



The effect of hydrogen peroxide concentration on the outcome of tooth whitening: an in vitro study

M. Sulieman, M. Addy, E. MacDonald, J.S. Rees*

Division of Restorative Dentistry, University of Bristol Dental School, Lower Maudlin Street, Bristol BS1 2LY, UK

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KEYWORDS

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Summary Aim. This in vitro study examined the effect that various concentration of hydrogen peroxide (5-35%) had on tooth whitening.

Method. Extracted third molars were sectioned and stained using a standardised tea solution to Vita shade C4. These stained specimens were then bleached with a series of gels containing 5, 10, 15 or 25% w/w hydrogen peroxide. Each specimen was bleached for a number of sessions with one session being defined as 3 × 10 min exposure.

Results. The number of applications of the various concentrations of bleaching gel varied from 12 applications for the 5% gel to one application for the 35% gel. Plotting the number of applications against hydrogen peroxide concentration showed an exponential response curve.

Conclusions. The concentration of hydrogen peroxide in a proprietary bleaching gel had a marked effect on the number of applications required to produce an optimal shade outcome.

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Introduction

Many patients are keeping their teeth for longer but dislike the physiological darkening process that follows the laying down of secondary dentine. Over the last decade or so there has been renewed interest in the topic of tooth whitening. The technique of bleaching or whitening teeth was first described in 1877.¹ However, contemporary techniques involve the application of hydrogen or carbamide peroxide based gels in a bleaching tray that is worn for up to 8 h each night. This so called

night guard vital bleaching technique tends to favour the use of 10% carbamide peroxide.² Also available are surgery-based products whose application needs to be supervised by a dental practitioner.³ These are usually applied under rubber dam and a strong light source is applied to the bleaching gel to accelerate the breakdown of hydrogen peroxide into oxygen and per-hydroxyl free radicals. These penetrate into the tooth to oxidise chromogens present. These so called 'power bleaching' techniques use concentrated hydrogen peroxide solutions of up to 35%.

A variety of case reports and small clinical studies have shown that a 10% carbamide peroxide gel used in a bleaching tray at night produces predictable results,⁴⁻¹² as do 6.5% hydrogen peroxide strips.¹³

*Corresponding author. Tel.: +44-117-928-4312; fax: +44-117-928-4780.

E-mail address: j.s.rees@bristol.ac.uk

Similarly, power bleaching agents using 35% hydrogen peroxide with or without light and/or heat activation has also been shown to be effective.¹⁴ A laboratory study has found that a 15% carbamide peroxide solution bleached more quickly than a 5 or 10% solution. However, all concentrations produced a bleaching effect if used for long enough.¹⁵

To date, there has been little scientific study of the effect that various concentrations of hydrogen peroxide have during the tooth whitening process. Recently, a reliable *in vitro* method that quantitatively assesses the effects of tooth whitening products has been developed.¹⁶ The aim of this study was to investigate the effect that variations in the concentration of hydrogen peroxide from 5 to 35% had on the tooth whitening process.

Materials and methods

Extracted teeth were stained with a standardised tea solution using the method of Sulieman et al.¹⁶ Third molar teeth, extracted from subjects aged between 18 and 30 years, were obtained from the Oral Surgery Department of Bristol Dental School. It is likely that the enamel of the teeth had matured, since apical closure of the teeth was complete or at an advanced stage. Prior to experimental use the teeth were examined for the absence of disease, cracks or other surface defects. The roots were removed and the crowns were sectioned vertically in half using a diamond saw (Microslice 2, Metals Research Ltd., Cambridge, UK) with copious water irrigation beginning at the level of the occlusal fissure. The specimens produced had a naturally curved outer enamel surface with a flat dentine surface beneath. The exposed dentine surface was polished using a 1000 grit silicon carbide paper (Kemet International, Parkwood Trading Estate, Maidstone, Kent, UK) in a lapping and polishing unit (Kemet International, Parkwood Trading Estate, Maidstone, Kent, UK) with water irrigation. Following polishing, the dentine was etched with 35% phosphoric acid etching gel for 60 s (3M Dental Products, St Paul, MN, USA). The etching gel was then removed by rinsing in water for a further 30 s. This was carried out to remove the smear layer, expose the tubule system and thereby encourage stain uptake into the tooth.

Stain development was monitored daily over a 6 day period using three assessment techniques:

- Visual assessment with a standard Vita shade guide (Vita, Zahnfabrik, Germany).

- Shade Vision System (SVS, X-rite, 3100 44th St SW, Grandville, MO, USA).
- Chromometer (Minolta CR 221, Minolta UK, 1-3, Blakelands North, Milton Keynes, UK)

The Shade Vision System is a commercially available shade taking system that provides an accurate coloured 'contour map' image of the tooth. It is essentially a colorimeter that utilises image-grabbing technology. It comprises a hand-held measuring device that is used to scan the tooth surface together with a docking station linked to a computer and associated software. The Shade Vision system was used to give a mean Vita shade for each of the specimens.

The electronic chromometer (Minolta CR 221, Minolta UK, 1-3, Blakelands North, Milton Keynes, UK) is a compact tristimulus colour analyser that electronically measures the reflective colours of surfaces. It has a 3 mm diameter measuring area with a 45° illumination angle and a 0°, viewing angle. An internal pulsed xenon arc lamp in a mixing chamber provided diffuse, even illumination of the sample surface. Six high-sensitivity silicon photo-cells, filtered to match the Commission Internationale de l'Eclairage (CIE) standard observer response, were used by the meter's double-beam feedback system to measure both incident and reflected light. The chromometer detected any slight deviation in the spectral power distribution of the pulsed xenon arc lamp, and compensated for this automatically. The image of the tooth specimen is automatically transformed to derive a set of numerical values in terms of the $L^*a^*b^*$ system.¹⁷ This system allows colour specification within a three dimensional space. The L^* -axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a^* plane represents the degree of green/red colour, while the b^* plane represents the degree of blue/yellow colour in the sample. The chromometer was applied to the outer surface of the enamel and the shade of each specimen was recorded from nine separate locations taken across the full width of the specimen working from right to left. The system operates in a similar manner to a reflectance spectrophotometer collecting light from the outer surface and is independent of background. Values for L^*, a^*, b^* for each specimen were recorded prior to application of the bleaching agents and at the end of the bleaching process.

To develop a stained specimen each tooth section was immersed in 5 ml of a standard tea solution at room temperature ($22 \pm 2^\circ\text{C}$) in screw capped plastic universal containers. The tea

solution was produced by boiling 2 g of tea (Marks and Spencer's Extra Strong tea, Marks and Spencer, London, UK) in 100 ml of distilled water, for 5 min and filtered through gauze to remove the tea from the infusion. The tea solutions were renewed each day and 25 specimens were stained to a uniform shade of C4 as judged visually and by the SVS instrument. Specimens were kept fully hydrated by immersion in distilled water when not in use.

Five different bleaching gels with varying concentrations of hydrogen peroxide were freshly prepared and used within 8 h. The parent gel was a commercially available product containing a powder of fumed silica and a photoactive colourant (Quick white bleaching powder, Quickwhite, Lombard House, 12-17, Upper Bridge St., Canterbury), which is usually mixed with 35% hydrogen peroxide liquid. The liquid was diluted with distilled water to produce solutions containing 25, 15, 10 and 5% hydrogen peroxide. Stained teeth were allocated randomly to each experimental group using a random number chart.

Teeth were bleached by the application of a 2-3 mm thickness of the gel and then activated for 6 s with a plasma arc lamp (Apolite II, DMDS UK, Lombard House 12-17 Upper Bridge St., Canterbury) placed just above the surface of the gel. The gel was left on the specimen surface for a period of 10 min. The gel was then removed with a piece of damp gauze, refreshed using a fresh mix and immediately illuminated for a further 6 s and left for a further 10 min. This cycle was repeated once more, so that each specimen was bleached using three 10 min passes, as suggested by the manufacturer for the clinical use of the product, with a minimal delay between applications of the bleaching gel. For the purposes of this study one clinical application period was assumed to be a bleaching period of 30 min (3 × 10 min). Shade assessments were made immediately after the bleaching gel had been removed.

All teeth were bleached to a uniform shade of B1 as judged by visual assessment and the SVS instrument. The number of applications required to reach the shade of B1 was noted.

As mentioned above, values for L^* , a^* and b^* were recorded for each specimen prior to application of the bleaching agent. Values for L^* , a^* and b^* were also recorded at the end of the bleaching process when a uniform shade of B1 had been achieved. The difference between L^* , a^* and b^* at the beginning and end of the experiment were expressed as ΔL^* , Δa^* and Δb^* . In addition to these values, the overall colour change of each specimen (ΔE) were calculated following

Table 1 Number of applications required to produce uniform bleaching.

Concentration of H ₂ O ₂ (%)	Number of applications
35	1
25	2
15	4
10	7
5	12

the following expression:

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

Results

Table 1 shows the number of applications required to produce the maximum change in tooth colour with the various concentrations of hydrogen peroxide. The number of applications ranged from one for the 35% solution to 12 applications for the 5% solution. This data is also shown graphically in Fig. 1, which shows a possible exponential relationship between hydrogen peroxide concentration and the number of applications required for an optimal clinical result. The data was explored further using non-linear regression. The resulting curve is shown in Fig. 2 and represents the exponential relationship:

$$Y = Y_0 + A e^{(-x/T)}$$

where $Y_0 = 0.806$, $A = 20.4926$, $T = 8.278$. This produced an r^2 value of 0.999.

Table 2 shows the chromometer readings (ΔL^* , Δa^* , Δb^*) for each of the five different concentrations of hydrogen peroxide together with

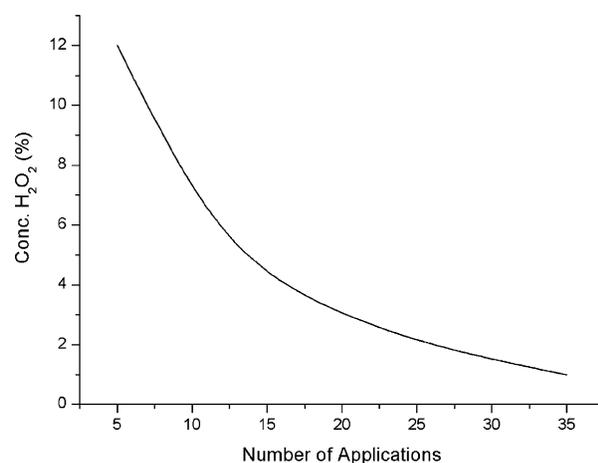


Figure 1 Relationship between concentration of hydrogen peroxide and number of applications.

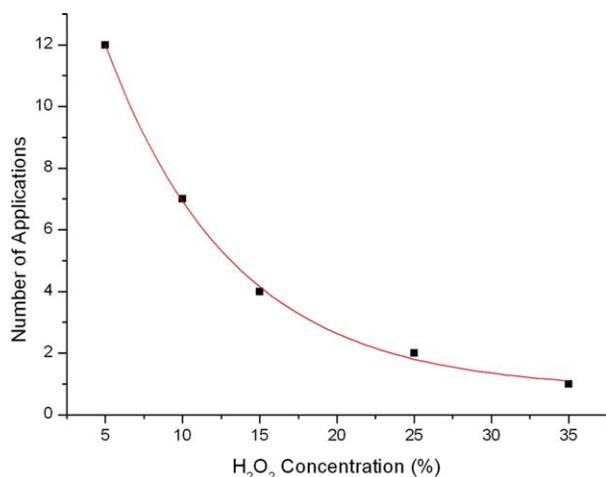


Figure 2 Exponential relationship between concentration of hydrogen peroxide and number of applications.

the composite value (ΔE) for overall colour. As can be seen, the numerical values for each of these readings were very similar for each different concentration of bleaching value. This strongly suggests that each tooth had been bleached to the same degree. Statistical comparison for each of the four different types of readings was undertaken using analysis of variance and no differences were found.

Discussion

The aim of this study was to assess what effect varying the concentration of bleaching agent would have on the response of stained teeth to bleaching. Not surprisingly, the study found that the gels with a higher peroxide concentration needed fewer applications to produce a bleaching effect. In common with previous studies, it also seems that it is possible to bleach teeth to a terminal point (Vita shade B1 in this study) and not beyond it. However, the surprising finding was that the relationship between peroxide concentration and the number of applications was not linear but exponential.

The diffusion of a liquid into a substrate is governed by Fick's law¹⁸ which relates the distance

x that a material will diffuse into a substrate by the relationship:

$$X = \sqrt{Dt}$$

where D is the diffusion coefficient and t is time.

This would partly explain the shape of the curve found in this study (Fig. 1), but Fick's law relates to small molecules such as water. The bleaching gel system is further complicated by the addition of thickening agents that would hinder the diffusion process. However, it may be argued that this represents a very simplistic approach to the interaction of bleaching agent and tooth. Non-linear regression was therefore used to represent the data producing the exponential relationship (Fig. 2). It is likely that this more accurately reflects the complex interactions between tooth and bleaching agent that involves diffusion and reaction of the peroxide moieties with the chromogens.

Colour changes of the specimens were also assessed with the spectrophotometer, particularly ΔL^* , Δa^* , Δb^* and ΔE . Table 2 summarises the changes in these parameters with the different concentrations of hydrogen peroxide. As can be seen, the ΔL^* value changed by 17-18 units showing that the lightness of the tooth increased. The Δa^* values increased by 1.6-2.6 units and the Δb^* values increased by 7-10 units. The Δa^* parameter measures greenness and the Δb^* parameter blueness and these changes are similar to those reported previously. The ΔE also showed an increase of 19-21 units and this is not surprising as it is a composite value derived from ΔL^* , Δa^* and Δb^* values.

Previous clinical studies have often used ΔL^* to assess changes in the lightness of a tooth.⁸⁻¹⁰ This study found that it was possible using the various bleaching regimes to produce changes of around 20 units. This is very similar to the values found in a previous laboratory study¹⁶ but other clinical and laboratory studies have reported smaller changes. For example, an in vitro study on dentine discs by White et al.¹⁹ reported a change of ΔL^* of seven units when a 10.5% carbamide peroxide gel was used for 30 h. Similarly, Gerlach and Zhou¹³ reported an improvement of ΔL^* of 2 units with

Table 2 ΔL^* , Δa^* , Δb^* and ΔE values for varying concentrations of hydrogen peroxide (standard deviations in parentheses).

Concentration of H ₂ O ₂ (%)	ΔL^*	Δa^*	Δb^*	ΔE
35	17.44 (2.37)	2.58 (1.04)	8.34 (3.00)	19.13 (3.28)
25	17.11 (4.91)	2.66 (1.61)	8.75 (3.06)	19.64 (4.47)
15	16.41 (7.50)	2.70 (1.36)	8.26 (1.46)	21.63 (1.52)
10	17.10 (2.79)	1.70 (0.45)	7.98 (2.23)	19.06 (2.92)
5	18.38 (4.49)	1.66 (1.04)	10.35 (2.34)	21.36 (4.24)

a whitening strip product. However, a carbamide peroxide concentration of 10.5% equates to a hydrogen peroxide concentration of 3.8% w/w. This study only used a concentration of 5% or higher, which may partly explain the greater change in the ΔL^* found in this study. Furthermore, many of the earlier clinical studies¹¹⁻¹³ used teeth that were not as heavily stained as in this study, so that the starting points of these clinical studies may well be different.

The change in Δa^* values represents a reduction in redness an effect reported by others.²⁰ The change in Δb^* values showed a reduction in yellowness of 8-10 units that is somewhat higher than the mean change of 2-4 units reported by Gerlach and Zhou.¹³

However, the changes in L^* , a^* , b^* and E^* values needs to be interpreted with a certain amount of caution. In the clinical setting in vital teeth, there is a continuous outward movement of fluid through the dentinal tubules and porous enamel, which would tend to flush out any applied bleaching agent. The use of extracted teeth that were devoid of dentinal fluid probably allowed the bleaching agent to permeate the tooth more quickly than would be the case clinically. Furthermore, this in vitro model used tea stain, which is a different chromophore to that may be found inside the tooth.

In spite of this reservation, the relationship between the number of applications and the concentration of the bleaching gel showed an exponential rather than a linear relationship.

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