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# Evaluation of a combined thiourea and hydrogen peroxide regimen to bleach bloodstained teeth

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**Abstract**

***Background:*** Current techniques for intra-coronal bleaching of stained root-filled teeth employ oxidative bleaching with hydrogen peroxide. However, concern over the potential for invasive cervical resorption following the use of hydrogen peroxide has been expressed by many researchers, and recommendations have been made to limit the use of this agent. A reductive-oxidative bleaching process using a thiourea and hydrogen peroxide regimen is proposed as an effective and safer bleaching combination. The efficacy of this novel bleaching regimen is evaluated in this study.

***Methods:*** The study involved a quantitative and qualitative spectrophotometric assessment of the ability of two amine (bleaching) agents, aqueous thiourea and acidified thiourea, to alter the absorption spectra of haemoglobin and methaemoglobin compared to hydrogen peroxide. In addition, extracted premolar teeth discoloured by blood were subjected to different bleaching regimens using amine reducing agents and hydrogen peroxide. The change in the colour of the bloodstained dentine samples was measured at each stage of the bleaching process with a Photometer and Reflectance Densitometer. Comparisons of different treatments were made using a method of least significant difference and/or analysis of variance.

***Results:*** Spectrophotometric studies showed that acidified thiourea solution greatly reduced the colour of the haemoglobin and methaemoglobin in the visible range (330–760nm). Aqueous thiourea had no effect on the presence of haemoglobin and methaemoglobin. Reflection Densitometer and Photometer scores indicate that the greatest bleaching effect was achieved by the combined acidified thiourea and hydrogen peroxide regimen.

***Conclusion:*** The recognition that bleaching discoloured teeth is a chemical process, which can be achieved by both reducing and oxidizing agents, offers the possibility of developing new and safer clinical bleaching protocols. It is concluded that the bleaching regimen which employs the sequential use of 0.1M acidified thiourea and 30% w/v hydrogen peroxide is as effective at bleaching bloodstained dentine as 30% w/v hydrogen peroxide alone. However, the addition of thiourea to the bleaching regimen has the potential benefit of reducing the level of damaging hydroxyl radicals and achieving a safer bleaching process.

***Key words:*** Thiourea, hydrogen peroxide, intra-coronal bleaching.

***Abbreviations and acronyms:*** ANSI = American National Standard Institute; DDW = distilled deionized water; LSD = least significant difference.

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## INTRODUCTION

Intra-coronal bleaching of stained teeth has been practised for over 100 years.[1] Currently, the most commonly used procedure is the "combination walking bleach" technique; utilizing hydrogen peroxide and sodium perborate as the bleaching agents. Sodium perborate releases hydrogen peroxide when activated, hence, irrespective of the bleaching technique used, current regimens rely on oxidative bleaching with hydrogen peroxide. For many years, intra-coronal bleaching with hydrogen peroxide was considered a safe and effective treatment.

However, Harrington and Natkin[2] questioned this belief when they reported seven cases in which resorption was observed in root-filled teeth that had been bleached with hydrogen peroxide. Other authors also have reported bleaching related root resorption in root-filled teeth.[3-7] Some of these authors suggested that the cause of this resorption could be leakage of bleaching agents from the pulp chamber through patent dentinal tubules into the cervical periodontium. Rotstein *et al.*[8] demonstrated the passage of hydrogen peroxide from the pulp chambers of root-filled teeth to

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the external surface. Dalhstrom *et al.*[9] demonstrated that hydrogen peroxide can act on haem pigment from bloodstained dentine to generate hydroxyl radicals that can diffuse through radicular dentine to the outer root surface. Hydroxyl radicals are extremely reactive and can alter the cell membrane and deoxyribonucleic acid, and have the potential to destroy connective tissue components, collagen and hyaluronic acid.[10] It has been proposed that the production of hydroxyl radicals during bleaching of bloodstained teeth with hydrogen peroxide may be a causative factor in the development of root resorption in traumatized and root-filled teeth.[9] The association between invasive cervical resorption and bleaching root-filled teeth has led many authors to caution against the use of hydrogen peroxide as a bleaching agent.[11-12]

Reports in the dental literature into alternative bleaching agents are sparse. Non-traditional bleaching agents have been proposed but, to date, these have limited or no success.[13] As early as 1924, Prinz[14] utilized a reducing agent, sulphur dioxide, to successfully bleach stained teeth when oxidative bleaching failed. The current authors propose that a reductive-oxidative bleaching process using a combination of thiourea (Thiourea (AnalaR), BDH Lab Supplies, England) and hydrogen peroxide be used for the intra-coronal bleaching of stained root-filled teeth.

In biological systems, thiourea is highly cell permeable and a scavenger of hydrogen peroxide and hydroxyl radicals,[15] and it is proposed that the combination of thiourea and hydrogen peroxide has the potential to provide superior bleaching with the potential benefit of removing damaging hydroxyl radicals. The aims of this research project were to: (1) determine the effectiveness of thiourea as a reductive bleaching agent compared to hydrogen peroxide; and (2) quantify the efficacy of a combined hydrogen peroxide-thiourea bleaching regime of bloodstained teeth.

## MATERIALS AND METHODS

### Part A: Spectrophotometric studies

A "Lambda-5" UV/VIS spectrophotometer (Perkin Elmer) and matched quartz cuvettes were used for all spectrophotometric recordings. Packed red blood cells* were diluted 1:40 with demineralized water to provide haemolysis. These were placed in ultracentrifuge tubes and spun using a L8-80 Ultracentrifuge (Beckman) at 35 000 rpm for one hour to sediment the red blood cell ghosts from the haemoglobin solution. The supernatant was drawn off with a pipette providing a clear red solution of haemoglobin. A dilution of 1:400 haemoglobin was used in the spectrophotometric study, and the absorption spectrum, in the visible range ($\lambda$=330-760nm), was recorded. A stock solution of methaemoglobin was prepared by dissolving 1.35gm of methaemoglobin powder in 10ml of distilled water. The resultant dark brown methaemoglobin solution was diluted 1:400, and the absorption spectrum, in the visible range, was recorded.

The effect of adding 0.1M aqueous thiourea, 0.1M acidified thiourea, and 30% w/v hydrogen peroxide (positive control) to eight test tubes containing 2ml haemoglobin and methaemoglobin solution at 1:400 dilution was assessed by spectrophotometric evaluation in the visible range ($\lambda$=330–760nm).

### Part B: Photometer and light reflectance density studies

#### *Preparation of dentine samples*

Five single rooted premolar teeth, extracted from orthodontic patients aged between 12–18 years, were used in this study. Approval for this research project was received by the Ethics Committee of The University of Adelaide. All teeth were stored in phosphate buffered isotonic saline (pH 7.4) at 4°C until required. An access cavity was made through the occlusal surface of the teeth with a cross-cut tungsten carbide fissure bur at high speed. The pulp was extirpated with a round bur at low speed and the root canal filed to a size 35-40 Hedström file. The root canal was irrigated with 1–2ml sterile 0.9% saline (154mM sodium chloride); a solution which is unlikely to have a bleaching effect or react with any of the bleaching solutions. The teeth were discoloured by a technique, initially devised by Freccia and Peters[16] and modified by Marin *et al.*[17] The prepared teeth were placed crown first individually in centrifuge tubes of an IEC Centra-MP4R high speed bench centrifuge and immersed in packed red blood cells. They were centrifuged at 5500rpm twice daily for 30 minutes over three consecutive days. In the interim, the teeth were stored at 37°C and 100 per cent humidity.

Following centrifugation the bloodstained teeth were sectioned longitudinally at 1mm thickness using a Van Moppes diamond cutting disk at 12 000rpm with water spray. Representative samples of these teeth were further sectioned until $1mm^3$ specimens of stained teeth were produced. It was important to ensure all the sections were of the same dimensions and it was assumed that all the different teeth were of the same density with the only variation being the colour.

Light absorbance by the dentine samples was measured, prior to them being subjected to various bleaching protocols, with a 502M Photometer (Photovolt) and light reflectance with a "Speedmaster" R75-CP Reflection Densitometer (Electronic Systems Engineering Co.). The various test solutions were placed into the wells of a culture plate as per the bleaching regimens in Table 1.

A dentine sample was removed at random from one of the centrifuge tubes, blotted on a damp filter paper

*The blood products were made available by courtesy of the Australian Red Cross, Adelaide. Blood products were tested negative for HIVAb, HCVAb, HTLV-I Ab, HBSAg. Concentration of Hb = 13.5gm/dl.

**Table 1. Bleaching regimens applied to bloodstained dentine**

| Bleaching regimen | Stage 1 | Stage 2 | Stage 3 |
|---|---|---|---|
| A1 | acidified thiourea | 5% NaOCl* | hydrogen peroxide |
| A2 | acidified thiourea | distilled water†† | hydrogen peroxide |
| A3 | acidified thiourea | acidified thiourea | hydrogen peroxide |
| A4 | acidified thiourea | acidified thiourea | acidified thiourea |
| B1 | aqueous thiourea | aqueous thiourea | hydrogen peroxide |
| B2 | aqueous thiourea | aqueous thiourea | Aqueous thiourea |
| D1-Positive control | hydrogen peroxide | hydrogen peroxide | hydrogen peroxide |
| E1 | hydrogen peroxide | acidified thiourea | acidified thiourea |
| E2 | hydrogen peroxide | acidified thiourea | aqueous thiourea |
| F1-Negative control | distilled water†† | distilled water†† | distilled water†† |

*5% NaOCl was adjusted to pH 11.7.
††Distilled deionized water.

to remove excess blood but left moist. Each sample was placed on the standard white calibration plaque and recorded by the probe of the Reflection Densitometer. Then the sample was immediately transferred to the Photometer and the optical density (absorbance-OD units) recorded at zero time (T0).

The dentine sample was then immersed in the test bleaching agent (Stage 1) for five minutes, blotted as before, and the reflectance density and absorbance measured. Then it was immersed in the second agent (Stage 2) for 60 seconds, blotted but left moist, and the reflectance density and absorbance measured as before. The procedure was repeated for the third agent (Stage 3) and measured after one hour (Stage 3 (i)) and repeated at 24 hours (Stage 3 (ii)). This procedure was repeated producing four samples for each of the 10 different bleaching regimens. The results obtained using the Photometer and Reflection Densitometer readings were analysed using a method of least significant difference (LSD) and/or analysis of variance.

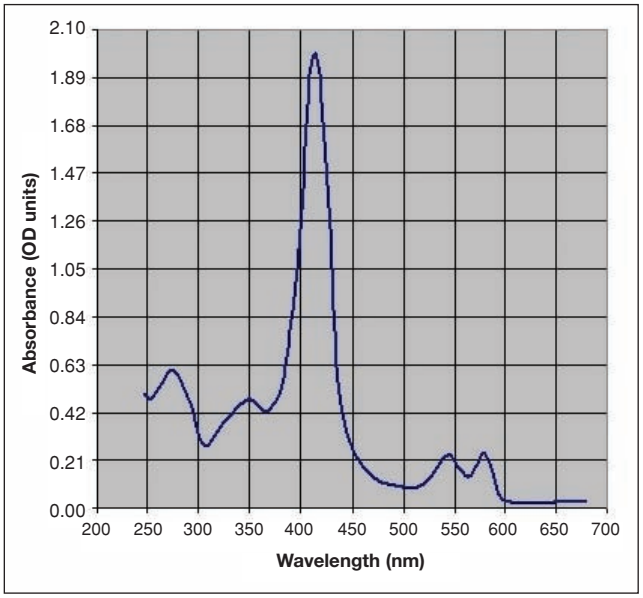


**Fig 1.** Absorption spectrum of haemoglobin derived from a sample of packed red blood cells.

## RESULTS

The densitometer measures the reflectance density on a linear scale from 0 to 2.5. It is calibrated so that the American National Standard Institute (ANSI) Standard White on the calibration plaque registers 0.80 and ANSI Standard Black registers 1.86. Thus a fall in this value towards 0.80 represents a reduction in colour, i.e., bleaching. The 502M Photometer measures the amount of incident light transmitted through a sample, i.e., the optical density of a sample, recorded in absorbance (OD) units. The darker stained dentine samples record a lower OD value, while the higher OD value represents a superior bleaching result.

### Part A: Spectrophotometric studies

#### *Absorption spectra of haemoglobin and methaemoglobin*

The absorption spectrum of the haemoglobin derived from the packed red blood cells (Fig 1) shows a peak at 415nm (Soret band) and α and β bands at absorption peaks at λ=540nm and λ=580nm. These bands are responsible for haemoglobin pigmentation and they appear in the visible range λ=330-760nm. In addition, there are two minor absorption peaks (non-visible and near ultra-violet range) at λ=276 and 344nm This is consistent with the absorption spectrum for oxyhaemoglobin as reported by Weisbluth.[18] The absorption spectrum of methaemoglobin (Fig 2) shows the Soret band at λ=405nm and two minor bands (α and β) at λ=500nm and λ=630nm, as reported by Weisbluth.[18]

#### *Absorption spectra of bleaching solutions*

The absorption spectrum of the solutions containing thiourea was compared to that of a 1:200 diluted solution of 30% w/v hydrogen peroxide in distilled deionized water (DDW). The positive control of 30% w/v hydrogen peroxide rendered the haemoglobin and methaemoglobin colourless and no absorption band could be recorded in the visible wavelength 330–760nm (Fig 3 and 4). Hydrogen peroxide proved to be the most effective bleaching agent.

The aqueous thiourea did not produce any changes in the absorption spectrum of the solution of haemoglobin (Fig 5) as compared with the absorption spectrum of haemoglobin derived from packed red blood cells (Fig 1). Similarly, the aqueous thiourea did not affect the absorption spectrum of methaemoglobin

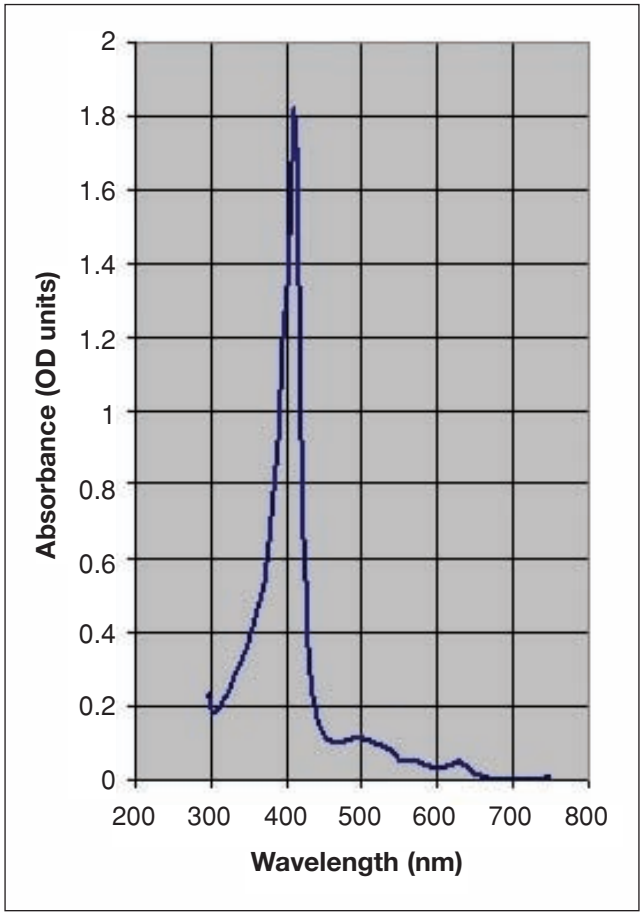


**Fig 2.** Absorption spectrum of methaemoglobin.

(Fig 6) compared with the absorption spectrum of methaemoglobin derived from packed red blood cells control (Fig 2).

The 0.1M acidified thiourea solution reduced the absorption spectrum of haemoglobin at the near Soret band (λ=405nm) by almost 55 per cent and greatly reduced absorption at the wavelength 450-650nm (Fig 7). The 0.1M acidified thiourea solution almost eliminated the absorption spectrum of methaemoglobin at the Soret band (λ=405nm) and greatly reduce the absorption spectrum at the wavelength 450-650nm (Fig 8). Deionized distilled water (negative control) had

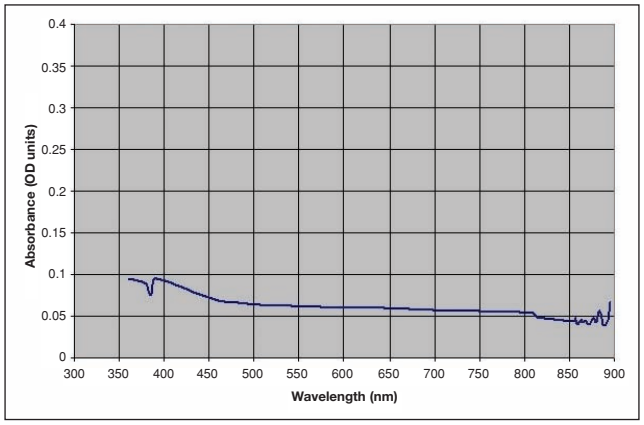


**Fig 3.** Absorption spectrum of haemoglobin after the addition of 30% w/v hydrogen peroxide.

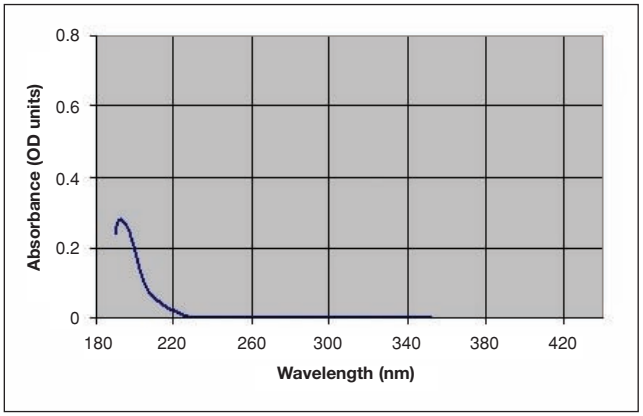


**Fig 4.** Absorption spectrum of methaemoglobin after the addition of 30% w/v hydrogen peroxide.

no effect on the elimination of colour from both haemoglobin and methaemoglobin.

### Part B: Photometer and Reflection Densitometer studies

Tables 2 and 3 represent the raw data obtained using the Photometer and Reflection Densitometer for the 10 different bleaching regimens from T0 to Stage 3(ii)–24 hours. A summary of the means of Photometer and

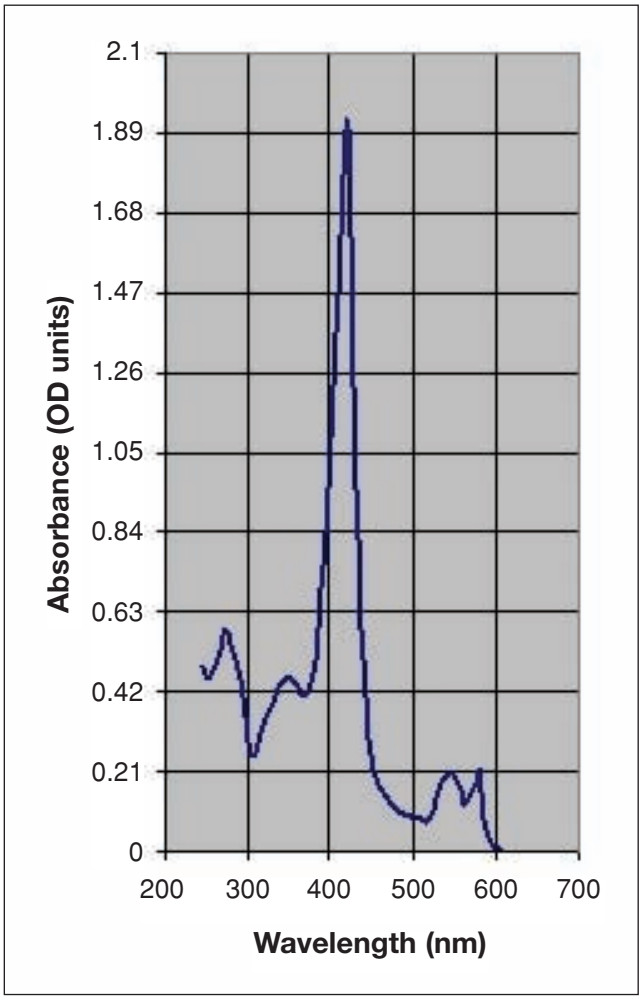


**Fig 5.** Absorption spectrum of haemoglobin after the addition of 0.1M aqueous thiourea.

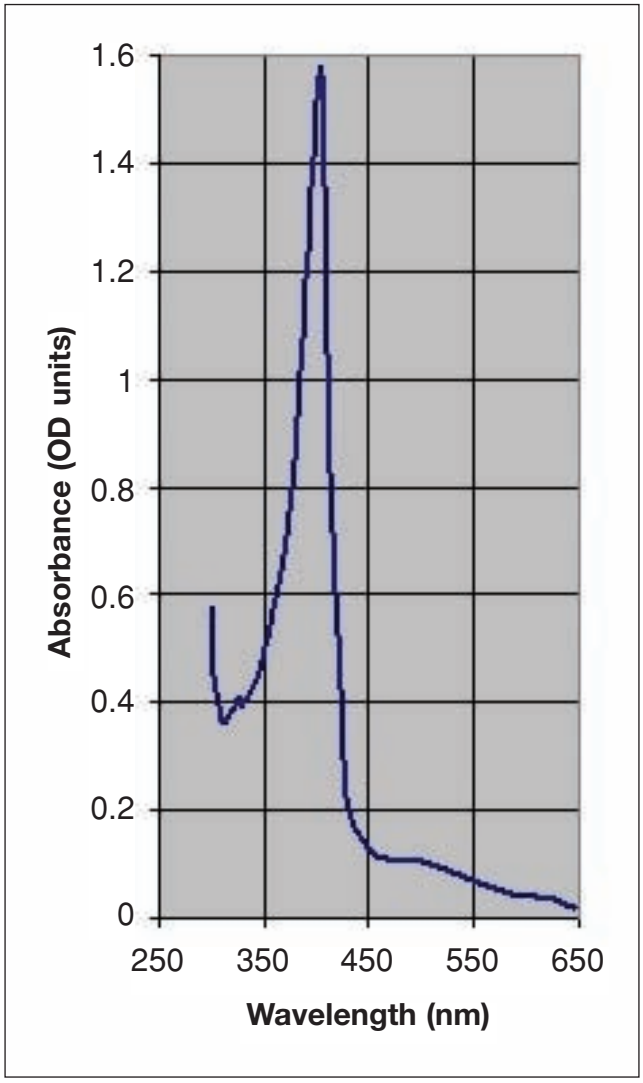


**Fig 6.** Absorption spectrum of methaemoglobin after the addition of 0.1M aqueous thiourea.

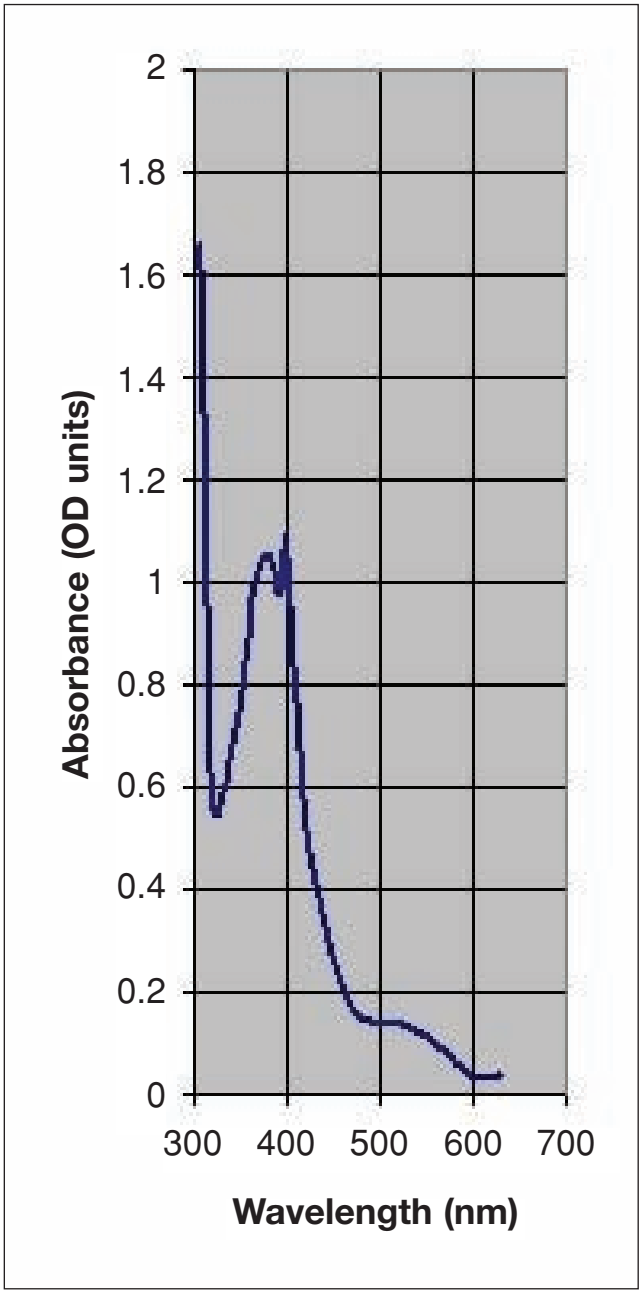


**Fig 7.** Absorption spectrum of haemoglobin after the addition of 0.1M acidified thiourea.

Reflection Densitometer scores at the end of Stage 3(ii)–24 hours is presented in Table 4. The complete statistical analysis of the means and standard deviations/s.e.m. of the bleaching regimens, for the Photometer data and for the Refection Densitometer data are presented in Table 5. The means of the different bleaching regimens were compared using an analysis of variance or the LSD value where appropriate.

### *Photometer studies*

The highest mean value for the photometer at 24 hours was 34.8, i.e., the positive control – Group Di (Table 4). That is, the lightest dentine samples were achieved by the use of hydrogen peroxide at each stage. The Photometer data indicate that all the bleaching regimens which included 30% w/v hydrogen peroxide at the third stage of the bleaching process (i.e., A1–A3, B1 and D1) were all similar with means approximately 30–40 OD units. The results of this group equate in clinical terms to successful bleaching of the bloodstained dentine.

### *Reflection Densitometer studies*

Complete statistical analysis of the means and standard deviations for the Reflection Densitometer data at each stage of the bleaching process from T0–Stage 3(ii) are presented in Table 3. The bleaching regimen, Group D1, which had 30% w/v hydrogen peroxide as the Stage 3(i) (immersion in hydrogen peroxide for one hour) of the bleaching process, showed a statistically significant reduction in colour at this time. The final stage of the bleaching with hydrogen peroxide did not continue at the same rate and did not show a statistically significant difference between Stage 3(i)–1 hour and Stage 3 (ii)–24 hours. The results of this study indicate that, in general, 30% w/v hydrogen peroxide bleached the bloodstained dentine quickly and reached its maximum effectiveness within one hour.

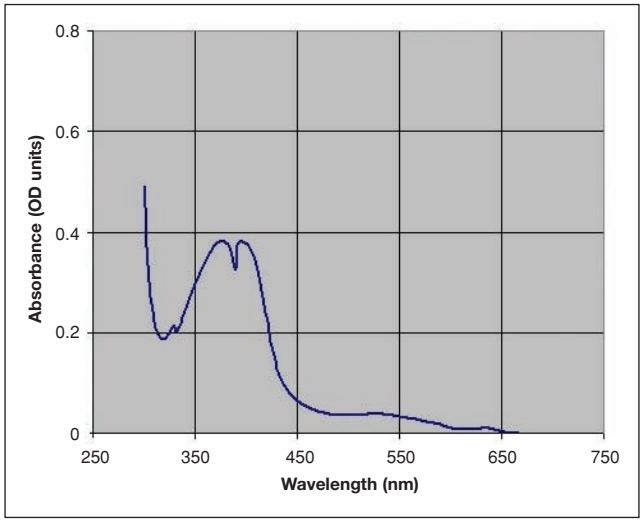


**Fig 8.** Absorption spectrum of methaemoglobin after the addition of 0.1M acidified thiourea.

The spectrophotometric study showed that aqueous thiourea had no effect on the absorption spectra of haemoglobin or methaemoglobin. This preliminary finding was confirmed by the second study of the discolouration of bloodstained dentine. Reflection Densitometer studies indicate that all the bleaching regimens with 30% w/v hydrogen peroxide at the third stage–24 hours namely, A1–A3, B1 and D1 were the most effective at bleaching the bloodstained dentine. The means of the photometer data (Table 4) indicate that the application of acidified thiourea/acidified thiourea/ hydrogen peroxide combination was equally effective at bleaching bloodstained dentine at Stage 3(ii) as the sole application of 30% w/v hydrogen peroxide.

The comparison of the means of the results of bleaching sets at 24 hours was consistent between the Photometer and Reflection Densitometer, and indicates that both instruments were measuring the same quality, i.e., bleaching.

The raw data for Reflection Densitometer scores indicate that the greatest bleaching effect (i.e., the lowest mean score for reflection densitometer of 0.820±0.008) was achieved by Group A3, the combined acidified thiourea and hydrogen peroxide regimen. However, it was not statistically significant.

**Table 2. Means of Photometer data at each stage of bleaching (n=4). An increase in value indicates a loss of colour**

| Bleaching regimen | T0 | Stage 1 | Stage 2 | Stage 3 (i) | Stage 3 (ii) |
|---|---|---|---|---|---|
| A1 | 13.50 | 14.75 | 13.25 | 25.75 | 28.50 |
| A2 | 17..00 | 16.825 | 16.25 | 31.00 | 32.50 |
| A3 | 12.125 | 14.50 | 14.50 | 23.125 | 30.75 |
| A4 | 13.75 | 14.2.5 | 14.25 | 17.00 | 16.50 |
| B1 | 12.25 | 11.00 | 11.00 | 26.25 | 30.25 |
| B2 | 5.75 | 6.25 | 6.25 | 8.625 | 9.12 |
| D1 | 13.375 | 19.625 | 19.625 | 26.00 | 37.7 |
| E1 | 4.75 | 10.75 | 10.75 | 15.625 | 15.375 |
| E2 | 5.875 | 9.125 | 9.125 | 14.75 | 18.625 |
| F1 | 16.00 | 15.75 | 15.75 | 18.00 | 23.25 |

**Table 3. Means of Reflection Densitometer data at each stage of bleaching (n=4). A decrease in value indicates a loss of colour**

| Bleaching regimen | T0 | Stage 1 | Stage 2 | Stage 3 (i) | Stage 3 (ii) |
|---|---|---|---|---|---|
| A1 | 0.965 | 0.9225 | 0.9225 | 0.860 | 0.8225 |
| A2 | 0.925 | 0.925 | 0.920 | 0.840 | 0.8225 |
| A3 | 0.9375 | 0.915 | 0.915 | 0.8425 | 0.820 |
| A4 | 0.930 | 0.9075 | 0.9075 | 0.900 | 0.8925 |
| B1 | 0.9425 | 0.930 | 0.930 | 0.845 | 0.8350 |
| B2 | 0.990 | 0.995 | 0.995 | 0.9875 | 0.9525 |
| D1 | 0.95 | 0.8975 | 0.8975 | 0.8475 | 0.8325 |
| E1 | 0.995 | 0.935 | 0.935 | 0.8975 | 0.9025 |
| E2 | 0.9725 | 0.920 | 0.920 | 0.890 | 0.8975 |
| F1 | 0.9975 | 0.8975 | 0.8975 | 0.880 | 0.903 |

## DISCUSSION

In the first part of the study, the efficacy of various bleaching amine solutions was compared to hydrogen peroxide by quantitative and qualitative assessment using spectrophotometric analysis. The results of this study showed that aqueous thiourea has no effect on the absorption of haemoglobin or methaemoglobin and, hence, no effect on the colour reduction. It was concluded that aqueous thiourea is ineffective as a bleaching agent for haem pigments.

Acidified thiourea (pH 2.0) significantly reduced the haemoglobin in the visible wavelength and greatly reduced the absorption spectrum of methaemoglobin in the visible wavelength. It has been noted that oxidative denaturation of haemoglobin is increased as pH is lowered.[19] Also, the denaturation of haemoglobin is more effective when hydrochloric acid is used prior to discolouration with oxidative agents.[20] It is reasonable to conclude that the change noted in the absorption spectra of haemoglobin and methaemoglobin is most likely due to denaturation of haemoglobin and methaemoglobin in response to the 0.1M addition of hydrochloric acid to the thiourea bleaching solution (0.1M acidified thiourea; pH 2.0). This effect of low pH on haemoglobin has not been considered previously in bleaching bloodstained teeth. However, acid etching of dentine with 30% phosphoric acid prior to intra-coronal bleaching has been recommended in order to open up the dentinal tubules and enhance penetration by the bleaching agent.[21] The benefit of dentinal etching

**Table 4. Raw data at 24 hours for Photometer and Reflection Densitometer**

| Bleaching regimen | Photometer (OD units) | Reflection densitometer (Range 0–2.5) |
|---|---|---|
| | Mean ± sem (n=4) | |
| A1 | 28.5 ± 2.2 | 0.823 ± 0.006 |
| A2 | 32.5 ± 2.8 | 0.823 ± 0.008 |
| A3 | 30.8 ± 2.0 | 0.820 ± 0.008 |
| A4 | 16.5 ± 3.2 | 0.893 ± 0.016 |
| B1 | 30.3 ± 3.5 | 0.835 ± 0.009 |
| B2 | 9.1 ± 0.4 | 0.953 ± 0.014 |
| D1 | 34.8 ± 6.8 | 0.833 ± 0.005 |
| E1 | 14.5 ± 2.2 | 0.903 ± 0.013 |
| E2 | 18.6 ± 3.0 | 0.898 ± 0.012 |
| F1 | 23.3 ± 0.9 | 0.903 ± 0.020 |

**Table 5. Summary of means and standard deviations/s.e.m. (n=4) at 24 hours**

| Bleaching regimen | Photometer data | | | Densitometer data | | |
|---|---|---|---|---|---|---|
| | Mean | St Dev | s.e.m. | Mean | St Dev | s.e.m. |
| A1 | 28.5 | 4.435 | 2.212 | 0.823 | 0.013 | 0.006 |
| A2 | 32.50 | 5.568 | 2.784 | 0.0823 | 0.017 | 0.008 |
| A3 | 30.75 | 3.594 | 1.797 | 0.820 | 0.008 | 0.008 |
| A4 | 16.50 | 6.351 | 3.176 | 0.893 | 0.032 | 0.016 |
| B1 | 30.25 | 6.946 | 3.473 | 0.835 | 0.019 | 0.009 |
| B2 | 9.13 | 0.854 | 0.427 | 0.953 | 0.028 | 0.014 |
| D1 | 34.75 | 13.574 | 6.787 | 0.833 | 0.010 | 0.005 |
| E1 | 14.5 | 4.466 | 2.233 | 0.903 | 0.026 | 0.013 |
| E2 | 18.63 | 5.963 | 2.982 | 0.898 | 0.024 | 0.012 |
| F1 | 23.25 | 1.708 | 0.854 | 0.903 | 0.040 | 0.020 |
| LSD | 8.949 | | | 0.0350 | | |

has been disputed and Casey *et al.*[22] have reported no significant difference in bleaching between un-etched and etched dentinal tubules.

Hydrogen peroxide induces oxidative denaturation of haemoglobin *in vitro*.[23] In the present study, hydrogen peroxide oxidized haemoglobin and methaemoglobin to a colourless product. This occurs if the 18-member conjugated system of haem-responsible for the red colour is disrupted. This indicates that an irreversible change has occurred, i.e., the bleaching is permanent and neither haemoglobin nor methaemoglobin can be expected to re-form. It has been reported that some colour return or "relapse" can occur following successful bleaching of root-filled teeth[21,24] and it has been suggested that this is due to a return or re-formation of the original pigment. The present spectrophotometric study has shown that hydrogen peroxide is a very effective bleaching agent for haem pigments and supports previous reports,[5,13,22,25,26] that, in the event of successful bleaching of bloodstained teeth, regression should not occur.

While there have been recommendations to limit the use of hydrogen peroxide,[5,6] other authors have recommended the placement of a base over the root filling, prior to bleaching, to limit the diffusion of hydrogen peroxide down the root filling.[27,28] However, after an extensive review of bases placed over the root-filling, prior to intra-coronal bleaching, MacIssac *et al.*[29] concluded that no base is ideal and all have the potential to leak. Also, the use of a catalase, an enzyme that breaks down hydrogen peroxide following intra-coronal bleaching, has been suggested to reduce the diffusion of the hydrogen peroxide to the outer root surface.[30] Hydroxyl radicals, produced when teeth stained with blood products are bleached with hydrogen peroxide, are extremely reactive and can destroy connective tissue products and may be a causative factor in bleaching related resorption. Rather than reduce the damage caused by hydrogen peroxide, it is more desirable to add an agent that minimizes the damaging effect of these hydroxyl radicals prior to the application of hydrogen peroxide. Thiourea is highly cell permeable and a scavenger of hydrogen peroxide and hydroxyl radicals.[15] Thiourea acts as a reductive bleaching agent, and it has been used in the textile industry in combination with hydrogen peroxide to bleach wool and ground wood pulp with results superior to hydrogen peroxide alone.

## CONCLUSION

All bleaching regimens which employed the sequential use of acidified thiourea/acidified thiourea/hydrogen peroxide combination were effective at bleaching the bloodstained dentine samples as 30% w/v hydrogen peroxide alone. Statistical analysis indicates that the addition of acidified thiourea does not reduce the efficacy of hydrogen peroxide to bleach bloodstained teeth. However, the development of a combined reductive-oxidative bleaching protocol for stained root-filled teeth, using acidified thiourea and hydrogen peroxide, offers the possibility of producing superior clinical bleaching which may have the added benefit of scavenging potentially damaging hydroxyl radicals.

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