using CIE L*C*h colour space

To evaluate the ΔE between the white spot lesion and the surrounding sound enamel, images of the samples after generating the WSL and after the application of the remineralisation protocol were obtained by using a digital flatbed scanner with 1200dpi resolution (Scanjet 3670, Hewlett Packard, USA), under standardised scanning conditions. A mould of the samples fabricated with heavy silicone (Panasil® Putty, Kettenbach Gmb H & Co., Germany) and painted black in order to obtain a higher contrast was used to ensure reproducibility of the position of the samples at both moments (Fig. 2). Images obtained were analysed with Image Pro-Plus 4.52 (Media Cybernetics, Massachusetts, USA).

To assess the colour difference, a specific area of the WSL and of the surrounding sound enamel was delimited before and after treatment (Fig. 3). In the delimited areas, colour

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parameters L, C and h were measured by image analysis software according to the CIE L*C* h* colour space model. With the values of these parameters, the colorimetric distance between the WSL and the healthy surrounding enamel was calculated before and after treatment using the equation ΔE*CMC (Colorimetric Distance according to Colour Measurement Committee).

$$\Delta E = \sqrt{(\Delta L/lS_L)^2 + (\Delta C/cS_C)^2 + (\Delta H/S_H)^2}$$

ΔE values are expressed as the mean±SE. ΔE variations before and after treatment, as well as separately for each parameter (L, C h), were analysed using Student's t test for paired samples, considering significant differences when p was less than 0.05. For the statistical analysis SPSS version 15 (IBM, USA) was used.

RESULTS.

Table 1 shows the values of the colorimetric distance (ΔE CMC) in the WSL area before and after treatment. Treatment with CPP-ACP caused a significant decrease of ΔE with respect to the pre-treatment stage, although it remains a noticeable value in the fixed experimental conditions (Fig. 4).

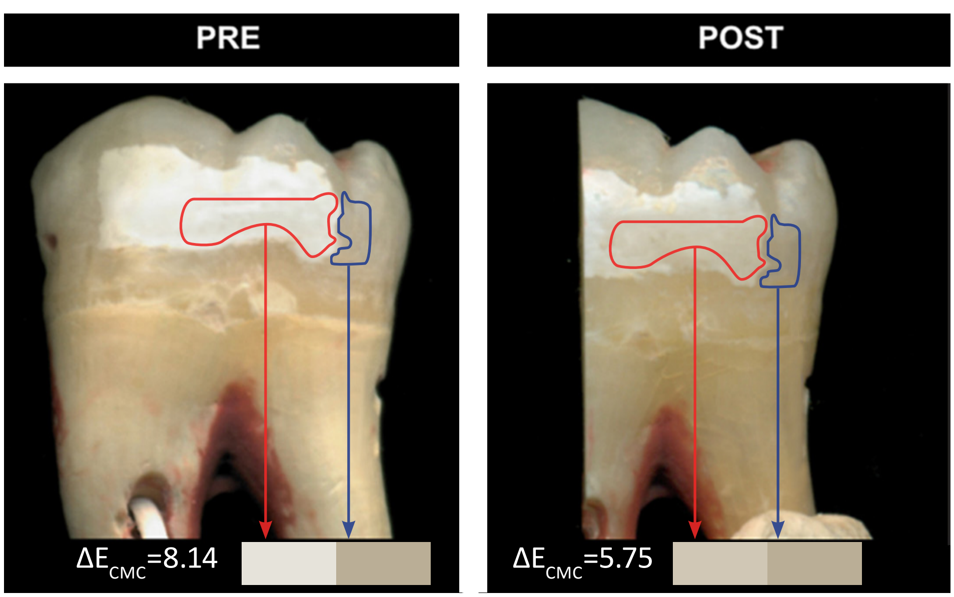
When the detailed analysis of each of the parameters of the colour was performed, it was observed that differences in brightness (ΔL) and hue (ΔH) between the WSL and the sound enamel decreased in the post-treatment stage (p<0.01

Table 1. Parameter values that define colour in the CIE L*C*h* colour system in the WSL area before and after treatment.

Colour parameters	Pre-treatment stage	Post-treatment stage
ΔE	8.24±0.67	5.60±0.46***
ΔL	7.04±0.55	5.40±0.53**
ΔH	3.60±0.53	2.14±0.40***
ΔC	2.25±0.58	1.63±0.34

Values are expressed as mean±SE. Sample size n=10 and (**) p<0.01; (***) p <0.001.

Figure 4. . Illustrative images scanned or digitalised from the same sample before and after treatment with CPP-ACP. WSL area (red area) and an area of sound enamel (blue area) were delimited. The lower panel shows colours extracted from both areas and the value of ΔE CMC for each stage.



and p<0.001, respectively). Moreover, although the CPP-ACP treatment caused a drop in the values of chromaticity (ΔC), these differences were not significant (Table 1).

DISCUSSION.

The optical properties of the tooth are affected by variations in the mineral density, crystal size and orientation of the enamel prisms. Furthermore, the translucency of the enamel is determined by the refractive index of hydroxyapatite and water that accumulates in intercrystalline spaces[8,9]; in the case of sound enamel these spaces are very small and have a very low water content, so that the overall translucency of the enamel is not affected[10].

When the WSL happens as a consequence of the demineralisation process, there is an increased porosity at subsurface level that affects how light is absorbed in that region resulting in the white chalky appearance that characterizes this lesion[11] (Fig. 3).

There are only few studies that assess the ability of mineralising substances to improve the colour of WSL and blend the lesion with the sound enamel. Besides, these studies use different methods of capturing images and measuring colour[12].

To determine objectively the difference between two perceived colours in this study a mathematical equation, in which the colorimetric distance (ΔE) is obtained within the colour space CIE L*C*h*, was used. This colour space is derived from another previously developed colour space (CIE L*a*b*) and has been used to measure the colour of teeth. However, CIE L*C*h* surpasses the previous versions, since its colour representation has a better correlation with the perception and interpretation of the human eye. Gomez *et al.*[13] using CIE L *C*h* showed that the natural colour of the teeth had a strong inverse correlation between the age of the teeth and the parameters of brightness and hue. Our work shows for the first time the application of CIE L*C*h* in assessing the colour of teeth after a mineralising treatment.

In this study the enamel surface was not worn so it could reflect its natural topography, keeping the surface layer properly preserved. The position of the specimen was carefully maintained in every measurement, using an *ad hoc* mould fabricated with heavy silicone for each tooth (Fig. 2). Moreover, image acquisition was performed by scanning the samples with manual adjustment of the image capture parameters in order to keep identical measurement values for both stages (before and after treatment).

While we generated an incipient carious lesion which resembles the white spot lesions observed in clinical practice, this *in vitro* study does not cover issues related to the influence of biofilm and its metabolic products, among other factors, which may influence the optical properties of an *in vivo*[14] incipient carious lesion.

When the colorimetric distance (ΔE) between the WSL and the surrounding sound enamel was compared (pre-stage), a marked colour difference between the two areas was observed (8.24±0.67) (Table 1). In the post-stage, after the application of CPP-ACP, it was shown that treatment caused a significant decrease in ΔE with respect to the pre-stage, making it more similar to the colour of sound enamel. Detailed analysis of each of the parameters that make up the colour in the treated WSL area in relation to sound enamel showed that the major differences in brightness (ΔL) and hue (ΔH) decreased after treatment with CPP-ACP. In addition, a decrease of colour intensity (ΔC) was observed after treatment, although these differences were not significant (Table 1 and Fig. 4).

In this *in vitro* study, the colour of the artificial WSL was perceived as an area of white chalky appearance. Remineralisation of this area using CPP-ACP caused a noticeable change in its colour. However, it did not completely reach the values of sound enamel. These results are not consistent with those of Yuan *et al.*[15], who found no significant effect on the recovery of the colour of a WSL after application of CPP-ACP. This difference could be explained by the treatment time; Yuan *et al.*[15] used 40 days, whereas in this study treatment lasted for 60 days.

Rocha *et al.*[16], in their study of WSL masking, quantified colour taking into account only the value of the L* parameter. The axis L* represents the brightness level of the area studied ranging from 0 (black) to 100 (white), so that high values of L* correspond to whiter lesions and could be related to a higher level of demineralisation. They found that while the values of ΔL between WSL and surrounding sound enamel after weekly application of 2% neutral NaF gel for 8 weeks had decreased, this decrease was not significant.

Furthermore, Kim *et al.*[17], by using the CIE L*a*b* system and different concentrations of a NaF solution (1000 and 5000ppm) during one hour for 7 days, concluded that the colorimetric distance between WSL and sound enamel pre- and post-treatment was significantly lower in the group treated with 5000ppm NaF solution with respect to the control group (demineralised samples immersed in distilled water).

The process of remineralisation of caries is not simply the precipitation of minerals on the surface of the enamel, but a repair of the subsurface area of the lesion[18]. Our results show that the remineralising substance penetrated to the depth of the WSL, making it more similar to the appearance of the sound enamel. However, it is possible that mineral phases different from the original structure may form and that the pores may not have been filled completely; and as a result, the colour of the white spot area does not blend with the sound enamel.

Medición del cambio de coloración del esmalte dental en la remineralización *in vitro* de la lesión incipiente de caries.

Resumen: La determinación objetiva del color se basa en el cálculo de la distancia colorimétrica (ΔE) dentro de un espacio cromático. Hasta el momento el más usado en odontología ha sido CIE L*a*b (Comission Internationale de l´Éclairage). Recientemente se ha desarrollado CIE L*C*h*, que tiene mayor correspondencia con la percepción del ojo humano. Objetivo: evaluar la capacidad de una sustancia remineralizante de mimetizar el color de la mancha blanca (MB) *in vitro* con el esmalte sano, determinando ΔE mediante el espacio cromático CIE L*C*h*. Método: se generó la MB *in vitro* sumergiendo 10 muestras obtenidas de terceros molares humanos en solución desmineralizante durante 72 h., luego se aplicó pasta con fosfato de calcio amorfo estabilizado por fosfopéptidos de caseína (CPP-ACP) durante 60 días manteniendo las muestras en saliva artificial a 37ºC. Para evaluar el color, se obtuvieron imágenes de las muestras ubicadas en moldes de silicona confeccionadas *ad hoc* luego de generar la MB (etapa pre) y del protocolo remineralizante (etapa post) mediante escáner y se aplicó la ecuación de Distancia colorimétrica según Color Measurement Committee. (ΔE*CMC). Resultados: el tratamiento con CPP-ACP provocó una disminución significativa de ΔE respecto de la etapa pre ($p<0,001$) mientras que el análisis de los parámetros que componen el color mostró disminución en la diferencia del tono (ΔH) ($p<0,001$) y la luminosidad (ΔL) ($p<0,01$) luego de aplicar CPP-ACP. Discusión: CPP-ACP penetró a la profundidad de la lesión, acercando la apariencia de la MB a la del esmalte sano, probablemente por la formación de fases minerales distintas a las de la estructura original aun cuando lo poros no hayan sido completamente rellenados.

Palabras clave: *Lesión de mancha blanca, Remineralización, Color del esmalte*

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