Efficacy and Safety of Various Tooth-Whitening Products, with Special Reference to the Three Dimensional Colour Space (L*a*b*) Measurements and the Microhardness Tests

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Efficacy and Safety of Various Tooth-Whitening Products, with Special Reference to the Three Dimensional Colour Space (L*a*b*) Measurements and the Microhardness Tests

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CIE L*a*b*
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Dentist-supervised home bleaching
In-office bleaching
Over-the-counter bleaching
DECLARATION

I, the undersigned hereby declare that “Efficacy and Safety of Various Tooth-Whitening Products, with Special Reference to the Three Dimensional Colour Space (L*a*b*) Measurements and the Microhardness Tests” is my own original work; that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Abdul Majeed

August, 2011

Signed:………………………………………

UNIVERSITY of the WESTERN CAPE
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LIST OF TERMS AND ABBREVIATIONS

Bleaching: Bleaching or whitening is a decolourisation process that can occur in a solution or on a surface. The process is commonly used in different industries such as clothing, paper and wood. A number of products are also available for humans to use as disinfectant or to bleach hair, skin and teeth. In this thesis the word "bleaching" refers to tooth-bleaching or tooth-whitening.

Efficacy: The degree of change in colour following tooth-bleaching (whitening effect) measured subjectively (shade guide) or objectively (spectrophotometer).

Safety: This will cover pH, peroxide concentration and the effects of tooth-whitening products on human enamel.

CP: Carbamide peroxide
HP: Hydrogen peroxide
DSHB: Dentist-supervised home bleaching
IOB: In-office bleaching
OTC: Over-the-counter
L*a*b*: Colour coordinates of three dimensional colour space established by CIE in 1976
L*: Lightness of the colour (L* = 0 yields black and L* = 100 indicates white)
a*: Represents position between red and green (+a* indicates reddish and -a* indicates greenish).
b*: Represents position between yellow and blue (+b* indicates yellowish and -b* indicates bluish).
△L*: Difference between two L* values
△a*: Difference between two a* values
△b*: Difference between two b* values

In this study, △L*, △a* and △b* indicates changes in L*, a* and b* coordinates from baseline following bleaching treatment and at different follow-up visits.

\(\Delta E_{ab}\): The total colour change (difference) which takes into account individual differences in L*, a* and b* coordinates and is represented by a single value.

CABA: Concentration of active bleaching agent
ACP: Amorphous calcium phosphate
PF: Potassium nitrate and fluoride

Relapse: Loss or reversal of whitening effect (colour improvements) over time also called rebound in tooth colour.
CHAPTER 1

SCOPE OF THESIS

Tooth-whitening or tooth-bleaching has become an integral part of modern dental practice. Today, a large number of whitening products are available on the market which are commonly categorized into dentist-supervised home bleaching, in-office bleaching and over-the-counter bleaching products according to their mode of application. This thesis looks into safety and efficacy of various tooth-whitening products and methods.

The thesis consists of 10 chapters in total. The basics of colour science, types of tooth discolourations and tooth-whitening are introduced in CHAPTER 2. The literature review gives a broad overview of tooth-whitening techniques and their effectiveness, CIE L*a*b* three dimensional colour space, and side effects including tooth hypersensitivity and changes in enamel microhardness.

General aims and objectives of the current project are outlined in CHAPTER 3. In CHAPTER 4 pH of various tooth-whitening products has been evaluated to determine their acidity levels and possible potential of enamel demineralization. CHAPTER 5 presents the determinations of hydrogen peroxide concentration in various dentist-supervised home bleaching products, in-office bleaching products and over-the-counter bleaching products. Determinations are based-on oxy-reduction titration reaction.

As part of safety evaluations, effects of various dentist-supervised home bleaching products and in-office bleaching products containing carbamide peroxide and/or hydrogen peroxide on enamel microhardness are evaluated in
CHAPTER 6. The study in CHAPTER 7 is a continuation of CHAPTER 6 and evaluates the effects of four over-the-counter tooth-whitening products on enamel microhardness.

As part of efficacy evaluations, CHAPTER 8 presents an in vitro study evaluating the efficacy of various tooth-whitening products and methods. The whitening effect is measured on stained human maxillary central incisors with a spectrophotometer based on CIE L*a*b* three dimensional colour space.

In CHAPTER 9 a randomized, single centre, observer-blind clinical trial is presented. The study evaluates the efficacy of three different tooth-whitening products using subjective and objective evaluation methods. Rebound in colour over six month period is measured using a spectrophotometer. Oral side effects including tooth sensitivity and gingival irritation are also evaluated using a sensitivity form and visual examination.

CHAPTER 10 represents a summary of all previous investigations with a short discussion and ends with conclusions and recommendations for future studies.

The list of publications, conference presentations and research awards is given as Appendix 2 and 3.
CHAPTER 2

LITERATURE REVIEW

2.1- INTRODUCTION

Dental aesthetics including tooth colour is of great importance for the majority of people and any discolouration or stain on them will affect their aesthetic qualities. The colour of teeth is influenced by a combination of their intrinsic and extrinsic shades. The intrinsic colour is influenced during tooth mineralization of dentine and enamel. The extrinsic colour is influenced by the presence of stains that may form on the tooth surface due to various factors like smoking, intake of tannin-rich foods (e.g. red wine, tea) and the use of chlorhexidine or metal salts such as tin and iron (Kihn, 2007; Watts and Addy, 2001; Nathoo, 1997). A number of methods such as professional cleaning and polishing to remove stains, whitening toothpastes, internal bleaching of non-vital teeth, external bleaching of vital teeth, micro-abrasion of enamel, crowns or veneers can be used to improve tooth colour (Joiner, 2006; Sarrett, 2002).

Composite or ceramic veneers/crowns and tooth-whitening or bleaching are two most commonly used procedures for the treatment of discoloured teeth. However, the placement of veneers or crowns is an invasive procedure that requires removal of sound tooth structure. It is also relatively expensive and time consuming. On the contrary, tooth-whitening is minimally invasive requiring no removal of tooth structure, cheaper and less time consuming.

During the past decades, the demand for a whiter and brighter smile has increased dramatically and so has the number of tooth whitening products and
published literature. This is illustrated by an internet search of the term “tooth
whitening” in two databases. The PubMed search of the above term revealed
528 scientific papers from 01/01/1990 to 01/01/2000 and 1071 from 02/01/2000
to 01/03/2010 while a Google search of the term revealed 5,010,000 strikes.
Furthermore a large number of tooth-whitening products are available to the
public, as over-the-counter (OTC) from supermarkets, pharmacies and over the
internet. Most of these products have not undergone testing for quality, safety
and effectiveness.

In order to prevent the reader from reading into irrelevant information, this
literature review is divided into different sections. The first few sections give a
broad overview about tooth colour, tooth discolouration and tooth-whitening
and the latter sections are about tooth colour measurements and adverse
effects.

The sections immediately following will cover tooth colour (2.2) and tooth
discolourations (2.3). Thereafter tooth-whitening (2.4), historical background
(2.5), bleaching chemistry (2.6), types of bleaching (2.7) and vital tooth-
bleaching methods (2.8) are described. The last few sections are about
measurement of tooth colour (2.9), adverse effects (2.10), and toxicity (2.11).
Finally the findings are summarized in section 2.12.

2.2- COLOUR SCIENCE AND TOOTH COLOUR

Colour has attracted man’s attention since time immemorial. The first theories
of colour and aesthetics flourished in 250-500BC during the Hellenism
civilization and persisted until the seventeenth century. The current concepts of
colour are based on the theoretical and scientific understanding of colour developed during the nineteenth and twentieth centuries (Burkinshaw, 2004).

Colour is a three dimensional system and can be described according to the Munsell terms of *hue*, *value* and *chroma* (Munsell, 1981). *Hue* is the term used to distinguish between different families of colour and refers to the name of colour or colour family such as red, blue, yellow, orange. *Value* refers to the relative lightness or darkness of a colour on a scale from black to white. *Chroma* is the quality used to describe the degree of colour saturation and strength of a colour as it changes, such as, intense or dull (Watts and Addy, 2001). This enables one to describe colours more accurately (Figure 2.1).

![Figure 2.1: The three dimensions of colour](image)

The science of colour involves chemistry, biology and physics. However, the science of colour differs from other areas of science in that while intrinsic properties of an object such as mass or volume are identical for all observers, the description of the colour refers to a sensation experienced by a particular observer. In other words, the perception of colour of a particular reflective object depends upon the combination of a light source, an object and an observer (Figure 2.2) (Burkinshaw, 2004).
Light is an electromagnetic radiation capable of stimulating the human sense of sight, the term covers a narrow band in the total spectrum of electromagnetic emissions, the wavelength being 380 nm to 760 nm (Burkinshaw, 2004). Light sources can be natural such as sun and flames or artificial such as incandescent lamps and fluorescent lights. The colour of light emitted by different sources varies from bluish white of daylight to the yellowish white of tungsten light (Burkinshaw, 2004).

When light interacts with an object it can be reflected from the surface of an object or it can be absorbed by the object or transmitted through the object (Figure 2.3). Colour of an object is determined by the relative extent of reflection, absorption and transmission of light.

Figure 2.2: The three components of colour

Figure 2.3: Simplified representation of reflection, absorption and transmission of light through a translucent material.
The colour vision apparatus which includes human eye and brain system forms the observer part. Although human eye/brain system can distinguish about 3-5 million different colours, the verbal description of these colours is an entirely different and extremely difficult situation (Burkinshaw, 2004).

Teeth are composed of a number of colours and individual tooth colour also varies from the gingival margin to the incisal edge (Watts and Addy, 2001). Tooth colour is a combination of intrinsic and extrinsic colourations. Light scattering and absorption properties of enamel and dentine are associated with intrinsic tooth colour while adsorption of materials e.g. tea, coffee, chlorhexidine etc. onto the tooth surface forms the extrinsic part (Joiner, 2004).

Natural teeth are translucent which means that when illuminating light encounters a tooth surface it is only partly absorbed. The non-absorbed light is then transmitted inside the tooth and follows highly irregular light paths (scattering) through the tooth structure (enamel and dentine) before emerging at the surface and reaching the eye of the observer (O’Brien et al., 1990). Natural teeth also exhibit high specular (gloss) reflection especially when wet which is perceived as white reflected light. Ko et al., (2000) reported that hydroxyfluoroapatite crystals and dentinal tubules were the predominant causes of light scattering in enamel and dentine while demineralization increased the light scattering coefficient of enamel because of changes in the microstructure. The scattering of light through enamel is small, mostly at wavelengths in the blue range. Ten Bosch and Coops (1995) demonstrated a strong correlation between the colours of 28 teeth from different patients before and after removal of the labial enamel and concluded that the major part of
visible tooth colour was produced by dentine while enamel only played a minor role in light scattering at wavelengths in the blue range. Therefore, proper understanding of the elements of tooth colour and discolouration, colour perception and influence of light sources is an important part of aesthetic dentistry. The next section (2.3) will describe types of tooth discolouration and staining.

2.3- TOOTH DISCOLOURATION

The aetiology of tooth discolouration is multifactorial and it varies in appearance and localization, severity and adherence to the tooth structure (Watts and Addy, 2001; Greenwall, 2001). Tooth discolouration can be classified as intrinsic, extrinsic, and internalised discolouration.

2.3.1- Intrinsic Tooth Discolouration

Intrinsic discolouration occurs during tooth development and is incorporated into the tooth structure, altering the light transmitting properties of enamel and dentine. A number of metabolic disorders such as alkaptonuria, congenital erythropoietic porphyria and systemic factors including administration of tetracycline during development, ingestion of excessive fluoride etc. affect the developing dentition and cause tooth discolouration. In addition, local factors such as traumatic injury or root resorption can also cause intrinsic tooth staining (Watts and Addy, 2001).

2.3.2- Extrinsic Tooth Discolouration

Extrinsic discolouration or staining is found on the tooth surface or in the pellicle. The cause of extrinsic discolouration can be divided into two
categories: direct staining by compounds which are incorporated into the pellicle and produce a stain as a result of the basic colour of chromogens, and indirect staining due to chemical interaction with another compound at the tooth surface which produces stain (Sulieman, 2005).

Direct Staining

Direct staining is caused by the dietary components, beverages, tobacco, medicines, spices, and vegetables. Chromogens derived from dietary sources such as tea and coffee get absorbed into the plaque or acquired pellicle and their natural colour imparts the stain onto the tooth. Chromogens incorporated into the pellicle act as a sponge and can hold fluids (Sulieman, 2005). Extrinsic staining caused by chromogenic bacteria has also been cited in children. Green and orange stains have been found in children with poor oral hygiene and black/brown stains in children with good oral hygiene and low caries experience (Watts and Addy 2001).

Indirect Staining

Indirect staining occurs due to the chemical interaction of cationic antiseptics and metal salts with another compound at the tooth surface. Different metal salts are associated with different discolouration, e.g., black discolouration of teeth is seen in people using iron supplements and iron foundry workers occupationally exposed to these metal salts. Mouth rinses containing potassium permanganate cause violet to black discolouration while mouth rinses containing copper salts produce green stains. Prolonged use of cationic antiseptics such as chlorhexidine produces brown to black discolouration (Sulieman, 2005; Watts and Addy, 2001).
2.3.3- Internalised Tooth Discolouration

Developmental defects or trauma can result in penetration of extrinsic tooth discolouration into enamel and dentine. Wear and tear of teeth from function or parafunction, dental caries and restorative materials can all cause tooth discolouration directly or indirectly. Loss of enamel due to abrasion, erosion and attrition exposes dentine to extrinsic chromogens. As dentine is more porous than enamel, it takes up extrinsic stains quickly. Loss of enamel also results in darker teeth as the yellow colour of dentine becomes more apparent. Teeth with enamel cracks and/or exposed dentine are prone to internalisation of extrinsic stains (Sulieman, 2005; Watts and Addy, 2001).

During progression of carious lesions various stages are recognised by the change in colour from initial white spot lesions to black arrested lesions which take up stain from extrinsic sources (Thylstrup and Fejerskov, 1995).

Some restorative materials used in dentistry can also discolour teeth. For example, grey to black discoloration of dentine seen around a long standing amalgam restoration. Eugenol-containing medicaments used during root canal treatment cause orange/yellow staining of dentine (Sulieman, 2005).

The literature indicates that tooth discolouration and origins of different types of stains are well understood.
2.4- TOOTH-WHITENING

Tooth-whitening or commonly known tooth-bleaching is not a recent development in dentistry. Dentists have been trying numerous materials and methods to remove various types of tooth discolouration for the last 200 years. However, many of the early attempts were not successful and bleaching techniques were considered experimental and unpredictable. It was not until the first publication by Haywood and Heymann in 1989 described successful bleaching of teeth with 10% carbamide peroxide worn in a tray overnight, that tooth-whitening became a popular more conservative alternative for the treatment of discoloured teeth. The next section describes a brief history or evolution of tooth bleaching in general from mid-19th century.

2.5- HISTORICAL BACKGROUND

Non-vital tooth bleaching with chloride of lime was practised in 1848. In 1860, Truman introduced the most effective technique for bleaching non-vital teeth, which used chlorine derived from a solution of calcium hydrochlorite and acetic acid, known as Labarraque’s solution (Sulieman, 2004). Numerous other bleaching agents including aluminium chloride, oxalic acid, pyrozone (ether-peroxide), hydrogen peroxide, sodium peroxide, sodium hypophosphate, sulphuric acid and cyanide of potassium were also employed successfully for non-vital tooth bleaching (Haywood, 1992). Although non-vital bleaching is not the focus of the present review, it has been mentioned only for the purpose of completeness.
By 1860, vital tooth bleaching using oxalic acid had been reported in the literature (Sulieman, 2004). In 1877, Chapple also proposed oxalic acid as the material of choice for vital tooth bleaching. Shortly after, Taft suggested calcium hypochlorite as an effective whitening solution (McLaughlin and Freedman, 1991). The first peroxide whitening material, namely hydrogen dioxide, was introduced by Harlan in 1884 (McLaughlin and Freedman, 1991). The aqueous solution of hydrogen dioxide and 3% solution of Pyrozone (ether-peroxide) were considered safe as a mouthwash both for children and adults as early as 1893 (Haywood, 1992).

In 1918, Abbot reported that use of heat or light could greatly enhance the bleaching action of superoxol (McLaughlin and Freedman, 1991). No major developments in bleaching techniques took place till the late 1950s. In 1961, Sapasser described a mixture of sodium perborate and water for non-vital bleaching. The mixture was sealed in the pulp chamber for one week and the patient would return to have the procedure repeated until the desired lightening effect was reached (Sulieman, 2004). Nutting and Poe (1967) modified this technique to what is known as “walking bleach” by using a combination of 30% hydrogen peroxide and sodium perborate sealed in the pulp chamber for one week.

In late 1960s, Klusmier described the use of Gly-Oxide (Marion Merrel Dow, Inc., Kansas City, Missouri), a 10% carbamide peroxide oral antiseptic, in a custom-fitting mouth tray for home bleaching (Kihn, 2007; Haywood, 1992). Later in 1972, he switched to Proxigel, which also contained 10% carbamide peroxide, because its viscosity allowed it to stay in the custom-fitting tray.
In 1989, Omni International (Arkansas, USA) developed and marketed the first 10% carbamide peroxide on a commercial basis (Kihn, 2007). Haywood and Heymann (1989) published the first clinical study on the Nightguard Vital Bleaching technique using 10% carbamide peroxide. In this technique, the bleaching solution (10% carbamide peroxide) is placed in a custom-fitting tray and the patient wears it overnight for several days or weeks until the desired effect is achieved. The continued scientific research into this technique has demonstrated its safety, efficacy and success and is the most commonly used take-home whitening procedure among the dental community.

2.6- BLEACHING CHEMISTRY

Whitening or bleaching is a decolourisation process that can occur in a solution or on a surface. Three prominent commercial bleaching processes are peroxide, chlorine and chloride. Peroxide bleaching is the most commonly used process because it requires less time. Bleaching occurs by oxidation-reduction reaction also known as “redox reaction”. In this reaction the oxidizing agent (e.g. hydrogen peroxide) loses free radicals with unpaired electrons and becomes reduced, whereas the reducing agent (the substance being bleached) becomes oxidized by accepting the electrons (Frysh, 1995).

2.6.1- Hydrogen Peroxide Bleaching

Hydrogen peroxide can oxidize a wide variety of organic and inorganic compounds by forming free radicals (HO$_2^+$ + O$^-$) which are very reactive (Joiner, 2006). In order to increase shelf life, pure aqueous solutions of hydrogen peroxide are made weakly acidic. The ionization of weakly acidic
aqueous solution of hydrogen produces a large number of weak (O·) free radicals (Figure 2.4) while ionization of buffered hydrogen peroxide at an alkaline pH of 9.5 to 10.8 produces a large number of stronger per-hydroxyl (HO₂·) free radicals (Figure 2.5) which results in a greater bleaching effect in the same time as at other pH levels (Frysh, 1995).
In the presence of decomposition catalysts and enzymes the reaction is modified and no free radicals are produced which renders hydrogen peroxide ineffective as bleaching agent. These enzymes, which are also present in the oral cavity, are a natural defence of the body against oxygen toxicity.

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

2.6.2- Mechanism of Dental Bleaching

Hydrogen peroxide is the most commonly used active ingredient in tooth-bleaching. Hydrogen peroxide can be applied directly to the tooth surface, or is produced in a chemical reaction from carbamide peroxide. Carbamide peroxide (\(\text{CH}_6\text{N}_2\text{O}_3\), or \(\text{CH}_4\text{N}_2\text{O}.\text{H}_2\text{O}_2\)) upon contact with water dissociates into hydrogen peroxide (\(\text{H}_2\text{O}_2\)) and urea (\(\text{N}_2\text{H}_4\text{CO}\)). Urea further breaks down into ammonia and carbon dioxide (Figure 2.6). It has been reported that 10% carbamide peroxide usually produces 3 – 3.35% hydrogen peroxide.

![Figure 2.6: Chemical breakdown of carbamide peroxide](image)

The exact mechanism of tooth-bleaching is not fully understood (Sulieman, 2004; Hannig et al., 2003). It is believed that hydrogen peroxide initially diffuses into and through enamel to dentine, producing free radicals (Kihn, 2007; Hannig et al., 2003). The free radicals with unpaired electrons are extremely
unstable and react with highly pigmented organic (carbon-rings) molecules found within the tooth structure and breaks them down into smaller, less pigmented components (carbon-chains). The smaller molecules reflect less light, thus creating a reduction in colour or “whitening effect” (Nixon et al., 2007).

As the tooth-bleaching process continues, a point is reached where only colourless hydrophilic structures exist. This is known as the “saturation point” and the bleaching process slows down (Frysh, 1995). Continued use of a bleaching product after this could result in breakdown of the enamel matrix (Figure 2.7).

![Figure 2.7: Tooth bleaching, saturation point, at which optimal bleaching occurred and result of continued bleaching. (Lightening natural teeth. ADEPT Report: 1991:2:1-24).](image)
2.7- TYPES OF TOOTH BLEACHING

Tooth-bleaching or –whitening is broadly classified into non-vital and vital bleaching. Non-vital bleaching as the name indicates is for non-vital teeth and vital bleaching is for vital teeth. In non-vital bleaching a medicament is placed in the pulp chamber for 3 to 7 days or bleaching material can be applied both externally and internally known as inside/outside technique (Sulieman, 2004). However, non-vital bleaching is not part of the present study and is mentioned for completeness purposes only. The next section (2.8) describes different approaches used for vital tooth-bleaching.

2.8- External or Vital Tooth Bleaching

The three commonly used approaches or methods for vital tooth-bleaching include: 1) dentist-supervised home bleaching, 2) in-office bleaching and 3) over-the-counter whitening products for self-application. These methods are described separately in the following sub-sections.

2.8.1- Dentist-supervised Home Bleaching (At-home Bleaching)

Dentist-supervised home bleaching or nightguard vital bleaching has become the most commonly used whitening technique because of its relative low-cost, high success rate, ease of use, and safety (Swift et al., 1999; Rosenstiel, Gegaff and Johnston, 1996; Russell et al., 1996; Gegaff et al., 1993; Reinhard et al., 1993). The technique has been regarded as the gold standard by which other techniques are judged (Sulieman, 2005).
The treatment involves the construction of a bleaching tray (nightguard) usually worn by the patient at night. The trays are fabricated from the stone models of a patient’s teeth and may or may not contain a reservoir for bleaching material. The original technique as described by Haywood and Heymann (1989) required wearing a custom fitting guard filled with 10% carbamide peroxide for 6 to 8 hours nightly for 2 to 6 weeks. In a clinical trial Matis et al., (2002) reported that the presence or absence of reservoirs in a bleaching tray did not influence the outcome of 15% carbamide peroxide.

Since the introduction of this technique, the technique has undergone a number of modifications, improvements and variations (Leonard et al., 2001; Garber et al., 1991; Haywood, 1991). Different products are available containing as little as 6% and as high as 15% hydrogen peroxide as well as 5%, 10% (equivalent to ~3% hydrogen peroxide), 15%, 20%, 35%, or 36% carbamide peroxide. Application time for different products varies from 30 minutes to 8 hours per day for 2 to 6 weeks. Bleaching products with high concentrations of peroxide are claimed to produce quicker whitening of teeth compared to the products with lower peroxide concentrations (Kihn et al., 2000). However, 10% carbamide peroxide solution is still the most commonly used at-home bleaching concentration (Basting et al., 2003), due to its reported safety and effectiveness (Alonso de La Pena and Cabrita, 2006; Cavalli, Giannini and Carvalho, 2004; Haywood, 1992; Haywood and Heymann, 1991). A high success rate of 98% for non-tetracycline stained teeth and 86% for tetracycline stained teeth has been reported in the literature for nightguard vital bleaching (Sulieman, 2005).
Disadvantages

However, this technique is associated with a high dropout rate since it requires active patient compliance for optimal results, which is a major disadvantage (Kihn, 2007; Sulieman, 2005). The most common problem is that some patients do not wear the tray daily while others continue bleaching for longer periods which frequently causes thermal sensitivity (67%) and gingival irritation (Haywood, 1992).

Efficacy

Nightguard vital bleaching using 10% carbamide peroxide is the most widely used and extensively researched tooth-bleaching technique. The American Dental Association has awarded its seal of acceptance to a number of dentist-supervised home bleaching products containing 10% carbamide peroxide (Hasson et al., 2007). The nightguard vital bleaching technique has been effective for lightening teeth stained by aging, mild fluorosis, trauma, inherent discolourations and tetracycline (Kihn, 2007; Sulieman, 2005; Haywood, 1994). According to the American Dental Association (1994) guidelines for the acceptance of peroxide-containing oral hygiene products, the clinical efficacy may be demonstrated by a change of two value oriented shade increments and a perceptible colour must be maintained in 50% of the recall population at 6 months compared to the control, to reflect the duration of efficacy using a shade guide. In a long-term clinical trial, Leonard et al., (2001) reported whitening of teeth in 98% of the participants by 10% carbamide peroxide and 82% of the participants retained the whitening effect up to 47 months post treatment.
A meta-analysis of the clinical trials from 1989-1999 on dentist-supervised home bleaching products using 10% carbamide peroxide suggested that only 73% of the population will show a colour change of two units or greater and 50% retain colour at 6 months post bleaching (Niederman et al., 2000).

Higher carbamide peroxide concentrations (15% and 20%) available for home-bleaching may whiten teeth slightly quicker than 10% carbamide peroxide during the early phase of treatment. However, the whitening effect shows some relapse after the cessation of active bleaching treatment before the colour is stabilized. Teeth treated with 10% carbamide peroxide stabilize in colour two weeks following the cessation of the treatment but the higher-concentration products take much longer (Matis et al., 2000). However, it is claimed that rapid whitening shown by the higher-concentration products is temporary and following rebound there will be no difference (Browning, 2007).

Hydrogen peroxide and carbamide peroxide tooth-bleaching products with equivalent peroxide concentrations demonstrate similar whitening efficacy with few side effects (Ziebolz et al., 2007; Berga-Caballero et al., 2006; Alonso de la Pena and Cabrita, 2006).

2.8.2- In-office Bleaching

In-office vital tooth bleaching also known as power bleaching has been regarded as a reliable technique for rapid lightening of discoloured teeth (Zekonis et al., 2003). In-office bleaching is advocated for use in specific situations such as severe discolouration, discolouration of a single tooth, lack of patient compliance or if a rapid treatment is desired (Buchalla and Attin, 2007;
Sulieman, 2005a). Usually a high concentration of hydrogen peroxide 30-50% is used with proper soft tissue protection either by rubber dam or light-curing isolating pastes (Pretty et al., 2006; Baik, Rueggeberg and Liewehr, 2001). The duration of one treatment is generally 30 to 60 minutes and the bleaching procedure might be repeated several times during one session. Multiple appointments may also be required to obtain desired results (Sulieman, 2005a).

An activating method such as heat, light or laser can be used to enhance or expedite the whitening effect (Buchalla and Attin, 2007; Kihn, 2007). Application of heat, light or laser increases the temperature of the bleaching agent which accelerates the release of hydroxyl radicals from peroxide. This in turn increases the rate of change of tooth colour (Baik et al., 2001). Certain bleaching products contain specific colorants to increase light absorption and as a result heat conversion (Buchalla and Attin, 2007).

Quartz-tungsten-halogen (QTH) lamps, plasma arc (PAC) lights, light emitting diodes (LEDs), argon lasers, metal halide and xenon halogen lights have been proposed for activation of bleaching agents (Buchalla and Attin, 2007; Kihn, 2007; Sulieman, 2005a; Baik et al., 2001). In addition some systems are chemically activated by mixing of two gels, while others utilize a dual activation system.

**Combination or Assisted Bleaching**

In-office application of a high-concentration hydrogen peroxide could also be used as a supplement or boost therapy, which may be supplemented by a
dentist-supervised home bleaching procedure until the desired result is achieved (Kihn, 2007; Perdigao, Baratieri and Arcari, 2004).

Bleaching products with high concentrations of carbamide peroxide 30-45% are also available for use in assisted bleaching (Pretty et al., 2006). The patient wears a custom fitting tray filled with bleaching material and is requested to sit in the waiting room for about 30 minutes to an hour. The gel is rinsed off the teeth after the time has elapsed. The procedure is also known as “waiting room bleach” (Pretty et al., 2006; Sulieman, 2005a).

Advantages of in-office bleaching include rapid whitening of teeth that can be used to motivate the patient to continue with at-home bleaching and patient-compliance is not a problem.

Disadvantages

Major disadvantages of this treatment are increased chair time, high costs, multiple visits to obtain optimal results and dehydration of the teeth which may give a false whitening effect immediately after treatment.

Efficacy

In-office bleaching procedures are performed using higher hydrogen peroxide (30%-38%) concentrations at chair-side under the close supervision of a dentist. A number of clinical studies have demonstrated the effectiveness of in-office bleaching alone (Matis et al., 2007a; Al Shethri et al., 2003; Gallagher et al., 2002) or in combination with the afterwards take-home bleaching products (Wetter et al., 2008; Deliperi et al., 2004). Auschill et al., (2005) in a randomized clinical trial comparing the efficacy of at-home, over-the-counter
and in-office bleaching techniques reported that all treatment methods were able to achieve a similar whitening effect but the treatment times were significantly different with the in-office bleaching technique requiring the least time and the most accepted method amongst the patients was the at-home bleaching technique. However, in contrast to these results, another study showed that treatment with an in-office bleaching product (35% hydrogen peroxide) was less effective as compared to a 14-day application of 10% carbamide peroxide in a tray (Zekonis et al., 2003).

A few studies have reported the acceleration or enhancing effect of different light/laser sources on in-office bleaching treatments (Luk et al., 2004; Tavares et al., 2003; Gallagher et al., 2002; Nakamura et al., 2001), while other studies reported no effect of light-activation on the final outcome of in-office bleaching with hydrogen peroxide (Marson et al., 2008; Wetter et al., 2008; Matis et al., 2007a, Kugel et al., 2006). Hein et al., (2003) investigated the contribution of three bleaching lights (LumaArch, Optilux 500, and Zoom!) to act as catalysts for whitening teeth in a split-arch clinical study. He reported that neither the heat produced from lights, nor the light outputs per se were responsible for catalytic activity and the tested lights did not lighten teeth more than their bleaching gels alone. In spite of contradictory reports in the literature, to date there is no concrete evidence to show that these devices improve the final outcome of in-office bleaching treatment (Marson et al., 2008; Ritter, 2006; Papathanasiou et al., 2002).

Although in-office bleaching products are accepted by the American Dental Association, no “ADA Seal” is issued to these products because the
professional component of the ADA Seal Program was discontinued on December 31, 2007 (American Dental Association, 2008).

2.8.3- Over-the-counter Bleaching

Over-the-counter products are available direct to the consumer and include complete whitening kits with preformed soft trays, paint-on gels, dentifrices and whitening strips. Most of the over-the-counter whitening products contain peroxide in some form as an active ingredient (Zantner et al., 2006). Sodium chlorite (NaClO₂) has also been used as a bleaching agent in over-the-counter products (Attin et al., 2005).

Whitening toothpastes contain mild abrasives to remove surface stains and some may contain a minimal amount of peroxide. The whitening effect of toothpastes is normally very limited because of the minimal exposure time and low peroxide concentration (Kihn, 2007).

Whitening Strips

Whitening strips, a novel “trayless” bleaching system, was introduced at the beginning of this century (Gerlach and Zhou, 2001). It uses a flexible polyethylene strip to deliver hydrogen peroxide to the anterior teeth (Sagel et al., 2000). The concentration of hydrogen peroxide on whitening strips ranges from 5.3 to 6.5% and the recommended wearing time is 30 minutes twice daily for two weeks or more (Gerlach and Zhou, 2001). The trayless delivery system offers advantages with respect to overall peroxide dose, contact time, and ease-of-use compared to other delivery systems (Gerlach, 2000). The strips
are disposed after use thus eliminating the need for tray cleaning, storage and maintenance.

**Paint-on Gels/Liquids**

Non-tray, paint-on whitening gels/liquids are also available on the market. These may contain 18% (5.9% hydrogen peroxide) to 25% (8.7% hydrogen peroxide) carbamide peroxide as an active ingredient. Duration and number of applications vary among different products (Kihn, 2007).

Contact time for over-the-counter products is significantly less (5 to 30 minutes per day) compared to the dentist-supervised home bleaching products (8 hours per day). Therefore, whitening strips and paint-on or other over-the-counter products need to be used for longer periods to obtain desired results (Kihn, 2007).

**Disadvantages**

The risk of inappropriate use is high because these products can be bought and used indiscriminately by the patients. Another disadvantage of over-the-counter products is that the trays provided with whitening kits are not custom-made. Formulations and results obtained from these products also vary (Kugel, 2003).

**Efficacy**

A large number of “over-the-counter” or “direct-to-consumer” whitening products including whitening strips or “trayless” whitening systems, paint-on-gels, gels with prefabricated trays and whitening toothpastes have become
increasingly popular in recent years because of low cost and overwhelming marketing by companies. Whitening strips usually contain 6%-14% hydrogen peroxide in gel form. An integrated clinical summary of nine randomized clinical trials reported the efficacy of whitening strips containing 14% hydrogen peroxide similar to popular tray-based bleaching systems (Gerlach and Barker, 2004). A clinical comparison of two brush-applied whitening systems showed that a 19% sodium percarbonate system that dries to form an adherent film provided significant improvement in tooth colour compared to 18% carbamide peroxide gel (Barlow et al., 2003). Zantner et al., (2006) reported that a new bleaching lacquer containing 8% carbamide peroxide for self-application without the use of a mouth guard produced two shade improvements in tooth colour.

A recent systematic review (Hasson, Ismail and Neiva, 2007) of home-based chemically-induced whitening of teeth demonstrated that dentist-supervised home bleaching systems and over-the-counter products (paint-on gels and whitening strips) were effective when compared to placebo/no treatment and the efficacy varied because of the different levels of active ingredients. However, the majority of studies are either sponsored or conducted by the manufacturers and are short term. Furthermore, tooth-whitening products are not regulated in many countries and most of these products have not undergone clinical evaluation for safety and effectiveness. Therefore, there is a great need for independent clinical trials or alternatively, laboratory studies which could provide a good indication of what could be expected in practice.
2.9- MEASUREMENT OF TOOTH COLOUR

Currently, reproducible and valid determination of tooth colour to evaluate the efficacy of a tooth-whitening product remains a challenge (Li, 2003). Commonly used colour assessment methods can be broadly divided into two categories: visual and instrumental (Okubo et al., 1998). In the following text both methods are described briefly with more emphasis on spectrophotometer in instrumental determinations.

2.9.1- Visual Colour Determination (Subjective)

Visual tooth colour determination using standard tooth shade guides is the most routinely applied method in clinical dentistry (Paravina, 2008). Visual determination is highly subjective and colour is perceived rather than measured by the observer (Hugo, Witzel and Klaiber, 2005). Therefore several factors such as external light conditions, experience, angle of perception, age, fatigue of the human eye and physiological variables such as colour blindness may lead to inconsistencies (Bayindir et al., 2007; Okubo et al., 1998). A study evaluating visual colour determinations using shade guides reported inconsistencies among individual dentists to match natural tooth shades and some dentists were even unable to reliably duplicate their own shade selections between two occasions (Culpepper, 1970). Other studies also reported inconsistencies in shade determinations by visual means (Okubo et al., 1998; Freedman, 1997). Furthermore, differences in colour, shape, structure and gloss between different parts of a tooth may be interpreted differently by individuals (Bayindir et al., 2007).
In addition to human variables, there are several other disadvantages of visual shade determinations using shade guides. Shade guides lack an adequate range of shades to represent the varying colour of natural teeth. Distribution of shades is illogical, non-uniform and inconsistent with natural tooth colour (Karamouzos et al., 2007). The lack of uniformity in distribution of shades throughout the colour space of natural teeth results in close matches for some shades and gross mismatches for others (Bayindir et al., 2007).

2.9.2- Instrumental Colour Measurement (Objective)

Difficulties and limitations associated with subjective visual colour determinations make an objective method desirable. The use of colour assessment electronic devices for various purposes in dentistry has been reported since the early 1970s (Dozic et al., 2007). A large number of computer-based instruments such as spectrophotometers/colorimeters for shade selection have become available since 1990s. Recent advances in these instruments have increased their use in dental research and provided the ability for objective and rapid colour determinations (Cal, Guneri and Kose, 2006).

Spectrophotometers measure the reflectance of light within the entire visible spectrum whereas colorimeters evaluate the reflected light only through red, green and blue wavelengths (Karamouzos et al., 2007). Instrumental measurements allow quantification and precise communication of colour (Okubo et al., 1998). The two most common systems for describing colour are Munsell’s System and the International Commission on Illumination (CIE) L*a*b* colour system. Only the latter system (CIE L*a*b*) is described below because it will be used for efficacy evaluation purposes in this study.
CIE L*a*b* three dimensional colour space

CIE L*a*b is a three dimensional colour model established by Commission Internationale de l’Eclairage in 1976, to describe all the colours visible to the human eye (Figure 2.8). The dimensions of colour are usually described as value (lightness/brightness), hue (colour name) and chroma (pale to strong) (Paravina, 2008). In this colour space lightness is represented by $L^*$ on a scale of 0 for black to 100 for white. The hue and chroma are represented on an $a^*$ versus $b^*$ plot where $a^*$ represents the red/green coordinate and $b^*$ represents the yellow/blue coordinate. $+a^*$ indicates red and $-a^*$ indicates green similarly $+b^*$ indicates yellow and $-b^*$ indicates blue (Burkinshaw, 2004).

Figure 2.8: CIE L*a*b* three dimensional colour space
The CIE L* a* b* colour system adequately represents visually uniform coverage of the colour space. Differences or changes in colour components (L*, a* and b*) of an object between two measurements can be calculated which are represented by \( \Delta L^* \), \( \Delta a^* \) and \( \Delta b^* \) respectively. Then total colour change (\( \Delta E_{ab}^* \)) can be calculated using the following formula:

\[
\Delta E_{ab}^* = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2}
\]

Dozic et al., (2007) investigated the performance of five commercially available tooth colour measuring devices and reported that under in vitro and clinical settings the spectrophotometer was the most reliable colour determination instrument compared to colorimeters and digital camera. Clinical studies comparing the visual and spectrophotometric colour determination methods on human teeth reported that spectrophotometer shade determinations were more accurate and reproducible than visual shade determinations (Derdilopoulou et al., 2007). Although spectrophotometers revealed predictable and reproducible colour determinations, disagreements with human colour perceptions were found (Hugo et al., 2005). Kielbassa et al., (2009) suggested that spectrophotometers should be used as an alternative to, or in conjunction with visual shade determinations for objectivity and standardization.
2.10- ADVERSE EFFECTS

Adverse effects of vital tooth-bleaching procedures on hard and soft tissues of the oral cavity have been reported in the literature (Jorgensen and Carroll, 2002). Tooth sensitivity and gingival or mucosal irritation are the most common side effects of vital tooth-bleaching. Other effects include minor orthodontic tooth movement, temporomandibular dysfunction due to long term tray use and sore throat (Pohjola et al., 2002).

2.10.1- Tooth Sensitivity

Tooth sensitivity is a common clinical side effect of tooth-whitening and occurs in two-thirds of patients treated with home bleaching products. The majority (55%) may experience mild sensitivity, whereas 10% may experience moderate and only 4% may experience severe sensitivity (Jorgensen and Carroll, 2002). Symptoms are noticed early in the treatment usually after 2-3 days and may persist 3-4 hours following removal of the tray (Sulieman, 2005) and disappear shortly after the treatment ends (Kihn, 2007). Other studies also reported transient tooth sensitivity with bleaching treatments which lasted up to 4 days after the cessation of treatment (Schulte et al., 1994; Cohen, 1979). However, duration of mild sensitivity might last for as long as 39 days after bleaching treatment according to Leonard, Haywood and Phillips (1997). Ishikawa-Nagai et al., (2004) compared two different brands of 10% carbamide peroxide and reported that 40 to 42% of the subject experienced reversible tooth sensitivity.

The use of bleaching products with higher peroxide concentrations also increases the risk of tooth sensitivity (Jacobsen and Bruce, 2001). Nathanson
(1997) reported higher incidence 65%-75% of tooth sensitivity following bleaching treatment using higher peroxide concentration exceeding 30% hydrogen peroxide and heat application. Bizhang et al., (2009) compared at-home, in-office and over-the-counter bleaching methods and reported that all methods caused mild tooth sensitivity and gingival irritation which disappeared without requiring interruption of treatment.

The aetiology of tooth sensitivity following bleaching treatment is multifactorial and is poorly understood (Pretty et al., 2006). Sensitivity is thought to be caused by the diffusion of by-products produced during hydrogen peroxide and carbamide peroxide breakdown through dentinal tubules (Gokay, Tuncbilek and Ertan, 2000). Glycerine, used as a carrier in most bleaching agents, is hydrophilic and causes dehydration of tooth structure during bleaching treatment. This can also result in tooth sensitivity (Leonard et al., 1997).

Fluoride, potassium nitrate and amorphous calcium phosphate (ACP) have been introduced in recent bleaching products to prevent either hypersensitivity and/or enamel demineralization (Chen et al., 2008). However, the literature available on the beneficial effects of these agents is contradictory. Giniger et al., (2005) reported a significant reduction in tooth hypersensitivity for a 16% carbamide peroxide bleaching agent with amorphous calcium phosphate (ACP) than that without ACP. In another study Tam, (2001) reported that presence of potassium nitrate and fluoride in a 10% carbamide peroxide bleaching product produced less tooth sensitivity than did a control bleaching agent during a 2-week at-home bleaching treatment. Matis et al, (2007) compared two bleaching products with different desensitizing agents and reported that 15% carbamide
peroxide with potassium nitrate and fluoride showed no significant difference in sensitivity compared to 16% carbamide peroxide with amorphous calcium phosphate. Tay et al., (2009) reported that use of a gel containing 5% potassium nitrate and 2% sodium fluoride before in-office bleaching procedure with 35% hydrogen peroxide reduced tooth sensitivity.

Cardoso et al., (2010) reported that a shorter application time of 1 hour per day instead of 8 hours per day for 10% carbamide peroxide bleaching treatment resulted in reduced tooth sensitivity. Gallo et al., (2009) reported that presence of potassium nitrate had little effect on sensitivity when 30% carbamide peroxide was applied for a shorter duration of 1 hour per day for 10 days.

In summary, it is evident from the literature that tooth sensitivity is still a common clinical side effect of tooth-whitening. However, sensitivity is transient and disappears shortly after the discontinuation of bleaching treatment. Addition of desensitizing compounds such as potassium nitrate, fluoride and amorphous calcium phosphate (ACP) do not completely eliminate tooth sensitivity but might be beneficial in reducing the sensitivity.

2.10.2- Gingival and Mucosal Irritation

Some patients may experience gingival or mucosal irritation during home bleaching procedures. Clinical studies using 10% carbamide peroxide in custom-fitting trays reported gingival irritation in 25 to 40% of the patients during treatment (Leonard et al., 1997; Tam, 1999). Soft tissue irritation may be caused by an ill-fitting tray impinging on the gingiva and/or the use of excess material (Sulieman, 2005). Management includes simply adjusting and
polishing the tray and instructing the patient to use less material. Hydrogen peroxide is a strong oxidizing agent and higher concentrations (from 30 to 35%) can cause burns of the gingival or mucosal tissue (Pretty et al., 2006). In-office bleaching procedures involve the use of higher hydrogen peroxide concentrations. Therefore, soft tissue protection with a rubber dam or light-cured resin material provided by the manufacturer is recommended during in-office bleaching procedures.

2.10.3- Effects on Tooth Structure

Bleaching of vital teeth involves direct contact with the enamel surface for an extensive period of time which differs between manufacturers. This fact increased concerns about the possible adverse effects of such a strong oxidizing agent on the enamel/dentine. Effects of various tooth-whitening products have been extensively investigated in the literature. The majority of these investigations were carried out in vitro on extracted human or bovine teeth. The evaluated concentrations of carbamide peroxide and hydrogen peroxide ranged from 10 to 37% and 5.3 to 38%, respectively. This part has collated these studies into investigations on the surface morphology and surface microhardness of enamel and dentine.

Surface Morphology

Effects of bleaching treatment on surface morphology of enamel have been analysed using the scanning electron microscope and profilometer. Scanning electron microscopy is a rapid and convenient method to qualitatively analyse the surface morphology of enamel. The profilometer determines quantitative
changes in surface roughness and loss of surface material by measuring pre- and post-treatment profiles (Joiner, 2007).

For the studies that employed these techniques, the reported findings are contradictory. The majority of the studies reported no significant changes in surface morphology of enamel following bleaching with low concentrations of carbamide peroxide (Zantner et al., 2007; Moraes et al., 2006; Cobankara et al., 2004; Justino et al., 2004; White et al., 2003; Lopes et al., 2002; Haywood et al., 1991; Haywood et al., 1990) and hydrogen peroxide (Duschner et al., 2006; White et al., 2003; Joiner et al., 2004; Nucci et al., 2004). Similarly studies evaluating the effects of higher concentrations of hydrogen peroxide (35%) and carbamide peroxide (35%) also reported no significant changes in enamel surface morphology (Sulieman et al., 2004; Worschech et al., 2003). In a clinical study, Leonard et al., (2001) evaluated the casts made from impressions of teeth bleached with 10% carbamide peroxide 8 hours per day for 14 days and reported none or minimal changes in the surface texture of enamel. In another study, Turkun et al., (2002) reported a slight increase in the porosity of enamel following bleaching treatment with 10% carbamide peroxide for 14 days, which disappeared within 3 months following the treatment.

In contrast, other studies reported changes in surface morphology of enamel following bleaching with carbamide peroxide and/or hydrogen peroxide products (Yeh et al., 2005; Cavalli et al., 2004; Pinto et al., 2004; Spalding et al., 2003; Smidt et al., 1998; Ernst et al., 1996; Ben-Amar et al., 1995; Bitter and Sanders, 1993; Shannon et al., 1993). Ben-Amar et al., (1995) described these changes as slight and Yeh et al., (2005) as mild surface pitting at
localized areas. Hegedűs et al., (1999) in an atomic force microscopy study demonstrated that carbamide peroxide and hydrogen peroxide were capable of causing mild changes in enamel surface. In an in vitro study, Spalding et al., (2003) observed minor changes in enamel surface morphology after bleaching with 35% hydrogen peroxide for 20 minutes followed by 10% carbamide peroxide for 12 hours per day for 1 week but considered these changes to be within the normal variations existing in natural teeth.

The contradictions found among different studies may be due to the differences in their in vitro protocols, lack of simulation of in vivo environment and/or the low pH of the products used. For example, Yeh et al., (2005) and Ben-Amar et al., (1995) used distilled water as storage medium between bleaching treatments instead of artificial or human saliva, thus negating any remineralization from salivary factors. Another study demonstrated that negative effects observed for in vitro bleached specimens when stored in water were not seen when similarly treated specimens were placed on an intraoral device and worn in the mouth and thus exposed to saliva.

The changes in surface morphology of enamel might be related to the low (acidic) pH of some bleaching products. Shannon et al., (1993) reported most severe changes in enamel topography following bleaching treatment with 10% carbamide peroxide products of lower pH (4.3 and 4.9). Similarly, other studies evaluating the bleaching products that used acidic pre-rinses containing citric acid or acetic acid prior to the peroxide application also demonstrated changes in enamel surface morphology. However, it is most likely that these changes
were caused by acidic pre-rinses rather than with bleaching products (Bitter, 1992; Bitter and Sanders, 1993).

**Surface Microhardness**

Surface microhardness measurement is a simple test to determine the mechanical properties of enamel and dentine. In the microhardness test, a fixed load is applied to a flat polished tooth surface using a diamond indenter. After unloading, the dimensions of the resultant indentation on the surface are measured with the help of a microscope. Two commonly used diamond indenters are Vickers diamond squared pyramid and Knoop elongated diamond pyramid.

Microhardness tests have been commonly used to determine the loss or gain of mineral (demineralization/remineralization) of tooth structure following bleaching treatments (Burrows, 2009; Joiner, 2007). The literature reported conflicting findings regarding the effects of tooth-whitening products on enamel and dentine microhardness. The majority of the studies reported no significant change in the microhardness of enamel and dentine following bleaching treatment (Ferreira et al., 2006; Ünlü et al., 2004; Seghi and Denry, 1992). For example, Sulieman et al., (2004) reported that bleaching with 35% hydrogen peroxide for 30 minutes did not reduced microhardness of enamel and dentine. Other studies evaluating the bleaching products containing 10% carbamide peroxide also showed no reduction in enamel microhardness (Leonard et al., 2005; Lopes et al., 2002; Cimilli and Pameijer, 2001; Murchinson, Charlton and Moore, 1992). In a cyclic experiment on enamel and dentine with 10 and 15% carbamide peroxide, Ünlü et al., (2004) found no significant reduction in
microhardness values following bleaching treatments. However, in this study and studies by Ferreira et al., (2006) and Lopes et al., (2002) treatment times were shorter (2-4 hours per day) than the manufacturers’ recommended treatment times (8 hours per day). Another cyclic experiment with 12% hydrogen peroxide where treatment times were 7 hours per day for 14 days demonstrated no effect on surface morphology and microhardness of enamel (Pugh et al., 2005).

In contrast, other studies reported a decrease in enamel and/or dentine microhardness following bleaching treatment (Dadoun and Bartlett, 2007; Ulukapi, 2007; Basting et al., 2005; Rodrigues et al., 2005 Pinto et al., 2004; Basting et al., 2003). In an in vitro study, Majeed et al., (2008) found that four different Opalescence teeth whiteners damaged enamel. The highest damage was done by the 10% and 20% carbamide peroxide products because of the much longer exposure period of 112 hours in comparison to only 7 hours for the Opalescence Quick PF 45% carbamide peroxide. Lewinstein et al., (2004) reported that in-office bleaching products i.e. 35% hydrogen peroxide and 35% carbamide peroxide, reduced hardness of enamel and dentine more than the home bleaching products i.e. 10% carbamide peroxide but the application of 0.05% fluoride solution for five minutes completely restored the softened tooth structure. Similarly Basting et al., (2005) reported a small reduction in enamel surface microhardness following bleaching treatment with 10% carbamide peroxide for 8 hours per day for 42 days which recovered to above baseline following 7 days in remineralization solution. A small reduction in dentine surface microhardness following exposure to 10% carbamide peroxide in situ was reported by Arcari et al., (2005) but they concluded that this might be
clinically insignificant. Other studies also reported reduction in enamel microhardness following bleaching with up to 35% hydrogen peroxide or 35% carbamide peroxide (Attin et al., 2004; de Oliveira, Paes Leme and Giannini, 2005; Pinheiro-Junior et al., 1996).

In an in vitro study Sulieman et al., (2004) reported that 35% hydrogen peroxide did not damage enamel or dentine and the adverse effects reported in the literature may be related to the pH of the products used. Smidt et al., (1998) observed slight reduction in enamel microhardness following treatment with three different 10% carbamide peroxide products applied for 6 hours per day for 16 days with pH values in the range of 4.3 to 5.5. Zantner et al., (2007) and Attin et al., (2005) reported that over-the-counter bleaching products containing sodium chlorite and citric acid with pH of 3.7 showed significantly higher reduction in enamel microhardness than those containing peroxide, possibly because of the acidic pH.

The conflicting data may be due to the differences in methodology and different pH levels of the products. The available data demonstrated that experiments differed in the type of teeth (human/bovine or erupted/unerupted) and bleaching agent used, treatment times and storage solutions. For example, Attin et al., (2004) used bovine enamel which has been reported to have faster lesion progression than human enamel (Featherstone and Melberg, 1981). Some studies used distilled water while others used artificial saliva as storage medium. Furthermore, human enamel exhibits large regional variations in structure related to the differences in local chemistry (varying levels of mineralization, organic matter and water) and microstructure (fractions of
inorganic crystals and organic matrix) (Braly et al., 2007; Spalding, Taveira and de Assis, 2003). Therefore, enamel microhardness may vary from area to area (Braly et al., 2007). This might be another reason for controversies found in the literature because experiments also differed in the position of hardness indents and the amount of forces applied as well as the type of diamond indenter used (Table 2.1).

Microhardness experiments require a flat polished surface in order to make indentations and to measure them. However, there is no standardized method of grinding and polishing the specimens. Polishing of the enamel surface removes the resilient hyper-mineralised layer, thus making the enamel more prone to the softening or demineralization effect of bleaching products (Zantner et al., 2007).

A review comparing the different hardness studies reported that the impact of bleaching agents on enamel microhardness was influenced by the study design. The experiments which closely simulated the intraoral conditions showed less reduction in enamel microhardness following bleaching treatments compared to those which did not simulate intraoral conditions (Attin et al., 2009).

In summary, the current review of literature also points out a number of differences in the methodology of various microhardness studies. Some of these studies are summarized in Table 2.1. There is a greater need to develop a standardized protocol to evaluate the effects of tooth-whitening products on the microhardness of enamel and dentine.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Bleaching Agent</th>
<th>pH</th>
<th>Duration of Bleaching</th>
<th>Storage Solution</th>
<th>Study design</th>
<th>Substrate (teeth)</th>
<th>Polishing</th>
<th>Hardness Test</th>
<th>No of Indents</th>
<th>Distance between pre- &amp; post-bleaching indents</th>
<th>Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seghi and Denry, 1992</td>
<td>10% CP</td>
<td>-</td>
<td>12 h</td>
<td>Saline (no remineralising solution)</td>
<td>in vitro</td>
<td>Human molar</td>
<td>Hand polished</td>
<td>Vickers</td>
<td>5</td>
<td>Widely separated</td>
<td>9.8 N for 15 sec</td>
</tr>
<tr>
<td>Potocnik et al., 2000</td>
<td>10% CP</td>
<td>6.6</td>
<td>42 x 8 h</td>
<td>No remineralising solution</td>
<td>In vitro</td>
<td>Human premolar, molar</td>
<td>1000 grit</td>
<td>Vickers</td>
<td>-</td>
<td>40-80 µm</td>
<td>200 g for 10 sec</td>
</tr>
<tr>
<td>Rodrigues et al., 2001</td>
<td>10% CP</td>
<td>-</td>
<td>8 h/d for 42 d</td>
<td>Artificial saliva</td>
<td>In vitro</td>
<td>Human molar</td>
<td>1000 grit</td>
<td>Knoop</td>
<td>3</td>
<td>200 µm</td>
<td>50 g for 20 sec</td>
</tr>
<tr>
<td>Lopes et al., 2002</td>
<td>10% CP</td>
<td>6.0</td>
<td>3 h/d for 14 d</td>
<td>Artificial Saliva</td>
<td>in vitro</td>
<td>Human molar</td>
<td>600 grit</td>
<td>Vickers</td>
<td>6</td>
<td>0.1 mm</td>
<td>100 g for 30 sec</td>
</tr>
<tr>
<td>Attin et al., 2003</td>
<td>10% CP+F</td>
<td>7.0</td>
<td>1 x 8 h</td>
<td>No remineralising solution</td>
<td>in vitro</td>
<td>Bovine</td>
<td>1200 grit</td>
<td>Knoop</td>
<td>5</td>
<td>-</td>
<td>Not given</td>
</tr>
<tr>
<td>Basting et al., 2003</td>
<td>10% CP</td>
<td>7.5</td>
<td>8 h/d for 42 d</td>
<td>Artificial Saliva</td>
<td>in vitro</td>
<td>Human molar</td>
<td>1000 grit</td>
<td>Knoop</td>
<td>3</td>
<td>-</td>
<td>25 g for 5 sec</td>
</tr>
<tr>
<td>Joiner et al., 2004</td>
<td>6% HP</td>
<td>-</td>
<td>2 x 20 min/d for 14 d</td>
<td>Sterile human saliva</td>
<td>In vitro</td>
<td>Human incisor</td>
<td>1200 grit</td>
<td>Knoop</td>
<td>5</td>
<td>50 µm</td>
<td>50 g</td>
</tr>
<tr>
<td>Lewinstein et al., 2004</td>
<td>35% HP</td>
<td>5.0</td>
<td>3 x in 35 min</td>
<td>No remineralising solution</td>
<td>in vitro</td>
<td>Human molar</td>
<td>1200 grit</td>
<td>Knoop</td>
<td>3</td>
<td>100 µm</td>
<td>100 g for 20 sec</td>
</tr>
<tr>
<td>Pinto et al., 2004</td>
<td>10% CP</td>
<td>-</td>
<td>6 h/d for 14 d</td>
<td>Artificial saliva</td>
<td>in vitro</td>
<td>Human molar</td>
<td>1200 grit</td>
<td>Knoop</td>
<td>3</td>
<td>-</td>
<td>25 g for 5 sec</td>
</tr>
<tr>
<td>Study</td>
<td>Percentage</td>
<td>Duration</td>
<td>Remineralisation Solution</td>
<td>Location</td>
<td>Grit</td>
<td>Hardness</td>
<td>Notes</td>
<td></td>
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<tr>
<td>Suleiman et al., 2004</td>
<td>35% HP</td>
<td>3 x 10 min</td>
<td>No remineralising solution</td>
<td>In vitro</td>
<td>Human molar</td>
<td>800 grit</td>
<td>Vickers 5</td>
<td>-</td>
<td>300 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teixeira et al., 2004</td>
<td>6%, 6.5%, 7.5%, 9.5% HP, 10% CP</td>
<td>-</td>
<td>HP: 30 min/d for 14d CP: 6 h/d for 14 d</td>
<td>Artificial saliva</td>
<td>In vitro</td>
<td>Human molar</td>
<td>1200 grit</td>
<td>Knoop 3</td>
<td>-</td>
<td>50 g for 15 sec</td>
<td></td>
</tr>
<tr>
<td>Unlu et al., 2004</td>
<td>10% CP 15% CP</td>
<td>1 x 4 h or 7 x 4 h</td>
<td>No remineralising solution</td>
<td>In vitro</td>
<td>Human incisor</td>
<td>-</td>
<td>Vickers 3</td>
<td>Widely separated</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leonard et al., 2005</td>
<td>10% CP 5.3% HP 8.75% HP</td>
<td>-</td>
<td>8 h/d for 14-21 d</td>
<td>Artificial saliva</td>
<td>In vitro</td>
<td>Human molar</td>
<td>1200 grit</td>
<td>Knoop 3</td>
<td>-</td>
<td>50 g for 15 sec</td>
<td></td>
</tr>
<tr>
<td>Rodrigues et al., 2005</td>
<td>37% CP+10% CP 37% CP+Placebo Placebo+10% CP</td>
<td>-</td>
<td>37% CP: 2 x 30 min at day 1, 7 &amp; 14 10% CP: 6-8 h/d = 21 d</td>
<td>Human saliva</td>
<td>In situ</td>
<td>Human molar</td>
<td>1000 grit</td>
<td>Knoop 5</td>
<td>30 µm</td>
<td>25 g for 5 sec</td>
<td></td>
</tr>
<tr>
<td>Ferreira et al., 2006</td>
<td>10% CP 3.5%, 4.5%, 5.5%, 7.5% HP</td>
<td>2 x 30 min/d for 14 d</td>
<td>Artificial saliva</td>
<td>In vitro</td>
<td>Human molar</td>
<td>600 grit</td>
<td>Vickers 6</td>
<td>0.1 mm</td>
<td>100 g for 30 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metz et al., 2007</td>
<td>15% CP with PF 15% CP without PF</td>
<td>-</td>
<td>8 h/d for 4 d or 14 d</td>
<td>Human saliva</td>
<td>In vivo</td>
<td>Human premolars</td>
<td>Carbide bur 169L</td>
<td>Knoop 5</td>
<td>-</td>
<td>50 g for 15 sec</td>
<td></td>
</tr>
<tr>
<td>Rodrigues et al., 2007</td>
<td>10% CP with carbopol or poloxamer</td>
<td>6 h/d for 7-28 d</td>
<td>Artificial Saliva</td>
<td>In vitro</td>
<td>Bovine</td>
<td>1000 grit</td>
<td>Knoop 5</td>
<td>-</td>
<td>300 µm</td>
<td>25 g for 5 sec</td>
<td></td>
</tr>
<tr>
<td>Ulukapi, 2007</td>
<td>10% CP 35% HP (heat)</td>
<td>-</td>
<td>14 x 8 h Not described</td>
<td>No remineralising solution</td>
<td>In vitro</td>
<td>Human molar</td>
<td>600 grit</td>
<td>Vickers 3</td>
<td>-</td>
<td>500 g for 10 sec</td>
<td></td>
</tr>
<tr>
<td>Faraoni-Romano et al., 2008</td>
<td>10% CP 7.5% HP 38% HP 18% HP/22% CP</td>
<td>8 h/d for 21 d 1 h/d for 21 d 15 min/w for 3 w 30 min/w for 3 w</td>
<td>Artificial saliva</td>
<td>In vitro</td>
<td>Bovine</td>
<td>1200 grit</td>
<td>Knoop 5</td>
<td>500 µm</td>
<td>Not given</td>
<td>25 g for 20 sec</td>
<td></td>
</tr>
<tr>
<td>Maia et al., 2008</td>
<td>10% CP 7.5% HP</td>
<td>1 h/d for 21 d</td>
<td>Human saliva</td>
<td>In situ</td>
<td>Human molar</td>
<td>1200-2000 grit</td>
<td>Knoop 3</td>
<td>100 µm</td>
<td>-</td>
<td>50 g for 5 sec</td>
<td></td>
</tr>
<tr>
<td>Majeed et al., 2008</td>
<td>10% CP 20% CP 45% CP 10% HP</td>
<td>-</td>
<td>8 h/d for 14 d 8 h/d for 14 d 30 min/d for 14 d 30 min/d for 14 d</td>
<td>Artificial Saliva</td>
<td>In vitro</td>
<td>Human molar</td>
<td>1200 grit</td>
<td>Vickers 4</td>
<td>-</td>
<td>10 µm</td>
<td>300 g for 15 sec</td>
</tr>
</tbody>
</table>
2.10.4- Effects on Restorative Materials

The increased use of highly oxidizing bleaching agents has raised concerns about their effects on different restorative materials. Several in vitro studies have evaluated the effects of carbamide peroxide (10-16%) and hydrogen peroxide (30-35%) whitening products on physical properties, surface morphology and colour of different restorative materials (Attin et al., 2004). Haywood (1992) and Swift (1997) reported that the nightguard vital bleaching technique had no significant effect on the colour and physical properties of porcelain, amalgam and gold. Increase in surface roughness of porcelain, microfilled composite and resin modified glass ionomer following treatment with 10-16% carbamide peroxide was reported by Turker and Biskin (2003). Modified glass ionomer also showed increased surface porosity and cracks in certain areas.

Controversy exists about the influence of external pre- and post-operative bleaching on microleakage of composite restorations. Crim (1992) reported that pre-restorative bleaching with 10% carbamide peroxide did not affect the marginal seal of subsequently placed restorations. Ulukapi et al., (2003) reported that pre- and post-operative bleaching with carbamide peroxide increased microleakage of resin composite restorations at enamel and dentine margins. In contrast other studies did not report increased microleakage rates at enamel margins (Owens et al., 1998).

The oxidation of surface pigments and amine compounds by bleaching agents can alter the colour of restorative materials. The oxidizing effect on the polymer-matrix of resin-based materials also increases surface porosities (Attin
et al., 2004). There is no clear evidence whether the changes in tooth-coloured restorative materials are superficial or deep. However, polishing of resin composite fillings is advisable following bleaching procedures to decrease the adherence of certain cariogenic microorganisms.

Bleaching agents also cause increased release of mercury from amalgam restorations (Rotstein et al., 2004; Rotstein et al., 2000). Coating of amalgam restorations with a protective varnish such as copalite before the bleaching procedure has been reported to reduce release of mercury into the surrounding environment (Rotstein et al., 2000). The corrosion potential of amalgam is also decreased if restorations are polished prior to the bleaching therapy.

2.10.5- Effects on Bond Strength

The effect of various bleaching procedures on shear or tensile bond strength of composites to enamel and dentine has been studied extensively. The majority of the studies reported that the bond strengths of composite restorative materials to enamel (Shinohara et al., 2005; Stokes et al., 1992; van der Vyver et al., 1997; Dishman et al., 1994; Stokes et al., 1992; Sung et al., 1999) and dentine (Toko and Hisamitsn, 1993; Spyrides et al., 2000; Far et al., 2003; Shinohara et al., 2005) was significantly reduced when applied immediately after bleaching with hydrogen peroxide or carbamide peroxide. Josey et al., (1996) reported no negative effects of 10% carbamide peroxide bleaching on composite-enamel bond strength. However, it is not clear whether the bleaching affects the bond strengths of alcohol- and acetone- based bonding agents to enamel and dentine similarly (Attin et al., 2004).
Several factors are responsible for the reduction in composite bond strengths to enamel and dentine. Polymerization inhibition of the resin adhesive systems due to the presence of oxygen released by the bleaching process on the enamel surface and within the dentinal tubules is the likely mechanism for the reduction in bond strengths (Barbosa et al., 2008; Dishman et al., 1994). Significant loss of enamel calcium and phosphorus content and morphological alterations of the majority of the crystals of the surface layer caused by the peroxide-based bleaching agents also adversely affects the bond strengths (Attin et al., 2004; Perdigão et al., 1998). Adebayo et al., (2007) reported that the use of conditioners prior to bonding with self-etching adhesive systems to bleached enamel may significantly improve bond strengths. However, the reduction in bond strength is time-dependent and returns to normal after a few days, when the residual oxygen is liberated. The recommended waiting time before performing bonding procedures after tooth bleaching ranges from 3-7 days (McGuckin et al., 1992), 7-14 days (Barbosa et al., 2008; Shinohara et al., 2005) to 3-weeks (Cavalli et al., 2001). Therefore, it is advisable to wait for a while before performing bonding procedures after bleaching.

2.11- TOXICITY OF WHITENING PRODUCTS

Hydrogen peroxide is normally present in many human tissues such as lungs, liver and salivary cells. Both intra- and extra-cellular mechanisms protect tissues against hydrogen peroxide-induced cytotoxicity (Marshall et al., 2001). Enzymes present in the body cells such as catalase, peroxidases, glutathione peroxidase and superoxide dismutase decompose hydrogen peroxide into oxygen and water (Carlson, 1987). The oral cavity contains salivary
peroxidases and catalase in adequate amounts to combat any adverse effects of reactive oxygen species (Carlson, 1987; Ericson and Bratt, 1987). An in vitro evaluation reported that the toxicological potential of bleaching agent containing 10% carbamide peroxide or 4% hydrogen peroxide was lower or comparable to commonly used dental materials like eugenol, composites and mouth rinses (Li, 1997).

During the bleaching process carbamide peroxide breaks down into hydrogen peroxide and urea. Further break down of urea produces ammonia and carbon dioxide, which is considered beneficial by being anti-cariogenic, stabilizing hydrogen peroxide, elevating the pH levels of the solution and stimulating saliva flow (Christensen, 1998). No risk of local and general toxicity has been reported for hydrogen peroxide released from carbamide peroxide (Kelleher and Roe, 1999).

Systemic toxic effects of hydrogen peroxide and carbamide peroxide depend upon the concentration and amount of hydrogen peroxide ingested (Dahl and Pallesen, 2003). However, local toxic effects are determined by the total dose, not the concentration of peroxide (Burrows, 2009). Dahl and Becher (1995) reported that daily exposure of carbamide peroxide should not exceed 10mg/kg of body weight. The average amount of bleaching agent used in one application has been calculated to be 502 mg. Even if an average person (60 kg) swallows all the gel, it would not exceed 8.37 mg/kg of body weight (Li, 1997). This suggests that the safety factor of the bleaching agent is high.
2.12- SUMMARY

Different treatment modalities available to the patient include dentist-supervised take-home bleaching, in-office bleaching, a combination of both and over-the-counter bleaching systems. Tooth sensitivity and gingival or mucosal irritation are the most common side effects of vital tooth-bleaching. However, known products tend to include agents to minimize or prevent these side effects. In general, findings stated in the literature are contradictory as far as the damaging effect of bleaching agents towards enamel is concerned. There is a lack of standardized protocols for in vitro experiments. However, well-performed hardness tests showed at least some kind of softening (damage) towards enamel. Whether this softening is insignificantly small or similar to damage by certain soft drinks or fruit juices, still needs to be determined. Tooth-bleaching procedures also reduce bond strengths to enamel and dentine. Therefore, it is advisable to wait for a while before performing bonding procedures after bleaching. Nowadays, the market is flooded with different products available to the general public, which are not tested for their efficacy or possible enamel damage and further research on these products is needed.

It also becomes clear that the best way to assess a bleaching agent is by performing a clinical study and ideally the lasting effect should be evaluated over a long period. CIE \( L^*a^*b^* \) colour space adequately represents the human eye in all three dimensions of the colour space and computer-based instruments (spectrophotometers) provide more objective, reproducible and reliable measurement of colour changes following bleaching treatment as compared to visual methods (shade guide).
Nightguard vital bleaching utilizing 10% carbamide peroxide is still considered the most cost-effective, safe and effective whitening procedure. In-office bleaching may be an acceptable method for patients who require rapid whitening or those who cannot tolerate home bleaching procedures but it is not necessarily more effective than dentist-supervised home bleaching. To date there is little evidence to show greater efficacy of light-activated systems over chemically activated in-office bleaching systems.

There is an urgent need for independent laboratory and clinical studies to evaluate the long-term effectiveness and harmful effects of vital tooth-bleaching products, to enable the dentist to inform their patients about the benefits and risks of different whitening modalities based on the current scientific evidence and to suggest the best treatment option based on proper diagnosis.
REFERENCES


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CHAPTER 3

AIMS AND OBJECTIVES OF THE STUDY

3.1- AIMS OF THE STUDY

The aim of this study was to evaluate the efficacy and safety of various tooth-whitening products, categorized into dentist-supervised home bleaching\(^1\), in-office bleaching\(^2\) and over-the-counter bleaching\(^3\) products according to their mode of application, with special reference to the three dimensional colour space (CIE L*a*b*) measurements and the microhardness tests.

3.2- HYPOTHESES

1. Tooth-whitening products are safe to use and do not cause any damage to the enamel when applied according to the manufacturer’s instructions.

2. All tooth-whitening products and techniques are equally effective in terms of the amount of bleaching or whitening.

3.3- OBJECTIVES

In order to test the hypotheses, this study was divided into in vitro and in vivo investigations. In vitro investigations evaluated the different aspects of tooth-whitening products related to the safety and effectiveness. In vivo investigation evaluated the efficacy, colour stability and oral side effects associated with selective tooth-whitening products.

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\(^1\) Dentist-supervised home bleaching (DSHB): Professional bleaching material supplied by the dentist and applied by patients at home, at night or during the day usually in custom-fitting trays.

\(^2\) In-office bleaching (IOB): Bleaching procedure is carried out by the dentist in the dental office using higher peroxide concentration.

\(^3\) Over-the-counter bleaching (OTC): Available to the public from pharmacies, stores, internet for at home self-application.
The objectives of in vitro investigations were:

1. To evaluate the pH and hydrogen peroxide concentration of various tooth-whitening products.

2. To evaluate the effects of various professional and over-the-counter tooth-whitening products on the surface microhardness of enamel using a standardized protocol.

3. To compare the efficacy of various tooth-whitening products and methods on stained human incisor teeth, using a spectrophotometer in a standardized in vitro environment.

The objectives of in vivo investigation were:

1. To evaluate and compare the degree of colour change of teeth and colour relapse associated with two at-home and one in-office tooth-whitening product using a spectrophotometer.

2. To evaluate the safety and tolerability by recording the oral side effects associated with the use of above mentioned products.
CHAPTER 4

THE pH OF VARIOUS TOOTH-WHITENING PRODUCTS

4.1- INTRODUCTION

Tooth-whitening has become increasingly popular in recent years. Peroxides in the form of hydrogen peroxide or carbamide peroxide are the most common active ingredients in tooth-whitening products. Several studies have evaluated the safety, effectiveness and side effects of whitening products on intraoral soft and hard tissues (Alonso de la Peña and Balboa Cabrita, 2006; Bitter, 1992; Cavalli, Giannini and Carvalho, 2004; Haywood and Heymann, 1991; Leonard, Haywood and Phillips, 1997). The American Dental Association has granted its seal of acceptance to a number of products. However, the current available whitening products vary in composition, concentration and type of active ingredients. Some products have been reported to have a pH as low as 3.67 (highly acidic), while others have been reported to have a pH of 11.13 (highly alkaline) (Price, Sedarous and Hiltz, 2000). Weiger, Kuhn and Lost (1993) reported that the greater the peroxide concentration, the lower the pH of the whitening product.

During the bleaching procedure teeth and soft tissues are exposed to a low or high pH for a varying period of time which may cause adverse effects. Some studies reported demineralization of enamel at a pH below 5.2 (Driessens et al., 1986), while others reported a pH of 5.5 as the critical pH for dental enamel (Barron et al., 2003). Dawes (2003) reported that the critical pH below which enamel is dissolved varies over a wide range (5.1 to 6.5) and depends on the
concentrations of calcium and phosphate in the solution. Available literature suggests that low pH and high acid concentrations cause enamel erosion (Hunter et al., 2000; Hughes et al., 2000).

Scanning electron microscope studies have reported changes in surface morphology of enamel following bleaching with carbamide peroxide (Pinto et al., 2004; Smidt, Weller and Roman, 1998; Ben-Amar et al., 1995; Bitter and Sanders, 1993; Shannon et al., 1993; Ernst, Morroquin and Willershausen-Zonnchen, 1996). Hegedüs et al., (1999) in an atomic force microscopy study demonstrated that carbamide peroxide and hydrogen peroxide were capable of causing alterations in enamel surface. Other investigators reported contradicting evidence that there were no changes in enamel morphology following exposure to bleaching agents (Zantner et al., 2007, Haywood et al., 1990; Haywood, Houck and Heymann, 1991). A decrease in enamel microhardness following bleaching treatment has also been reported in the literature (Majeed et al., 2008; Pinto et al., 2004; Basting et al., 2003).

Bleaching products may affect the bond strengths and properties of composite restorative materials. However, it is not known if the effects are related to the pH or peroxide concentration of the bleaching products or both (Price et al., 2000). The majority of the studies reported that the bond strength of composite restorative materials to enamel was significantly reduced when bonding procedure was carried out immediately after bleaching treatment (Dishman, Covey and Baughan, 1994; Shinohara et al., 2005; Sung et al., 1999; van der Vyver, Lewis and Marais, 1997). Polymerization inhibition of the resin adhesive systems due to the presence of oxygen released by the bleaching process on
the enamel surface and within the dentinal tubules is the likely mechanism for
the reduction in bond strengths (Barbosa et al., 2008; Dishman et al., 1994).
Alternatively, loss of mineral content of the enamel may also adversely affect
the bond strengths (Attin et al., 2004; Perdigão et al., 1998).

A large number of whitening products are available on the market. One would
expect that these products should have neutral or near neutral pH levels.

4.2- AIM

The purpose of this in vitro study was to determine the pH levels of 21
commercially available tooth-whitening products containing carbamide
peroxide, hydrogen peroxide or other active ingredient.

4.3- OBJECTIVES

1. To evaluated the pH levels of five dentist-supervised home bleaching
   products.

2. To evaluate the pH levels of five in-office bleaching products.

3. To evaluate the pH levels of four over-the-counter whitening products

4. To evaluate the pH levels of seven whitening toothpastes.

4.4- HYPOTHESIS

The 21 tested whitening products have similar and neutral or near neutral pH
levels.
4.5- MATERIALS AND METHODS

Twenty one tooth-whitening products commercially available in South Africa were evaluated in this study (Figure 4.1 and Tables 4.1 to 4.4). The selection was based on the availability of different whitening products from dental suppliers, supermarkets and pharmacies at the time of study. The products were divided into four categories: dentist supervised-home bleaching (DSHB) products (n=5); in-office bleaching (IOB) products (n=5); over-the-counter (OTC) whitening products (n=4) and whitening toothpastes (WT) (n=7).

The pH was measured using an Orion Expandable ion Analyzer EA940 and an Orion 9165BNWP Sure-Flow®, Epoxy-body combination pH electrode, (Thermo Fisher Scientific Inc., Beverly, MA, USA). The pH electrode was calibrated using two buffering solutions of pH 4 and pH 7 (Beckman Instruments, Irvine, CA, USA) and the electrode response to the buffering solutions was checked after each 6 sample measurements. The products were placed in disposable cups and stirred with the pH electrode to have a uniform contact with the electrode tip. The electrode tip was left in contact for about five minutes at room temperature to allow the pH value to stabilize. Three samples of each product were measured and in between samples, the electrode was thoroughly washed and rinsed with distilled water to completely remove the material. The excess of water at the bottom tip of the electrode was sucked away by a piece of tissue paper before placing it into the next sample. One product required more than one step to complete the bleaching process hence each step was measured separately and the combination of both steps was also measured. The pH of a
commonly utilised carbonated soft drink (Coca Cola) was also measured for reference purposes.

4.6- STATISTICAL ANALYSIS

Simple descriptive statistics such as mean, minimum, maximum and standard deviation were calculated. The pH levels of tooth-whitening products between different categories were compared using Kruskal-Wallis one-way ANOVA significant at p<0.05.
Figure 4.1(A-J): Various tooth-whitening products evaluated in the study
4.7- RESULTS

The mean pH values of whitening products are given in Tables 4.1 to 4.4 and represented in graphical form in Figure 4.2.

Overall, the pH levels of all tooth-whitening products ranged from 3.76 ± 0.07 (highly acidic) to 9.68 ± 0.03 (highly alkaline).

The five dentist-supervised home bleaching products had a mean pH of 6.21 ± 0.76 and range from 4.88 to 6.81 (Table 4.1).

The five in-office bleaching products had a mean pH of 6.26 ± 1.19 and range from 5.30 to 7.85 (Table 4.2).

The four over-the-counter whitening products had a mean pH of 5.07 ± 1.74 and range from 3.76 to 8.03 (Table 4.3) and the seven whitening toothpastes had a mean pH of 7.66 ± 1.19 and range from 6.61 to 9.68 (Table 4.4).

There was a significant difference between the pH values of the four categories (Kruskal-Wallis one-way ANOVA; p<0.05). The pH level of the over-the-counter category was significantly lower than all other categories (p<0.05). Rapid White (over-the-counter) had an acidic pH of 3.76 ± 0.07 and Colgate Advanced Whitening toothpaste showed an alkaline pH of 9.68 ± 0.03.

The pH level of Coca Cola soft drink was 2.62 ± 0.04. It was not discussed further, as it was used as a reference only.
Table 4.1: Mean pH of dentist-supervised home-bleaching (DSHB) products.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Manufacturer</th>
<th>Lot No.</th>
<th>Application Time</th>
<th>Mean pH (±SD*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nite White® ACP 10% CP</td>
<td>Discus Dental, Culver City, CA, USA</td>
<td>9068046</td>
<td>8 hrs/d</td>
<td>4.88 ± 0.02</td>
</tr>
<tr>
<td>Yotuel® Patient 10% CP</td>
<td>Biocosmetic Laboratories, Madrid, Spain</td>
<td>Z311</td>
<td>8 hrs/d</td>
<td>6.43 ± 0.02</td>
</tr>
<tr>
<td>Opalescence® PF 10% CP</td>
<td>Ultradent Products, Inc., South Jordan, UT, USA</td>
<td>B2CR6</td>
<td>8 hrs/d</td>
<td>6.46 ± 0.02</td>
</tr>
<tr>
<td>Opalescence® PF 20% CP</td>
<td>Ultradent Products, Inc., South Jordan, UT, USA</td>
<td>B33RW</td>
<td>8 hrs/d</td>
<td>6.81 ± 0.03</td>
</tr>
<tr>
<td>Opalescence® Trèswhite Supreme 10% HP</td>
<td>Ultradent Products, Inc., South Jordan, UT, USA</td>
<td>B2FJF</td>
<td>30 min/d</td>
<td>6.45 ± 0.17</td>
</tr>
</tbody>
</table>

SD = Standard deviation, CP = carbamide peroxide, HP = hydrogen peroxide

Table 4.2: Mean pH of in-office bleaching (IOB) products.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Manufacturer</th>
<th>Lot No.</th>
<th>Application Time</th>
<th>Mean pH (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yotuel® 10 Minutes 30% CP</td>
<td>Biocosmetic Laboratories, Madrid, Spain</td>
<td>A023/Z321</td>
<td>10 min/d</td>
<td>5.38 ± 0.07</td>
</tr>
<tr>
<td>Opalescence® PF Quick 35% CP</td>
<td>Ultradent Products, Inc., South Jordan, UT, USA</td>
<td>-</td>
<td>30 min/d</td>
<td>7.23 ± 0.04</td>
</tr>
<tr>
<td>Opalescence® PF Quick 45% CP</td>
<td>Ultradent Products, Inc., South Jordan, UT, USA</td>
<td>B3LWK</td>
<td>30 min/d</td>
<td>5.54 ± 0.00</td>
</tr>
<tr>
<td>Yotuel® Special 35% HP</td>
<td>Biocosmetic Laboratories, Madrid, Spain</td>
<td>08YT35005</td>
<td>45-60 min/session</td>
<td>5.30 ± 0.09</td>
</tr>
<tr>
<td>Opalescence® Boost 38% HP</td>
<td>Ultradent Products, Inc., South Jordan, UT, USA</td>
<td>B3MDL</td>
<td>60-80 min/session</td>
<td>7.85 ± 0.13</td>
</tr>
</tbody>
</table>

SD = Standard deviation, CP = carbamide peroxide, HP = hydrogen peroxide

Table 4.3: Mean pH of over-the-counter (OTC) bleaching products.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Manufacturer</th>
<th>Lot No.</th>
<th>Application Time</th>
<th>Mean pH (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid White Step 1 Accelerator</td>
<td>Rapid White Products, Tonawanda, NY, USA</td>
<td>LK429</td>
<td>-</td>
<td>8.03 ± 0.30</td>
</tr>
<tr>
<td>Rapid White Step 2 Whitening gel</td>
<td>Rapid White Products, Tonawanda, NY, USA</td>
<td>LK429</td>
<td>5-10 min/d</td>
<td>3.76 ± 0.07</td>
</tr>
<tr>
<td>Rapid White combination of step 1 &amp; 2</td>
<td>Rapid White Products, Tonawanda, NY, USA</td>
<td>LK429</td>
<td>5-10 min/d</td>
<td>3.76 ± 0.03</td>
</tr>
<tr>
<td>Absolute White™</td>
<td>Dr. Fresh, Inc. La Mirada, CA, USA</td>
<td>-</td>
<td>30 min/d</td>
<td>3.94 ± 0.02</td>
</tr>
<tr>
<td>Speed White</td>
<td>CCA Industries, Inc. E Rutherford, NJ, USA</td>
<td>7610</td>
<td>5-10 min/d</td>
<td>4.65 ± 0.04</td>
</tr>
<tr>
<td>White Glo</td>
<td>Barros Laboratories Pty Ltd. NSW, Australia</td>
<td>11615</td>
<td>20 min/d</td>
<td>6.30 ± 0.03</td>
</tr>
</tbody>
</table>

SD = Standard deviation
### Table 4.4: Mean pH of whitening toothpastes

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Manufacturer</th>
<th>Lot No.</th>
<th>Application Time</th>
<th>Mean pH (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colgate Advanced Whitening</td>
<td>Colgate-Palmolive (Pty) Ltd. Boksburg, RSA</td>
<td>7134ZA10</td>
<td>2-4 min/d</td>
<td>9.68 ± 0.03</td>
</tr>
<tr>
<td>Plus White Xtra with peroxide whitening</td>
<td>CCA Industries, Inc. E Rutherford, NJ, USA</td>
<td>27243X</td>
<td>2-4 min/d</td>
<td>6.61 ± 0.08</td>
</tr>
<tr>
<td>Opalescence Whitening TP</td>
<td>Ultradent Products, Inc. South Jordan Utah, USA</td>
<td>B2CR6</td>
<td>2-4 min/d</td>
<td>6.70 ± 0.02</td>
</tr>
<tr>
<td>Plus White Xtra Cool Mint TP</td>
<td>CCA Industries, Inc. E Rutherford, NJ, USA</td>
<td>-</td>
<td>2-4 min/d</td>
<td>8.69 ± 0.06</td>
</tr>
<tr>
<td>Rapid White Whitening TP</td>
<td>Rapid White Products, Tonawanda, NY, USA</td>
<td>LK429</td>
<td>2-4 min/d</td>
<td>7.04 ± 0.04</td>
</tr>
<tr>
<td>White Glo Whitening TP</td>
<td>Barros Laboratories Pty Ltd. NSW, Australia</td>
<td>-</td>
<td>2-4 min/d</td>
<td>8.11 ± 0.10</td>
</tr>
<tr>
<td>Aqua Fresh White &amp; Shine TP</td>
<td>GlaxoSmithKline South Africa, Bryanston, RSA</td>
<td>-</td>
<td>2-4 min/d</td>
<td>6.81 ± 0.06</td>
</tr>
</tbody>
</table>

SD = Standard deviation

![Figure 4.2: The pH of the whitening products in various categories](image-url)

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4.8- DISCUSSION

The pH of various tooth-whitening products evaluated in this study ranged from as low as 3.76 to as high as 9.68. The tested hypothesis was rejected because the range was far from the expected neutral pH of 7.0. In a previous study, Price et al., (2000) also reported that the pH of 26 commercially available tooth-whitening products ranged from 3.67 to 11.13. Exposure of oral soft and hard tissues to highly acidic or alkaline solutions may result in detrimental effects. However, the degree of damage depends on the contact time and the frequency of application of a bleaching agent as far as pH is concerned. The products evaluated in the present study differed greatly in treatment time and frequency of application; therefore, it is important to consider these factors when evaluating the possible side effects of a product.

The recommended application time for the various products ranged from 5 minutes per day to 8 hours per day. Most dentist-supervised home bleaching products and over-the-counter products are in contact with the teeth for an extensive period of time. The pH was nearly neutral for most dentist-supervised home bleaching products ranging from 6.43 to 6.81 except for one product (Nite White ACP) which had slightly lower pH of 4.88 (Table 4.1). The low pH of Nite White ACP might be due to the chemical reaction involved in the formation and precipitation of amorphous calcium phosphate.

However, an increase in pH has been reported during nightguard home bleaching with 10% carbamide peroxide (Leonard et al., 1994). It has been reported that 10% aqueous solution of carbamide peroxide (CH₆N₂O₃) decomposes into 3.35% solution of hydrogen peroxide (H₂O₂) and 6.65% of
urea (CH$_4$N$_2$O) (Zantner et al., 2007). Subsequently, urea breaks down into ammonia and carbon dioxide that increases the intraoral pH (Leonard et al., 1994; Rodrigues et al., 2005). Hydrogen peroxide further breaks down into water, oxygen and free radicals (Figures 2.4 and 2.5). Free radicals result in oxidation of the pigments in the tooth structure producing a whitening effect (Leonard, Sharma and Haywood, 1998; Smidt et al., 1998). Leonard et al., (1994) evaluated the pH levels of plaque, saliva and 10% carbamide peroxide in four subjects during 2 hours bleaching period and reported a significant increase in pH levels for the duration of the study. Furthermore, dentist-supervised home bleaching products are generally applied in custom-fitting trays that prevent the contact of bleaching product to the soft tissue and minimize the risk of injury. In contrast, over-the-counter products use prefabricated standard trays and there is greater risk of deglutition of the bleaching agent by the patients and soft tissue injury. In-office bleaching products are applied in the dental office and contain high concentrations of peroxide. Therefore, the manufacturers recommend the use of a protective barrier, either rubber dam or other resin based light-cured materials to protect oral soft tissues.

The pH of 5.2 to 5.8 has been reported as the critical pH for enamel (Barron et al., 2003). The pH of a solution below this level can cause demineralization of enamel (Barron et al., 2003; Driessens et al., 1986). The critical pH is the pH at which a solution is just saturated with respect to the mineral, for example, enamel. If the pH of the solution is above the critical pH, the solution is supersaturated with the mineral and more mineral will tend to precipitate out. On the other hand, if the pH of the solution is below the critical pH, the solution
is unsaturated, and the mineral will tend to dissolve until the solution becomes saturated.

Dental enamel is primarily composed of hydroxyapatite (HA), \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \), along with several other impurities such as carbonate and fluoride. A small amount of tooth (hydroxyapatite) can slowly dissolve in distilled water (about 30 mg/L) at neutral pH of 7, releasing calcium, phosphate and hydroxyl ions because water is unsaturated and contains no calcium or phosphate ions. The process will continue until water becomes saturated with tooth mineral. Similarly tooth can dissolve to some extent in any solution which does not contain calcium and phosphate ions. In contrast, saliva and plaque fluid, are normally supersaturated with calcium, phosphate and hydroxyl ions because the pH is higher than the critical pH. Therefore, teeth do not dissolve in saliva or under plaque until or unless the pH is reduced to less than the critical pH (Dawes, 2003).

In the present study, two over-the-counter whitening products namely Rapid White and Absolute White showed the lowest pH of 3.76 and 3.94 respectively (Table 4.3). Rapid White is a non-peroxide whitening product containing sodium chlorite and citric acid among other ingredients. A complete bleaching procedure requires the application of an accelerator onto the teeth followed by wearing of a prefabricated tray containing the bleaching gel for 5-10 minutes. On the other hand, Absolute White contains hydrogen peroxide and is a paint-on whitening gel applied directly onto the labial surfaces of teeth for 30 minutes per day for 14 days without the use of a tray. Previous studies also reported low pH values for over-the-counter whitening products containing sodium
chlorite. Zantner et al., (2007) reported a low pH of 3.7 for Odol-med3 Beauty-Kur (GlaxoSmithKline, PA, USA) while Price et al., (2000) reported a pH of 5.09 for Natural-White®, 5-Minute® non-peroxide tooth whitening system (Natural White Inc., NY, USA). Consequently these low pH products may cause enamel demineralization and soft tissue injury. In an in vitro study, Attin et al., (2005) reported a significant decrease in subsurface microhardness of enamel and dentine following application of Rapid White containing sodium chlorite for 10 minutes twice a day for 10 days when compared to the other products tested containing carbamide peroxide or hydrogen peroxide as active ingredient. Zantner et al., (2007) also reported a significant decrease in enamel microhardness and increased surface cracks along enamel prisms following treatment with a product (Odel-med3 Beauty-Kur) containing similar components as Rapid White. The pH of Coca Cola was 2.62, which is highly acidic and many patients consume soft drinks even during the bleaching treatments. The low pH of such products can further increase damage to the tooth surfaces. However, the buffering capacity\(^1\) of soft drinks is low (Grobler, Senekal and Laubscher, 1990). On the other hand no information is available about the buffering capacity of over-the-counter whitening products.

The solutions with low pH levels are known to soften enamel and produce dental erosion (Attin et al., 2003; Lussi et al., 2000; Lussi et al., 1995). In an in vitro study, Hunter et al., (2000) observed that increasing the frequency of exposure to a low pH drink resulted in a non-proportional increase in dental erosion. However, reducing the frequency of exposure to half did not result in

\(^1\) Buffering capacity is the ability of a solution to resist a change in pH (ability to maintain pH). The higher the buffering capacity of a solution, the higher is the erosive potential.
similar reduction in tissue loss. Although products such as Nite White ACP (pH 5.33) do not have a highly acidic pH, an application of 8 hours per day for 14 days, may be enough to cause damage. The mean pH of in-office bleaching products in the present study ranged from 5.30 to 7.85. In-office bleaching products are applied for shorter durations as compared to the dentist-supervised home bleaching products. For example, recommended time for Opalescence PF Quick 35% and 45% carbamide peroxide products is 30 minutes per application whereas 2 to 3 applications of 10 to 20 minutes are carried out for Opalescence Boost 38% hydrogen peroxide in one session. To determine the possible damage caused by in-office bleaching products at these pH levels and length of exposure requires further investigation. In-office bleaching treatments are generally repeated 3 to 7 days after the first application only if desired results have not been achieved. Furthermore, fluoride, amorphous calcium phosphate (ACP) and potassium nitrate have been introduced in recent bleaching products to prevent either demineralization or hypersensitivity or to remineralize damaged area (Chen et al., 2008).

The mean pH of whitening toothpastes investigated in this study ranged from 6.61 to 9.68 (Table 4.4). Plus White Xtra Cool Mint and Colgate Advanced Whitening toothpastes had basic pH levels of 8.69 and 9.68, respectively. It is expected that whitening toothpastes have neutral pH of 7.0 because they are used twice daily at least for 1-2 minutes as regular brushing toothpastes. The effects of repeated and prolonged brushing with such basic toothpastes require further investigation.
The pH levels of 21 commercially available tooth-whitening products were measured in this study. A wide range of pH levels was observed in different categories of whitening products which requires further investigation. Additionally, the pH of whitening products changes inside the oral cavity during the bleaching process. For example, it has been reported that breakdown of carbamide peroxide during bleaching process moves the oral environment to a more basic pH within 15 minutes (Leonard et al., 1994; Rodrigues et al., 2005). However, it is not known if the changes in pH occur at the same rate for products containing hydrogen peroxide or carbamide peroxide, or if the pH changes adversely affect oral soft and hard tissues (Price et al., 2000).

Though in this study every effort was made to carry out all measurements at standardized room temperature (23 ± 2°C), the pH of whitening products can be affected by the higher intraoral temperatures. For example, the use of external light sources during in-office bleaching increases the intraoral temperature and can influence the pH of bleaching product.
4.9- CONCLUSIONS

1. The pH of all tooth-whitening products ranged from 3.76 (highly acidic) to 9.68 (highly alkaline).

2. Most of the dentist-supervised home-bleaching products, in-office bleaching products and whitening toothpastes had near neutral pH and therefore can be considered safe for application as far as the pH is concerned.

3. Over-the-counter whitening products showed the lowest pH levels (3.76 to 6.30) as compared to the other categories. This is of concern as the general public does not have the knowledge to recognize the damaging effects of these products when used wrongly.

4. The low pH levels of over-the-counter whitening products suggest further investigations to evaluate peroxide concentration, effects on enamel microhardness and whitening efficacy.
REFERENCES


CHAPTER 5

CONCENTRATION OF HYDROGEN PEROXIDE IN VARIOUS TOOTH-WHITENING PRODUCTS

5.1- INTRODUCTION

Peroxides are widely used as industrial reagents due to their oxidising properties. Most common uses include the paper industry, domestic bleach, textiles, wine cork and food industry. Hydrogen peroxide is also used in disinfectant and cosmetic products. However, the concentration of peroxide in these products is low. Cosmetic products such as teeth whiteners, toothpastes and mouth washes are meant for oral application. Therefore, the concentration of peroxide in these products is critical (Ertas et al., 2000). Due to growing demand for whiter teeth, use of peroxide tooth whitening products has increased dramatically in recent years.

It has been reported that successful bleaching of teeth depends on a number of factors such as bleaching agent, type of stain and how long it has been present (Martin et al., 2007). However, efficacy of bleaching agent has been related to the peroxide concentration, frequency of application and time it stays in contact with the tooth surface (Leonard, Sharma and Haywood, 1998).

Bleaching products with high concentrations of peroxide are claimed to produce quicker whitening of teeth compared to the products with lower peroxide concentrations (Kihn et al., 2000). Studies evaluating the efficacy of different concentrations of carbamide peroxide found that although lower concentration of peroxide takes longer to whiten teeth, it eventually achieves the same result
as that achieved by higher concentrations (Matis et al., 2000; Leonard et al., 1998). An in vitro study, compared the efficacy of 5%, 10% and 16% carbamide peroxide applied for 8 hours per day for 2 weeks using a value oriented shade guide and demonstrated quicker two-tab colour improvement for 10% and 16% carbamide peroxide groups than 5% group. However, the colour improvement reached similar to the 2-week 10% and 16% values when the treatment in the 5% group was continued for a third week (Leonard et al., 1998).

A large number of whitening products are commercially available. These products have different compositions and concentrations and are made by different manufacturers. The retail price of a product and economic situation of a patient can be a determining factor in choosing a whitening product, without considering the quality guarantee of the product (Martin et al., 2007). Previous studies reported that even commercially produced whitening products had lower concentrations of active bleaching agents than those specified by the manufacturers (Al Shethri et al., 2003; Matis et al., 2003). Martin et al., (2007) compared the concentration of four bleaching agents prepared by dispensing pharmacies to that of a commercially available product containing 16% carbamide peroxide and reported that neither the bleaching agents dispensed by pharmacies nor the commercial product showed expected concentration of 16% carbamide peroxide.

However, no study was found which compared the real concentration of hydrogen peroxide in professional and over-the-counter tooth-whitening products to that specified by the manufacturers for a specific product containing hydrogen- or carbamide- peroxide. Furthermore, manufacturers do not provide
details about the concentration of hydrogen peroxide or carbamide peroxide in most over-the-counter tooth-whitening products.

5.2- AIM
The purpose of this in vitro study was to analyse the hydrogen peroxide concentration in various professional and over-the-counter tooth-whitening products, so as to check whether it corresponds to the respective concentration of the product given by the manufacture.

5.3- OBJECTIVES
1. To evaluated the hydrogen peroxide concentration of five dentist-supervised home bleaching products.

2. To evaluate the hydrogen peroxide concentration of four in-office bleaching products.

3. To evaluate the hydrogen peroxide concentration of three over-the-counter whitening products.

4. To evaluate the hydrogen peroxide concentration of four whitening toothpastes.

5.4- MATERIALS AND METHODS
Sixteen commercially available tooth-whitening products containing various concentrations of carbamide peroxide or hydrogen peroxide were investigated in the study. Samples of all products were measured in triplicate from three different batches.
The peroxide concentration was determined by oxy-reduction titration method. The process is based on the principal that the reaction of hydrogen peroxide with excess potassium iodide in the presence of an ammonium molybdate catalyst produces triiodide ions, which are then titrated with a standard thiosulphate solution (Solvay, 2004).

All reagents used in the study were of analytical grade and freshly prepared before starting the experiment. All chemical analyses were carried out at the Oral and Dental Research Institute, Faculty of Dentistry, University of the Western Cape, under the supervision of an experienced scientist.

5.4.1- Reagents used in the study

1. Potassium iodide 10% solution in deionised water.
2. Ammonium molybdate acid mixture: 0.18 g of ammonium molybdate \([(NH_4)_6 Mo_7O_{24} \cdot 4H_2O]\), 750 ml of water and 300 ml of concentrated sulphuric acid (H_2SO_4).
3. Potassium iodate solution (0.1N)
4. Starch solution (10 g/L)
5. Standardized sodium thiosulfate solution (Na_2S_2O_3 \cdot 5H_2O) (0.1N)

5.4.2- Procedure

A weighed sample of whitening product was dissolved in 200 ml of de-ionised water in a 500 ml Erlenmeyer flask using a Bibby HC502 magnetic stirrer (Bibby Sterilin Ltd, Staffordshire, UK). Potassium iodide solution and acid mixture were added to the solution and mixed well. The flask was covered with a stopper and left to stand for five minutes. The colour of the solution became
light yellow to dark brown depending on the peroxide concentration of the sample. The mixed sample solution was titrated with standardized sodium thiosulphate solution (0.1N) in a 50 ml burette, until a light straw colour was achieved. Then a few drops of starch solution were added and titration continued until the colour changed sharply from blue to colourless. The volume of sodium thiosulphate used for titration was recorded as “A”.

The titration was repeated without the addition of a whitening product (blank) and the volume of sodium thiosulphate used was recorded as “B”.

The hydrogen peroxide concentration was calculated according to the following formula (Solvay, 2004):

\[
\text{Hydrogen peroxide % w/w} = \frac{(A-B)(N)(1.7007)}{\text{Sample weight}}
\]

Where

- A = titration volume of sodium thiosulphate for sample.
- B = titration volume of sodium thiosulphate for blank.
- N = normality of sodium thiosulphate

5.5- STATISTICAL ANALYSIS

Simple descriptive statistics such as means, standard deviations, minimum and maximum were calculated. The hydrogen peroxide concentration of 3 to 3.35% in 10% carbamide peroxide was used as a standard for comparison purposes. The hydrogen peroxide concentration of various tooth-whitening products was represented graphically (Figure 5.1).
5.6- RESULTS

Details of products, active ingredients, expected hydrogen peroxide and mean hydrogen peroxide concentration as measured in this study for various tooth-whitening products are given in Tables 5.1 to 5.4. Figure 5.1 shows hydrogen peroxide determinations in bar graph for various products.

The hydrogen peroxide concentration in various dentist-supervised home bleaching products and in-office bleaching products ranged from 3.02% to 37.08% (Tables 5.1, 5.2 and Figure 5.1).

The hydrogen peroxide concentration in over-the-counter whitening products ranged from 1.24% for White Glo to 5.57% for Speed White (Table 5.3 and Figure 5.1).

Colgate Plax whitening rinse showed more than 1% hydrogen peroxide concentration while it was lower than 0.05% in other whitening toothpastes and oral rinses (Table 5.4 and Figure 5.1).

<table>
<thead>
<tr>
<th>Products</th>
<th>Active Ingredient</th>
<th>Expected $\text{H}_2\text{O}_2$</th>
<th>Mean $\text{H}_2\text{O}_2$ Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yotuel® Patient</td>
<td>10% CP</td>
<td>3 – 3.35</td>
<td>3.02</td>
</tr>
<tr>
<td>Opalescence® PF</td>
<td>10% CP</td>
<td>3 – 3.35</td>
<td>3.40</td>
</tr>
<tr>
<td>Nite White® ACP</td>
<td>10% CP</td>
<td>3 – 3.35</td>
<td>3.75</td>
</tr>
<tr>
<td>Opalescence® PF</td>
<td>20% CP</td>
<td>6 – 6.67</td>
<td>6.31</td>
</tr>
<tr>
<td>Opalescence® Trèswhite Supreme</td>
<td>10% HP</td>
<td>10</td>
<td>8.98</td>
</tr>
</tbody>
</table>

CP = carbamide peroxide, HP = hydrogen peroxide
Table 5.2: Mean peroxide concentration of In-office bleaching products

<table>
<thead>
<tr>
<th>Product</th>
<th>Active Ingredient</th>
<th>Expected H₂O₂%</th>
<th>Mean H₂O₂ Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yotuel® 10 Minutes</td>
<td>30% CP</td>
<td>9-10</td>
<td>9.93</td>
</tr>
<tr>
<td>Opalescence® PF Quick</td>
<td>45% CP</td>
<td>13.5-15</td>
<td>16.24</td>
</tr>
<tr>
<td>Yotuel® Special</td>
<td>35% HP</td>
<td>35</td>
<td>27.19</td>
</tr>
<tr>
<td>Opalescence® Boost</td>
<td>38% HP</td>
<td>38</td>
<td>37.08</td>
</tr>
</tbody>
</table>

CP = carbamide peroxide, HP = hydrogen peroxide

Table 5.3: Mean peroxide concentration of Over-the-counter bleaching products

<table>
<thead>
<tr>
<th>Products</th>
<th>Active Ingredient</th>
<th>% H₂O₂</th>
<th>Mean H₂O₂ Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Glo</td>
<td>CP</td>
<td>Not provided</td>
<td>1.24</td>
</tr>
<tr>
<td>Absolute White</td>
<td>HP</td>
<td>Not provided</td>
<td>3.20</td>
</tr>
<tr>
<td>Speed White</td>
<td>HP</td>
<td>Not provided</td>
<td>5.57</td>
</tr>
</tbody>
</table>

CP = carbamide peroxide, HP = hydrogen peroxide

Table 5.4: Mean peroxide concentration of Whitening toothpastes and rinses

<table>
<thead>
<tr>
<th>Products</th>
<th>Active Ingredient</th>
<th>Expected H₂O₂%</th>
<th>Mean H₂O₂ Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plus+White whitening pre-rinse</td>
<td>-</td>
<td>-</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Plus+White with peroxide</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pearl Drops Whitening Tooth Polish</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Colgate Plax whitening rinse</td>
<td>-</td>
<td>-</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Figure 5.1: Hydrogen peroxide concentration of whitening products in different categories
5.7- DISCUSSION

A large number of tooth-whitening products are available on the market. On one hand, within professional tooth-whitening products, one has a choice between dentist-supervised home bleaching products and in-office bleaching products or combinations of the two. On the other hand, numerous over-the-counter whitening products are available direct to the consumer for self-application. Furthermore, the concentrations of active ingredients and exposure times, as well as application methods also vary greatly. Cost of whitening product/treatment might be an important factor for a consumer when deciding tooth-whitening treatment without taking the quality into consideration.

The purpose of this study was to determine the concentration of hydrogen peroxide in various professional and over-the-counter tooth-whitening products, so as to check whether it corresponds to the respective concentration of the product given by the manufacture.

It has been reported that 10% carbamide peroxide dissociates into a hydrogen peroxide concentration of 3 to 3.5% (Zantner et al., 2007, Basting et al., 2003). Therefore carbamide peroxide based whitening products were expected to have the corresponding hydrogen peroxide concentrations. The results of the present study showed that the hydrogen peroxide concentrations of the professional tooth-whitening products were different from the expected concentrations. However, deviations were small and most of the products were close to expected range except for Yotuel Speical in-office bleaching product (Tables 5.1 and 5.2). Yotuel Special was expected to have 35% hydrogen
peroxide concentration when freshly prepared. However, the concentration found in the present study was only 27.19%.

Although active ingredients were labelled on over-the-counter whitening products tested in the present study, no concentrations were provided by the manufacturers. Hydrogen peroxide concentrations for these products ranged from 1.24% for White Glo to 5.57% for Speed White (Table 5.3). As for whitening toothpastes and oral rinses, only Colgate Plax whitening rinse showed peroxide concentration of 1.50% whilst it was almost nil in the remaining products.

There are no studies available in the literature which determined real hydrogen peroxide concentrations in professional and over-the-counter tooth-whitening agents. This fact makes it difficult to compare the results of the present study with data from the literature. However, the few studies that assessed the carbamide peroxide concentrations of professional tooth-whitening products showed that the concentrations of these products were lower than those expected (Matis et al., 2003; Al Shethri et al., 2003; Matis et al., 2000). In an in vitro study, Marin et al., (2007) evaluated the concentrations of 16% carbamide peroxide in products produced commercially or dispensed by pharmacies and showed that the concentrations were different from those expected in all products. However, the commercially produced bleaching product showed the best mean concentration close to the expected 16%. The findings are in agreement with the results of the present study, which also found that concentrations of bleaching products were marginally different from those expected.
In the present study hydrogen peroxide concentrations of three 10% carbamide peroxide products from different manufacturers were determined. The results revealed marginal differences among these products. Yotuel Patient showed the lowest hydrogen peroxide concentration of 3.02% and Nite White ACP showed the highest concentration of 3.75% whereas hydrogen peroxide concentration for Opalescence PF was 3.40%. This might be due to the differences in formulations, manufacturing and quality of the products.

The rate of bleaching is related to peroxide concentration and application time (Martin et al., 2007; Rodrigues et al., 2005) and lower concentrations require longer treatment times than higher peroxide concentrations. Matis et al., (2000) reported that a higher carbamide peroxide concentration of 15% resulted in a quicker and greater whitening than 10% carbamide peroxide during the early phase of treatment. However, the whitening effect showed some relapse after the cessation of active bleaching treatment and no significant differences were found after 6 weeks. The results of the present study revealed lower than expected peroxide concentrations for some professional tooth-whitening products such as Opalescence Trèswhite Supreme and Yotuel Special. Therefore, these products would probably require longer treatment times than those recommended by the manufacturers.

In spite of the results achieved further studies should be carried out to investigate the efficacy and side effects of these products on tooth structure, particularly enamel.
5.8- CONCLUSIONS

1. Professional (dentist-supervised home bleaching and in-office bleaching) products showed peroxide concentrations that differed marginally from the desired values.

2. The concentration of hydrogen peroxide in various over-the-counter tooth-whitening products ranged from 1.24% to 5.57%.

3. Hydrogen peroxide concentration in whitening toothpastes and rinses was negligible except Colgate Plax whitening rinse which had 1.50% hydrogen peroxide.
REFERENCES


CHAPTER 6

EFFECT OF VARIOUS PROFESSIONAL TOOTH-WHITENING PRODUCTS ON ENAMEL MICROHARDNESS

6.1- INTRODUCTION

Tooth-bleaching or tooth-whitening has become an increasingly popular dental procedure to lighten discoloured teeth. Over time, different bleaching techniques have been advocated, such as at-home bleaching, in-office bleaching and over-the-counter tooth-whitening products for self-application.

The active ingredients of bleaching products are mainly hydrogen peroxide or carbamide peroxide but may also be one of a few other products such as oxalic acid, chlorine and muriatic acid (Teixeira et al., 2004). Lower concentrations of peroxide are being used for at-home bleaching with a hydrogen peroxide concentration of up to 10% or a carbamide peroxide concentration of 10 to 22%. However, much higher peroxide concentrations (30 to 35%) are being used for in-office procedures (Cavalli, Giannini and Carvalho, 2004). Bleaching products with high concentrations of peroxide are claimed to ensure quicker whitening of teeth as compared to products with lower peroxide concentrations (Kihn et al., 2000). Currently a wide range of whitening products containing varying concentrations of CP and /or HP is available on the South African market.

It is stated that a 10% carbamide peroxide solution is still the most commonly used at-home bleaching concentration (Basting, Rodrigues and Serra, 2003; Kihn et al., 2000) because of its reported safety and effectiveness (Alonso de la
Pena and Balboa Cabrita, 2006; Basting et al., 2001; Cavalli et al., 2004; Haywood, 1992). Ten percent carbamide peroxide dissociates into a hydrogen peroxide concentration of 3 - 3.5% (Zantner et al., 2007; Alonso de la Pena and Balboa Cabrita, 2006).

Tooth-whitening involves direct contact of bleaching agent with the enamel surface for an extensive period of time which differs between manufacturers. These differences are a cause for increased concerns about the possible adverse effects of bleaching products on the enamel/dentine. The available literature is contradictory. Some scanning electron microscope studies reported changes in surface morphology of enamel following bleaching with carbamide peroxide (Pinto et al., 2004; Smidt, Weller and Roman, 1998; Ben-Ammar et al., 1995; Bitter and Sanders, 1993; Shannon et al., 1993) and/or hydrogen peroxide products (Pinto et al., 2004; Ernst, Morroquin and Willershausen-Zonnchen, 1996) while others reported no alterations in the enamel morphology (Zantner et al., 2007; Haywood, Houch and Heymann, 1991; Haywood et al., 1990). For example, Hegedüs et al., (1999) in an atomic force microscopy study, demonstrated that carbamide peroxide and hydrogen peroxide were capable of causing alterations in enamel surface.

In terms of microhardness tests, conflicting results have also been reported in the literature. Some studies reported that tooth-bleaching decreased enamel microhardness (Basting et al., 2003; Pinto et al., 2004; Rodrigues et al., 2005; Basting et al., 2005), while others reported no detrimental effects of bleaching on enamel (Ferreira et al., 2006; Ünlü et al., 2004). Lewinstein et al., (2004) reported that in-office bleaching products i.e. 35% HP and 35% CP, reduced
hardness of enamel and dentine significantly more than the home bleaching products with lower concentrations i.e. 10% CP, but stated that the application of 0.05% fluoride solution for five minutes completely restored the softened tooth structure. Faraoni-Romano et al., (2008) reported that bleaching of enamel with varying concentrations of CP and/or HP did not alter the microhardness and surface roughness of enamel.

The differences in results reported in the literature may be due to the differences in methodology. The available data demonstrated that experiments differed in the type of teeth (human/bovine or erupted/unerupted) and bleaching agent used, treatment times and storage solutions, in the position of hardness indents and the amount of forces applied as well as the type of diamond indenter used. For example, Attin et al., (2004) used bovine enamel which has been reported to have faster lesion progression than human enamel (Featherstone and Mellberg, 1981). Some studies used distilled water while others used artificial saliva as storage medium (remineralization solution). From the literature it becomes clear that different products, different concentrations as well as different bleaching agents will all influence the effect on enamel or dentine differently.
6.2- AIM

The purpose of this in vitro study was to evaluate the effect of various professional tooth-whitening products containing carbamide peroxide or hydrogen peroxide on enamel microhardness using a standardized protocol.

6.3- OBJECTIVES

1- To determine the effect of carbamide peroxide and hydrogen peroxide tooth-whitening products on enamel surface microhardness.

2- To determine the effect of whitening treatment times on enamel microhardness.

6.4- NULL HYPOTHESIS

There is no difference in the effect of tooth-whitening products containing carbamide peroxide or hydrogen peroxide on enamel microhardness.

6.5- MATERIALS AND METHODS

6.5.1- Specimen Preparation

Freshly extracted, non-caries human molar teeth were collected and stored in water with a few thymol crystals. The roots were removed approximately 2-3 millimetres apical to the cemento-enamel junction using a double-sided diamond saw in a slow-speed motor. Enamel blocks of 5x5 mm² were
sectioned longitudinally to the crowns. These enamel blocks were then examined under a stereomicroscope at 25x magnification and those with stains or cracks were discarded. One hundred of the selected enamel blocks were individually embedded in acrylic in PVC rings with a length of 1 cm (cut from a 25mm diameter electrical tubing) with the enamel surface exposed above the acrylic and 90º to the PVC ring. The exposed enamel surfaces of the specimens were polished with water cooled carbide paper up to 1200 grit fineness (3M, St. Paul, MN, USA) using a universal polisher (Metaserv, Betchworth, Surrey, UK). The specimens were randomly divided into ten treatment groups (1-10) with 10 specimens each.

For the treatment of enamel blocks, individual bleaching trays were fabricated (to simulate the in vivo bleaching procedure) for Groups 2 to 5 and 8 using the impressions of the enamel blocks. The manufacturers do not suggest the fabrication of trays for other materials. Models were poured in yellow stone and light-cured resin block-out material (Ultradent LC Block-out Resin, Ultradent Products Inc., South Jordan, UT, USA) was used to create a reservoir for bleaching materials, with the exception of Group 2, as suggested by the manufacturer. The trays were fabricated with a 0.035” thick, 5x5” soft tray material (Ultradent Products Inc., South Jordan, UT, USA) in a heat/vacuum tray-forming machine. The trays were trimmed to fit each specimen perfectly.
Treatments were performed as follows:

**Group 1 (Control) (n=10):** The enamel blocks were stored in the prepared artificial saliva (Table 6.1) at 37°C without any whitening treatment.

**Group 2 (Nite White® ACP 10% CP) (n=10):**

The bleaching trays were filled with a layer (approx. 1 mm thick) of this bleaching gel and applied to the enamel surfaces of the blocks for 8 hours/day for 14 days, as suggested by the manufacturer. During the treatment period specimens were kept in 100% relative humidity at 37°C. After each bleaching procedure, the bleaching gel was gently removed from the enamel surfaces using a paper towel and then thoroughly rinsed and stored in the artificial saliva at 37°C until the next treatment. The artificial saliva was replaced on a daily basis.

**Group 3 (Yotuel® Patient 10% CP) (n=10):**

The treatment procedure in this group was exactly as in the Group 2 except for the bleaching agent.

**Group 4 (Opalescence® PF 10% CP) (n=10):**

The treatment procedure in this group was exactly as in the Group 2 except for the bleaching agent.

**Group 5 (Opalescence® PF 20% CP) (n=10):**

The treatment procedure in this group was exactly as in the previous groups, except that 20% CP was used.
Group 6 (Opalescence® Trèswhite Supreme 10% HP) (n=10):

The treatment procedure in this group was also as for Group 2, except that 10% HP gel was used for 30 minutes/day for 14 days, as suggested by the manufacturer.

Group 7 (Yotuel® Patient 30% CP) (n=10):

The treatment procedure in this group was also as for Group 2, except that 30% CP gel was used for 10 minutes/day for 14 days without bleaching trays, as suggested by the manufacturer.

Group 8 (Opalescence® Quick PF 45% CP) (n=10):

The treatment procedure in this group was also as for Group 2, except that 45% CP gel was used for 30 minutes/day for 14 days, as suggested by the manufacturer.

Group 9 (Yotuel® Special 35% HP) (n=10):

The bleaching material was freshly prepared as suggested by the manufacturer and applied onto the polished enamel surfaces of all specimens in a 0.5 to 1.0 mm thick layer for 20 minutes. The material was agitated after 10 minutes with a brush soaked in activator. After 20 minutes the bleaching gel was removed gently from the enamel surfaces using a paper towel. The specimens were thoroughly rinsed with distilled water and air dried. The bleaching procedure was performed three times in one session. The specimens were stored in artificial saliva at 37°C until the next treatment. The artificial saliva was
replaced on a daily basis. After 7 days, the above bleaching procedure was repeated.

**Group 10 (Opalescence® Boost 38% HP) (n=10):**

The bleaching material was freshly mixed according to the manufacturer’s instructions and applied onto the polished enamel surfaces of all specimens in a 0.5 to 1.0 mm thick layer for 20 minutes. The material was agitated every 5 minutes. After 20 minutes, the bleaching gel was removed gently using a paper towel. The specimens were thoroughly rinsed with distilled water and air dried. The bleaching material was applied four times in one session. The specimens were stored in artificial saliva at 37°C until the next treatment. The artificial saliva was replaced on a daily basis. After 7 days, the above bleaching procedure was repeated.

**Table 6.1: Composition of artificial saliva***

<table>
<thead>
<tr>
<th>Composition</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>10.0</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>30.0</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.844</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.052</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.146</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.342</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*prepared in the laboratory (Cipla Medpro, Bellville, RSA)
Table 6.2: General information about the bleaching products according to the manufacturers.

<table>
<thead>
<tr>
<th>Products</th>
<th>Manufacturers</th>
<th>Groups</th>
<th>Composition</th>
<th>Type</th>
<th>Treatment Time</th>
<th>Treatment (Total hours)</th>
<th>Equivalent % H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nite White® ACP 10% CP</td>
<td>Discus Dental Culver City, CA, USA</td>
<td>2</td>
<td>Propylene glycol, glycerine, water, dicetyl phosphate, cetaryl alcohol, ceteth-10 phosphate, silica, carbamide peroxide, hydrogen peroxide, hydroxypropylecellulose, potassium nitrate, flavour, sodium phosphate, calcium nitrate, calcium carbonate, potassium hydroxide</td>
<td>At-home (Custom tray)</td>
<td>Carbamide peroxide 8 hrs/day</td>
<td>112</td>
<td>3.35</td>
</tr>
<tr>
<td>Yotuel® Patient 10% CP</td>
<td>Biocosmetics Laboratories, Madrid, Spain</td>
<td>3</td>
<td>Glycerin, urea peroxide, xylitol, potassium citrate, carbomer, aroma, potassium fluoride, sodium saccharin</td>
<td>At-home (Custom tray)</td>
<td>Carbamide peroxide 8 hrs/day</td>
<td>112</td>
<td>3.35</td>
</tr>
<tr>
<td>Opalescence® PF 10% CP</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>4</td>
<td>Carbamide peroxide, potassium nitrate, 0.11% ion fluoride, carbopol, glycerine, flavour.</td>
<td>At-home (Custom tray)</td>
<td>Carbamide peroxide 8 hrs/day</td>
<td>112</td>
<td>3.35</td>
</tr>
<tr>
<td>Opalescence® PF 20% CP</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>5</td>
<td>Carbamide peroxide, potassium nitrate, 0.11% ion fluoride, carbopol, glycerine, flavour.</td>
<td>At-home (Custom tray)</td>
<td>Carbamide peroxide 8 hrs/day</td>
<td>112</td>
<td>6.7</td>
</tr>
<tr>
<td>Opalescence® Tréswhite Supreme 10% HP</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>6</td>
<td>Carbamide peroxide, hydrogen peroxide, sodium fluoride, potassium nitrate, fillers, flavour</td>
<td>At-home (Prefabricated tray)</td>
<td>Hydrogen peroxide 30 min/day</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Yotuel® 10 Minutes 30% CP</td>
<td>Biocosmetic Laboratories, Madrid, Spain</td>
<td>7</td>
<td>Gel: Glycerine, aqua, urea peroxide, triethanolamine, xylitol, carbomer, aroma, potassium fluoride, diazolidinyl urea Activator: potassium fluoride, xylitol</td>
<td>In-office</td>
<td>Carbamide peroxide 10 min/day</td>
<td>2h 20min</td>
<td>11</td>
</tr>
<tr>
<td>Opalescence® PF Quick 45% CP</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>8</td>
<td>Carbamide peroxide, potassium nitrate, 0.11% ion fluoride, carbopol, glycerine.</td>
<td>In-office (Custom tray)</td>
<td>Carbamide peroxide 30 min/day</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Yotuel® Special 35% HP</td>
<td>Biocosmetics Laboratories, Madrid, Spain</td>
<td>9</td>
<td>Gel: Aqua, hydrogen peroxide, carbomer, triethanolamine, xylitol, sodium hydroxide, potassium fluoride, diazolidinyl urea Activator: potassium fluoride, xylitol</td>
<td>In-office</td>
<td>Hydrogen peroxide 60 min/session</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td>Opalescence® Boost 38% HP</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>10</td>
<td>Gel: Hydrogen peroxide Activator: potassium hydroxide, 1.1% fluoride and 3% potassium nitrate</td>
<td>In-office</td>
<td>Hydrogen peroxide 80 min/session</td>
<td>2h 40min</td>
<td>38</td>
</tr>
</tbody>
</table>
6.5.2- Microhardness Measurements

Surface microhardness of the enamel blocks were measured using a digital hardness tester (Zwick Roell Indentec, ZHV; Indentec UK) with a Vicker’s diamond indenter. The saliva soaked specimens were wiped gently with a tissue paper, rinsed with distilled water and tissue blot dried before each microhardness measurement. Before any treatment, 4 indentations were made (baseline hardness values) on the polished enamel surface of each enamel block (10 blocks, Figures 6.1 and 6.2) with a 300g load applied for 15 seconds. The indents were repeated after 14 days of active bleaching treatment close to the above mentioned baseline indents (about 10 µm away from where the base-line indent was made, Figure 6.1). All data were saved as Vickers hardness values (HV) for statistical analysis.

Figure 6.1: Schematic diagram showing the position of control (baseline) and post-bleaching indentations on polished enamel.
Vickers hardness is calculated according to the following formula:

\[ HV = 1.854 \times \frac{F}{d^2} \]

F = Applied load in kg
\( d \) = Arithmetic mean of the two diagonals, \( d_1 \) and \( d_2 \) in mm
HV = Vickers hardness

**Figure 6.2:** A, Digital Hardness Tester with Vickers diamond indenter. B, Specimen in place and hardness test in progress. C, The shape of indent indicating the two diagonal measurements (\( d_1 \) and \( d_2 \)) used for hardness calculation. D, Diagonal measurements in micron as seen on display. E, Vickers hardness value (HV)
6.5.3- Scanning Electron Microscope Observations

Three enamel blocks of each test group were also polished, as described above. One hardness indent was then made on each block and the position of the indent was marked. The blocks were now subjected to the full 14-day treatment as described above, thoroughly rinsed under tap water, blot dried and another indent made next to the previous one (10 µm away). This gave a treated as well as untreated indent. Scanning Electron Microscope images of the baseline and post-bleaching indents were taken to compare the demineralization effect on the indents.

6.6- STATISTICAL ANALYSIS

Data were captured in MS Excel spread sheet and analysed using the NCSS 2007 statistical software package (NCSS, Kaysville, UT, USA).

For each group baseline and post-bleaching microhardness values were compared using the Wilcoxon Signed Rank Sum Test significant at p<0.05. For multiple comparisons, differences in Vickers microhardness values (HV) were calculated between measurements at baseline and post-bleaching. Then data were analysed using the Kruskal-Wallis one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test for differences amongst the different groups (significance level was 5%). The findings of Scanning Electron Micrographs were elaborated.
6.7- RESULTS

General information about the composition, treatment period and hydrogen peroxide concentration of different bleaching products is given in Table 6.2, according to the manufacturers.

Comparison of the baseline and post-bleaching values showed that all whitening products tested in this study decreased enamel microhardness significantly (p<0.05, Table 6.3) except two in-office bleaching products (Opalescence Boost 38% HP and Yotuel Special 35% HP).

<table>
<thead>
<tr>
<th>Products</th>
<th>Groups</th>
<th>Baseline</th>
<th>Post-bleaching</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva Control</td>
<td>1</td>
<td>341.61 ± 20.68</td>
<td>342.11 ± 26.56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nite White ACP 10% CP</td>
<td>2</td>
<td>349.53 ± 19.67</td>
<td>192.45 ± 31.57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Yotuel Patient 10% CP</td>
<td>3</td>
<td>341.08 ± 20.21</td>
<td>284.85 ± 22.16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Opalescence PF 10% CP</td>
<td>4</td>
<td>337.65 ± 21.46</td>
<td>317.85 ± 17.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Opalescence PF 20% CP</td>
<td>5</td>
<td>333.13 ± 23.66</td>
<td>314.85 ± 27.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Opalescence Trèswhite Supreme 10% HP</td>
<td>6</td>
<td>321.73 ± 22.95</td>
<td>310.90 ± 22.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Yotuel 10 Minutes 30% CP</td>
<td>7</td>
<td>342.13 ± 18.34</td>
<td>300.53 ± 32.68</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Opalescence Quick 45% CP</td>
<td>8</td>
<td>351.68 ± 27.44</td>
<td>334.90 ± 19.16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Yotuel Special 35% HP</td>
<td>9</td>
<td>343.60 ± 17.59</td>
<td>336.55 ± 31.54</td>
<td>n.s.</td>
</tr>
<tr>
<td>Opalescence Boost 38% HP</td>
<td>10</td>
<td>331.83 ± 27.97</td>
<td>333.85 ± 31.16</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

p-values for significant differences are highlighted in (italic), non-significant differences are given (= n.s.).

CP = carbamide peroxide, HP = hydrogen peroxide

Groups 2, 3, 4, 5 & 7 (Figure 6.3 and Table 6.3) showed statistically significant decreases in enamel microhardness when compared to the control group (p<0.05). Groups 2, 3 & 7 also showed a statistically significant decrease in enamel microhardness compared to all the other groups (p<0.05). Group 2
(Nite White® ACP 10% CP) showed the highest damage and also differed significantly from Groups 3 & 7 ($p<0.05$).

Figure 6.3 depicts the Box-and-Whisker plots of the median Vickers microhardness differences between the base-line and the post-treatment hardness values for the control and treatment groups. In each diagram, the top line shows the maximum and the bottom line the minimum hardness values, while the box part shows the location of 50% of the values and the line in the box the median hardness value of the difference for the specific group.

![Box-and-Whisker plots of the Vickers microhardness differences for the control and treated groups. Groups that differ significantly from the control group (artificial saliva) are marked with an asterisk (*).](image)

**Figure 6.3:** Box-and-Whisker plots of the Vickers microhardness differences for the control and treated groups. Groups that differ significantly from the control group (artificial saliva) are marked with an asterisk (*).

(* = Nite white ACP, Yotuel Patient, Opalescence PF10, Opalescence PF 20 and Yotuel 10 Minutes)
Representative scanning electron microscope images of indent marks on enamel are illustrated in Figures 6.4 to 6.7. Figure 6.4 represents an indent mark on enamel which was not exposed to any bleaching treatment. Figures 6.5 to 6.7 represent indent marks exposed to Yotuel® Patient 10% CP for 112 hours, Opalescence® Trèswhite Supreme 10% HP for 7 hours, and Yotuel® Special 35% HP for 2 hours, respectively. Comparing these images, it can be clearly seen that the indent marks in Figures 6.5 and 6.6 faded because of demineralization of the enamel for 14 days when compared to the indent mark in Figure 6.4, which was not subjected to any bleaching process. Indent mark in Figure 6.7 showed relatively less damage. These images correspond to the results found in their hardness values (Figure 6.3).
**Figure 6.4:** Scanning Electron Micrograph of indentation on enamel that was not exposed to any bleaching treatment, x500 final magnification. Arrows indicating the smooth well defined indent mark.

**Figure 6.5:** Scanning Electron Micrograph of indentation on enamel that was exposed to Yotuel Patient 10% CP for 112 hours, x500 final magnification. Arrows indicating the damage caused by bleaching agent to the indent mark.

**Figure 6.6:** Scanning Electron Micrograph of indentation on enamel that was exposed to Opalescence Trèswhite Supreme for 7 hours, x500 final magnification. Arrow indicating the barely visible affected indent mark.

**Figure 6.7:** Scanning Electron Micrograph of indentation on enamel that was exposed to Yotuel Special 35% HP for 2 hours, x500 final magnification. Arrow indicating the well-defined relatively unaffected indent mark.
6.8- DISCUSSION

The null hypothesis that there is no difference in the effect of tooth-whitening products on enamel microhardness was rejected because the results of the present study showed significant differences between different groups. Tooth-bleaching agents may damage dental enamel even under prescribed conditions. The guidelines by the American Dental Association for any bleaching agent recommended that enamel hardness should be evaluated to ensure that no substantial changes in the morphology and/or properties of enamel would occur during tooth-bleaching treatment according to the product’s usage instructions (ADA, 2006). Following bleaching treatment, a decrease in surface hardness of enamel has been reported (Rodrigues et al., 2005; Pinto et al., 2004; Basting et al., 2003). Surface-softening lesions have been identified as the initial stage of caries lesion formation and dental erosion can occur easily in softened enamel (Ulukapi, 2007). Furthermore, it is now generally accepted that microhardness determinations give a reliable indication of the demineralization (damage) of enamel or dentine and are commonly used for this purpose (Joiner, 2007; Shannon et al., 1993; Koulourides and Volker, 1994; Feagin, Koulourides and Pigman, 1969).

One of the main reasons for the controversial results in microhardness studies might be due to the differences in the study design. Human enamel exhibits large regional variations in structure related to the differences in local chemistry (varying levels of mineralization, organic matter and water) and microstructure (fractions of inorganic crystals and organic matrix) (Braly et al., 2007; Spalding, Taveira and de Assis, 2003). Enamel microhardness may consequently vary
from area to area (Braly et al., 2007). From the results (Figure 6.3), it can be seen that the damaging effect on enamel could be relatively small as far as the decrease in the hardness value is concerned. Therefore, good planning as to the exact position of the indentations to be compared was necessary to keep the site variations of the hardness values small. The solution to this problem was to have the baseline indent and the test indent as close as possible to each other without the one interfering with the other (Figures 6.1 and 6.8). This is even more important when the hardness variation is very small and could consequently be easily masked when different areas on enamel were used.

Figure 6.8 demonstrates the scanning electron micrograph of polished enamel surface showing the position of pre-treatment (baseline) and post-treatment hardness indents.

![Figure 6.8: Scanning electron micrograph of pre- & post- treatment indents](image)

Black arrows indicate pre-treatment indents while white arrows indicate post-treatment hardness indents. Larger sizes of the post-treatment indents compared to the pre-treatment indents indicate softness of enamel or demineralization due to the bleaching treatment.
The control group (Figure 6.3), which consisted of enamel blocks stored in artificial saliva for the entire experimental period (14 days), showed almost no hardness change over 14 days (median difference between the start and the end was only 1.0). This finding showed that the artificial saliva solution did not alter the hardness of sound enamel either positively or negatively and could therefore be rightfully used as a soaking medium in the experiment.

The difference in the hardness had a negative value when the bleaching treatment resulted in a softer enamel surface (Figure 6.3). In general, it can be seen that all bleaching agent treatments (except Group 10) resulted in lower hardness values which indicated damage to enamel. In general, carbamide peroxide whitening products (Groups 2-5,7,8) showed more damage than hydrogen peroxide products (Groups 6,9,10) but the treatment periods also influenced the hardness values. It seemed that a combination of a short treatment period with a high peroxide concentration (Groups 6,8-10) gave rise to a lower degree of damage to enamel when compared to a combination of a low concentration treatment (Groups 2-5; 3.35% and 6.7%) with a long period (112 hours). Group 7 (Yotuel® 30% CP) is an exception with a relatively short treatment time period (140 minutes) and showed a significant decrease in enamel microhardness. In contrast to our study (8 hours/day), no change in enamel microhardness was found when 10% CP was used for a shorter treatment period of 2 or 3 hours per day for 14 days (Ferreira et al., 2006; Lopes et al., 2002). These results underline the negative effect of longer treatment periods.
However, differences amongst different groups with similar peroxide concentrations (10% CP, Groups 2, 3 and 4) and treatment times (112 hours) indicated that such negative effects may not only be related to the peroxide concentration and exposure time. Changes in enamel microhardness following tooth-bleaching may also depend on the composition of the product (Zantner et al., 2007). The composition of the bleaching products investigated in this study is given in Table 6.2. Groups 2 to 6 are at-home bleaching products and groups 7 to 10 are in-office bleaching products with high peroxide concentrations. Nite White® ACP (Group 2) 10% CP gel contained amorphous calcium phosphate (ACP), potassium nitrate but no fluoride, while Yotuel® 10% CP (Group 3), 30% CP (Group 7) and 35% HP (Group 9) gels contained potassium fluoride. Opalescence® 10% CP (Group 4), 20% CP (Group 5), 45% CP (Group 8), 10% HP (Group 6) and 38% HP (Group 10) gels contained fluoride and potassium nitrate. Thus, the absence of fluoride in Nite White ACP might have a negative influence on the results obtained for this product in the present study. Pinheiro Junior et al., (1996) also reported that Opalescence CP bleaching agent showed significantly less reduction in enamel microhardness than Nite White 10% CP.

Fluoride, potassium nitrate and amorphous calcium phosphate (ACP) have been introduced in recent bleaching products to prevent either hypersensitivity or demineralization effects (Chen et al., 2008). Fluoride is known to act as a remineralising agent by forming a calcium fluoride layer on enamel which inhibits demineralization or a decrease in microhardness values (Featherstone et al., 1982) whereas amorphous calcium phosphate (ACP) undergoes rapid hydrolysies to form apatite similar to the carbonated apatite of tooth mineral
(Tung and Eichmiller, 2004). However, in this study, a decrease in enamel microhardness was observed for the products containing fluoride, potassium nitrate or ACP. The findings are in agreement with other previous studies (Lewinstein et al., 2004; de Oliveira, Paes Leme and Giannini, 2005; da Costa and Mazur, 2007).

Although some studies have reported no significant changes in enamel microhardness after bleaching with Opalescence® 10% (Ferreira et al., 2006, Teixeira et al., 2004; Smidt et al., 1998; Murchison, Charlton and Moore, 1992), 20% CP (Mielczarek et al., 2008), Xtra Boost 38% HP (Polydorou, Hellwig and Hahn, 2008), Nite White® Excel 2Z 10% CP (Araujo et al., 2003), Yotuel® 10% CP (Akal et al., 2001), other studies have found a decrease in enamel microhardness after treatment with Opalescence® 10% (Attin et al., 2005; Basting et al., 2003; Basting et al., 2001; da Costa and Mazur, 2007), 20% (Basting et al., 2001) and 35% CP (Pinto et al., 2004), Xtra Boost 38% HP (Attin et al., 2005), and Nite White® ACP 10% CP (da Costa and Mazur, 2007; Pinheiro Júnior et al., 1996).

The damaging effect was confirmed by the scanning electron microscope images of the indents made with the hardness tester before and after the bleaching treatment process (Figures 6.4 to 6.7). Scanning electron microscope images were done for all the products but only a few images were shown because they are representative of different degrees of damage by the various bleaching products.
**6.9- CONCLUSIONS**

1. All products tested in this study decreased enamel microhardness except Opalescence Boost 38% HP.

2. The products containing carbamide peroxide were more damaging to enamel due to the longer application times of the bleaching agents.

3. Longer treatment periods influenced the enamel microhardness values negatively.

4. Nite White ACP 10% CP showed the highest reduction in enamel microhardness as compared to other products tested.

5. The presence of additives such as fluoride and/or amorphous calcium phosphate in the bleaching products did not prevent a decrease in enamel microhardness completely, but might have minimized the extent of damage to enamel.

**6.10- RECOMMENDATION**

From the results it seems that it may be safer to use whitening products with higher peroxide concentrations for shorter periods to prevent damage to enamel. During bleaching treatment additional use of remineralization solutions such as fluoridated toothpast or oral rinse should be encouraged to minimize damage to the enamel.

See Appendix 2 for publications.
REFERENCES


CHAPTER 7

EFFECT OF FOUR OVER-THE-COUNTER TOOTH- WHITENING PRODUCTS ON ENAMEL MICROHARDNESS

7.1- INTRODUCTION

Vital tooth bleaching has become increasingly popular in recent years. Two commonly used methods for vital tooth bleaching include nightguard vital bleaching introduced by Haywood and Heymann in 1989 and in-office bleaching (Kihn, 2007). In the late 1990s, manufacturers’ introduced a new range of bleaching products available to the public for self-application in the USA and later in Europe. These products are commonly known as over-the-counter bleaching products and include whitening strips or “trayless” whitening systems, paint-on-gels, gels with prefabricated trays and whitening toothpastes. Although most over-the-counter whitening products contain hydrogen peroxide or carbamide peroxide in some form as an active ingredient, sodium chlorite (NaClO₂) with an acid activator has also been used in over-the-counter products. The latter liberates chlorine dioxide (ClO₂) in the presence of acid which results in bleaching (Zantner et al., 2007).

During the whitening process, the bleaching agent is in direct contact with the enamel surface for an extensive period of time, which may have harmful effects. Although some previous studies investigating the effects of various professional whitening products containing carbamide peroxide and/or hydrogen peroxide on tooth structure reported contradictory results (Basting,
Rodrigues and Serra, 2003; Pinto et al., 2004; Rodrigues et al., 2005; Ferreira et al., 2006; Ünlü et al., 2004)**, the effects of over-the-counter products were not studied thoroughly before their introduction into the market (Zantner et al., 2007).

In terms of effects of over-the-counter products on enamel microhardness, the available literature is very limited. In an in vitro study, Zantner et al., (2007) investigated the effects of two home bleaching products containing carbamide peroxide and three over-the-counter products containing hydrogen peroxide or sodium chlorite on enamel microhardness following a 14-day treatment and after 6-week storage in artificial saliva. They reported a significant decrease in enamel microhardness for all three over-the-counter whitening products after a 14-day treatment as compared to the home bleaching products. The decrease in hardness remained significant for one over-the-counter product (Odel-med3 Beauty-Kur) containing sodium chlorite even after 6-week storage (remineralization) in artificial saliva. Another study reported significant decrease in subsurface enamel and dentine microhardness following bleaching with an over-the-counter product (Rapid White) containing sodium chlorite, while in the case of a hydrogen peroxide based over-the-counter product (Whitestrips) and other professional whitening products (Opalescence), the reduction was limited to enamel only (Attin et al., 2005).

Rodrigues et al., (2001) reported that both the composition of the bleaching product and its pH values can affect microhardness of enamel. It has been reported that with the use of acidic solutions there is more chance of alteration

**Studies described in chapter 6, page number 106-108
in enamel surface (Shannon et al., 1993). Therefore, low pH over-the-counter bleaching products should be seen as a high risk to cause damage to enamel and dentine in vivo.

Currently, over-the-counter whitening products are widely available to the public at pharmacies, supermarkets and over the internet (Demarco, Meireles and Masotti, 2009). The risk of harmful effects on soft and hard tissues is high because these products can be bought and used indiscriminately by patients.

7.2- AIM

The purpose of this in vitro study was to evaluate the effect of four over-the-counter tooth-whitening products containing hydrogen peroxide, carbamide peroxide or sodium chlorite on enamel microhardness.

7.3- OBJECTIVES

1. To evaluate the effect of three over-the-counter whitening products containing hydrogen peroxide or carbamide peroxide on enamel microhardness.

2. To evaluate the effect of over-the-counter whitening product containing sodium chlorite and citric acid on enamel microhardness.

7.4- NULL HYPOTHESIS

Over-the-counter tooth-whitening products do not reduce enamel microhardness when applied according to the manufacturers’ instructions.
7.5- MATERIALS AND METHODS

7.5.1- Specimen Preparation

Freshly extracted, non-carious human molar teeth were collected and stored in water with a few thymol crystals. The roots were removed approximately 2-3 millimetres apical to the cemento-enamel junction using a double-sided diamond saw in a low-speed motor. Enamel blocks of 5x5 mm² were sectioned longitudinally to the crowns. These enamel blocks were then examined under a stereomicroscope at 25x magnification and those with stains or cracks were discarded. Fifty of the selected enamel blocks were individually embedded in acrylic in PVC rings with a length of 1 cm (cut from a 25 mm diameter electrical tubing) with the enamel surface exposed above the acrylic and 90º to the PVC ring. The exposed enamel surfaces of the specimens were polished with water cooled carbide paper up to 1200 grit fineness (3M, St. Paul, MN, USA) using a universal polisher (Metaserv, Betchworth, Surrey, UK). The specimens were randomly divided into five treatment groups (1-5) with 10 specimens each.

7.5.2- Bleaching Procedure

Treatments were performed as follows:

Group 1 (Control) (n=10):

The enamel blocks were stored in the prepared artificial saliva (Table 7.1) at 37°C without any whitening treatment.
Table 7.1: Composition of artificial saliva*

<table>
<thead>
<tr>
<th>Composition</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>10.0</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>30.0</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.844</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.052</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.146</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.342</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*prepared in the laboratory (Cipla Medpro, Bellville, RSA)

Group 2, Rapid White (n=10):

Firstly, Rapid White accelerator was applied to the polished enamel surface. Thereafter the Rapid White whitening gel was applied in a layer (approx. 1 mm thickness) onto the enamel surfaces of the blocks for 10 minutes per day for 14 days, as suggested by the manufacturer. During the treatment period (bleaching) the specimens were kept in 100% relative humidity at 37 ºC. After each bleaching procedure, the bleaching gel was removed gently from the enamel surfaces using a paper towel and then thoroughly rinsed and stored in the artificial saliva at 37 ºC until the next treatment. The artificial saliva was replaced on a daily basis.

Group 3, Absolute White (n=10):

Absolute White paint-on-gel was applied to the enamel blocks using a brush for 30 minutes per day for 14 days, as suggested by the manufacturer. The rest of the procedure was exactly as for group 2.
**Group 4, Speed White (n=10):**

In this group, the Speed White gel was applied to the enamel blocks for 5 minutes per day for 14 days, as suggested by the manufacturer. The rest of the procedure was exactly as for group 2.

**Group 5, White Glo (n=10):**

In this group, the White Glo gel was applied to the enamel blocks for 20 minutes per day for 14 days, as suggested by the manufacturer. The rest of the procedure was exactly as for group 2.

<table>
<thead>
<tr>
<th>Table 7.2: General information about the bleaching products according to the manufacturers’</th>
<th>Products</th>
<th>Manufacturer</th>
<th>Group</th>
<th>Composition</th>
<th>Treatment Time</th>
<th>Active Ingredient</th>
<th>% H₂O₂*</th>
<th>pH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid White</td>
<td>Rapid White Products, Tonawanda, NY, USA</td>
<td>2</td>
<td>Accelerator: aqua, sodium chlorite Whitening gel: aqua, glycerine, carbomer 974P, polysorbate 20, citric acid, sodium hydroxide, aroma, methylparaben</td>
<td>10 min/day</td>
<td>Sodium chlorite</td>
<td>-</td>
<td>3.76</td>
<td></td>
</tr>
<tr>
<td>Absolute White</td>
<td>Dr. Fresh, Inc. La Mirada, CA, USA</td>
<td>3</td>
<td>Hydrogen peroxide, glycerin, SD alcohol 40-B, water, carbomer, PEG-8, triethanolamine, PEG-2M phosphoric acid, sodium phosphate, BHT</td>
<td>30 min/day</td>
<td>Hydrogen peroxide</td>
<td>3.20</td>
<td>3.94</td>
<td></td>
</tr>
<tr>
<td>Speed White</td>
<td>CCA Industries, Inc. E Rutherford, NJ, USA</td>
<td>4</td>
<td>Aqua, poloxamer 407, glycerine, hydrogen peroxide, methly salicylate, sodium saccharin, phosphoric acid</td>
<td>5 min/day</td>
<td>Hydrogen peroxide</td>
<td>5.57</td>
<td>4.65</td>
<td></td>
</tr>
<tr>
<td>White Glo</td>
<td>Barros Laboratories Pty Ltd. NSW, Australia</td>
<td>5</td>
<td>Propylene glycol, glycerine, carbamide peroxide, carbomer 940, triethanolamine, peppermint oil,</td>
<td>20 min/day</td>
<td>Carbamide peroxide</td>
<td>1.24</td>
<td>6.30</td>
<td></td>
</tr>
</tbody>
</table>

* pH and hydrogen peroxide concentration as measured in the laboratory (Chapter 4 & 5).
7.5.3- Microhardness Measurements

Surface microhardness of the enamel blocks was measured using a digital hardness tester with a Vickers diamond indenter. The saliva soaked specimens were wiped gently with a tissue paper, rinsed with distilled water and tissue blot dried before each microhardness measurement. Before any treatment, 4 indentations were made (baseline hardness values) on the polished enamel surface of each enamel block (10 blocks) with a 300g load applied for 15 seconds. The indents were repeated after 1, 7 and 14 days of active bleaching treatment close to the above mentioned baseline indents†† (about 10 µm away from where the baseline indent was made) (Majeed et al., 2008). All data were saved as Vickers hardness values (HV) for statistical analysis.

7.6 - STATISTICAL ANALYSIS

Data were captured in MS Excel spread sheet and analysed using the NCSS 2007 statistical software package (NCSS, Kaysville, UT, USA).

For each group baseline and post-bleaching microhardness values at day 1, 7 and 14 were compared using the Wilcoxon Signed Rank Sum Test significant at p<0.05. For multiple comparisons, differences in Vickers microhardness values were calculated between measurements at baseline and 1, 7 and 14-day post-treatment. The microhardness data were then analysed using the Kruskal-Wallis one-way ANOVA followed by the Tukey-Kramer Multiple Comparison Test for differences amongst the different groups (significance level was 5%).

†† See Figures 6.1, 6.2 and 6.8 in Chapter 6, page numbers 115, 116 and 123
7.7- RESULTS

Average baseline microhardness measurements were in the range of 313.03 ± 16.76 to 341.08 ± 15.19. After 14 days of treatment, the microhardness values were in the range of 275.98 ± 29.53 to 341.53 ± 18.04 for all groups (Table 7.3 and Figure 7.1).

Table 7.3: Means and standard deviations of enamel surface microhardness values at different time intervals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>1 Day</th>
<th>7 Days</th>
<th>14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva Control</td>
<td>341.08 ± 15.19</td>
<td>344.13 ± 17.64</td>
<td>356.25 ± 13.94</td>
<td>341.53 ± 18.04</td>
</tr>
<tr>
<td><strong>Rapid White</strong></td>
<td><strong>319.80 ± 41.91</strong></td>
<td><strong>304.68 ± 40.17</strong></td>
<td><strong>296.95 ± 36.04</strong></td>
<td><strong>275.98 ± 29.53</strong></td>
</tr>
<tr>
<td>Absolute White</td>
<td>313.03 ± 16.76</td>
<td>310.18 ± 21.98</td>
<td>300.45 ± 16.94</td>
<td>305.15 ± 18.84</td>
</tr>
<tr>
<td><strong>Speed White</strong></td>
<td><strong>319.33 ± 27.41</strong></td>
<td><strong>325.78 ± 18.99</strong></td>
<td><strong>332.03 ± 16.88</strong></td>
<td><strong>338.48 ± 20.65</strong></td>
</tr>
<tr>
<td>White Glo</td>
<td>325.50 ± 28.52</td>
<td>316.48 ± 25.96</td>
<td>334.80 ± 26.96</td>
<td>325.18 ± 30.01</td>
</tr>
</tbody>
</table>

*Rapid White group showed continuous decrease and Speed White group showed continuous increase in enamel microhardness over time.

**Figure 7.1**: Vickers microhardness values at baseline and after 14 days of treatment.
Figure 7.2 depicts the Box-and-Whisker plots of the median Vickers microhardness differences between the base-line and the post-treatment hardness values for the control and treatment groups. In each diagram, the top line shows the maximum and the bottom line the minimum hardness values, while the box part shows the location of 50% of the values and the line in the box the median hardness value of the difference for the specific group.

*Group 2 differed significantly from the control and treatment groups after 14 days of bleaching.
A one-way ANOVA showed significant differences amongst the groups (p<0.05). The post hoc Tukey-Kramer multiple comparison test showed that Rapid White differed significantly from the control, Absolute White and Speed White groups at 1 day of treatment (p<0.05). At 7 treatment days, Rapid White and Absolute White had significantly lower microhardness values than the control, White Glo and Speed White groups. At 14 treatment days, only the Rapid White group showed significantly lower hardness values than the control and other treatment groups (p<0.05). No statistically significant differences were found among the control, Absolute White and White Glo groups. The Speed White showed an increase in enamel microhardness after 14 days of treatment and was significantly different from the Rapid White and Absolute White groups (p<0.05).

Paired comparison between baseline and 1, 7 and 14 days was also carried out using the Wilcoxon Signed Rank Sum test to evaluate the effect of treatment within each group. Whitening treatment with Rapid White showed a significant reduction in enamel microhardness from baseline to 1, 7 and 14-day time periods (p<0.05). A statistically significant difference was observed for the Absolute White group at 7 treatment days only but not at 14 treatment days. The White Glo group showed a statistically significant reduction in enamel microhardness from baseline to 1 day treatment period. However no significant differences were found from baseline to 7 and 14-day treatment periods. No significant changes in enamel microhardness were observed for the control group at different time intervals.
7.8- DISCUSSION

Over-the-counter tooth-whitening products reduced (affected) enamel microhardness except Speed White where enamel microhardness showed some increase. The results of the present study indicate that at some point during the treatment period three over-the-counter whitening products (Rapid White, Absolute White and White Glo) reduced enamel microhardness (Table 7.3, Figures 7.1 and 7.2). Therefore the null hypothesis was rejected. However, only one product that significantly reduced enamel microhardness after 14 days of active treatment was Rapid White.

Microhardness determinations (also employed in this study) give a reliable indication of changes in the mineral content (demineralization) of enamel or dentine (Joiner, 2007; Shannon et al., 1993). Microhardness experiments have been used in the past to evaluate the effects of whitening products on tooth structure and restorative materials (Grobler et al., 2009; Leonard et al., 2005; Teixeira et al., 2004). The American Dental Association also recommends that enamel hardness should be evaluated to ensure that exposure to tooth-whitening products does not produce substantial changes in the structure and/or properties of enamel when applied according to the manufacturer’s instructions (ADA, 2006).

The difference in hardness had a negative value when bleaching resulted in a softer enamel surface (Figure 7.2). Treatments with Rapid White, Absolute White and White Glo resulted in lower hardness values which indicated damage to enamel. However, only the Rapid White group showed statistically significant reduction in enamel microhardness after 14 days of active bleaching.
treatment. The findings are in agreement with the results reported by Attin et al., (2005). In their study a significant decrease in subsurface microhardness of enamel and dentine was observed when Rapid White was applied for 10 minutes twice a day for 10 days as compared to the other products tested containing carbamide peroxide or hydrogen peroxide.

Rapid White is a non-peroxide whitening product containing sodium chlorite and citric acid among other ingredients (Table 7.2). Sodium chlorite (NaClO₂) liberates chlorine dioxide (ClO₂) in the presence of acid which results in bleaching. Zantner et al., (2007) reported a significant decrease in enamel microhardness and increased surface cracks along enamel prisms following treatment with a product (Odel-med3 Beauty-Kur) containing similar components as Rapid White (sodium chlorite and citric acid). Absolute White was the second product which resulted in relatively more reduction in enamel microhardness as compared to White Glo, Speed White and control groups. Absolute White is a hydrogen peroxide based paint-on whitening product.

In the present study every effort was made to simulate in vivo conditions. Bleaching procedures were carried out in a humid environment at 37°C and specimens were stored in artificial saliva until the next treatment. However, this may not simulate the intra-oral situation completely. Under clinical situations, small amounts of saliva might diffuse into the applied material and cause some dilution and buffering, especially in case of over-the-counter products with prefabricated trays which do not fit tightly. The situation might be worse in the case of paint-on gels such as Absolute White. In the laboratory, paint-on gel was applied to the dried teeth and stayed there for 30 minutes every day.
However, it did not take into consideration the mechanical removal of the material by lips, cheeks and tongue and dilution by saliva expected during intra-oral use.

Several studies evaluated the relationship between the concentration of hydrogen peroxide and the decrease in enamel microhardness (Zantner et al., 2007; Attin et al., 2005; Wandera et al., 1994). Zantner et al., (2007) reported significantly more softening of enamel for products containing 5.9% hydrogen peroxide than for products containing 2.7% and 3.3% hydrogen peroxide. In contrast, in the present study more damage was observed for Absolute White and White Glo containing 3.2% and 1.24% hydrogen peroxide respectively than Speed White containing 5.57% hydrogen peroxide where the latter showed increase in enamel microhardness over time. This indicated that the hydrogen peroxide is not solely responsible for the damage to enamel. One can argue that the increase in enamel microhardness in Speed White group may be due to the shorter treatment time (5 minutes per day) and relatively higher pH levels (4.65) compared to the Rapid White and Absolute White (Table 7.2).

Another factor could be the acidic pH of bleaching products. The results of pH determinations of whitening products in the previous chapter‡‡ demonstrated an acidic pH of 3.76 and 3.94 for Rapid White and Absolute White respectively. Obvious demineralization of enamel has been reported for pH lower than 5.2-5.8 (Barron et al., 2003; Driessens et al., 1986). Exposure to acidic whitening products can result in softening and/or even abrasion of enamel under in vivo conditions.

‡‡Chapter 4, page number 78 and 79.
Exposure time has also been reported to effect enamel microhardness. Grobler et al., (2009) reported that lower peroxide concentrations applied for longer treatment periods resulted in more damage to enamel than higher peroxide concentrations applied for shorter time periods. Although application times for over-the-counter products are shorter, low pH of these whitening products might result in irreversible damage to enamel. It was reported that exposure of enamel to low pH solutions even for seconds caused severe damage to enamel (Grobler, Senekal and Kotze, 1989).

Microhardness experiments require a flat polished surface in order to make indentations and to measure them. However, there is no standardized method of grinding and polishing the specimens. Polishing of the enamel surface removes the resilient hyper-mineralised layer, making the enamel more prone to the softening or demineralization effect of bleaching products (Zantner et al., 2007). Therefore, in the present study, enamel surface of specimens was polished very carefully and minimally to prevent the excessive loss of surface and subsurface enamel (Figure 7.3).

![Enamel surface sequentially polished up to 1200-grit](image)

**Figure 7.3:** Scanning electron micrograph of enamel block showing the polished area prepared for microhardness test (x100 magnification).

It is known that saliva plays an important role in the remineralization of enamel (Attin et al., 2000). Thus, artificial saliva containing calcium and phosphate was
used as a remineralization solution in the present study to simulate physiological conditions. In vitro studies have demonstrated artificial saliva as an effective agent in the re-hardening of softened enamel (Zantner et al., 2007; Meyer-Lueckel et al., 2004; Buskes et al., 1985). Therefore, it could be argued that changes in the microstructure of enamel might be reversed due to the remineralization potential of salivary components (de Freitas et al., 2002). However, remineralization studies of initial enamel lesions in artificial saliva demonstrated that the regaining of microhardness was dependent on the storage period in the remineralization solution (Meyer-Lueckel et al., 2004).

In the present study, reduction in enamel microhardness was seen in Rapid White, Absolute White and White Glo groups after 1 treatment day. The values obtained after 14 days of active bleaching treatment and storage in artificial saliva in between treatments showed almost full recovery in hardness values for the White Glo group and only a slight gain for the Absolute White group while the Rapid White group showed continuous softening of enamel until the end of whitening treatment (Table 7.3). Thus, it can be hypothesized that in spite of the remineralization potential of saliva, softening of enamel as a result of acid attacks as seen with the Rapid White bleaching product might be so serious that it cannot be repaired within a short time in clinical situations and will require long remineralization period. This makes enamel more susceptible to surface loss due to abrasive influences such as tooth-brushing (Attin et al., 1997; Davis and Winter, 1980).

The numbers of whitening products on the shelves are increasing day by day and the majority of over-the-counter products differ in their chemical
composition, preparation and application methods. The effect of different components such as carbomer, glycerine, and acids requires further investigations.

7.9- CONCLUSIONS

1. Over-the-counter tooth-whitening products may adversely affect enamel microhardness depending upon the type of product selected.

2. Rapid White, containing sodium chlorite in combination with citric acid as an activator, reduced enamel microhardness significantly possibly due to its low pH.

3. The Speed White group showed significant increase in enamel microhardness after 14 days of treatment.

4. Although saliva act as a buffering and remineralization solution, acidity and lack of control over the use of over-the-counter whitening products may result in excessive loss of enamel structure.

5. Repeated use of over-the-counter bleaching products should be discouraged.

7.10- RECOMMENDATION

Numerous over-the-counter products are available on the market. Dentists should caution patients about possible side effects these products might have on enamel, especially the product containing sodium chlorite due to its combination with citric acid and low pH.
REFERENCES


CHAPTER 8

IN VITRO SPECTROPHOTOMETRIC EVALUATION OF THE EFFICACY OF VARIOUS TOOTH-WHITENING PRODUCTS AND METHODS

8.1- INTRODUCTION

Vital tooth bleaching has become an integral part of aesthetic dentistry in recent years due to increased demand from patients for whiter and brighter teeth (Al Machot, Noack and Hoffmann, 2010). Tooth-whitening products are commonly divided into three categories known as dentist-supervised home bleaching products, in-office bleaching products and over-the-counter whitening products.

Hydrogen peroxide or its precursor carbamide peroxide are the most common active ingredients in tooth-whitening products which might also contain one of a few other products such as oxalic acid, chlorine and muriatic acid (Teixeira et al., 2004; Fasanaro, 1992). Beside the active ingredients, tooth-whitening products contain a few other components such as catalysts, stabilizers, desensitizing and flavouring agents (Dietschi, Benbachir and Krejci, 2010). Lower concentrations of carbamide peroxide and hydrogen peroxide are used for home bleaching whereas higher peroxide concentrations are used for in-office procedures (Cavalli et al., 2004). In-office bleaching products are either chemically activated or use external light sources such as halogen, plasma arc, ultraviolet light, laser, or a light emitting diode (LED) to activate and accelerate the degradation of the bleaching gel (Dietschi et al., 2010).
Attempts have been made to compare different products and methods to provide information regarding their efficacy and safety to dentists and the general public. Current literature demonstrates that 10% carbamide peroxide solution is still the most commonly used at-home bleaching concentration due to its reported safety and effectiveness (Alonso de la Pena and Cabrita, 2006; Ritter et al., 2002; Haywood, 1992). Other studies have demonstrated the effectiveness of in-office bleaching products alone (Matis et al., 2007; Al Shethri et al., 2003; Gallagher et al., 2002) or in combination with home bleaching products (Wetter et al., 2008; Deliperi et al., 2004). However, the information available about over-the-counter products is limited (Dietschi et al., 2010).

Although whitening products are marketed with excellent efficiency claims by manufacturers, there is no clear evidence that all available products and techniques are equally effective (Dietschi et al., 2010). The fact that diffusion of peroxide into dental tissue is related to the concentration and application time (Hanks et al., 1993) suggests that the whitening products with different concentrations and application methods will vary in efficiency.

In an in vitro study evaluating various at-home and over-the-counter bleaching products, Dietschi et al., (2010) reported that tray-based home bleaching products showed faster and better whitening of teeth than over-the-counter bleaching products and that the bleaching effect was dependent on the mode of application, concentration and application time. Other studies reported that higher peroxide concentrations might produce faster whitening with major changes in lightness and chroma. However, the final result with both high- and
low-peroxide concentration products was similar in the long run (Braun, Jepsen and Krause, 2007; Matis et al., 2000). Differences in efficacy have also been reported for various bleaching agents with different compositions and application methods (Kielbassa et al., 2009).

Bleaching studies utilise different methods to assess the effectiveness of tooth-whitening products such as comparison to shade guides, patients’ self-appreciation, pre- and post-bleaching photographs, computer-based instruments (spectrophotometers/colorimeters). Studies show variations in time intervals for the post-treatment colour evaluations (Kielbassa et al., 2009; Lee et al., 2007; Amaechi and Higham, 2002; Bentley et al., 1999). Colour change following bleaching treatment is most commonly determined by visual comparison of standard shade guides against teeth. However, colour determination using a shade guide is highly subjective and influenced by a number of factors such as lighting conditions, the human eye and brain (Al Machot et al., 2010) while computer-based instruments are more objective and reliable (Kielbassa et al., 2009). In addition, the lack of consensus about how the efficiency of tooth-whitening should be evaluated makes it difficult to compare the results of studies using different products and methods.

Over the past few years the tooth-whitening industry has grown tremendously and manufacturers are continuously launching new products in the market. Today dentists and patients are faced with a large selection of tooth-whitening products without any knowledge about their relative effects. Therefore, the purpose of this study was to measure the lightening potential of various tooth-whitening products and techniques under a controlled in vitro environment.
8.2- AIM

The purpose of this in vitro study was to evaluate the effects of 13 tooth-whitening products with different peroxide concentrations, active agents and application times on previously stained human incisor teeth, using a spectrophotometer.

8.3- OBJECTIVES

1. To evaluate the efficacy of dentist-supervised home bleaching products containing various concentrations of carbamide peroxide and/or hydrogen peroxide.

2. To evaluate the efficacy of in-office bleaching products.

3. To evaluate the efficacy of over-the-counter bleaching products.

4. To compare the effectiveness of different bleaching techniques.

8.4- NULL HYPOTHESES

1. There is no difference in the bleaching efficacy of various tooth-whitening products.

2. There is no difference amongst dentist-supervised home bleaching, in-office bleaching and over-the-counter bleaching techniques.
8.5- MATERIALS AND METHODS

8.5.1- Preparation of Specimens

Freshly extracted, non-carious, human maxillary central incisors were collected and stored in water with a few thymol crystals. All adherent tissue was removed from the teeth using a scalpel. Each tooth was cleaned with pumice paste using a slow hand-piece to remove any external stains. Teeth were then examined under a stereomicroscope at 25x magnification and those with defects such as fluorosis and hypoplasia were discarded. In total one hundred and thirty teeth were selected for the study.

8.5.2- Staining of Specimens

All teeth were stained in a staining broth described by the American Dental Association (2008). Staining broth was a mixture of coffee, tea, mucin powder, sterilized trypticase soya broth, FD&C Red and Yellow colour along with red wine and 24-hour culture of Micrococcus Luteus (Table 8.1). Teeth were kept in staining broth for two weeks at room temperature with light stirring. Following the staining procedure, each tooth was cleaned again with pumice paste for 5 seconds using a slow hand-piece and rinsed with distilled water. Teeth were stored at 4°C in distilled water with few crystals of thymol until further use.

<table>
<thead>
<tr>
<th>Table 8.1: Composition of staining broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Finely divided Instant Coffee</td>
</tr>
<tr>
<td>Finely Ground Instant Tea</td>
</tr>
<tr>
<td>Finely ground Gastric Mucin</td>
</tr>
<tr>
<td>Sterilized Trypticase Soy Broth</td>
</tr>
<tr>
<td>FD&amp;C Red 40*</td>
</tr>
<tr>
<td>FD&amp;C Yellow 5*</td>
</tr>
<tr>
<td>A 24-hour Micrococcus Luteus culture</td>
</tr>
<tr>
<td>Red Wine</td>
</tr>
</tbody>
</table>
8.5.3- Bleaching Procedure

Teeth were randomly divided into 13 groups (1-13) with 10 teeth in each group. Teeth were embedded in autopolymerising acrylic with the crowns exposed above the acrylic. The groups belonging to different bleaching techniques were as follows:

- Groups 1 to 5: Dentist-supervised home bleaching products
- Groups 6 to 9: In-office bleaching products
- Groups 10-13: Over-the-counter bleaching products

Bleaching trays were fabricated for Groups 1 to 4 and 7 to simulate the in vivo bleaching procedure, as suggested by the manufacturers’. Impressions were recorded of the embedded teeth using rubber based impression material (Lab-Putty, Coltène/Whaledent®, Switzerland). Models were poured in yellow stone and a light-cured resin block-out material (Ultradent LC Block-out Resin, Ultradent Products Inc., South Jordan, UT, USA) was used to create a reservoir on the labial side for bleaching material, with the exception of Group 2, as suggested by the manufacturer. The trays were fabricated with a 0.035” thick, 5x5” soft tray material (Ultradent Products Inc., South Jordan, UT, USA) in a heat/vacuum tray-forming machine. The trays were trimmed to fit specimens perfectly.

General information about the bleaching materials, active bleaching agents, application times and percentage of hydrogen peroxide is given in Table 8.2.
Treatments were performed as follows:

**Dentist-supervised Home Bleaching**

**Group 1 (Nite White® ACP 10% CP) (n=10):**

A small amount (gel drop) of this bleaching gel was placed in the centre of each tooth compartment in the bleaching tray and applied to the teeth for 8 hours per day for 14 days, as suggested by the manufacturer. During the treatment period specimens were kept in 100% relative humidity at 37ºC. After each bleaching procedure, the bleaching tray was removed and teeth were wiped gently using a paper towel. Teeth were then thoroughly rinsed with distilled water and stored in the artificial saliva at 37ºC until the next treatment. The artificial saliva was replaced on a daily basis. The bleaching tray was also cleaned and stored in a cool place.

**Group 2 (Yotuel® Patient 10% CP) (n=10):**

The treatment procedure in this group was exactly as in Group 1 except for the bleaching agent.

**Group 3 (Opalescence® PF 10% CP) (n=10):**

The treatment procedure in this group was exactly as in Group 1 except for the bleaching agent.

**Group 4 (Opalescence® PF 20% CP) (n=10):**

The treatment procedure in this group was exactly as in the previous groups, except that 20% carbamide peroxide gel was used.
Group 5 (Opalescence® Trèswhite Supreme 10% HP) (n=10):

The bleaching material was applied to the teeth using the prefabricated trays for 30 minutes per day for 14 days. The rest of the procedure was the same as in the previous groups except that the prefabricated bleaching tray was discarded after single use.

In-office Bleaching

Group 6 (Yotuel® 10 Minutes 30% CP) (n=10):

The bleaching material was dispensed onto a glass slab. Eight to ten drops of activator were added to the gel and mixed thoroughly with a plastic spatula, according to the manufacturer’s instructions. The activated gel was applied to the labial surfaces of teeth in a thin layer (approx. 1mm). After 10 minutes, the gel was removed and teeth were thoroughly cleaned with distilled water for 2 minutes. Teeth were air dried and the above procedure was repeated again. Following two applications of 10 minutes, teeth were cleaned and stored in artificial saliva at 37°C until the next treatment. In total 4 bleaching sessions (2x10 minutes per session) were performed at 3 day intervals over a period of two weeks.

Group 7 (Opalescence® Quick PF 45% CP) (n=10):

A small amount of this bleaching gel was placed in the centre of each tooth compartment in the bleaching tray and applied to the teeth for 30 minutes per session, as suggested by the manufacturer. During the treatment period specimens were kept in 100% relative humidity at 37°C. After each bleaching procedure, the bleaching tray was removed and teeth were wiped gently using
a paper towel. Teeth were then thoroughly rinsed with distilled water and stored in the artificial saliva at 37ºC until the next treatment. The artificial saliva was replaced on a daily basis. The bleaching tray was also cleaned and stored in a cool place. In total 4 bleaching sessions (30 minutes per session) were performed at 3 day intervals over a period of two weeks.

**Group 8 (Yotuel® Special 35% HP) (n=10):**

Yotuel Special vials containing powder and liquid in separate compartments were used in the present study. The powder and liquid were mixed by shaking the bottle for 30 seconds until a homogenous solution was obtained. Fifteen drops of activator solution were then added to the solution and mixed well using a plastic spatula until a gel like consistency was obtained. The whole mixing procedure was performed according to the manufacturer’s instructions.

The freshly prepared bleaching material was applied onto the labial surfaces of the teeth in a 0.5 to 1.0 mm thick layer for 20 minutes. The material was agitated after 10 minutes with a brush soaked in activator solution. After 20 minutes the bleaching gel was removed gently from the enamel surfaces using a paper towel. The specimens were thoroughly rinsed with distilled water and air dried. Fifteen drops of activator solution were added to the remaining bleaching material again and mixed thoroughly to reactivate the gel. The above bleaching procedure was repeated again. In total three bleaching treatments (3x20 minutes) were performed during one session. Following the bleaching treatment, the teeth were cleaned and stored in artificial saliva at 37ºC until the next treatment. The artificial saliva was replaced on a daily basis. After 7 days, the above bleaching procedure was repeated.
Group 9 (Opalescence® Boost 38% HP) (n=10):

Opalescence Boost is supplied in two syringes and utilizes syringe-to-syringe jet mixing. The red syringe contains concentrated hydrogen peroxide and the clear syringe contains chemical activator with 1.1% fluoride and 3% potassium nitrate. Both syringes were attached to each other and the chemical was pressed from the red syringe to the clear syringe. Then the clear syringe plunger was pressed forcefully into the middle clear barrel to rupture the internal plunger membrane. This allowed mixing of the activator and bleaching agent. The activator and the hydrogen peroxide were thoroughly mixed by rapidly pressing the plungers 12-13 times in each direction. Then the mixed chemical was pressed into the red syringe. The clear syringe was removed and the Micro 20g FX tip (Ultradent Products Inc., South Jordan, UT, USA) was twisted onto the red syringe.

The freshly mixed bleaching gel was applied onto the labial surfaces of teeth in a 0.5 to 1.0 mm thick layer for 20 minutes, according to the manufacturer’s instructions. The material was agitated every 5 minutes. After 20 minutes, the bleaching gel was removed gently using a paper towel. The teeth were thoroughly rinsed with distilled water and air dried. The above bleaching procedure was repeated again. In total the bleaching treatment was performed four times in one session (4x20 minutes). Following the bleaching treatment, teeth were cleaned and stored in artificial saliva at 37°C until the next treatment. The artificial saliva was replaced on a daily basis. After 7 days, the above bleaching procedure was repeated again.
Over-the-counter Bleaching

**Group 10, Rapid White (n=10):**

Firstly, Rapid white accelerator was applied to the labial surfaces of the teeth. Secondly, the Rapid White whitening gel was applied in a layer of 1 mm for 10 minutes per day for 14 days, as suggested by the manufacturer. After each bleaching procedure, the bleaching gel was removed from the teeth using a paper towel. Teeth were then thoroughly rinsed with distilled water and stored in the artificial saliva until the next treatment. The artificial saliva was replaced on a daily basis.

**Group 11, Absolute White (n=10):**

Absolute White paint-on-gel was applied to the teeth using a brush supplied with the material for 30 minutes per day for 14 days, as suggested by the manufacturer. The rest of the procedure was exactly as for the previous group.

**Group 12, Speed White (n=10):**

In this group, the Speed White gel was applied to the teeth for 5 minutes per day for 14 days, as suggested by the manufacturer. The rest of the procedure was exactly as for group 10.

**Group 13, White Glo (n=10):**

In this group, the White Glo gel was applied to the teeth for 20 minutes per day for 14 days, as suggested by the manufacturer. The rest of the procedure was exactly as for group 10.
Table 8.2: General information about the bleaching products, application times, active ingredient and concentration of hydrogen peroxide

<table>
<thead>
<tr>
<th>Products</th>
<th>Codes</th>
<th>Manufacturers</th>
<th>Groups</th>
<th>Application Type</th>
<th>Treatment Time</th>
<th>Active Bleaching Agent</th>
<th>CABA‡ (% H₂O₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nite White® ACP</td>
<td>NW10</td>
<td>Discus Dental, Culver City, CA, USA</td>
<td>1</td>
<td>At-home bleaching</td>
<td>14 x 8 hrs.</td>
<td>Carbamide peroxide</td>
<td>10 (3.35)</td>
</tr>
<tr>
<td>Yotuel® Patient</td>
<td>YP10</td>
<td>Biocosmetic Laboratories, Madrid, Spain</td>
<td>2</td>
<td>At-home bleaching</td>
<td>14 x 8 hrs.</td>
<td>Carbamide peroxide</td>
<td>10 (3.35)</td>
</tr>
<tr>
<td>Opalescence® PF</td>
<td>OP10</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>3</td>
<td>At-home bleaching</td>
<td>14 x 8 hrs.</td>
<td>Carbamide peroxide</td>
<td>10 (3.35)</td>
</tr>
<tr>
<td>Opalescence® 20% PF</td>
<td>OP20</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>4</td>
<td>At-home bleaching</td>
<td>14 x 8 hrs.</td>
<td>Carbamide peroxide</td>
<td>20 (6.7)</td>
</tr>
<tr>
<td>Opalescence® Trèswhite Supreme</td>
<td>TresW</td>
<td>Ultradent Products Inc., South Jordan, UT, USA</td>
<td>5</td>
<td>At-home bleaching</td>
<td>14 x 30 min</td>
<td>Hydrogen peroxide</td>
<td>10 (10)</td>
</tr>
</tbody>
</table>

Yotuel® 10 Minutes  Y10Min Biocosmetic Laboratories, Madrid, Spain 6  In-office  4 x 20 min  Carbamide peroxide  30 (11)

Opalescence® PF Quick OP45 Ultradent Products Inc., South Jordan, UT, USA 7  In-office  4 x 30 min  Carbamide peroxide  45 (15)

Yotuel® Special YS Biocosmetic Laboratories, Madrid, Spain 8  In-office  2 x 60 min  Hydrogen peroxide  35 (35)

Opalescence® Boost OB Ultradent Products Inc., South Jordan, UT, USA 9  In-office  2 x 80 min  Hydrogen peroxide  38 (38)

Rapid White RW Rapid White Products, Tonawanda, NY, USA 10  OTC (prefabricated tray)  14 x 10 min  Sodium chlorite  -

Absolute White AW Dr. Fresh, Inc. La Mirada, CA, USA 11  OTC (paint-on)  14 x 30 min  Hydrogen peroxide  -(3.20)

White Glo WG Barros Laboratories Pty Ltd. NSW, Australia 12  OTC (prefabricated tray)  14 x 20 min  Carbamide peroxide  -(1.24)

Speed White SW CCA Industries Inc., E Rutherford, NJ, USA 13  OTC (prefabricated tray)  14 x 5 min  Hydrogen peroxide  -(5.57)

‡ Concentration of active bleaching agent.
8.5.4- Measurement of Tooth Colour

Tooth colour measurements were performed using a spectrophotometer (CM-2600d, Konica Minolta Sensing, Inc., Japan; Figure 8.1) with a 6 mm diameter probe. The spectrophotometer (Konica Minolta Sensing, Inc., Japan) measured the colour of teeth based on the CIE L* a* b* colour space system defined by the Commission Internationale de l’Eclairage (CIE 1976). In this colour space (Figure 8.2) lightness is represented by L* on a scale of 0 for black to 100 for white. The hue and chroma are represented on a* versus b* plot where a* represents the red/green coordinate and b* represents the yellow/blue coordinate where +a* indicates red and -a* indicates green similarly +b* indicates yellow and -b* indicates blue (Burkinshaw, 2004).

A positioning jig was fabricated for each group using a light-cured acrylic material (Megatray, Megadenta Dentalprodukte, GmbH, Germany), in order to measure the colour of the same area of tooth at each time interval. Before use, the spectrophotometer was calibrated using a white standard calibration disk provided by the manufacturer. The colour of an area (6 mm) at the centre of the crown of each tooth was measured three times with the spectrophotometer. The average of three measurements was considered as the measured value. The measurements were performed after the staining prior to bleaching (baseline) and 24 hours after the completion of active bleaching treatment (post-treatment).
8.6- STATISTICAL ANALYSIS

All data were saved as CIE L*, a* and b* values and analysed using the NCSS 2007 statistical software package (NCSS, Kaysville, UT, USA).

Baseline (pre-bleaching) spectrophotometric measurements (L*, a* and b*) of 13 groups were compared using the Kruskal-Wallis one-way ANOVA for any
significant differences between groups (significance level was 5%). Chroma
(C*) was also calculated for all groups from baseline (pre-bleaching) a* and b*
values using the formula $C^* = (a^{*2} + b^{*2})^{1/2}$.

For each group L*, a* and b* values at baseline and 24 hours after-treatment
were compared using the Wilcoxon Signed Rank Sum Test significant at
$p<0.05$.

For the multiple comparison of the efficacy of different products in each
category, differences in individual colour components ($\Delta L^*$, $\Delta a^*$ and $\Delta b^*$) and
total colour difference ($\Delta E_{ab}^*$) from baseline were calculated. Whitening effect
or colour improvement was represented by a positive $\Delta L^*$ (increased
brightness), negative $\Delta a^*$ (reduction in redness) and negative $\Delta b^*$ (reduction in
yellowness). The total colour changes due to treatment were calculated using
the formula:

$$\Delta E_{ab}^* = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2}.$$

Then the data were analysed using the Kruskal-Wallis one-way ANOVA
followed by the Tukey-Kramer Multiple Comparison Test for differences
amongst the different groups (significance level was 5%).

8.7- RESULTS

The analysis of base-line tooth colour values ($L^*a^*b^*$) of all groups showed a
similar colour distribution with no statistically significant differences ($p>0.05$).
The distribution of baseline tooth colour for all groups was also surveyed graphically by plotting $L^*$ versus $C^*$ (chroma) values (Figure 8.3).

For all groups, $L^*$, $a^*$ and $b^*$ values (median and interquartile range) before and after bleaching treatments are given in Tables 8.3, 8.4 and 8.5. Figures 8.4, 8.5 and 8.6 depict Box-and-Whisker plots of median $\Delta E_{ab}^*$ values for Groups 1 to 5 (dentist-supervised home bleaching products); Groups 6 to 9 (in-office bleaching products) and Groups 10 to 13 (over-the-counter bleaching products) respectively, after the completion of active bleaching treatment. In each diagram, the top line shows the maximum and the bottom line the minimum $\Delta E_{ab}^*$ values, while the box part shows the location of 50% of the values and the line in the box the median $\Delta E_{ab}^*$ value for the specific group.

Figure 8.3: Distribution map showing chroma $C^*$ versus $L^*$ values of teeth before treatment for all groups.
8.7.1: Dentist-supervised Home Bleaching (Groups 1 to 5)

All bleaching treatments produced significant changes in $L^*$, $a^*$ and $b^*$ values when compared to the baseline values (Wilcoxon Signed Rank Sum Test; $p<0.05$). Teeth in all groups showed an increase in $L^*$ values (lighter/brighter) from 60.02 to 70.44, a decrease in $a^*$ values (less reddish) from 0.54 to -1.10 and a decrease in $b^*$ values (less yellowish) from 8.04 to 3.49 (Table 8.3).

<table>
<thead>
<tr>
<th>Product</th>
<th>Baseline</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$L^*$</td>
<td>$a^*$</td>
</tr>
<tr>
<td>NW10</td>
<td>60.59</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>(4.70)</td>
<td>(1.04)</td>
</tr>
<tr>
<td>YP10</td>
<td>63.75</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>(4.64)</td>
<td>(1.57)</td>
</tr>
<tr>
<td>OP10</td>
<td>60.02</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>(4.20)</td>
<td>(1.03)</td>
</tr>
<tr>
<td>OP20</td>
<td>61.59</td>
<td>-0.29</td>
</tr>
<tr>
<td></td>
<td>(3.16)</td>
<td>(1.75)</td>
</tr>
<tr>
<td>TS10</td>
<td>60.72</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>(4.00)</td>
<td>(0.84)</td>
</tr>
</tbody>
</table>

See table 8.2 for product code explanation.

All products showed varying degrees of total colour change ($\Delta E_{ab}$) after 14 days of active bleaching treatment (Figure 8.4). Opalescence PF 20% carbamide peroxide (OP20, Group 4) produced significantly better bleaching (whitening) effect ($\Delta E_{ab}$ 9.68) as compared to all other products in this category ($p<0.05$). Trèswhite Supreme (TS10, Group 5) produced the least whitening effect ($\Delta E_{ab}$ 4.11) and differed significantly from all other groups ($p<0.05$). No significant differences were found between Nite White ACP (NW10, $\Delta E_{ab}$ 6.19), Yotuel Patient (YP10, $\Delta E_{ab}$ 6.48) and Opalescence PF 10 (OP10, $\Delta E_{ab}$ 6.77).
Products ranked according to the median $\Delta E_{ab}^*$ values in descending order:

Opalescence PF 20 > Opalescence PF 10 > Yotuel Patient > Nite White ACP > Trèswhite Supreme

Highest--------------------------Lowest

Figure 8.4: Box-and-Whisker plots of $\Delta E_{ab}^*$ values for different dentist-supervised home bleaching products. Group 4 (OP20) showed a significantly higher and Group 5 (TresW) showed a significantly lower bleaching effect (whitening) as compared to all other groups and are marked with (*).

8.7.2: In-office Bleaching (Groups 6 to 9)

All bleaching products produced significant changes in $L^*$, and $b^*$ values when compared to the baseline while $a^*$ values changed significantly only in groups 8 and 9 (Wilcoxon Signed Rank Sum Test; $p<0.05$). The $L^*$ values showed an
increase (lighter/brighter) from 60.47 to 67.42; and b* values showed a decrease (less yellowish) from 6.11 to 3.53 (Table 8.4).

<table>
<thead>
<tr>
<th>Product</th>
<th>Baseline</th>
<th>Post-treatment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>Y10Min</td>
<td>61.24 (4.04)</td>
<td>0.37 (1.59)</td>
<td>7.08 (5.08)</td>
</tr>
<tr>
<td>OP45</td>
<td>63.12 (7.67)</td>
<td>-0.32 (0.79)</td>
<td>6.11 (4.52)</td>
</tr>
<tr>
<td>YS35</td>
<td>60.47 (3.82)</td>
<td>0.40 (1.67)</td>
<td>8.04 (4.12)</td>
</tr>
<tr>
<td>OB38</td>
<td>63.13 (4.08)</td>
<td>0.16 (0.44)</td>
<td>6.73 (3.33)</td>
</tr>
</tbody>
</table>

See table 8.2 for product code explanation.

The total colour change ($\Delta E_{ab}^*$) varied between groups according to the product used (Figure 8.5). Opalescence Boost (OB) showed more pronounced bleaching effect ($\Delta E_{ab}^*$ 5.24) than Opalescence Quick (OP45, $\Delta E_{ab}^*$ 3.48), Yotuel Special (YS35, $\Delta E_{ab}^*$ 3.46) and Yotuel 10 Minutes (Y10Min, $\Delta E_{ab}^*$ 2.44) which also differed significantly from Opalescence Boost ($p<0.05$). No significant differences were found among other groups.

Products ranked according to the median $\Delta E_{ab}^*$ values:

Opalescence Boost > Opalescence PF 45 > Yotuel Special > Yotuel 10 Minutes

Highest--------------------------------------------------------------- Lowest
Only Group 6 (Y10Min) showed a significantly lower bleaching effect as compared to Group 9 (OB) and is marked with (*).

8.7.3: Over-the-counter Bleaching (Groups 10 to 13)

All bleaching treatments produced significant changes in L* and b* values when compared to the baseline while no significant changes were seen in a* values (Wilcoxon Signed Rank Sum Test; p<0.05). Teeth in all groups showed an increase in L* values (lighter, brighter) from 60.52 to 63.55 and a decrease in b* values (less yellowish) from 7.24 to 3.61 (Table 8.5).

Table 8.5: Median L*a*b* values and interquartile ranges (IQR) before and after bleaching for over-the-counter bleaching products

<table>
<thead>
<tr>
<th>Product</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RW</td>
<td>61.21 (6.38)</td>
<td>-0.06 (1.65)</td>
<td>7.24 (4.15)</td>
<td>63.17 (6.29)</td>
<td>-0.06 (1.80)</td>
<td>5.05 (3.26)</td>
</tr>
<tr>
<td>AW</td>
<td>61.09 (6.57)</td>
<td>0.13 (1.01)</td>
<td>6.75 (3.17)</td>
<td>62.51 (7.25)</td>
<td>0.24 (0.72)</td>
<td>3.78 (4.21)</td>
</tr>
<tr>
<td>WG</td>
<td>60.52 (3.57)</td>
<td>-0.25 (2.17)</td>
<td>6.48 (3.74)</td>
<td>62.39 (4.64)</td>
<td>-0.31 (1.86)</td>
<td>5.70 (2.80)</td>
</tr>
<tr>
<td>SW</td>
<td>62.60 (2.44)</td>
<td>-0.33 (0.79)</td>
<td>3.97 (3.35)</td>
<td>63.55 (3.09)</td>
<td>-0.24 (0.83)</td>
<td>3.61 (2.86)</td>
</tr>
</tbody>
</table>

See table 8.2 for product code explanation.
All products showed varying degrees of total colour change ($\Delta E^{*\text{ab}}$) after 14 days of active bleaching treatment (Figure 8.6). Rapid White (RW) showed a greater bleaching effect ($\Delta E^{*\text{ab}}$ 3.85) than Absolute White (AW, $\Delta E^{*\text{ab}}$ 3.08), White Glo (WG, $\Delta E^{*\text{ab}}$ 2.37) and Speed White (SW, $\Delta E^{*\text{ab}}$ 1.59) in this category. Speed White group showed the least bleaching effect and differed significantly from Rapid White ($p<0.05$). No significant differences were found between the other groups.

Products ranked according to the median $\Delta E^{*\text{ab}}$ values in descending order:

Rapid White > Absolute White > White Glo > Speed White

Highest---------------------------------------------------------------Lowest

Figure 8.6: Box-and-Whisker plots of $\Delta E^{*\text{ab}}$ values for over-the-counter bleaching products. Only Group 13 (SW) showed a significantly lower bleaching effect as compared to Group 10 (RW) and is marked with (*).
8.7.4: Comparison among Bleaching Techniques

In Figure 8.7, Box-and-Whisker plots of $\Delta E_{ab}^*$ values for all whitening products are grouped together according to the application methods (dentist-supervised home bleaching, in-office bleaching or over-the-counter for self-application). The Kruskal-Wallis one-way ANOVA showed significant differences between different products and methods ($p<0.05$). Overall tray-based dentist-supervised home bleaching products; Nite White ACP 10% CP ($\Delta E_{ab}^* 6.19$), Yotuel Patient 10% CP ($\Delta E_{ab}^* 6.48$), Opalescence PF 10% CP ($\Delta E_{ab}^* 6.77$) and Opalescence PF 20% CP ($\Delta E_{ab}^* 9.68$) showed a more pronounced bleaching effect than all in-office and over-the-counter bleaching products. Furthermore, in-office bleaching products were more effective than over-the-counter bleaching products.

Opalescence Boost ($\Delta E_{ab}^* 5.24$) and Opalescence Quick 45% CP ($\Delta E_{ab}^* 3.48$), Yotuel Special ($\Delta E_{ab}^* 3.46$) and Rapid White ($\Delta E_{ab}^* 3.85$) showed a more pronounced bleaching effect than Yotuel 10 Minutes ($\Delta E_{ab}^* 2.44$), Absolute White ($\Delta E_{ab}^* 3.08$), White Glo ($\Delta E_{ab}^* 2.37$) and Speed White ($\Delta E_{ab}^* 1.59$).

After the completion of the active bleaching treatment, Opalescence PF 20% CP ($\Delta E_{ab}^* 9.68$) was the most effective product significantly superior to all other bleaching products ($p<0.05$).
Figure 8.7: Box-and-Whisker plots of $\Delta E_{ab}^*$ values for dentist-supervised home bleaching, in-office bleaching, over-the-counter bleaching products

1 = Nite White ACP 10% carbamide peroxide (NW10)
2 = Yotuel Patient 10% carbamide peroxide (YP10)
3 = Opalescence PF 10% carbamide peroxide (OP10)
4 = Opalescence PF 20% carbamide peroxide (OP20)
5 = Opalescence Trèswhite Supreme 10% hydrogen peroxide (TS10)
6 = Yotuel 10 Minutes 30% carbamide peroxide (Y10Min)
7 = Opalescence PF Quick 45% carbamide peroxide (OP45)
8 = Yotuel Special 35% hydrogen peroxide (YS35)
9 = Opalescence Boost 38% hydrogen peroxide (OB)
10 = Rapid White (RW)
11 = Absolute White (AW)
12 = White Glo (WG)
13 = Speed White (SW)
8.8- DISCUSSION

A number of different staining techniques have been reported in previous in vitro efficacy studies. Kielbassa et al., (2009) stained extracted human teeth in a (1:1) mixture of red wine and black tea. Dietschi et al., (2010) used human blood to obtain uniform staining of bovine enamel and dentine fragments. The staining technique applied in this study has been recommended by the American Dental Association (2008) for in vitro experiments. The purpose of staining was to allow a more discriminative comparison of the effectiveness of different bleaching products and methods. The absence of significant differences in the baseline L*, a* and b* values of stained teeth between different groups indicates a reasonably uniform distribution and staining of samples (Figure 8.3).

In the present study colour measurements were recorded in the L*a*b* three dimensional colour space established by the Commission Internationale de l’Eclairage (CIE) in 1976. The CIE L*a*b* colour space is a mixture of hue (green, red, blue, yellow etc.), lightness (bright colours and dark colours) and saturation (vivid colours and dull colours) and adequately represents the colour perception of the human eye in all 3 dimensions of colour space (Kuehni, 1976). Where L* indicates lightness/darkness (white/black), the a* value varies from the negative side (more greenish) to the positive side (more reddish), while the b* value varies from the more blue side (negative side) to the more yellow side (positive side).

A relatively high class spectrophotometer (CM-2600d) was used to determine the colour changes of teeth as recommended by the American Dental
Association (2008). Cheap spectrophotometers or colorimeters tend to have a high deviation between measurements and the results should be treated with caution. The standard deviation when total colour change ($\Delta E_{ab}^*$) is measured with the present spectrophotometer is low and claimed to be within 0.04, with an inter-instrument agreement of 0.2 (MAV/SCI). The instrument numerically measures the quantity of colour in a three dimensional colour space $L^*a^*b^*$ (Figures 8.1 and 8.2). To obtain a single value like the shade guide, overall colour change ($\Delta E_{ab}^*$) can be calculated using the formula $\Delta E_{ab}^* = [(\Delta L^{*})^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ are the changes which occurred in these components (CIE, 1978). $\Delta E_{ab}^*$ was used to compared the efficacy of different products and techniques. It has been reported that a total colour change ($\Delta E_{ab}^*$) value of 3.3 represents a moderate difference which is visually noticeable (Um and Ruyter, 1991).

Further analysis of the individual components of three dimensional colour space showed that the $L^*$ (lightness) component was a more significant parameter for comparison between products than the $a^*$ and $b^*$ values. Bleaching treatment with all products resulted in an increase in $L^*$ (lightness) values proportional to the effectiveness of the product used (Tables 8.3 to 8.5). The change in the $L^*$ ($\Delta L^*$) component ranged from 1.04 for the Speed White to 8.63 for the Opalescence PF 20% carbamide peroxide. Only a few products showed a decrease in $a^*$ values while most of the bleaching products did not produce any significant changes. All products showed a decrease in $b^*$ values (less yellow) proportional to the product used with $\Delta b^*$ ranging from -0.75 for Speed White to -3.0 for Opalescence PF 20% carbamide peroxide. However, only a few significant differences were found between products.
The products tested in this study were based on home bleaching techniques, dentist-supervised or over-the-counter for self-application and in-office bleaching techniques. All bleaching products were applied according to the manufacturers' instructions. In order to simulate an in vivo situation and to standardize experimental conditions, teeth were stored in artificial saliva at 37°C between treatments. This also prevented colour changes due to dehydration effects.

The null hypotheses were rejected because major differences were observed in the present study between dentist-supervised home bleaching products (Opalescence PF 20% CP, Opalescence PF 10% CP, Nite White ACP 10% CP and Yotuel Patient 10% CP), in-office bleaching products (Yotuel 10 Minutes 30% CP, Opalescence Quick 45% CP and Yotuel Special 35% HP) and over-the-counter bleaching products (Rapid White, Absolute White, White Glo, Speed White).

Dentist-supervised home bleaching products showed a more pronounced whitening effect as compared to all other products. Ten percent carbamide peroxide is the most common active ingredient in dentist-supervised home bleaching products. It has been reported that 10% carbamide peroxide dissociated into 3.33% hydrogen peroxide and 6.67% urea. Hydrogen peroxide undergoes further breakdown to generate free radicals (see page 15 for reaction) which penetrate into the tooth structure and oxidize the colour pigments (Zantner et al, 2007; Alonso de la Pena and Balboa Cabrita, 2006). The potential of carbamide peroxide in terms of its penetration through enamel and uniform bleaching of dentine has been reported in previous studies.
(Dietschi et al., 2006; Ritter et al., 2002; McCaslin et al., 1999). Safety and effectiveness of carbamide peroxide bleaching material used for tray-based dentist-supervised home bleaching technique has been well documented in short- and long-term clinical trials (Grobler et al., 2010; Meireles et al., 2008; Ritter et al., 2002; Leonard et al., 2001). In vitro studies also reported similar efficiency for this technique (Dietschi et al., 2010; McCaslin et al., 1999). The findings of the present study also reported more pronounced bleaching efficacy for tray-based home bleaching products: Nite White ACP 10% CP ($\Delta E_{ab}^{*}$ 6.19), Yotuel Patient 10% CP ($\Delta E_{ab}^{*}$ 6.48), Opalescence PF 10% CP ($\Delta E_{ab}^{*}$ 6.77) and Opalescence PF 20% CP ($\Delta E_{ab}^{*}$ 9.68).

Although slight variations in bleaching effect were seen among different 10% carbamide peroxide products, 20% carbamide peroxide showed significantly better bleaching effect. In an in vitro study, Sulieeman (2004) investigated tooth-bleaching efficiency of different concentrations (10, 15, 20, 22 and 30%) of carbamide peroxide bleaching gels. He reported that the bleaching effect was dependent upon the concentration of carbamide peroxide and duration of exposure where a maximum bleaching effect was observed earlier with higher concentrations of carbamide peroxide than lower peroxide concentrations. However, the final bleaching effect after 14 days of treatment was independent of the concentration and time was a dominant variable.

It can be argued that the use of high concentrations of carbamide peroxide or hydrogen peroxide results in large amount of free radicals and faster penetration through enamel and dentine causing quicker bleaching (Hanks et al., 1993). Clinical studies also reported that 15% carbamide peroxide
bleaching gel showed significantly better whitening than 10% carbamide peroxide after 2 weeks of treatment (Kihn et al., 2000; Matis et al., 2000). However, 4 weeks post-treatment evaluation did not reveal any difference between the two products (Matis et al., 2000). In contrast to the findings of the present study and above mentioned studies, Dietschi et al., (2006) reported that higher concentrations (15, 16 and 20%) of carbamide peroxide did not show increased bleaching effect on stained specimens of bovine dentine with either Opalescence or Nite White products.

In-office bleaching products were less effective than dentist-supervised home bleaching products. Opalescence Boost 38% HP showed the highest colour change ($\Delta E_{ab}^* 5.24$) as compared to Opalescence Quick 45% CP ($\Delta E_{ab}^* 3.48$), Yotuel Special 35% HP ($\Delta E_{ab}^* 3.46$) and Yotuel 10 Minutes 30% CP ($\Delta E_{ab}^* 2.44$). Although the dentist-supervised home bleaching technique is well documented, there is insufficient information available about the effectiveness of in-office bleaching techniques (Dietschi et al., 2006; Lee et al., 2007; Luk, Tam and Hubert, 2004; Sulieman et al., 2005).

In an in vitro study Dietschi et al., (2006) evaluated the efficacy of different bleaching methods and products on stained-bovine enamel and dentine specimens using a colorimeter. They found that in-office bleaching products (Opalescence Xtra Boost 35% HP, Opalescence Quick 35% CP and Brite Smile 15% HP) were less effective than dentist-supervised home bleaching products (Opalescence 10, 15, 20% CP and Nite White Excel2 10, 16% CP) in removing stains from dentine. The findings are in agreement with the present study.
In contrast, Wiegand et al., (2004) reported that both home bleaching products (Opalescence 10 and 15% CP) and in-office bleaching products (Opalescence Xtra Boost 35% HP and Opalescence Quick 35% CP) were equally effective in bleaching bovine enamel and dentine specimens. However, in their study specimens were not stained which might have produced different results. The results of another in vitro study (Lee et al., 2007) evaluating the efficacy of 10% and 35% carbamide peroxide using human premolar and molar teeth demonstrated that both products showed a similar bleaching effect. However, the darkest teeth showed more change with 35% than with 10% carbamide peroxide.

The fact that diffusion of peroxide depends on the diffusion coefficient, duration of application and concentration of the active bleaching agent (Hanks et al., 1993) might explain the reason for reduced efficacy of in-office bleaching products. As the exposure time for in-office bleaching products is far less compared to dentist-supervised home bleaching products, it can be assumed that addition of chemical activators or use of external heat/light sources as well as use of higher concentrations of hydrogen peroxide does not fully compensate for the reduced contact time between the bleaching agent and the tooth structure (Dietschi et al., 2006). Therefore manufacturers’ recommend some form of home bleaching to stabilize or reinforce the in-office bleaching effect (Kugel et al., 1997).

Over-the-counter tooth-whitening products are relatively new on the market. There is large variation in relation to the concentration of active bleaching agents, application methods (paint-on, strips, prefabricated trays etc.) and
exposure times. Although numerous clinical studies demonstrated the efficiency of various over-the-counter tooth-whitening products (Brunton, Ellwood and Davies, 2004; Gerlach and Zhou, 2001; Gerlach, Gibb and Sagel, 2000; Zantner et al., 2006a), there is no long-term evidence about the safety and stability of the bleaching effect of several new products.

Over-the-counter tooth bleaching products proved least effective as compared to dentist-supervised home bleaching products and in-office bleaching products (Figure 8.7). Rapid White ($\Delta E_{ab}^* 3.85$) was the most effective product in this category and Speed White ($\Delta E_{ab}^* 1.59$) was the least effective while Absolute White ($\Delta E_{ab}^* 3.08$) and White Glo ($\Delta E_{ab}^* 2.54$) showed an intermediate effect. However, no significant differences were found among different over-the-counter products.

Absolute White and Speed White were based on hydrogen peroxide while White Glo contained carbamide peroxide as active ingredient. No studies were found about these particular products for comparison purposes. However, the findings of studies evaluating various other over-the-counter whitening products also demonstrated poor bleaching efficiency for over-the-counter products (Dietschi et al., 2010; Zantner et al., 2006b; Wiegand et al., 2004).

Rapid White was the only non-peroxide bleaching product evaluated in this study. Rapid White contains aqueous sodium chlorite which react with the citric acid and generates chlorine dioxide as active bleaching agent (Kielbassa et al., 2009). Previous studies reported a negligible degree of change in tooth colour following treatment with this agent (Kielbassa et al., 2009; Zantner et al., 2006b). In a controlled in vitro study, the bleaching effect of Rapid White
containing sodium chlorite and citric acid proved significantly inferior to Opalescence 10 & 15% CP, Opalescence Quick 35% CP and Opalescence Xtra Boost 35% HP (Wiegand et al., 2004). This is in agreement with the findings of the present study.

The duration of single application for over-the-counter products was relatively short ranging from 5 minutes per day to 30 minutes per day. Shorter treatment times or contact duration with the tooth structure and low peroxide concentrations might be a possible reason for reduced bleaching efficiency of over-the-counter products.

Overall dentist-supervised home bleaching products proved to be more effective than in-office and over-the-counter bleaching products.

8.9- CONCLUSIONS

The results of the present study suggest the following conclusions:

1. Following bleaching treatments, major changes were observed in the L* (lightness/brightness) and b* (yellow/blue) components of the CIE L*a*b* colour space while a* values showed only minor changes.

2. The L* values (lightness) showed an increase of varying amplitude according to the product used and the b* values showed a decrease (less yellowish). Changes in L* (ΔL*) proved to be the most significant parameter for comparison between products.
3. Tray-based dentist-supervised home bleaching products (Nite White ACP 10%, Yotuel Patient 10%, Opalescence PF 10 and 20%) showed more pronounced bleaching effects than in-office (Yotuel 10 Minutes 30% CP, Opalescence Quick 45% CP, Yotuel Special 35% HP and Opalescence Boost 38% HP) and over-the-counter (Rapid White, Absolute White, White Glo, Speed White) bleaching products. Furthermore, in-office bleaching products were more effective than over-the-counter bleaching products.

4. Overall, Opalescence PF 20% carbamide peroxide showed significantly higher bleaching effect ($\Delta E_{ab}^* 9.68$) possibly because of the higher peroxide concentration and longer exposure time (8 hours per day).

5. Over-the-counter whitening products were the least effective bleaching agents.

6. In summary, dentist-supervised home bleaching technique was more effective than in-office and over-the-counter bleaching techniques.
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CHAPTER 9

CLINICAL EVALUATION OF THE EFFICACY AND ORAL SIDE EFFECTS OF THREE DIFFERENT TOOTH-WHITENING PRODUCTS

9.1- INTRODUCTION

Vital tooth bleaching has become a common procedure in dentistry to lighten stained or discoloured teeth. A number of different techniques are available to the dentist for tooth-whitening. However, at-home bleaching and in-office bleaching are two widely used methods (Joiner, 2006).

The active ingredient in a bleaching product is mainly hydrogen peroxide or carbamide peroxide but might also be one of a few other products such as oxalic acid, chlorine and muriatic acid (Teixeira et al., 2004, Fasanaro, 1992). Lower peroxide concentrations are used for at-home bleaching with a hydrogen peroxide (HP) concentration of up to 10% or a carbamide peroxide (CP) concentration between 10% and 22% while much higher hydrogen peroxide concentrations (30-35%) are being used for in-office procedures (Cavalli et al., 2004). Bleaching products with high concentrations of peroxide are claimed to ensure quicker whitening of teeth as compared to products with lower peroxide concentrations (Kihn et al., 2000). However, at-home bleaching using 10% carbamide peroxide is still the most widely used technique because of its relative safety, low-cost and ease of use (Grobler et al., 2010, Meireles et al., 2008; dos Santos and de Lima., 2008; Goo et al., 2004; Ritter et al., 2002).
Studies have reported that in-office bleaching techniques are as effective as at-home bleaching (Bernardon et al., 2010; Bizhang et al., 2009). However, a rapid rebound in colour has also been reported for in-office bleached teeth (Matis et al., 2007). In a clinical study over a 3-month period, Zekonis et al., (2003) compared the change in tooth colour, colour relapse and gum and tooth sensitivity following at-home bleaching treatment with 10% carbamide peroxide for 8 hours per day for 14 days and in-office bleaching treatment with 35% hydrogen peroxide for 60 minutes (two sessions, each with three 10-minute applications). The results demonstrated that at-home bleaching with 10% carbamide peroxide was significantly more effective than in-office bleaching with 35% hydrogen peroxide at all post-treatment follow-up visits. Both regimes showed colour relapse following the completion of active bleaching treatment which stabilized by six weeks. No significant differences were found between the treatments for tooth sensitivity.

A common clinical side effect of tooth bleaching is thermal tooth sensitivity and gingival irritation which varies from patient to patient and from product to product (Matis et al., 2007). A clinical study on 24 patients, who bleached their teeth with Nite White Excel, Platinum Professional and Opalescence 10% carbamide peroxide products for 8 hours per day for two weeks, reported similar tooth whitening by all products. The results were based on the whitening effect as perceived by the patients because no evaluations were performed by the operator using a shade guide or computer-based instruments. Tooth sensitivity was the most common side effect reported in 64% of the patients. However, it was temporary and disappeared after cessation of the treatment (Tam, 1999).
Bizhang et al. (2009) compared the efficacy and side effects of three different bleaching methods over a 3-month period: 1) home-bleaching with Illumine Home 10% carbamide peroxide eight hours per day for two weeks; 2) in-office bleaching with Illumine Office 15% hydrogen peroxide in a tray for 45 minutes, three times over three weeks; and 3) over-the-counter bleaching product Whitestrips 6% hydrogen peroxide for 30 minute, twice a day for two weeks. The results demonstrated significantly better whitening efficiency for home-bleaching ($\Delta E$, 6.57) and in-office bleaching ($\Delta E$, 5.77) compared to over-the-counter ($\Delta E$, 3.58) bleaching method. Colour relapse was seen in all groups; however, home-bleaching and in-office bleaching groups maintained superior results for up to three months. Mild and transient tooth hypersensitivity was reported in 72% of the participants with home-bleaching, 64% with in-office bleaching, and 60% with whitening strips, which disappeared spontaneously without requiring any further treatment.

Manufacturers have introduced different compounds such as fluoride, potassium nitrate and amorphous calcium phosphate (ACP) in bleaching products to prevent either hypersensitivity or demineralization effects (Chen et al., 2008). Browning et al. (2008) evaluated the effect of potassium nitrate and sodium fluoride on tooth sensitivity following bleaching treatment by comparing 10% carbamide peroxide with or without potassium nitrate and sodium fluoride in a placebo-controlled double-blind randomized clinical trial. The addition of a small percentage of potassium nitrate (0.5%) alone or with sodium fluoride (0.25%) to 10% carbamide peroxide significantly reduced tooth sensitivity with only 36% to 45% of the participants experienced tooth sensitivity during the 14-day treatment compared to 62% who used 10% carbamide peroxide without
potassium nitrate and sodium fluoride. In a clinical trial, Giniger et al., (2005) reported that subjects who used 16% carbamide peroxide bleaching gel with added amorphous calcium phosphate for three hours per day for 14 days experienced significantly less thermal and tactile tooth sensitivity than those who used 16% carbamide peroxide gel without amorphous calcium phosphate.

Most of the clinical studies evaluated the efficacy of tooth-bleaching using dental shade guides. Although it is a simple method to use, it is not very reliable and is highly subjective (Lima et al., 2009). Variables such as observer's experience, eye fatigue, ambient light conditions and the background against which a tooth is compared may lead to inconsistencies (Ishikawa-Nagai et al., 2004). To overcome these problems spectrophotometric assessment of tooth shade has been recommended (Kielbassa et al., 2009; Derdilopoulou et al., 2007; Joiner, 2004; Paul et al., 2002).

9.2- AIMS

The aims of the study were:

1. To evaluate and compare the whitening efficacy and colour rebound/relapse associated with two at-home and an in-office tooth-whitening products using a spectrophotometer over a six-month period.

2. To evaluate the oral side effects including tooth sensitivity and gingival irritation experienced during tooth-whitening treatments with the above-mentioned products.
9.3- OBJECTIVES

1. Objective evaluation of whitening efficacy of Nite White ACP 10% CP, Opalescence Trèswhite Supreme 10% HP and Opalescence Boost 38% HP by comparing changes in L*a*b* (ΔL*, Δa*, Δb*) and total colour changes (ΔE*ab) immediately after the completion of treatment using a spectrophotometer.

2. Subjective evaluation of the whitening efficacy of Nite White ACP 10% CP, Opalescence Trèswhite Supreme 10% HP and Opalescence Boost 38% HP immediately after the completion of treatment using a Vita Lumen shade guide.

3. To evaluate and compare rebound in tooth colour after 2-week post-treatment, 3-month and 6-month follow-up periods using a spectrophotometer.

4. To determine the amount of tooth sensitivity and gingival irritation caused by tooth-whitening products using a sensitivity form and visual examination.

9.4- NULL HYPOTHESES

1. There is no difference in the whitening efficacy of Nite White ACP 10% CP, Opalescence Trèswhite Supreme 10% HP and Opalescence Boost 38% HP.

2. Tooth-whitening products do not cause tooth-sensitivity or gingival irritation.
9.5- MATERIALS AND METHODS

This clinical study evaluated the effectiveness and side effects of two at-home tooth-whitening products viz Nite White ACP 10% carbamide peroxide, Opalescence Trèswhite Supreme 10% hydrogen peroxide, and one in-office tooth-whitening product viz Opalescence Boost 38% hydrogen peroxide. The detailed information about the composition, mode of application, treatment time and manufacturer is given in Table 9.1.

<table>
<thead>
<tr>
<th>Products</th>
<th>Groups</th>
<th>Composition</th>
<th>Treatment Time</th>
<th>Application Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nite White® ACP 10% CP Discus Dental, Culver City, CA, USA</td>
<td>1</td>
<td>Propylene Glycol, Glycerin, Water, Dicetyl Phosphate, Cetearyl Alcohol, Coeth-10 Phosphate, Silica, Carbamide Peroxide, Hydrogen Peroxide, Hydroxypropylecellulose, Potassium Nitrate, Flavor, Sodium Phosphate, Calcium Nitrate, Calcium Carbonate, Potassium Hydroxide</td>
<td>8 hrs/day for 14 days</td>
<td>At-home bleaching with custom made trays</td>
</tr>
<tr>
<td>Opalescence® Trèswhite Supreme 10% HP Ultradent Products Inc., South Jordan, UT, USA</td>
<td>2</td>
<td>Carbamide peroxide, hydrogen peroxide, sodium fluoride, potassium nitrate, fillers, flavour</td>
<td>1 hr/day for 14 days</td>
<td>At-home bleaching with prefabricated trays</td>
</tr>
<tr>
<td>Opalescence® Boost, 38% HP Ultradent Products Inc., South Jordan, UT, USA</td>
<td>3</td>
<td>Gel: Hydrogen peroxide, Activator: Potassium hydroxide, 1.1% fluoride and 3% potassium nitrate</td>
<td>1 hr (3 x 20 min)</td>
<td>In-office bleaching</td>
</tr>
</tbody>
</table>

9.5.1- Study Design

Sixty two subjects seeking tooth-whitening treatment were selected to participate in this randomized, single centre, observer-blind, 3-group clinical trial. The study and the consent form were approved by the Ethics Committee of the University of the Western Cape. All participants included in the study signed an informed consent form after full verbal and written explanation of the project.
9.5.2- Selection of Subjects

Sixty two volunteers (students, faculty staff and patients) willing to have their teeth whitened were enrolled in the study. The subjects enrolled in the study met the following inclusion and exclusion criteria.

Subject inclusion criteria:

1. 18 years of age or older
2. wanted to have their teeth whitened
3. who had sound maxillary incisors, free of caries, restorations, and crowns with tooth colour A2 or darker as determined by Vita Lumin Vacuum shade guide

Subject exclusion criteria:

1. subjects with generalized tooth sensitivity and poor oral hygiene
2. presence of fluorosis or tetracycline staining
3. restorations in the maxillary anterior teeth
4. on any medical treatment
5. previous use of bleaching products
6. pregnant or lactating women

After the screening, appropriate subjects who met the inclusion and exclusion criteria were selected. Each participant was given oral hygiene instructions and professional tooth cleaning was performed using ultrasonic instruments. Tooth polishing was carried out using a rubber cup and a fluoride containing prophylaxis paste, Nupro Supreme (Dentsply Int, York, PA, USA) to remove the external stains at least two weeks prior to the beginning of the study. All
participants were also asked about their daily consumption of tea, coffee, red wine and cigarette smoking.

Participants were instructed to brush twice a day with Colgate Fluoride Toothpaste containing 0.76% sodium monofluorophosphate and 0.1% sodium fluoride (Colgate-Palmolive (Pty) Ltd., South Africa) provided to standardize the fluoride levels and oral hygiene.

The subjects were then randomly allocated into one of the following three groups by a simple randomization process. The Excel random number generator function was used which supplied random numbers between 0 and 1 with uniform distribution. The random numbers assigned to each subject contain at least five decimal digits and are greater than 0 and less than 1 for example 0.710133, 0.481232, 0.016972 etc.

**Group 1 (n=21):** Subjects in this group were to receive at-home bleaching with Nite White® ACP containing 10% carbamide peroxide.

**Group 2 (n=21):** Subjects in this group were to receive at-home bleaching with Opalescence® Trèswhite™ Supreme containing 10% hydrogen peroxide.

**Group 3 (n=20):** Subjects in this group were to receive in-office bleaching with Opalescence® Boost PF 38% hydrogen peroxide.

Two subjects in Group 2 discontinued treatment after two days and were replaced. The reason for discontinuation is discussed in the results and discussion section.
9.5.3- Treatment Procedure

The subjects allocated for Groups 1 and 2 (at-home bleaching) collected their bleaching materials from the study assistant. The assistant was also responsible for the appointments of Group 3 (in-office bleaching) and the management of recall (follow-up) visits for all groups, in order to prevent bias and keep the observer blinded to the treatment assignment. Although only the bleaching of maxillary anterior teeth was part of the study, full bleaching treatment of mandibular teeth was also provided to all the participants of the three groups.

The bleaching procedure in each group was carried out according to the manufacturers’ instructions as follows:

**Group 1: Nite White® ACP 10% carbamide peroxide (At-home bleaching)**

At the initial examination, alginate impressions (Blueprint® cremix, Dentsply DeTrey, GmbH, Germany) were recorded and models were poured in yellow stone. Study casts were trimmed and the maxillary bleaching trays were fabricated from 1 mm soft tray material (Discus Dental, Culver City, CA, USA) using a vacuum forming technique. The labial surfaces of teeth on models were not blocked, to produce a reservoir for the bleaching material as suggested by the manufacturer. Trays were trimmed on the labial and lingual surfaces incisal to the free gingival margin, creating a scalloped pattern (Figure 9.1). The bleaching material (Nite White® ACP 10% CP) was administered at home overnight (8 hours/day) for 14 days using the customized bleaching trays. All participants were given verbal and written instructions about the use of the
material (see Appendix 4). The complete treatment process was according to
the manufacturer’s instructions.

Figure 9.1(A-G): Nite White ACP 10% carbamide peroxide home bleaching kit (A). Stone
model of upper arch (B) for bleaching tray construction. Model is in place on a vacuum-forming
machine and the tray material is heated (C). The formed bleaching tray (D & E). The bleaching
tray is cut to fit from right first premolar to left first premolar (F). On the labial and palatal
aspects tray is trimmed slightly incisal to the free gingival margin (G).
Group 2: Opalescence® Trèswhite™ Supreme 10% hydrogen peroxide
(At-home bleaching)

In this group, the subjects administered the bleaching material (Opalescence Trèswhite Supreme 10% HP) 60 minutes per day for 14 days using the prefabricated bleaching trays containing the bleaching material, according to the manufacturer’s instructions (Figure 9.2). All participants were also given verbal and written instructions about the use of the material (see Appendix 5).

Figure 9.2 (A-F): Opalescence Trèswhite Supreme 10% hydrogen peroxide (A & B) comes in prefabricated trays and different flavours. Upper bleaching trays are bigger than the lower trays. Green trays (C) are used as carriers only for soft clear trays (D) containing the standard amount of bleaching material along the labial and palatal/lingual surfaces in a thin line (E & F). Bleaching material is limited to the anterior teeth only on the palatal/lingual side (F). Once the bleaching trays are positioned in the mouth, green trays are removed and discarded.
Group 3: Opalescence® Boost PF 38% hydrogen peroxide
(In-office bleaching)

The in-office bleaching procedure was performed by an experienced dentist who was not involved in any other part of the study. At the bleaching appointment, cheek retractors were placed to keep the skin and lips away from the treatment area. Vaseline was applied to the lips and around the corners of the patient’s mouth. A bite block (IsoBlock®, Ultradent Products Inc., South Jordan, UT, USA) was placed in the patient’s mouth to keep the tongue away from the treatment area. In order to protect the maxillary gingiva as well as any exposed dentine or cementum, a light-cured gingival mask (OpalDam®, Ultradent Products Inc., South Jordan, UT, USA) was applied to the entire maxillary gingiva according to the manufacturer’s instructions and cured with a halogen light curing unit (Demetron LC, sdskerr, USA). The operator and the patient wore protective eyeglasses during the procedure.

The bleaching material was freshly mixed according to the manufacturer’s instructions and applied onto the labial surfaces of maxillary teeth in a 0.5 to 1.0 mm thick layer for 20 minutes (Figure 9.3). The material was agitated for a few seconds every 5 minutes. After 20 minutes, the bleaching gel was removed using a high volume suction and the teeth were rinsed thoroughly with water. A layer of fresh bleaching material was applied again. The entire procedure was repeated three times in the same session. The total in-office bleaching application time was 60 minutes (3 x 20 minutes). A waiting period of 30 minutes was allowed for rehydration of teeth before any tooth colour measurements were performed.
Figure 9.3 (A-G): Contents of Opalescence Boost in-office bleaching kit (A): IsoBlock bite block, OpalDam gingival mask, two red syringes of bleaching material attached to the clear syringes containing red activator material and tips for dispensation of OpalDam and bleaching material (B). Firstly, small plunger (C) is pressed to break the seal releasing the activator. Secondly, plungers D and E are pressed alternatively (12-13 times in each direction) to mix the activator and bleaching gels until a red homogenised gel (F) is achieved. Activated bleaching gel is pushed into the red syringe and clear syringe is then discarded. IsoBlock bite block (G) and OpalDam gingival mask (H) in place to prevent tongue and gingiva from bleaching material. Opalescence Boost 38% hydrogen peroxide bleaching gel placed on teeth (I) during the bleaching process.
9.5.4- Tooth Colour Measurements

All tooth colour assessments were carried out by a trained examiner in the same dental office setting blinded as to the treatment assignment.

Objective Evaluation (Spectrophotometer)

Objective tooth colour measurements of maxillary central incisors were taken using a contact type spectrophotometer (CM-2600d, Konica Minolta Sensing, Inc., Japan) with a 6 mm diameter probe. The spectrophotometer (Konica Minolta Sensing, Inc., Japan) measured the colour of teeth based on the CIE L* a* b* colour space system defined by the International Commission on Illumination (Figure 9.1).

![Figure 9.4: CIE L*a*b* three dimensional colour space](image)

In this colour space lightness is represented by L* on a scale of 0 for black to 100 for white. The hue and chroma are represented on an a* versus b* plot where a* represents the red/green coordinate and b* represents the yellow/blue
coordinate, while +a* indicates red and -a* indicates green, and similarly +b* indicates yellow and -b* indicates blue.

Before use, the spectrophotometer was calibrated using a white standard calibration disk provided by the manufacturer. The colour of an area (6 mm) at the centre of the crown of each maxillary central incisor (11 and 21) was measured three times with the spectrophotometer. The average of three measurements was considered as the measured value. The measurements were performed after the prophylaxis prior to bleaching (baseline), then immediately after the completion of active bleaching treatment (post-treatment), and later after 2-weeks post-treatment, 3-months and 6-months.

**Subjective Evaluation (Shade Guide)**

In addition to the objective colour measurements using the spectrophotometer, the tooth colour was also determined subjectively at the middle third of the labial surfaces of central incisors using the Vita Lumin Vacuum shade guide (Vident, Brea, CA, USA) at baseline (pre-treatment) and post-treatment intervals. The Vita Lumin shade guide is widely used in daily clinical practice for colour determinations. It consists of the 16 most common shade tabs which were arranged by value order according to the shade from light to dark (Table 9.2). The shade evaluations were carried out every time immediately after the spectrophotometer measurements in the dental office and by the same clinician using the same shade guide under similar lightening conditions. At each visit, tooth colour was recorded on a separate form to prevent the possibility that the examiner could be influenced by the previous determinations. A photograph
was also taken with the evaluated tooth shades of the tooth shade guide. The tooth colour improvement following bleaching treatment was calculated by subtracting the shade tab value corresponding to the baseline shade from that of the final evaluation.

<table>
<thead>
<tr>
<th>Lightest</th>
<th>Darkest</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>A1</td>
</tr>
<tr>
<td>B2</td>
<td>D2</td>
</tr>
<tr>
<td>A2</td>
<td>C1</td>
</tr>
<tr>
<td>C2</td>
<td>D4</td>
</tr>
<tr>
<td>A3</td>
<td>D3</td>
</tr>
<tr>
<td>B3</td>
<td>A3.5</td>
</tr>
<tr>
<td>B4</td>
<td>C3</td>
</tr>
<tr>
<td>A4</td>
<td>C4</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
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<td>3</td>
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<td>14</td>
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<tr>
<td>15</td>
<td>16</td>
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</tbody>
</table>

9.5.5- Oral Side Effects

A sensitivity sheet was given to all participants to record any tooth sensitivity experienced during the treatment period in one of five categories: 1- none, 2- mild, 3- moderate, 4- considerable, 5- severe. In Group 3, the bleaching treatment was performed in a dental chair in one session, and the subjects recorded sensitivity information during the next 7 days. The participants were asked to take note of any gingival irritation during the treatment period. An examination was carried out to see the presence or absence of gingival irritation after 7 days and at the end of active bleaching treatment.
9.6- STATISTICAL ANALYSIS

The data were captured in an Excel spreadsheet and analysed using a statistical software package NCSS 2007 (NCSS, LLC, Kaysville, UT, USA).

Baseline (pre-treatment) L*, a* and b* values of three groups were compared using the Kruskal-Wallis one-way ANOVA for any significant differences between groups (significance level was 5%).

For each group L*, a* and b* and shade guide values at baseline and immediately after-treatment were compared using the Wilcoxon Signed Rank Sum Test significant at p<0.05.

Changes in individual colour components (ΔL*, Δa* and Δb*) and total colour change (ΔE*\textsubscript{ab}) from baseline were calculated at the different time intervals. Whitening effect or colour improvement was represented by a positive ΔL* (increased brightness), negative Δb* (reduction in yellowness). The total colour differences were calculated using the formula: \( \Delta E*_{\text{ab}} = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2} \). Chroma (C*) was also calculated for all groups from baseline (pre-bleaching) a* and b* values using the formula \( C* = (a*^2+b*^2)^{1/2} \).

For multiple comparison among groups ΔL*, Δa*, Δb*, ΔE*\textsubscript{ab} and changes in shade guide values were analysed using the Kruskal-Wallis one-way ANOVA and Tukey-Kramer Multiple Comparison Test on a significance level of 5%.

Tooth sensitivity data was represented graphically and compared using the Wilcoxon Rank Sum Test significant at p<0.05. Gingival irritation data was analysed using Fisher’s Exact Test (p<0.05).
9.7- RESULTS

9.7.1- Demographic Characteristics

Sixty two subjects selected for the study included 41 females and 21 males with a mean age of 29.0 (11.2) years. The general distribution of age and gender in each group and information about the use of coffee, tea and tobacco is given in Table 9.3.

<table>
<thead>
<tr>
<th></th>
<th>Nite White ACP (G1, n=21)</th>
<th>Trèswhite Supreme (G2, n=21)</th>
<th>Opalescence Boost (G3, n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>28.7 (11.9)</td>
<td>27.8 (11.2)</td>
<td>30.6 (10.7)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12 (57.1%)</td>
<td>14 (66.7%)</td>
<td>15 (75.0%)</td>
</tr>
<tr>
<td>Male</td>
<td>9 (42.9%)</td>
<td>7 (33.3%)</td>
<td>5 (25.0%)</td>
</tr>
<tr>
<td>Coffee/Tea</td>
<td>14 (66.7%)</td>
<td>15 (71.4%)</td>
<td>15 (75.0%)</td>
</tr>
<tr>
<td>Smokers</td>
<td>3 (14.3%)</td>
<td>7 (33.3%)</td>
<td>7 (35.0%)</td>
</tr>
</tbody>
</table>

9.7.2- Efficacy of Tooth-whitening Products

The analysis of baseline tooth colour values of the three groups showed a similar colour distribution with no statistically significant differences (p>0.05). The distribution of baseline tooth colour for the three groups was also surveyed graphically by plotting L* versus C* (chroma) values (Figure 9.5).
Before treatment (at baseline), no significant differences were found in L*, a* and b* values between tooth 11 and 21 for all groups (Wilcoxon Signed Rank Sum Test, p>0.05). Therefore, the values of tooth 11 and 21 were subsequently pooled for any further analysis.

In the following section, the results for each group are initially explained separately and then a comparison of all groups regarding their efficacy and oral side effects is given.

9.7.3- Efficacy of Nite White® ACP 10% carbamide peroxide (Group 1)

Objective Evaluation (Spectrophotometer)

For all three components (L*, a* and b*) statistically significant differences were found in the values between baseline (pre-treatment) and post-treatment (14 days), baseline and 2-weeks post-treatment (30 days), as well as baseline and the 3-month and 6-month periods (Wilcoxon Signed Rank Sum Test, p<0.05).
All participants showed statistically significant whitening of teeth after 14 days of bleaching treatment with Nite White ACP 10% carbamide peroxide (p<0.05). When comparing the baseline L*a*b* values to the post-treatment values; all subjects showed an increase in the L* value (lighter and brighter) and decrease in the a* value (less reddish and more greenish) and b* value (less yellow).

The results obtained at the 2-weeks post-treatment follow-up visit indicated a significant relapse or rebound in colour. The L* value decreased while the a* and b* values increased significantly (Wilcoxon Signed Rank Sum Test, p<0.05). However, no significant differences were found in the median values of the three components amongst 2-weeks post-treatment, 3-month and 6-month periods.

The median decrease in $\Delta L^*$ from immediately after treatment to 2-weeks post-treatment (30 days) was about 37% with a further decrease to ~46% after 3-months where it more or less stabilized for at least 6-months (Figure 9.6).

![Figure 9.6](image.png)

**Figure 9.6:** The 25th percentile, median and 75th percentile differences in the L* values ($\Delta L^*$) between the baseline and post-treatment (14 days), 2 weeks post-treatment, after 3-months and 6-months for Nite White ACP 10% carbamide peroxide.
The median $\Delta a^*$ value decreased (relapsed) over time from ~12% after 1-month to 33% after 3 months and ~35% after 6 months (Figure 9.7).

![a* Differences ($\Delta a^*$) over Time](image)

*Figure 9.7:* The 25th percentile, median and 75th percentile differences in the $L^*$ values ($\Delta L^*$) between the baseline and post-treatment (14 days), 2 weeks post-treatment, after 3-months and 6-months for Nite White ACP 10% carbamide peroxide.

The median $\Delta b^*$ value also decreased by ~29% after 2-weeks post-treatment, by ~41% after 3 months and by ~42% after 6 months (Figure 9.8).

![b* Differences ($\Delta b^*$) over Time](image)

*Figure 9.8:* The 25th percentile, median and 75th percentile differences in the $L^*$ values ($\Delta L^*$) between the baseline and post-treatment (14 days), 2 weeks post-treatment, after 3-months and 6-months for Nite White ACP 10% carbamide peroxide.

Although there was a rebound in whitening effect, the comparison between the values obtained at baseline and 6-month follow-up period showed that the teeth still appeared significantly ($p<0.05$) whiter 6 months after bleaching than initially.
In terms of total colour change (Figure 9.9), the median $\Delta E^*_{ab}$ value was 5.29 immediately after 14 days of active bleaching treatment ($\Delta E_1$). All participants showed a significant rebound in tooth colour at the 2-weeks post-treatment follow-up visit with the median $\Delta E^*_{ab}$ decreased to 3.67 ($\Delta E_2$) (Wilcoxon Signed Rank Sum Test, $p<0.05$). After that the tooth colour became more or less stabilized for at least 6 months. The median $\Delta E^*_{ab}$ value was 3.32 after 3 months and ($\Delta E_3$) and 3.87 after 6 months ($\Delta E_4$).

Statistically significant differences were found in the median $\Delta E^*_{ab}$ values between $\Delta E_1$ and $\Delta E_2$, $\Delta E_3$ as well as $\Delta E_4$. However, no significant differences were found amongst $\Delta E_2$, $\Delta E_3$ and $\Delta E_4$ values.

The median decrease in $\Delta E^*_{ab}$ from post-treatment was 30% after 2-weeks, 37% after 3 months and 28% after 6 months (Figure 9.9).

Figure 9.9: The 25th percentile, median and 75th percentile $\Delta E^*_{ab}$ values between the baseline and post-treatment (14 days), 2 weeks post-treatment, after 3-months and 6-months for Nite White ACP 10% carbamide peroxide.
Subjective Evaluation (Shade Guide)

The comparison between baseline and post-treatment scores of the subjective tooth colour evaluation using Vita Lumin shade guide also showed statistically significant improvement in tooth colour after 14 days of bleaching treatment (Wilcoxon Signed Rank Sum Test, p<0.05). The mean $\Delta$ Vita shade tab improvement in tooth colour was $6 \pm 2.15$ units. No shade guide evaluations were performed at follow-up visits.

9.7.4- Oral Side Effects of Nite White ACP 10% Carbamide Peroxide

Tooth Sensitivity

Of the 21 subjects, 12 (57%) experienced tooth sensitivity at some point during the active bleaching period of 14 days. The majority of the subjects intermittently experienced only mild sensitivity up to 6 days or less with an average duration of 38 minutes per day. Only 4 subjects experienced sensitivity for more than 9 days with moderate to severe sensitivity of short duration (10 minutes) for 2 to 3 days. The duration of sensitivity was more in the first week of bleaching than in the second week.

Gingival Irritation

Only 4 subjects had gingival irritation during the first week and 1 subject reported throat irritation during 2 weeks of bleaching.

Of the total of 294 days of bleaching for the 21 subjects (14 days per subject) there were 72 days (24.5%) of sensitivity and 28 days (9.5%) of gingival irritation. The sensitivity was mild for 60 days (20%) and moderate to severe for 12 days (4%).
Group 1: Nite White ACP 10% carbamide peroxide

Clinical photographs of some patients before and after bleaching treatment (Figures 9.10 to 9.12).

**Figure 9.10:** Maxillary anterior teeth of a patient before treatment (A) and after 14 days of bleaching treatment with Nite White ACP 10% carbamide peroxide (B).

**Figure 9.11:** Maxillary anterior teeth of a patient before treatment (A) and after 14 days of bleaching treatment with Nite White ACP 10% carbamide peroxide (B).

**Figure 9.12:** Maxillary anterior teeth of a patient before treatment (A) and after 14 days of bleaching treatment with Nite White ACP 10% carbamide peroxide (B).
9.7.5- Efficacy of Opalescence Trèswhite Supreme 10% Hydrogen Peroxide (Group 2)

Objective Evaluation (Spectrophotometer)

For all three components (L*, a* and b*) statistically significant differences were found in the values between baseline (pre-treatment) and post-treatment (14 days), baseline and 2-weeks post-treatment (30 days), as well as baseline and the 3-month and 6-month periods (Wilcoxon Signed Rank Sum Test, p<0.05).

All participants showed statistically significant whitening of teeth after 14 days of bleaching treatment with Opalescence Trèswhite Supreme 10% hydrogen peroxide (p<0.05). When comparing the baseline values to the post-treatment values, all subjects showed an increase in the L* values (lighter and brighter) and decrease in the a* values (less reddish and more greenish) and b* values (less yellow).

The results obtained at the 2-weeks post-treatment follow-up visit indicated a relapse or rebound in tooth colour. The L* values decreased while the b* values increased. Statistically significant differences were found in the median ∆a* and ∆b* values between post-treatment and 3-months follow-up periods, in the median ∆L* and ∆b* values between post-treatment and 6-months follow-up periods and in the median ∆b* values between 2-weeks post-treatment period and 3-months as well as 6-months follow-up periods (Wilcoxon Signed Rank Sum Test, p<0.05). However, no significant differences were found in the median values of the three components between post-treatment and 2-weeks post-treatment period and between 3-month and 6-month periods follow-up visits.
The median decrease in $\Delta L^*$ from post-treatment to 2-weeks post-treatment was about 23%, to 3-months 19% and to 6-months 26% (Figure 9.13).

![Figure 9.13: The 25th percentile, median and 75th percentile differences in the L* values ($\Delta L^*$) between the baseline and after treatment (14 days), 2 weeks post-treatment, after 3-months and 6-months for Opalescence Trèswhite Supreme 10% hydrogen peroxide.](image)

The median $\Delta a^*$ value showed a slight decrease (17%) only after 6-months (Figure 9.14).

![Figure 9.14: The 25th percentile, median and 75th percentile differences in the a* values ($\Delta a^*$) between the baseline and after treatment (14 days), 2 weeks post-treatment, after 3-months and 6-months for Opalescence Trèswhite Supreme 10% hydrogen peroxide.](image)
The median $\Delta b^*$ values also decreased by 7% after 3 months, and by 16% after 6 months (Figure 9.15).

![Figure 9.15: The 25th percentile, median and 75th percentile differences in the $b^*$ values ($\Delta b^*$) between the baseline and after treatment (14 days), 2 weeks post-treatment, after 3-months and 6-months for Opalescence Trèswhite Supreme 10% hydrogen peroxide.](image)

Although there was a rebound in whitening effect, the comparison between the values obtained at baseline and 6-month follow-up period showed that the teeth still appeared significantly ($p<0.05$) whiter 6 months after bleaching than initially.

In terms of total colour change (Figure 9.16), the median $\Delta E^*_{ab}$ value was 4.09 after 14 days of bleaching treatment with Opalescence Trèswhite Supreme 10% hydrogen peroxide ($\Delta E_5$). All participants showed a rebound (relapse) in tooth colour at the 2-weeks post-treatment follow-up visit with the median $\Delta E^*_{ab}$ decreased to 3.02 ($\Delta E_6$). After that the tooth colour became more or less stabilized for at least 6 months. The median $\Delta E^*_{ab}$ value was, 3.19 after 3 months ($\Delta E_7$) and 2.94 after 6 months ($\Delta E_8$).

Statistically significant differences were found in the median $\Delta E^*_{ab}$ values for the following pairs: $\Delta E_5-\Delta E_7$, $\Delta E_5-\Delta E_8$ and $\Delta E_6-\Delta E_8$ (Wilcoxon Signed Rank...
Sum Test, p<0.05). However, no significant differences were found between the $\Delta E_5-\Delta E_6$, $\Delta E_6-\Delta E_7$ and $\Delta E_7-\Delta E_8$ pairs.

The median decrease in $\Delta E^*_{ab}$ from post-treatment was 26% after 2-weeks, 22% after 3-months and 28% after 6-months (Figure 9.16).

Subjective Evaluation (Shade Guide)

The comparison between baseline and post-treatment scores of the subjective tooth colour evaluation using Vita Lumin shade guide also showed statistically significant improvement in tooth colour after 14 days of bleaching treatment (Wilcoxon Signed Rank Sum Test, p<0.05). The mean $\Delta$ Vita shade tab improvement in tooth colour was $4.54 \pm 2.43$ units. No shade guide evaluations were performed at follow-up visits.
9.7.6- Oral Side Effects of Opalescence Trèswhite Supreme 10% Hydrogen Peroxide

Tooth Sensitivity

Of the 21 subjects, 15 (71%) experienced tooth sensitivity at some point during the active bleaching period of 14 days. The majority of the subjects experienced only mild sensitivity with an average duration of 30 minutes per day. Only 4 subjects experienced moderate sensitivity and 2 subjects experience severe sensitivity of short duration.

Gingival Irritation

The bleaching material comes in standard pre-fabricated soft trays. Size and softness of trays was a major reason for soft tissue irritation. Two subjects stopped bleaching treatment after two applications due to burns on the palate and gingiva.

Seven subjects had gingival irritation during the bleaching period. One subject reported burning sensations on the tongue and one subject showed burning of the palatal tissue (sloughing or white peels).

Of the total of 294 days of bleaching for the 21 subjects (14 days per subject) there were 124 days (42%) of sensitivity and 98 days (33.3%) of gingival irritation. The sensitivity was mild for 85 days (29%), moderate for 35 days (12%) and severe for 4 days (1%).
Group 2: Opalescence Trèswhite Supreme 10% hydrogen peroxide

Clinical photographs of some patients before and after bleaching treatment (Figures 9.17 to 9.19).

Figure 9.17: Maxillary anterior teeth of a patient before treatment (A) and after 14 days of bleaching treatment with Opalescence Trèswhite Supreme 10% hydrogen peroxide (B).

Figure 9.18: Maxillary anterior teeth of a patient before treatment (A) and after 14 days of bleaching treatment with Opalescence Trèswhite Supreme 10% hydrogen peroxide (B).

Figure 9.19: Maxillary anterior teeth of a patient before treatment (A) and after 14 days of bleaching treatment with Opalescence Trèswhite Supreme 10% hydrogen peroxide (B).
9.7.7- Efficacy of Opalescence Boost 38% Hydrogen Peroxide (Group 3)

Objective Evaluation (Spectrophotometer)

For all three components (L*, a* and b*) statistically significant differences were found in the values between baseline (pre-treatment), and post-treatment as well as the 2-weeks post-treatment period (Wilcoxon Signed Rank Sum Test, p<0.05). However, after 3 months, significant differences were found only in L* and b* values. For all three components (L*, a* and b*) no statistically significant differences were found between baseline and the 6-month follow-up.

All participants showed statistically significant whitening of teeth after 60 minutes of bleaching treatment with Opalescence Boost 38% hydrogen peroxide (p<0.05). When comparing the baseline values to the post-treatment values; all subjects showed an increase in the L* values (lighter and brighter) and a decrease in the a* values (less reddish and more greenish) and b* values (less yellow).

The results obtained at 2 weeks post-treatment, 3-month and 6-month follow-up visits showed a continued relapse or rebound in tooth colour over time. The L* values decreased while the b* values increased significantly (Wilcoxon Signed Rank Sum Test, p<0.05). The a* values showed a further significant decrease (improvement) at the 2-weeks post-treatment visit and then started to relapse till the 6-month evaluation.
The median decrease in $\Delta L^*$ from post-treatment to the 2-weeks post-treatment period was about 61% with a further decrease to ~74% after 3 months and 81% after 6 months (Figure 9.20).

![Figure 9.20: The 25th percentile, median and 75th percentile $\Delta L^*$ values between the baseline and after treatment, 2 weeks post-treatment, after 3-months and 6-months for Opalescence Boost 38% hydrogen peroxide.](image)

The median $\Delta a^*$ value decreased (relapsed) over time by 29% after 3 months and by 53% after 6 months (Figure 9.21).

![Figure 9.21: The 25th percentile, median and 75th percentile $\Delta a^*$ values between the baseline and after treatment, 2 weeks post-treatment, after 3-months and 6-months for Opalescence Boost 38% hydrogen peroxide.](image)
The median $\Delta b^*$ values also decreased by $\sim 18\%$ after 2-weeks post-treatment, by $\sim 58\%$ after 3 months and by $69\%$ after 6 months (Figure 9.22).

![Figure 9.22: The 25th percentile, median and 75th percentile $\Delta b^*$ values between the baseline and after treatment, 2 weeks post-treatment, after 3-months and 6-months for Opalescence Boost 38% hydrogen peroxide.](image)

In terms of total colour change (9.23), the median $\Delta E_{ab}^*$ value was 4.05 after 60 minutes of bleaching treatment with Opalescence Boost 38% hydrogen peroxide ($\Delta E_9$). All participants showed continuous significant rebound in tooth colour with the median $\Delta E_{ab}^*$ decreased to 2.36 ($\Delta E_{10}$) at the 2-weeks post-treatment follow-up visit, 1.7 after 3 months ($\Delta E_{11}$) and 1.43 after 6 months ($\Delta E_{12}$).

Statistically significant differences were found in the median $\Delta E_{ab}^*$ values amongst $\Delta E_9$, $\Delta E_{10}$, $\Delta E_{11}$ (Wilcoxon Signed Rank Sum Test, $p<0.05$).

The median decrease in $\Delta E_{ab}^*$ from post-treatment was 42% after 2-weeks post-treatment, 58% after 3 months and 65% after 6 months (Figure 9.23).
Subjective Evaluation (Shade Guide)

The comparison between baseline and post-treatment scores of the subjective tooth colour evaluation using the Vita Lumin shade guide also showed statistically significant improvement in tooth colour after 60 minutes of in-office bleaching treatment (Wilcoxon Signed Rank Sum Test, p<0.05). The mean ΔVita shade tab improvement in tooth colour was 6.93 ± 2.74 units. No shade guide evaluations were performed at other follow-up visits.

9.7.8- Oral Side Effects of Opalescence Boost 38% hydrogen peroxide

Tooth Hypersensitivity

Of the 20 subjects, 17 (85%) experienced tooth sensitivity on the day of in-office treatment. The majority of the subjects experienced moderate to severe sensitivity on different teeth with an average duration of 8 hours. However, the sensitivity was not generalized and disappeared in most cases. On the second day after treatment only 9 (45%) subjects experienced mild to moderate
sensitivity with an average duration of 2.25 hours. Only 2 subjects reported mild sensitivity on the third day.

**Gingival Irritation**

The gingival irritation was very low in this group because the bleaching procedure was carried out in the dental office under a controlled environment and gingiva was covered with a light-cured material (OpalDam). However, in 1 subject the material leaked under the protective mask and produced slight inflammation on the interdental papilla in some maxillary teeth.

In summary, the bleaching procedure was completed in one session in the dental office and subjects were asked to record sensitivity for the following 7 days. Of the total 140 days of sensitivity recording for the 20 subjects (7 days per subject) there were 29 days (20.7%) of sensitivity. The sensitivity was mild for 12 days (8.6%), moderated for 11 days (7.9%) and severe for only 6 days (4.3%). Gingival irritation was noted only in 1 subject around few teeth due to the leakage of the material during bleaching and disappeared after 2 days (Figure 9.24).

*Figure 9.24:* The burn spots around the gingival margins of teeth indicated by the arrows caused by the leakage of bleaching material
Group 3: Opalescence Boost 38% hydrogen peroxide

Clinical photographs of two patients before and after bleaching treatment (Figures 9.25 and 9.26).

Figure 9.25: Maxillary anterior teeth of a patient before treatment (A), immediately after 60 minutes of bleaching with Opalescence Boost 38% hydrogen peroxide (B) and 2 weeks post-treatment (C).

Figure 9.26: Maxillary anterior teeth of a patient before treatment (A), immediately after 60 minutes of bleaching with Opalescence Boost 38% hydrogen peroxide (B) and 2 weeks post-treatment (C).
9.8- Comparison of Efficacy and Colour Relapse amongst Nite White ACP, Opalescence Trèswhite Supreme and Opalescence Boost

9.8.1- Comparison of Objective Evaluations (Spectrophotometer)

The median values for $\Delta L^*$, $\Delta a^*$, $\Delta b^*$ and $\Delta E_{ab}^*$ for all groups are summarized in Table 9.4. Significant differences in colour changes ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$ and $\Delta E_{ab}^*$) were found amongst three groups at time intervals: baseline to immediately after completion of active bleaching treatment (post-treatment), baseline to 2-weeks post-treatment and baseline to 3- and 6-month follow-up periods.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Median $\Delta L^*$</th>
<th>Median $\Delta a^*$</th>
<th>Median $\Delta b^*$</th>
<th>Median $\Delta E_{ab}^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW TW OB NW TW OB NW TW OB NW TW OB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>4.17 2.79 2.97 -0.60 -0.23 -0.17 -3.26 -2.06 -2.10</td>
<td>5.29 4.09 4.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-weeks post-treatment</td>
<td>2.60 2.15 1.16 -0.48 -0.23 -0.40 -2.31 -2.15 -1.72</td>
<td>3.67 3.02 2.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>2.25 2.27 0.77 -0.39 -0.23 -0.12 -1.93 -1.99 -0.89</td>
<td>3.32 3.19 1.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>2.24 2.06 0.56 -0.38 -0.19 -0.08 -1.89 -1.81 -0.65</td>
<td>3.87 2.94 1.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NW= Nite White ACP, TW= Opalescence Trèswhite Supreme, OB= Opalescence Boost

Figures 9.27 to 9.29 depict Box-and-Whisker plots of median differences between baseline and post-treatment, 2-weeks post-treatment and 3-month follow-up for all three components ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$) and $\Delta E_{ab}^*$. In each diagram, the top line shows the maximum and the bottom line the minimum values, while the box part shows the location of 50% of the values and the line in the box the median value for each component for a specific group.
There is no Box-and-Whisker plot for 6-month follow-up because major colour changes took place during the first 3 months. The whitening effect became more or less stable in Groups 1 and 2 after 3-months and only a slight further relapse in colour was observed for Group 3 after 6 months (Table 9.4).

**Comparison of changes in L* (Lightness/brightness) component**

The colour changes in brightness ($\Delta L^*$) between baseline and post-treatment were significantly greater for bleaching treatment in Group 1 as compared to the bleaching treatment in Group 2 (Tukey-Kramer Multiple Comparison Test, $p<0.05$). The colour changes in Group 3 were not significantly different from Group 1 and Group 2 (Figure 9.27).

At 2-weeks post-treatment follow-up, all groups showed a decrease in brightness ($\Delta L^*$). However, the colour changes $\Delta L^*$ remained significantly greater for Group 1 as compared to Group 3 (Tukey-Kramer Multiple Comparison Test, $p<0.05$). Group 2 did not differ significantly from Group 1 and Group 3 in $\Delta L^*$ (Figure 9.28).

At 3-month and 6-month follow-ups, the colour changes ($\Delta L^*$) remained significantly greater for Groups 1 and 2 as compared to Group 3 (Tukey-Kramer Multiple Comparison Test, $p<0.05$). No significant differences were found between Groups 1 and 2 (Figure 9.29).

**Comparison of changes in a* (red-green) component**

The colour changes along the red-green axis ($\Delta a^*$) between baseline and post-treatment were significantly greater for the treatment in Group 1 as compared
to the treatment in Group 3 (Tukey-Kramer Multiple Comparison Test, p<0.05). Group 2 did not show significant differences from Groups 1 and 3 (Figure 9.27).

Although the colour changes remained greater for Groups 1 and 2 at 2-weeks post-treatment, 3-month and 6-month follow-up periods, no significant differences were found amongst Groups 1, 2 and 3 in $\Delta a^*$ (Figures 9.28 and 9.29).

**Comparison of changes in $b^*$ (yellow-blue) component**

The colour changes along the yellow-blue axis ($\Delta b^*$) between baseline and post-treatment were significantly greater for the treatment in Group 1 as compared to the treatment in Group 3 (Tukey-Kramer Multiple Comparison Test, p<0.05). Group 2 did not show significant differences from Groups 1 and 3 (Figure 9.27).

At 2-weeks post-treatment, 3-month and 6-month follow-ups, the colour changes were significantly greater for Groups 1 and 2 as compared to Group 3 (Tukey-Kramer Multiple Comparison Test, p<0.05). No significant differences were found between Group 1 and Group 2 (Figures 9.28 and 9.29).

**Comparison of total colour changes ($\Delta E_{ab}^*$)**

The total colour changes between baseline and post-treatment revealed significantly better whitening efficacy for Group 1 (Nite White ACP, $\Delta E_{ab}^*$ 5.29) as compared to Group 2 (Opalescence Trèswhite Supreme, $\Delta E_{ab}^*$ 4.09) and Group 3 (Opalescence Boost, $\Delta E_{ab}^*$ 4.05) (Tukey-Kramer Multiple Comparison Test, p<0.05). No significant differences were found between Groups 2 and 3 (Figure 9.27).
All three bleaching treatments showed a significant relapse in colour at the 2-weeks post-treatment follow-up visit (Wilcoxon Signed Rank Sum Test, p<0.05). However, the total colour change of Group 1 ($\Delta E_{ab}^* 3.67$) remained significantly better than that of Group 3 ($\Delta E_{ab}^* 2.36$). Group 2 ($\Delta E_{ab}^* 3.02$) did not differ significantly from Groups 1 and 3 (Figure 9.28).

Groups 1 and 2 did not show further relapse in tooth colour and became more or less stable till 6 months while Group 3 showed continued relapse in colour till 6 months. At 3-month and 6-month follow-up visits, the total colour changes of Groups 1 and 2 remained significantly greater than that of Group 3. No significant differences were found between Groups 1 and 2 in $\Delta E_{ab}^*$ (Table 9.4 and Figure 9.29).

**Figure 9.27:** Box-and-Whisker plots showing colour changes ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$ and $\Delta E_{ab}^*$) between baseline and immediately after completion of bleaching treatment (post-treatment).
Figure 9.28: Box-and-Whisker plots showing colour changes ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$ and $\Delta E_{ab}^*$) between baseline and 2-weeks post-treatment follow-up.

Figure 9.29: Box-and-Whisker plots showing colour changes ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$ and $\Delta E_{ab}^*$) between baseline and 3-month follow-up.
The number of subjects who showed total colour change ($\Delta E_{ab}^*$) $\geq$ 3.3 are given in Table 9.5 and Figure 9.30.

**Table 9.5: Number of subjects with $\Delta E_{ab}^* \geq 3.3$ in each group at different time intervals.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Post-bleaching (n, %)</th>
<th>2-week post-bleaching (n, %)</th>
<th>3 months (n, %)</th>
<th>6 months (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, Nite White</td>
<td>15 (71%)</td>
<td>12 (57%)</td>
<td>11 (52%)</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>2, Trèswhite</td>
<td>14 (67%)</td>
<td>10 (48%)</td>
<td>9 (43%)</td>
<td>7 (33%)</td>
</tr>
<tr>
<td>3, Boost</td>
<td>11 (55%)</td>
<td>5 (25%)</td>
<td>2 (10%)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 9.30:** Percentage of subjects in each group with $\Delta E_{ab}^* \geq 3.3$ at different time intervals.

### 9.8.2 - Comparison of Subjective Evaluations (Shade Guide)

Comparison of the baseline and post-treatment ranks for evaluated shade tabs showed statistically significant improvement in colour for all three groups (Wilcoxon Signed Rank Sum Test, $p<0.05$). The mean $\Delta$ Vita shade tab
improvement for Groups 1, 2 and 3 was $6 \pm 2.15$, $4.5 \pm 2.43$ and $6.9 \pm 2.74$ units respectively. However, no significant differences were found amongst the three groups (Tukey-Kramer Multiple Comparison Test, $p<0.05$).

9.9- Comparison of Oral Side Effects amongst Nite White ACP, Opalescence Trèswhite Supreme and Opalescence Boost

Table 9.6 and Figure 9.31 demonstrate the overall information about the sensitivity and gingival irritation for all groups. The percentage of subjects with tooth sensitivity ranged from 57% to 85%. Although the total number of days on which subjects experienced tooth sensitivity were more in Groups 1 and 2 (Nite White ACP and Opalescence Trèswhite Supreme) than Group 3 (Opalescence Boost), the differences were not statistically significant (Wilcoxon Rank Sum Test, $p<0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Nite White ACP 10% CP (Group 1, n=21)</th>
<th>Trèswhite Supreme 10% HP (Group 2, n=21)</th>
<th>Opalescence Boost 38% HP (Group 3, n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tooth Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occurrence in subjects</td>
<td>12 (57%)</td>
<td>15 (71%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>(n, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of days</td>
<td>72 (24.5%)</td>
<td>124 (42%)</td>
<td>29 (20.7%)</td>
</tr>
<tr>
<td>with any sensitivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gingival Irritation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occurrence in subjects</td>
<td>4 (19%)</td>
<td>7 (33.3%)</td>
<td>1 (5.0%)</td>
</tr>
<tr>
<td>(n, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of days</td>
<td>28 (9.5%)</td>
<td>98 (33.3%)</td>
<td>2 (1.4%)</td>
</tr>
</tbody>
</table>
Figure 9.31: Average tooth sensitivity scores for different treatment groups

Studying the graph of tooth sensitivity (Figure 9.31), it was observed that Group 3 displayed the most severe initial sensitivity of the three treatment groups as measured by the crude indicatory measure of sensitivity (average of 1 to 4 sensitivity scale). The other two treatments (Groups 1 and 2) demonstrated a much lower initial sensitivity than Group 3. However, the initial sensitivity experienced in Group 3 dissipated mostly at Day 3 and disappeared completely at Day 5. Due to the treatment regime of the two at-home bleaching products, the sensitivity experienced remained at a constant level from Day 3 to Day 14. For this period Group 2 displayed slightly “more” sensitivity than Group 1.

Four subjects in Group 1 and 7 subjects in Group 2 experienced gingival irritation during the bleaching treatment period (Table 9.6). No significant differences were found between Groups 1 and 2 (Fisher’s Exact Test).
9.10- DISCUSSION

9.10.1- Efficacy

In this study a relatively sophisticated spectrophotometer was used to determine the objective colour changes of teeth. This allowed for more standardized readings compared to the cheap spectrophotometers or colorimeters which tend to have a high deviation between measurements. The results from such instruments should be treated with caution. The standard deviation when $\Delta E_{ab}^*$ is measured with the present spectrophotometer is low and claimed to be within 0.04, with an inter-instrument agreement of 0.2 (MAV/SCI). In this study, the CIE system of colour (Minolta, 1994) was used which is a mixture of hue (green, red, blue, yellow etc), lightness (bright colours and dark colours) and saturation (vivid colours and dull colours). The instrument numerically measures the quantity of colour in a three dimensional colour space ($L^*a^*b^*$), where $L^*$ indicates lightness/darkness and varies from 0 for black to 100 for pure white, the $a^*$ value varies from a negative side (more greenish) to the positive side (more reddish), while the $b^*$ value varies from the more blue side (negative side) to the more yellow side (positive side).

![Figure 9.1: CIE L*a*b* three dimensional colour space](image_url)
Many studies used shade guides to measure the colour of teeth (Browning et al., 2008; dos Santos et al., 2008). However, the shade guide has many limitations of which the major ones are (Horn, 1998):

- it is observer sensitive
- It does not cover the whole spectrum of the natural colour of teeth
- it only gives an overall colour value which is not broken down into L*, a* and b* components, as with the spectrophotometer.
- it is also reported that intra-evaluator agreement for the shade guide can be as low as 60%.

In this study the Vita Lumin Vacuum shade guide (Vident, Brea, CA, USA) was used to measure the colour changes between baseline and immediately after the completion of the bleaching treatment only. All groups demonstrated statistically significant whitening effect. The results are in accordance with the previous studies with at-home bleaching products containing 10% carbamide peroxide or 10% hydrogen peroxide and in-office bleaching products containing 35% or 38% hydrogen peroxide (Gurgan et al., 2010; Zekonis et al, 2003; Toko et al, 1998; Marques et al, 2008).

The amount of colour change as described by the change in Vita shade guide ranks (Table 9.2) was 6 ± 2.15 on average for Nite White ACP 10% CP, 4.54 ± 2.43 for Opalescence Trèswhite Supreme 10% HP and 6.93 ± 2.74 for Opalescence Boost 38% HP. No significant differences were found amongst the three groups when compared for Vita Shade tab changes (p>0.05).
No studies were found which compared the whitening efficacy of Nite White ACP, Opalescence Trèswhite Supreme and Opalescence Boost using the shade guide. However, the studies evaluating either the previous versions of these products or comparing them with different products reported slightly different results for visual shade evaluation. In a clinical study Toko et al., (1998) evaluated Nite White Excel 10% carbamide peroxide used for 5 hours per day for two weeks and reported an average improvement of 6.9 in Vita Shade tab, while another study with the same product and similar application time reported an average improvement of 4.28 in Vita Shade tab (Hagiwara et al., 1999).

Two clinical studies (Marques et al., 2008 and Li et al., 2005) found for Opalescence Trèswhite Supreme (10% HP) and Opalescence Trèswhite (9% HP) reported an average Vita Shade tab improvement of 6.6 and 6.1 respectively which was slightly higher than that of 4.54 observed in the present study. Gurgan, et al., (2010) reported an average shade tab improvement of 8.7 in subjects with A3 or darker teeth bleached with 38% HP in-office bleaching product (Opalescence Xtra Boost) for 30 minutes. Other studies also reported 6 to 9 shade tab improvement with Opalescence Xtra Boost 38% HP bleaching product (Auschill et al., 2005; Gallagher et al., 2002). However, in these studies subjects with a baseline colour of A3 or darker were included as opposed to the present study where the inclusion criteria was A2 or darker. It has been reported that the darker the teeth at baseline, the better the whitening outcome (Gerlach and Zhou, 2001).
It is possible to measure the overall colour change ($\Delta E_{ab}^*$) with the spectrophotometer as one value (like the shade guide). The formula then is:

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

where $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ are the changes which occurred in these components (CIE, 1978). However, this would limit the information about the real colour of the tooth or the real change in colour which took place during a treatment with a whitener, which might vary from one to the other.

The results obtained for $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ in this study indicated that all bleaching products increased $L^*$ values (lighter and brighter) while decreasing the $a^*$ (less reddish), and $b^*$ (less yellow) values. The comparison of the baseline values of the three groups indicated similar colour distribution with no statistical significant differences (Figure 9.5). However the values obtained for $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ post-bleaching indicated wider distribution for Group 1 (Nite White ACP 10% CP) as compared to Groups 2 and 3 (Figures 9.27 to 9.29). This might suggest that Nite White ACP 10% CP had a more pronounced whitening effect which was statistically significant compared to Opalescence Trèswhite Supreme 10% HP and Opalescence Boost 38% HP immediately after the completion of the active bleaching treatment.

As in this study, most studies also found relatively small changes in the $a^*$ values and the major changes during tooth whitening took place in the $L^*$ and $b^*$ values (Grobler et al., 2010; Meireles et al., 2008; Ishikawa-Nagai et al., 2004; ADA, 2006; Ferrari et al., 2004).

Although no significant differences were found amongst the three groups with shade guide evaluation, the comparison of total colour change ($\Delta E_{ab}^*$) obtained
from spectrophotometric evaluation showed that Nite White ACP 10% CP (Group 1) resulted in significantly greater whitening (median $\Delta E^{*}_{ab} = 5.29$) immediately after completion of the bleaching treatment as compared to Opalescence Trèswhite Supreme 10% HP and Opalescence Boost 38% HP (Groups 2 and 3) with median $\Delta E^{*}_{ab}$ of 4.09 and 4.05 respectively. Previous studies also reported significantly better whitening with 14-day at-home bleaching using 10% CP than 60 minutes of in-office bleaching using 35% HP (Zekonis et al., 2003) while others reported similar results for tooth bleaching with different techniques (home bleaching, in-office bleaching with or without light source and a combination of in-office and home bleaching) after a 2-week period (Bernardon et al., 2010). The differences found in the two methods of shade evaluation (shade guide and spectrophotometer) correspond with the findings of other studies (Gurgan et al., 2010; Marson et al., 2008; Al Shethri et al., 2003).

No clinical trials could be found for Nite White 10% ACP. However, for the other previous 10% carbamide peroxide Nite White products it varied from a total unit colour improvement of 2.5; 6.0; 8.3; 8.81 up to 9.6 units respectively (Gerlach and Zhou, 2002; Callan et al., 2008; Toko et al., 1998; Hagiwara et al., 1999; Ishikawa-Nagai et al., 2004), in comparison to the 5.3 units found in this study immediately after 14 days of bleaching treatment (Figure 9.9).

The improvement as a result of the Nite White ACP treatment was over 4 units in the $L^*$ value, 3.3 units in the $b^*$ value and only 0.6 units in the $a^*$ value (Figures 9.6 to 9.8). In another spectrophotometric study approximately the same distribution was reported for Nite White Excel and Opalescence PF 10%
carbamide peroxide used for 4 hours daily for 14 days with a total colour change ($\Delta E_{ab}^*$) of 3.89 and 3.77, respectively (Ishikawa-Nagai et al., 2004). They reported a significant change of more than 3.6 units in $\Delta E_{ab}^*$ (for both Opalescence 10% PF and Nite White Excel) which was lower than the 5.25 found for Nite White ACP (treated overnight for 14 days) in this study. Furthermore, a $\Delta E_{ab}^*$ increase of 3.66 units (Grobler et al, 2010) (overnight for 14 days) and 3 units (dos Santos Medeiros et al., 2008) (overnight for 14 days) was also reported for Opalescence PF 10% carbamide peroxide.

In contrast to this study, many studies did not apply the manufacturer’s recommendation for the treatment period (overnight for 14 days). It also seems that the treatment period has an effect on the results and that a longer period of treatment and stronger peroxide solution is associated with better whitening (Meireles et al., 2008; dos Santos Medeiros and de Lima., 2008; Goo et al., 2004; Ritter et al., 2002).

At the stage of compiling this dissertation no clinical studies could be found in the literature to compare the whitening effect and colour relapse of Opalescence Trèswhite Supreme 10% hydrogen peroxide (Group 2) observed in the present study over six months using a spectrophotometer. However, the studies using the shade guide for colour evaluation have been mentioned earlier.

It is difficult to compare the results of the in-office bleaching product (Opalescence Boost 38% HP, Group 3) in the present study with the available data from other studies with the previous product containing 38% HP (Opalescence Xtra Boost) due to the differences in methodology and time.
intervals at which colour measurements were carried out. Gurgan, et al., (2009) reported a total colour improvement ($\Delta E_{ab}^*$) of 5.54 units with Opalescence Xtra Boost 38% HP after 2 applications of 15 minutes each. However, the colour measurements were carried out one week after the bleaching treatment. In a study by Al Shethri et al., (2003) demonstrated $\Delta E_{ab}^*$ of 2.54 units for 38% HP (Opalescence Xtra Boost) when the bleaching treatment was performed in two sessions of 30 minutes each 7 days apart and the final colour changes were measured one week after the last bleaching session using a chromameter. However, in the present study bleaching treatment with 38% HP was performed for 60 minutes in one session and total colour improvement ($\Delta E_{ab}^*$) of 4.05 units was found 30 minutes after bleaching.

All groups (Nite White ACP, Opalescence Trèswhite Supreme and Opalescence Boost) showed a major relapse in colour at 2-week follow-up (14 days after the completion of treatment). The decrease was significant for Nite White ACP and Opalescence Boost. From the results it can be seen that the decrease (relapse) in the whiteness/brightness ($\Delta L^*$) for Nite White ACP and Opalescence Trèswhite Supreme was approximately 37% and 23% respectively at the 2-weeks post-treatment follow-up visit while the $\Delta L^*$ values decreased by 61% in the Opalescence Boost group (Figures 9.6, 9.13 and 9.20). The yellowness ($\Delta b^*$) values decreased (relapsed) by 29% and 18% for Nite White ACP and Opalescence Boost (Figures 9.8 and 9.22) while Opalescence Trèswhite Supreme did not show any decrease (Figure 9.15). The results indicated that further decrease (relapse) was less in the $L^*$ and more in the $b^*$ components at 3- and 6-months follow-ups. Opalescence Boost showed the highest decrease (relapse) of 69% in $\Delta b^*$ after 6 months.
On the other hand, from the Figures 9.9 and 9.16 it can be seen that the overall major $\Delta E_{ab}$ decrease (relapse of whitening effect) of ~32% and 26% for Nite White ACP and Opalescence Trèswhite Supreme respectively (at-home bleaching) also took place during the 2 weeks after the completion of the bleaching treatments and then became stable for at least 6 months. The relapse after 1 month (which is 2 weeks after the 14 day treatment) was already 26% to 32% which showed that the decrease just after treatment is quite fast. However, there was still about a 70% improvement which lasted for at least 6 months. The decrease (relapse) in $\Delta E_{ab}$ for Opalescence Boost (in-office bleaching product) was 42% at 2-weeks post-treatment follow-up which was higher than the other two groups and it further increased (relapsed) to 58% after 3 months (Figure 9.23). In the Opalescence Boost group only 42% improvement could be seen after 3 months as compared to approximately 70% for the other groups. Therefore it seems that a single in-office bleaching treatment of 60 minutes with Opalescence Boost 38% hydrogen peroxide produced a significant whitening effect immediately after the bleaching treatment, but the rebound (relapse) in colour was also fast resulting in almost complete reversal to baseline in 3 to 6 months (Figure 9.23).

The colour relapse observed in the present study corresponds to the findings of the previous studies. Matis et al., (1998), in a 6-month clinical study, reported that most colour relapse in teeth bleached with 10% CP occurred during the first month. In a clinical study, Matis et al., (2000) showed that the colour relapse started when the subjects discontinued the bleaching treatment with 10% and 15% CP and was highest after 1 week post-bleaching and then continued slowly till 4 weeks post-bleaching. In contrast Meireles et al., (2008)
reported maintenance of bleaching effect of 10% and 16% CP for up to six months and Bernardon et al., (2010) showed no rebound in colour for 10% CP up to 4 months.

For the in-office bleaching, the findings of the present study corroborate the findings of the previous studies which also found short-term colour rebound (Al Shethri et al., 2003; Matis et al., 2007; Marson et al., 2008). Matis et al., (2007) reported 51% reduction in $\Delta E_{ab}^*$ after one week and up to 65% reduction six weeks after bleaching for various in-office bleaching products. Conversely, no rebound in colour was reported for the 35% HP in-office bleaching product by others (Bernardon et al., 2010).

It has been suggested that variations in $\Delta E_{ab}^*$ from 3.3 to 3.7 produces clinically noticeable colour changes (Vichi, Ferrari and Davidson, 2004). Although all groups produced total colour differences $> 3.7$ immediately after the completion of the bleaching treatment, only Nite White ACP 10% carbamide peroxide demonstrated $\Delta E_{ab}^*> 3.7$ after 6 months. The median $\Delta E_{ab}^*$ value for Opalescence Trèswhite Supreme 10% hydrogen peroxide was 2.94 and only 1.43 for Opalescence Boost 38% hydrogen peroxide after 6 months. If $\Delta E_{ab}^*$ of 3.3 is considered as the minimum cut point, Nite White ACP showed the most stable result where 12 (57%) subjects had $\Delta E_{ab}^* \geq 3.3$ after 6 months while Opalescence Trèswhite Supreme had only 7 (33%) subjects. On the other hand, no subjects were observed with $\Delta E_{ab}^* \geq 3.3$ after 6 months in Opalescence Boost (Table 9.5 and Figure 9.30).

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1 When using 3.7 as the upper value for clinically noticeable colour change range ($\Delta E_{ab}^*$).
2 When using 3.3 as the lower value for clinically noticeable colour change range ($\Delta E_{ab}^*$).
9.10.2- Side Effects

Tooth sensitivity is a common clinical side effect of tooth bleaching and is strongly related to the treatment period, peroxide concentration, and type of bleaching agent (Marson et al., 2008; Deliperi, Bardwell and Papanasiou, 2004). The exact mechanism responsible for bleaching-related tooth sensitivity is also not fully understood (Bernardon et al., 2010). Therefore, it is difficult to compare tooth sensitivity results reported in the literature with our results. In this study, tooth sensitivity was noticed in all groups. Although the number of subjects who experienced sensitivity and the number of days with sensitivity were different for each group, no significant differences were found amongst the groups (Table 9.6 and Figure 9.31). The oral side effects were transient and reversible in all groups.

Karpinia et al., (2002) reported tooth sensitivity or gingival irritation during the treatment period for 35-40% of the subjects who used Nite White Excel2 10% carbamide peroxide for 2 hours per day for 14 days. Leonard et al., (2001) reported tooth sensitivity or gingival irritation for 66% of the patients who bleached their teeth with Nite White Classic 10% carbamide peroxide during the active treatment period. On the other hand, Callan et al., (2008) reported only 16% sensitivity for Nite White Excel 2Z (2-week treatment period), but that was over a 302 days period. This can be expected as it was also found in this study that the sensitivity disappeared at the end of the active treatment period. Pohjola et al., (2002) reported a 25% gingival sensitivity for Nite White Excel 2Z when treated for 83 days but did not report sensitivity for any one of the products. Again it was calculated over a long period and what really happened
over the treatment period could be masked. Thus, these values are well removed from the 25% found for Nite White 10% carbamide peroxide in the present study during the 14 day treatment period.

The reasons for more tooth and gingival sensitivity in Group 2 (Opalescence Trèswhite Supreme 10% HP) might be due to high peroxide concentration and use of prefabricated bleaching trays as compared to custom-made properly fitting bleaching trays for Group 1 (Nite White ACP 10% CP). Li et al., (2005) reported no significant differences between a placebo and Opalescence Trèswhite (9% HP) bleaching product as far as gingival irritation and tooth sensitivity was concerned.

The sensitivity experienced by the subjects in Group 3 (Opalescence Boost 38% HP) was moderate to severe in most cases and it was more severe on the day of gel application. This might be due to the high concentration of the bleaching agent. However, the sensitivity dissipated mostly by Day 2 and disappeared completely by Day 4. The findings are in agreement with the previous studies with in-office bleaching products using 35% HP (Bernardon et al., 2010; Marson et al., 2008). Al Shethri et al., (2003) reported slight gingival irritation and tooth sensitivity with 38% HP (Opalescence Xtra Boost) which disappeared after two days. Auschill et al., (2005) also reported similar findings.

Some believe that tooth sensitivity can be decreased by the addition of various chemicals such as potassium nitrate, fluoride and amorphous calcium phosphate (ACP) (Chen et al., 2008). In this sense a significant reduction in the hypersensitivity was reported with the addition of amorphous calcium phosphate (ACP) to a 16% carbamide whitener (Giniger et al., 2005). However,
in another tooth whitening study it was found that the presence of amorphous calcium phosphate relative to the presence of potassium nitrate and fluoride could not make a difference in the sensitivity over a three month period. Matis et al., (2007) compared two bleaching products with different desensitizing agents and reported that 15% carbamide peroxide with potassium nitrate and fluoride showed no significant difference in sensitivity compared to 16% carbamide peroxide with amorphous calcium phosphate. Tam (2001) reported that the addition of potassium nitrate and fluoride to 10% carbamide peroxide bleaching gel resulted in less sensitivity compared to the bleaching gel without any desensitizing agent.

The above clinical data indicated no difference in tooth sensitivity between amorphous calcium phosphate on the one hand and potassium nitrate plus fluoride on the other. However, it is possible that a combination of all three might have a combined effect. One of the products under investigation in the present study (Nite White ACP 10% carbamide peroxide with amorphous calcium phosphate, potassium nitrate, and fluoride) with all three mentioned chemicals showed lower tooth sensitivity (57%) than its predecessors without it for example 66% for Nite White Classic® (Leonard et al., 2001) and 62% for Nite White Excel (Tam, 1999).
9.11- CONCLUSIONS

1. All products effectively whitened teeth when evaluated by subjective and objective methods immediately after bleaching treatment.

2. Objective evaluation using the spectrophotometer showed significantly better whitening efficacy for Nite White ACP 10% ($\Delta E_{ab}^* = 5.29$) immediately after completion of bleaching treatment as compared to Opalescence Trèswhite Supreme 10% HP ($\Delta E_{ab}^* = 4.09$) and Opalescence Boost 38% HP ($\Delta E_{ab}^* = 4.05$). However, subjective (shade guide) evaluation did not indicate any significant differences in the efficacy among the three groups.

3. Major changes were observed in the $L^*$ and $b^*$ components of CIE L*a*b* while the $a^*$ values showed only minor changes.

4. All groups displayed major rebound in colour during the two weeks after the completion of the bleaching treatment. The median $\Delta E_{ab}^*$ decrease was 30% for Nite White ACP, 26% for Opalescence Trèswhite Supreme and 42% for Opalescence Boost. The whitening effect became stable for at least six months in subjects treated with Nite White ACP and Opalescence Trèswhite Supreme. However, subjects treated with Opalescence Boost showed a further significant rebound in colour.

5. All products resulted in tooth hypersensitivity. Side effects were transient and reversible. Higher tooth sensitivity was observed for Opalescence Boost 38% HP immediately after the bleaching treatment but it was not significantly different from the other products.
6. Opalescence Trèswhite Supreme with prefabricated ill-fitting trays and higher peroxide concentration caused greater gingival irritation/burning than Nite White ACP with custom-fabricated trays and low peroxide concentration.

7. Opalescence Boost caused only minor gingival irritation because the procedure was performed in the dental office under a controlled environment with soft tissue protection.

8. Overall Nite White ACP demonstrated significant tooth-whitening (unit increase= 5.29) with a low tooth sensitivity probably due to the presence of amorphous calcium phosphate, potassium nitrate, and fluoride. The whitening effect decreased the most after one month and then maintained well even after a 6-month period (units 3.87).

Dentist-supervised home bleaching with 10% carbamide peroxide using a custom-fitting tray is safe and effective tooth-whitening procedure. Colour improvement (whitening effect) is well maintained for longer periods without the need for re-bleaching.

In-office bleaching procedure although effective, short term colour relapse is high. In order to attain long term and stable tooth-whitening results, one will need either two or more in-office bleaching sessions or a combination of an in-office and dentist-supervised home bleaching procedure.
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CHAPTER 10
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

10.1- SUMMARY AND DISCUSSION

In Chapter 2, as an introduction to the main topic of study, a major aspect of aesthetic dentistry was presented. During the past decade, tooth-whitening or –bleaching has become one of the fastest growing areas of aesthetic dentistry. Although the exact mechanism of tooth-bleaching is not fully understood, it is presumed to be an oxidation-reduction process which affects both enamel and dentine. Hydrogen peroxide diffuses initially into and through enamel to dentine, producing free radicals. The free radicals with unpaired electrons are extremely unstable and react with highly pigmented organic (carbon-rings) molecules found within the tooth structure and breaks them down into smaller, less pigmented components (carbon-chains). The smaller molecules reflect less light, thus creating a reduction in colour or “whitening effect”.

Literature revealed that tooth-whitening is not a new field and a number of products and methods are available, the most common being the nightguard vital bleaching method. Other methods include in-office or power bleaching and recently introduced so-called over-the-counter products such as whitening strips and paint-on gels. A number of studies have investigated the efficacy of dentist-supervised home bleaching products. However, little information is available about in-office and over-the-counter bleaching products. However, efficacy studies demonstrated great variation in design, methodology and post-
treatment evaluation periods and lacked standardized protocol to measure colour changes.

Although the use of a shade guide to measure colour improvements following bleaching treatment is the most common method, it is subjective and has poor validity. Recently a large number of computer-based instruments (spectrophotometers and colorimeters) have become increasingly popular. These instruments provide objective measurements in three dimensional colour space (CIE L*a*b*) and are not prone to observer influences. CIE L*a*b* measurements adequately represent the colour perception of the human eye in all 3 dimensions of colour space.

Finally, the possible side effects of tooth-whitening products were reviewed with particular emphasis on tooth-sensitivity and effects on dental enamel. Tooth sensitivity appeared to be the most common clinical side effect of tooth-whitening treatment and occurs in 55 to 75% of the patients. Although exact causes of tooth sensitivity are not fully understood, it is commonly related to tooth dehydration due to bleaching treatment. In order to reduce the occurrence of tooth sensitivity, fluoride, potassium nitrate and amorphous calcium phosphate have been introduced in recent bleaching products. The available literature is contradictory and reported little or no benefit of these substances.

Tooth-whitening involves direct contact of the bleaching agent to the enamel surface for an extensive period of time which differs between manufacturers. This fact increased concerns about the possible adverse effects of such a strong oxidizing agent on the enamel/dentine. Some studies reported alterations in surface morphology and microhardness of enamel following
bleaching treatment while others reported no effect. The literature suggests that microhardness experiments also lack standardized protocols; differ in the preparation of specimens, the position of hardness indents, forces applied and exposure times.

In **Chapter 4**, the issue of safety of various tooth-whitening products was addressed. The purpose of this study was to measure the pH of 21 commercially available tooth-whitening products using Orion Expandable Ion Analyzer with a combination semi-micro pH electrode. Tooth-whitening products were grouped into four categories: dentist-supervised home bleaching products, in-office bleaching products, over-the-counter bleaching products and whitening toothpastes or rinses. It was hypothesized that tooth-whitening products should have neutral (7.0) or near neutral pH.

Three samples of each product were measured at room temperature. One product required more than one step to complete the bleaching process hence each step was measured separately and a combination of both steps was also measured. The pH of a commonly consumed carbonated soft drink (Coca Cola) was also measured for reference purposes.

The pH of five dentist-supervised home bleaching products ranged from 4.88 to 6.81 with a mean pH of 6.21 ± 0.76. The pH of five in-office bleaching products ranged from 5.30 to 7.85 with a mean pH of 6.26 ± 1.19. The four over-the-counter whitening products had a mean pH of 5.07 ± 1.74 and range from 3.76 to 8.03 and the pH of seven whitening toothpastes ranged from 6.61 to 9.68 with a mean pH of 7.66 ± 1.19.
The over-the-counter product category demonstrated significantly lower pH values than all other categories \((p<0.05)\). Rapid White and Absolute White showed a low (acidic) pH of 3.76 and 3.94 respectively, while Colgate Advanced Whitening toothpaste showed an alkaline pH of 9.68 \((0.03)\). The pH of the Coca Cola drink was 2.62 \((0.04)\) and it was not used in the analysis.

The hypothesis that tooth-whitening products have a neutral or near neutral pH was partially rejected because highly acidic and alkaline pH levels were recorded for some products. During the bleaching procedure teeth and soft tissues are exposed to a low or high pH for an extensive period of time which may cause adverse effects. Highly acidic solutions are known to soften enamel (demineralize) and produce dental erosion. Demineralization of enamel has been reported for pH lower than 5.2 to 5.8.

The lower (acidic) pH levels recorded for over-the-counter bleaching products compared to dentist-supervised home bleaching products and in-office bleaching products in the present study, demonstrate the potential damage these products can cause to the tooth structure. Furthermore, over-the-counter products are applied either using ill-fitting prefabricated trays or directly brushed onto the tooth surface (paint-on). Therefore, there is a greater risk of soft tissue injury and deglutition of bleaching agent by the patients. In contrast, dentist-supervised home bleaching products are applied in custom-fitting trays and in-office bleaching procedures are carried out with a soft-tissue protective barrier that protects the soft tissue and minimizes the risk of injury.

The degree of damage also depends upon the exposure time and the frequency of application of a bleaching agent as far as the pH is concerned.
The products evaluated in the present study differed greatly in treatment time and frequency of application; therefore, it is important to consider these factors when evaluating the possible side effects of a product.

The study in Chapter 5 investigated the concentration level of hydrogen peroxide in various tooth-whitening products. Peroxides are widely used as industrial reagents due to their oxidising properties. Cosmetic products such as teeth whiteners, toothpastes and mouth washes are meant for oral application. Therefore, the concentration of hydrogen peroxide in these products is critical. The purpose of this study was to determine the concentration of hydrogen peroxide in various professional and over-the-counter tooth-whitening products and to check whether it corresponds to the respective concentration of the product given by the manufacturer.

Sixteen commercially available tooth-whitening products containing various concentrations of carbamide peroxide or hydrogen peroxide were investigated in the study. Samples of all products were measured in triplicate from three different packages. The peroxide concentration was determined by the oxy-reduction titration method. Means and standard deviations were calculated for all determinations.

The mean concentration level of hydrogen peroxide in various dentist-supervised home bleaching and in-office bleaching products was: Nite White® ACP 10% CP = 3.75%; Yotuel® Patient 10% CP = 3.02%; Opalescence® PF 10% CP = 3.40%; Opalescence® PF 20% CP = 6.31%; Opalescence® Trèswhite Supreme 10% HP = 8.98%; Yotuel® 10 Minutes 30% CP = 9.93%;
Opalescence® Quick 45% CP = 16.24%, Yotuel® Special 35% HP = 27.19 and Opalescence® Boost 38% HP = 37.08%.

The concentration level of hydrogen peroxide in over-the-counter whitening products was: White Glo = 1.24%, Absolute White = 3.20% and Speed White = 5.57%. Only Colgate Plax whitening rinse showed 1.50% hydrogen peroxide while it was almost negligible (< 0.05%) in other whitening toothpastes and oral rinses.

It has been reported that 10% carbamide peroxide dissociates into a hydrogen peroxide concentration of 3 to 3.5%. Therefore, carbamide peroxide based whitening products were expected to have the corresponding hydrogen peroxide concentrations. Although the concentrations of hydrogen peroxide in most of the dentist-supervised home bleaching products and in-office bleaching products were close to the expected range, the results revealed lower than expected concentrations in Opalesce nce Trèswhite Supreme and Yotuel Special. Opalescence Trèswhite Supreme and the freshly prepared gel of Yotuel Special were supposed to have 10% and 35% hydrogen peroxide respectively but the concentrations found in the present study were only 8.98% and 27.19% respectively. Lower peroxide concentrations will result in reduced efficacy of these products.

Efficacy of bleaching agent has been related to the peroxide concentration, frequency of application and time it stays in contact with the tooth surface. Therefore, these products would probably require longer treatment times than those recommended by the manufacturers.
Slight differences were observed in the hydrogen peroxide concentration of three 10% carbamide peroxide products (Nite White = 3.75%, Opalescence PF = 3.40% and Yotuel Patient = 3.02%). This might be due to the differences in formulations, manufacturing and quality of the products.

The study in Chapter 6 addressed the issue of safety of tooth-whitening products with respect to enamel microhardness. The purpose of this in vitro study was to evaluate the effect of various dentist-supervised home bleaching products and in-office bleaching products containing carbamide peroxide (CP) or hydrogen peroxide (HP), on enamel microhardness.

All whitening products tested in this study decreased enamel microhardness except Opalescence Boost 38% HP. Groups 2,3,4,5 &7 (Nite White ACP 10% CP, Yotuel Patient 10% CP, Opalescence PF 10% CP, Opalescence PF 20% CP and Yotuel 10 Minutes 30% CP) showed a significant decrease in enamel microhardness as compared to the control group (p<0.05). Groups 2, 3 and 7 also differed significantly from all other test groups (p<0.05). The highest damage was recorded for Group 2 (Nite White), which also differed significantly from Groups 3 and 7. SEM images also showed damage to enamel.

Enamel microhardness varies from area to area due to large regional variations in mineralization and structure. Therefore, the base-line and the post-treatment indents were made as close as possible to each other (~ 10 µm apart). Furthermore, the effect of some bleaching treatments on the hardness of enamel was relatively small and could easily be masked if different areas on enamel were used.
In general, carbamide peroxide based whitening products (Groups 2,3,4,5,7 & 8) showed more damage than hydrogen peroxide based products (Groups 6,9,10) but the treatment periods also influenced the hardness values. It seemed that a combination of a shorter treatment period (2-7 hours) with a higher peroxide concentration (Groups 6,8,9,10; 10% to 38%) gave rise to a lower degree of damage to enamel when compared to a combination of a lower concentration treatment (Groups 2,3,4,5; 3.35% and 6.7%) for a longer period (112 hours). In contrast to our study (8 hours/day), other studies reported no change in enamel microhardness when 10% CP was used for a shorter treatment period of 2 or 3 hours per day for 14 days. These results underline the negative effect of longer treatment periods.

Fluoride, potassium nitrate and amorphous calcium phosphate (ACP) have been introduced in recent bleaching products to prevent either hypersensitivity or demineralization effects. Fluoride protects enamel against demineralization challenges by forming a calcium fluoride layer while rapid hydrolyses of ACP forms an apatite similar to the carbonated apatite of tooth mineral to protect enamel. However, in this study, a decrease in enamel microhardness was observed for the products containing fluoride, potassium nitrate or ACP. The findings are in agreement with other previous studies.

The damaging effect was also confirmed by scanning electron micrographs of pre- and post-treatment indents which correspond to the results found in their hardness values.
The study in Chapter 7 was complimentary to that in Chapter 6. The purpose of this study was to evaluate the effects of four over-the-counter tooth-whitening products on enamel microhardness.

Enamel Blocks were exposed to: Rapid White (Group 2, n=10); Absolute White (Group 3, n=10); White Glo (Group 4, n=10) and Speed White (Group 5, n=10) according to the manufacturers’ instructions. The control (Group 1, n=10) was enamel blocks kept in artificial saliva at 37°C without any treatment. The microhardness values were obtained before exposure (baseline) and after 1, 7 and a 14-day treatment period. Specimens were kept in artificial saliva at 37°C between treatments. Data were analysed using the Wilcoxon Signed Rank Sum Test, Kruskal-Wallis one-way ANOVA and Tukey-Kramer Multiple Comparison Test significant at p<0.05.

Over-the-counter tooth-whitening products reduced (affected) enamel microhardness at some point during the treatment except Speed White where enamel microhardness showed an increase. A significant reduction in enamel microhardness was recorded for the White Glo group after 1 treatment day and for the Absolute White group after 7 treatment days only. The Rapid White group showed continuous reduction in enamel microhardness after 1, 7, and 14 treatment days and differed significantly from the control and other treatment groups (p<0.05). No statistically significant differences were found amongst the control, Absolute White and White Glo groups after 14 days of active bleaching treatment.

The results of the present study demonstrated significant reduction in enamel microhardness for Rapid White after 14 days. Rapid White is a non-peroxide
whitening product containing sodium chlorite and citric acid amongst other ingredients. Sodium chlorite reacts with citric acid and produces chlorine dioxide as active bleaching agent. The results were in agreement with previous studies which also reported significant decrease in enamel microhardness following treatment with bleaching products containing sodium chlorite and citric acid. Absolute White based on hydrogen peroxide was the second product which resulted in relatively more reduction in enamel microhardness as compared to White Glo, Speed White and the control group, although differences were not statistically significant. The results of pH determinations in Chapter 4 demonstrated an acidic pH of 3.76 and 3.94 for Rapid White and Absolute White respectively. Enamel demineralization has been reported to be obvious for pH levels lower than 5.8 to 5.2. Although exposure times for over-the-counter whitening products were relatively shorter (5 to 30 minutes per day), the acidic pH of these products might have resulted in damage to enamel.

In vitro studies demonstrated artificial saliva as an effective agent in the re-hardening (remineralization) of softened enamel. However, remineralization studies of initial enamel lesions in artificial saliva demonstrated that the regaining of microhardness was dependent on the storage periods. After 14 days of bleaching treatment and storage in artificial saliva, the results of the present study demonstrated almost full recovery for the White Glo group and partial regaining of enamel microhardness in the Absolute White group. However, the Rapid White group showed a continuous decrease in enamel microhardness.
Thus, it can be hypothesized that in spite of the remineralization potential of saliva, softening of enamel as a result of acid attacks as seen with the Rapid White bleaching product might be so serious that it cannot be repaired within a short time in clinical situations. This makes enamel more susceptible to surface loss due to abrasive influences such as tooth-brushing.

The study in Chapter 8 addressed the question of the efficacy of different tooth-whitening products and methods. Today, a large number of tooth-whitening products are commercially available to dentists and consumers with little information about their relative effectiveness. Therefore, the purpose of this in vitro study was to evaluate the effects of 13 tooth-whitening products with different peroxide concentrations, active agents and application times on previously stained human incisor teeth, using a spectrophotometer. It was hypothesized that there is no difference in the efficacy of different tooth-whitening products and methods.

Tooth colour measurements were performed at baseline (pre-treatment) and 24 hours after completion of bleaching treatment using a spectrophotometer. The spectrophotometer measured the colour of teeth based on the CIE L*a*b* colour space system defined by the Commission Internationale de l’Eclairage (CIE 1976).

Changes in individual colour components ($\Delta L^*$, $\Delta a^*$ and $\Delta b^*$) and total colour changes ($\Delta E^*_{ab}$) from baseline were analysed using the Kruskal-Wallis one-way ANOVA followed by the Tukey-Kramer Multiple Comparison Test for differences amongst the different groups (significance level was 5%).
The null hypothesis was rejected because tooth-whitening products showed significant difference in bleaching efficacy. Tray-based dentist-supervised home bleaching products; Nite White ACP 10% ($\Delta E_{ab}^*$ 6.19), Yotuel Patient 10% ($\Delta E_{ab}^*$ 6.48), Opalescence PF 10% ($\Delta E_{ab}^*$ 6.77) and Opalescence PF 20% ($\Delta E_{ab}^*$ 9.68) showed a more pronounced bleaching effect than all in-office and over-the-counter bleaching products. Opalescence PF 20% was the most effective product, significantly superior to all other bleaching products ($p<0.05$).

Furthermore, in-office bleaching products: Opalescence Boost ($\Delta E_{ab}^*$ 5.24), Opalescence Quick 45% ($\Delta E_{ab}^*$ 3.48), Yotuel Special ($\Delta E_{ab}^*$ 3.46) and over-the-counter bleaching product, Rapid White ($\Delta E_{ab}^*$ 3.85) showed better whitening than Yotuel 10 Minutes ($\Delta E_{ab}^*$ 2.44), Absolute White ($\Delta E_{ab}^*$ 3.08), White Glo ($\Delta E_{ab}^*$ 2.37) and Speed White ($\Delta E_{ab}^*$ 1.59).

Further analysis showed that the $L^*$ (lightness) component was a more significant parameter for comparison between products than the $a^*$ and $b^*$ values. Bleaching treatment with all products resulted in an increase in $L^*$ (lightness) values and a decrease in $b^*$ (yellowness) values proportional to the effectiveness of the product used. Only a few products showed a decrease in $a^*$ values while most of the bleaching products did not produce significant changes in $a^*$.

Tray-based dentist-supervised home bleaching products showed a more pronounced whitening effect as compared to all other products. These results are in agreement with the findings of other clinical and in vitro studies. The potential of carbamide peroxide in terms of its penetration through enamel and uniform bleaching of dentine has been well documented in literature. It has
been reported that the bleaching effect is dependent on the concentration of carbamide peroxide and duration of exposure. Bleaching agents with higher concentrations of carbamide peroxide produce better whitening than those with lower carbamide peroxide concentrations. In the present study, Opalescence PF containing 20% carbamide peroxide also showed significantly better whitening than the products containing 10% carbamide peroxide.

During the bleaching process breakdown of hydrogen peroxide releases free radicals which penetrate through enamel into dentine and oxidize the colour pigments. The diffusion of peroxide depends on the diffusion coefficient, duration of application and concentration of active bleaching agent and this might explain the reason for the reduced efficacy of in-office and over-the-counter bleaching products.

Although in-office bleaching products contained higher peroxide concentrations, the exposure times were far shorter (1.33 to 2.67 hours) than tray-based dentist-supervised home bleaching products (112 hours). It can be assumed that addition of chemical activators as well as the use of higher concentrations of hydrogen peroxide (10% to 38%) did not fully compensate for the reduced contact time between the bleaching agent and the tooth structure. Similarly, over-the-counter products had shorter exposure times (1.16 to 7 hours) as well as lower hydrogen peroxide concentrations (1.24% to 5.57%), except for Rapid White which contained sodium chlorite.

The study in Chapter 9 was complementary to that in Chapter 8 and the in vivo issues of whitening potential, stability of whitening effect and oral side
effects associated with three different tooth-whitening products were addressed.

The purpose of this study was to evaluate the efficacy, rebound in whitening effect over time and side effects of two dentist-supervised home bleaching products (Nite White ACP 10% CP and Opalescence Trèswhite Supreme 10% HP) and an in-office bleaching product (Opalescence Boost 38% HP).

A randomized, single centre, observer blind clinical trial was conducted. Sixty one volunteers with an A2 or darker shade, willing to have their teeth whitened and who signed an informed consent form, were enrolled in this study. All subjects received complete prophylaxis at least two weeks before the start of treatment. Subjects were randomly divided into three groups and received treatment according to the manufacturers’ instructions: Nite White ACP 10% CP applied in custom-fitting trays for 8 hours overnight for 14 days (Group 1, N=21), Opalescence Trèswhite Supreme 10% HP applied for 60 minutes per day for 14 days (Group 2, N=21) and Opalescence Boost 38% HP applied for 60 minutes (3 x 20 minutes) in one session (Group 3, N=19).

Objective tooth colour measurements of maxillary central incisors were taken using a spectrophotometer at baseline (pre-treatment), after the completion of active bleaching treatment (post-treatment), as well as 2 weeks post-treatment, after 3-months and 6-months. The spectrophotometer measured the colour of teeth in the CIE L*a*b* colour space. Changes in tooth colour were also determined subjectively with a Vita Lumin Vacuum shade guide at baseline and after completion of bleaching treatment (post-treatment). A sensitivity sheet
was given to all participants to record any tooth sensitivity experienced during the treatment period. Gingival irritation was noted as present or absent.

Both shade guide and spectrophotometer evaluations demonstrated significant colour improvements for all groups immediately after the completion of bleaching treatments. Nite White ACP 10% CP ($\Delta E_{ab}^* 5.29$) showed significantly better whitening than Opalescence Trèswhite Supreme 10% HP ($\Delta E_{ab}^* 4.09$) and Opalescence Boost 38% HP ($\Delta E_{ab}^* 4.05$).

All groups showed significant relapse in tooth colour (whitening effect). The relapse was highest during the first two weeks after the completion of the active bleaching treatments. The median decrease (relapse) in total colour change ($\Delta E_{ab}^*$) was 28% for Nite White ACP, 28% for Trèswhite Supreme and 65% for Opalescence Boost after 6 months. Colour improvements in the Nite White ACP and Opalescence Trèswhite Supreme groups remained significantly better when compared to baseline and Opalescence Boost after 6 months.

The percentage of subjects with tooth sensitivity ranged from 57% to 81%. Although the total number of days on which subjects experienced tooth sensitivity were more in the Nite White and Opalescence Trèswhite groups than in the Opalescence Boost group, the differences were not statistically significant ($p>0.05$). Four subjects in the Nite White group and 7 subjects in the Opalescence Trèswhite group experienced gingival irritation during the bleaching treatment period.

The application of 10% carbamide peroxide in a custom-fitting tray for 8 hours per day for 2 weeks demonstrated significantly better whitening than the application of 10% hydrogen peroxide in prefabricated ill-fitting trays for 1 hour.
per day for 2 weeks or 38% hydrogen peroxide in a dental chair for 1 hour. This study confirms the findings of the previous clinical studies showing better effectiveness of tray-based home bleaching products. The in vitro study in Chapter 9 also demonstrated better whitening for Nite White ACP 10% CP when compared to Opalescence Trèswhite Supreme 10% HP and Opalescence Boost 38% HP.

The colour relapse observed in the present study corresponds to the findings of the previous studies. A 6-month clinical study reported that most colour relapse in teeth bleached with 10% CP occurred during the first month. Another study showed that the colour relapse was highest after 1 week post-bleaching and then continued slowly till 4 weeks post-bleaching, while some studies reported no rebound in colour for 10% CP up to 4 months. The findings of the present study corroborate the findings of the previous studies which found short-term colour relapse with 51% reduction in $\Delta E_{ab}$ after one week and up to 65% reduction six weeks after bleaching for various in-office bleaching products.

Tooth sensitivity is a common clinical side effect of tooth bleaching and is strongly related to the treatment period, peroxide concentration and type of bleaching agent. In this study, tooth sensitivity was noticed in all groups. Although the number of subjects who experienced sensitivity and the number of days with sensitivity were different for each group, no significant differences were found amongst the groups. The oral side effects (tooth sensitivity and gingival irritation) were transient and disappeared after the cessation of treatment.
10.2- CONCLUSIONS

From the findings of the above studies, the following conclusions can be drawn:

1. Most of the tested dentist-supervised home-bleaching products, in-office bleaching products and whitening toothpastes had near neutral pH. Over-the-counter whitening products showed the lowest pH (acidic) levels as compared to all other products. Safety of these products is questionable because strong acidic products will demineralise enamel and dentine and also damage soft tissues of the oral cavity.

2. Although slight variations in the concentration of hydrogen peroxide were observed for dentist-supervised home bleaching products and in-office bleaching products, most of the products were close to the manufacturers’ suggested values. The concentration of hydrogen peroxide in various over-the-counter whitening products ranged from 1.24\% to 5.57\%.

3. The hydrogen peroxide concentration in whitening toothpastes and rinses was negligible except Colgate Plax whitening rinse which had 1.50\% hydrogen peroxide.

4. The tested dentist-supervised home bleaching products, in-office bleaching products and over-the-counter bleaching products decreased enamel microhardness, except Opalescence Boost 38\% HP and Speed White.
5. The products containing carbamide peroxide showed more damage to enamel probably because of the longer application times of the bleaching agents.

6. Scanning electron micrographs revealed undesirable negative effects on enamel.

7. Rapid White containing sodium chlorite in combination with citric acid as an activator reduced enamel microhardness significantly more, possibly due to its low pH.

8. Although saliva acts as a buffering and remineralization solution, acidity and lack of control over the use of over-the-counter whitening products may result in excessive loss of enamel structure due to abrasion and/or erosion.

9. Tray-based dentist-supervised home bleaching products showed more pronounced whitening effect than in-office and over-the-counter bleaching products. Over-the-counter bleaching products were the least effective bleaching agents and their use may be of little clinical significance.

10. The clinical trial showed significantly better whitening efficacy for Nite White ACP 10% CP ($\Delta E_{ab}^* = 5.29$) immediately after bleaching as compared to Opalescence Trèswhite Supreme 10% HP ($\Delta E_{ab}^* = 4.09$) and Opalescence Boost 38% HP ($\Delta E_{ab}^* = 4.05$).
11. Post-bleaching relapse in colour started as soon as the bleaching treatment was discontinued and was highest during the first 2 weeks after the completion of active treatment.

12. The whitening effect became stable for at least six months in subjects treated with Nite White ACP 10% CP and Opalescence Trèswhite Supreme 10% HP. However, subjects treated with Opalescence Boost 38% HP showed further significant relapse in colour.

13. The tested products resulted in tooth hypersensitivity. Higher tooth sensitivity was observed for Opalescence Boost 38% HP immediately after the bleaching treatment but it was not significantly different from the other products. Side effects were transient and reversible.

14. Opalescence Trèswhite Supreme with prefabricated ill-fitting trays and higher peroxide concentration caused greater gingival irritation/burning than Nite White ACP with custom-fabricated trays and low peroxide concentration.

15. The presence of amorphous calcium phosphate, potassium nitrate and/or fluoride in bleaching products might have reduced the tooth sensitivity but did not result in its complete prevention.
10.3- SHORT SUMMARY

Bleaching is an effective tooth whitening procedure. Dentists should choose a product and technique which is most suitable for their patients. Dentist-supervised home bleaching (nightguard vital bleaching) using 10% carbamide peroxide bleaching agent in a custom-fitting tray should be the procedure of choice in most cases.

In-office bleaching procedure can be used in patients who are not comfortable with wearing bleaching trays or in those who require faster results (in combination with dentist-supervised home bleaching). When used alone two or more in-office bleaching sessions (few days apart) will be required to achieve sustainable long term results.

Tooth sensitivity and gingival irritation are temporary side effects and usually disappear shortly after the completion of the bleaching treatment. Bleaching does result in softening (demineralization) of enamel. These side effects can be minimized by using bleaching products with additives such as fluoride, potassium nitrate and amorphous calcium phosphate and fluoride toothpastes or gels.

Relapse in whitening effect (tooth colour) is the highest during first two weeks after the completion of the bleaching treatment. Therefore, following bleaching treatment a waiting period of 2 to 4 weeks is recommended before carrying out any aesthetic restorative procedures such composite fillings, crowns etc.

The use of over-the-counter bleaching products which lack scientific evidence to support their safety and effectiveness should be discouraged by dentists.
Over-the-counter bleaching products tested in this study showed low (acidic) pH levels, softening of enamel and lower whitening effect compared to the professional bleaching product. Extended use of over-the-counter bleaching products can result in excessive loss of tooth structure (enamel) and soft tissue injury.
10.4- RECOMMENDATIONS FOR FUTURE RESEARCH PROJECTS

1. The low pH observed for over-the-counter bleaching products suggests further investigation of other over-the-counter products for possible side effects and whitening efficacy of these products.

2. The microhardness evaluation protocol used in the present investigations enabled us to record even small changes in enamel microhardness. Future investigation should be carried out using this protocol to evaluate post-bleaching remineralization potential of fluoride and artificial saliva.

3. More independent long-term clinical trials should be carried out to evaluate the efficacy and stability of colour for other tooth-whitening products and techniques not evaluated in this study.
Appendix 1

Scanning Electron Microscope Images of Human Enamel

The enamel surfaces were exposed to various treatments and scanning electron micrographs were obtained. These images are presented here for interest sake and they do not represent any conclusive evidence of positive or negative effects of whitening treatments.

Figure 1: Scanning electron micrograph of enamel surface of maxillary central incisor not exposed to any whitening treatment (x2000 original magnification). The surface appears normal.

Figure 2: Scanning electron micrograph of enamel surface of maxillary molar not exposed to any whitening treatment (x2000 original magnification). Note surface pits indicating demineralized or damaged enamel. It can be hypothesized that enamel was hypomineralized in this area and it was damaged as a result of exposure to acidic substances during everyday life.

Figure 3: Scanning electron micrograph of enamel surface of maxillary central incisor not exposed to any whitening treatment (x2000 original magnification). Similar to Figure 2 with less apparent damage.
**Figure 4:** Scanning electron micrograph of enamel surface of human molar tooth polished up to 1200 grit fineness not exposed to any whitening treatment (x1000 original magnification). Note scratch marks of polishing paper on the surface.

**Figure 5:** Scanning electron micrograph of enamel surface of human molar tooth polished up to 1200 grit fineness and exposed to Coca Cola soft drink for 5 minutes per day for 14 days (x1000 original magnification). Note flat surface with no visible scratch marks and exposed enamel prisms. This shows enamel erosion caused by a low pH soft drink.

**Figure 6:** Scanning electron micrograph of enamel surface of human molar tooth (unpolished) exposed to Coca Cola soft drink for 5 minutes per day for 14 days (x1000 original magnification).
Rapid White Over-the-counter Whitening Product

Figure 7: Scanning electron micrograph of control enamel surface of maxillary central incisor not exposed to any whitening treatment (x2000 original magnification).

Figure 8: A, Scanning electron micrograph of enamel surface bleached with Rapid White containing sodium hypochlorite with citric acid for 10 minutes (x2000 original magnification).

Figure 8: B, Scanning electron micrograph of enamel surface bleached with Rapid White for 10 minutes at higher magnification (x20000 original magnification). Arrows indicate cracks along enamel prisms.
Opalescence PF 10% carbamide peroxide At-home whitening product

**Figure 11:** Scanning electron micrograph of control enamel surface of maxillary central incisor not exposed to any whitening treatment (x2000 original magnification).

**Figure 12:** A, Scanning electron micrograph of enamel surface bleached with Opalescence PF 10% carbamide peroxide for 8 hours (x2000 original magnification). The enamel surface appears similar to the control image.

**Figure 12:** B, Scanning electron micrograph of enamel surface bleached with Opalescence PF 10% carbamide peroxide for 8 hours (x20000 original magnification). Arrows point out the increased roughness of the enamel surface visible at higher magnification as compared to the control.
Figure 13: Scanning electron micrograph of control enamel surface of maxillary central incisor not exposed to any whitening treatment (x2000 original magnification)

Figure 14: A, Scanning electron micrograph of enamel surface bleached with Opalescence PF 20% carbamide peroxide for 8 hours (x2000 original magnification).

Figure 14: B, Scanning electron micrograph of enamel surface leached with Opalescence PF 20% carbamide peroxide for 8 hours (x20000 original magnification). Arrows indicate loss of interprismatic substance (demineralization) of enamel.
Figure 15: Scanning electron micrograph of enamel surface of human molar tooth polished up to 1200 grit fineness and exposed to Opalescence PF 20% carbamide peroxide for 8 hours per day for 14 days (x2000 original magnification).

Figure 16: Scanning electron micrograph of enamel surface of human molar tooth (unpolished) exposed to Opalescence PF Quick 45% carbamide peroxide for 30 minutes per day for 14 days (x2000 original magnification).
Appendix 2

LIST OF PUBLICATIONS AND PRESENTATIONS

- ARTICLES -


ABSTRACTS


• CONFERENCE PRESENTATIONS

1. Effect of over-the-counter tooth-whitening products on enamel microhardness. 43rd Annual Scientific Meeting of the South African Division of IADR, September, 2010, Pretoria, South Africa.

2. Effect of various tooth-whitening products on enamel microhardness. 88th General Session of IADR, July 2010, Barcelona, Spain (Unilever/IADR Hatton Competition).

3. Effect of various tooth-whitening products on enamel microhardness. 2nd Scientific Meeting of the African Middle East Region of IADR, September 2009, Mombasa, Kenya.


5. Effect of four different Opalescence tooth-whitening products on enamel microhardness. 42nd Annual Scientific Meeting of the South African Division of IADR, August, 2008, Cape Town, South Africa.

6. The pH evaluation of various tooth-whitening products. 41st Annual Scientific Meeting of the South African Division of IADR; September, 2007, Centurion, South Africa.
Appendix 3

RESEARCH AWARDS

- **IADR/Unilever Hatton Divisional Award, 2010,**
  88\textsuperscript{th} General Session of IADR, July, 2010 Barcelona, Spain.

- **Colgate Postgraduate Award, 2009** (South African Division)
  African Middle East Regional Conference, September, 2009; Whitesands Hotel, Mombasa, Kenya.

- **Hugo Retief Award** (Best Oral Presentation Dental Materials)
  African Middle East Regional Conference of IADR, September, 2009; Whitesands Hotel, Mombasa, Kenya.

- **Professor Cornelis H Pameijer Fellowship, 2008**
Appendix 4

INSTRUCTION LEAFLET FOR NITE WHITE ACP GROUP

1- Floss & brush your teeth.
2- In a counter clockwise motion, twist and pull off the clear plastic cap from the end of the syringe.
3- Place the mixing nozzle on the end of the syringe and secure by twisting the mixing nozzle in a clockwise motion.
4- To fill the custom trays, place one dot of whitening gel in the center of each tooth compartment. Do not overfill the trays.
5- Place the tray with the gel in your mouth. As you insert the tray, be careful not to push the gel out of the tray. You may see “bubbling” within your trays while wearing them. This bubbling is actually part of the whitening process.
6- Use extra care to avoid getting gel on your gums. Remove excess gel with cotton swab or a dry toothbrush.
7- Wear tray overnight (6-8 hrs). After whitening rinse the trays with cold water. If necessary, use a toothbrush to remove any residual gel. Store the tray in a cool and dry place.
8- Rinse and brush your teeth to remove excess gel.

Note:
1- Place tip cap on syringe nozzle after each use to avoid potential product leakage.
2- Try to minimize consumption of coffee, tea, red wine and tobacco because these substances may re-stain teeth during and after the whitening process.
3- Continue good oral hygiene throughout the treatment.
4- Store remaining whitening gel in a cool dry place for later use.
5- Occasionally some parts of teeth may bleach faster or less evenly than others.
6- Results may be varied. In approximately 5% of the population, teeth may be resistant to bleaching.
4- Acidic foods/juices may cause sensitivity, if consumed shortly after bleaching.
5- Do not use tobacco products or eat while bleaching.
6- Try to minimize consumption of coffee, tea, red wine and tobacco because these substances may re-stain the teeth during and after the whitening process.
7- Not intended for use by pregnant women.

Warning: Some users may experience sensitivity, which is most often mild and transient, but in some cases may require treatment. If you experience significant discomfort, stop using this product and contact XXXXX or XXXX
INSTRUCTION LEAFLET FOR TRÈSWHITE SUPREME GROUP

1- Floss & brush your teeth
   Centre tray on arch

2- Gently suck down on the tray

3- Remove outer tray

4- Lightly tap tray

5- Wear tray for 60 minutes per day. When finished remove bleaching tray and brush teeth

Note:
1- This product is not for night time use.
2- Occasionally some parts of teeth may bleach faster or less evenly than others.
3- Results may be varied. In approximately 5% of the population, teeth may be resistant to bleaching.
4- Acidic foods/juices may cause sensitivity, if consumed shortly after bleaching.
5- Do not use tobacco products or eat while bleaching.
6- Try to minimize consumption of coffee, tea, red wine and tobacco because these substances may re-stain the teeth during and after the whitening process.
7- Not intended for use by pregnant women.
8- Keep gel out of heat/sunlight. Do not freeze.

Warning: Some users may experience sensitivity, which is most often mild and transient, but in some cases may require treatment. If you experience significant discomfort, stop using this product and contact XXXXX or XXXXX.