# ANALYSIS OF CORONAL DISCOLORATION FROM COMMONLY USED OBTURATION MATERIALS

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# Analysis of coronal discoloration from commonly used obturation materials

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**KEYWORDS**

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Root canal sealers

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Spectrophotometer

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## ABSTRACT

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A major cause of coronal tooth discoloration may be attributed to remnants of obturation materials left in the pulp chamber following root canal therapy. Endodontic materials that contain certain compounds such as eugenol, phenol, and silver additives may lead to colour changes in coronal tooth structure when they come in contact with dentine. The degree of staining in such cases varies according to the material used and is usually challenging to manage. Several studies evaluated the discoloration potential of sealers and the changes over a period of time. Most of the previous studies used digital imaging as a method of colour measurement, and focused on limited products only.

**Title:** Analysis of coronal discoloration from commonly used obturation materials. **Aim and Objectives:** The objective of this study was to assess coronal discoloration due to four commonly used endodontic sealers with gutta-percha, using spectrophotometric analysis. **Materials and Methods:** Extracted human teeth were obturated with the experimental sealers and GP. The sealers that were tested included AH Plus™, EndoRez™, Sealapex™, and Kerr Pulp Canal Sealer™. The teeth were maintained in a moist environment at 37°C. Immediate pre-treatment readings of the crowns of the extracted teeth with a spectrophotometer were used as baseline data. Subsequent readings were taken every two weeks for two months. **Results:** Results were analysed using a Wilcoxson Signed Rank Sum test and Kruskal Wallis test.

October, 2007.

## DECLARATION

I hereby declare that *Analysis of Coronal Discoloration from Commonly Used Obturation Materials* is my own 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.

***Mohamed M.A. Elkhazin***

**October, 2007**

**Signed:** ……

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## DEDICATION

*To my mother Mona and my father Mohyeldin Elkhazin for their continuous encouragement, support, and sacrifice for me, their only son, throughout my education.*

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Chapter 1

# INTRODUCTION

A major cause of coronal tooth discoloration may be attributed to remnants of obturation materials left in the pulp chambers following root canal therapy. Endodontic materials that contain certain compounds such as eugenol, phenol, and silver additives may lead to colour changes in coronal tooth structure when they come in contact with dentine. The degree of staining in such cases varies according to the material used and is usually challenging to manage. Several studies evaluated the discoloration potential of sealers and the changes over a period of time. Most of the previous studies used digital imaging as a method of colour measurement, and focused on certain products.

## 1.1 Definition of terms

For the purpose of this study, the following terms will be defined as follows:

- **Discoloration:** a change in the original or proper colour of something giving it an unpleasant, faded, or darkened appearance.

- **Endodontic sealers:** are root canal sealers used to seal the interface between the dentinal wall and the obturating core material.

Sealers also fill voids and irregularities in the root canal, lateral and accessory canals, and spaces between gutta-percha points used in lateral condensation. Sealers also serve as lubricants during the obturation process.

- **Gutta-percha:** is a naturally occurring latex extracted from tropical trees. Gutta-percha points or cones are used as a core obturation material and contain only 20% of gutta-percha.

- **Obturation:** the process of occluding or filling a cavity. In endodontics, it is the filling of the prepared root canal system. Obturation materials include the core filling material (gutta-percha), sealers and cements, and medicated pastes.

- **Spectrophotometer:** a spectrophotometer is a photometry device used for the measurement of spectral transmission, reflectance, or relative emissions.

- **Stain:** discoloration of a tooth surface as a result of ingested materials, bacterial action, tobacco, and/or other substances. This may be extrinsic, intrinsic, acquired or inflicted.

- **Staining potential:** the capacity or ability of a stain to produce future discoloration.

Chapter 2

# LITERATURE REVIEW

## 2.1 Introduction

The appearance of the dentition is of concern to a large number of people seeking dental treatment and the colour of teeth is of particular cosmetic importance. There has been a recent increase in interest in the management of tooth staining and discoloration by many dental practitioners. A good understanding of the aetiology of tooth discoloration is important in order to make the correct diagnosis. Remnants of obturation materials in the pulp chamber following root canal therapy are believed to be a major cause of discoloration. Several studies evaluated the staining ability of different sealers and the colour changes that occurred over time (Van der Burgt *et al.* 1986, Parsons *et al.* 2001, Davis *et al.* 2002, Partovi *et al.* 2006). The objective of this study is to evaluate the discoloration potential of commonly used endodontic sealers and gutta-percha, using a spectrophotometer to measure the colour changes if any.

## 2.2 Tooth colour

A basic understanding of the elements of tooth colour is necessary in many aspects of aesthetic dentistry. The colour of natural teeth is affected by several factors. These include the thickness, composition, and structure of the dental hard tissues, parameters that evolve considerably throughout life, thus affecting the natural colour of the tooth over time (Touati *et al.* 1999, Watts and Addy 2001). The tooth consists of three main tissues, the pulp, dentine, and enamel.

### 2.2.1 The pulp

The pulp has a dark-reddish colour that can be observed in the centre of the tooth. The volume occupied by the pulp has a great influence on the overall colour of the tooth (Touati *et al.* 1999, Watts and Addy 2001); therefore younger teeth with larger pulps exhibit a more pinkish appearance.

### 2.2.2 Dentine

Dentine is the most important dental tissue in terms of colour. The low mineral content of dentine compared to enamel and the high organic component explains the relative opacity of dentine. The dentinal tubules play an important role in the selective diffraction of light (reflection and absorption of rays), resulting in the opaque nature of dentine (Touati *et al.* 1999, Watts and Addy 2001).

The optical properties of dentine are also affected by the physiologic evolution of dentine (Touati *et al.* 1999). Teeth become darker as a result of ageing; this may be partly due to the laying down of secondary dentine, incorporation of extrinsic stains and gradual wear of the overlying enamel allowing a greater influence on the colour of the tooth by the underlying dentine. Secondary dentine has a higher mineral content compared to primary dentine and thus manifests less opacity. On the other hand, sclerotic dentine displays a more saturated shade and is limited to the site of the insult (Touati *et al.* 1999, Watts and Addy 2001).

### 2.2.3 Enamel

Enamel has a high mineral content and a specific crystalline arrangement, making it more translucent. The optical property of enamel is affected by a number of factors including thickness, composition, structure, and surface texture, all of which are altered as a result of ageing. The incisal third has the thickest enamel and no underlying dentine, thus being more translucent compared to the thinner middle and cervical portions of enamel (Touati *et al.* 1999, Watts and Addy 2001).

### 2.2.4 Natural tooth colour

Natural teeth are typically composed of a number of colours, generally in the yellowish-white range (Joiner 2004, Touati *et al.* 1999). The colour varies among individuals and even among teeth within the same arch. In addition, an individual tooth also exhibits a gradation of colour from the gingival margin to the incisal edge of the tooth. The gingival margin often has a darker appearance because of the close approximation of the dentine below the enamel (Joiner 2004, Touati *et al.* 1999). This variation has been explained by many factors that can influence natural tooth colour including hereditary/genetic factors, environmental factors (tetracycline and exogenous stains), nutritional factors (calcium and vitamin D), and endocrine/hormonal factors (pituitary gland, thyroid and parathyroid secretions) (Joiner 2004, Touati *et al.* 1999, Scully and Began 2004, Watts and Addy 2001).

## 2.3 Tooth discoloration: Aetiology and classification

Discoloration of crowns especially of the anterior teeth is an aesthetic problem to both the patient and the dentist. Causes of coronal tooth discoloration can be natural/acquired or iatrogenic/inflicted (Parsons *et al.* 2001, Davis *et al.* 2002, Walton and Rotstein 1996). Natural causes occur as a result of disturbances during tooth development, or from patient behaviour, caries, or traumatic injuries. Iatrogenic causes result from dental procedures, or from certain restorative materials (Parsons *et al.* 2001, Davis *et al.* 2002, Walton and Rotstein 1996).

Tooth discoloration can also be classified according to the location of the stain, which may be intrinsic, extrinsic, or internalised (Partovi *et al.* 2006, Watts and Addy 2001).

### 2.3.1 Intrinsic discoloration

Intrinsic discoloration is attributed to the incorporation of a chromatogenic material into the enamel or dentine during odontogenesis (***pre-eruptive discoloration***) or following tooth eruption (***post-eruptive discoloration***) (Watts and Addy 2001, Grossman *et al.* 1988, Dahl and Pallesen 2003).

***Pre-eruptive tooth discoloration*** can result from the exposure to high levels of fluoride, administration of certain drugs (tetracycline), inherited developmental disorders (dentineogenesis imperfecta), or trauma to the developing tooth (Watts and Addy 2001, Grossman *et al.* 1988, Dahl and Pallesen 2003, Scully and Began 2004).

***Post-eruptive tooth discoloration*** of an intrinsic nature can be due to ageing, pulp necrosis, and iatrogenic causes (Dahl and Pallesen 2003).

### 2.3.2 Extrinsic discoloration

Extrinsic discoloration occurs outside the tooth substance and lies on the tooth surface or in the acquired pellicle (Watts and Addy 2001, Grossman *et al.* 1988). The origin of the stain is exogenous, such as, from dietary sources (coffee, tea, red wine, carrots, and oranges) or from substances habitually placed in the mouth such as occurs in tobacco chewing and smoking (Watts and Addy 2001, Grossman *et al.* 1988).

### 2.3.3 Internalised tooth discoloration

Internalised discoloration of the tooth is due to the incorporation of an extrinsic stain into the tooth substance following tooth development (Partovi *et al.* 2006). This category includes discoloration following dental caries, tooth wear, recession, and from the placement of some restorative materials (Partovi *et al.* 2006, Watts and Addy 2001, Grossman *et al.* 1988, Dahl and Pallesen 2003, Attin *et al.* 2003).

### 2.3.4 Discoloration related to drug administration

Drugs such as chlorhexidine, fluorides, and iron can result in surface tooth discoloration. Other drugs such as some antibiotics and essential oils may also cause discoloration. Intrinsic discoloration is prominent when tetracycline is given to children under 12 years of age, resulting in a cosmetically unacceptable dentition (Scully and Began 2004, Wray and Welbury 2001).

## Fluorosis

This may arise endemically from naturally occurring fluoride containing water supplies or from fluoride delivered in mouth rinses, tablets or toothpastes when used as a supplement (Adair 2006). The severity is related to age and dose, with the primary and secondary dentitions both being affected in endemic fluorosis. The enamel is often affected and may vary from areas of flecking to diffuse opacious mottling, whilst the colour of the enamel ranges from chalky white to a dark brown/black appearance. The brown/black discoloration is post-eruptive and probably caused by the internalisation of an extrinsic stain into the porous enamel (Watts and Addy 2001).

## Tetracycline staining

Systemic administration of tetracycline during development is associated with the deposition of tetracycline within bone and the dental hard tissues. Tetracycline and its homologues have the ability to form complexes with calcium ions on the surface of the hydroxyapatite crystals within bone and in the dental tissues. Dentine has been shown to be more heavily stained than enamel. Tetracycline has the ability to cross the placental barrier and should be avoided from 29 weeks *in utero* until full term to prevent incorporation into the deciduous dental tissues. Since the permanent teeth continue to develop in the infant and young child until 12 years of age, tetracycline administration should be avoided in children below this age as well as in breast-feeding and expectant mothers. The most critical time to avoid the administration of tetracycline for the deciduous dentition is 4 months *in utero* to 5 months post-partum, especially with regard to the deciduous incisor and canine teeth. In the permanent dentition, for the incisor and canine teeth, this

period is from 4 months post-partum to approximately 7 years of age. The colour changes involved depend upon the precise medication used, the dosage and the period of time over which the medication was administered. Teeth affected by tetracycline staining have a yellowish or brown-grey appearance which is worse on eruption and diminishes with time (Scully and Began 2004, Wray and Welbury 2001, Watts and Addy 2001).

### 2.3.5 Discoloration related to endodontic treatment

According to Nicholls (cited by Van der Burgt *et al.* 1986), the main causes of intrinsic tooth discoloration related to endodontic treatment include decomposition of necrotic pulp tissue, haemorrhage into the pulp chamber, and remnants of endodontic drugs and filling materials in the pulp chambers following endodontic therapy.

#### Decomposition of pulpal tissues

Gradual discoloration due to the decomposition of pulpal tissue following bacterial, mechanical, or chemical irritation to the pulp is very common, particularly if the pulp becomes necrotic (Walton and Rotstein 1996, Grossman *et al.* 1988, Attin *et al.* 2003, Rotstein 2002). Inadequate removal of the roof of the pulp chamber during access cavity preparation may leave fragments of pulp tissue within the pulp chamber or pulp horns. Subsequent decomposition of the proteins present in this necrotic pulp tissue may cause gradual discoloration perhaps due to the slow formation of colour-producing compounds (Walton and Rotstein 1996, Grossman *et al.* 1988, Attin *et al.* 2003). The degree of discoloration depends on how long the pulp had been necrotic: the longer the discoloration compounds are present in the pulp chamber, the greater is the discoloration (Rotstein 2002).

## Pulpal haemorrhagic products

Excessive and persistent haemorrhage during pulp extirpation usually indicates the presence of vital pulp fragments in the root canal. Rupture of blood vessels following traumatic injury of the teeth, may also cause profuse haemorrhage (Walton and Rotstein 1996, Grossman *et al.* 1988, Dahl and Pallesen 2003, Attin *et al.* 2003, Watts and Addy 2001). Blood components may then disseminate into the dentinal tubules causing the discoloration of the tooth concerned (Grossman *et al.* 1988, Dahl and Pallesen 2003, Attin *et al.* 2003). Initially a dark pinkish hue of the crown is detected, which then turns pinkish brown some days after the incident. Iron is then released from the blood degradation products during haemolysis. Iron is also converted into black ferric sulphate by the action of bacterial enzymes, causing a greyish stain of the crown. Therefore, the pulp chamber and root canal must be thoroughly irrigated after pulp extirpation to prevent discoloration, by removing the blood remnants from the dentinal tubules (Walton and Rotstein 1996, Grossman *et al.* 1988).

## Endodontic drugs and filling materials

Incomplete removal of endodontic filling materials from the pulp chamber or pulp horns can also lead to subsequent staining of the tooth structure (Walton and Rotstein 1996, Grossman *et al.* 1988, Attin *et al.* 2003). Endodontic materials that contain certain compounds such as eugenol, phenol, tetracycline medicaments, and silver additives can lead to colour changes when placed in contact with dentine (Davis *et al.* 2002, Walton and Rotstein 1996, Grossman *et al.* 1988, Attin *et al.* 2003, Van der Burgt *et al.* 1986). The degree of staining in such cases varies according to the material used and is the most challenging to manage

post-endodontically. Some materials stain the tooth directly, whereas others stain only when decomposing or combining with other agents used in endodontic treatment (Grossman *et al.* 1988, Attin *et al.* 2003, Van der Burgt *et al.* 1986). Careful selection of intracanal medicaments and obturation materials is essential in order to prevent unnecessary consequential staining of the remaining tooth structure (Van der Burgt *et al.* 1986).

## 2.4 Obturation materials

Root canal filling materials include the following:

- Core filling materials (solids and semi-solids)
- Sealers and cements
- Medicated pastes.

The standard root canal obturation procedure is a combination of sealer cement with a central core filling material. The function of the core material is to act as a piston on the flowable sealer, causing it to spread and fill voids, and to wet and attach to the instrumented dentinal wall (Ørstavik 2005). With intent, it is the sealer that should come into contact with the canal walls and base of the pulpal space; only occasionally does the gutta-percha protrude from the sealer and touch the dentine, pulp or periodontal tissues. Therefore, the sealer should possess many of the critical properties of the root canal filling material (Grossman *et al.* 1988, Ørstavik 2005).

## Properties of an ideal obturation material

Grossman's criteria for an ideal root canal filling material is considered a classic and is the most frequently listed in endodontic textbooks (Grossman *et al.* 1988). He listed ten requirements (Table 2.1) which although considered desirable properties; cannot be entirely fulfilled by any product commercially available at present.

| Grossman's criteria for an ideal core filling material |
|---|
| **It should be easily introduced into the canal** |
| **It should seal the canal laterally as well as apically** |
| **It should not shrink after being inserted** |
| **It should be impervious to moisture** |
| **It should be bacteriostatic or at least not encourage bacterial growth** |
| **It should be radiopaque** |
| **It should not stain tooth structure** |
| **It should not irritate periapical tissues** |
| **It should be sterile, or quickly and easily sterilised before insertion** |
| **It should be easily removed from the root canal if necessary** |

**Table 2.1** Grossman's criteria for an ideal core filling material (Grossman 1988).

Figdor (cited by Ørstavik 2005) assigned three primary functions to a root canal filling material, which he believed are more practical and technical properties that must be possessed by all obturation materials. Figdor's primary requirements of a root filling material are illustrated in Figure 2.1:

- Sealing against ingrowths of bacteria from the oral cavity;
- Entombment of remaining micro-organisms;
- Complete obturation to prevent stagnant fluid from accumulating and serving as nutrients for bacteria from any source.

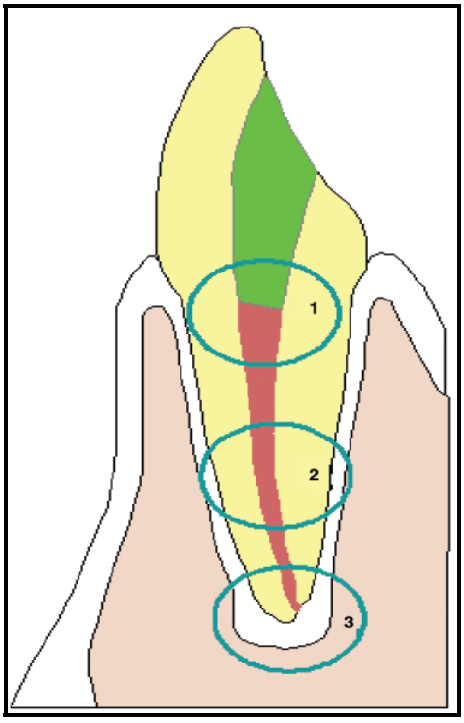


**Figure 2.1** Primary functions of a root canal filling according to Figdor (cited by Ørstavik 2005). 1, stop coronal leakage; 2, entomb surviving micro-organisms; 3, prevent accumulation of stagnant fluid.

### 2.4.1 Core filling materials

Core filling materials include gutta-percha, silver cones, and resin-based core filling materials.

### Gutta-percha (GP)

Gutta-percha is the most commonly used root canal filling material. GP points (Table 2.2) contain 20% gutta-percha and up to 75% zinc oxide filler (Regan 2004, Himel *et al.* 2006, Carrotte, 2004, Gatewood 2007, Ingle *et al* 2002). The remainder is composed of additives such as metal salts (radiopacifiers), resins and wax, added to enhance the plasticity of the GP (plasticizers). Some manufacturers add antimicrobials, such as calcium hydroxide, chlorhexidine, or iodoform, to promote some disinfectant properties to the materials (Ørstavik 2005).

GP exists in two crystalline forms, the alpha (α) phase and the beta (β) phase. The α-phase appears in nature; the β-phase occurs during refining and is dominant in the products used in endodontics (Regan 2004, Himel *et al.* 2006, Carrotte, 2004, Gatewood 2007, Ingle *et al.* 2002, Ørstavik 2005).

When the naturally occurring α-phase GP is heated it transforms into a pliable form, which is more flowable under pressure. When allowed to cool slowly (0.5 °C per hour) it can re-crystallize back into the α-phase, but a faster cooling of the material will re-crystallize it into the β-phase (Gatewood 2007). In the unheated β-phase, the material is a solid mass that can only be compacted. A disadvantage of the alpha phase is the shrinkage after setting of the material (Johnson and Gutmann 2006, Himel *et al.* 2006, Carrotte, 2004). However, some authors suggested that the dimensional stability of the α-phase GP is improved if it is not warmed above 45°C (Johnson and Gutmann 2006).

GP is considered to have acceptable biocompatibility with a low degree of toxicity (Hauman and Love 2003). An ideal obturating material should not cause staining of tooth structure, but it has been demonstrated that GP does show some degree of staining, although its staining effect is low when compared to that of endodontic sealers (Partovi *et al.* 2006).

| Gutta-percha cones | |
|---|---|
| **Gutta-percha** | **(19%-22%)** |
| **Zinc oxide** | **(59%-79%)** |
| **Heavy metal salts** | **(1%-17%)** |
| **Wax or Resin** | **(1%-4%)** |

**Table 2.2** Composition of GP for endodontic use (Carrotte, 2004).

## Silver points

Silver points (SP) were up to a few years ago the most commonly used solid core filling material, specifically indicated for narrow and curved canals of mature teeth (Ingle *et al.* 2002, Ørstavik 2005). Failure of SP was attributed to misuse of the material that led to the bad reputation of the material. SP are flexible but quite stiff, and have the advantage of being more easily inserted in cases where the canals are narrow and curved (Regan 2004, Himel *et al.* 2006, Ingle *et al.* 2002, Ørstavik 2005).
Case reports and clinical experience with signs and symptoms of apical periodontitis associated with these fillings brought SP into some discredit. Corrosion of the point with release of toxic products from the metal was believed to initiate or support periapical inflammatory

reactions. In addition, doubts on the sealing ability of these fillings that subsequently developed tooth and gingival staining emerged. Thus SP are not recommended for use as an obturation material currently (Regan 2004, Himel *et al.* 2006, Ingle *et al.* 2002, Ørstavik 2005).

## Resin-based core filling materials

The search for a resin-based alternative to GP was the centre of attention of many investigators in the past decades. The introduction of the Resilon™ material points (Pentron Clinical Technologies, USA), presented a possible alternative to GP in clinical practice.

Resilon is a synthetic polyester core material with bioactive glass, bismuth and barium salts as fillers (Johnson and Gutmann 2006, Himel *et al* 2006, Ørstavik 2005, Gatewood 2007). It is presented as cones for master point and accessory point placement with the lateral condensation technique and as pellets designed for the thermoplastic and vertical condensation technique. With physical and handling characteristics similar to gutta-percha, the main advantage of thermoplastic resin as core material will be the extent to which it will bond to the sealer used. The sealer used with Resilon is Epiphany™ Root Canal Sealant (Pentron Clinical Technologies, USA). It is a dual-curable composite resin sealer (Johnson and Gutmann 2006, Himel *et al* 2006, Gatewood 2007). A root canal system obturated with this technique is said to create a 'mono-block', in which the Resilon bonds to the Epiphany sealer, which in turn bonds to the dentinal wall.

There are various advantages of the Resilon-Epiphany system including the high sealing ability, low micro-leakage, and increased fracture resistance. This advancement of dentine bonding into the root canals provided an efficient seal between the sealer-wall interface and the

sealer-core interface. This in turn would compensate for the micro-leakage possibility that was greater when GP was used as the core filling material. The system also showed an increased resistance to fracture, when compared to the conventional GP obturation systems (Johnson and Gutmann 2006, Himel *et al* 2006, Gatewood 2007).

### Resin coated gutta-percha

Resin coated GP (Ultradent, USA) was developed in an attempt to achieve bonding at the GP-sealer interface. The manufacturer placed a uniform layer of resin over the GP that formed a resin bond when contacting a resin-based sealer, such as EndoRez™ (Ultradent, USA). The manufacturer claimed inhibition of leakage between the sealer and the core filling material (Johnson and Gutmann 2006, Himel *et al* 2006). This novel and promising product requires more research to test the efficacy of it before it can be substituted with the current GP systems.

## 2.4.2 Root canal sealers, cements, and pastes.

The principal functions of the final root filling materials include providing a fluid-tight seal of the root canal system, elimination of remaining bacteria and the filling of voids and irregularities in the prepared canal. It is the properties of the root canal sealers that are responsible for the fulfilment of these requirements (Ørstavik 2005, Gatewood 2007). Due to this, the sealer has as much or more importance than the core material in providing a successful clinical outcome (Gatewood 2007). Grossman (1988) described a number of properties that should be found in an ideal sealer. Although no sealer possesses all these properties, some have more than others. Grossman's criteria for an ideal sealer are outlined in Table 2.3.

| Properties of an ideal root canal sealer |
|---|
| **It should be tacky when mixed to provide good adhesion between it and the canal wall when set.** |
| **It should make a hermetic seal.** |
| **It should be radiopaque so that it can be visualized in the radiograph.** |
| **The particles of powder should be very fine so that they can mix easily with the liquid.** |
| **It should not shrink upon setting.** |
| **It should not stain tooth structure.** |
| **It should be bacteriostatic or at least not encourage bacterial growth.** |
| **It should set slowly.** |
| **It should be insoluble in tissue fluids.** |
| **It should be tissue tolerant, that is, non-irritating to peri-radicular tissue.** |
| **It should be soluble in a common solvent if it is necessary to remove the root canal filling.** |

**Table 2.3** Grossman's requirements of an ideal root canal sealer (Grossman 1988)

## Classification of root canal sealers

Endodontic sealers may be generally divided into two main groups, according to their constituents: (Carrotte, 2004)

- Eugenol based sealers
- Non-eugenol based sealers.

Eugenol based sealers are mainly zinc oxide-eugenol cements that are manufactured according to various formulae (Rickert's formula and Grossman's formula). These basic formulations will be discussed in detail later.

Non-eugenol sealers include resin-based, calcium hydroxide based, silicon-based, and glass ionomer sealers.

## Eugenol-based sealers

The zinc oxide-eugenol (ZOE) sealers may be divided into sealers based on the Rickert's formula (introduced in 1931) and those based on the subsequent Grossman's formula (introduced in 1958). The essential difference between the two groups is that Rickert's sealer contains precipitated silver and Grossman's sealer has barium and bismuth salt as the radiopacifier. Table 2.4 lists the constituents as prescribed by Grossman and Table 2.5 gives a classification of endodontic sealers according to chemistry and type (Ingle *et al.* 2002, Ørstavik 2005, Carrotte, 2004).

Rickert's sealer is available commercially in the form of Kerr™ Pulp Canal Sealer (Kerr, Romulus, MI, USA). This sealer admirably met the requirements set down by Grossman except for severe staining. The silver, added for radiopacity, caused a dark grey discoloration of the teeth, thus creating an undesirable public image for endodontics (Ingle *et al.* 2002).

Grossman's sealer emerged as a non-staining ZOE-based cement and has several commercial variants, such as Roth™ sealer (Roth Inc., Chicago, USA) and ProcoSol™ (Den-tal-ez, PA, USA).

Some manufacturers added paraformaldehyde for antibacterial activity, as in Endomethasone™ (Septodont, France). ZOE-based sealers have some antibacterial activity of their own, but will also exhibit some cytotoxicity when placed directly on vital tissues (Ingle *et al.* 2002).

| Grossman's formula | |
|---|---|
| **Powder** | |
| **Zinc oxide** | 42% |
| **Staybelite resin** | 27% |
| **Bismuth subcarbonate** | 15% |
| **Barium sulphate** | 15% |
| **Sodium borate (anhydrous)** | 1% |
| **Liquid** | |
| **Eugenol** | 100% |

**Table 2.4** Grossman's sealer (Carrotte 2004)

## Non-eugenol sealers

Non-eugenol sealers (Table 2.5) can be classified into the following groups: (Ingle *et al.* 2002, Carrotte, 2004, Regan 2004, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007)

- Calcium hydroxide-based materials
- Resin-based sealers
- Glass ionomer sealers
- Silicone-based sealers.

| Type | Brand | Principle component | Manufacturer |
|---|---|---|---|
| Zinc oxide-Eugenol | **Roth** | ZnO-Eugenol, colophony, Bismuth & Barium salts | Roth Inc., Chicago, USA |
| | **Kerr PCS** | ZnO-Eugenol, Thymol & Silver | Kerr, Romulus, MI, USA |
| | **ProcoSol** | ZnO-Eugenol, colophony, Bismuth & Barium salts | Den-tal-ez , PA, USA |
| | **Endomethasone** | ZnO-Eugenol, Paraformaldehyde | Septodont, France |
| Resin | **AH Plus** | Epoxy-bis-phenol resin, adamantine | Dentsply Maillefer, Switzerland |
| | **EndoRez** | UDMA | Ultradent, UT, USA |
| | **Epiphany** | BisGMA, UDMA & hydrophilic methacrylates | Pentron,Wallingfor, USA |
| | **Acroseal** | Epoxy-bis-phenol resin, metheneamine, enoxolone, calcium hydroxide | Septodont, France |
| Glass ionomer | **KetacEndo** | Polyalkenoate cement | 3M ESPE, St. Paul, MN, USA |
| Silicone | **RoekoSeal** | Polydimethylsiloxane, silicone oil, zirconium oxide | Roeko/Coltene/Whaledent, Germany |
| | **GuttaFlow** | Polydimethylsiloxane, silicone oil, zirconium oxide, gutta-percha | Roeko/Coltene/Whaledent, Germany |
| Calcium hydroxide | **Sealapex** | Toluene salicylate, calcium oxide | Kerr, Romulus, MI, USA |
| | **Apexit** | Salicylates, calcium hydroxide | Ivoclar-Vivadent, Schaan, Liechtenstein |

**Table 2.5** Classification of endodontic sealers: chemistry and types (Ørstavik 2005)

## Calcium hydroxide-based sealers

Calcium hydroxide has proved to be a successful pulp protecting and capping agent and as an effective inter-appointment dressing in endodontics. This has further encouraged its use as a root canal sealer and warranted it being added in some cement formulations (Table 2.5). Sealapex™ (Kerr, Romulus, MI, USA) and Apexit™ (Ivoclar Vivadent, Schaan, Liechtenstein) are well known brand names of this class of material (Ingle *et al.* 2002, Regan 2004, Valera *et al.* 2004, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007).

The bioactive potential (osteogenic effect) of calcium hydroxide when placed adjacent to vital tissue in pulp capping or apexification has made the material attractive for use in endodontics. However, to be effective in this respect, calcium hydroxide must dissociate into calcium and hydroxyl ions. For this to occur, it would require some degree of dissolution of the sealer. If dissolution of the calcium hydroxide component occurred, the likelihood of the sealing ability being compromised would increase (Gatewood 2007). Thus, the calcium hydroxide content may dissolve leaving behind obturation voids and impairing the primary function of the sealer.

In addition, calcium hydroxide sealers have the disadvantage of lacking stability and may exhibit remarkable leakage over time. The material also has shown lack of physical strength. Thorough condensation of gutta-percha is especially important to minimize the risk of the root filling loosening during post space preparation (Ørstavik 1988, Ørstavik 2005).

Calcium hydroxide is also added to cements of other chemical compositions, such as resins and ZOE-based sealers, but there is limited evidence for any benefit derived from its inclusion in these formulations (Ingle *et al.* 2002, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007).

## Resin-based sealers

Resin-based sealers have a long history of use and possess the advantage of providing good adhesive properties. Epoxy resins and a polyketone compound are examples of polymers used as endodontic sealers.

AH26 (Dentsply Maillefer, Switzerland) is an example of an epoxy resin-based material that has good handling characteristics and good adhesion to dentine. However, it exhibits significant toxicity in the unset state, causes severe tooth staining, but still having adequate sealing ability (Ørstavik 1988, Ingle *et al.* 2002, De Moor and Hommez 2002, Regan 2004, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007). This bi-phenol resin utilised methenamine for polymerization. As methenamine gives off some formaldehyde during the setting reaction, a substitute was necessary. It was found that a mixture of amines could polymerise the material without the formation of formaldehyde and preserving the natural tooth colour. AH Plus (Dentsply Maillefer, Switzerland) was the result of this product development (Ingle *et al.* 2002, Regan 2004, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007).

Diaket™ (3M ESPE, St. Paul, MN, USA) is a polyketone sealer. The material is a resin-reinforced chelate formed between zinc oxide and diketone. The material has a tacky consistency that provides good adhesion to dentine and contributes to its difficult handling characteristics (Ørstavik 1988, Ingle *et al.* 2002, Regan 2004, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007).

EndoRez™ (Ultradent, South Jordan, UT, USA) is based on urethane dimethacrylate (UDMA). It has some hydrophilic properties assumed to improve performance even if moisture is present. Recently, EndoRez has been marketed in conjunction with resin-coated GP, which through bonding to the sealer supposedly gives better adhesion and seal throughout the filling mass in the root canal.

## Glass-ionomer sealers (GIS)

A glass-ionomer sealer such as Ketac-Endo™ (3M ESPE, St. Paul, MN, USA) has the advantage of chemically bonding to dentine, fluoride ion release, and an antimicrobial effect (Czarnecka *et al.* 2007). This offers the potential of improving the seal and possibly strengthening the root against fracture. Some studies have shown that canals obturated using GP with GIS were more resistant to fracture than when other sealers were used, whereas other studies showed no difference (Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007).

Glass-ionomer materials tend to show good biocompatibility (Valera *et al.* 2004). The GIS is viscous and has a shorter working time than many other sealers. Due to its hardness and relative insolubility in GP solvents, re-treatment can be more difficult (Ingle *et al.* 2002, Regan 2004, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007).

## Silicone-based sealers

Endo-Fill™ (Lee Pharmaceuticals, El Monte, CA, USA) was an early attempt in utilizing the water repellent, chemical stability and adhesive properties of silicone materials in endodontics (Ørstavik 2005, Himel *et al.* 2006).

RoekoSeal™ (Roeko/Coltene/Whaledent, Germany) is a more recent formulation that can polymerize without shrinkage. It consists of polydimethyl siloxane, silicone oil, paraffin-base oil, hexachloroplatinic acid (catalyst), and zirconium dioxide (radiopaque material). It is supplied ready to use in a dual-barrel syringe. The material shows impressive biological performance, documented by testing according to international standards and clinical follow-up studies (Gencoglu *et al.* 2003, Ørstavik 2005, Himel *et al.* 2006).

With Gutta-Flow™ (Roeko/Coltene/Whaledent, Germany), an attempt has been made to incorporate the filling qualities of GP in the sealer. GP was milled to a low grain size and mixed into components of the silicone sealer. In the paste fill technique advocated, the GP is then carried with the sealer to fill the entire root canal system (Ørstavik 2005, Himel *et al.* 2006).

According to Grossman's (1988) requirements of an ideal root canal sealer, none of the above mentioned materials should stain tooth structure. However, this condition is evidently being violated by a number of sealers (Parsons *et al.* 2001, Davis *et al.* 2002, Partovi *et al.* 2006, Van der Burgt *et al.* 1986, Rotstein 2002). Van der Burgt and her associates (1986) reported that Grossman's cement, zinc oxide–eugenol, endomethasone, and N2 induced a moderate orange-red stain in the crowns of upper premolar teeth. Furthermore it was found that Diaket and Tubli-Seal caused a mild pink discoloration, while AH-26 gave a distinct colour shift towards grey (Table 2.6). As far as the staining ability of other materials is concerned, Van der Burgt and associates (1986), found that gutta-percha caused a mild pinkish tooth discoloration and that AH-26 Silver-Free induced a distinct colour shift towards grey. No discoloration was recorded for teeth filled with glass ionomer cements.

Sealers that contain silver as a radiopacifier, such as Kerr's Pulp Canal Sealer or the original AH-26, are major tooth stainers. They cause a greyish stain analogous to amalgam-stained teeth (Parsons *et al.* 2001, Van der Burgt *et al.* 1986, Carrotte, 2004). Chemically improved products that do not contain silver can also stain dentine, and in those cases it was proved that eugenol was the primary offender (Parsons *et al.* 2001, Walton and Rotstein 1996, Partovi *et al.* 2006, Van der Burgt *et al.* 1986). It was demonstrated that free or bound eugenol oxidises and darkens over time (Parsons *et al.* 2001). Therefore, it seems wise to avoid leaving any sealers or staining cements in the pulp chamber following root canal therapy.

| Sealer | Stain | Study |
|---|---|---|
| **1. ZnO-Eugenol (Rickert's formula)** | **Gray to gray-black** | Van der Burgt *et al*, 1986 |
| **1. ZnO-Eugenol (Grossman's cement)** | **Orange red** | Van der Burgt *et al*, 1986<br>Partovi *et al*, 2006 |
| **2. Diaket™** | **Mild pink** | Van der Burgt *et al*, 1986 |
| **3. AH 26™** | **Gray to gray-black** | Van der Burgt *et al*, 1986<br>Parsons *et al*, 2001<br>Davis *et al*, 2002<br>Partovi *et al*, 2006 |
| **4. TubliSeal™** | **Mild pink** | Van der Burgt *et al*, 1986 |
| **5. Gutta-percha** | **Mild pink** | Van der Burgt *et al*, 1986<br>Partovi *et al*, 2006 |

**Table 2.6** Summary of previous studies that assessed discoloration from endodontic sealers.

## 2.5 Tooth colour analysis

Many methods are currently used to assess tooth colour. These range from visual (subjective) comparisons using paper, coloured porcelain or acrylic resin shade guides, to instrumental (objective) measurements using spectrophotometers, colorimeters and digital image analysis techniques (Joiner 2004).

### 2.5.1 Digital image analysis

Recent advances in photography and computing have resulted in the widespread use of the digital camera for colour imaging. This new device is capable of recording digital data from an object, which may subsequently be viewed as an image on a computer screen and transmitted via the Internet. Digital images can be analysed with appropriate imaging software enabling the collection of colour values from the whole or parts of such images. This is a much cheaper process than the use of traditional colour measurement devices such as spectrophotometers or colorimeters (Jarad *et al.* 2005, Chu and Tarnow 2001, Cal *et al.* 2006).

### 2.5.2 Spectrophotometry

A spectrophotometer is a photometry device used for the measurement of spectral transmission, reflectance, or relative emissions (Joiner 2004, Guan *et al.* 2005, Cal *et al.* 2006). It is equipped with a high-precision sensor that can receive reflected light from an object and transmit this information to a built-in microcomputer. The microcomputer will determine the spectral reflectance based on the information received

from the sensor and the results will be displayed as a numerical value or on a spectral reflectance graph. Spectrophotometers are considered highly accurate when compared to other types of colorimeters (Joiner 2004, Guan *et al.* 2005, Cal *et al.* 2006).

### 2.5.3 Commission Internationale de l'E'clairage

The Commission Internationale de l'E´clairage (CIE), an organisation devoted to standardisation in areas such as colour and appearance defined a colour space, CIE L*a*b*, that supports the accepted theory of colour perception based on three separate colour receptors (red, green and blue) in the eye and is currently one of the most popular colour systems used in dental research (Joiner 2004, Guan *el al.* 2005, Cal *et al.* 2006). The CIE Lab colour space (Figure 2.2, Figure 2.3) represents a uniform colour space, with equal distances corresponding to equal perceived colour differences (Baltzer and Kaufmann-Jinoian 2004).

Difference in colour can be measured from values obtained by the spectrophotometer using the CIE L*a*b* colour space (Guan *et al.* 2005). The advantage of the CIE L*a*b* colour space system is that colour differences can be expressed in units that can be related to visual perception and clinical significance.

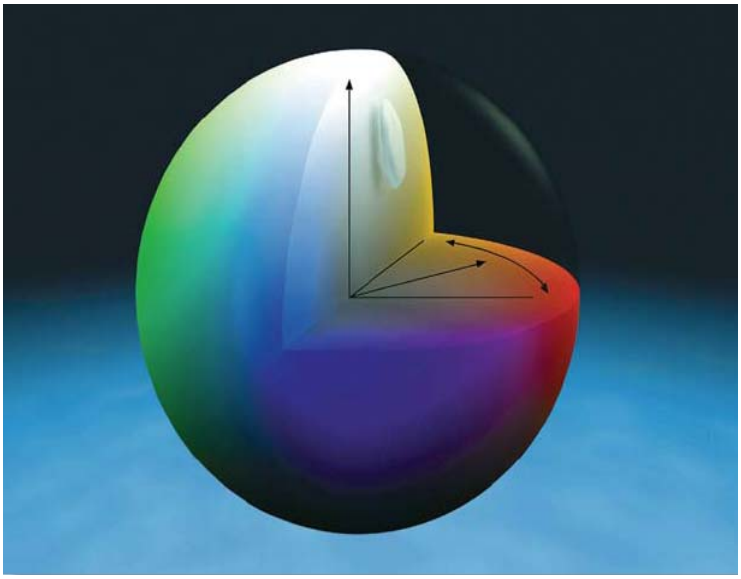

**Figure 2.2** CIE L*a*b* colour space

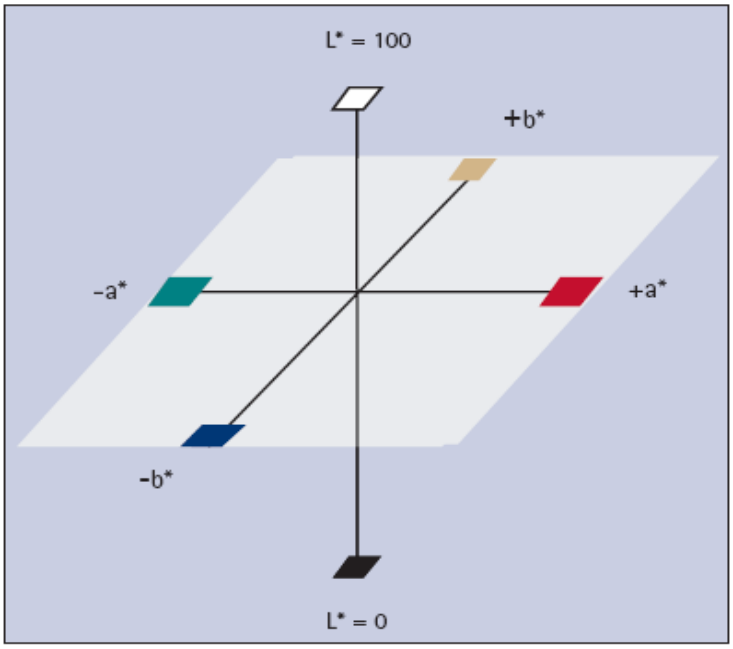


**Figure 2.3** CIE L*a*b* colour co-ordinates (Baltzer and Kaufmann-Jinoian 2004).

Several studies evaluated the discoloration potential of sealers and the changes over a period of time (Van der Burgt *et al.* 1986, Parsons *et al.* 2001, Davis *et al.* 2002, Partovi *et al.* 2006). Most of the previous studies used digital imaging as a method of colour measurement, and focused on certain products only. Furthermore, previous studies did not mimic a clinical situation. The pulp chambers were filled in bulk with the tested sealer through an apical access without using a core filling material. The reason why the material was placed in bulk was to induce staining of the dentine that could be detectable by the visual colour inspectors or by the digital images. In this study, the GP will be sealed with the tested material through a coronal access, thus simulating the clinical situation. The objective of this study is to assess coronal discoloration by some commonly used endodontic sealers and gutta-percha, using spectrophotometric analysis.

Chapter 3

# AIMS AND OBJECTIVES

## 3.1 Aim

The aim of this study was to assess the degree of staining of crowns of teeth by commonly used obturation materials using a spectrophotometric method of colour analysis.

## 3.2 Objectives

- To compare coronal discoloration by some commonly used endodontic sealers and gutta-percha.
- To relate the staining potential of the constituents present in the endodontic sealers and gutta-percha with the resultant tooth discoloration.

## 3.3 Null Hypothesis

There is no significant difference in the discoloration caused by the different sealers when used with gutta-percha in the obturation of root canals.

Chapter 4

# MATERIALS AND METHODS

## 4.1 Study Design

This was an *in vitro* experimental study. A pilot study was carried out before the main study to standardize the obturation technique and coronal seal. The study was conducted in the Dental Research Institute, Tygerberg Oral Health Centre, University of the Western Cape.

## 4.2 Sample size

Sixty (60) human premolar teeth, extracted for orthodontic reasons, were used in this study. The teeth were collected from the Oral Health Centres of the Faculty of Dentistry, University of the Western Cape.

## 4.3 Inclusion criteria

- Sound human premolar teeth extracted for orthodontic purposes.

## 4.4 Exclusion criteria

- Teeth which are extracted due to decay or fractures.
- Teeth with restorations.

## 4.5 Materials
### 4.5.1 Experimental teeth

The extracted teeth were collected from the Oral Health Centres of the Faculty of Dentistry located in Tygerberg and Mitchells Plain. The teeth were preserved in jars containing a solution of normal saline and one percent (1.0%) thymol crystals. Thymol was used as an antiseptic, fungicide, and a preservative to ensure that there was no growth of any organisms on the experimental teeth.

Sixty teeth that fulfilled the inclusion criteria were cleaned with a rubber cup and fluoridated pumice (Glitter™ Premier, USA) to remove debris and extrinsic stains from the surface of the crowns. The rubber cups were used on a slow speed handpiece revolving at a speed of 5000 revolutions per minute (Figure 4.1). the rubber cup was replaced after every five teeth.


**Figure 4.1** Removal of extrinsic debris using a rubber cup and pumice.

### 4.5.2 Endodontic sealers

Several studies have evaluated the coronal discoloration resulting from different root canal sealers (Van der Burgt *et al.* 1986, Parsons *et al.* 2001, Davis *et al.* 2002, Partovi *et al.* 2006). Recent products introduced to the dental market have not yet been tested for the discoloration they may cause and as such were included in the study (Table 4.1).

- AH Plus™ (Dentsply, Switzerland) is an epoxy resin-based sealer and is the successor to AH26 (Figure 4.2). The previously marketed AH26 was proven to cause discoloration (Van der Burgt *et al.* 1986, Parsons *et al.* 2001, Davis *et al.* 2002, Partovi *et al.* 2006). AH Plus is not supposed to cause discoloration according to the manufacturer. There are no reports to the contrary in the literature and as such it was included amongst the sealers to be tested.

- EndoRez™ (Ultradent, South Jordan, UT, USA) is a UDMA resin-based sealer, introduced recently to the profession. This material is gaining wide interest with the evolution of resin bonding systems in endodontics and as such it was included amongst the sealers to be tested (Figure 4.3).

- Sealapex™ (Sybron Kerr, Romulus, MI, USA) is the sealer of choice used at the Faculty of Dentistry, University of the Western Cape. Accordingly, this calcium hydroxide-based material was incorporated in the study (Figure 4.4).

- Zinc oxide- eugenol based sealers are very widely used in Sudan. Hence it was the investigator's personal interest to observe the staining potential of this category of endodontic sealers. Pulp canal sealer™ (Sybron Kerr, Romulus, MI, USA) is the most popular zinc oxide-based sealer available commercially in South Africa, and widely used in the Paedodontics department at the Faculty of Dentistry, University of the Western Cape (Figure 4.5).

| Sealer tested | Manufacturer |
|---|---|
| **1. AH Plus** | DeTrey, Dentsply (Switzerland ) |
| **2. EndoRez** | Ultradent (UT, USA) |
| **3. Sealapex** | SybronEndo, Kerr (MI, USA) |
| **4. Pulp Canal Sealer** | SybronEndo, Kerr (MI, USA) |

**Table 4.1** List of sealers used in the study.


**Figure 4.2** AH Plus (DeTrey, Dentsply, Switzerland)


**Figure 4.3** EndoRez (Ultradent, USA)


**Figure 4.4** Sealapex (SybronEndo, Kerr, USA)


**Figure 4.5** PCS (SybronEndo, Kerr, USA)

### 4.5.3 Spectrophotometer

A spectrophotometer (Figure 4.6) was used to measure the CIE L*a*b* values of all the crowns of the teeth used in the study at baseline and every two weeks thereafter for the eight weeks of the study. The spectrophotometer (SP CM-2600d Konica Minolta Sensing, Japan) was calibrated using a white background specimen supplied by the manufacturer before the readings were taken. A probe with an aperture measuring 2mm in diameter was placed against the tooth surface with the aid of a custom made silicone index that would allow repositioning of the probe in exactly the same position over the tooth for the multiple readings for that tooth.

**Figure 4.6** Spectrophotometer with probe attached.

## 4.6 Methodology

After the extracted teeth were sifted according to the inclusion criteria, all the teeth were cleaned using a rubber cup and pumice to remove surface debris and stains. Sixty teeth were included in the experiment. The teeth were randomly assigned to the four experimental and the two control groups (Flowchart in Figure 4.7). Forty eight teeth were used as the experimental teeth, which were obturated with GP and randomly sealed with the four materials being tested (twelve teeth per group). The remaining twelve teeth were used as the control teeth with six teeth as positive controls and six teeth as negative controls. The six positive control teeth were filled with an amalgam filling material (Permite

C™/SDI, USA) in the access opening and sealed with composite (Z100™, 3M-ESPE, USA).

The six negative control teeth were only instrumented and sealed with a composite (Z100™, 3M-ESPE, USA). Permite C™ and Z100™ were used to fill the access cavities of the positive and negative control teeth as they are the current filling materials of choice used in the Faculty of Dentistry, University of the Western Cape to seal access cavities in the student clinics.

A coronal access cavity was created in all the teeth using a fissure carbide bur (No: 009, Dentsply-Maillefer Instruments, Switzerland) in a turbine hand-piece until the roof of the pulp chamber was just penetrated. A safe-tipped endodontic access bur (Dentsply-Maillefer Instruments, Switzerland) was then used to remove the entire roof and horns of the pulp chamber. The root canal was then prepared using the Profile system (Dentsply-Maillefer, Switzerland) to standardize the preparation technique (Figure 4.8). Thorough irrigation with 2.5% sodium hypochlorite (Milton™, Figure 4.9) followed by EDTA (RC Prep™, Premier, USA, Figure 4.10) was used throughout the preparation procedure according to the standard irrigation protocol recommended in the literature (Schafer 2007, Zehnder 2006). The canals were then dried with paper points and cotton pellets. This was followed by obturation using the tested sealer and GP (Dentsply-Maillefer, Switzerland). The coronal access was sealed with composite resin filling material (Z100™, 3M-ESPE, USA)

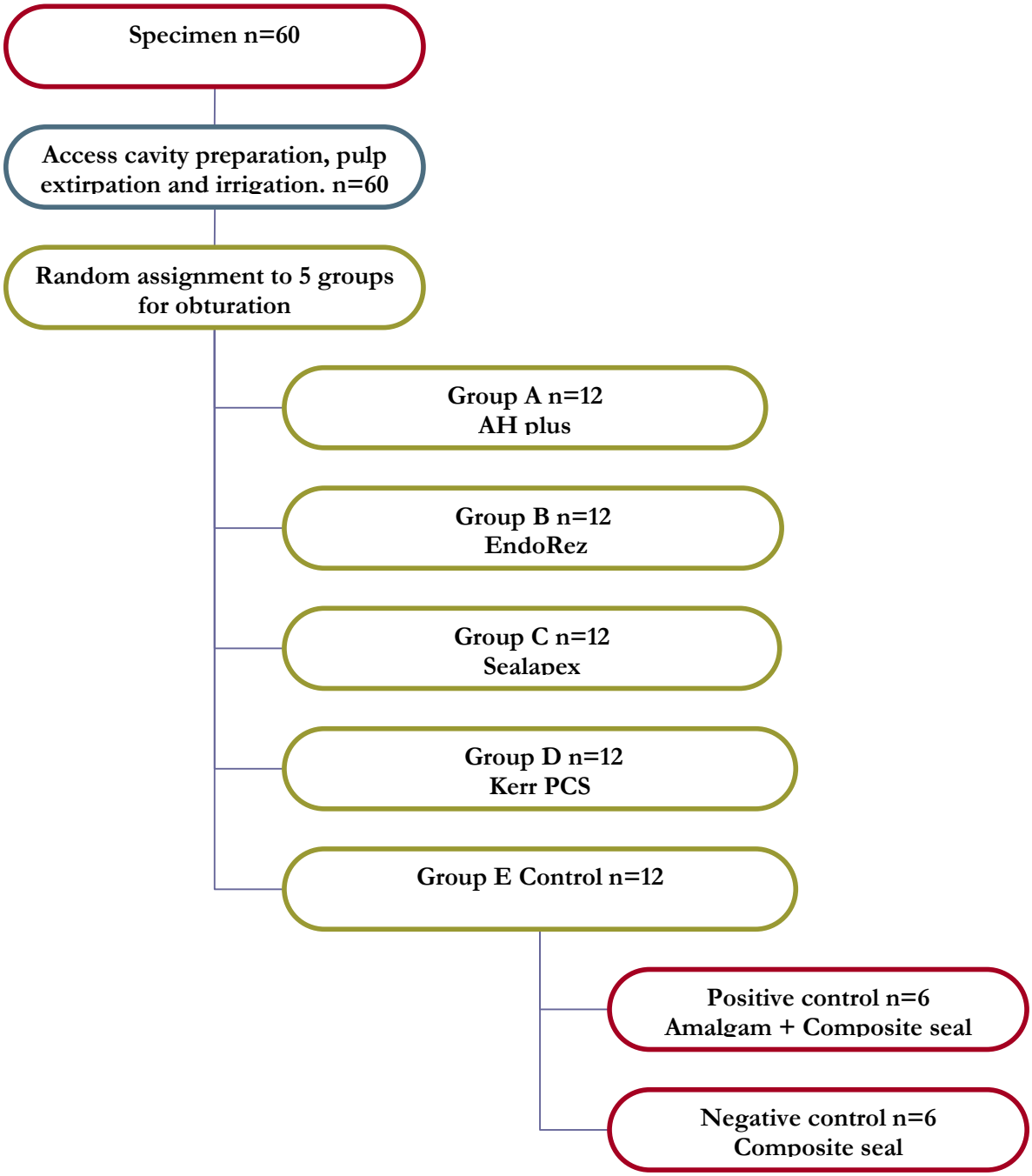


**Figure 4.7** Flowchart depicting the study design

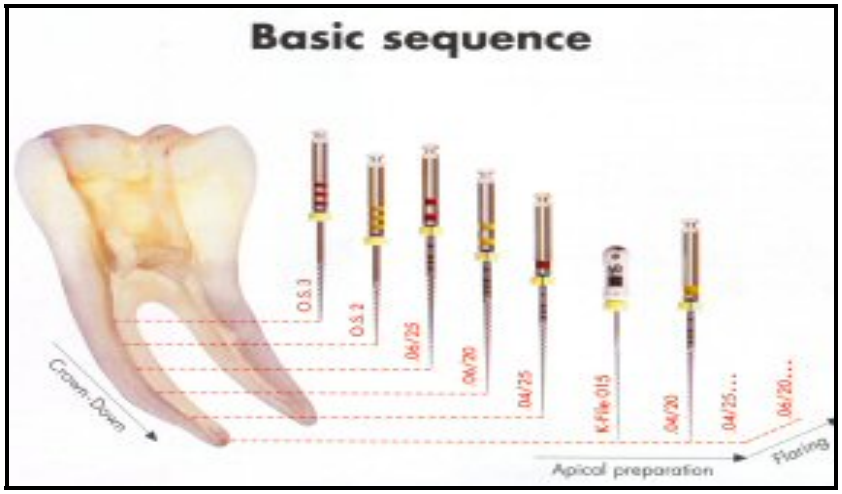


**Figure 4.8a** Basic sequence of root canal preparation using the Profile system as recommended by the manufacturer (From Dentsply International).

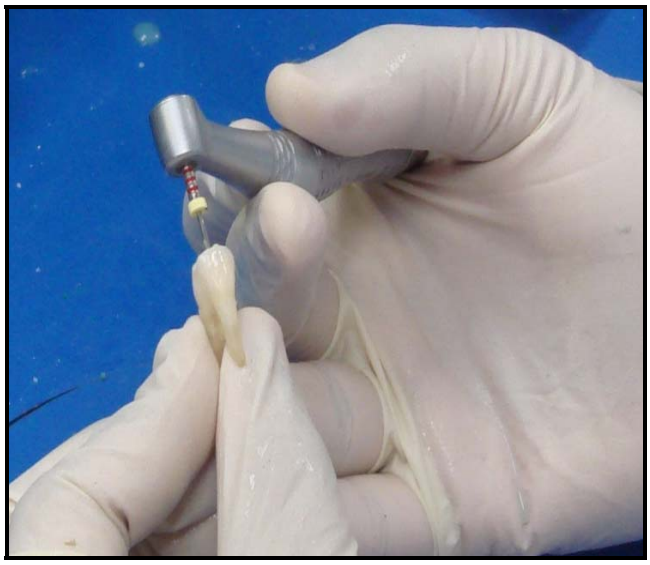

**Figure 4.8b** Root canal preparation with Profile rotary system (Dentsply).


**Figure 4.8c** Profile rotary system (Dentsply).


**Figure 4.9** Milton (2.5% Sodium hypochlorite solution used for root canal irrigation)


**Figure 4.10** RC Prep (Premier, USA) EDTA

Teeth were then stored partially submerged in sterile water in individually marked vials (Figure 4.11) in an incubator at 37°C (Memmert Schwartbach, Germany, Figure 4.12).

A custom-made index (Figure 4.13) was fabricated for each tooth using silicone impression putty (President™, Coltene-Whaledent, Germany). The index was constructed by moulding the impression putty around the 2mm aperture of the spectrophotometer when the probe was in the desired place on the tooth. The indices acted as a guide for the probe to ensure that it captured the CIE L*a*b* reading from exactly the same position every time the measurements were recorded.

After obturation, and at subsequent intervals (2, 4, 6, and 8 weeks), the teeth were evaluated for their colour co-ordinates utilising the spectrophotometer (Figure 4.14) and data was recorded in a data capture sheet (Appendix I).

The CIE L*a*b* values, where L* represents lightness, and a* and b* describe chroma, in which red is +a, and green is –a, yellow is +b, and blue is –b, obtained from the spectrophotometer readings were used to measure the colour change if any between the readings represented by ΔE in the following formula:

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

(From O'Brien 2002)

ΔL is the difference in lightness obtained by deducting the L* reading obtained from the spectrophotometer at a point from the previous L* reading. As such ΔL can be computed between any two L* readings and between any point of reference during the experiment and the baseline values recorded for L*. Δa and Δb are also calculated in the same manner as explained above. After calculating ΔL, Δa, and Δb values, ΔE can be determined using the formula according to O'Brien (2002).


**Figure 4.11** Teeth partially submerged in saline in individually marked vials. Note thermometer left inside incubator to control temperature.


**Figure 4.12a** Incubator set at 37˚C. Memmert™ (Germany)


**Figure 4.12b** Marked vials inside incubator.

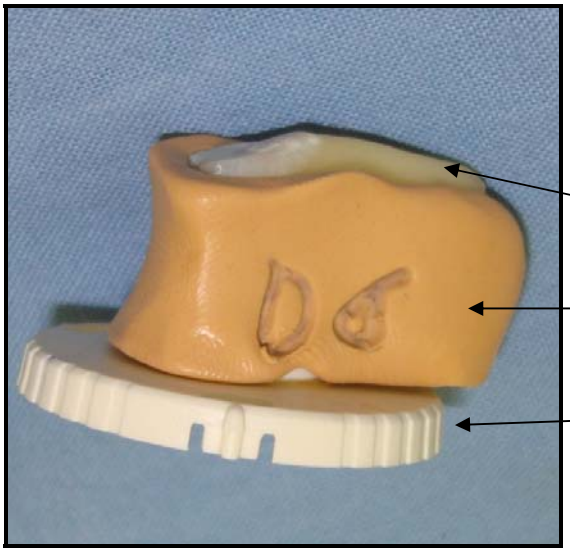


**Figure 4.13a** Silicone putty index with tooth and probe in place. Note each index is marked for referencing each tooth.

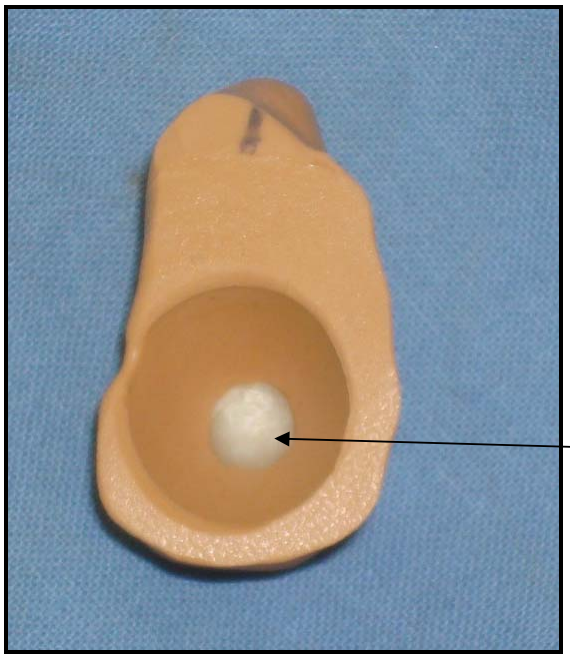


**Figure 4.13b** Inside view of index showing tooth position in relation to aperture.

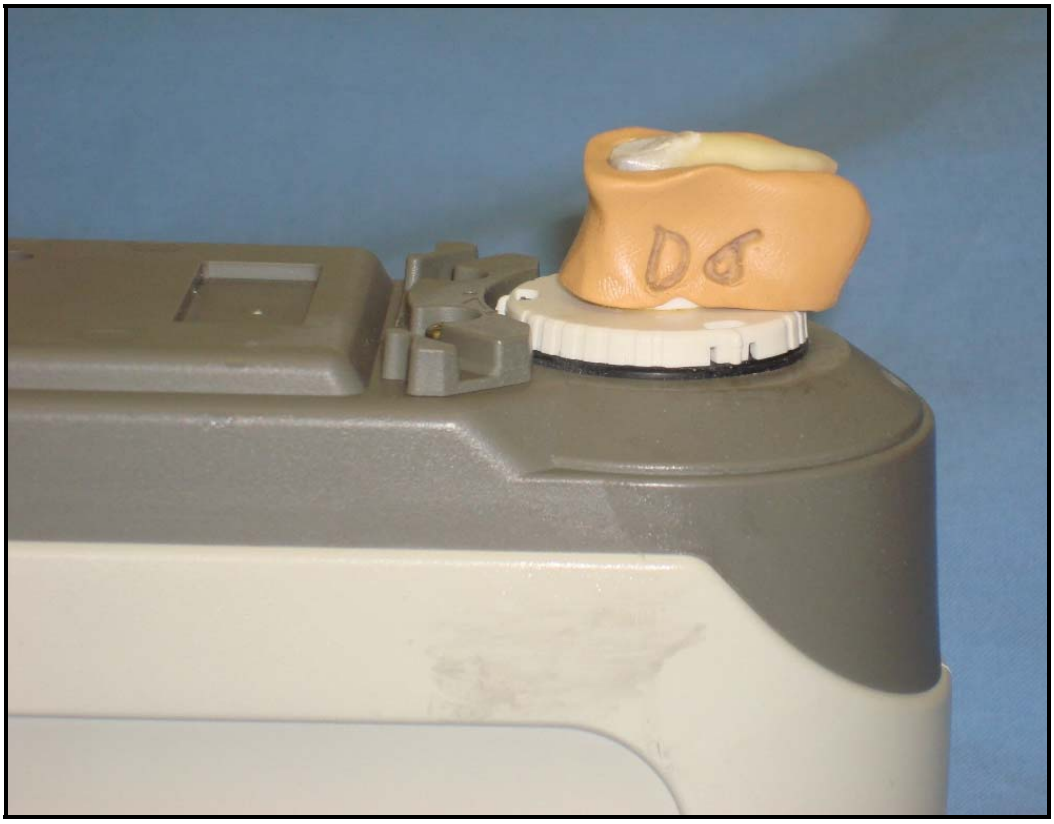


**Figure 4.14** Spectrophotometer measuring tooth colour with index.

## Chapter 5

# DATA ANALYSIS

The CIE L*a*b* values for each experimental tooth were obtained from the spectrophotometer. Baseline measurements were first recorded followed by subsequent readings at 2, 4, 6, and 8 weeks. These readings were digitally displayed in the spectrophotometer screen and recorded manually in a data capture sheet. The measurements were then transferred to an Excel spreadsheet (Microsoft Corporation, USA) for further analysis (Appendix I).

After the data was collected, a Wilcoxson Signed Rank Sum Test (non-parametric test for paired data) and a Kruskal Wallis Test (non-parametric one way analysis of variance) was used to determine statistically significant differences if any, in the L*a*b* values between the teeth at base line and subsequently at 2, 4, 6 and 8 weeks. P-values less than 0.05 were regarded as statistically significant. ΔE values greater than or equal to 3.5 are considered clinically observable changes (O'Brien 2002). All statistical analysis were carried out using SPSS 14.0 for windows (SPSS©, Inc. Chicago, IL, USA) and Microsoft Excel 2007 (Microsoft Corporation, USA).

## Chapter 6

# RESULTS

All measurements at baseline and subsequent readings at two, four, six, and eight weeks were transferred to an Excel spreadsheet (Microsoft Corporation, USA). The raw data (Appendix I) refers to L*a*b* values over the experimental period. The colour change represented by ΔE was computed using the following formula:

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

(From O'Brien 2002)

Where ΔL is the difference in lightness calculated by differences in the L* readings between two periods. This can be calculated for any period between baseline and at two, four, six, and eight weeks. Δa and Δb refers to the difference in chroma and are also obtained in the same manner as for ΔL. Similarly Δa and Δb represent the differences in a* and b* readings between any two periods. This can be calculated for any period between baseline and at two, four, six, and eight weeks.

Appendix II represents the calculations of ΔE for all the experimental groups between baseline and at two, four, six, and eight weeks.

According to O'Brien (2002), a ΔE value ranging between 3.3 and 3.5 is considered a clinically observable colour change. For convenience and for the purposes of this study, a ΔE value greater than or equal to 3.5 was considered a clinically detectable colour change.

## 6.1 Descriptive Analysis

The means, standard deviation, range (minimum and maximum values) for each experimental group at two, four, six, and eight weeks were calculated using Microsoft Excel (Microsoft Corporation, USA).

Table 6.1, 6.2, 6.3, and 6.4 outline the descriptive data of all the experimental groups at the four measurement intervals respectively.

### Colour changes (ΔE) at two weeks:

Table 6.1 summarises the colour changes of the experimental groups at two weeks from baseline. The data from Table 6.1 are illustrated in the Box plot graph (Figure 6.1). At 2 weeks, Pulp Canal Sealer showed the highest discoloration with a mean ΔE of 7.68, followed by Sealapex and EndoRez with a mean ΔE of 7.41 and 5.89 respectively. AH Plus exhibited the least discoloration with a mean ΔE of 5.68 (Table 6.1 and Figure 6.1). According to the guidelines of O'Brien (2002) all the changes that occurred by the end of two weeks after obturation could be clinically perceptible as the ΔE was greater than 3.5.

| | Sealer | | | | | |
|---|---|---|---|---|---|---|
| **Data** | **AH Plus (1)** | **EndoRez (2)** | **Sealapex (3)** | **PCS (4)** | **Positive (5)** | **Negative (6)** |
| **Count of ΔE** | 12 | 12 | 12 | 12 | 6 | 6 |
| **Mean of ΔE** | 5.68 | 5.89 | 7.41 | 7.68 | 6.90 | 4.04 |
| **SD of ΔE** | 1.76 | 1.60 | 1.71 | 2.28 | 1.62 | 2.42 |
| **Min of ΔE** | 3.24 | 3.33 | 4.83 | 4.32 | 4.81 | 1.47 |
| **Max of ΔE** | 8.70 | 8.18 | 11.31 | 10.85 | 9.27 | 7.55 |

**Table 6.1 Analysis of ΔE at two weeks**

From Figure 6.1 it is evident that an outlier exists in the Sealapex experimental group. This outlier is the 35th reading which corresponds to the maximum colour change that was measured in the Sealapex group at two weeks from baseline (ΔE of 11.31). The next highest ΔE in the Sealapex group at two weeks is a ΔE of 8.99 (Appendix II) which is almost a ΔE value of 3 lower than the highest ΔE. this implies that the colour difference between these two specimens within the same group at two weeks could be clinically perceptible.

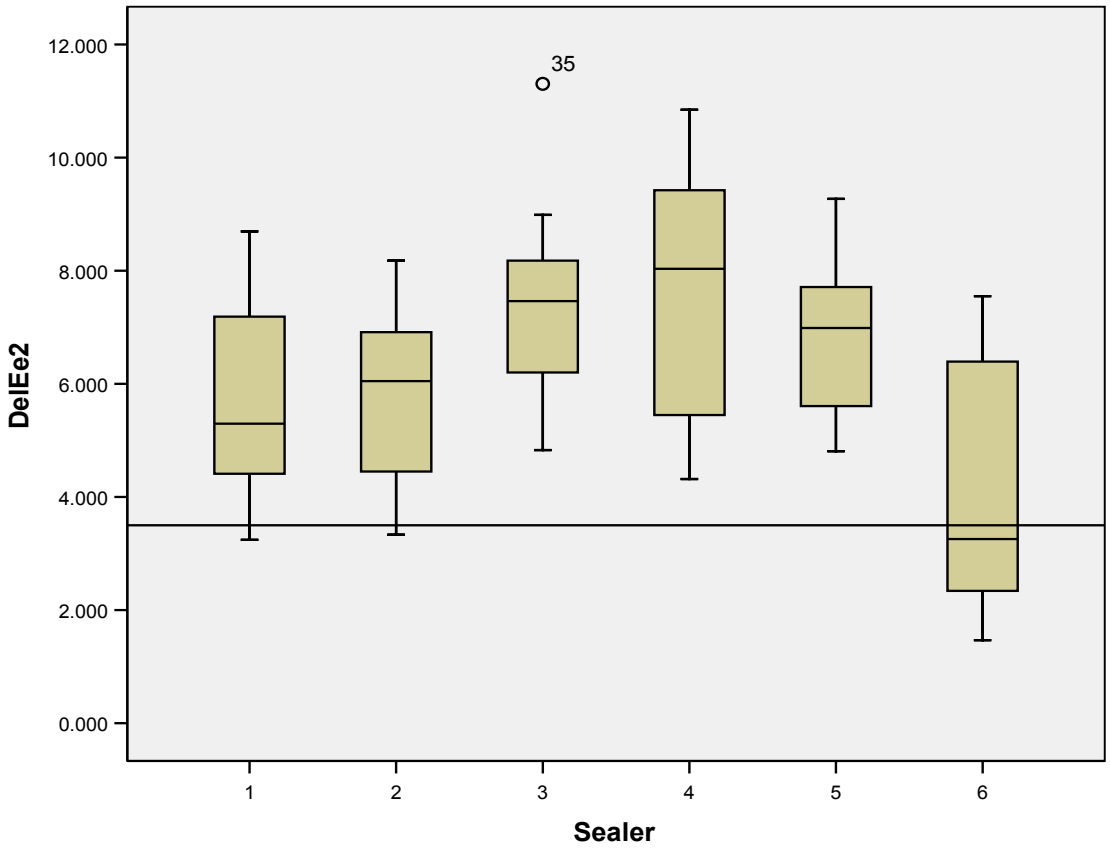


**Figure 6.1 Box plot of colour changes represented by ΔE at two weeks.**

## Colour changes (ΔE) at four weeks:

The colour changes from baseline to four weeks of the different experimental groups are tabulated in Table 6.2 and graphically illustrated in Figure 6.2. At 4 weeks AH Plus and PCS showed the greatest discoloration with a mean ΔE of 6.30 and 6.28 respectively. Sealapex presented less colour change with a mean ΔE of 5.42, whilst EndoRez with a mean ΔE of 4.92, exhibited the least colour change according to the spectrophotometric readings. However, all these colour changes according to O'Brien (2002) would be clinically perceptible as the ΔE is greater than 3.5.

| | Sealer | | | | | |
|---|---|---|---|---|---|---|
| **Data** | **AH Plus (1)** | **EndoRez (2)** | **Sealapex (3)** | **PCS (4)** | **Positive (5)** | **Negative (6)** |
| **Count of ΔE** | 12 | 12 | 12 | 12 | 6 | 6 |
| **Mean of ΔE** | 6.30 | 4.92 | 5.42 | 6.28 | 6.27 | 4.23 |
| **SD of ΔE** | 1.95 | 0.80 | 1.23 | 1.47 | 2.56 | 1.82 |
| **Min of ΔE** | 3.89 | 3.16 | 3.05 | 4.14 | 3.03 | 2.64 |
| **Max of ΔE** | 10.26 | 6.66 | 7.28 | 8.50 | 8.82 | 7.35 |

**Table 6.2 Analysis of ΔE at four weeks**

It is evident from Figure 6.2 that two outliers exist that relate to the 22$^{nd}$ and 24$^{th}$ readings in the EndoRez experimental group. These values correspond to the maximum and minimum colour change that occurred at four weeks from baseline in the experimental group that was sealed with EndoRez (ΔE of 3.16 and 6.66 respectively). The rest of the readings for EndoRez computed to a narrow spread around the mean ΔE of 4.9.

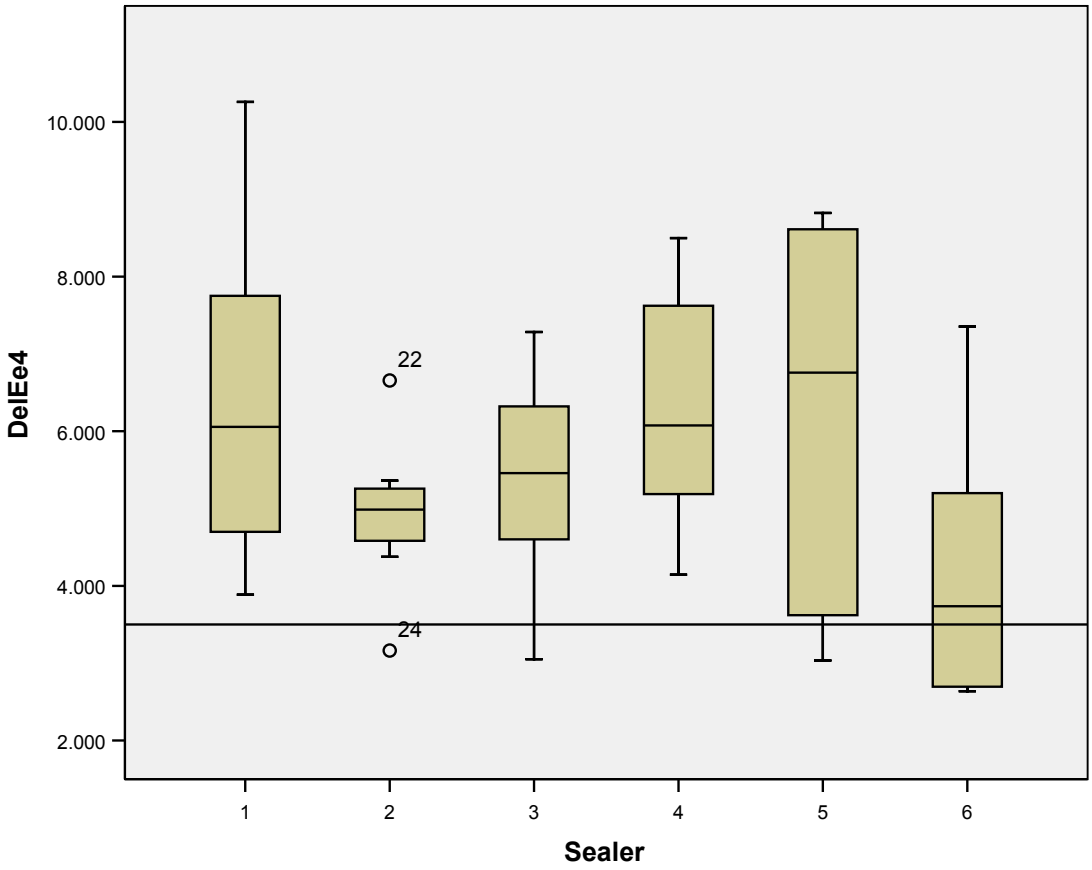


**Figure 6.2 Box plot of colour changes represented by ΔE at four weeks**

## Colour changes (ΔE) at six weeks:

Coronal discoloration measured at six weeks from baseline is summarised in Table 6.3 and depicted graphically in Figure 6.3. At week six Sealapex had the greatest discoloration amongst the experimental groups with a mean ΔE of 17.13. All the other groups also demonstrated a dramatic increase in the degree of discoloration ranging from a mean ΔE of 11 to 14.5, except for the negative control which only had a mean ΔE of 8.25. However at this stage the colour changes in all the specimens from baseline would have been clinically perceptible.

| | Sealer | | | | | |
|---|---|---|---|---|---|---|
| **Data** | **AH Plus (1)** | **EndoRez (2)** | **Sealapex (3)** | **PCS (4)** | **Positive (5)** | **Negative (6)** |
| **Count of ΔE** | 12 | 12 | 12 | 12 | 6 | 6 |
| **Mean of ΔE** | 13.98 | 14.52 | 17.13 | 14.19 | 11.03 | 8.25 |
| **SD of ΔE** | 3.15 | 2.62 | 2.68 | 4.47 | 2.99 | 1.65 |
| **Min of ΔE** | 9.10 | 10.24 | 12.78 | 8.77 | 8.66 | 5.20 |
| **Max of ΔE** | 17.64 | 17.89 | 20.80 | 21.01 | 16.42 | 9.89 |

**Table 6.3 Analysis of ΔE at six weeks**

An outlier corresponding to the 55$^{th}$ reading existed at six weeks. This value corresponds to the minimum colour change that occurred in the negative control group at six weeks from baseline (ΔE of 5.20). The rest of the readings for EndoRez computed to a narrow spread around the mean ΔE of 8.25.

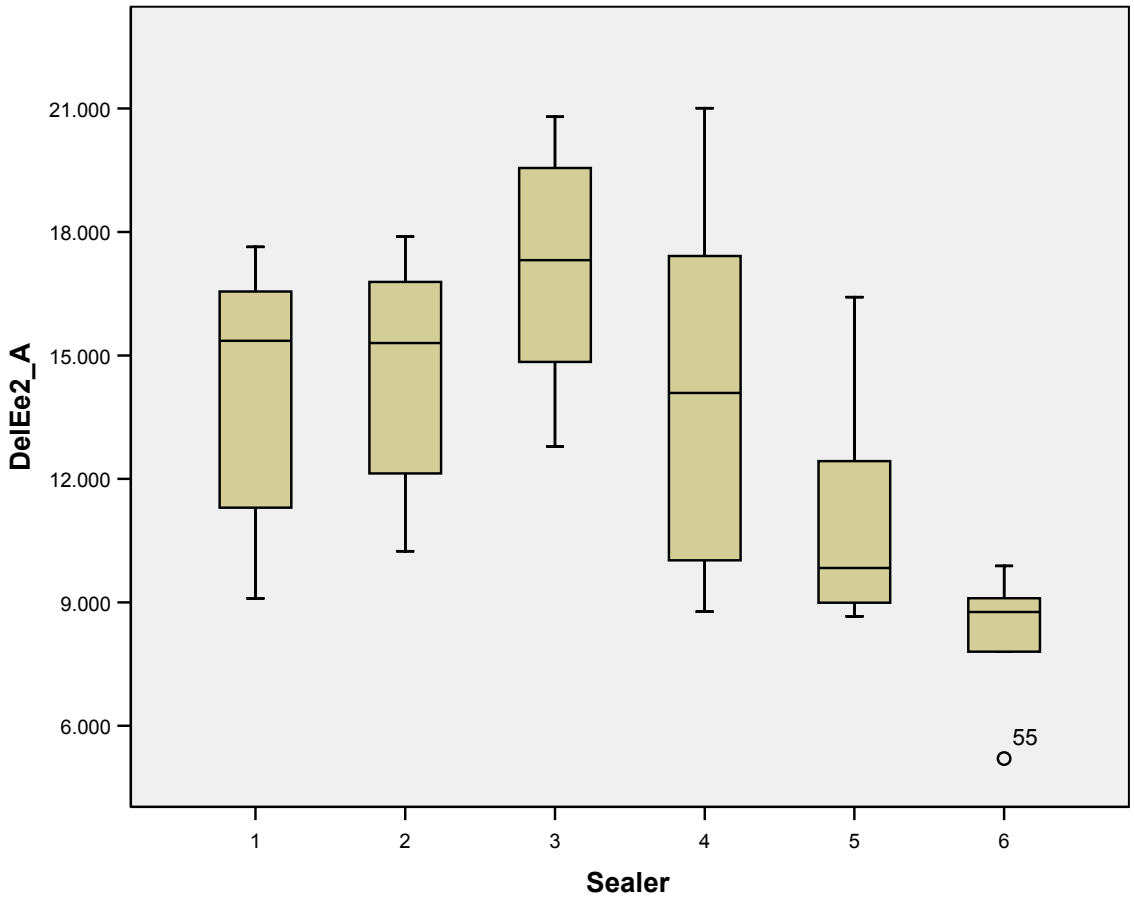


**Figure 6.3 Box plot of colour changes represented by ΔE at six weeks**

## Colour changes (ΔE) at eight weeks:

The final readings at week 8, revealed that Sealapex, PCS, and AH Plus demonstrated the highest discoloration with a mean ΔE of 8.89, 8.79, and 8.70 respectively, which are all very similar. EndoRez at week 8 showed the least colour change amongst the experimental sealers with a mean ΔE of 7.29 (Table 6.4 and Figure 6.4). At this stage, according to O'Brien (2002), the colour changes from baseline would have been clinically perceptible.

| | Sealer | | | | | |
|---|---|---|---|---|---|---|
| **Data** | **AH Plus (1)** | **EndoRez (2)** | **Sealapex (3)** | **PCS (4)** | **Positive (5)** | **Negative (6)** |
| **Count of ΔE** | 12 | 12 | 12 | 12 | 6 | 6 |
| **Mean of ΔE** | 8.70 | 7.29 | 8.89 | 8.79 | 9.01 | 6.43 |
| **SD of ΔE** | 2.85 | 1.52 | 1.03 | 1.31 | 1.31 | 1.90 |
| **Min of ΔE** | 3.75 | 5.23 | 7.00 | 6.34 | 7.35 | 3.08 |
| **Max of ΔE** | 11.92 | 9.97 | 10.29 | 10.52 | 11.23 | 8.37 |

**Table 6.4 Analysis of ΔE at eight weeks**

At week eight, an outlier was detected which related to the 49$^{th}$ reading as evident from Figure 6.4. This outlier corresponds to the maximum colour change recorded for the positive control group (ΔE of 11.23).

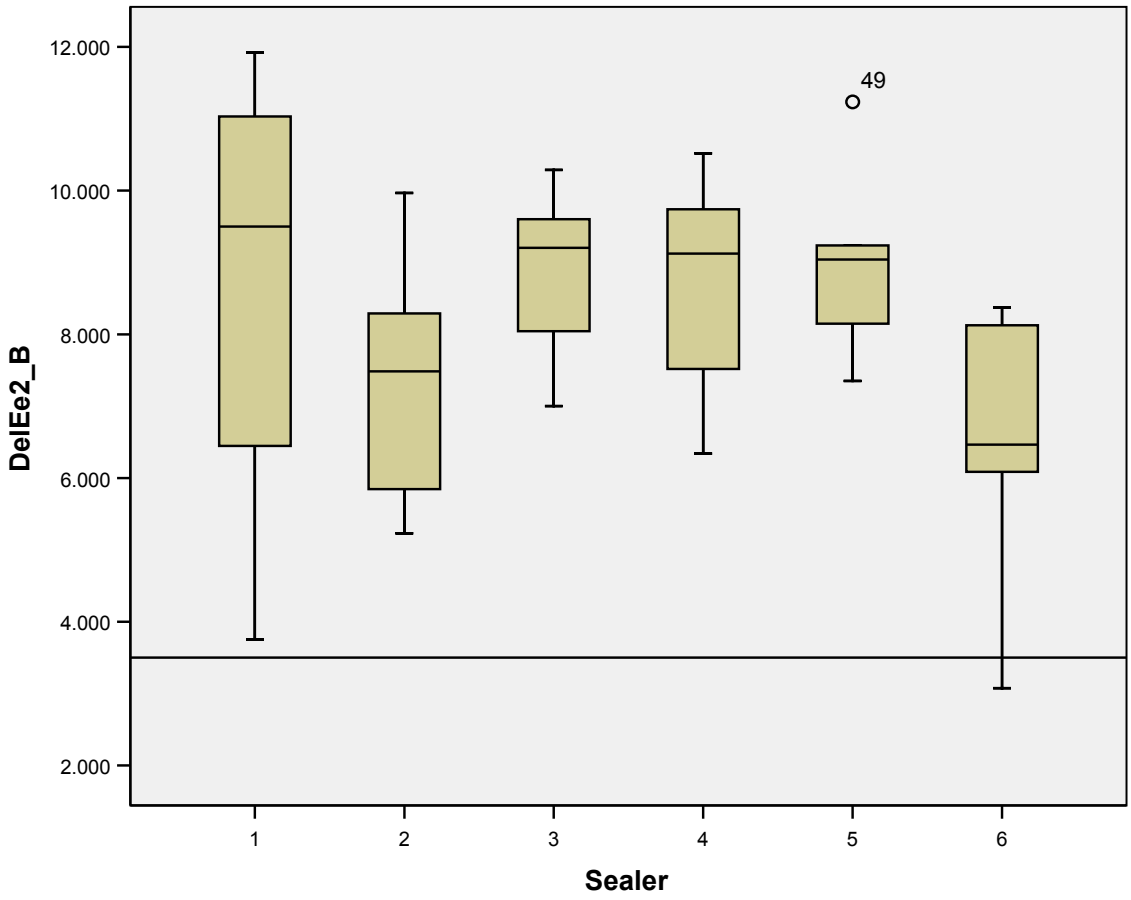


**Figure 6.4 Box plot of colour changes represented by ΔE at eight weeks.**

## 6.2 Degree of tooth discoloration

The degree of tooth discoloration during the experimental period for each group is summarised in Table 6.5. The table outlines the mean colour changes (Mean ΔE) for each experimental group and the two control groups from baseline at two, four, six, and eight weeks.

| | Mean ΔE | | | |
|---|---|---|---|---|
| **Group** | Week 2 | Week 4 | Week 6 | Week 8 |
| **AH Plus** | 5.68291 | 6.29581 | 13.98057 | 8.70249 |
| **EndoRez** | 5.89312 | 4.91879 | 14.52186 | 7.28729 |
| **Sealapex** | 7.41300 | 5.41874 | 17.12936 | 8.89295 |
| **Pulp Canal Sealer** | 7.68388 | 6.27591 | 14.18858 | 8.78864 |
| **Positive control** | 6.89561 | 6.26744 | 11.02848 | 9.00774 |
| **Negative control** | 4.04376 | 4.22695 | 8.25325 | 6.43201 |

**Table 6.5 Mean colour changes from baseline at 2, 4, 6, and 8 weeks as reflected by ΔE.**

The data from Table 6.5 is depicted graphically in Figure 6.5 and Figure 6.6. As evident from Table 6.5, Figure 6.5 and Figure 6.6, the negative controls had the least tooth discoloration throughout the experimental period with a mean ΔE ranging from 4.0 at the end of 2 weeks to a high of 8.3 at the end of 6 weeks which then decreased to 6.43 at the end of 8 weeks. Overall the negative control group had a mean ΔE of 6.4 at the end of the experimental period (week 8) which according to O'Brien was sufficient to be perceived clinically as a colour change.

The positive control group also had an immediate discoloration with a mean ΔE ranging from 6.89 at the end of 2 weeks to a high of 11.0 at the end of 8 weeks, which was in the range of the other experimental groups.

The experimental group Ah Plus exhibited an immediate discoloration with a mean ΔE ranging from as low as 5.68 at 2 weeks and as high as 13.98 at 6 weeks which then declined to 8.7 at week 8. The overall degree of discoloration was ΔE of 8.7 at the end of the observation period, which according to O'Brien (2002) would be sufficient to be perceived clinically as a colour change.

EndoRez revealed an immediate colour change at two weeks from baseline (ΔE=5.68) which then slightly declined to a ΔE of 4.92 at the end of week 4. An abrupt increase to as high as 14.52 at week six and 7.29 at week 8 was recorded. According to O'Brien (2002) the overall change in colour for EndoRez was 7.28 at the end of the experimental period, which could be regarded as a clinically perceptible change.

Sealapex displayed an immediate change in colour ranging from a ΔE of 7.41 at week two to as high as ΔE of 17.13 at week six, declining to 8.89 at the end of the observation period. This overall change in colour from baseline to week 8 (ΔE=8.89) would be regarded as clinically perceptible as ΔE is greater than 3.5 (O'Brien 2002).

Kerr's Pulp Canal Sealer demonstrated colour changes (ΔE) in the range of 7.68 at 2 weeks to as high as 14.19 at week 6, regressing to 8.79 at week 8. The overall colour change from baseline to week 8 was ΔE=8.79, which would be sufficient to be perceived clinically (O'Brien 2002).

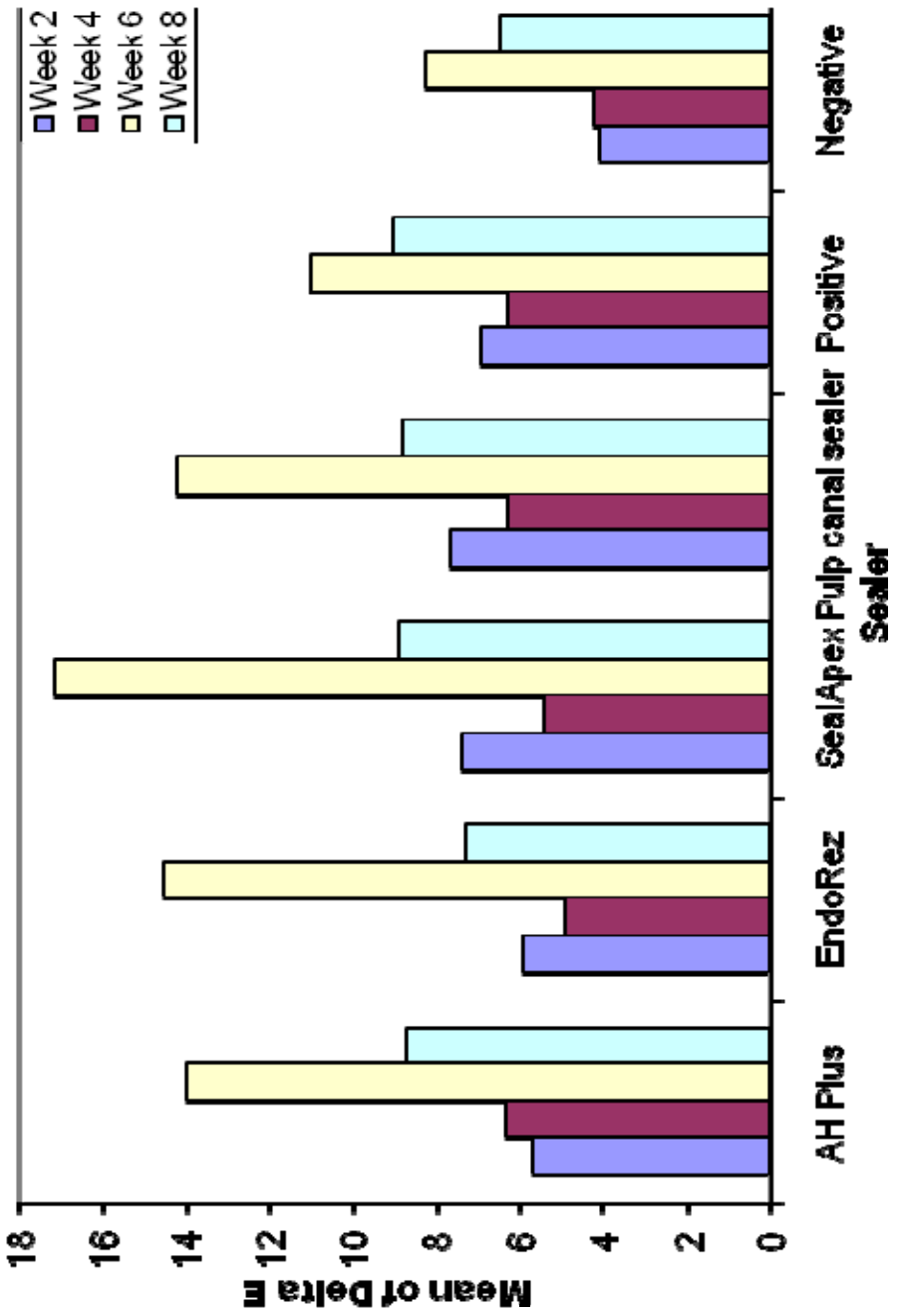


**Figure 6.5 Column chart displaying mean colour changes (ΔE) over time.**

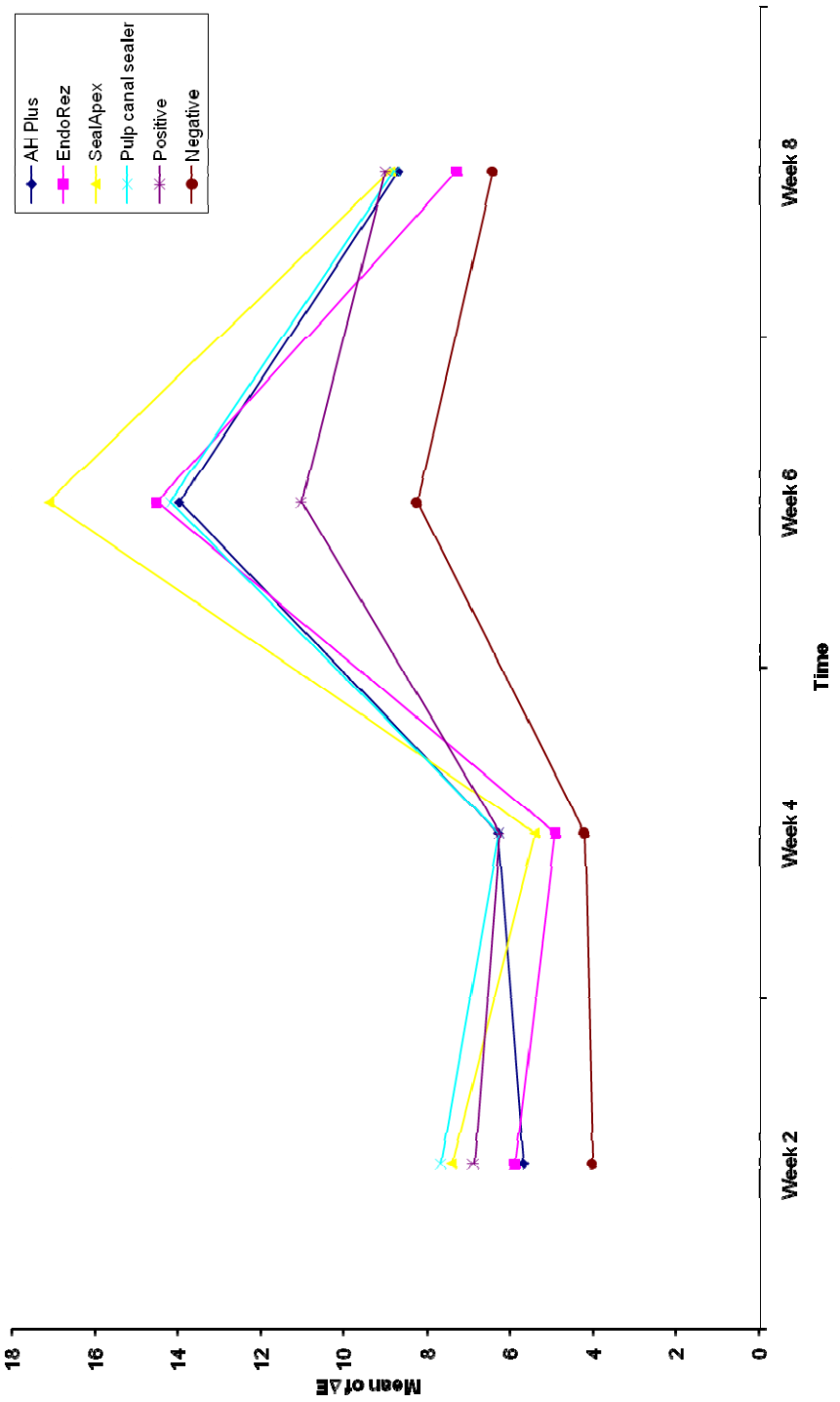


**Figure 6.6 Line graph demonstrating mean colour change ΔE over time.**

## 6.3 Differences in discoloration within weeks

Table 6.6 represents the differences in the mean colour change that took place from two weeks to four weeks, four weeks to six weeks, and finally from six to eight weeks. These measurements demonstrate the difference in discoloration within weeks. It is evident from Table 6.6 that slight discoloration occurred between weeks two and four, ranging from -1.99 to 0.6. This would not have been clinically perceptible as the threshold is a ΔE of 3.5 (O'Brien 2002). The greatest discoloration was evident between week four and week six, ranging from 4.02 to 11.7. The colour changes would have been clinically perceptible in all the groups as ΔE is greater than 3.5. Regression or an improvement in colour was again evident between week six and week eight, with a ΔE ranging from -8.23 to -1.82. This was especially true for the experimental groups implying that products influencing the colour of the tooth were neutralised or not as influential, as the colour seems to be improving.

| Differences in the mean colour change Δ (Mean ΔE) | | | |
|---|---|---|---|
| **Group** | Δ (Δ E4-ΔE2) | Δ (Δ E6-ΔE4) | Δ (Δ E8-ΔE6) |
| **AH Plus** | 0.6129 | 7.68476 | -5.27808 |
| **EndoRez** | -0.97433 | 9.60307 | -7.23457 |
| **Sealapex** | -1.99426 | 11.71062 | -8.23641 |
| **Pulp Canal Sealer** | -1.40797 | 7.91267 | -5.39994 |
| **Positive control** | -0.62817 | 4.76104 | -2.02074 |
| **Negative control** | 0.18319 | 4.0263 | -1.82124 |

**Table 6.6 Differences in the mean colour change within observation periods.**

## 6.4 Analysis of colour difference (ΔE)

The measurements of each experimental group was then analysed with a non-parametric paired test, Wilcoxson Signed Rank Sum test. This test compared the differences between the colour change (ΔE) at two weeks with the subsequent colour changes at four, six, and eight weeks (Appendix III).

Table 6.6 summarizes the results of the Wilcoxson Signed Rank Sum Test.

| Group | Median ΔE2 | Median ΔE4 | P-value | Median ΔE6 | P-value | Median ΔE8 | P-value |
|---|---|---|---|---|---|---|---|
| **AH Plus** | 5.298 | 6.056 | 0.2094 | 15.356 | 0.0022* | 9.501 | 0.0229* |
| **EndoRez** | 6.049 | 4.986 | 0.0597 | 15.301 | 0.0022* | 7.483 | 0.0096* |
| **Sealapex** | 7.463 | 5.457 | 0.0076* | 17.315 | 0.0022* | 9.204 | 0.0186* |
| **PCS** | 8.033 | 6.075 | 0.0712 | 14.087 | 0.0022* | 9.124 | 0.0281* |
| **Positive** | 6.987 | 6.757 | 0.4631 | 12.432 | 0.0277* | 9.198 | 0.0277* |
| **Negative** | 3.256 | 3.736 | 0.7532 | 9.080 | 0.0277* | 8.250 | 0.0464* |

***Changes in colour statistically significant at P<0.05**

**Table 6.7 Summary of Wilcoxson Signed Rank Test.**

### AH Plus:

As depicted in Table 6.7, there is a colour change (ΔE) from two weeks (5.298) to four weeks (6.056) in the AH Plus experimental group. Results of the Wilcoxson Signed Rank Sum test indicate that this change in colour is not statistically significant (*P-value=0.2094*).

However there is a statistically significant change in colour (*P-value=0.002*) at six weeks, as well as at eight weeks (*P-value=0.023*).

### EndoRez:

The experimental group which was sealed with EndoRez revealed a similar pattern compared to the AH Plus group. There was no statistically significant colour difference from week two to week four (*P-value=0.056*), however there was a statistically significant colour change at week six and week eight (*P-values 0.002* and *0.009* respectively).

### Sealapex:

Sealapex displayed a colour change which was statistically significant throughout the experimental period. A statistically significant colour change was noticed at week four (*P-value=0.007*), week six (*P-value=0.002*) and at week eight (*P-value=0.018*).

### Pulp Canal Sealer (PCS):

The experimental group PCS revealed not statistically significant changes in colour between week two and week four (*P-value=0.07*). Measurements of colour change were statistically significant at the subsequent weeks (*P-values 0.002* at week 6 and *0.028* at week 8 respectively).

### Control groups:

The positive control group demonstrated a statistically significant colour change at weeks six and eight (*P-value=0.02* at both recording periods), when compared to the colour change at week two (*P-value=0.463*).

The negative control showed the least colour change at week four and this was not statistically significant from week two with a *P-value=0.753*. Although this group displayed the least colour change at subsequent weeks, the colour change was statistically significant when compared to week two (*P-value=0.028* at week 6 and *0.046* at week 8 respectively).

## 6.5 Analysis of colour difference (ΔE) between groups

A non-parametric one way analysis of variance test was used to compare the colour differences that occurred between the experimental groups. The test used to describe this comparison between the groups is the Kruskal Wallis test (Appendix IV). The summary of this test is presented in Table 6.8.

| Kruskal Wallis Test | | | | |
|---|---|---|---|---|
| **Sealer** | ***Sample*** | ***Rank Sum*** | ***Sample Size*** | ***Test Statistic*** |
| **AH Plus** | 1 | 336 | 12 | |
| **EndoRez** | 2 | 184 | 12 | *H = 6.8912* |
| **Sealapex** | 3 | 332 | 12 | *P-Value = 0.0754* |
| **PCS** | 4 | 324 | 12 | |

**Table 6.8 Summary of the Kruskal Wallis test.**

The results of the Kruskal Wallis test indicate that there is no statistically significant difference in colour change (ΔE) (*P-value=0.0754*) between the experimental sealers. However, from the results in Table 6.8, it is evident that EndoRez had the least rank (184) when compared to the other sealers and may be regarded the best amongst the other experimental sealers.

## 6.6 Conclusion

In conclusion, the results obtained from the Wilcoxson Rank Sum test and the Kruskal Wallis tests indicate that there is a considerable effect of time and sealer type on the discoloration. The discoloration in the groups does change over time. Overall there was no statistically significant difference in the degree of discoloration between the experimental groups, however there was a statistically significant difference within the groups between the different recording periods.

Chapter 7

# DISCUSSION

## 7.1 Colour analysis

Evaluation of tooth colour can be divided into either subjective or objective analysis, depending on the measuring medium. A subjective method of tooth colour analysis can be conducted via visual shade guides which are commercially available (Vitapan® Classic and VITA™ 3D-Master®, Vident Incorporation, Germany). The main disadvantage of the visual method is the difficulty in achieving a perfect colour match. This is further affected by interfering variables, such as the observer's interpretation and environmental influences such as the light source. Colour perception varies amongst individuals, and colour fatigue is a common phenomena resulting from exposure to a constant colour stimulus that might decrease the response of the eye to that specific colour. Other factors that can affect colour perception include ageing, emotional status of the observer, and metamerism (Cal *et al* 2006, O'Brien 2002).

Spectrophotometry is an objective (instrumental) alternative to the subjective (visual) method of assessing colour. This device eliminates the uncontrolled variables during the colour matching process, thus providing a more accurate result. Spectrophotometers are extremely sensitive devices, and can be very useful in determining minute colour changes. Unlike the human eye, a reflectance spectrophotometer can readily record colour changes that are not even clinically observable. These colour changes are also detected much earlier when compared to the traditional visual assessment of tooth colour. For these reasons, it

was decided to use spectrophotometric analysis for evaluating tooth discoloration in this study (Guan *et al* 2005).

## 7.2 Preparation technique

The previous studies that analysed tooth discoloration from endodontic materials (Van der Burgt *et al.* 1986, Parsons *et al.* 2001, Davis *et al* 2002, Partovi *et al.* 2006) performed similar obturation techniques. Preparation of the root canals was via an apical access cavity, which is not performed clinically. In this study, a coronal access cavity was created to obturate the root canal system, simulating the clinical scenario. Furthermore, the previous studies placed the tested sealer in bulk in the pulp chambers. Although every effort should be performed to remove all the excess sealer from the pulp chamber following root canal obturation, there is often little or no attempt by the dentist to remove this excess. Thus, in this study, no attempt was made to remove this excess sealer from the pulp chamber.

## 7.3 Effect of time

The exact time interval for tooth discoloration to occur resulting from root canal therapy is still not documented. Previous studies revealed that coronal tooth discoloration resulting from endodontic materials takes place form seven weeks after obturation (van der Burgt *et al* 1986) to several months (Parsons *et al* 2001, Davis *et al* 2002). Differences in the results of the previous studies could be attributed to the methodologies employed. The amount of time to lapse for discoloration to be clinically observable depends on many factors that include the thickness of the remaining dentine, the quality and quantity of the sealer, and the presence of the smear layer (Grossman *et al* 1988).

A similar study by van der Burgt *et al* (1986) illustrated measurable discoloration only after seven weeks. Although, in both the van der Burgt study and this study the smear layer was removed, discoloration of the teeth by the different sealers in this study was measurable at two weeks. This difference in time to discoloration could be attributed to the criteria of colour analysis utilised. In the van der Burgt (1986) study trained visual inspectors analysed the colour difference between samples. This subjective method of colour analysis was prone to error due to the factors (individual and environmental) that might intervene with the perception of colour. In the present study, a more accurate approach was used to measure the colour at the different times. A spectrophotometer can detect colour without the interference of any uncontrolled factors. In addition, this instrument is very sensitive thus not requiring a long experimental time period (Cal *et al* 2006, O'Brien 2002).

The investigations of Parsons *et al* (2001) and Davis *et al* (2002) revealed a contradictory outcome. In both those studies, tooth discoloration occurred only after several months. This could be largely explained by the methodology utilised to prepare the experimental samples and the method of colour analysis. No attempt was made to remove the smear layer in both the studies. In a clinical situation, it is almost impossible to limit the effect of sodium hypochlorite and EDTA to the root canal space only, without removing the smear layer of the pulp chamber as well. Therefore, leaving behind the smear layer in the pulp chamber will occlude the dentinal tubules, and will dramatically reduce the rate of sealer penetration through dentine. This may explain why discoloration in these two studies was only evident after several months even though the studies utilised digital imaging which is a reliable method to analyse tooth colour (Guan *et al* 2005).

Figure 7.1 is a modification of the line graph in Figure 6.6. The following section will use this modified line graph to explain the trends in colour changes that took place over time.

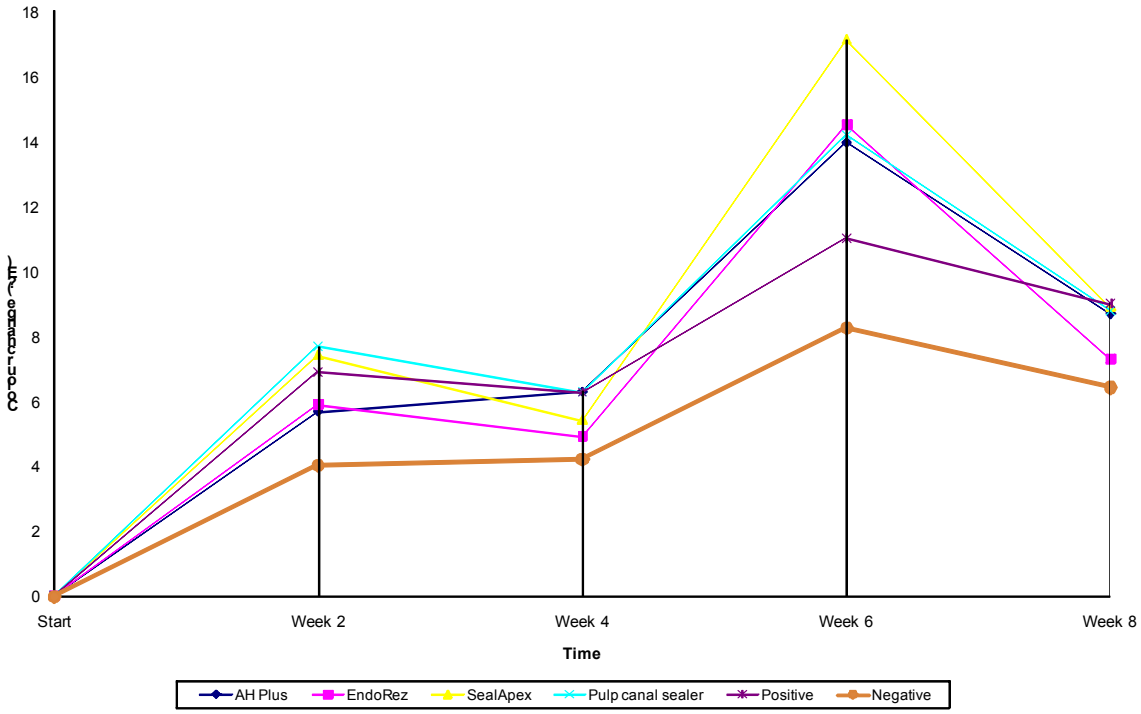


**Figure 7.1 Line graph demonstrating mean colour change (ΔE) over time.**

## Colour change at two weeks

At two weeks, all experimental sealers exhibited a mean colour change (ΔE) which was clinically perceptible (ΔE≥3.5) from baseline, ranging from 5.68 to 7.68 (Table 6.1 and Figure 7.1).

Pulp Canal Sealer (PCS) exhibited the greatest discoloration with a mean ΔE of 7.68. This initial discoloration of PCS can be attributed to the silver constituents in the powder and the eugenol content of the liquid.

An outlier existed in the Sealapex experimental group (Figure 6.1) which corresponded to the maximum value recorded at two weeks (ΔE=11.31). This outlier could have resulted from external factors such as the improper placement of the silicone index for that tooth (C35). Another

reason could be the sensitivity of the spectrophotometer to external factors such as temperature, external sources of light, or the improper placement of the measuring probe.

## Colour change at four weeks

Minimal change in colour (ΔE) occurred between week 2 and week 4 (Figure 7.1). The colour change (ΔE) ranged from as low as -1.99 (Sealapex) to as high as 0.6 (AH Plus). Although the values indicate an improvement in colour, they were all clinically not perceptible, as the values were all less than ±3.5. However, when the measurements at week 4 are compared from baseline, AH Plus and PCS exhibited the greatest discoloration which were clinically observable changes, with a mean ΔE of 6.30 and 6.28 respectively. EndoRez showed the least discoloration at four weeks from baseline (mean ΔE of 4.92). Although the difference in ΔE of EndoRez from the other groups is not great, the standard deviation is considerably small (SD=0.80) and the range in ΔE varied from 3.16 to 6.66. Thus, the results suggest that EndoRez was a more predictable material when compared to the other experimental groups (Figure 6.2).

## Colour change at six weeks

A gradual increase was noticed for all the experimental and control groups in ΔE from week 4 to week 6 (Figure 7.1). The colour changes (ΔE) ranged from 4.03 to 11.71, which were all regarded as clinically detectable changes. Comparisons from baseline indicate that colour changes at six weeks were greatest for Sealapex with a mean ΔE of 17.13 and a standard deviation of 2.68. Although EndoRez had a mean ΔE of 14.5 the SD was 2.62 and the ΔE ranged from 10 to 17, thus having the

least variability when compared to the other groups (Table 6.3 and Figure 6.3).

## Colour change at eight weeks

The data from Table 6.6 represents the differences in the mean colour change that took place from week 2 to week 4, week 4 to week 6, and finally from 6 to 8 weeks. These measurements demonstrate the difference in discoloration within weeks. The reason for these measurements was to exclude all factors that could have attributed to the colour changes such as the initial shade of the tooth at baseline. Although all teeth were randomly assigned to each experimental group, there was a chance that some groups had a greater number of darker teeth than others. It is evident from Table 6.6 and Figure 7.1 that slight discoloration occurred between weeks 2 and 4, ranging from -1.99 to 0.6. These differences in colour changes were all less than 3.5, thus were not regarded as clinically perceptible. The greatest discoloration was evident between week 4 and week 6, ranging from 4.02 to 11.7. Readings between week 4 and week 6 were all greater than 3.5 thus implying a clinically detectable colour difference. Regression was evident between week 6 and week 8 for all the experimental sealers, with a $\Delta E$ ranging from -8.23 to -5.28. These negative values indicate improvement in colour from the previous readings which are all regarded as clinically perceptible values ($\Delta E$ greater than or equal to $\pm 3.5$).

As depicted in Figure 7.1 there appears to be a decrease in discoloration between weeks two and four and again between weeks six and eight. This gradual decrease can to a great extent be related to the amount of time required by the sealer to disintegrate into smaller particles and penetrate through the dentinal tubules (Davis *et al* 2002).

Kraus and Jordan (cited by Davis *et al* 2002) demonstrated that the pathway by which staining materials diffuse from the root canal space is through the dentinal tubules. Thus the patency of the dentinal tubules is critical. As a result, the mechanism of diffusion of the sealer will be greatly influenced by the presence or absence of the smear layer. Consequently, if the smear layer is removed the sealer will readily diffuse through the dentinal tubules causing discoloration. The anatomy of the dentinal tubules may also alter the rate of sealer diffusion. The diameter of the dentinal tubules gets narrower as it approaches the dentino-enamel junction. Therefore the sealer might require some degree of disintegration into smaller particles that are able to diffuse through the narrower pathways. It can be suggested that the disintegration of the sealer might be facilitated by the dentinal fluid present in the tubules that might dissolve or have a washing effect on the sealer. From this implication, it may explain why Sealapex had the greatest increase and regression in tooth colour compared to the other sealers tested. It is extensively documented in the literature that calcium hydroxide-based sealers (such as Sealapex) lack stability. The calcium hydroxide is readily soluble in tissue fluids and disintegrates far more readily when compared to resin-based sealers such as AH Plus and EndoRez (Ingle *et al.* 2002, Regan 2004, Valera *et al.* 2004, Ørstavik 2005, Himel *et al.* 2006, Gatewood 2007). This explains the abrupt behaviour of the calcium hydroxide-based sealer Sealapex when compared to the other classes of endodontic sealers used in this study.

## 7.4 Effect of sealer type

Even though, Sealapex does not contain silver, or any other heavy metals that might cause staining, it displayed a considerable degree of tooth discoloration with a mean ΔE of 8.89 after eight weeks which was statistically significant (*P-value=0.02*). The degree of discoloration observed by Sealapex was slightly greater than Pulp Canal Sealer (mean ΔE=8.78) and AH Plus (mean ΔE=8.70) after eight weeks of experimentation (Table 6.4 and Table 6.7). This difference could be explained by the eugenol content in the catalyst of the two paste system of Sealapex. Eugenol is unstable and oxidises whether it is free or bound, thus having a darkening effect over time (Parsons *et al* 2001, Davis *et al* 2002).

The experimental teeth which were sealed with Pulp Canal Sealer exhibited severe discoloration after eight weeks (mean ΔE=8.78) which was statistically significant from baseline (*P-value=0.02*) (Table 6.4 and Table 6.7). These results were similar to the findings of several studies including van der Burgt *et al* (1986), Parsons *et al* (2001), and Davis *et al* (2002). Kerr's Pulp Canal Sealer is manufactured according to Rickert's formula that utilises precipitated silver as a radiopacifier and a strengthening agent. The discoloration could be attributed to the silver constituents of this sealer. The silver can corrode by oxidation giving a grey-black hue analogous to amalgam staining (Grossman *et al* 1988). Another possible contributing factor is the presence of eugenol. As mentioned earlier, free or even bound eugenol oxidises over time, and hence darkens the PCS more (Parsons *et al.* 2001, Walton and Rotstein 1996, Partovi *et al.* 2006, Van der Burgt *et al.* 1986).

Although AH Plus is silver-free, and advertised as non-staining compared to its predecessor AH26, it caused discoloration in this study (mean ΔE=8.70 and *P-value=0.03*). Therefore, it can be argued that the silver ions were not the sole reason for tooth discoloration caused by AH26 (Partovi *et al* 2006). The literature lacks evidence regarding the staining potential of AH Plus, therefore additional research is required to investigate the constituents of AH Plus that might be responsible for the discoloration of endodontically treated teeth (Table 6.4 and Table 6.7).
EndoRez with a mean ΔE of 7.28 displayed the least discoloration after eight weeks. This novel resin-based sealer has only recently been introduced commercially to the profession. From the results of this study, it can be stated that the staining potential of EndoRez at eight weeks after obturation is low and although the discoloration resulting from it is statistically significant (*P-value=0.01*), EndoRez demonstrated the least rank in contrast to the other experimental groups.

The results of this study support the null hypothesis that there is no significant difference in the discoloration caused by the different sealers when used with gutta-percha in the obturation of root canals. In addition, according to this study it can be suggested that there is a significant effect of time on discoloration within each experimental group.

## Chapter 8

# LIMITATIONS OF THE STUDY

Laboratory studies are dependent on various factors that can affect the outcome of the study. Thus, controlling all these external factors that might play a role on the end result can be difficult. The primary limitation in this study was the difficulty to control the absolute environmental factors such as light and temperature during the spectrophotometric readings. Although random sampling was carried out, the initial tooth colour was another internal factor that might have affected the results. The presence of several outliers can be attributed to these uncontrollable factors.

The inability to reproduce an exact clinical situation is another limitation of this study. Unlike all previous studies, the preparation and obturation procedures performed in this study followed the standard protocol for endodontic treatment, thus mimicking the clinical situation. The fact that the experimental teeth were overfilled with the various sealers and no attempt was made to remove the excess sealer limited the replication of an ideal clinical situation.

Statistically, the greater the sample size the more reliable the results. The sample size for each group in this study was relatively small (n=12). The duration of the experiment was also relatively short. These factors could have further limited the outcome of this *in vitro* study.

Although all these factors that might be considered as limitations to *in vitro* studies, the importance of this type of research in predicting the clinical outcome must not be ignored as it is an indicator of what could happen in the clinical setting.

Chapter 9

# CONCLUSION AND RECOMMENDATIONS

## Conclusion

The results of this study support the null hypothesis that there is no statistically significant difference in the discoloration caused by the different sealers when used with gutta-percha in the obturation of root canals. In addition, according to this study it can be suggested that there is a significant effect of time on discoloration within each experimental group.

## Recommendations

On the basis of the results of this study, it is difficult to recommend a particular sealer for endodontic therapy, since each sealer caused a measurable tooth discoloration. EndoRez produced the least discoloration, although not statistically significant when compared to the other experimental sealers. Therefore, it is difficult to recommend a particular sealer even if it produced the least discoloration.

Future research in this field is required, utilising a larger sample size and a longer experimental period for more precise and accurate results that can aid in predicting the clinical outcome. Investigating the chromatogenic ingredients of the different sealers can also be of future research interest. Further research in this field can help manufacturers in eliminating such ingredients from future refined products.

Spectrophotometric analysis is attracting researchers in the field of colour and discoloration, and more future research utilising this sophisticated instrument is likely.

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## Appendix I

# RAW DATA

### Baseline readings (Base)

| Sealer | Tooth | Base Date | L_Base | a_Base | b_Base |
|---|---|---|---|---|---|
| AH Plus | A1 | 06/07/2007 | 74.32 | -0.03 | -0.58 |
| AH Plus | A2 | 06/07/2007 | 74.79 | -0.46 | -0.55 |
| AH Plus | A3 | 06/07/2007 | 74.33 | 0.13 | -0.1 |
| AH Plus | A4 | 06/07/2007 | 74.42 | 0.22 | 1.41 |
| AH Plus | A5 | 06/07/2007 | 74.14 | -0.02 | -1.31 |
| AH Plus | A6 | 06/07/2007 | 74.25 | -0.08 | -0.88 |
| AH Plus | A7 | 06/07/2007 | 75.29 | -0.14 | -0.85 |
| AH Plus | A8 | 06/07/2007 | 73.08 | -0.29 | -1.09 |
| AH Plus | A9 | 06/07/2007 | 74.28 | -0.03 | -0.53 |
| AH Plus | A10 | 06/07/2007 | 74.75 | 0.41 | 0.4 |
| AH Plus | A11 | 06/07/2007 | 75.96 | 0.65 | 0.5 |
| AH Plus | A12 | 06/07/2007 | 77.26 | 0.05 | -0.17 |
| EndoRez | B1 | 06/07/2007 | 73.15 | 0.39 | 0.02 |
| EndoRez | B2 | 06/07/2007 | 74 | -0.11 | -0.48 |
| EndoRez | B3 | 06/07/2007 | 71.55 | 0.31 | -0.74 |
| EndoRez | B4 | 06/07/2007 | 74.56 | -0.04 | -1.04 |
| EndoRez | B5 | 06/07/2007 | 74.68 | 0.31 | -0.1 |
| EndoRez | B6 | 06/07/2007 | 73.4 | 0.24 | 0.43 |
| EndoRez | B7 | 06/07/2007 | 74.72 | -0.1 | -0.59 |
| EndoRez | B8 | 06/07/2007 | 72.86 | 1.95 | 2.89 |
| EndoRez | B9 | 06/07/2007 | 73.16 | 0.62 | 0.96 |
| EndoRez | B10 | 06/07/2007 | 77.06 | 0.14 | 1.04 |
| EndoRez | B11 | 06/07/2007 | 72.94 | 0.68 | -0.79 |
| EndoRez | B12 | 06/07/2007 | 72.56 | 0.8 | 0.51 |
| Sealapex | C1 | 06/07/2007 | 72.03 | 0.11 | -1.84 |
| Sealapex | C2 | 06/07/2007 | 75.14 | 0.04 | -0.73 |
| Sealapex | C3 | 06/07/2007 | 74.87 | -0.23 | -1.66 |
| Sealapex | C4 | 06/07/2007 | 75.69 | -0.43 | -1.27 |
| Sealapex | C5 | 06/07/2007 | 74.23 | 0.6 | -0.16 |
| Sealapex | C6 | 06/07/2007 | 75.69 | -0.38 | -1.57 |
| Sealapex | C7 | 06/07/2007 | 71.61 | 0.52 | -0.96 |
| Sealapex | C8 | 06/07/2007 | 76.78 | -0.27 | -1.03 |
| Sealapex | C9 | 06/07/2007 | 74.26 | 0.61 | 0.58 |
| Sealapex | C10 | 06/07/2007 | 74.96 | 0.08 | 0.18 |
| Sealapex | C11 | 06/07/2007 | 74.43 | 0.25 | -0.99 |
| Sealapex | C12 | 06/07/2007 | 74.85 | 0.17 | -1.03 |

## Baseline readings: (continued)

| Sealer | Tooth | Base Date | L_Base | a_Base | b_Base |
|---|---|---|---|---|---|
| PCS | D1 | 06/07/2007 | 73.07 | 0.27 | 0.33 |
| PCS | D2 | 06/07/2007 | 74.07 | 0.13 | -0.17 |
| PCS | D3 | 06/07/2007 | 74.4 | 0.22 | 0.79 |
| PCS | D4 | 06/07/2007 | 76.1 | 0.03 | 1.06 |
| PCS | D5 | 06/07/2007 | 74.77 | 0.36 | 0.78 |
| PCS | D6 | 06/07/2007 | 77.09 | -0.12 | -0.28 |
| PCS | D7 | 06/07/2007 | 73.65 | 0.14 | -0.71 |
| PCS | D8 | 06/07/2007 | 74.2 | 0.16 | 0.8 |
| PCS | D9 | 06/07/2007 | 73.6 | 0.43 | -0.82 |
| PCS | D10 | 06/07/2007 | 75.01 | 0.13 | -0.14 |
| PCS | D11 | 06/07/2007 | 75.44 | 0.38 | 1.06 |
| PCS | D12 | 06/07/2007 | 73.3 | -0.16 | -1.93 |
| Positive | F1 | 06/07/2007 | 73.18 | -0.4 | -2.75 |
| Positive | F2 | 06/07/2007 | 73.79 | -0.12 | -0.37 |
| Positive | F3 | 06/07/2007 | 73.04 | 0.4 | 1.33 |
| Positive | F4 | 06/07/2007 | 75.8 | 0.78 | 1.59 |
| Positive | F5 | 06/07/2007 | 75.22 | -0.6 | -3.93 |
| Positive | F6 | 06/07/2007 | 74.46 | -0.12 | 0.02 |
| Negative | G1 | 06/07/2007 | 72.1 | 0.28 | 0.63 |
| Negative | G2 | 06/07/2007 | 71.88 | 0.28 | -0.05 |
| Negative | G3 | 06/07/2007 | 74.75 | 0.02 | 0.19 |
| Negative | G4 | 06/07/2007 | 73.57 | 0.12 | 0.29 |
| Negative | G5 | 06/07/2007 | 72.96 | 0.14 | 0.73 |
| Negative | G6 | 06/07/2007 | 73.49 | -0.11 | -0.92 |

## Readings at 2 weeks (2_W):

| Sealer | Tooth | 2_w Date | L_2w | a_2w | b_2w |
|---|---|---|---|---|---|
| AH Plus | A1 | 20/07/2007 | 71.09 | 1.28 | 2.26 |
| AH Plus | A2 | 20/07/2007 | 72.68 | 0.77 | 4.13 |
| AH Plus | A3 | 20/07/2007 | 72.52 | 1.1 | 4.19 |
| AH Plus | A4 | 20/07/2007 | 72.5 | 0.94 | 8.45 |
| AH Plus | A5 | 20/07/2007 | 73.04 | 1.52 | 5.34 |
| AH Plus | A6 | 20/07/2007 | 68.06 | 2.28 | 1.57 |
| AH Plus | A7 | 20/07/2007 | 72.07 | 1.59 | 1.13 |
| AH Plus | A8 | 20/07/2007 | 65.59 | 1.73 | 2.57 |
| AH Plus | A9 | 20/07/2007 | 71.69 | 1.37 | 1.36 |
| AH Plus | A10 | 20/07/2007 | 70.16 | 2.21 | 1.74 |
| AH Plus | A11 | 20/07/2007 | 69.91 | 2.28 | 4.25 |
| AH Plus | A12 | 20/07/2007 | 71.94 | 0.21 | 0.69 |
| EndoRez | B1 | 20/07/2007 | 66.55 | 2.27 | 1.55 |
| EndoRez | B2 | 20/07/2007 | 66.45 | 2.62 | 1.55 |
| EndoRez | B3 | 20/07/2007 | 71.04 | 1.63 | 3.52 |
| EndoRez | B4 | 20/07/2007 | 69.19 | 2.04 | 1.64 |
| EndoRez | B5 | 20/07/2007 | 74.19 | 1.72 | 2.98 |
| EndoRez | B6 | 20/07/2007 | 66.84 | 0.21 | 0.94 |
| EndoRez | B7 | 20/07/2007 | 73.39 | 1.71 | 3.62 |
| EndoRez | B8 | 20/07/2007 | 69.27 | 2.83 | 5.7 |
| EndoRez | B9 | 20/07/2007 | 69.05 | 0.87 | 2.13 |
| EndoRez | B10 | 20/07/2007 | 70.55 | 2.41 | 4.33 |
| EndoRez | B11 | 20/07/2007 | 67.28 | 2.21 | 1.72 |
| EndoRez | B12 | 20/07/2007 | 68.37 | 2.64 | 3.6 |
| Sealapex | C1 | 20/07/2007 | 67.37 | 1.34 | -0.27 |
| Sealapex | C2 | 20/07/2007 | 67.94 | 2.12 | 1.37 |
| Sealapex | C3 | 20/07/2007 | 69.46 | 1.65 | 0.79 |
| Sealapex | C4 | 20/07/2007 | 70.36 | 1.5 | 1.9 |
| Sealapex | C5 | 20/07/2007 | 66.73 | 1.77 | 2.09 |
| Sealapex | C6 | 20/07/2007 | 68.81 | 1.46 | 1.64 |
| Sealapex | C7 | 20/07/2007 | 65.3 | 1.69 | 0.29 |
| Sealapex | C8 | 20/07/2007 | 70.29 | 1.27 | 0.3 |
| Sealapex | C9 | 20/07/2007 | 67.54 | 2.51 | 3.22 |
| Sealapex | C10 | 20/07/2007 | 66.86 | 2 | 3.39 |
| Sealapex | C11 | 20/07/2007 | 65.41 | 2.48 | 6.44 |
| Sealapex | C12 | 20/07/2007 | 66.46 | 0.82 | 1.08 |

## Readings at 2 weeks (continued)

| Sealer | Tooth | 2_w Date | L_2w | a_2w | b_2w |
|---|---|---|---|---|---|
| PCS | D1 | 20/07/2007 | 65.71 | 2.14 | 3.77 |
| PCS | D2 | 20/07/2007 | 65.65 | 2.28 | 2.17 |
| PCS | D3 | 20/07/2007 | 65.21 | 1.93 | 3.22 |
| PCS | D4 | 20/07/2007 | 66.37 | 2.09 | 4.33 |
| PCS | D5 | 20/07/2007 | 65.68 | 2.55 | 4.44 |
| PCS | D6 | 20/07/2007 | 70.11 | 1.53 | 1.33 |
| PCS | D7 | 20/07/2007 | 68.88 | 1.33 | -0.15 |
| PCS | D8 | 20/07/2007 | 66.55 | 2.33 | 4.07 |
| PCS | D9 | 20/07/2007 | 67.74 | 1.57 | 0.35 |
| PCS | D10 | 20/07/2007 | 71.95 | 1.84 | 2.52 |
| PCS | D11 | 20/07/2007 | 69.72 | 2.44 | 4.54 |
| PCS | D12 | 20/07/2007 | 69.06 | 0.59 | -1.77 |
| Positive | F1 | 20/07/2007 | 64.15 | 1.49 | -0.91 |
| Positive | F2 | 20/07/2007 | 69.42 | 1.03 | 1.65 |
| Positive | F3 | 20/07/2007 | 68.57 | 1.95 | 3.01 |
| Positive | F4 | 20/07/2007 | 70.06 | 1.53 | 2.61 |
| Positive | F5 | 20/07/2007 | 68.01 | 1.14 | -2.11 |
| Positive | F6 | 20/07/2007 | 67.12 | 1.57 | 1.18 |
| Negative | G1 | 20/07/2007 | 70.63 | -0.18 | 1.76 |
| Negative | G2 | 20/07/2007 | 70.84 | 0.15 | -1.03 |
| Negative | G3 | 20/07/2007 | 70.94 | 0.12 | 0.02 |
| Negative | G4 | 20/07/2007 | 66.62 | -0.35 | 2.91 |
| Negative | G5 | 20/07/2007 | 66.64 | 0.04 | 0.96 |
| Negative | G6 | 20/07/2007 | 71.21 | 0.11 | -1.43 |

## Readings at 4 weeks (4_w):

| Sealer | Tooth | 4_w Date | L_4w | a_4w | b_4w |
|---|---|---|---|---|---|
| AH Plus | A1 | 03/08/2007 | 72.25 | 1.67 | 3.11 |
| AH Plus | A2 | 03/08/2007 | 72.88 | 1.07 | 3.62 |
| AH Plus | A3 | 03/08/2007 | 72.32 | 1.22 | 4.18 |
| AH Plus | A4 | 03/08/2007 | 71.64 | 1.1 | 9.11 |
| AH Plus | A5 | 03/08/2007 | 69.05 | 1.71 | 1.13 |
| AH Plus | A6 | 03/08/2007 | 66.62 | 2.45 | 2.04 |
| AH Plus | A7 | 03/08/2007 | 71.93 | 1.52 | 1.71 |
| AH Plus | A8 | 03/08/2007 | 67.6 | 1.72 | 3.24 |
| AH Plus | A9 | 03/08/2007 | 72.12 | 1.49 | 2.32 |
| AH Plus | A10 | 03/08/2007 | 68.8 | 2.32 | 2.01 |
| AH Plus | A11 | 03/08/2007 | 71.41 | 2.12 | 4.46 |
| AH Plus | A12 | 03/08/2007 | 68.13 | 2.65 | 3.72 |
| EndoRez | B1 | 03/08/2007 | 70.1 | 2.13 | 2.9 |
| EndoRez | B2 | 03/08/2007 | 69.76 | 2.14 | 1.72 |
| EndoRez | B3 | 03/08/2007 | 70.77 | 1.66 | 4.39 |
| EndoRez | B4 | 03/08/2007 | 71.22 | 1.78 | 1.81 |
| EndoRez | B5 | 03/08/2007 | 72.57 | 1.83 | 3.72 |
| EndoRez | B6 | 03/08/2007 | 68.92 | 1.76 | 2.24 |
| EndoRez | B7 | 03/08/2007 | 73.2 | 1.78 | 3.78 |
| EndoRez | B8 | 03/08/2007 | 68.42 | 2.84 | 5.52 |
| EndoRez | B9 | 03/08/2007 | 69.59 | 2.36 | 3.96 |
| EndoRez | B10 | 03/08/2007 | 71.5 | 2.33 | 3.97 |
| EndoRez | B11 | 03/08/2007 | 69.47 | 2.01 | 1.52 |
| EndoRez | B12 | 03/08/2007 | 72.02 | 2.23 | 3.28 |
| Sealapex | C1 | 03/08/2007 | 69.78 | 1.23 | -0.11 |
| Sealapex | C2 | 03/08/2007 | 71.03 | 1.83 | 1.12 |
| Sealapex | C3 | 03/08/2007 | 70.01 | 1.55 | 1.16 |
| Sealapex | C4 | 03/08/2007 | 71.35 | 1.69 | 2.18 |
| Sealapex | C5 | 03/08/2007 | 70.97 | 1.73 | 1.77 |
| Sealapex | C6 | 03/08/2007 | 71.33 | 1.4 | 1.12 |
| Sealapex | C7 | 03/08/2007 | 66.6 | 1.75 | 0.92 |
| Sealapex | C8 | 03/08/2007 | 69.81 | 1.41 | 0.25 |
| Sealapex | C9 | 03/08/2007 | 68.31 | 2.59 | 3.18 |
| Sealapex | C10 | 03/08/2007 | 71.81 | 1.65 | 2.74 |
| Sealapex | C11 | 03/08/2007 | 71.13 | 2.09 | 4.55 |
| Sealapex | C12 | 03/08/2007 | 69.97 | 1.54 | 0.51 |

## Readings at 4 weeks (continued)

| Sealer | Tooth | 4_w Date | L_4w | a_4w | b_4w |
|---|---|---|---|---|---|
| PCS | D1 | 03/08/2007 | 65.37 | 2.56 | 3.1 |
| PCS | D2 | 03/08/2007 | 68.95 | 2.16 | 1.97 |
| PCS | D3 | 03/08/2007 | 68.85 | 1.75 | 2.85 |
| PCS | D4 | 03/08/2007 | 70.82 | 1.87 | 3.33 |
| PCS | D5 | 03/08/2007 | 67.82 | 2.51 | 3.56 |
| PCS | D6 | 03/08/2007 | 69.43 | 1.73 | 1.86 |
| PCS | D7 | 03/08/2007 | 69.74 | 1.44 | -0.02 |
| PCS | D8 | 03/08/2007 | 69.93 | 2.15 | 2.94 |
| PCS | D9 | 03/08/2007 | 70.07 | 1.5 | 1.07 |
| PCS | D10 | 03/08/2007 | 71.35 | 1.86 | 3.12 |
| PCS | D11 | 03/08/2007 | 69.65 | 2.4 | 3.63 |
| PCS | D12 | 03/08/2007 | 66.11 | 0.86 | -0.23 |
| Positive | F1 | 03/08/2007 | 64.94 | 1.59 | -0.3 |
| Positive | F2 | 03/08/2007 | 65.6 | 1.32 | 1.87 |
| Positive | F3 | 03/08/2007 | 70.61 | 1.83 | 2.45 |
| Positive | F4 | 03/08/2007 | 68.13 | 1.47 | 3.57 |
| Positive | F5 | 03/08/2007 | 70.64 | 1.1 | -1.27 |
| Positive | F6 | 03/08/2007 | 71.36 | 1.29 | 1.25 |
| Negative | G1 | 03/08/2007 | 68.98 | -0.2 | 1.34 |
| Negative | G2 | 03/08/2007 | 69.27 | 0 | -0.31 |
| Negative | G3 | 03/08/2007 | 70.53 | -0.36 | 0.14 |
| Negative | G4 | 03/08/2007 | 66.23 | 0.03 | 0.75 |
| Negative | G5 | 03/08/2007 | 67.93 | 0.18 | 2.05 |
| Negative | G6 | 03/08/2007 | 70.8 | 0.03 | -0.82 |

## Readings at 6 weeks (6_w):

| Sealer | Tooth | 2_w Date | L_6w | a_6w | b_6w |
|---|---|---|---|---|---|
| AH Plus | A1 | 17/08/2007 | 65.92 | 1.84 | 3.92 |
| AH Plus | A2 | 17/08/2007 | 65.44 | 1.18 | 5.68 |
| AH Plus | A3 | 17/08/2007 | 60.96 | 1.55 | 8.12 |
| AH Plus | A4 | 17/08/2007 | 69.47 | 0.9 | 9.01 |
| AH Plus | A5 | 17/08/2007 | 66.55 | 1.78 | 7.09 |
| AH Plus | A6 | 17/08/2007 | 58.25 | 3.08 | 3.02 |
| AH Plus | A7 | 17/08/2007 | 61.15 | 1.95 | 3.54 |
| AH Plus | A8 | 17/08/2007 | 57.36 | 1.91 | 5.4 |
| AH Plus | A9 | 17/08/2007 | 63.87 | 1.84 | 3.28 |
| AH Plus | A10 | 17/08/2007 | 58.82 | 2.86 | 3.08 |
| AH Plus | A11 | 17/08/2007 | 61.44 | 2.81 | 7.57 |
| AH Plus | A12 | 17/08/2007 | 60.68 | 3.25 | 4.93 |
| EndoRez | B1 | 17/08/2007 | 57.74 | 3.2 | 5.35 |
| EndoRez | B2 | 17/08/2007 | 57.69 | 2.72 | 3.5 |
| EndoRez | B3 | 17/08/2007 | 62.88 | 1.93 | 6.19 |
| EndoRez | B4 | 17/08/2007 | 62.44 | 2.33 | 2.85 |
| EndoRez | B5 | 17/08/2007 | 66.37 | 2.15 | 5.6 |
| EndoRez | B6 | 17/08/2007 | 56.22 | 2.83 | 4.69 |
| EndoRez | B7 | 17/08/2007 | 61.71 | 2.49 | 7.35 |
| EndoRez | B8 | 17/08/2007 | 58.23 | 3.87 | 8.02 |
| EndoRez | B9 | 17/08/2007 | 59.37 | 3.08 | 6.71 |
| EndoRez | B10 | 17/08/2007 | 60.61 | 3.16 | 5.68 |
| EndoRez | B11 | 17/08/2007 | 59.88 | 2.46 | 2.12 |
| EndoRez | B12 | 17/08/2007 | 62.64 | 3.02 | 5.48 |
| Sealapex | C1 | 17/08/2007 | 57.57 | 1.54 | 0.65 |
| Sealapex | C2 | 17/08/2007 | 61.09 | 2.41 | 2.19 |
| Sealapex | C3 | 17/08/2007 | 62.73 | 1.67 | 1.85 |
| Sealapex | C4 | 17/08/2007 | 59.91 | 2.12 | 5.1 |
| Sealapex | C5 | 17/08/2007 | 60.07 | 2.15 | 4.35 |
| Sealapex | C6 | 17/08/2007 | 58.86 | 2.18 | 2.21 |
| Sealapex | C7 | 17/08/2007 | 51.39 | 2.68 | 3.21 |
| Sealapex | C8 | 17/08/2007 | 56.37 | 1.55 | 1.36 |
| Sealapex | C9 | 17/08/2007 | 54.66 | 3.92 | 6.7 |
| Sealapex | C10 | 17/08/2007 | 60.09 | 2.42 | 5.02 |
| Sealapex | C11 | 17/08/2007 | 59.29 | 3.04 | 9.23 |
| Sealapex | C12 | 17/08/2007 | 57.83 | 2.29 | 2.03 |

## Readings at 6 weeks (continued):

| Sealer | Tooth | 2_w Date | L_6w | a_6w | b_6w |
|---|---|---|---|---|---|
| PCS | D1 | 17/08/2007 | 56.63 | 2.98 | 5.78 |
| PCS | D2 | 17/08/2007 | 53.97 | 3.17 | 5.13 |
| PCS | D3 | 17/08/2007 | 58.68 | 2.43 | 5.44 |
| PCS | D4 | 17/08/2007 | 59.68 | 2.5 | 5.93 |
| PCS | D5 | 17/08/2007 | 55.6 | 3.49 | 8.42 |
| PCS | D6 | 17/08/2007 | 62.22 | 2.01 | 2.43 |
| PCS | D7 | 17/08/2007 | 65.02 | 1.57 | -0.05 |
| PCS | D8 | 17/08/2007 | 65.34 | 2.41 | 3.58 |
| PCS | D9 | 17/08/2007 | 63.57 | 1.74 | 0.86 |
| PCS | D10 | 17/08/2007 | 65.65 | 2.17 | 4.07 |
| PCS | D11 | 17/08/2007 | 63.83 | 2.67 | 6.22 |
| PCS | D12 | 17/08/2007 | 63.67 | 0.83 | -0.49 |
| Positive | F1 | 17/08/2007 | 57.17 | 1.79 | 0.16 |
| Positive | F2 | 17/08/2007 | 65.16 | 1.12 | 2.1 |
| Positive | F3 | 17/08/2007 | 64.5 | 1.95 | 3.67 |
| Positive | F4 | 17/08/2007 | 63.63 | 1.52 | 4.02 |
| Positive | F5 | 17/08/2007 | 64.95 | 1.05 | -1.85 |
| Positive | F6 | 17/08/2007 | 66.03 | 1.39 | 1.31 |
| Negative | G1 | 17/08/2007 | 66.91 | -0.026 | 0.85 |
| Negative | G2 | 17/08/2007 | 64.12 | -0.03 | -0.79 |
| Negative | G3 | 17/08/2007 | 65.79 | -0.08 | 1.5 |
| Negative | G4 | 17/08/2007 | 64.6 | -0.26 | 1.77 |
| Negative | G5 | 17/08/2007 | 63.12 | 0.06 | 1.69 |
| Negative | G6 | 17/08/2007 | 65.02 | -0.14 | -1.12 |

## Readings at 8 weeks (8_w):

| Sealer | Tooth | 8_w Date | L_8w | a_8w | b_8w |
|---|---|---|---|---|---|
| AH Plus | A1 | 31/08/2007 | 68.02 | 1.95 | 4.24 |
| AH Plus | A2 | 31/08/2007 | 71.1 | 1.29 | 4.27 |
| AH Plus | A3 | 31/08/2007 | 69.96 | 0.1 | 0.15 |
| AH Plus | A4 | 31/08/2007 | 70.96 | 0.95 | 0.15 |
| AH Plus | A5 | 31/08/2007 | 69.02 | 1.67 | 7.65 |
| AH Plus | A6 | 31/08/2007 | 63.46 | 2.76 | 2.99 |
| AH Plus | A7 | 31/08/2007 | 66.73 | 2.29 | 4.61 |
| AH Plus | A8 | 31/08/2007 | 62.67 | 1.81 | 4.32 |
| AH Plus | A9 | 31/08/2007 | 68.95 | 1.52 | 2.99 |
| AH Plus | A10 | 31/08/2007 | 65.95 | 2.4 | 2.41 |
| AH Plus | A11 | 31/08/2007 | 67.71 | 2.22 | 5.47 |
| AH Plus | A12 | 31/08/2007 | 66.66 | 2.66 | 3.77 |
| EndoRez | B1 | 31/08/2007 | 66.45 | 2.31 | 3.37 |
| EndoRez | B2 | 31/08/2007 | 65.53 | 2.17 | 2.17 |
| EndoRez | B3 | 31/08/2007 | 69.06 | 1.67 | 4.22 |
| EndoRez | B4 | 31/08/2007 | 69.77 | 1.86 | 1.99 |
| EndoRez | B5 | 31/08/2007 | 71.03 | 1.8 | 3.9 |
| EndoRez | B6 | 31/08/2007 | 65.14 | 1.96 | 3.09 |
| EndoRez | B7 | 31/08/2007 | 69.51 | 1.99 | 4.72 |
| EndoRez | B8 | 31/08/2007 | 67.23 | 2.81 | 6.03 |
| EndoRez | B9 | 31/08/2007 | 67.01 | 2.37 | 5.13 |
| EndoRez | B10 | 31/08/2007 | 68.21 | 2.45 | 5 |
| EndoRez | B11 | 31/08/2007 | 66.16 | 2.04 | 1.65 |
| EndoRez | B12 | 31/08/2007 | 69.19 | 2.42 | 4.17 |
| Sealapex | C1 | 31/08/2007 | 64.8 | 1.29 | 0.04 |
| Sealapex | C2 | 31/08/2007 | 66.99 | 1.99 | 1.6 |
| Sealapex | C3 | 31/08/2007 | 67.65 | 1.5 | 1.42 |
| Sealapex | C4 | 31/08/2007 | 67.47 | 1.88 | 2.95 |
| Sealapex | C5 | 31/08/2007 | 67.76 | 1.72 | 2.27 |
| Sealapex | C6 | 31/08/2007 | 66.84 | 1.55 | 1.84 |
| Sealapex | C7 | 31/08/2007 | 62.74 | 2.04 | 1.46 |
| Sealapex | C8 | 31/08/2007 | 66.84 | 1.32 | 0.12 |
| Sealapex | C9 | 31/08/2007 | 65.51 | 2.66 | 3.11 |
| Sealapex | C10 | 31/08/2007 | 67.81 | 1.91 | 3.39 |
| Sealapex | C11 | 31/08/2007 | 67.14 | 2.38 | 5.95 |
| Sealapex | C12 | 31/08/2007 | 66.17 | 1.74 | 1.16 |

## Readings at 8 weeks (continued):

| Sealer | Tooth | 8_w Date | L_8w | a_8w | b_8w |
|---|---|---|---|---|---|
| PCS | D1 | 31/08/2007 | 64.45 | 2.42 | 3.81 |
| PCS | D2 | 31/08/2007 | 64.6 | 2.32 | 2.47 |
| PCS | D3 | 31/08/2007 | 65.69 | 1.99 | 3.39 |
| PCS | D4 | 31/08/2007 | 66.99 | 2.04 | 4.2 |
| PCS | D5 | 31/08/2007 | 65.37 | 2.59 | 4.94 |
| PCS | D6 | 31/08/2007 | 68.57 | 1.96 | 3.71 |
| PCS | D7 | 31/08/2007 | 67.98 | 1.65 | 1.69 |
| PCS | D8 | 31/08/2007 | 67.92 | 2.44 | 4.63 |
| PCS | D9 | 31/08/2007 | 67.3 | 1.77 | 2.58 |
| PCS | D10 | 31/08/2007 | 67.97 | 2.24 | 4.96 |
| PCS | D11 | 31/08/2007 | 72.69 | 3.28 | 9.11 |
| PCS | D12 | 31/08/2007 | 66.79 | 0.99 | 1.25 |
| Positive | F1 | 31/08/2007 | 62.97 | 1.85 | 1.36 |
| Positive | F2 | 31/08/2007 | 65.7 | 1.5 | 3.78 |
| Positive | F3 | 31/08/2007 | 66.52 | 2.11 | 4.26 |
| Positive | F4 | 31/08/2007 | 67.41 | 1.71 | 4.35 |
| Positive | F5 | 31/08/2007 | 67.25 | 1.3 | 0.25 |
| Positive | F6 | 31/08/2007 | 67.05 | 1.6 | 2.94 |
| Negative | G1 | 31/08/2007 | 73.61 | 0.52 | 3.3 |
| Negative | G2 | 31/08/2007 | 63.59 | 0.21 | 1.13 |
| Negative | G3 | 31/08/2007 | 68.77 | 0.13 | 2.99 |
| Negative | G4 | 31/08/2007 | 66.47 | 0.2 | 4.24 |
| Negative | G5 | 31/08/2007 | 67.98 | 0.38 | 4.22 |
| Negative | G6 | 31/08/2007 | 67.35 | 0.16 | 0.58 |

**Code guide:**

**1. Group code:** there are six groups incorporated in this study (A-G). Each group consists of twelve teeth sealed with the specific material tested.

**Group A:** AH plus

**Group B**: Endo-Rez

**Group C**: Sealapex

**Group D**: Kerr pulp canal sealer

**Group E**: Positive Control

**Group F:** Negative control

For example A1 means tooth number 1 in the AH plus group.

**2. L*a*b* values are obtained from spectrophotometer readings at 0, 2, 4, 6, and 8 weeks interval.**

**3. ΔE is the colour difference measured by using the following formula:**

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

ΔL is the difference in lightness obtained by deducting the L* reading obtained from the spectrophotometer at a point from the previous L* reading. As such ΔL can be computed between any two L* readings and between any point of reference during the experiment and the baseline values recorded for L*. Δa and Δb are also calculated in the same manner as explained above. After calculating ΔL, Δa, and Δb values, ΔE can be determined using the formula according to O'Brien (2002).

**Appendix II**

# Calculation of colour change (ΔE)

| Sealer | ΔE2 | ΔE4 | ΔE6 | ΔE8 |
|---|---|---|---|---|
| AH Plus | 4.154 | 4.560 | 9.711 | 8.176 |
| AH Plus | 4.798 | 4.835 | 11.355 | 6.318 |
| AH Plus | 4.666 | 4.852 | 15.759 | 4.377 |
| AH Plus | 8.695 | 8.234 | 9.095 | 3.754 |
| AH Plus | 5.665 | 5.904 | 11.463 | 10.457 |
| AH Plus | 6.808 | 8.552 | 16.769 | 11.810 |
| AH Plus | 3.826 | 4.539 | 14.953 | 10.440 |
| AH Plus | 8.172 | 7.268 | 17.149 | 11.918 |
| AH Plus | 3.243 | 3.886 | 11.242 | 6.573 |
| AH Plus | 5.228 | 6.453 | 16.339 | 9.243 |
| AH Plus | 7.571 | 6.208 | 16.294 | 9.758 |
| AH Plus | 5.367 | 10.259 | 17.639 | 11.606 |
| EndoRez | 7.035 | 4.541 | 16.546 | 7.733 |
| EndoRez | 8.177 | 5.280 | 17.025 | 9.163 |
| EndoRez | 3.794 | 5.362 | 11.217 | 5.714 |
| EndoRez | 5.988 | 4.753 | 12.948 | 5.978 |
| EndoRez | 3.333 | 4.621 | 10.244 | 5.616 |
| EndoRez | 6.627 | 5.065 | 17.889 | 8.847 |
| EndoRez | 4.260 | 4.994 | 15.460 | 7.727 |
| EndoRez | 6.794 | 5.237 | 15.622 | 6.504 |
| EndoRez | 4.636 | 4.977 | 15.142 | 7.634 |
| EndoRez | 8.141 | 6.655 | 17.357 | 9.967 |
| EndoRez | 6.110 | 4.376 | 13.498 | 7.333 |
| EndoRez | 5.823 | 3.164 | 11.315 | 5.232 |
| Sealapex | 4.827 | 3.051 | 14.742 | 7.563 |
| Sealapex | 7.619 | 4.850 | 14.545 | 8.698 |
| Sealapex | 5.782 | 5.894 | 12.779 | 8.038 |
| Sealapex | 5.979 | 5.936 | 17.207 | 9.524 |
| Sealapex | 7.873 | 3.953 | 14.941 | 7.001 |
| Sealapex | 7.308 | 5.423 | 17.438 | 9.679 |
| Sealapex | 6.424 | 5.491 | 20.758 | 9.319 |
| Sealapex | 6.677 | 7.283 | 20.630 | 10.132 |
| Sealapex | 7.690 | 6.788 | 20.798 | 9.336 |
| Sealapex | 8.988 | 4.352 | 15.812 | 8.048 |
| Sealapex | 11.305 | 6.706 | 18.478 | 10.288 |
| Sealapex | 8.484 | 5.297 | 17.422 | 9.089 |

## Colour change (ΔE)... continued

| Sealer | ΔE2 | ΔE4 | ΔE6 | ΔE8 |
|---|---|---|---|---|
| PCS | 8.957 | 8.497 | 17.531 | 9.541 |
| PCS | 9.887 | 5.909 | 21.008 | 10.072 |
| PCS | 10.847 | 6.114 | 16.542 | 9.261 |
| PCS | 10.351 | 6.035 | 17.304 | 9.843 |
| PCS | 7.295 | 7.788 | 20.872 | 10.518 |
| PCS | 4.918 | 8.166 | 15.264 | 9.635 |
| PCS | 8.933 | 4.178 | 8.773 | 6.339 |
| PCS | 5.980 | 5.174 | 9.555 | 7.701 |
| PCS | 4.317 | 4.145 | 10.254 | 7.283 |
| PCS | 7.588 | 5.198 | 10.464 | 8.946 |
| PCS | 4.655 | 6.649 | 12.910 | 8.987 |
| Positive | 9.270 | 7.458 | 9.787 | 7.336 |
| Positive | 4.811 | 8.824 | 16.419 | 11.234 |
| Positive | 5.607 | 8.612 | 9.062 | 9.236 |
| Positive | 6.350 | 3.034 | 8.989 | 7.350 |
| Positive | 7.711 | 7.951 | 12.432 | 8.881 |
| Positive | 7.624 | 5.563 | 10.608 | 9.198 |
| Negative | 2.339 | 3.621 | 8.661 | 8.148 |
| Negative | 1.469 | 3.236 | 5.204 | 3.077 |
| Negative | 3.811 | 2.638 | 7.801 | 8.374 |
| Negative | 7.549 | 4.237 | 9.056 | 6.604 |
| Negative | 6.393 | 7.355 | 9.099 | 8.125 |
| Negative | 2.700 | 5.200 | 9.887 | 6.086 |

## Appendix III

# Wilcoxson Signed Rank Sum Test

### Paired data analysis of differences between ΔE2 and ΔE4

| Sealer | ΔE2 | ΔE4 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| AH Plus | 4.154 | 4.560 | *Number of Nonzero Differences = 12* |
| AH Plus | 4.798 | 4.835 | *T+ = 23* |
| AH Plus | 4.666 | 4.852 | *T- = 55* |
| AH Plus | 8.695 | 8.234 | *Large Sample Approximation* |
| AH Plus | 5.665 | 5.904 | *Test Statistic Z = -1.255* |
| AH Plus | 6.808 | 8.552 | *P-Value = 0.2094* |
| AH Plus | 3.826 | 4.539 | |
| AH Plus | 8.172 | 7.268 | |
| AH Plus | 3.243 | 3.886 | |
| AH Plus | 5.228 | 6.453 | |
| AH Plus | 7.571 | 6.208 | |
| AH Plus | 5.367 | 10.259 | |
| | | | |
| **minimum** | 3.243 | 3.886 | |
| **Q1st** | 4.538 | 4.766 | |
| **Median** | 5.298 | 6.056 | |
| **Q3rd** | 6.999 | 7.509 | |
| **Maximum** | 8.695 | 10.259 | |
| | | | |
| EndoRez | 7.035 | 4.541 | *Number of Nonzero Differences = 12* |
| EndoRez | 8.177 | 5.280 | *T+ = 63* |
| EndoRez | 3.794 | 5.362 | *T- = 15* |
| EndoRez | 5.988 | 4.753 | *Large Sample Approximation* |
| EndoRez | 3.333 | 4.621 | *Test Statistic Z = 1.883* |
| EndoRez | 6.627 | 5.065 | *P-Value = 0.0597* |
| EndoRez | 4.260 | 4.994 | |
| EndoRez | 6.794 | 5.237 | |
| EndoRez | 4.636 | 4.977 | |
| EndoRez | 8.141 | 6.655 | |
| EndoRez | 6.110 | 4.376 | |
| EndoRez | 5.823 | 3.164 | |
| **minimum** | 3.333 | 3.164 | |
| **Q1st** | 4.542 | 4.601 | |
| **Median** | 6.049 | 4.986 | |
| **Q3rd** | 6.854 | 5.248 | |
| **Maximum** | 8.177 | 6.655 | |

| Sealer | ΔE2 | ΔE4 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| Sealapex | 4.827 | 3.051 | *Number of Nonzero Differences = 12* |
| Sealapex | 7.619 | 4.850 | *T+ = 73* |
| Sealapex | 5.782 | 5.894 | *T- = 5* |
| Sealapex | 5.979 | 5.936 | *Large Sample Approximation* |
| Sealapex | 7.873 | 3.953 | *Test Statistic Z = 2.667* |
| Sealapex | 7.308 | 5.423 | *P-Value = 0.0076* |
| Sealapex | 6.424 | 5.491 | |
| Sealapex | 6.677 | 7.283 | |
| Sealapex | 7.690 | 6.788 | |
| Sealapex | 8.988 | 4.352 | |
| Sealapex | 11.305 | 6.706 | |
| Sealapex | 8.484 | 5.297 | |
| **minimum** | 4.827 | 3.051 | |
| **Q1st** | 6.313 | 4.725 | |
| **Median** | 7.463 | 5.457 | |
| **Q3rd** | 8.026 | 6.128 | |
| **Maximum** | 11.305 | 7.283 | |
| | | | |
| PCS | 8.478 | 8.497 | *Number of Nonzero Differences = 12* |
| PCS | 8.957 | 5.909 | *T+ = 62* |
| PCS | 9.887 | 6.114 | *T- = 16* |
| PCS | 10.847 | 6.035 | *Large Sample Approximation* |
| PCS | 10.351 | 7.788 | *Test Statistic Z = 1.804* |
| PCS | 7.295 | 8.166 | *P-Value = 0.0712* |
| PCS | 4.918 | 4.178 | |
| PCS | 8.933 | 5.174 | |
| PCS | 5.980 | 4.145 | |
| PCS | 4.317 | 5.198 | |
| PCS | 7.588 | 6.649 | |
| PCS | 4.655 | 7.458 | |
| **minimum** | 4.317 | 4.145 | |
| **Q1st** | 5.715 | 5.192 | |
| **Median** | 8.033 | 6.075 | |
| **Q3rd** | 9.189 | 7.541 | |
| **Maximum** | 10.847 | 8.497 | |

| Sealer | ΔE2 | ΔE4 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| **Positive** | 9.270 | 8.824 | *Number of Nonzero Differences = 6* |
| **Positive** | 4.811 | 8.612 | *T+ = 14* |
| **Positive** | 5.607 | 3.034 | *T- = 7* |
| **Positive** | 6.350 | 7.951 | *Large Sample Approximation* |
| **Positive** | 7.711 | 5.563 | *Test Statistic Z = 0.734* |
| **Positive** | 7.624 | 3.621 | *P-Value = 0.4631* |
| **minimum** | 4.811 | 3.034 | |
| **Q1st** | 5.793 | 4.106 | |
| **Median** | 6.987 | 6.757 | |
| **Q3rd** | 7.689 | 8.447 | |
| **Maximum** | 9.270 | 8.824 | |
| | | | |
| **Negative** | 2.339 | 3.236 | *Number of Nonzero Differences = 6* |
| **Negative** | 1.469 | 2.638 | *T+ = 9* |
| **Negative** | 3.811 | 4.237 | *T- = 12* |
| **Negative** | 7.549 | 7.355 | *Large Sample Approximation* |
| **Negative** | 6.393 | 5.200 | *Test Statistic Z = -0.314* |
| **Negative** | 2.700 | 2.695 | *P-Value = 0.7532* |
| **minimum** | 1.469 | 2.638 | |
| **Q1st** | 2.429 | 2.831 | |
| **Median** | 3.256 | 3.736 | |
| **Q3rd** | 5.748 | 4.960 | |
| **Maximum** | 7.549 | 7.355 | |

## Paired data analysis of differences between ΔE2 and ΔE6

| Sealer | ΔE2 | ΔE6 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| AH Plus | 4.154 | 9.711 | *Number of Nonzero Differences = 12* |
| AH Plus | 4.798 | 11.355 | *T+ =* |
| AH Plus | 4.666 | 15.759 | *T- = 78* |
| AH Plus | 8.695 | 9.095 | *Large Sample Approximation* |
| AH Plus | 5.665 | 11.463 | *Test Statistic Z = -3.059* |
| AH Plus | 6.808 | 16.769 | *P-Value = 0.0022* |
| AH Plus | 3.826 | 14.953 | |
| AH Plus | 8.172 | 17.149 | |
| AH Plus | 3.243 | 11.242 | |
| AH Plus | 5.228 | 16.339 | |
| AH Plus | 7.571 | 16.294 | |
| AH Plus | 5.367 | 17.639 | |
| **minimum** | 3.243 | 9.095 | |
| **Q1st** | 4.538 | 11.326 | |
| **Median** | 5.298 | 15.356 | |
| **Q3rd** | 6.999 | 16.446 | |
| **Maximum** | 8.695 | 17.639 | |
| | | | |
| EndoRez | 7.035 | 16.546 | *Number of Nonzero Differences = 12* |
| EndoRez | 8.177 | 17.025 | *T+ =* |
| EndoRez | 3.794 | 11.217 | *T- = 78* |
| EndoRez | 5.988 | 12.948 | *Large Sample Approximation* |
| EndoRez | 3.333 | 10.244 | *Test Statistic Z = -3.059* |
| EndoRez | 6.627 | 17.889 | *P-Value = 0.0022* |
| EndoRez | 4.260 | 15.460 | |
| EndoRez | 6.794 | 15.622 | |
| EndoRez | 4.636 | 15.142 | |
| EndoRez | 8.141 | 17.357 | |
| EndoRez | 6.110 | 13.498 | |
| EndoRez | 5.823 | 11.315 | |
| **minimum** | 3.333 | 10.244 | |
| **Q1st** | 4.542 | 12.540 | |
| **Median** | 6.049 | 15.301 | |
| **Q3rd** | 6.854 | 16.666 | |
| **Maximum** | 8.177 | 17.889 | |

| Sealer | ΔE2 | ΔE6 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| Sealapex | 4.827 | 14.742 | *Number of Nonzero Differences = 12* |
| Sealapex | 7.619 | 14.545 | *T+ =* |
| Sealapex | 5.782 | 12.779 | *T- = 78* |
| Sealapex | 5.979 | 17.207 | *Large Sample Approximation* |
| Sealapex | 7.873 | 14.941 | *Test Statistic Z = -3.059* |
| Sealapex | 7.308 | 17.438 | *P-Value = 0.0022* |
| Sealapex | 6.424 | 20.758 | |
| Sealapex | 6.677 | 20.630 | |
| Sealapex | 7.690 | 20.798 | |
| Sealapex | 8.988 | 15.812 | |
| Sealapex | 11.305 | 18.478 | |
| Sealapex | 8.484 | 17.422 | |
| **minimum** | 4.827 | 12.779 | |
| **Q1st** | 6.313 | 14.892 | |
| **Median** | 7.463 | 17.315 | |
| **Q3rd** | 8.026 | 19.016 | |
| **Maximum** | 11.305 | 20.798 | |
| | | | |
| PCS | 8.478 | 17.531 | *Number of Nonzero Differences = 12* |
| PCS | 8.957 | 21.008 | *T+ =* |
| PCS | 9.887 | 16.542 | *T- = 78* |
| PCS | 10.847 | 17.304 | *Large Sample Approximation* |
| PCS | 10.351 | 20.872 | *Test Statistic Z = -3.059* |
| PCS | 7.295 | 15.264 | *P-Value = 0.0022* |
| PCS | 4.918 | 8.773 | |
| PCS | 8.933 | 9.555 | |
| PCS | 5.980 | 10.254 | |
| PCS | 4.317 | 10.464 | |
| PCS | 7.588 | 12.910 | |
| PCS | 4.655 | 9.787 | |
| **minimum** | 4.317 | 8.773 | |
| **Q1st** | 5.715 | 10.137 | |
| **Median** | 8.033 | 14.087 | |
| **Q3rd** | 9.189 | 17.361 | |
| **Maximum** | 10.847 | 21.008 | |

| Sealer | ΔE2 | ΔE6 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| **Positive** | 9.270 | 16.419 | *Number of Nonzero Differences = 6* |
| **Positive** | 4.811 | 9.062 | *T+ =* |
| **Positive** | 5.607 | 8.989 | *T- = 21* |
| **Positive** | 6.350 | 12.432 | *Large Sample Approximation* |
| **Positive** | 7.711 | 10.608 | *Test Statistic Z = -2.201* |
| **Positive** | 7.624 | 8.661 | *P-Value = 0.0277* |
| **minimum** | 4.811 | 8.661 | |
| **Q1st** | 5.793 | 9.062 | |
| **Median** | 6.987 | 12.432 | |
| **Q3rd** | 7.689 | 16.419 | |
| **Maximum** | 9.270 | 21.008 | |
| | | | |
| **Negative** | 2.339 | 5.204 | *Number of Nonzero Differences = 6* |
| **Negative** | 1.469 | 7.801 | *T+ =* |
| **Negative** | 3.811 | 9.056 | *T- = 21* |
| **Negative** | 7.549 | 9.099 | *Large Sample Approximation* |
| **Negative** | 6.393 | 9.887 | *Test Statistic Z = -2.201* |
| **Negative** | 2.700 | 8.472 | *P-Value = 0.0277* |
| **minimum** | 1.469 | 5.204 | |
| **Q1st** | 2.429 | 8.618 | |
| **Median** | 3.256 | 9.080 | |
| **Q3rd** | 5.748 | 11.796 | |
| **Maximum** | 7.549 | 21.008 | |

## Paired data analysis of differences between ΔE2 and ΔE8

| Sealer | ΔE2 | ΔE8 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| AH Plus | 4.154 | 8.176 | *Number of Nonzero Differences = 12* |
| AH Plus | 4.798 | 6.318 | *T+ = 10* |
| AH Plus | 4.666 | 4.377 | *T- = 68* |
| AH Plus | 8.695 | 3.754 | *Large Sample Approximation* |
| AH Plus | 5.665 | 10.457 | *Test Statistic Z = -2.275* |
| AH Plus | 6.808 | 11.810 | *P-Value = 0.0229* |
| AH Plus | 3.826 | 10.440 | |
| AH Plus | 8.172 | 11.918 | |
| AH Plus | 3.243 | 6.573 | |
| AH Plus | 5.228 | 9.243 | |
| AH Plus | 7.571 | 9.758 | |
| AH Plus | 5.367 | 11.606 | |
| **minimum** | 3.243 | 3.754 | |
| **Q1st** | 4.538 | 6.509 | |
| **Median** | 5.298 | 9.501 | |
| **Q3rd** | 6.999 | 10.744 | |
| **Maximum** | 8.695 | 11.918 | |
| | | | |
| EndoRez | 7.035 | 7.733 | *Number of Nonzero Differences = 12* |
| EndoRez | 8.177 | 9.163 | *T+ = 6* |
| EndoRez | 3.794 | 5.714 | *T- = 72* |
| EndoRez | 5.988 | 5.978 | *Large Sample Approximation* |
| EndoRez | 3.333 | 5.616 | *Test Statistic Z = -2.589* |
| EndoRez | 6.627 | 8.847 | *P-Value = 0.0096* |
| EndoRez | 4.260 | 7.727 | |
| EndoRez | 6.794 | 6.504 | |
| EndoRez | 4.636 | 7.634 | |
| EndoRez | 8.141 | 9.967 | |
| EndoRez | 6.110 | 7.333 | |
| EndoRez | 5.823 | 5.232 | |
| **minimum** | 3.333 | 5.232 | |
| **Q1st** | 4.542 | 5.912 | |
| **Median** | 6.049 | 7.483 | |
| **Q3rd** | 6.854 | 8.011 | |
| **Maximum** | 8.177 | 9.967 | |

| Sealer | ΔE2 | ΔE8 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| Sealapex | 4.827 | 7.563 | *Number of Nonzero Differences = 12* |
| Sealapex | 7.619 | 8.698 | *T+ = 9* |
| Sealapex | 5.782 | 8.038 | *T- = 69* |
| Sealapex | 5.979 | 9.524 | *Large Sample Approximation* |
| Sealapex | 7.873 | 7.001 | *Test Statistic Z = -2.353* |
| Sealapex | 7.308 | 9.679 | *P-Value = 0.0186* |
| Sealapex | 6.424 | 9.319 | |
| Sealapex | 6.677 | 10.132 | |
| Sealapex | 7.690 | 9.336 | |
| Sealapex | 8.988 | 8.048 | |
| Sealapex | 11.305 | 10.288 | |
| Sealapex | 8.484 | 9.089 | |
| **minimum** | 4.827 | 7.001 | |
| **Q1st** | 6.313 | 8.046 | |
| **Median** | 7.463 | 9.204 | |
| **Q3rd** | 8.026 | 9.563 | |
| **Maximum** | 11.305 | 10.288 | |
| | | | |
| PCS | 8.478 | 9.541 | *Number of Nonzero Differences = 12* |
| PCS | 8.957 | 10.072 | *T+ = 11* |
| PCS | 9.887 | 9.261 | *T- = 67* |
| PCS | 10.847 | 9.843 | *Large Sample Approximation* |
| PCS | 10.351 | 10.518 | *Test Statistic Z = -2.197* |
| PCS | 7.295 | 9.635 | *P-Value = 0.0281* |
| PCS | 4.918 | 6.339 | |
| PCS | 8.933 | 7.701 | |
| PCS | 5.980 | 7.283 | |
| PCS | 4.317 | 8.946 | |
| PCS | 7.588 | 8.987 | |
| PCS | 4.655 | 7.336 | |
| **minimum** | 4.317 | 6.339 | |
| **Q1st** | 5.715 | 7.610 | |
| **Median** | 8.033 | 9.124 | |
| **Q3rd** | 9.189 | 9.687 | |
| **Maximum** | 10.847 | 10.518 | |

| Sealer | ΔE2 | ΔE8 | Wilcoxson Signed Rank Sum Test |
|---|---|---|---|
| **Positive** | 9.270 | 11.234 | *Number of Nonzero Differences = 6* |
| **Positive** | 4.811 | 9.236 | *T+ =* |
| **Positive** | 5.607 | 7.350 | *T- = 21* |
| **Positive** | 6.350 | 8.881 | *Large Sample Approximation* |
| **Positive** | 7.711 | 9.198 | *Test Statistic Z = -2.201* |
| **Positive** | 7.624 | 8.148 | *P-Value = 0.0277* |
| **minimum** | 4.811 | 7.350 | |
| **Q1st** | 5.793 | 8.881 | |
| **Median** | 6.987 | 9.198 | |
| **Q3rd** | 7.689 | 9.687 | |
| **Maximum** | 9.270 | 11.234 | |
| | | | |
| | | | |
| **Negative** | 2.339 | 3.077 | *Number of Nonzero Differences = 6* |
| **Negative** | 1.469 | 8.374 | *T+ = 1* |
| **Negative** | 3.811 | 6.604 | *T- = 20* |
| **Negative** | 7.549 | 8.125 | *Large Sample Approximation* |
| **Negative** | 6.393 | 6.086 | *Test Statistic Z = -1.992* |
| **Negative** | 2.700 | 6.326 | *P-Value = 0.0464* |
| **minimum** | 1.469 | 3.077 | |
| **Q1st** | 2.429 | 6.396 | |
| **Median** | 3.256 | 8.250 | |
| **Q3rd** | 5.748 | 9.119 | |
| **Maximum** | 7.549 | 11.234 | |

**Appendix IV**

# Kruskal Wallis Test (Non-parametric one-way ANOVA)

## Comparing end point colour change at week 8 (ΔE8) between groups.

| Sealer | AH Plus | EndoRez | Sealapex | PCS |
|---|---|---|---|---|
| 1 | 8.176 | 7.733 | 7.563 | 9.541 |
| 2 | 6.318 | 9.163 | 8.698 | 10.072 |
| 3 | 4.377 | 5.714 | 8.038 | 9.261 |
| 4 | 3.754 | 5.978 | 9.524 | 9.843 |
| 5 | 10.457 | 5.616 | 7.001 | 10.518 |
| 6 | 11.810 | 8.847 | 9.679 | 9.635 |
| 7 | 10.440 | 7.727 | 9.319 | 6.339 |
| 8 | 11.918 | 6.504 | 10.132 | 7.701 |
| 9 | 6.573 | 7.634 | 9.336 | 7.283 |
| 10 | 9.243 | 9.967 | 8.048 | 8.946 |
| 11 | 9.758 | 7.333 | 10.288 | 8.987 |
| 12 | 11.606 | 5.232 | 9.089 | 7.336 |
| minimum | 3.754 | 5.232 | 7.001 | 6.339 |
| Q1st | 6.509 | 5.912 | 8.046 | 7.610 |
| Median | 9.501 | 7.483 | 9.204 | 9.124 |
| Q3rd | 10.744 | 8.011 | 9.563 | 9.687 |
| Maximum | 11.918 | 9.967 | 10.288 | 10.518 |

**Kruskal Wallis Test**

| Sealer | *Sample* | *Rank Sum* | *Sample Size* | *Test Statistic* |
|---|---|---|---|---|
| AH Plus | 1 | 336 | 12 | *H = 6.8912* |
| EndoRez | 2 | 184 | 12 | *P-Value = 0.0754* |
| Sealapex | 3 | 332 | 12 | |
| PCS | 4 | 324 | 12 | |